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Enhancing the Nutritive Value of Corn DDGS for Pigs

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ENHANCING THE NUTRITIVE VALUE OF CORN DDGS FOR PIGS

BY

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ENHANCING THE NUTRITIVE VALUE OF CORN DDGS FOR PIGS

This thesis is approved as a creditable and independent investigation by a candidate for the Master of Science degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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ABBREVIATIONS

AA	Amino acid(s)
ADF	Acid detergent fiber
AID	Apparent ileal digestibility
ATTD	Apparent total tract digestibility
BCVFA	Branched chain volatile fatty acid(s)
BW	Body weight
CP	Crude protein
CA	Citric acid
d	Day
DDGS	Dried distillers grains with solubles
DE	Digestible energy
DM	Dry matter
EE	Ether extract
g	Gram
GE	Gross energy
h	Hour
IVDDM	In vitro digestibility of dry matter

kg	Kilogram(s)
ME	Metabolizable energy
min	Minute
NDF	Neutral detergent fiber
NE	Net energy
NSP	Non-starch polysaccharide(s)
OIVDDM	Overall in vitro digestibility dry matter
SDSU	South Dakota State University
SID	Standard ileal digestibility
UI	International unit(s)
VFA	Volatile fatty acid(s)
Wet DG	Wet distillers' grains
WS	Whole stillage

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ABSTRACT

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Corn dried distillers' grains with solubles (DDGS) can be a good alternative feedstuff to the traditional corn soybean meal diets since it has high fat and protein content. However, it has a high fiber content, which is not well digested by pigs and can reduce nutrient utilization by encapsulation. The nutritive value of fibrous feedstuffs like DDGS can be improved by supplementation with fiber-degrading enzymes. However, fiber-degrading enzymes have not been effective in improving digestibility of DDGS. The overall goal of this thesis research to was to unravel why pigs poorly digest DDGS and to develop strategies that can increase the digestibility of DDGS in pigs.

Objective 1: to determine the effects of supplemental cocktail of fiber-degrading enzymes (multi-enzyme) on porcine in vitro porcine digestion and fermentation characteristics of corn DDGS and wet distillers' grains (Wet DG). With the goal of determining whether or not the drying of Wet DG into DDGS results in reduced digestibility of DDGS by pigs, and in reduced effect of fiber-degrading enzymes on digestibility of DDGS by pigs. Samples of DDGS and wet DG without or with the supplemental multi-enzyme in 2 × 2 factorial arrangement were hydrolyzed in 2 steps using pepsin and pancreatin. Undigested residues were incubated in a buffer solution with minerals and fresh pig feces as inoculum for determination of volatile fatty acid production and kinetics of gas production. The DDGS and Wet DG did not differ in porcine in vitro digestibility and fermentability. In addition, multi-enzyme did not affect porcine in vitro digestion and fermentation characteristics of DDGS or Wet DG. Thus, it appears that the drying of Wet DG into DDGS does not affect the digestibility of DDGS by pigs, and that effect of fiber-

degrading enzymes on the digestibility of DDGS by pigs is not influenced by drying of Wet DG into DDGS.

Objective 2: To determine the effects of pretreatment and predigestion technologies on in vitro digestion and fermentation characteristics of whole stillage (WS; slurry material that remains after distillation of fermented corn mash, which is subsequently centrifuged to obtain Wet DG that is dried into DDGS); thus, establishing if the poor digestibility of corn DDGS' fiber can improve the digestibility of the DDGS in pigs. This is because pretreatment and predigestion technologies can increase susceptibility of (the otherwise highly indigestible) fiber to digestion or fermentation. The WS was either untreated or pretreated with heat (at 160^{o}C and 70 psi for 20 min) alone or in combination with citric acid (10 g/L; CA), sulfuric acid (90 mM; H_2SO_4) or ammonia (1%; NH_3). Untreated WS and pretreated WS were un-predigested or predigested with multi-enzyme (for 24 h) in 5 × 2 factorial arrangement to give 10 treatment combinations. Pre-digested samples together with untreated and pretreated samples were freeze-dried and subjected to porcine in vitro digestion and fermentation as described in Objective 1. Pre-treatment of WS with heat, CA, H_2SO_4 or NH_3 increased ($P < 0.01$) in vitro digestibility of DM (IVDDM) by a mean of 13.2%. Also, multi-enzyme predigestion of untreated or pretreated WS increased ($P < 0.01$) IVDDM by a mean of 13.9%. Pretreatment of WS with heat, CA, or NH_2 did not affect total gas production. However, pretreatment of WS with H_2SO_4 decreased ($P < 0.01$) total gas production. Pretreatment of WS with heat, CA, H_2SO_4 or NH_2 decreased ($P < 0.01$) total VFA production. The results showed that the poor digestibility of DDGS fiber by pigs could be due to recalcitrance of DDGS fiber to enzymatic hydrolysis or fermentation, and that pretreatment and predigestions technologies can be used to improve nutritive value of WS and hence DDGS. Heat and CA pretreatment technologies can be attractive methods of improving the digestibility of DDGS because heat pretreatment is relatively cheaper than alkali or acid pretreatment, and CA is less corrosive than H_2SO_4 or NH_2.

Objective 3: to determine the effects of pretreating WS heat or CA on nutrient digestibility of the resulting DDGS for growing pigs. The WS was untreated or pretreated with heat (160^{o}C at 70 psi for 20 min) alone (heat) or with the heat plus CA (12 g/L; heat+CA). Untreated and pretreated WS were paddle-dried before their inclusion in diets. Five diets were fed; they included cornstarch-based containing DDGS, untreated WS, heat-pretreated WS, or CA-pretreated WS as the sole source of protein; and N-free diet. The DDGS diet was included for comparison. The 5 diets were fed to 10 ileal-cannulated barrows (57 ± 1.53 kg BW) in a replicated 5 × 5 Latin square to give 10 replicates/diet. Untreated WS had greater ($P < 0.001$) apparent ileal digestibility of GE than DDGS. Pretreatment of WS with heat or CA improved ($P < 0.001$) apparent ileal digestibility of GE, CP, and ether extract in diet. Pretreatment of WS with heat or CA reduced ($P < 0.001$) standardized ileal digestibility of most AA. Thus, pretreatment and drying of WS at conditions employed in the current study can improve energy digestibility, but reduce AA availability of the resulting DDGS for pigs.

Overall, it appears that the low digestibility of DDGS by pigs and limited effect of fiber-degrading enzymes on the digestibility of DDGS by pigs. Mainly because of recalcitrance of DDGS fiber in corn to enzymatic hydrolysis, and not because of drying of Wet DG into DDGS because pretreatment technologies that increase susceptibility of fiber to enzymatic degradation improved the digestibility of DDGS. Heat and CA pretreatment technologies can be attractive methods of improving the digestibility DDGS, but optimal conditions for the pretreatment of WS with heat and CA, and for drying the pretreated WS need to be identified.

GENERAL INTRODUCTION

Corn dried distillers' grains with solubles (**DDGS**) is a co-product ethanol industry that is available for livestock. The DDGS is produced large quantities in United States and other countries. For example, 23.3 million tons of DDGS were produced in 2016 in the United States (USDA, March 2017). In 2007, President George W. Bush announced the need for alternative fuel sources to alleviate foreign gas importation; this resulted in the use of corn for producing ethanol, an hence DDGS that is now used nationwide. Thus, DDGS can be a valuable feedstuff for livestock like pigs.

During the production of ethanol and DDGS from corn grain, starch in the corn grain is fermented into ethanol and carbon dioxide, which are removed to leave behind concentrated amounts of other nutrients in DDGS. Thus, DDGS compared with corn grain, has a higher content of protein (amino acids) and P (NRC, 2012), which are, respectively, the second and third most expensive nutrients in swine diets. In addition, DDGS has a greater content of fat than corn. However, like protein and P content, DDGS has greater content of fiber than corn grain (Stein and Shurson, 2009). Fiber is poorly digested by pigs, and can reduce nutrient utilization in pigs partly by reducing nutrient digestibility. In addition, amino acids, especially lysine can react with reducing sugars in DDGS to form Maillard reaction products during the drying of DDGS, leading to reduced availability of amino acids in DDGS.

The negative effects of fiber in DDGS can potentially be alleviated through supplementation with fiber-degrading enzymes (carbohydrases; (Yáñez et al., 2011) . However, carbohydrases have not been so effective in improving the nutrient digestibility of DDGS. For example, Jha et al. (2015) observed improved nutrient digestibility of

fibrous feedstuffs in pigs due to carbohydrase supplementation, whereas Yáñez et al. (2011) did not report improved digestibility of DDGS in pigs due to carbohydrase supplementation (Yáñez et al., 2011; de Vries et al., 2013; Woyengo et al., 2015). Thus, there is need to unravel why carbohydrases are not effective in digestion of DDGS.

It was hypothesized that fiber in corn DDGS combines with other nutrients during the drying of DDGS to form a complex that cannot be broken down by carbohydrases. This is because of the Maillard reaction, which results in heat damaged DDGS, leading to reduced nutrient digestibility (Jha et al., 2015). Jaworski and Stein (2017) reported that DDGS has greater content of fiber (non-starch polysaccharides; **NSP**) than wheat or wheat middlings, and that cellulose constitute greater proportion of NSP than wheat or wheat middlings. For instance, corn DDGS contained 12.95% cellulose, whereas wheat middlings contained only 6.6% cellulose (Jaworski and Stein, 2017). Cellulose is an insoluble NSP that is crystalline in nature, and hence it is poorly fermented in hindgut of pigs (Kootstra et al., 2009). Thus, it was also hypothesized that the fiber in corn DDGS is more resistant to enzymatic hydrolysis due to the high proportion of cellulose in its NSP.

If reason for lack of effect of fiber-degrading enzymes on digestibility of DDGS in pigs is due to drying process of DDGS, then the fiber-degrading enzymes would have a greater effect on wet distillers' grain (**wet DG:** wet slurry corn material for distilled corn residue) compared to DDGS. If reason for lack if effect carbohydrases on digestibility of DDGS in pigs is due to the high proportion of cellulose in corn DDGS fiber, then pretreatment of DDGS with heat or diluted acids or alkalis could result in the increase in susceptibility of fiber to enzymatic degradation. This is because the pretreatment of fibrous materials with heat, or diluted acids or alkalis resulted in a release of sugars from

fiber, implying that pretreatment can increase the availability of sugars within fiber for digestion in pigs (Kootstra et al., 2009; de Vries et al., 2013; de Vries et al., 2014). The pretreatment technology can be a good method of improving nutritive value DDGS for pigs because it can be integrated into the ethanol producing plants, leading to decreased cost of pretreating DDGS. Whole stillage (**WS)**, which is the slurry material that remains after distillation of fermented corn mash, and which is subsequently centrifuged and dried into DDGS) would be subjected to pretreatment, and then processed through the existing steps of centrifugation and drying. However, there is lack of information on influence of drying wet DG into DDGS on the effects of carbohydrase on digestibility of DDGS in pigs. Also, there is lack of information on effects of pre-treating WS with heat or diluted acids or alkalis on digestibility of DDGS in pigs. The overall objective of this thesis research was to unravel why pigs poorly digest DDGS and develop strategies to improve digestibility of DDGS in pigs.

1.0 LITERATURE REVIEW

1.1 Dried Distillers' Grains with Solubles

In United States, approximately 23.2 million tons of biofuel ethanol is produced from cereal grain by dry milling process, whereas 15.3 million tons is produced from cereal grain by wet milling process (Song and Shurson, 2013). The DDGS is the major co-product that is produced in dry mill plants, whereas corn gluten meal, corn gluten feed, and corn germ meal are the co-products that are produced in wet mill plants. The DDGS is also a co-product from ethanol beverage industry; however, its production from the ethanol beverage industry is less than 1% of the total DDGS produced in the United States. The DDGS from the ethanol beverage industry is often characterized as having a darker color and is more variable in nutrient composition than the "new generation" DDGS (which is DDGS from biofuel ethanol plants that were built after 1990) that is primarily used as feedstuff in the livestock industry.

Yellow dent corn is the most commonly used cereal grain for production of DDGS. Starch content constitutes approximately two thirds of corn. However, during the fermentation and distillation processes used in the dry mill ethanol plants, most of the starch is converted into ethanol and carbon dioxide to leave co-product (DDGS). This product has low concentration of starch and high concentration of non-starch components of corn grain such as fiber, amino acids, fat, and P. Corn DDGS has traditionally been used to formulate diets for ruminants due to its high fiber content and variable nutrient composition (Singh et al., 2005). However, it is becoming increasingly more popular in formulating non-ruminant diets because of its relatively high content of amino acids, fat (energy) and P (Singh et al., 2005; Belyea et al., 2010). The DDGS produced in new

generation modern ethanol plants has greater digestible energy and nutrient contents than DDGS that was produced in traditional ethanol plants (Shurson, 2003). The DDGS from the new generation ethanol plants located in the upper Midwest have particularly higher levels of digestible energy and nutrient content than old generation (Whitney and Shurson, 2004). These plants use enzymes and yeast to increase the conversion of starch to ethanol, and they use low temperature drying techniques that improve the nutritional value of DDGS for swine (Whitney and Shurson, 2004). Thus, the new generation DDGS can potentially be a good source of nutrients for non-ruminants.

1.2 Economic Impact of DDGS as Livestock Feed

Historically, DDGS was not used extensively in formulation of swine diets due to its low quality and high variability in nutrient content. The DDGS has high fiber content, which cannot be efficiently digested by pigs because pigs do not produce enzymes that are capable of digesting fiber. In addition, amino acids in DDGS are poorly digested in pigs due to overheating of the DDGS during the drying process, leading to the damage of amino acids.

Higher fuel prices combined with the Energy Policy Act of 2005, and the Energy Independence and Security Act of 2007, have partially stimulated United States' ethanol production growth. The usage of new generation DDGS in United States for swine feeding increased from approximately 30,000 tons in 2000 to more than 80,000 tons in 2002 (Shurson, 2003). Between 2001 and 2003, the percentage of DDGS used in the swine and poultry industry increased from 4 to 11% (Shurson and Noll, 2005). Due to high prices of conventional feedstuffs (such as corn, soybean meal, and di-calcium phosphorous), along with the abundance of the ethanol co-products, DDGS can be an

economically viable alternative to corn, soybean meal, and di-calcium phosphorous in the swine diets (Shurson and Noll, 2005; Belyea et al., 2010). Use of DDGS in formulating pig diets resulted in reduction in P excretion via manure and in an increase in number of weaned pigs per sow (Shurson and Noll, 2005), further indicating that DDGS can be an economically and environmentally viable feedstuff for pigs. The pork industry is very flexible; however, the industry has yet to capitalize on the DDGS market in an efficient way.

1.3 Physical Characteristics and Nutrient Composition of DDGS

The physical appearance, chemical composition, and nutrient digestibility of DDGS vary considerably depending on the source, processing method, and drying procedures. Rosentrater and Muthukumarappan (2006) reported that water activity in DDGS is highly correlated with bulk density and moderately correlated with thermal properties. Color is known to moderately to highly correlate with most other physical properties, such as moisture content, water activity, product conductivity, resistivity, diffusivity, and bulk density. Smell and color of DDGS correlate with its nutritional value for non-ruminants (Cromwell et al., 1993). Smell of DDGS can range from sweet to smoky and burnt, or musty smell. Smoky smell is due to overheating DDGS, whereas musty smell is associated with spoiled DDGS due to incomplete drying. The color of DDGS can range from light golden, which is ideal for feed usage; to dark brown, which is an indicator of heat damage of nutrients. For instance, DDGS with lighter color has greater digestible amino acid content than DDGS with darker color (Belyea et al., 2010) because of heat damage of amino acids in dark-colored DDGS products (Shurson and Noll, 2005; Stein, 2007).

The nutrient composition of DDGS has been extensively studied. The dry matter content of DDGS is around 89%, whereas the average CP, and crude fat contents in DDGS are 27%, and 8.9%, respectively (NRC, 2012). The average P and NDF contents in DDGS are 0.6% and 30%, respectively (NRC, 2012). Shurson and Noll (Shurson and Noll) reported that DDGS has higher total and available P contents than corn. Availability of P in corn was 14%, whereas availability of P in DDGS ranged from 80 to 90% (Gaines et al., 2007). Thus, DDGS could partially replace expensive inorganic sources of P that are commonly added in swine diets leading to reduction in cost of feed. The additional benefit of high availability of P in DDGS is a reduction of P that is excreted in the manure. In addition to the higher total and available P contents, DDGS has higher total amino acid content than corn. Corn has low lysine and tryptophan contents, and hence DDGS has relatively lower level of the same amino acids than other conventional protein feedstuffs such as soybean meal (Shurson and Noll, 2005). Lysine in DDGS can further be reduced by overheating DDGS during its production. This excessive heating often leads to darker-colored DDGS products; hence, golden-colored DDGS products that are not heat-damaged are of high nutritive value because they have amino acid (Song and Shurson, 2013). The total lysine methionine, threonine, and tryptophan content of DDGS are 0.9%, 0.57%, 0.99%, and 0.2%, respectively (NRC, 2012). However, the total contents of lysine, methionine, threonine and tryptophan content of corn are 0.25%, 0.18%, 0.28%, and 0.06%, respectively (NRC, 2012).

Ethanol plants have recently started to extract the oil from DDGS, resulting in de-oiled DDGS. Typically, the regular DDGS' oil content is between 6 and 9 % (NRC, 2012), however, de-oiled DDGS that has lower than 4% oil is currently available for

livestock feeding (NRC, 2012). Regular DDGS compared with de-oiled DDGS has lower NDF content (30.5% vs. 33.8%), but similar crude protein (NRC, 2012).

The DDGS has a higher content of fiber than most other cereal grain co-products such as wheat middlings, which reduces its digestion in pigs (Jaworski et al., 2015). For instance, corn DDGS contain 12.1% cellulose and 33.89% NDF, whereas wheat middlings contained 6.62% cellulose and 33.97% NDF (Jaworski et al., 2015). Urriola et al. (2010) reported that DDGS digestibility and metabolizable energy (ME) values are similar to those of corn. The GE, DE, ME, and NE values of corn are 3,933, 3,451, 3,395, and 2,672 kcal/kg, respectively (NRC, 2012). Corn DDGS has a slightly higher GE value (4,710 kcal/kg) than corn, however the DE value (3582 kcal/kg and ME value (3396 kcal/kg) values of corn DDGS are similar the corn values, whereas the NE value (2343 kg/kg) of corn DDGS is lower than that for corn (NRC, 2012).

At this time, no industry quality standards exist for DDGS due to the variability in composition of corn used; and high variability in ethanol production process, and storage and handling of the product across facilities. For instance, inside the ethanol plant, the processing technologies such as the type of yeast used for fermenting, fermentation and distillation times, quantity of solubles blended with the distillers' grains, and drying temperatures and time have the potential to alter the nutrient composition of DDGS (Kerr and Shurson, 2013).

Overall, DDGS is similar to corn in DE and ME values, has higher total amino acid and P contents than corn, and has high content of available P than corn. However, DDGS have lower NE and is more variable in quality than corn due to the high variation in processes it goes through to during its production.

1.4 Effect Dietary DDGS on Growth Performance of Pigs

1.4.1 Nursery Pigs

Dried distillers' grains with solubles can be included in diets fed to pigs starting at weaning stage. The United States Grain Council (2010) recommended an inclusion rate of up to 20% of DDGS for nursery diets. However, some studies have reported a temporary reduction in feed intake by nursery pigs due to dietary inclusion of DDGS (Avelar et al., 2010). While research varies on the maximum inclusion rate, the commercial standard is no more than 25% for nursery pigs with body weight of up to 7 kg (Whitney and Shurson, 2004; Shurson and Noll, 2005). The inclusion of DDGS in nursery diets at 10% has been shown to improve gut health (Shurson and Noll, 2005). However, when DDGS is included in diets to replace corn and soybean meal, there is need to supplement the diets with synthetic amino acids to ensure adequate dietary supply of amino acids to the pigs because DDGS has a lower amino acid content than a combination of corn and soybean meal. In addition to the lower amino acid content in DDGS than in a combination of corn and soybean meal, DDGS has lower density than corn or soybean meal, which limits its inclusion in weaned pig diets that are nutrient dense; therefore, 5% dietary inclusion rate of DDGS is preferred within industry (Whitney and Shurson, 2004).

Due to conflicting results from previous studies, optimal dietary levels of DDGS for nursery pigs have not been clearly identified. For instance, Stein (2007) reported that dietary inclusion of DDGS up to 7.5% DDGS did not negatively affect growth performance of nursery pigs immediately after weaning. Furthermore, others have suggested that DDGS could be included in diets at 30% 2 to 3 weeks of pigs to minimize the negative effects of DDGS on growth performance when it is included in diets before

2 weeks post-weaning (Hoffman and Baker, 2011). Senne et al. (1996) observed no effect of including 20% of DDGS in isocaloric diets for nursery pigs on the rate and efficiency of gain. Moreover, the inclusion of DDGS at rate of 20% did not affect ADG, ADFI, or G:F of nursery pigs (Senne et al., 1996). Pelleting diets for nursery pigs containing 30% DDGS did not effects on ADF, ADFI, or G:F. However, pelleting process improved the energy utilization in late nursery pigs fed the diet containing 30% DDGS (Zhu et al., 2010).

Seabolt et al. (2010) determined the preferences of nursery pigs to a non-DDGS diet, a DDGS diet, or high-protein DDGS, and observed reduction in preference of diets containing DDGS or high-protein DDGS even at lower inclusion rates. In their study, there was a negative correlation between preference and crude fiber, possibly due to low energy density of higher fiber diets. Fiber present in a feed can also affect the texture of the feed, which in turn, can influence feed preference (Hastad et al., 2005).

Overall, the inclusion of DDGS nursery pig diets is not so common; however, when included, the dietary inclusion rate typically does not get above 10%. The biggest concern for the inclusion of DDGS in nursery diets is the need for additional synthetic amino acids, high fiber content in DDGS and low palatability of DDGS-containing diets.

1.4.2 Grow-Finish Pigs.

The effect of including DDGS in diets for grow-finish pigs has been determined in several studies. Majority of studies reported no differences between pigs fed DDGS-containing diets and those fed corn-soybean meal based diets with regard to growth performance. However, some studies reported reduced growth performance of grow-finish pigs due to dietary inclusion of DDGS. Senne et al. (1996) observed no effect of including 30% of DDGS in isocaloric diets for grow finish pigs on the rate and efficiency

of gain. Stein (2007) conducted several experiments in which inclusion of 30% DDGS in diets for grow-finish pigs did not affect growth performance of the pigs. Hoffman and Baker (2011) reported a reduced grow-finish pig growth performance when DDGS was included in diets at 30%. Whitney and Shurson (2004), reported that pigs fed 25% DDGS had performed same as pigs fed diets without DDGS with regard to growth rate. Other studies also reported no effect of a 25% inclusion rate of DDGS on ADG, ADFI, G:F (Cook et al., 2005; DeDecker et al., 2005). Cook et al. (2005) showed a decrease in pig mortality as the level of DDGS in the diet increased from 0 to 30%. However, a decrease in ADG and G:F was observed as the inclusion rate of DDGS was increased from 20% to 30% (Whitney et al., 2006; (Benz et al., 2010). Hastad et al. (2005) observed that preferences of DDGS by grow finish pigs decreased linearly as its inclusion rate increased from 0% to 30% in the diets.

The effect of dietary inclusion of DDGS on growth performance of grow-finish pigs has been inconsistence, and the reasons for the inconsistence can only be speculated. This inconsistence may be due to batch to batch or plant to plant variation in drying methods, levels of residual sugars, or grain quality (Hastad et al., 2005; Belyea et al., 2010). It is hypothesized that the reduced growth performance of pigs due to dietary inclusion of DDGS is due to reduced feed intake because of low DDGS quality or palatability. If the DDGS added in diet has a low lysine content and digestibility, pig performance would be expected to decline since lysine is the first limiting amino acid in practical diets for pigs. It is impossible to determine if the performance decline is due to DDGS in the diet or increased crude protein. However, inclusion of crystalline lysine or tryptophan in pig diets may reduce the negative impact of increasing crude protein

through dietary inclusion of DDGS (Stein, 2007). Whitney and Shurson (2004) reported that the reduction in growth rate of the pigs because of dietary inclusion of DDGS might be partially due to reduction in dietary energy concentration. High fiber content in DDGS may also have reduced the growth performance through reduction in nutrient digestibility (Whitney and Shurson, 2004).

In studies where DDGS and corn were compared, several differences became relevant. For instance, GE intake, along with fecal and urine excretion GE were greater for diets containing DDGS than in those that were based on corn (Benz et al., 2010). A greater N absorption in pigs fed DDGS-based diets than in those fed corn-based diet was also observed; however, the percentage of nitrogen retained did not differ among diets. Phosphorus intake, fecal excretion, and fecal retention were greater in diets with DDGS based diets compared with corn diets (Benz et al., 2010).However, there were no differences among DDGS diets on phosphorus intake and fecal or urine excretion or retention of P (Benz et al., 2010).

In general, DDGS can be included diets up to 30% without significant effects on growth performance for grow finish. However, inclusion of DDGS in diets for grow-finish pigs at levels greater than 30% results in reduced growth performance likely due to high fiber content and varied nutrient content from various drying standards. In addition, the low lysine content in DDGS limit its inclusion in diets for grow-finish pigs.

1.4.3 Effects of Dietary DDGS on Meat Quality

In recent studies, dietary DDGS has had varying effects on carcass traits of pigs. Benz et al, (2010) reported that loin depth of pigs fed DDGS-based diets were not affected when DDGS was included at in diets at 20%. Carcass weight, percent yield,

backfat, and fat-free lean index decreased as the dietary level of DDGS increased beyond a 20% inclusion rate (Benz et al., 2010). Diets with 30% to 45% DDGS did not have a major effect on growth performance, but resulted in softer bellies (Cromwell et al., 2011). After reviewing data from several studies, Stein and Shurson (2009) concluded that growth performance does not change when DDGS is included in diets at 30%, but carcass characteristics such as carcass yield and jowl iodine values are adversely when DDGS is included in diets at ≥30%. It was suggested that these adverse effects are due to the high fiber and unsaturated fatty acid contents in DDGS (Stein and Shurson, 2009). Graham et al. (2014) reported a decrease in carcass yield and hot carcass weight with an increase in dietary inclusion level from 0 to 45% DDGS from 73.98 to 71.84% and 93.39 to 88.52%. They also determined an increasing effect of DDGS on jowl (70.2 to 76.3%), and observed an increase in iodine value due to an increase in level of DDGS' oil in diet. Bergstrom et al. (2014) reported decreased final body weights, hot carcass weight, and backfat but also increased jowl iodine value of pigs due to increasing dietary DDGS from 20 to 60%. Also, iodine values of backfat of grow-finish pigs were linearly increased with a linear increase in dietary level of DDGS from 58.4 to 72.1% and 61.1 to 82.2% and hence linear increase in dietary level of unsaturated fatty acids (Xu et al., 2008; Cromwell et al., 2011).

Recently, (Xu et al., 2016) determined the effect of dietary de-oiled DDGS on meat and carcass quality of grow-finish pigs, and observed no effect of replacing regular DDGS with de-oiled DDGS on iodine value or belly fat. Theoretically, DDGS with low fat content may adversely affect carcass yield, but not carcass fat value if it is included at higher levels (greater than 30%). However, this was not observed in the study of (Xu et

al., 2016). Thus, more research on effects of including de-oiled DDGS in diets for grow-finish pigs on carcass traits should be conducted.

In summary, dietary DDGS negatively affect meat and carcass traits by increasing iodine values of jowl fat and backfat, and by decreasing loin depth and carcass yield. The increase in jowl fat and backfat iodine values of pigs because of dietary inclusion of DDGS is due to presence of unsaturated fatty acids in DDGS. The decrease in carcass yield of pigs because of dietary inclusion of DDGS is due to relatively high level of fiber in DDGS. Thus, inclusion of DDGS with low fat in diets for grow-finish may be limited by its high fiber content and not fat content.

1.5 Effects of Dietary DDGS on Gut Health

Minimal research has been conducted on the influence dietary DDGS gut health of pigs. The interest has been on the effect of dietary DDGS on growth performance; it is understood thoroughly that DDGS negatively affect energy and nutrient digestibility, leading to reduced growth performance. The fiber present in DGGS, which is mostly insoluble in nature, has the potential to provide gut health benefits in pigs (Jha and Berrocoso, 2015).

Selective inclusion of dietary fiber in diet can be used as a nutritional approach to improve the intestinal health of pigs, despite its lesser digestibility and significant negative effects on digestibility of other nutrients. Zijlstra et al. (2010) reported that soluble non-starch polysaccharides can reduce gastric release of digesta and reduce nutrient digestibility, leading to increased amount of digesta flow to large intestine and hence alteration in fermentation in large intestine. Owusu-Asiedu et al. (2006) noted that insoluble NSP increase gut fill due to reduced energy and protein digestibility, the

reduced digestibility is attributed to NSP overrides an expected marginal increase in energy and protein.

Nursery and grow-finish pigs are able to utilize moderate levels of fiber in their gastrointestinal tract, but there is a need to increase their ability to effectively utilize high amounts of fiber in DDGS along with the energy associated with the fiber (Kerr and Shurson, 2013). Kerr and Shurson (2013) observed an inverse relationship between fiber content and energy digestibility. The apparent ileal digestibility and total tract digestibility of dietary fiber in DDGS is similar to that in corn (Urriola et al., 2010). However, less than 50% of total dietary fiber is digested over the entire digestive tract; this indicates that more than 50% passes through the pigs without being fermented (Urriola and Stein, 2010).

Fiber alters the gastrointestinal tract of pigs in several ways. Some of these include increasing in the empty weight of the gastrointestinal tract, cell turnover rate and metabolic demand. It can also influence the gut health by decreasing the rate of gastric emptying via soluble fiber or by increasing rate of the passage of digesta (Kerr and Shurson, 2013).

The DDGS is aggregate of resistant non-fermentable starch and non-starch components that form during its production. Most starch and non-starch components of DDGS interact to form complexes that could be resistant to digestion by pepsin and pancreatic enzymes. Fiber fraction contains non-starch polysaccharides that the pig is unable to digest since pigs do not produce enzymes that digest fiber (Jones et al., 2010). Moreover, the total gas and volatile fatty acids and individual fatty acids production was greater for the undigested residue of corn DDGS compared to corn (Jones et al., 2010).

This could has been due to the increased fermentable substrate following in vitro digestibility of dry matter of corn DDGS, because corn DDGS contained more starch, in the form of resistant starch, which is highly fermentable (Jha et al., 2011b). Production of volatile fatty acids, especially butyric acid, in hindgut leads to improved gut health.

The effects of DDGS and other forms of insoluble fiber on gut health of pigs and other mammals have revealed possible mechanisms by which DDGS may alter gut health of pigs (Wilberts et al., 2014). Insoluble fiber increases digesta passage rate, leading to reduced available time for pathogenic microorganisms to proliferate and attach to gut mucosa (Molist et al., 2014). Insoluble fiber improved gut health of weaned pigs (Wellock et al., 2008; Molist et al., 2009).

It was noted that fermented feedstuffs tended to decrease the population of lactic acid bacteria and anaerobic bacteria mostly in the large intestine while increasing the pH of the lower gut in pigs (van Winsen et al., 2001). Widyaratne and Zijlstra (2007) hypothesized that reduced nutrient digestibility, feed intake, and ultimately reduce energy intake of diets containing co-products.

Based on results from these studies, it appears that DDGS can have an effect on the gut health of pigs. However, there is need of more research to determine the effects of dietary DDGS on gut health of pigs.

1.6 Feed Enzymes for DDGS-Based Diets

The main anti-nutritional factors in plant feedstuffs such as DDGS are phytate (which is a storage form of P in plant feedstuffs) and fiber (Woyengo et al., 2014). Non-ruminants such as pigs cannot digest phytate-bound P (Zijlstra and Beltranena, 2007). Furthermore, phytate has capacity to bind to other nutrients in the gut, leading to their

reduced digestibility (Woyengo et al., 2014). As previously mentioned, fiber is poorly digested by pigs and can reduce nutrient digestibility (Jha et al., 2011a).

The negative effects of phytate and fiber can be alleviated through supplementation with phytase and fiber-degrading enzyme, respectively. Phytase can breakdown phyate to release phytate-bound P for digestion and reduce the capacity of phyate to bind nutrients in the gut (Selle and Ravindran, 2007; Almeida and Stein, 2010). Supplementation of phytase to DDGS-based diets for grow-finish pigs increased standardized total tract digestibility (STTD) of P (Kiarie et al., 2010). However, low concentrations of phytate-bound P in DDGS may reduce the effectiveness of phytase in improving the digestibility of P in DDGS when compared with corn (Almeida and Stein, 2010). The increase in STTD of P due phytase in corn and corn germ could be predicted by a regression equation (Almeida and Stein, 2010). However, the increase in STTD of P for DDGS due to phytase cannot be accurately predicted by regression equations because of the limited effects of phytase on STTD of P for DDGS (Almeida and Stein, 2010). Others have reported increased energy digestibility in pigs due to supplemental phytase (Brady et al., 2002; Shelton et al., 2003; Jendza et al., 2005). Anderson et al. (2012) suggested that there is a possible effect of phytase on energy digestibility, but the effect could be relatively small and highly variable.

Commercial swine diets contain fibrous feedstuffs such as DDGS (Partridge and Marlborough, 2009). To improve the feed efficiency of pigs in a commercial setting, exogenous enzymes that degrade fiber (non-starch polysaccharides; **NSP**) have been widely added in commercial diets. The European swine industry has indeed found such enzymes to be beneficial in swine diets. Non-starch polysaccharides are complex

carbohydrates, other than starches, which are not digested in the small intestine of pigs. Carbohydrases such as α-galactosidease, β-1,4-mannanase, β-glucanase, and xylanase have been shown to breakdown NSP, leading to increased digestibility of the NSP and other dietary components.

Kiarie et al. (2010) reported that a combination of multi-carbohydrase and phytase improved nutrient digestibility of barley- and wheat-based diets for pigs. However, addition of protease to multi-carbohydrase-supplemented DDGS reduced in vitro degradation of DDGS (Woyengo et al., 2014). It has been hypothesized that the protease may degrade the microbial and supplemental multi-carbohydrase, leading to reduced nutrient digestibility in pigs (Yin et al., 2001). Jha et al. (Jha and Berrocoso) improved porcine in vitro digestibility of wheat DDGS due to supplemental multi-carbohydrase; however, the improvement in the digestibility of wheat DDGS by the supplemental multi-carbohydrase was lower for heat damaged wheat than for wheat DDGS that had not been heat damaged. After reviewing several articles, Jha and Berrocoso (2015) concluded that multicarbohydrases can effectively increase the fermentability of DDGS; however, the effectiveness varies depending on the heat damage of DDGS.

1.7 Supplementing DDGS-Based Diets with Enzymes on Growth Performance of Pigs.

The effects of supplemental fiber-degrading enzymes on growth performance of pigs fed DDGS-based diets have been determined in several studies. Supplementation of a carbohydrase product that contained α-galactosidase, or galactomannanase, orβ-glucanase, or xylanase to diet containing 30% DDGS did not improve weaned pig growth performance, but improved performance when it was added to corn-soybean meal-based diet (Jones et al., 2010). Ao et al. (2010) reported insignificant changes in

growth performance, but an increase in apparent ileal digestibility of N and amino acids in grow finish pigs due the multi-carbohydrase supplementation of DDGS-based diets. Lee et al. (2012) reported that supplementation of a combination of mannanase and phytase to DDGS-based diet for grow-finish pigs decreased ADG and ADFI. Yoon et al. (2010) observed that mannase supplementation to DDGS diets for grow-finish pigs resulted in an improved growth performance. Young et al. (1993) reported that supplementation of mannanase diets for containing 10 or 15% DDGS diets improved growth performance and ATTD of DM, GE and CP of grow-finish pigs. However, Wang et al. (2011) observed that β-mannanase did not improve the energy and nitrogen digestibility of DDGS-based diet for pigs.

Reasons why fiber-degrading enzymes have been inconsistent in improving nutrient availability in DDGS and hence performance of pigs has been suggested. They include Maillard reaction between AA and sugars (during the drying of DDGS) to form indigestible complexes; short retention time feed in the gastrointestinal tract, leading to reduced time of interaction between fiber and fiber-degrading enzymes; and the resistance of fiber to enzymatic hydrolysis (Kootstra et al., 2009; Woyengo et al., 2014).

1.8 Effects of Pre-digesting Lignocellulose Biomass with Enzymes on Nutrient Availability

Enzyme predigestion can be potentially improve the nutritive value of fibrous feedstuffs such as DDGS because of limited time of interaction between fiber degrading enzymes and DDGS in the gastrointestinal tract (Fan et al., 1987). Fastinger (2005) showed that a 24-hour saccharification with a cocktail of carbohydrases increased energy digestibility of DDGS by pigs from 69 to 85%. In addition, steeping of DDGS followed

by enzymatic predigestion of the DDGS significantly improved the amino acid and energy digestibility of the DDGS-based diets by pigs (Fastinger, 2005). Therefore, predigesting DDGS with enzyme prior to inclusion of the DDGS can result in greater improvement in nutrient utilization than simply adding the enzyme directly to the diet (Fastinger, 2005). There is need for more research to determine effects of enzymatic pre-digestion of DDGS on its nutritive value for pigs.

While enzymatic predigestion of DDGS seems to be a promising technology for improving the nutritive value of the DDGS, enzymatic predigestion of WS may be a more effective with regard to cost of enzymatic predigestion. This is because the enzymatic predigestion technology can be in integrated in ethanol plants. The WS would be predigested and then processed through the existing steps of centrifugation and drying, eliminating the cost of re-slurring DDGS for enzymatic predigestion and drying of predigested DDGS.

1.9 Effects of Pre-treatment of Lignocellulose Biomass on Fiber Digestion

As previously mentioned, supplemental fiber-degrading enzyme have not been effective in improving the DDGS digestibility in pigs. This lack of effect of fiber degrading enzymes on DDGS digestibility could partly be due to resistance of fiber in DDGS to enzymatic degradation. The DDGS has a relatively high content of cellulose, and like any other feedstuff of plant origin, it has lignin in its cell wall matrix. Cellulose is poorly fermented in non-ruminants because it is crystalline in nature. Enzymes that are produced by microorganisms during fiber fermentation cannot degrade lignin, and lignin reduces fermentation of fiber by chemically binding the fiber and by physically blocking the accessibility of the enzymes to the fiber. Fibrous feedstuffs or lignocellulose biomass

can be pretreated by various methods to release sugars, which are then fermented to produce ethanol (Jørgensen et al., 2007). The same pretreatment could be used to improve DDGS digestibility because DDGS contain cellulose and lignin. Pretreatment of lignocelluloses can result in disruption of the crystalline structure of cellulose (Taherzadeh and Karimi, 2007), de-polymerization of NSP, breakdown of bonds between lignin and NSP, and degradation of lignin. There are many methods of pretreating fibrous feedstuffs to release sugars for fermentation. The pretreatment methods are broadly classified as physical, chemical, and biological. Among them, chemical methods of pretreatment (hot water, dilute acid and dilute alkali hydrolysis) are the most commonly used methods of pretreatment (Hendriks and Zeeman, 2009). Esteghlalian et al. (1997) observed that diluted acids degraded large amounts of hemicellulose (80%) of corn stover, leading to increased accessibility of fiber-degrading enzymes to cellulose. The main disadvantage of dilute acid pretreatment is the necessity of neutralization of pH for the downstream enzymatic hydrolysis (Taherzadeh and Karimi, 2007). Alkali pretreatment results in solvation and saponification of lignocellulose biomass followed by swelling of the biomass, thus making it more accessible for enzymatic and bacterial degradation (Hendriks and Zeeman, 2009). Alkali pretreatment utilizes lower temperatures and pressures compared with acid hydrolysis (Esteghlalian et al., 1997).

Several studies have investigated the effects of various pretreatment technologies on release of sugars from lignocellulosic biomass. Diluted sulfuric acid was effective in hydrolysing cellulose in lignocellulosic biomass (Esteghlalian et al., 1997). Also, Sun and Cheng (2002) observed that pretreatment of lignocellulose biomass with sulfuric acid resulted in increased hydrolysis of fiber in the lignocellulose biomass. Pretreatment of

DDGS with diluted maleic acid increased the degradation of NSP in DDGS by at least 30% (de Vries et al., 2013). Thus, pretreatment technologies can used to improve the nutritive value of DDGS for pigs. Also, pretreatment technologies can be in integrated in ethanol plants, where WS can be pretreated and be processed into DDGS as previously described.

Pre-treatment of fibrous materials with inorganic acids can generate significant amounts of toxic compounds such as furans that inhibit activities of digestive enzymes (Kootstra et al., 2009). Furthermore, inorganic acids and alkalis are corrosive, and hence pretreatment of fibrous feedstuffs with inorganic acids and alkalis can be expensive because of the requirement special treatment reactors (Wyman et al., 2005). However, pre-treatment of fibrous materials with hot water (heat) or diluted organic acids at ≤170°C does not generate significant amounts of toxic compounds such as furans that inhibit activities of digestive enzymes (Kootstra et al., 2009). Thus, hot water and organic acid pretreatment technologies are potentially good methods of pretreating feedstuffs for livestock feeding.

1.10 Effects of Pre-digestion of Pretreated Lignocellulose Biomass on Nutrient Availability

Predigestion of pretreated fibrous feedstuffs can potentially enhance the nutritive value of the feedstuffs for pigs because the pretreatment can result in increased susceptibility of the fiber to enzymatic hydrolysis. Feng et al. (Feng et al.) reported that treatment of wheat straw at 200°C for 30 minutes with hot water, diluted acids (including sulfuric, oxalic, citric and acetic acids), or diluted ammonia resulted in >90% fiber hydrolysis following enzyme pretreatment. Predigestion of hot water- or ammonia fiber

expansion-pretreated DDGS with multi-carbohydrase resulted in increased hydrolysis of NSP, releasing over 90% of the total glucose yield in DDGS (Kim et al., 2008). Wang et al. (2011) investigated the effects of ammonia fiber explosion process pretreatment on the enzymatic hydrolysis of both wet and dry DDGS, and observed virtual completion of the conversion of cellulose to glucose after seventy-two hours of the predigestion. Dien et al. (2008) determined the effects of pretreating DDGS with hot water and ammonia fiber explosion process followed by predigestion with a mixture of commercial cellulase and β-glucosidase, and observed increased release of glucose from cellulose the pretreatments of DDGS followed by the enzymatic predigestion. Thus, it is apparent that pretreatment of fibrous feedstuffs such as WS followed by enzymatic predigestion can result in improved nutritive value of the resulting DDGS for pigs.

1.11 Conclusions

The DDGS is available in large quantities for livestock feeding. The DDGS has high content of AA, P and fat, and hence it can a good source of energy in swine diets. However, the inclusion of DDGS in swine diet is limited partly by its high fiber content, which reduces nutrient digestibility in DDGS. Fiber-degrading enzymes have not been effective in improving the digestibility of DDGS. It appears that the limited effect of fiber-degrading enzymes on the digestibility of DDGS for pigs is due to heat damage of DDGS during its drying or resistance of corn fiber to enzymatic hydrolysis or both. Pretreatment and predigestion technologies have been used to improve fiber degradation in fibrous feedstuffs including DDGS and crop residues such as wheat and rice straw. However, there is lack of information on the influence of drying Wet DG into DDGS on the effects of fiber-degrading enzymes on digestibility of DDGS. Also, the effects

pretreating and predigesting WS on nutritive of the resulting DGSS for pigs have not been reported. Pretreatment and predigestion of WS in ethanol plants is more economically viable method of improving the nutritive value of DDGS than pretreatment and predigestion of DDGS. Thus, there is need to fill this gap in knowledge.

2.0 Porcine in vitro digestion and fermentation characteristics of corn wet distillers' grains and DDGS without or with multi-enzyme

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2.1 ABSTRACT

A study was conducted to determine porcine in vitro digestion and fermentation characteristics of wet distillers' grains and DDGS without or with multi-enzyme (Superyme-CS, Canadian Bio-Systems Inc., Calgary, AB) that supplied 9,600 U of xylanase, 1,200 U of glucanase, 4,000 U of cellulase, 480 U of mannanase, 5,600 U of invertase, 40,000 U of protease, and 96,000 U of amylase/kilogram of feedstuff. Four gram samples were weighed into conical flasks (5 flasks per treatment) and hydrolyzed in 2 steps using pepsin and pancreatin. Subsequently, residues were incubated in a buffer solution with minerals and fresh pig feces as inoculum. Gas production was measured for 72 h, and modeled to estimate kinetics of gas production. Concentration of VFA per unit weight of residue incubated or feedstuff was measured in fermented solutions. On DM basis, the wet distillers' grains and DDGS contained 23.52 and 28.87% CP, and 6.25 and 10.99% ether extract, respectively. In vitro digestibility of DM (IVDDM) of wet distillers' grains (50.4%) was similar to that of DDGS (48.6%). Multi-enzyme supplementation did not affect the IVDDM of wet distillers' grains or DDGS. Total gas production of residue incubated for wet distillers' grains was similar to that of DDGS (120.7 vs. 115.8 mL/g DM). Multi-enzyme did not affect the total gas production of residue incubated for wet distillers' grains. Wet distillers' grains and DDGS were similar in degradation rate of incubated residue. There was no effect of multi-enzyme supplementation on degradation rate of incubated residue for wet distillers' grains or DDGS. Total VFA production of residue incubated for wet distillers' grains was similar to that of DDGS (5.55 vs. 5.33 mmol/g DM). Also, wet distillers' grains and DDGS were similar in individual VFA production of incubated residue. Multi-enzyme did not affect

the total or individual VFA production of residue incubated for wet distillers' grains or DDGS. In conclusion the wet distillers' grains and DDGS were similar in in vitro digestibility and fermentability. The multi-carbohydrase used in the current study has limited effect on porcine in vitro digestibility of DDGS or wet distillers' grains.

Key words: pig, DDGS, digestibility

2.2 INTRODUCTION

Dried distillers' grains with solubles (**DDGS**) is co-products from cereal grain ethanol industry. Compared with corn, DDGS has a higher content of AA and fat (Spiehs et al., 2002; NRC, 2012). Moreover, DDGS has energy value that is close to that of corn for pigs (Shurson et al., 2003; NRC, 2012). However, DDGS has high in fiber (non-starch polysaccharides, **NSP**), which is poorly digested by pigs, and decreases the utilization of nutrients by pigs (Stein and Shurson, 2009)

The NSP degrading enzymes could alleviate the negative effects that dietary fiber has for pigs. However, the enzymes have improved the digestibility of cereal grain-based diets (Zijlstra et al., 2010; Woyengo et al., 2015), but have not consistently improved the digestibility of DDGS (Yáñez et al., 2011; Woyengo et al., 2015). Jha et al. (2015) reported that starch granules in wheat grain were separated from other components of wheat, whereas starch granules in wheat-derived and corn-derived DDGS interacted with other components of DDGS to form complexed aggregates. Jha et al. (2015) also reported that and that the intensity of interaction between starch and other components of DDGS was less in DGGS with light-brown color than in DDGS with dark-brown color (which is

an indicator of Maillard reaction), implying that the interactions occurred during the drying stage of DDGS. Thus, we hypothesized that components of condensed distillers combine with fiber in wet distillers grains (**Wet DG**) to form complexes that are resistant to fiber degrading enzymes during the drying stage of producing DDGS from wet DG and condensed distillers. However, information is lacking on the effect of adding thin stillage to Wet DG and drying the resulting mixture into DGGS on response of NSP degrading enzymes with regard to digestion and fermentation characteristics of DDGS for pigs. Objective of this study was to determine the effects of supplemental cocktail of fiber-degrading enzymes (multi-enzyme) on porcine in vitro digestion and fermentation characteristics of DDGS and Wet DG.

2.3 MATERIALS AND METHODS

2.3.1 Feedstuffs

The DDGS) and wet DG were obtained from Dakota Ethanol (Wentworth, SD) from the same batch of corn grain. Wet DG was freeze dried since it was originally in liquid form. The DDGS and dried Wet DG samples were ground to pass through a 0.75 mm screen using a Thomas Wiley Laboratory Mill (Model 4; Thomas Scientific grinder (Swedesboro, NJ, USA)). The DDGS and Wet DG were unsupplemented or supplemented with multi-enzyme at 1% (v/w) in a 2 × 2 factorial arrangement to give 4 treatment combinations. The multi-enzyme product used was Superzyme-CS, (Canadian Bio-Systems Inc., Calgary, AB, Canada), and it supplied 24,000 U of xylanase, 3,000 U of glucanase, 10,000 U of cellulase, 1,200 U of mannanase, 14,000 U of invertase, 10,000 U of protease, and 24,000 U of amylase/kilogram of DDGS and Wet DG. The

unsupplemented and multi-enzyme-supplemented DDGS and Wet DG were subjected to in vitro digestion and fermentation as described below.

2.3.2 In vitro digestion

Samples were subjected to in vitro digestion as described by Woyengo et al. (2015). Four grams of samples were weighed into 500 mL conical flasks. A phosphate buffer solution (200 mL, 0.1 M, pH 6.0), HCl solution (80 mL, 0.2 M) and fresh pepsin (4 mL, 20 g/L porcine pepsin, P-0609; Sigma-Aldrich Corp.) were then added into the flasks with the samples. Additionally, 2 mL of chloramphenicol (C-0378; Sigma-Aldrich Corp., St. Louis, Mo) solution (0.5g/100 mL) was added in the flasks to prevent bacterial growth during the enzymatic hydrolysis. The samples were then placed into a water bath at 39 ^{o}C for 2 h under a gentle agitation (50 revolutions per min). Subsequently, phosphate buffer solution (80 mL, 0.2 M, pH 6.8), NaOH (20 mL, 0.6 M), and fresh pancreatin solution (8 mL, 100 g/L pancreatin; P-1750 Sigma-Aldrich Corp.) were added into the flasks, and digestion was continued for 4 h at the same conditions under which the samples were digested with pepsin. The residues of the samples after the digestion were collected by filtration on a nylon cloth (50 μm), and then washed with ethanol (2 × 25 mL 95% ethanol) and acetone (2 × 25 mL 99.5% acetone). The washed residues were dried for 12 h at 60 ^{o}C and weighed for determination of in vitro digestibility of DM digestibility (**IVDDM**). The in vitro digestion was done in 5 batches in order to sufficient amounts of undigested residues for in vitro fermentation. The experiment was conducted as a complete block design with the flask as experimental unit, and batch as block. The undigested residues from different batches were pooled together for each treatment for determining in vitro fermentation.

2.3.3 In vitro fermentation

The fermentation of undigested residues from the in vitro enzymatic digestion of DDGS and Wet DG without and with enzyme supplementation was evaluated in vitro using a cumulative gas-production technique that has been adapted to the pig (Bindelle et al., 2007; Jha et al., 2011a; Jha et al., 2015). Two hundred milligrams of the undigested residues were weighed into 125 mL-glass bottle (Wheaton^{TM} 223748, ThermoFischer Scientific, Waltham, MA) containing 30 mL buffer solution that contained macro- and micro-minerals (Menke and Steingass, 1988) and a fecal inoculum. The undigested residues were then incubated within a water bath at 39 ^{o}C with a slight agitation of 50 revolutions per min.

The fecal inoculum was obtained from three growing pigs from the South Dakota State University's Animal Science Complex, where they were fed a corn-DDGS-soybean meal grower diet with no antibiotics. Fecal samples were collected straight from the rectum and instantly placed in air-tight plastic syringes and kept in a water bath at 39 ^{o}C until used for fermentation, which started within 30 minutes after fecal collection. The inoculum prepared from the fecal samples was diluted 20 times using the buffer solution, and then filtered through a 250 µm screen (E.H. Sargent and Co., Chicago, IL). The inoculum was then transferred into the 150 ml bottles with the fermentation substrates. The bottles were sealed with a rubber stopper and placed within the water bath for incubation.

The anaerobic environment was constantly maintained throughout the experiment, from inoculum preparation until the incubation step by flushing with carbon dioxide gas. The gas generated during fermentation was measured at 0, 2, 5, 8, 12, 18, 24, 36, 48, and

72 h using a pressure transducer (SIN-54978; GP:50, Grand Island, NY, USA) (Mauricio et al., 1999) that was fitted with a digital data tracker (Blue Ribbon Corp., Grand Island, NY). The bottles were vented after each reading using a needle. After 72 h of incubation, fermentation was stopped by placing the bottles in ice. The contents of the bottles were collected and stored in a -20ºC freezer. The experimental scheme for in vitro fermentation was as follows: (4 treatments × 5 replicates/treatment) + (6 blanks) × (2 batches).

2.3.4 Sample Analysis

2.3.4.1 Chemical Analysis of Feedstuffs and Undigested Residues

Ground DDGS and Wet DG samples were analyzed for dry matter (Redmer et al., 2004), crude protein (**CP**), either extract (**EE**), neutral detergent fiber (**NDF**). Samples were analyzed for DM (method 930.15), CP (method 984.13), EE (method 920.39A) and NDF (method 2002.04) of AOAC (2005).

2.3.4.2 Volatile Fatty Acid Analysis

Samples collected from the bottles after fermentation were centrifuged at 3,000 g × for 30 min at 4 ºC. The supernatant was collected after centrifugation for VFA analysis, and the solid residue was freeze-dried and weighed for determination in vitro fermentability of DM (**IVFDM**). The concentration of VFA in the liquid phase of the fermented samples was determined using gas chromatography in a method described by Erwin et al. (1961) with some modifications. Briefly, 0.8 mL of sample was added into a 1.5 mL centrifuge tube that contained 0.2 mL of 25% phosphoric acid and 0.2 mL of internal standard solution (150 mg of 4-methyl-valeric acid, S381810, Sigma-Aldrich

Corp.) and vortexed for 1 min. Afterwards, the samples were analyzed for VFA (i.e., acetic, propionic, butyric, isobutyric, valeric, isovaleric, and caporic acids) using Gas Chromatograph (Trace 1310, ThermoFischer Scientific, Waltham, MA) with a Stabilwx-DA column (30-m x 0.25-mm i.d.; Restek, Bellefonte, PA). A flame-ionization detector was used with an injector temperature of 170 °C and a detector temperature of 190 °C. Branched-chain VFA content was calculated as the sum of the isobutyric and isovaleric acids.

2.3.5 Calculations

The IVDDM (%) after pepsin and pancreatin hydrolysis was calculated as follows:

$$\text{IVDDM} = \left(\frac{\text{dry weight of intact sample - dry weight of hydrolysed residue}}{\text{dry weight of intact sample}} \right) \times 100 \quad (1)$$

The IVFDM (%) after in vitro fermentation was calculated as follows:

$$\text{IVFDM} = \left(\frac{\text{dry weight of hydrolysed residue - dry weight of fermented residue}}{\text{dry weight of hydrolysed residue}} \right) \times 100 \quad (2)$$

Overall in vitro digestibility of DM (**OIVDDM**) was calculated as sum of IVDDM and IVFDM.

Gas pressure measurements were converted into gas volume (G, per gram DM) using the ideal gas law, assuming an atmospheric pressure of 101325 Pa and a temperature of 312.15 K. Gas accumulation curves recorded during the 72 h of fermentation were modelled according to France et al. (France et al.) (France et al., 1993):

G (mL g-1 DM) = 0, if $0 < t < L$ (4)

$$G \text{ (mL g}^{-1} \text{ DM)} = G_f\left(1 - \exp\{-\langle b\,[t - L] + c[\sqrt{t} - \sqrt{L}]\rangle\}\right),\ if\ t\ \geq L \quad (5)$$

where, G denotes the gas accumulation to time, G_f (mL/g DM) the maximum gas volume for t = ∞ and L (h) the lag time before the fermentation starts. The constants b (h^{-1}) and c ($h^{-1/2}$) determine the fractional rate of degradation of the substrate μ (h^{-1}), which is postulated to vary with time as follows:

$$\mu = b + \frac{c}{2\sqrt{t}}, \quad \text{if } t \geq L \quad (6)$$

Kinetics parameters (G_f, L, $\mu_{t=T/2}$ and T/2) were compared in the statistical analysis. The T/2 is the time to half-asymptote when $G = G_f / 2$.

2.3.6 Statistical Analysis

The IVDDM, IVFDM, total gas production, fermentation kinetics parameters and fermentation metabolites production were subjected to ANOVA using MIXED procedure of SAS (ver. 9.3, SAS Institute Inc., Cary, NC). Feedstuff means were separated by the least significant difference. To test the hypotheses, $P < 0.05$ was considered significant.

2.4 RESULTS

The DDGS had a higher content of CP and EE than Wet DG. However, Wet DG had higher amounts of NDF than DDGS (Table 2.1.). Wet DG did not differ from DDGS with regard to IVDDM (Figure 2.1.). Moreover, the multi-enzyme supplementation did not affect IVDDM of DDGS or Wet DG (Figure 2.1.). There were no interactions between feedstuff and enzyme on IVDDM (Figure 1).

Per unit weight of undigested residue or feedstuff, the IVFDM for DDGS was similar to that for Wet DG (Table 2.2.). Multi-enzyme supplementation did not affect the IVFDM and OIVDDM of DDGS or Wet DG. Lag time, half time, rate of degradation, and total gas production did not differ among all treatments (Table 2.2., Figure 2.2.).

There were no interactions between feedstuff and enzyme on IVFDM, OIVDDM and gas kinetics (Figure 2.1.).

Per unit weight of undigested residue or feedstuff, DDGS and Wet DG without or with multi-enzyme supplementation did not differ in total VFA production (Table 2.3.). Moreover, there were no effects of treatment on acetic acid, propionic acid, butyric acid and BCVFA (branched chain volatile fatty acid) production (Table 2.3.). Total VFA production did not differ as per unit weight of residue for treatments as well. Similar results were found with acetic acid, propionic acid, and branched-chain VFA production (Table 2.3).. However, butyric acid for Wet DG, but not for DDGS, tended to decrease ($P = 0.07$) due to multi-enzyme supplementation. There were no interactions between feedstuff and multi-enzyme on VFA production.

2.5 DISCUSSION

The objective of this study was to determine porcine in vitro digestion and fermentation characteristics of DDGS and Wet DG without and with multi-enzyme supplementation. Both Wet DG and DDGS are co-products of dry milling of cereal grains to obtain ethanol. During the production of ethanol from corn grain, most of the starch is converted into ethanol and carbon dioxide to leave a slurry material known as whole stillage. The whole stillage is centrifuged to separate it into solid and liquid phases. The solid material is known as Wet DG, whereas the liquid material is known thin stillage. The Wet DG has a relatively greater content of fiber and lower content of fat and soluble carbohydrates, protein, and minerals than thin stillage. The DDGS is produced by evaporating the thin stillage to form syrup, followed by mixing of the syrup with Wet DG and drying the mixture. The DDGS had lower moisture content than Wet DG, which is

expected because the former is dried, whereas the latter is not dried before it is marketed for use as a feedstuff. The DDGS had greater content of CP and EE and lower content of NDF than Wet DG, which is also expected because DDGS contain syrup, which has a greater content of EE and soluble protein and lower content of NDF than Wet DG. The CP (28.9%), EE (11.0%), and NDF (33.5%) values for DDGS were similar to the values (30.6% CP, 10.0 % EE, and 34.1% NDF, on DM basis) that were reported by NRC (NRC, 2012) for DDGS containing between 6 and 9% oil. The NDF value (46.4%, on DM basis) for Wet DG was also similar to the value (46.1%, on DM basis) that was reported by NRC (NRC, 2012) for Wet DG. However, CP and EE values (23.5 and 6.3%, respectively, on DM basis) for Wet DG were lower than the values (31.8% CP and 9.57% EE, on DM basis) that were reported by NRC (NRC, 2012) for Wet DG. These differences in chemical composition of Wet DG used in the current study and that reported by NRC (NRC, 2012) could have been due to differences among ethanol plants with regards to fermentation conditions and amounts of soluble nutrients that were removed from whole stillage during the production of Wet DG.

There was no significant difference between DDGS and Wet DG with regard to IVDDM. Fiber is indigestible by pepsin and pancreatic enzymes, whereas fat and soluble protein and carbohydrates such as simple sugars are highly digested in small intestine of pigs. The digestibility of Wet DG would be expected to be lower than that for DDGS because the former has greater content of fiber and lower content of soluble nutrients than the latter. However, it should be noted that Wet DG used in the current study was freeze-dried before it's in vitro digestion and fermentation, whereas the DDGS is produced in ethanol plants by drying the mixture of Wet DG and syrup at a relatively high

temperature. Drying of feedstuffs at high temperature results in a reaction (Maillard reaction) between amino acids and reducing sugars and amino acids to form complexes that are poorly digested by pepsin and pancreatic enzymes. Lysine to CP ratio (which is indicator of extent of Maillard reaction and hence amino acid availability) for corn grain (3.63%) was greater than that for corn DDGS (3.48%; Jaworski and Stein, 2017), indicating that the amino acid availability is indeed reduced during the production of DDGS from corn grain. Thus, the similarity between DDGS and Wet DG with regard to IVDDM could have been due to greater fiber content in Wet DG than in DDGS, and low digestibility of soluble sugars and amino acids in DDGS.

Multi-enzyme supplementation did not affect IVDDM. The digestibility of wheat grain in pigs was increased by supplementation of fiber-degrading enzymes, whereas the digestibility of wheat-derived DDGS in pigs was not improved by the supplementation of fiber-degrading enzymes (Yáñez et al., 2011). Jha et al. (2015) determined the matrix structure of wheat and wheat-derived DDGS, and observed that starch granules in wheat grain were separated from non-starch components, whereas starch granules in the wheat DDGS were combined with components of the DDGS such as protein and fiber to form complex aggregates. In their study, starch and non-starch components were more aggregated in dark-colored wheat DDGS than in light-colored wheat DDGS, implying that the intensity of formation of the aggregates was increased with an increase in drying temperature. Thus, it had been hypothesized that the multi-enzyme would increase the IVDDM of Wet DG (compared with the increase of that of DDGS) by greater magnitude because the components in Wet DG are less aggregated than those in DDGS. Thus the lack of effect of multi-enzyme on IVDDM of both Wet DG and DDGS imply that the

drying of a mixture of Wet DG and syrup into DDGS does not influence the effect of fiber-degrading enzymes on the digestibility of DDGS. However, it should be noted that DDGS has greater content of fiber than wheat and its milling by-products such as wheat middllings (NRC, 2012), and that cellulose constitute greater proportion of fiber in corn DDGS than in wheat or wheat middllings (Jaworski and Stein, 2017). For instance, corn DDGS contained 12.95% cellulose, whereas wheat middlings contained only 6.6% cellulose (Jaworski and Stein, 2017). Cellulose is relatively less susceptible to fiber-degrading enzymes because it is crystalline in nature (Kootstra et al., 2009). Thus, the lack of effect of multi-enzyme on the digestibility of Wet DG and DDGS could have been due to the recalcitrance of fiber (in these co-products) to multi-enzymatic hydrolysis. Indeed, Jaworski and Stein (2017) reported that wheat middlings, compared with corn DDGS, had greater digestibility of non-starch polysaccharides in small intestine and hindgut of pigs.

There was no significant difference in IVFDM between DDGS and Wet DG. Also, total gas and total VFA production for DDGS were not different from those for Wet DG. In a previous study, wheat DDGS in which starch and non-starch components were more aggregated was less extensively fermented in vitro than wheat DDGS in which starch and non-starch components were less aggregated (Jha et al., 2015). Thus it had been hypothesized that Wet DG would be more fermentable than DDGS. The lack of difference between Wet DG and DDGS with regard to their porcine in vitro fermentation could have been due to the recalcitrance of fiber in these 2 co-products to microbial degradation in hind gut of pigs. Butyric acid production for undigested residue of DDGS was greater than that of Wet DG, and the reason for this is not clear.

The IVFDM was unaffected by multi-enzyme supplementation. Also, total gas and VFA production for DDGS or Wet DG were not affected by multi-enzyme supplementation. It had been hypothesized that the multi-enzyme would have more positive effect on the degradation of undigested residue for Wet DG than of residue for DDGS. This lack of effect of multi-enzyme on fermentation of Wet DG and DDGS could be attributed to the recalcitrance of fiber in these 2 to enzymatic hydrolysis. The OIVDDM for DDGS was not different from that of Wet DG. Also, OIVDDM for DDGS or Wet DG was unaffected by multi-enzyme supplementation, which was due to the lack of differences between DDGS and Wet DG with regard to IVDDM and IVFDM.

In conclusion, the wet distillers' grains and DDGS were similar in in vitro digestibility and fermentability. The multi-enzyme did not affect porcine in vitro digestibility and fermentation characteristics of DDGS and Wet DG. Thus, the lack of effect of fiber-degrading enzymes on the digestibility of DDGS by pigs may not be due to drying of the mixture of Wet DG and syrup into DDGS.

2.6 TABLES AND FIGURES

Table 2.1. Analyzed composition (on a DM basis) of test feedstuffs

Item, %	DDGS	Wet DG
DM	91.56	80.23
CP	28.87	23.52
EE	10.99	6.25
NDF	33.51	46.37

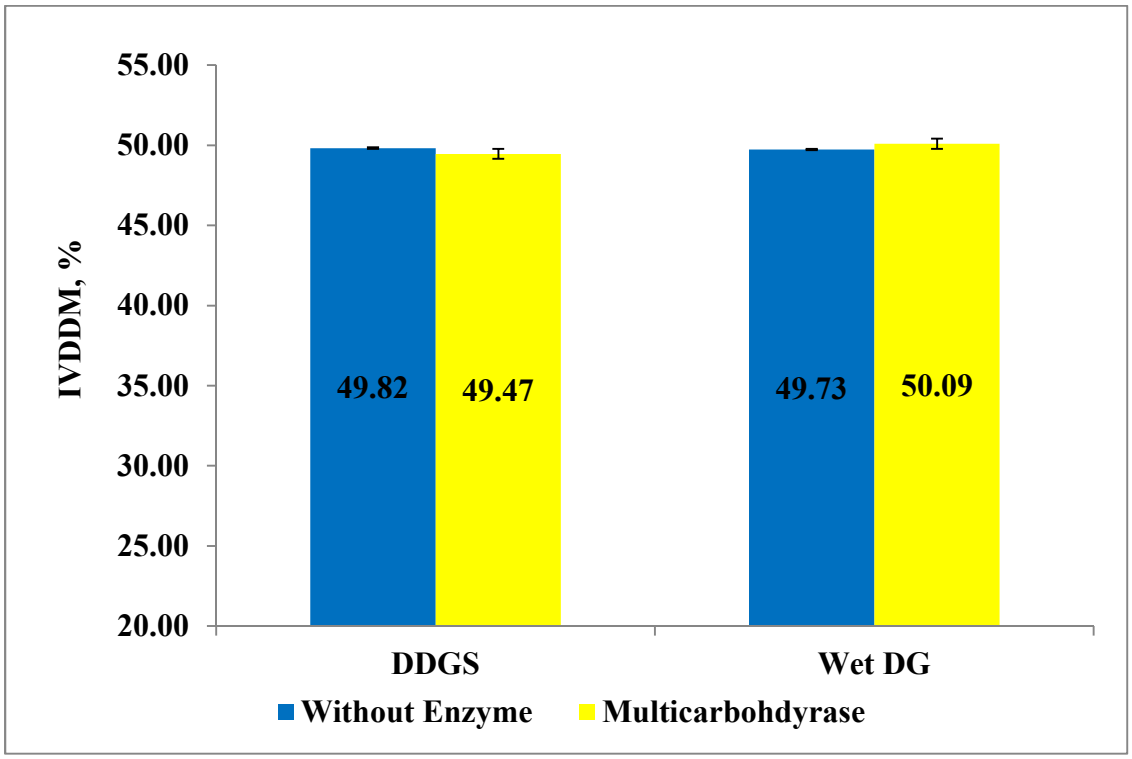

Figure 2.1. In vitro digestibility of DM of DDGs and Wet DG

[1-]Enzyme = without enzyme pre-digestion; +Enzyme = pre-treatment was followed by enzymatic hydrolysis for 24 h at pH 4.6 and 38ºC. The enzyme supplied 1,200 U of xylanase, 150 U of glucanase, 60 U of mannanase, 700 U of invertase, 5,000 U of protease, and 12,000 U of amylase/kg of feedstuff; Superzyme-CS, 500 ml/L . The solid loading rate was 10%.

[1]Enzyme = without enzyme pre-digestion; +Enzyme = pre-treatment was followed by enzymatic hydrolysis for 24 h at pH 4.6 and 38ºC. The enzyme supplied 48 U of xylanase, 6 U of glucanase, 2.4 U of mannanase, 28 U of invertase, 200 U of protease, and 480 U of amylase/kg of feedstuff; Superzyme-CS, 20 ml/L. The solid loading rate was 10%.

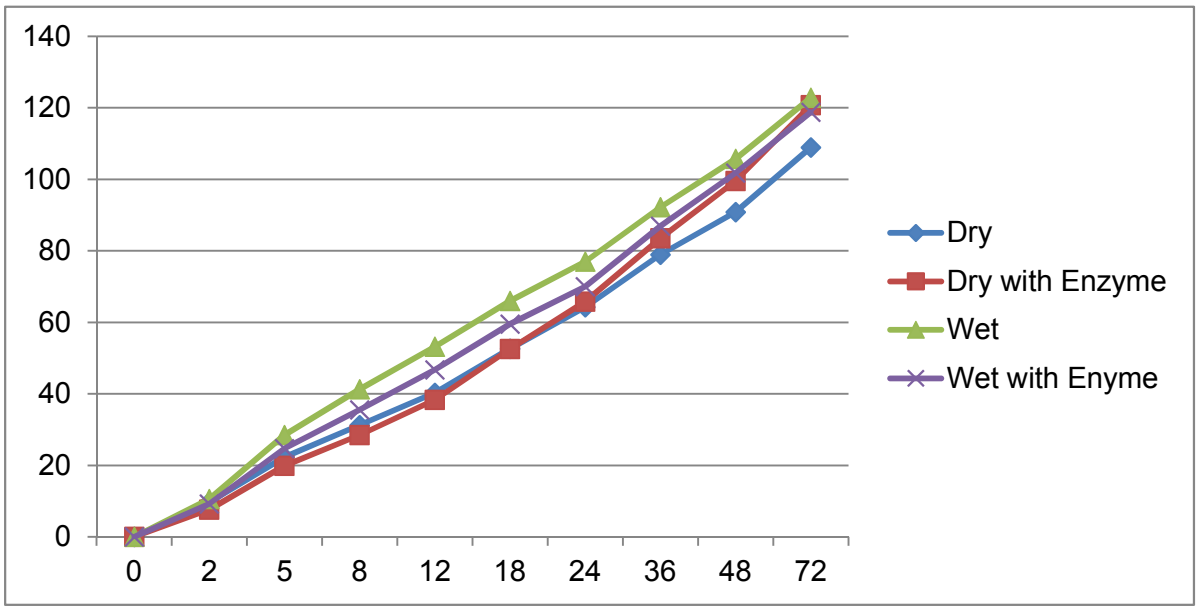

Figure 2.2. In vitro fermentation of DM of DDGs and Wet DG

Table 2.2 Fitted kinetics parameters (means) of gas accumulation after in vitro fermentation of DDGs and wet DG

	Without Enzyme		With Enzyme			P-value		
Variable	DDGS	Wet DG	DDGS	Wet DG	SEM	Feedstuff	Enzyme	Interaction
IVFDM, %								
Per unit weight of undigested residue	22.01	17.44	20.64	19.10	3.89	0.44	0.78	0.80
Per unit weight of feedstuff	33.74	31.80	34.47	31.07	1.64	0.40	0.15	0.51
OIVDDM	83.56	81.54	83.93	81.17	1.63	0.38	0.24	0.86
Fermentation kinetics								
Lag time	0.64	3.71	0.26	4.10	2.47	0.38	0.28	0.42
Half time	19.33	19.86	17.03	22.16	2.64	0.89	0.18	0.33
Rate of degradation	0.28	0.28	0.28	0.28	0.09	0.99	1.00	0.99
Total gas	115.79	120.71	116.88	119.63	4.06	0.40	0.63	0.24

[1]Enzyme = without enzyme pre-digestion; +Enzyme = pre-treatment was followed by enzymatic hydrolysis for 24 h at pH 4.6 and 38ºC. The enzyme supplied 48 U of xylanase, 6 U of glucanase, 2.4 U of mannanase, 28 U of invertase, 200 U of protease, and 480 U of amylase/kg of feedstuff; Superzyme-CS, 20 ml/L. The solid loading rate was 10%.

Table 2.3. VFA production after in vitro fermentation of DDGs and wet DG

	Without Enzyme		Multicarbohydrase			P-value		
Variable	DDGS	Wet DG	DDGS	Wet DG	SEM	Feedstuff	Enzyme	Interaction
VFA concentration, mmol/g DM feedstuff								
Total VFA	5.33	5.55	5.42	5.46	0.24	0.49	0.89	1.00
Acetic acid	2.49	2.62	2.56	2.56	0.15	0.49	1.00	0.95
Propionic Acid	1.52	1.57	1.54	1.54	0.05	0.55	0.99	0.87
Butryic Acid	1.53	1.57	1.55	1.54	0.05	0.64	0.90	0.76
BCVFA	0.14	0.12	0.13	0.13	0.01	0.06	0.88	0.83
VFA concentration, mmol/g DM undigested residue								
Total VFA	5.64	5.19	5.36	5.47	0.22	0.16	0.72	0.93
Acetic acid	2.67	2.42	2.53	2.56	0.13	0.19	0.84	0.94
Propionic Acid	1.60	1.48	1.53	1.55	0.05	0.11	0.87	0.92
Butryic Acid	0.61	0.48	0.55	0.55	0.05	0.07	0.98	0.77
BCVFA	0.14	0.13	0.13	0.13	0.00	0.07	0.99	0.97

[1]Enzyme = without enzyme pre-digestion; +Enzyme = pre-treatment was followed by enzymatic hydrolysis for 24 h at pH 4.6 and 38ºC. The enzyme supplied 48 U of xylanase, 6 U of glucanase, 2.4 U of mannanase, 28 U of invertase, 200 U of protease, and 480 U of amylase/kg of feedstuff; Superzyme-CS, 20 ml/L. The solid loading rate was 10%.

[2]Branched chain volatile fatty acids

Figure Legends

Figure 1. In vitro digestion of dry matter of DDGs and Wet DG samples. -Enzyme = without enzyme pre-digestion; +Enzyme = pre-treatment was followed by enzymatic hydrolysis for 24 h at pH 4.6 and 38ºC. The enzyme supplied 1,200 U of xylanase, 150 U of glucanase, 60 U of mannanase, 700 U of invertase, 5,000 U of protease, and 12,000 U of amylase/kg of feedstuff; Superzyme-CS, 500 ml/L . The solid loading rate was 10%.

Figure 2. Gas production kinetics of the undigested residue of soybean meal and canola co-products during a 72-h incubation with fecal inoculum of DDGs and Wet DG. . -Enzyme = without enzyme pre-digestion; +Enzyme = pre-treatment was followed by enzymatic hydrolysis for 24 h at pH 4.6 and 38ºC. The enzyme supplied 48 U of xylanase, 6 U of glucanase, 2.4 U of mannanase, 28 U of invertase, 200 U of protease, and 480 U of amylase/kg of feedstuff; Superzyme-CS, 20 ml/L. The solid loading rate was 10%.

3.0 Porcine in vitro digestion and fermentation characteristics of pretreated and predigested whole stillage[1]

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3.1 ABSTRACT

Inclusion of corn DDGS in swine diets is limited by its low NE:GE and nutrient digestibility due to its high fiber content. Pre-treatment of whole stillage with heat, diluted acids or alkalis, or fiber-degrading enzymes can potentially improve DDGS digestibility. Thus, a study was conducted to determine the effects of pretreating WS with heat, diluted citric acid, sulfuric acid or ammonia, without or with subsequent enzymatic hydrolysis, on porcine in vitro digestion and fermentation characteristics. The WS was either untreated or pretreated with heat (at 160ºC and 70 psi for 20 min) alone or in combination with citric acid (10 g/L; CA), sulfuric acid (90 mM; H_2SO_4) or ammonia (1%; NH_3). Parts of untreated sample and of each of the pretreated samples were further hydrolyzed with the multi-enzyme product Superzyme-CS at 10 ml/kg per DM WS for 24 h. This provided the following enzyme dosages per kg WS: 24,000 U of xylanase, 3,000 U of glucanase, 10,000 U of cellulase, 1,200 U of mannanase, 14,000 U of invertase, 10,000 U of protease, and 24,000 U of amylase/kg of WS. The untreated and pretreated samples were dried and digested in two steps using pepsin and pancreatin. Undigested residues were incubated in a buffer solution with fresh pig feces as inoculum for 72 h, and gas and VFA produced were measured. Dried untreated, heat-pretreated, CA-pretreated, H_2SO_4-pretreated, and NH_3-pretreated WS contained 31, 32, 33, 31, and 38% CP; and 23, 21, 12 19, and 18% total non-starch polysaccharides, respectively. Pre-treatment of WS with heat, CA, H_2SO_4 or NH_3 increased ($P < 0.01$) in vitro digestibility of DM (IVDDM) by 15.7, 15.1, 15.8, and 19.6%, respectively. Also, multi-enzyme hydrolysis of untreated and heat-, CA-, H_2SO_4-, or NH_3-pretreated WS increased ($P < 0.01$) IVDDM by a mean of 13.9%. Pretreatment of WS with H_2SO_4

reduced ($P < 0.05$) total gas production of residue incubated by a 15%. Pretreatment of WS with heat, CA, H_2SO_4 or NH_2 decreased ($P < 0.01$) total VFA production per unit weight of feedstuff by a mean of 36%. In conclusion, IVDDM of WS was improved by the heat, CA, H_2SO_4 or NH_3 pretreatment and multi-enzyme hydrolysis. Thus, heat pretreatment or multi-enzyme pre-digestion, or both can be attractive methods of improving the digestibility of WS and hence DDGS because heat pretreatment is relatively cheaper than alkali or acid pretreatment, and enzymes are often added in swine diets and hence their use for enhancing nutritive value of the DDGS will not significantly alter the feed cost.

Key words: pig, DDGS, predigestion, pretreatment, in vitro

3.2 INTRODUCTION

Corn dried distillers' grains with solubles (**DDGS**) has a high content of AA and oil and hence it can be a good source of AA and energy in swine diets (Spiehs et al., 2002; Jha et al., 2015). However, DDGS has a low NE:GE due to high fiber (insoluble non-starch polysaccharides; **NSP**) content, which reduces nutrient utilization in pigs (Stein and Shurson, 2009; Jaworski et al., 2015).

Supplemental NSP degrading enzymes (carbohydrases) may improve digestibility of fiber and other nutrients. However, dietary enzymes have not been effective in improving the digestibility of DDGS (Yáñez et al., 2011; Woyengo et al., 2015; Zangaro et al., 2017) likely due to the recalcitrance of insoluble NSP to hydrolysis or the short retention time of feed within the gastrointestinal tract for enzyme hydrolysis, or both. For

example, cellulose, which is an insoluble NSP that is crystalline in nature and hence not easily degraded by carbohydrases (Kootstra et al., 2009), constituted greater proportion of NSP in DDGS (38.2%) than of NSP in corn grain (16.0%) or in wheat millrun (19.5%; Jaworski and Stein, 2017). Apparent ileal digestibility of NSP (1.5%) for DDGS for pigs was lower than that (46.6%) for wheat millrun (Jaworski and Stein, 2017), implying that NSP in DDGS is indeed poorly degraded in pigs. Also, some NSP are complexed with lignin, which reduces their availability for digestion.

Pretreatment of whole stillage (**WS**; slurry material that remains after distillation of fermented corn mash, which is subsequently centrifuged and dried into DDGS) with heat, or with diluted acids or alkalis at high temperature and pressure may improve the nutritive value of DGGS. This is because the pre-treatment can result in destruction of hydrogen bonds among the NSP and depolymerization of NSP (Kootstra et al., 2009), thereby increasing the susceptibility of NSP to enzymatic hydrolysis (de Vries et al., 2014). The pretreatment of WS and not of DDGS can be an attractive technology of improving the nutritive value of the DDGS because this technology can be integrated into currently existing corn ethanol production facilities with minimal cost. The effect of pretreating DDGS with inorganic and organic acid at high temperature on NSP degradation and in vitro digestibility of DDGS has been reported (de Vries et al., 2013). However, information is lacking on the effect of pretreating WS with heat, diluted acids or alkalis, or enzyme on NSP composition, and digestion and fermentation characteristics of the resulting DDGS for pigs. The objectives of this study were to determine the effects of: (1) pretreating WS with heat, diluted citric acid, diluted sulfuric acid, and diluted ammonia on NSP composition, and porcine in-vitro digestion and fermentation

characteristics; and (2) pre-digestion of heat-, citric acid-, sulfuric acid-, and ammonia-pretreated WS with multi-enzyme on NSP composition, and porcine in-vitro digestion and fermentation characteristics. In vitro digestibility and fermentation techniques were used to achieve the objectives in this study because the in vitro assays are cheaper and faster, and hence they can be used to screen several treatments. Furthermore, in vitro digestion and fermentation characteristics of fibrous feedstuffs simulate their digestion gastrointestinal tract of pigs (Jha et al., 2015; Woyengo et al., 2016b).

3.3 MATERIALS AND METHODS

3.3.1 Whole Stillage and Pre-treatment

The WS was obtained from Dakota Ethanol (Wentworth, SD) in one lot. A portion of the WS was pretreated with heat (at 160^{o}C and 70 psi for 20 min) alone or in combination with citric acid (10 g/L; **CA**), sulfuric acid (90 mM; **H_2SO_4**), or ammonia (1%, w/w; **NH_3**) at the National Center for Agricultural Utilization Research (Peoria, IL) using the Techne Industrial Fluidized Sand Bath (model IFB-101, Princeton, NJ). Untreated WS and pretreated WS were un-predigested or predigested with multi-enzyme (Superzyme-CS; Canadian Bio-Systems, Alberta, Calgary, AB) in 5 × 2 factorial arrangement to give 10 treatment combinations with pretreatment (untreated WS, heat-pretreated WS, CA-pretreated WS, H_2SO_4-pretreated WS, and NH_3-pretreated WS) and multi-enzyme predigestion (un-predigestion and predigestion) as factors. Predigestion involved incubation of WS with at the multi-enzyme at 1% (v/w) in 100 mM acetate buffer solution (pH = 4.6) at 200 g of WS per 200 ml of the citrate buffer solution in 500-mL Erlenmeyer flasks in an incubator (Imperial III Incubator, 311M, Dubuque, IA) at

38ºC for 24 h. Two milliliters of chloramphenicol (C-0378; Sigma-Aldrich Corp., St. Louis, Mo) solution (0.5 g/100 mL) was added to the 500-ml flasks to prevent microbial growth during the pre-digestion. During incubation, the solutions in the flasks were stirred at 100 rpm on a stir plate. The multi-enzyme product (Superzyme-CS) supplied 24,000 U of xylanase, 3,000 U of glucanase, 10,000 U of cellulase, 1,200 U of mannanase, 14,000 U of invertase, 10,000 U of protease, and 24,000 U of amylase/kilogram of WS.

At the end of the incubation, the pre-digested samples together with untreated and pretreated samples were freeze-died and ground to pass through 0.75 mm screen using a Thomas Wiley Laboratory Mill (Model 4; Thomas Scientific Grinder, Swedesboro, NJ, USA). The ground samples were subjected to porcine in-vitro digestion as described below.

3.3.2 In Vitro digestion

The ground untreated and pretreated WS samples were subjected to in vitro digestion as described by Woyengo et al. (2015). Four grams of samples were weighed into 500 mL conical flasks. A phosphate buffer solution (200 mL, 0.1 M, pH 6.0), HCl solution (80 mL, 0.2 M) and fresh pepsin (8 mL, 20 g/L porcine pepsin, P-0609; Sigma-Aldrich Corp., St. Louis, MO) were added to the flasks. Additionally, 2 mL of chloramphenicol (C-0378; Sigma-Aldrich Corp., St. Louis, MO) solution (0.5 g/100 mL) was added in the flasks to prevent bacterial growth during the enzymatic hydrolysis. The samples were then placed into water bath at 39ºC for 2 h under gentle agitation (50 revolutions/min). Subsequently, phosphate buffer solution (80 mL, 0.2 M, pH 6.8),

NaOH (20 mL, 0.6 M), and fresh pancreatin solution (8 mL, 100 g/L pancreatin; P-1750 Sigma-Aldrich Corp.) were added to the flasks, and digestion was continued for 4 h under the conditions described above. Sample residues were collected following digestion by filtration using a nylon cloth (10 × 20 cm) with porosity of 50 ± 10 µm (ANKOM R1020 filter bags; ANKOM Technology, Macedon, NY, USA), and then washed with ethanol (2 × 25 mL 95% ethanol) and acetone (2 × 25 mL 99.5% acetone). The washed residues were dried for 18 h at 60^{o}C and weighed to determine in-vitro digestibility of DM (**IVDDM**). The enzymatic digestion was performed in 8 batches to obtain large amounts of residues for in vitro fermentation. The experiment was conducted as a complete randomized design with the flask as experimental unit, and untreated and pretreated WS samples without or with multi-enzyme predigestion as treatments. Pretreatments and multi-enzyme predigestion were fixed factors. The undigested residues from different batches were pooled for each treatment to determine in vitro fermentation. Pretreatment of WS with heat, diluted acids or ammonia followed by pre-digestion with multi-enzyme resulted in almost complete in vitro digestion of the WS. Thus, undigested residues for these samples were not generated for in vitro fermentation.

3.3.3 In-Vitro fermentation

Fermentation of undigested residues for untreated, or heat-, CA-, H_2SO_4- or NH_3-pretreated WS was conducted in vitro using a cumulative gas-production technique that has been adapted to the pig (Bindelle et al., 2008; Jha et al., 2015; Woyengo et al., 2015). Two hundred milligrams of the undigested residues were weighed into 125 mL-glass bottle (Wheaton^{TM} 223748, ThermoFischer Scientific, Waltham, MA). Buffer solution

(30 mL) that contained macro- and micro-minerals (Menke and Steingass, 1988) and a fecal inoculum was then added to each bottle, and the bottles were incubated within a water bath at 39ºC with a slight agitation of 50 rpm.

The buffer solution used contained disodium phosphate, monopotassium phosphate, magnesium sulfate, ammonium bicarbonate, calcium chloride, magnesium chloride, cobalt chloride, iron chloride, and resazurin. The fecal inoculum was obtained from 3 growing pigs from the South Dakota State University's Animal Science Complex, where they were fed a corn-DDGS-soybean meal grower diet with no antibiotics. Fecal samples were collected straight from the rectum and instantly placed in air-tight plastic syringes and kept in a water bath at 39ºC until when used for fermentation, which started approximately 30 min after fecal collection. Experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee at South Dakota State University (-069E).

The inoculum prepared from the fecal samples was diluted 20 times using the buffer solution, and then filtered through a 250 μm screen (E.H. Sargent and Co., Chicago, IL). The bottles were sealed with a rubber stopper and placed within the water bath for incubation. The anaerobic environment was constantly maintained throughout the experiment, from inoculum preparation until the incubation step by flushing with carbon dioxide gas. The gas that was generated during fermentation was measured at 0, 2, 5, 8, 12, 18, 24, 36, 48, and 72 h using a pressure transducer (SIN-54978; GP:50, Grand Island, NY, USA) (Mauricio et al., 1999) that was fitted with a digital data tracker (Blue Ribbon Corp., Grand Island, NY). The bottles were vented with a needle after each gas production reading. After 72 h of incubation, fermentation was stopped by placing the

bottles in ice. The contents of the bottles were collected and stored in a -20ºC freezer. The experimental scheme for *in vitro* fermentation was as follows: ([5 treatments × 5 replicates/treatment]) + 8 blanks) × 2 batches. The experiment was conducted as a complete randomized block design with the bottle as experimental unit, untreated and pretreated WS samples as treatments, and batch as block. Treatment was a fixed factor, whereas block was a random factor.

3.3.4 Sample Analysis

Ground samples of untreated and pretreated WS were analyzed for DM (method 930.15), CP (method 984.13), ether extract (method 920.39A) and Lys (method 994.12) by the AOAC (2006). The samples were also analyzed for NSP by gas-liquid chromatography (component neutral sugars) and by colorimetry (uronic acids). The neutral sugars were analyzed as described by Englyst and Cummings (Englyst and Cummings) with modifications (Slominski and Campbell, 1990), whereas uronic acids were determined using the procedure described by Scott (Scott).

Samples collected from the bottles after fermentation was centrifuged at 3,000 × g for 30 min at 4ºC. The supernatant of centrifuged samples was collected for VFA analysis, whereas the solid residue was freeze-dried and weighed to determine in-vitro fermentability of DM (**IVFDM**). The VFA concentration in the supernatant of the fermented samples was determined using gas chromatography using a method of Erwin et al. (1961) with some modifications. Briefly, 0.8 mL of sample was added into a 1.5 mL centrifuge tube that contained 0.2 mL of 25% phosphoric acid and 0.2 mL of internal standard solution (150 mg of 4-methyl-valeric acid, S381810, Sigma-Aldrich Corp.) and

vortexed for 1 minute. Afterwards, the samples were analyzed for VFA (i.e., acetic, propionic, butyric, isobutyric, valeric, isovaleric, and caporic acids) using gas chromatograph (Trace 1310, ThermoFischer Scientific, Waltham, MA) with a Stabilwx-DA column (30-m x 0.25-mm i.d.; Restek, Bellefonte, PA). A flame-ionization detector was used with an injector temperature of 170°C and a detector temperature of 190°C. Branched-chain VFA (**BCVFA**) content was calculated as the sum of the isobutyric and isovaleric acids.

3.3.5 Calculations

The IVDDM (%) was calculated as follows:

$$\text{IVDDM} = \left(\frac{\text{dry weight of intact sample - dry weight of hydrolysed residue}}{\text{dry weight of intact sample}} \right) \times 100$$

The IVFDM (%) was calculated as follows:

$$\text{IVFDM} = \left(\frac{\text{dry weight of hydrolysed residue - dry weight of fermented residue}}{\text{dry weight of hydrolysed residue}} \right) \times 100$$

Gas pressure measurements were converted into gas volume (G, per gram DM) using the ideal gas law, assuming an atmospheric pressure of 101325 Pa and a temperature of 312.15 K. Gas accumulation curves recorded during the 72 h of fermentation were modelled per France *et al*. (France et al.):

G (mL g-1 DM) = 0, if $0 < t < L$

$$G \text{ (mL g}^{-1} \text{ DM)} = G_f\left(1 - \exp\{-\langle b\,[t - L] + c[\sqrt{t} - \sqrt{L}]\rangle\}\right),\ if\ t\ \geq L$$

where, G denotes the gas accumulation to time, G_f (mL/g DM) the maximum gas volume for t = ∞ and L (h) the lag time before the fermentation starts. The constants b (h^{-1}) and c ($h^{-1/2}$) determine the fractional rate of degradation of the substrate μ (h^{-1}), which is postulated to vary with time as follows:

$$\mu = b + \frac{c}{2\sqrt{t}}, \quad \text{if } t \geq L \quad (6)$$

Kinetics parameters (G_f, L, $\mu_{t=T/2}$ and T/2) were compared in the statistical analysis. The T/2 is the time to half-asymptote when $G = G_f/2$.

3.3.6 Statistical Analysis

The IVDDM, IVFDM, fermentation kinetics parameters and fermentation metabolites produced were subjected to ANOVA using MIXED procedure of SAS (ver. 9.3, SAS Institute Inc., Cary, NC). The model included treatment as the fixed factor and batch as a random factor. Treatment means were separated by the least significant difference. Significance and tendencies were set at $P \leq 0.05$ and $P \leq 0.10$, respectively, for all statistical tests.

3.4 RESULTS

The heat-, CA-, H_2SO_4-, and NH_3-pretreated WS samples were similar in ether extract content (Table 3.1). However, the NH_3-pretreated WS contained more CP than untreated, or heat-, CA-, or H_2SO_4-pretreated WS. Lysine content for heat- or NH_3-pretreated WS was similar to that for the untreated WS. Lysine content for CA- or H_2S0_4-

pretreated WS was lower than that for the untreated WS. Lysine content as proportion of CP content for heat-pretreated WS was similar to that for the untreated WS. However, Lys content as proportion of CP content for CA-, H_2S0_4- or NH_3-pretreated WS was lower than that for the untreated WS. Pretreatment of WS with heat, CA, H_2SO_4 or NH_3 reduced its total NSP content (Table 2). The concentration of arabinose, xylose, mannose, galactose, and uronic acid sugars in NSP of WS was reduced by heat, CA, H_2SO_4 or NH_3 pretreatment. The NSP of CA-pretreated WS had lower concentration of arabinose and xylose sugars than the NSP of heat- H_2SO_4-, or NH_3-pretreated WS. The concentration of glucose sugar in NSP of WS was reduced by CA, H_2SO_4 or NH_3 pre-treatment. The magnitude by which the concentration of glucose sugar in NSP of WS was reduced by CA, H_2SO_4 or NH_3 pre-treatment was lower than that by which the concentration of arabinose and xylose sugars in NSP of WS was reduced by the same pretreatments. Heat pretreatment of WS did not reduce the concentration of glucose in its NSP. Furthermore, the magnitude by which NSP concentration in WS was reduced by heat pretreatment was lower than that by which NSP concentration of WS was reduced by CA, H_2SO_4 or NH_3 pretreatment. Predigestion of the untreated WS, and heat-, CA-, H_2SO_4-, or NH_3-pretreated WS with multi-enzyme reduced their total NSP concentration and the concentration of arabinose, xylose, mannose, galactose, glucose, and uronic acid sugars in their NSP. Nonetheless, the magnitude of reduction in NSP concentration of WS due to multi-enzyme pre-digestion was less for H_2SO_4 and NH_3 pretreatment than for the rest of pre-treatments. The pretreatment of WS with heat, CA, H_2SO_4, or NH_3 increased ($P < 0.001$) IVDDM (Figure 3.1.). Also, predigestion of untreated WS, or heat-, CA-, H_2SO_4-

or NH_3-pre-treated WS with multi-enzyme increased ($P < 0.001$) IVDDM. Pretreatment and multi-enzyme predigestion did not interact on IVDDM.

The lag time, half time, and rate of degradation did not differ among untreated and pretreated WS samples (Table 3.3.). Pretreatment of WS with heat, CA or NH_3 did not affect total gas production (Table 3.3. and Figure 3.2). Pretreatment of WS with H_2SO_4 reduced ($P < 0.05$) total gas production. Pretreatment of WS with H_2SO_4 or NH_3 did not affect IVFDM per unit weight of undigested residue. The pretreatment of WS with heat or CA reduced ($P < 0.05$) IVFDM per unit weight of undigested residue. Pretreatment of WS with heat or CA did not affect IVFDM per unit weight of feedstuff. However, pretreatment of WS with NH_3 reduced ($P < 0.05$) IVFDM per unit weight of feedstuff. In addition, pretreatment of WS with H_2SO_4 tended to reduce ($P < 0.10$) IVFDM per unit weight of feedstuff. The overall *in vitro* digestibility of DM (**OIVDDM**, which is IVDDM plus IVFDM per unit weight of feedstuff) of WS was increased ($P < 0.05$) by heat, CA, H_2SO_4, or NH_2 pretreatment.

Pretreatment of WS with heat, CA, or H_2SO_4 did not affect total VFA production per unit weight of undigested residue (Table 3.4).. Pretreatment of WS with NH_3 reduced ($P < 0.05$) total VFA production per unit weight of undigested residue. No differences were noted in acetic acid production (per unit weight of undigested residue) among pretreatments. Pretreatment of WS with heat, H_2SO_4, or NH_3 reduced ($P < 0.05$) propionic acid production per unit weight of undigested residue. Pretreatment of WS with CA did not affect propionic acid production per unit weight of undigested residue. Butyric acid production per unit weight of undigested residue was greater ($P < 0.05$) for CA-pretreated WS compared with the untreated WS. However, pretreatment of WS with

heat, H_2SO_4, or NH_3 did not affect butyric acid production per unit weight of undigested residue. The BCVFA production for untreated WS was greater ($P < 0.05$) than that for heat-, CA-, H_2SO_4-, or NH_3-pretreated WS.

Pretreatment of WS with heat, CA, H_2SO_4, or NH_3 decreased ($P < 0.05$) total VFA for WS per unit weight of feedstuff (Table 3.4.). Also, pretreatment of WS with heat, CA, H_2SO_4, or NH_2 decreased ($P < 0.05$) acetic acid, propionic acid, butyric acid, and BCVFA production per unit weight of feedstuff. The total VFA production for heat-pretreated WS was greater ($P < 0.05$) than that for H_2SO_4- or NH_3-pretreated WS. Per unit weight of feedstuff, total VFA, and acetic, propionic and butyric acids production for heat-pretreated WS did not differ from that for CA-pretreated WS; however, total VFA, and acetic, propionic and butyric acids production for heat-pretreated WS was greater ($P < 0.05$) than that for H_2SO_4- or NH_3-pretreated WS.

3.5 DISCUSSION

The objective of this study was to determine the effects of pretreating WS with heat alone, or combination with diluted CA, H_2SO_4, or NH_3, and predigesting untreated WS or heat-, CA-, H_2SO_4-, and NH_3-pretreated WS on NSP content, and in vitro digestion and fermentation characteristics of the resulting DGGS for pigs. Nutritive value of fibrous feedstuffs for ruminants was improved by pretreatment of the same feedstuffs with alkalis but without heat and pressure (Woyengo et al., 2004; Polyorach and Wanapat, 2015). However, pretreatment of feedstuffs with alkalis or acids alone (without heat and pressure) has been effective in improving nutritive value if pretreatment duration is long (more than 48 h; Feng et al., 2014; Kootstra et al., 2009; Woyengo et al., 2004).

Longer duration of pretreating fibrous feedstuffs with alkalis or acids require investment equipment and structures for pretreatment. Treatment technologies that involve short treatment duration of WS can easily be integrated in ethanol production plants, leading to reduced capital costs. Thus, in the current study, WS was pretreated with alkalis and acids under high temperature and pressure to reduce the pretreatment duration. Inorganic acids such as sulfuric acid and alkalis such as ammonia have been used to pretreat slurried fibrous feedstuffs. However, pretreatment of slurried fibrous materials with inorganic acids such as sulfuric acid results in generation of the toxic compounds such as furans that can inhibit the activity of digestive enzymes (Kootstra et al., 2009). Also, pretreatment of slurried fibrous materials with alkalis such as ammonia can result in degradation of lignin into acids such as ferulic and *p*-coumaric acids (Lee et al., 2014) that inhibit activities of digestive enzymes including α-glycosidase, α-amylase, lipase, pepsin, trypsin, and chymotrypsin (Martinez-Gonzalez et al., 2017). Citric acid is a noncorrosive organic acid, and pretreatment of slurried fibrous feedstuffs with diluted organic acids such as CA at ≤170°C did not result in production of toxic compounds that inhibit activity of digestive enzymes (Kootstra et al., 2009). Also, organic acids such as CA are added diets for weaned to improve their growth performance by lowering gastric pH, thereby optimizing nutrient digestion as well as preventing pathogen overgrowth in gastrointestinal tract (Heo et al., 2013). Thus, CA was included in this study for comparison with diluted sulfuric acid and ammonia. Heat treatment of feedstuffs (such as WS) that have high moisture content does not result in damage of AA through Maillard reaction (Schroeder et al., 1955). Thus, proposed pretreatment technologies can

potentially be attractive methods of increasing the nutritive value of DDGS for monogastric animals.

Pretreatment of WS with NH_3 resulted in an increased CP content of 25%, which was due to retention of some of the ammonia N by the WS. Kim et al. (Kim et al.) also observed increased CP content of DDGS due to ammonia fiber expansion (**AFEX**) pretreatment of the DDGS. Lysine content (1.09% of DM) in untreated WS was greater than the value (0.86% of DM) that was reported by (NRC, 2012) for DDGS containing more than 10% EE. Also, the Lys to CP ratio (3.51%) for untreated WS was greater than the value (2.82% of DM) that was reported by (NRC, 2012) for DDGS containing more than 10% EE. The Lys to CP ratio in feedstuffs is an indicator of intensity of Maillard reaction and hence heat damage of AA in the same feedstuff during its heat treatment (Kim et al., 2012). The WS used in the current study had EE value that was greater than 10%. Thus, the lower Lys to CP ratio for DDGS than for WS could be attributed to the fact some Lys in WS is damaged when the latter is dried into DDGS. The Lys to CP ratio for untreated WS was similar to that for the heat-pretreated WS, but greater than that for CA-, H_2S0_4- or NH_3-pretreated WS. It should be noted that the amounts of sugars that were released from WS during its pretreatment with CA, H_2S0_4 or NH_3, were greater than those that were released from WS during its pretreatment with heat. Intensity of Maillard reaction within feedstuffs during heat treatment is dependent on amount of reducing sugars present the same feedstuffs (Rizzi, 2003). Thus, the reduction in Lys to CP ratio for WS due to CA, H_2S0_4 or NH_3 pretreatment, but not due to heat pretreatment could have been due to greater amounts of available sugars in CA-, H_2S0_4- or NH_3-pretreated WS than in heat-pretreated WS. The reduction in Lys to CP ratio for WS due to NH_3

pretreatment could also have been due to the greater CP content in NH_3-pretreated WS than in untreated WS. Similarly, Kim et al. (2008) reported a reduction in Lys content of DDGS due to heat treatment or ammonia fiber expansion treatment of the DDGS.

The NSP content of WS was decreased by pretreatment with heat, CA, H_2SO_4, or NH_3, which was due to the degradation of some of the NSP into simple sugars by the pre-treatments. Pretreatment of slurried fibrous feedstuffs such as WS with heat, heat plus acids or heat plus alkalis at high pressure results in degradation of some NSP (Kootstra et al., 2009; Lee et al., 2014). Arabinoxylans and cellulose are the major NSP in corn and corn DDGS (Jaworski et al., 2015). The CA pretreatment compared with H_2SO_4 pretreatment resulted in release of greater amounts arabinose and xylose sugars from NSP of WS. It is not clear why CA was more effective than H_2SO_4 with regard to hydrolysis of arabinoxylans. However, Kootstra et al. (2009) reported greater production of furfural (which is derived from xylose) from wheat straw that had been pretreated with diluted sulfuric acid than from wheat straw that had been pretreated with diluted maleic acid (an organic acid) when pretreatment temperature was increased from 130 to 150 or 170°C. In the current study, WS was pretreated at 160°C. Thus, the release of lower amounts of arabinose and xylose release from WS by H_2SO_4 pretreatment could have been due to greater conversion of some of the released arabinose and xylose sugars into toxic products. de Vries et al. (2013) also reported release of greater amounts of arabinose and xylose sugars from NSP of DDGS due to pretreatment of the DDGS with diluted maleic acid than due to pretreatment of the same feedstuff with diluted sulfuric acid. The NH_3 pretreatment compared with CA pretreatment resulted in release of less amounts of arabinose and xylose sugars from NSP of WS. Feng et al. (2013) similarly reported

release of less amounts of xylose from NSP of wheat straw when the wheat straw was pretreated with diluted ammonia than when it was pretreated with diluted acids. Thus, the less effect of NH_3 pretreatment on release of arabinose and xylose sugars from NSP of WS could be attributed to the fact that ammonia pretreatment is not as effective as acid pretreatment with regard to degradation of NSP into simple sugars. The CA, H_2SO_4, or NH_3 pretreatment had less effect on the concentration of glucose in NSP than on concentration of arabinose and xylose sugars in NSP of WS. Cellulose, which is the main NSP in DDGS that yields glucose, was less affected by acid pretreatment of DDGS than arabinoxylans (de Vries et al., 2013). Thus, the less effect of CA, H_2SO_4, or NH_3 pretreatment on glucose content in NSP of WS could be attributed to the fact that cellulose compared with arabinoxylans is more resistant hydrolysis by diluted acids or alkalis. Heat pretreatment compared with CA, H_2SO_4, or NH_3 pretreatment had less effect on total NSP content in WS, implying that heat pretreatment alone is not as effective as acid or alkali treatment with regard to degradation of NSP into simple sugars. Also, heat pretreatment compared with CA, H_2SO_4, or NH_3 pretreatment did not affect glucose content in total NSP of WS, implying heat pretreatment alone cannot solubilize glucose-containing NSP such as cellulose.

The NSP content of untreated WS or heat-, CA-, H_2SO_4- or NH_3-pretreated WS was reduced by predigestion with multi-enzyme, which was due to the de-polymerization of the NSP within the WS by the multi-enzyme blend. The NSP content of unfermented (Jakobsen et al., 2015b) or fermented (Jakobsen et al., 2015a) wheat DDGS was also reduced due to carbohydrase enzyme predigestion. Predigestion of hot water- or ammonia fiber expansion-pretreated DDGS with multi-carbohydrase that contained cellulase and β-

glucosidase activities resulted in greater glucan hydrolysis than the predigestion of untreated DDGS (Kim et al., 2008). In the current study, the magnitude by which the NSP content of the untreated WS was reduced by multi-enzyme pre-digestion was greater than the magnitude by which the NSP content of the heat- or CA-pretreated, H_2SO_4-, or NH_3-pretreated WS was reduced by the pre-digestion, which was contrary to expectations. Apart from degradation of some NSP, pre-treatment of slurried fibrous materials such as WS with heat, CA, H_2SO_4-, or NH_3 is expected to result in deconstruction of NSP in the fibrous materials, leading to reduced crystallinity of cellulose and increased susceptibility of NSP to enzymatic hydrolysis (Lee et al., 2014). Also, pre-treatment of slurried fibrous materials with ammonia can result in degradation of lignin by breaking glycosidic ether bonds within lignin, leading to increased accessibility of digestive enzymes to NSP (Lee et al., 2014). Thus, it had been assumed that the response to multi-enzyme pre-treatment with regard to reduction in NSP content of WS would be greater for pretreated WS than for untreated WS. Kim et al. (2008) reported that the rate of glucan hydrolysis in DDGS due to multi-carbohydrase predigestion was greater when the DDGS had been pretreated with hot water than when it had been untreated. he rate of degradation of hot water-pretreated DDGS increased and then plateaued after 5 h of multi-carbohydrase predigestion, whereas that for untreated DDGS increased and plateaued after 72 h of the predigestion. Thus, the observation that the degradation of NSP from multi-enzyme on pretreated WS was less than that of untreated WS could have been due to longer multi-enzyme predigestion period (24 h). It will be interesting to see the effect of reducing multi-enzyme pre-digestion period on the reduction of NSP content in untreated and pretreated WS.

In the current study, the magnitude by which the NSP content of the H_2SO_4- or NH_3-pretreated WS was reduced by multi-enzyme pre-digestion was less than the magnitude by which the NSP content of the untreated WS, or heat- or CA-pretreated WS was reduced by the pre-digestion. It should be noted that pre-treatment of slurried fibrous materials such as WS with heat or diluted organic acids such as CA at ≤170°C does not generate significant amounts of toxic compounds such as furans that inhibit activities of digestive enzymes (Kootstra et al., 2009). However, pre-treatment of slurried fibrous materials with inorganic acids such as H_2SO_4 results in generation of the toxic compounds that can inhibit the activity of digestive enzymes (Kootstra et al., 2009). Also, pre-treatment of slurried fibrous materials with alkalis such as ammonia can result in degradation of lignin into acids such as ferulic and *p*-coumaric acids (Lee et al., 2014) that inhibit activities of digestive enzymes (Martinez-Gonzalez et al., 2017). Thus, the less effect of multi-enzyme pre-digestion on NSP content of the H_2SO_4- or NH_2-pretreated WS than on NSP content of the heat- or CA-pretreated WS could be attributed to presence of enzyme-inhibiting compounds in H_2SO_4- and NH_2-pretreated WS.

The disappearance of nutrients during *in vitro* digestion and fermentation procedures reflect the amount of nutrients that are available for digestion by animals in upper gut (stomach and small intestine) and hindgut, respectively. Thus, effects of processing technologies on *in vitro* digestibility and fermentability of feedstuffs indicate how the processing technologies can affect the digestion and fermentation of the same feedstuffs within the animals. In the current study, IVDDM of WS was increased by heat, CA, H_2SO_4, or NH_2 pre-treatment, implying that the pre-treatments increased availability of nutrients in the WS for digestion and absorption in the small intestine. As discussed

earlier, the degradation of NSP by the pretreatments could have contributed to the increase in digestibility in the simulated foregut. Fiber, and thus NSP content, is poorly digested in the small intestine of pigs and reduces the digestibility of AA and energy (Stein and Shurson, 2009). de Vries et al. (2014) similarly observed increased apparent ileal digestibility of DM in pigs due to hydrothermal treatment (extrusion) of maleic acid-pretreated DDGS. In the current study, the magnitude by which the IVDDM of WS was increased by heat pre-treatment was similar to the magnitude by IVDDM of WS was increased by CA, H_2SO_4, or NH_2 pre-treatment; this was despite that the fact that the NSP content of the WS was less affected by heat pretreatment than by CA, H_2SO_4, or NH_2 pre-treatment. This lack of difference between heat-pretreated WS and CA-, H_2SO_4-, or NH_2-pre-treated WS with regard to IVDDM indicates that heat pretreatment of WS results in increased availability of nutrients for digestion without significant degradation of NSP to monosaccharides.

The IVDDM for untreated WS or of heat-, CA-, H_2SO_4-, or NH_2-pretreated WS was increased by multi-enzyme pre-digestion, which was due to degradation of NSP in WS as evidenced by the reduced NSP content of the WS by the multi-enzyme pre-digestion. Fastinger and Mahan (2005) similarly observed increased apparent ileal digestibility of AA for DDGS in pigs due to pre-digestion of slurried DDGS with multi-enzyme that contained xylanase, cellulase and protease activities. Multi-enzyme product used in the current study contained protease, which can digest protein present in WS, leading to increased IVDDM. The pre-digestion of untreated WS with multi-enzyme increased IVDDM to that of WS that had been pretreated with heat, CA, H_2SO_4, or NH_2 followed by pre-digestion with multi-enzyme. This implies that pre-digestion of WS with

multi-enzyme for 24 h is as effective as pre-treatment of WS with heat, CA, H_2SO_4, or NH_2 followed by pre-digestion with multi-enzyme with regard to increasing the IVDDM for WS. It would be interesting to see the effect of reducing multi-enzyme pre-digestion period on digestibility of untreated and pretreated WS.

The rate of degradation of WS was not affected by pre-treatments, which was contrary to our expectations. Pre-treatment of fibrous materials is expected to result in degradation of NSP into mono- and oligosaccharides that are readily fermentable. For instance, addition of carbohydrase containing activity of xylanase and mannase to DDGS increased fermentation of the DDGS in the hindgut of pigs (Jakobsen et al., 2015b). Thus, the rate of degradation is expected to be higher for pretreated fibrous materials than for untreated fibrous materials, and the reason for the lack of effect of pre-treatment of WS on its rate of degradation in the current study is not clear. The total gas production for H_2SO_4-pretreated WS was less than that for untreated WS. In addition, the IVFDM (per unit weight of feedstuff) for untreated WS greater than that for Ammonia-pretreated WS and tended to be greater than that for H_2SO_4-pretreated WS. The lower fermentation of H_2SO_4-pretreated WS than for untreated WS could probably have been due to presence of toxic compounds such as furfurans in the undigested residue for H_2SO_4-pretreated WS. Moreover, the lower fermentation of NH_2-pretreated WS than for untreated WS could probably have been due to presence of toxic compounds such as ferulic and *p*-coumaric acids in the undigested residue for NH_2-pretreated WS.

Fiber fermentation in the hindgut of pigs results in the production of VFA, mostly acetic, propionic, and butyric acids, which serve as a source of energy for pigs. The VFA production (per unit weight of feedstuff) for the WS was reduced by pre-treatment of the

WS with heat, CA, H_2SO_4, or NH_2, which could have been due to reduced substrate availability for fermentation because of increased IVDDM due to the pre-treatments. Woyengo et al. (2016a) also reported a negative relationship between VFA production and IVDDM among various types of canola co-products. However, in the current study, the VFA production (per unit weight of feedstuff) for H_2SO_4- or NH_2-pretreated WS was lower than that for heat-treated WS, which could have been due to presence of afore-mentioned toxic compounds in undigested residues for H_2SO_4- and NH_2-pre-treated WS. The OIVDDM for untreated WS was lower than for heat-, CA- H_2SO_4- or NH_2-pre-treated WS, which was due to greater IVDDM for pre-treated WS than for untreated WS.

It appears that H_2SO_4 or NH_2 pretreatment technologies compared with heat and CA pre-treatment technologies are less effective in improving fiber digestion or fermentation, but do not affect WS digestion by gastric and pancreatic enzymes because of the following 3 reasons. First, pre-digestion of H_2SO_4- or NH_2- pretreated WS compared with pre-digestion of heat- or CA-pretreated WS with multi-enzyme (that had high activities of fiber-degrading enzymes) resulted in less reduction in NSP content of the WS. Second, incubation of H_2SO_4- or NH_2- pretreated WS compared with incubation of heat- or CA-pretreated WS with pig fecal inoculum resulted in less production of VFA. Pig feces contain microorganisms that produce fiber degrading enzymes (Bindelle et al., 2007). Lastly, the IVDDM of WS was not affected by type of chemical used for pre-treatment of the WS, implying that the gastric and pancreatic digestion of WS was not affected by type of pre-treatment.

In conclusion, the nutritive value of WS and hence DDGS can be improved by the pre-treatment and multi-enzyme pre-digestion technologies. Heat, when compared

with acids or alkalis, is cheap and non-corrosive. Furthermore, the heat pre-treatment and multi-enzyme pre-digestion technologies can be readily integrated into currently existing corn ethanol production facilities with minimal cost, thus minimizing the overall cost of the technology. Thus, heat and CA pre-treatment technologies can be attractive methods of increasing the susceptibility of DDGS for enzymatic digestion.

3.6 TABLES AND FIGURES

Table 3.1. Analyzed composition (on a DM basis) of test feedstuffs

Item, %	Control	Heat	Citrate	H_2SO_4	NH_3
Moisture	11.02	14.75	15.54	13.97	13.36
Crude protein	31.03	32.00	33.34	31.15	38.84
Ether extract	12.20	11.81	12.35	13.56	11.86
Lysine	1.09	1.03	0.90	0.87	1.04
Lysine per CP	3.51	3.23	2.70	2.80	2.67

[1]Control = untreated whole stillage; Heat = whole stillage heated at 70 psi and 160ºC for 20 min; Citrate = whole stillage pre-treated with citric acid (10 g/L) at 70 psi and 160ºC for 20 min; H_2SO_4 = whole stillage pretreated with sulfuric acid (90 mM) at 70 psi and 160ºC for 20 min; and NH_3 = whole stillage pretreated with NH_3 (1%) at 70 psi and 160ºC for 20 min.

Table 3.2. Effect of pre-treatment[1] and multi-enzyme pre-digestion[2] on non-starch polysaccharide (NSP) content of whole stillage and proportions of sugars that were released from NSP

	-Enzyme					+Enzyme				
Item	Control	Heat	CA	H_2SO_4	NH_3	Control	Heat	CA	H_2SO_4	NH_3
Content, %										
Arabinose	4.23	2.85	0.44	3.92	3.22	2.31	1.25	0.22	2.08	2.11
Xylose	6.84	6.76	2.73	5.12	5.21	3.42	3.29	0.87	4.51	4.81
Mannose	1.59	1.36	1.27	1.24	1.38	1.12	0.93	1.06	0.95	1.24
Galactose	1.22	1.14	0.54	0.96	0.98	0.66	0.71	0.31	0.81	0.97
Glucose	7.40	7.65	6.61	5.46	6.21	3.78	4.68	3.41	4.58	4.41
Uronic acid	1.36	1.24	0.80	1.13	1.07	0.61	0.54	0.34	0.76	0.84
Total NSP	23.34	21.00	12.38	18.78	18.07	11.90	11.40	6.11	13.75	15.39
Sugars released from NSP, %										
Arabinose	32.6	89.6	7.3	23.9	32.6	45.4	37.8	5.2	43.5	26.2
Xylose	1.2	60.1	25.1	23.8	1.2	50.0	50.7	27.2	8.9	5.8
Mannose	14.5	20.1	22.0	13.2	14.5	29.6	27.0	13.2	18.2	8.8
Galactose	6.6	55.7	21.3	19.7	6.6	45.9	35.2	18.9	12.3	0.8
Glucose	-3.4	10.7	26.2	16.1	-3.4	48.9	40.1	43.2	11.9	24.3
Uronic acid	8.8	41.2	16.9	21.3	8.8	55.1	51.5	33.8	27.2	16.9

[1]Control = untreated whole stillage; Heat = whole stillage heated at 70 psi and 160°C for 20 min; CA = whole stillage pre-treated with citric acid (10 g/L) at 70 psi and 160°C for 20 min; H_2SO_4 = whole stillage pretreated with sulfuric acid (90 mM) at 70 psi and 160°C for 20 min; and NH_3 = whole stillage pretreated with NH_3 (1%) at 70 psi and 160°C for 20 min.

[2]Enzyme = without enzyme pre-digestion; +Enzyme = pre-treatment was followed by enzymatic hydrolysis for 24 h at pH 4.6 and 38°C. The enzyme supplied 24,000 U of xylanase, 3,000 U of glucanase, 10,000 U of cellulase, 1,200 U of mannanase, 14,000 U of invertase, 10,000 U of protease, and 24,000 U of amylase/kilogram of WS; Superzyme-CS, 2 mL/ 400 mL of incubation medium.

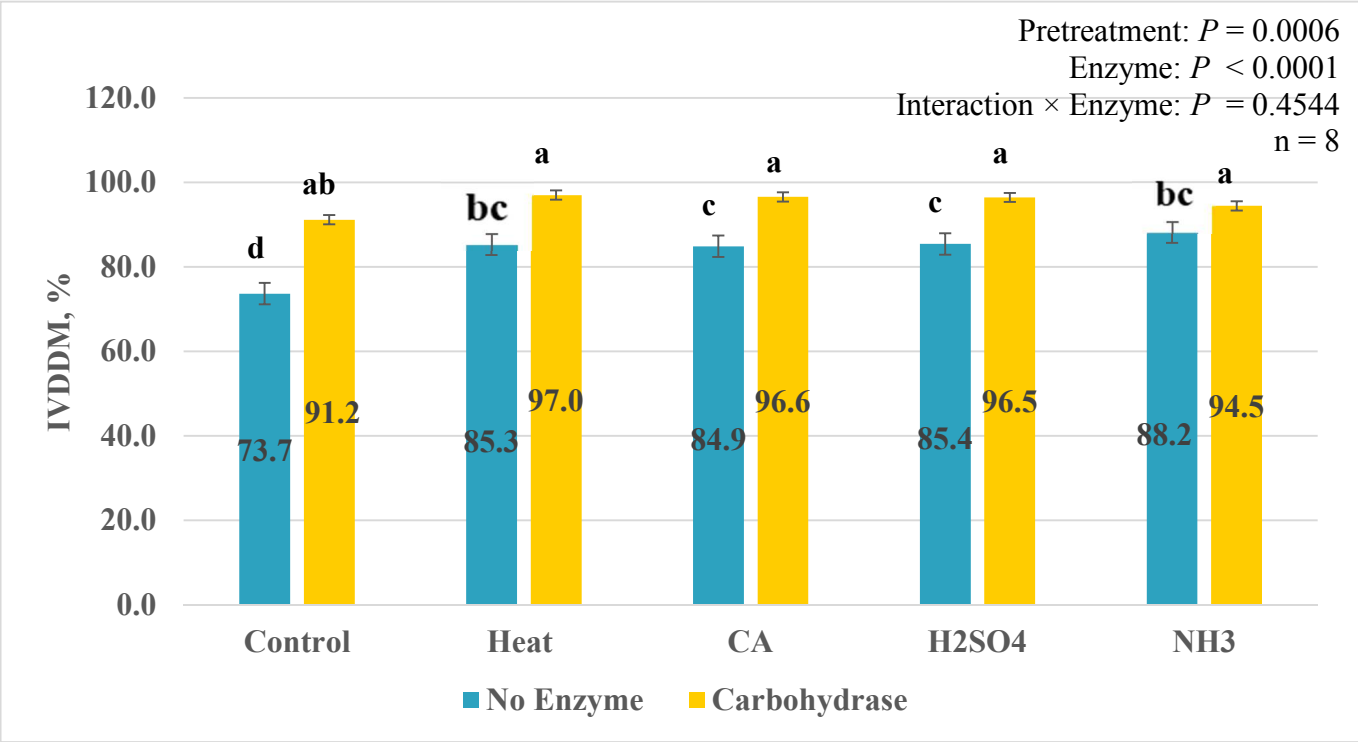

Figure 3.1. *In vitro* digestibility of DM of pre-treated and pre-digested whole stillage[1]

[a–c]Among all the 10 treatments, means without a common superscript differ ($P < 0.05$).

[1]Control = untreated whole stillage; Heat = whole stillage heated at 70 psi and 160ºC for 20 min; Citrate = whole stillage pre-treated with citric acid (10 g/L) at 70 psi and 160ºC for 20 min; H_2SO_4 = whole stillage pretreated with sulfuric acid (90 mM) at 70 psi and 160ºC for 20 min; NH_3 = whole stillage pretreated with NH_3 (1%) at 70 psi and 160ºC for 20 min; Enzyme = without enzyme pre-digestion; and +Enzyme = pre-treatment was followed by enzymatic hydrolysis for 24 h at pH 4.6 and 38ºC. The enzyme supplied 24,000 U of xylanase, 3,000 U of glucanase, 10,000 U of cellulase, 1,200 U of mannanase, 14,000 U of invertase, 10,000 U of protease, and 24,000 U of amylase/kilogram of WS; Superzyme-CS, 2 mL/ 400 mL of incubation medium. Data are a mean of 8 replicates.

Table 3.3. Fitted kinetics parameters (means) of gas accumulation and *in vitro* fermentability of DM of whole stillage

	Pre-treatment[2]						
Variable[1]	Control	Heat	Citrate	H_2SO_4	NH_3	SEM	*P*-value
Fermentation Kinetics							
Lag time[3]	17.3	14.3	13.5	11.8	9.01	3.05	0.41
Half time[4]	29.0	25.7	25.6	23.8	20.9	3.43	0.56
Degradation rate[5]	0.10	0.09	0.11	0.10	0.09	0.02	0.89
Total gas[6]	133^{ab}	143^{ab}	153^{a}	101^{c}	124^{bc}	10.2	0.01
IVFDM[7]							
% of undigested residue	53.8^{a}	29.7^{b}	33.2^{b}	41.4^{ab}	45.7^{a}	4.53	0.0025
% of feedstuff	12.1^{ab}	13.1^{a}	10.3^{bc}	9.95^{bc}	8.41^{c}	0.88	0.0033
OIVDDM[8], %	85.9^{b}	94.5^{a}	94.9^{a}	93.0^{a}	92.6^{a}	0.88	<0.001

[a–c]Within a row, means without a common superscript differ ($P < 0.05$).

[1]Data are a mean of 8 replicates

[2]Control = untreated whole stillage; Heat = whole stillage heated at 70 psi and 160°C for 20 min; Citrate = whole stillage pre-treated with citric acid (10 g/L) at 70 psi and 160°C for 20 min; H_2SO_4 = whole stillage pretreated with sulfuric acid (90 mM) at 70 psi and 160°C for 20 min; and NH_3 = whole stillage pretreated with NH_3 (1%) at 70 psi and 160°C for 20 min.

[3]Time taken to start fermentation (h).

[4]Half-time to asymptote (h, T/2).

[5]Factional rate of degradation (h^{-1}) at $t = T/2$.

[6]Cumulative gas volume (mL per g sample incubated for fermentation).

[7]IVFDM = *in vitro* fermentability of DM.

[8]OIVDDM= overall *in vitro* digestibility of DM.

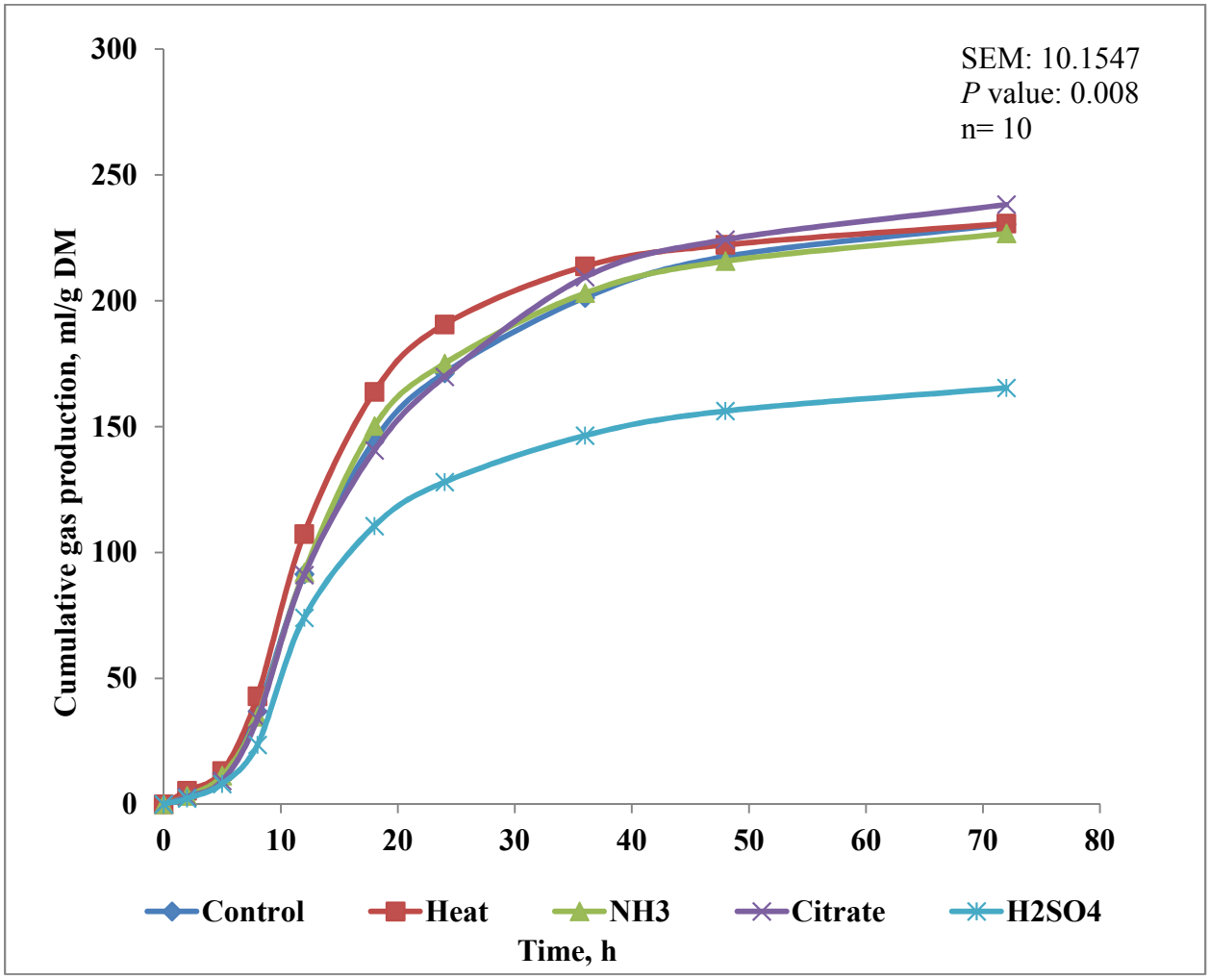

Figure 3.2. *In vitro* fermentation of DM of pre-treated and pre-digested whole stillage[1]

[1]Control = untreated whole stillage; Heat = whole stillage heated at 70 psi and 160ºC for 20 min; Citrate = whole stillage pre-treated with citric acid (10 g/L) at 70 psi and 160ºC for 20 min; H_2SO_4 = whole stillage pretreated with sulfuric acid (90 mM) at 70 psi and 160ºC for 20 min; and NH_3 = whole stillage pretreated with NH_3 (1%) at 70 psi and 160ºC for 20 min.

Table 3.4. Concentration of VFA in the solution after fermentation of undigested residue of pretreated whole stillage

	Pre-treatment[1]						
Item	Control	Heat	Citrate	H_2SO_4	NH_3	SEM	*P*-value
VFA concentration, mmol/g DM undigested residue							
Total VFA	8.01^{a}	7.67^{ab}	7.60^{ab}	7.28^{ab}	7.24^{b}	0.44	0.80
Acetic acid	3.50	3.66	3.51	3.43	3.40	0.27	0.97
Propionic acid	2.55^{a}	2.09^{b}	2.22^{ab}	2.00^{b}	2.01^{b}	0.123	0.02
Butryic acid	1.53^{b}	1.57^{ab}	1.59^{a}	1.57^{ab}	1.54^{ab}	0.023	0.21
BCVFA	0.37^{a}	0.28^{b}	0.29^{b}	0.28^{b}	0.26^{b}	0.019	0.002
VFA concentration, mmol/g DM feedstuff							
Total VFA	1.95^{a}	1.45^{b}	1.33^{bc}	1.07^{c}	1.14^{c}	0.098	<.0001
Acetic acid	0.89^{a}	0.68^{b}	0.60^{bc}	0.54^{bc}	0.52^{c}	0.056	<.0001
Propionic acid	0.53^{a}	0.39^{b}	0.38^{b}	0.31^{c}	0.32^{c}	0.021	<.0001
Butryic acid	0.41^{a}	0.29^{c}	0.27^{c}	0.25^{d}	0.24^{d}	0.004	<.0001
BCVFA[2]	0.10^{a}	0.05^{b}	0.05^{b}	0.05^{b}	0.04^{b}	0.004	<.0001

[1]Control = untreated whole stillage; Heat = whole stillage heated at 70 psi and 160^{o}C for 20 min; Citrate = whole stillage pre-treated with citric acid (10 g/L) at 70 psi and 160^{o}C for 20 min; H_2SO_4 = whole stillage pretreated with sulfuric acid (90 mM) at 70 psi and 160^{o}C for 20 min; and NH_3 = whole stillage pretreated with NH_3 (1%) at 70 psi and 160^{o}C for 20 min.

[2]BCVFA = branched chain volatile fatty acids.

4.0 Nutrient Digestibility of heat- or heat plus citric acid-pretreated DDGS for pigs

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4.1 ABSTRACT

A study was conducted to determine the effects of pretreating whole stillage (WS) with heat or heat plus diluted citric acid **(CA)** on nutrient digestibility of the resulting DDGS for growing pigs. The WS was untreated or pretreated with heat (160^{o}C at 70 psi for 20 min) alone (heat) or with the heat plus CA (12 g/L; heat+CA) at 70 psi for 20 min. Untreated and pretreated WS were paddle-dried before their inclusion in diets. Five diets were fed. The diets were cornstarch-based containing DDGS, untreated WS, heat-pretreated WS, or heat+CA-pretreated WS as the sole source of protein; and N-free diet, which was included for estimation of basal endogenous losses of AA. The DDGS diet was included for comparison. The 5 diets were fed to 10 ileal-cannulated barrows (57 ± 1.53 kg BW) in a replicated 5 × 5 Latin square to give 10 replicates/diet. On DM basis, DDGS contained 30.7% CP, 3.7% starch, 3.6% ether extract (EE), and 34.2% NDF; whereas untreated WS contained 37% CP, 4.5% starch, 9.5% EE, and 36.5% NDF. Pretreatment of WS with heat or heat+CA improved ($P < 0.001$) apparent ileal digestibility (AID) of GE in diet from 74.2 to 82.3 or to 79.7%, respectively; AID of CP in diet from 78.2 to 84.7 or to 82.0%, respectively; and AID of EE in diet from 84.4 to 89.2 or 90.4%, respectively. Pretreatment of WS with heat or heat+CA did not affect apparent total tract digestibility (ATTD) of GE in diet. The untreated WS diet had lower ($P < 0.001$) AID and ATTD of GE by 4 and 2% compared to DDGS, respectively. However, untreated WS diet had greater ($P < 0.001$) AID of EE than DDGS diet by 4%. Pretreatment of WS with heat or heat+CA reduced ($P < 0.001$) DE and NE values of the WS. Pretreatment of WS with heat reduced ($P < 0.001$) standardized ileal digestibility (SID) of Met, Thr, and Trp by 8.25, 8.88, and 4.73%, respectively, but did not affect SID

of Lys. Pretreatment of WS with heat+CA reduced ($P < 0.001$) standardized ileal digestibility (SID) of Met, Thr, and Trp by 9.88, 11.88, and 32.84%, respectively; and tended to reduce ($P = 0.062$). The untreated WS and DDGS did not differ in SID of AA. In conclusion, pretreatment of WS with heat or heat+CA improved energy digestibility, but reduced AA digestibility. Thus, pretreatment and drying of WS at conditions employed in the current study can improve energy digestibility, but reduce AA availability of the resulting DDGS for pigs.

Key words: DDGS, pretreatment, pig

4.2 INTRODUCTION

Traditionally, pork producers use corn and soybean meal in swine diets as the primary source of energy and protein, respectively. However, due to the fluctuating prices of these traditional cereal grains, alternative feedstuffs are being considered for formulating swine diets. In recent years, dried distillers' grains with solubles (**DDGS**), a by-product of the ethanol industry that is produced from corn, has been added to grow-finish pig diets to partially replace corn and soybean meal.

Compared with corn, DDGS has a higher GE, AA, and fat content (Spiehs et al., 2002; Stein and Shurson, 2009; NRC, 2012), and hence DDGS can potentially be a good source of energy and AA for pigs. However, DDGS has greater content of fiber, (non-starch polysaccharides (**NSP**) than corn (Spiehs et al., 2002; Stein and Shurson, 2009). Pigs, unlike ruminants are not efficient at digesting fiber (Stein and Shurson, 2009), which result in reduced nutrient utilization of DDGS for pigs. Thus, research needs to be done to increase the utilization of NSP in DDGS for pigs.

Pretreatment of whole stillage (**WS**; slurry material that remains after ethanol extraction for grain, which is further dried to create DDGS) with heat or diluted acids may improve the nutritive value of DDGS for pigs. Pre-treatment results in destruction of H bonds among the NSP, depolymerization of NSP and delignification of lignin (Kootstra et al., 2009). Indeed pretreatment of WS with heat or diluted citric acid reduced its NSP content and improved its porcine in vitro digestibility of dry matter (see Chapter 3) (Zangaro et al., 2017) (Zangaro et al., 2017) (Zangaro et al., 2017). However, information is lacking on the effect of pretreating WS with heat or diluted acids on digestion and fermentation characteristics of the resulting DDGS in vivo (in pigs). The objective of this study is to determine the effect of pretreating WS with heat or diluted citric acid on standardized ileal digestibility (**SID**) of AA, and DE and NE values of the resulting DDGS.

4.3 MATERIALS AND METHODS

Experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee at South Dakota State University (IACUC #: 15-029A).

4.3.1 Experimental Animals

Ten crossbred ileal-cannulated barrows (initial BW of 56.75 ± 1.53 kg; Duroc x Landrace × Large White; Pig Improvement Company) were used in the study. Pigs have been surgically fitted with a simple T-cannula at the distal ileum as described by Sauer and Ozimek (1986). Pigs were housed individually in grower pens (2.3 × 1.8 m) that allowed freedom to move in a temperature-controlled room (degrees Celsius). Each pen had fully metal-slated floor with one single-space dry feeder and a nipple drinker.

4.3.2 Experimental Diets

Diets included a cornstarch-based diet with untreated WS, heat-treated WS, CA treated WS, or standard DDGS; and N-free diet (Table 4.1). These diets contained titanium dioxide (0.4%) as an indigestible marker. The N-free diet was fed to estimate basal endogenous AA losses for determining SID of AA. The DDGS diet was included for comparison. The WS was the sole source of protein in the WS-containing diets, whereas DDGS was the sole source of protein in the DDGS-containing diet. The ratio of cornstarch to sugar and soybean oil in WS and DDGS-containing diets was identical to the N-free diet to allow calculation of energy digestibility of WS or DDGS using the difference method (Fan and Sauer, 1995). The WS and DDGS were obtained from POET Ethanol (Sioux Falls, SD). The WS was pretreated with heat (at 160ºC for 30 min) alone or in combination with citric acid (12.5 g/L; **CA**) at POET (Sioux Falls, SD). The untreated and pretreated WS were dried using paddle dryers. The DDGS was produced from the same batch of untreated and pretreated WS.

4.3.3 Experimental Design and Procedure

The 10 pigs were fed 5 diets in a replicated 5 × 5 Latin square design to give 5 replicates per diet. Each period consisted of 9 d; the first 5 d was for adaptation, then 2 d of fecal collection and then 2 d of ileal digesta collection. Pigs were fed at 3 times maintenance energy requirement (3 × 197 kcal for ME/ kg of $BW^{0.60}$; (NRC, 2012)) that was based on the BW at the beginning of each period. Daily feed allowance was offered in 2 equal portions at 0800 an 1530 h. Representative fecal samples were collected from each pen between 0800 and 1700 h daily. Ileal digesta was collected continuously for 12 h from 0800 to 2000 h daily (Nyachoti et al., 2002). The collected feces and digesta was pooled for each pig and period and stored frozen at -20ºC.

4.3.4 Sample Preparation and Analyses

Ileal digesta and fecal samples were freeze-dried. The freeze-dried ileal digest and fecal samples together with diet and feedstuff samples were ground to pass through a 0.75 mm screen using a Thomas Wiley Laboratory Mill (Model 4; Thomas Scientific grinder (Swedesboro, NJ, USA). The ground feedstuff samples were analyzed for DM, GE, CP, ether extract (**EE**), AA, NDF, ADF, and starch. The ground diet, fecal, and ileal digesta samples were analyzed for DM, GE, CP, EE and titanium dioxide. Diet and ileal digesta samples were additionally analyzed for AA.

Samples were analyzed for CP (method 984.13AD), EE (method 920.29A) as per AOAC (2006); and NDF (method 2002.04; AOAC, 2005); ADF (method 973.18; AOAC, 2007); and starch was analyzed using a Total Starch Assay kit (Megazyme, Chicago, IL). The GE was analyzed using an adiabatic bomb calorimeter (model AC600, Leco, St. Joseph, MI). Titanium dioxide in samples was determined by spectrophotometry (model Spectra MAX 190, Molecular Devices, Sunnyvale, CA) at 408 nm after ashing at 525°C for 10 h (Myers, 1997).

4.3.5 Calculations and Statistical Analysis

The apparent ileal digestibility (**AID**) and apparent total tract digestibility (**ATTD**) values of the diets were calculated using the indicator method (Eq. [2]; Stein et al., 2007). The SID for AA in diets was calculated for AID corrected for basal endogenous AA loss (Eq. [7]; Stein et al., 2007). The AA digestibility of the untreated or pretreated WS, DDGS was determined by the direct method. Energy digestibility of the untreated or pretreated WS, and DDGS was determined by difference method (Fan and Sauer, 1995) from N-free diet. The DE value of untreated or pretreated WS, and DDGS

was calculated by multiplying GE by the ATTD. The NE value of test feedstuff was calculated from the determined DE value and analyzed macronutrient content using Eq. % that was developed by Noblet et al. (1994) and has been adopted by NRC (NRC, 2012):

NE = 0.700 × DE + 1.61 × ether extract + 0.48 × starch – 0.91 × CP- 0.87 × ADF.

Data were analyzed using the MIXED procedure (SAS Inst. Inc., Cary, NC) with the diet as a fixed factor, and pig and period as random factors. Means were separated by probability of difference. To test the hypothesis, the significance level was set at 5%.

4.4 RESULTS

The analyzed composition of feedstuffs and diets are presented in Tables 4.2. and 4.3. respectively. The analyzed dietary CP values were greater than to the calculated values in Table 4.1.. The CP, AA, EE, starch, NDF, and ADF contents in DDGS were lower than those in untreated WS. The DGGS and untreated WS were similar in ADF. The untreated WS had greater GE value than DDGS. Pretreatment of WS with heat or CA increased CP content, but reduced starch, EE, and NDF contents of the WS. Pretreatment of WS did not affect its ADF content.

The AID and SID of CP and AA for feedstuffs are presented in Tables 4.4. and 4.5., respectively. All treatments were similar for SID of Lys, but lower ($P < 0.001$) SID of Met, Thr and Trp than untreated WS. Pretreatment of WS with heat or CA reduced ($P < 0.001$) the SID of Lys and Trp. In addition, pretreatment of WS with CA reduced ($P < 0.001$) the SID of Met and Thr. However, pretreatment of WS with heat did not affect the SID of Met and Thr.

The AID and ATTD of nutrients and DE values for diets and feedstuffs, and NE values for feedstuffs are presented in Table 4.6. Untreated WS diet had lower ($P < 0.001$) AID and ATTD of GE than DDGS diet, but greater ($P < 0.001$) AID of EE than DDGS diet. Pretreatment of WS with heat or CA improved ($P < 0.001$) AID of GE and EE in diet. However, pretreatment of WS with heat or CA did not affect ATTD of GE in diet. Untreated WS had greater ($P < 0.001$) AID of GE, and DE and NE values than DDGS. Pretreatment of WS with heat or CA increased ($P < 0.001$) its AID of GE, but reduced its DE and NE values.

4.6 DISCUSSION

The objective of this study was to determine the effect of pre-treating WS with heat or diluted citric acid (**CA**) on SID of AA, and DE and NE values of the resulting DDGS. Heat was used in this experiment due its relatively cheap process; citric acid was used because it is an organic acid that is not corrosive in nature. The DDGS, which is commonly added in diets for grow-finish swine diets (Stein and Shurson, 2009) was included in this study for comparison. The DDGS contained 30.75% CP, 3.11% EE, 34.2% NDF, which similar to the values (31.2% CP and 4 % EE, on DM basis) that were reported by NRC (NRC, 2012) for DDGS containing between 4% oil. However, the values of starch (3.71%) and NDF (34.2%) for the DDGS were lower than the values (11.2% starch and 37.8% NDF, on DM basis) that were reported by NRC (NRC, 2012) for DDGS containing between 4% oil. The differences in starch and fiber composition DDGS fed in the current study and that reported by NRC (NRC, 2012) could have been due to differences in fermentation conditions among ethanol plants. Untreated WS contained more AA, EE, and NDF than DDGS. Most of oil in the syrup that was

combined with Wet DG to form DDGS fed in the current study was removed before the mixing of the syrup with Wet DG. Oil was not removed from WS used in the current study. Thus, the greater content of EE in untreated WS than in DDGS was due to removal of most oil in syrup before the mixing of the latter with Wet DG to form DDGS. However, it is not clear why untreated WS had greater content of NDF and AA because the high amounts of oil in the WS is expected to dilute other nutrients. The DDGS had lower GE value than untreated WS, which was due to the higher oil content in the latter than in the former. Oil has higher energy value than other energy-yielding nutrients (protein and carbohydrates). Pretreatment of WS with heat or CA reduced its NDF, which could have been due to degradation of some of NSP in WS by the pretreatments. Treatment of slurried fibrous feedstuffs with heat under high pressure results in degradation of some of NSP in the feedstuffs (Kootstra et al., 2009; Lee et al., 2014). Bertipaglia et al. (2008) similarly reported a reduction in NDF content of soybeans and corn due to their pretreatment with heat at high pressure under moist conditions. Pretreatment of WS with heat or CA reduced its starch and AA content. The pretreated WS was darker in color than untreated WS; implying pretreatment resulted in Maillard reaction between sugars and AA. Thus, the lower starch and AA content in pretreated WS than in untreated WS could have been due to heat damage of AA and starch due to the pretreatment. The ADF content in WS was unaffected by heat or CA pretreatment. The NDF is mainly composed of hemicelluloses, cellulose, lignin and insoluble ash, whereas ADF is mainly composed of cellulose, lignin and insoluble ash. Thus, the reduction in NDF content, but ADF content of WS by the pretreatment indicate that the reduction in NDF due to pretreatment was mainly due to degradation of hemicelluloses.

Hemicelluloses are more susceptible to hydrothermal hydrolysis than cellulose (Kootstra et al., 2009; de Vries et al., 2013; Lee et al., 2014). Pretreatment of WS with heat or CA reduced its EE content, and the reason for this is not clear.

The AID of GE for DDGS (77.2%) was higher than the value that was reported by Urriola et al. (2014); which is most likely due to differences fermentation conditions and extent of oil extraction from the DDGS. The DDGS had lower AID of GE and AID of EE than untreated WS, which could have been due to the higher oil content in WS than in DDGS. As previously mentioned, oil has higher energy value, and it is more digestible in small intestine of pigs than other major components of DDGS such as fiber (Han and Liu, 2010). The AID of GE and EE for WS were increased by pretreatment of the WS with heat or CA, which could have been due to degradation of NSP into simple sugars that were digested in the small intestine, and to release of NSP-encapsulated nutrients. Also, de Vries et al. (2014) reported increased AID of DM in pigs due to hydrothermal treatment (extrusion) of maleic acid-pretreated DDGS.

The SID of AA values for DDGS were similar to the values that were reported by NRC (NRC, 2012) for DDGS with 4% oil content. The SID of AA values for DDGS was similar to all whole stillage treatment diets. Most of oil in corn and hence corn DDGS is composed of unsaturated fatty acids (NRC, 2012). Unsaturated fatty acids have been reported to increase ileal digestibility of AA in pigs (Li et al., 1994; Cervantes-Pahm and Stein, 2008), likely by reducing the rate of flow of digesta in the small intestine (Cervantes-Pahm and Stein, 2008). Pretreatment of WS with heat or CA reduced SID of Lys and some other AA, which was due to heat damage of the AA by the pretreated WS as evidenced by the low Lys content and hence low Lys to CP ratio in the pretreated WS.

As previously mention in the Chapter 3, the ratio of Lys to CP in feedstuffs is an indicator of extent of heat damage of AA in the feedstuffs. In the current study, WS was pretreated followed by its drying in paddle driers. Untreated WS was also dried in paddle driers. The heat damage of AA in pretreated WS could have been as a result of drying and not pretreatment because of the following 3 reasons. First, the color of untreated WS did not darken due to drying, whereas that for pretreated WS did not darken during the pretreatment, but darkened during the drying process, implying that the heat damage occurred during the drying process. Second, as previously mentioned in Chapter 3, heat pretreatment of slurried feedstuffs does not result in damage of AA through Maillard reaction. Lastly, in our study in which we determined the effects of pretreatment on in vitro digestion and fermentation characteristics of WS (see Chapter 3), pretreatment of WS followed by its freeze-drying did not severely reduce the Lys to CP ratio in the WS. The greater damage of AA in pretreated WS than in untreated WS due to drying could have been due to greater amounts of reducing (available) sugars in pretreated WS than in untreated WS as evidenced by the reduced NDF content of WS due to pretreatment. This is because, as previously mentioned on Chapter 3, the extent of AA acid damage in feedstuffs during heat treatment is dependent on amount of reducing sugars present in the same feedstuffs. Thus, there is a need for development of optimal conditions for drying pretreated WS.

The untreated WS had greater ATTD of GE value and hence greater DE and NE values than DDGS, which could have been due to the greater AID of GE and oil content in the former than in the latter. Pretreatment of WS with heat or CA did not result in increased ATTD of GE of WS despite the fact that pretreatment increased the AID of GE.

This could be attributed to the fact that energy-yielding components of untreated WS that escaped digestion in small were extensively fermented in the hindgut of the pigs, leading to similar or greater ATTD of GE values for untreated than for pretreated WS. Woyengo et al. (2016b) also observed greater porcine in vitro fermentation of fibrous feedstuffs that had low porcine in vitro digestibility of DM than those that had high porcine in vitro digestibility of DM. However, it should be noted that simple sugars, which are the end products of carbohydrate digestion in small intestine are more efficiently utilized by pigs as sources of energy than volatile fatty acids, which are the end products of carbohydrate fermentation in the hindgut. Thus, the increase in AID of GE by the pretreatments implies increased energy value of WS by the pretreatments. Pretreatment of WS with heat or CA did not result in increased DE value of WS, which was due to the failure of the pretreatments to improve ATTD of GE. In addition, pretreatment of WS with heat or CA did not result in increased NE value of WS, which was due to reduction in starch and EE content of the WS by the pretreatments, and to the failure of the pretreatments to improve DE of the WS. As previously described, NE values of feedstuffs were estimated from their DE values and macronutrients (CP, starch, EE and ADF) content. Starch and EE contents in feedstuffs are positively correlated with NE values of the same feedstuffs; whereas CP and ADF contents in feedstuffs are negatively correlated with NE values of the same feedstuffs (NRC, 2012).

In conclusion, WS greater energy and digestible nutrient content than DDGS. Pretreatment of WS with heat or CA improved AID of GE, but did not improve ATTD of GE, indicating that the pretreatments shifted energy digestibility from hindgut towards small intestine. However, pretreatment of WS with heat or CA reduced AA digestibility.

Because the shift in energy digestibility from hindgut to small intestine results in improved efficiency of energy utilization, the pretreatment and drying of WS at conditions employed in the current study can improve energy value, but reduce AA availability of the resulting DDGS for pigs.

4.7 TABLES AND FIGURES

Table 4.1. Composition of diets used in the study

Item	N free	DDGs	WS	WS Heated	WS heated with CA
Ingredient, %					
Corn DDGs	-	50	-	-	-
Whole Stillage	-	-	50	-	-
Whole Stillage heated	-	-	-	50	-
Whole Stillage heated with Citric Acid	-	-	-	-	50
Cornstarch	80	40.52	40.52	40.52	40.52
Limestone	0.7	1.42	1.42	1.42	1.42
Vegetable Oil	3.00	1.52	1.52	1.52	1.52
Monocal P	-	0.37	0.37	0.37	0.37
Dical P	1.7	-	-	-	-
Sucrose	10	5.066	6.066	7.066	8.066
Cellulose	3	-	-	-	-
Salt	0.5	0.5	1.5	2.5	3.5
KCO3	0.4	-	-	-	-
MgO	0.1	-	-	-	-
Vitamin Premix	0.05	0.05	0.05	0.05	0.05
Mineral Premix	0.15	0.015	0.015	0.015	0.015
Marker titanium	0.4	0.4	0.4	0.4	0.4
Calculated content					
DE, kcal/kg	3,815	3,743	-	-	-
CP, %	0	14	-	-	-
Digestible Lys, %	0	0.23	-	-	-
Digestible Met, %	0	0.23	-	-	-
Ca, %	0.69	0.66	-	-	-
Available P, %	0.26	0.31	-	-	-
Total P, %	0.32	0.44	-	-	-

Table 4.2. Analyzed feedstuff composition (on a DM basis)

Item, %	DDGS	WS	WS Heat	WS heated CA
Moisture	13.45	4.49	2.98	3.77
Crude protein	30.75	36.94	40.23	41.42
Ether Extract	3.11	9.04	9.56	7.36
Gross Energy, kcal/kg	4,431	4,744	4,663	4,661
Starch	3.71	4.51	1.69	1.1
NDF	34.21	36.46	26.11	30.97
ADF	16.00	16.13	17.59	15.54
Indispensable AA				
Arginine	1.23	1.38	0.86	0.87
Histidine	0.81	0.92	0.80	0.78
Isoleucine	1.28	1.51	1.66	1.77
Leucine	3.72	4.5	5.39	5.74
Lysine	1.00	1.03	0.49	0.49
Methionine	0.64	0.82	0.90	0.91
Phenylalanine	1.68	1.96	2.29	2.46
Threonine	1.10	1.29	1.27	1.34
Tryptophan	0.19	0.22	0.18	0.19
Valine	1.60	1.84	1.98	2.06
Dispensable AA				
Alanine	2.24	2.59	2.94	3.09
Aspartic Acid	1.93	2.28	1.70	1.63
Cysteine	0.64	0.77	0.66	0.72
Glutamic Acid	4.90	6.00	6.84	7.21
Glycine	1.20	1.3	1.34	1.37
Proline	2.51	2.94	3.29	3.30
Serine	1.21	1.37	1.47	1.47
Tyrosine	1.15	1.40	1.69	1.81

Table 4.3. Analyzed diet composition (on a DM basis)

Item, %	N-Free	DDGS	WS	WS Heat	WS heated CA
Moisture	8.48	10.92	6.08	6.19	8.23
Crude protein	0.00	21.59	18.63	22.07	20.98
Ether Extract	1.77	3.26	2.60	4.77	4.49
Gross Energy, kcal/kg	3,083	4,039	3,950	4,412	4,371
Indispensable AA					
Arginine	0.01	0.63	0.68	0.44	0.39
Histidine	0.00	0.42	0.45	0.42	0.39
Isoleucine	0.04	0.66	0.75	0.89	0.86
Leucine	0.03	1.91	2.22	2.86	2.77
Lysine	0.03	0.52	0.50	0.26	0.25
Methionine	-	0.32	0.38	0.46	0.41
Phenylalanine	0.03	0.82	0.94	1.17	1.16
Threonine	0.01	0.58	0.63	0.68	0.66
Tryptophan	< 0.02	0.10	0.12	0.09	0.05
Valine	0.01	0.81	0.90	1.05	1.03
Dispensable AA					
Alanine	0.02	1.17	1.28	1.58	1.53
Aspartic acid	0.02	1.04	1.14	0.93	0.80
Cysteine	0.01	0.33	0.38	0.35	0.33
Glutamic acid	0.04	2.68	3.09	3.79	3.60
Glycine	0.01	0.61	0.64	0.70	0.68
Proline	0.03	1.31	1.46	1.74	1.69
Serine	0.01	0.64	0.71	0.78	0.69
Tyrosine	0.02	0.58	0.69	0.83	0.53

Table 4.4. Apparent ileal digestibility of diet (per DM basis)

Item, %	DDGS	WS	WS Heat	WS heated CA	SEM	P-value
Crude Protein	80.89[c]	78.15[d]	84.70[a]	82.04[b]	0.475	<0.0001
Gross Energy	77.22[c]	74.24[d]	82.31[a]	79.65[b]	0.509	<0.0001
Ether Extract	81.08[d]	84.43[c]	89.16[b]	90.38[a]	0.183	<0.0001
Indispensable AA						
Arginine	78.15[b]	81.02[a]	72.50[c]	59.70[d]	0.398	<0.0001
Histidine	80.00[a]	79.84[a]	74.69[b]	68.63[c]	0.361	<0.0001
Isoleucine	80.80[b]	81.79[a]	78.55[c]	73.46[d]	0.325	<0.0001
Leucine	87.49[b]	88.04[a]	83.81[c]	81.10[d]	0.220	<0.0001
Lysine	57.45[a]	52.60[b]	37.20[c]	20.60[d]	0.847	<0.0001
Methionine	87.53[b]	88.43[a]	84.91[c]	79.95[d]	0.220	<0.0001
Phenylalanine	84.83[b]	85.76[a]	83.27[c]	80.27[d]	0.252	<0.0001
Threonine	72.43[b]	73.94[a]	73.37[a]	64.80[c]	0.451	<0.0001
Tryptophan	78.57[b]	81.01[a]	77.69[c]	28.36[d]	0.524	<0.0001
Valine	78.50[b]	79.84[a]	75.66[c]	70.81[d]	0.361	<0.0001
Dispensable AA						
Alanine	82.27[b]	82.69[a]	81.06[c]	76.68[d]	0.298	<0.0001
Aspartic Acid	74.89[b]	76.10[a]	70.73[c]	62.20[d]	0.436	<0.0001
Cysteine	74.53[b]	75.58[a]	69.72[c]	60.08[d]	0.449	<0.0001
Glutamic Acid	84.62[b]	85.08[a]	80.51[c]	75.20[d]	0.276	<0.0001
Glycine	52.26[c]	54.40[b]	61.00[a]	47.93[d]	0.7533	<0.0001
Proline	38.18[d]	48.98[b]	57.28[a]	41.19[c]	0.898	<0.0001
Serine	78.76[b]	79.53[a]	74.84[c]	63.52[d]	0.385	<0.0001
Tyrosine	85.87[b]	87.67[a]	85.74[b]	73.67[c]	0.253	<0.0001

Table 4.5. Standard ileal digestibility of AA of diet (per DM basis)

Item, %	DDGS	WS	WS Heat	WS heated CA	SEM	P-value
Indispensable AA, %						
Arginine	108.78[a]	108.01[a]	108.58[a]	106.43[b]	2.039	<0.0001
Histidine	89.31[a]	89.71[a]	77.12[b]	76.72[b]	0.793	<0.0001
Isoleucine	90.85[a]	91.65[a]	80.24[b]	79.62[b]	0.928	<0.0001
Leucine	92.81[a]	93.39[a]	83.08[b]	83.71[b]	0.775	<0.0001
Lysine	85.76[a]	84.21[ab]	75.68[bc]	73.56[bc]	5.424	<0.0001
Methionine	92.86[a]	93.72[a]	84.61[b]	82.98[b]	0.689	<0.0001
Phenylanine	92.51[a]	93.27[a]	84.17[b]	84.56[b]	0.788	<0.0001
Threonine	89.38[a]	90.74[a]	80.50[b]	77.50[c]	1.100	<0.0001
Tryptophan	100.20[a]	99.15[a]	95.47[b]	67.36[c]	2.117	<0.0001
Valine	91.13[a]	92.20[a]	78.86[b]	79.02[b]	0.864	<0.0001
Dispensable AA, %						
Alanine	95.31[a]	95.01[a]	85.60[b]	85.27[b]	0.947	<0.0001
Aspartic Acid	89.64[a]	88.28[b]	87.19[b]	81.34[c]	1.360	<0.0001
Cysteine	84.85[a]	85.93[a]	70.91[b]	67.67[c]	0.935	<0.0001
Glutamine	91.75[a]	92.19[a]	80.38[b]	79.12[c]	0.729	<0.0001
Glycine	114.31[a]	111.22[a]	104.14[b]	100.15[b]	4.080	<0.0001
Proline	170.07[a]	158.86[b]	144.57[c]	139.54[d]	8.050	<0.0001
Serine	94.10[a]	94.14[a]	80.64[b]	75.64[c]	1.120	<0.0001
Tyrosine	94.37[a]	95.39[a]	87.83[b]	81.41[c]	0.767	<0.0001

Table 4.6. Apparent total tract digestibility (ATTD) of GE and CP, and DE values of diets and feedstuffs, and NE values for feedstuffs (per DM basis)

Item, %	DDGS	WS	WS Heat	WS heated CA	SEM	P-value
Diet						
ATTD of CP	93.37[a]	92.51[b]	91.93[c]	92.61[b]	0.1666	<0.0001
ATTD of GE	91.95[a]	91.37[b]	91.34[b]	91.25[b]	0.1097	<0.0001
DE	3,714[c]	3,610[d]	4,030[a]	3,989[b]	4.4776	<0.0001
Feedstuff						
ATTD of GE	4,431[d]	4,744[a]	4,663[b]	4,661[c]	7.1455	<0.0001
DE	4,110[b]	4,045[c]	4,271[a]	4,261[a]	9.1345	<0.0001
NE	2,833[d]	2,989[a]	2,941[b]	2,911[c]	6.3941	<0.0001

5.0 GENERAL DISCUSSION

The digestibility of fiber in corn DDGS by pigs is generally lower than that of most other common feedstuffs for pigs. For instance, ileal and hindgut digestibilities of NSP in wheat middlings for pigs were greater than that for corn DDGS (Jaworski and Stein, 2017). Dietary fiber can also reduce nutrient utilization in pigs partly by encapsulating the nutrients. Fiber-degrading enzymes have been used to improve fiber digestibility and hence alleviate the negative effects of fiber in diets for pigs (Kiarie et al., 2010). However, the effects of fiber-degrading enzymes on digestibility of DDGS by pigs have been inconsistent. The objective of this thesis research was to establish why fiber in corn DDGS is inadequately degraded in pigs and to identify means of improving the digestibility of DDGS in pigs.

Dietary fiber can be soluble or insoluble; however, pigs poorly digest insoluble fiber. Corn DDGS has a higher content of insoluble fiber than most other feedstuffs for pigs (Jaworski et al., 2015; Jaworski and Stein, 2017). It was hypothesized that the high content of insoluble fiber and hence poor digestibility of DDGS by pigs is because fiber in corn DDGS combines with other nutrients during the drying of Wet DG into DDGS to form insoluble complexes. This is hypothesis was supported by results from the study by Jha and Berrocoso (2015), which showed that starch granules in wheat grain were separated from other components of wheat grain, whereas starch granules in wheat-derived DDGS were tightly combined with other non-starch components to form complexed aggregates. Results from the study by Jha and Berrocoso (2015) also showed that intensity of interaction between starch and other components of DDGS was greater in DGGS that had been subjected to a lot of heat during its drying than in DGGS that had

been subjected to less heat during its drying. It was also hypothesized that high insoluble fiber content in DDGS is because cellulose that constitute high proportion of NSP in corn fiber. This hypothesis was supported by the fact that corn's NSP contained more cellulose than other cereal grains such as wheat (Jaworski et al., 2015), and that cellulose is crystalline in nature (Kootstra et al., 2009), and hence it is poorly digested or fermented in pigs (Jaworski and Stein, 2017).

To test the first hypothesis, a study was conducted to determine porcine in vitro digestion and fermentation characteristics of both DDGS and wet DG without or with fiber-degrading enzymes. The DDGS and Wet DG were similar in porcine in vitro digestibility and fermentability, and porcine in vitro porcine digestion and fermentation characteristics of DDGS or Wet DG were unaffected by fiber-degrading enzymes. The conditions for drying Wet DG into DDGS have continuously been optimized. For instance, the Lys to CP ratio in DDGS that was recently produced was 3.5% (Jaworski and Stein, 2017), whereas the Lys to CP ratio in various samples of DDGS that was produced several years ago averaged 2.8% (Pedersen et al., 2007). Thus, it appears that the drying of Wet DG into DDGS does not affect the digestibility of the currently produced DDGS.

As previously mentioned, cellulose is poorly digested by pigs. Pretreatment of fibrous feedstuffs that have high content of cellulose and lignin with heat alone or in combination with diluted acids or alkalis resulted in increased release of simple sugars from cellulose and other NSP in the fibrous feedstuffs (de Vries et al., 2013). Also, predigestion of the fibrous feedstuffs with fiber-degrading enzymes resulted in release of simple sugars from cellulose and other NSP in the fibrous feedstuffs (Kim et al., 2008).

This implies that pretreatment of DDGS with heat alone or in combination with diluted acids or alkalis can potentially improve its digestibility in pigs. In addition, predigestion of DDGS with fiber-degrading enzyme can improve the nutritive value of the DDGS. Thus, to test the second hypothesis, a study was conducted to determine the effects of pretreatment and predigestion technologies on porcine in vitro digestion and fermentation of WS. Pre-treatment of WS with heat alone or in combination with CA, H_2SO_4 or NH_3 increased in vitro digestibility of WS. In addition, predigestion of untreated or pretreated WS with fiber-degrading enzymes increased in vitro digestibility of WS. Thus, results from the second study indicate that low digestibility of fiber in DDGS by pigs is partly due to recalcitrance fiber in DDGS to fermentation, and that the digestibility of DDGS can be increased through use pretreatment and predigestions technologies. The proposed pre-treatment and pre-digestion technologies can be readily integrated into currently existing corn grain ethanol production facilities with minimal cost, thus minimizing the overall cost of the technology. Whole stillage was be subjected to the pre-treatment and pre-digestion processes, and then processed through the existing steps of centrifugation and drying.

Heat pretreatment technology can be attractive method of increasing the susceptibility of DDGS for enzymatic digestion because it is cheaper than the other pretreatment technologies. Also, CA pre-treatment, compared with H_2SO_4 or NH_3 pretreatment, can be a better method of increasing the susceptibility of DDGS for enzymatic digestion because CA is less corrosive than H_2SO_4 or NH_3 and is routinely added in diets for pigs to improve gut health. Thus, the last study was conducted to determine the effects of pretreating WS with heat alone or in combination with CA on

nutrient digestibility in growing pigs. The DDGS was included in this study for comparison. The digestibility of untreated WS was greater than that for DDGS. Pretreatment of WS improved its energy digestibility, but decreased its amino acid digestibility. Thus, energy digestibility of DDGS for pigs can be improved, whereas AA digestibility of the same DDGS can be reduced by pretreating and drying of WS at conditions used in the current study.

It should be noted that untreated and pretreated WS that was used in this third study were dried at conditions (relatively high temperature) at which Wet DG is dried into DDGS in ethanol plants, whereas untreated and pretreated WS that were used in the second study were freeze-dried before they were subjected to in vitro digestion and fermentation. In both the second and third study, pretreatment of WS did not darken the color of the WS. Also, the Lys to CP ratio in WS used in the second study did not significantly change due to the pretreatments. However, the color of pretreated WS (and not of untreated WS) fed in the third study was darkened by its drying. Also, the Lys to CP ratio in pretreated WS fed in the third study was lower than that of untreated WS fed in the same study, implying that the drying of pretreated WS fed in the third study resulted in heat damage of AA (Maillard reaction), leading to reduced AA digestibility. The intensity of Maillard reaction in a feedstuff increases with increase in concentration of available sugars in the feedstuff. Thus, the reduction in AA digestibility of pretreated WS due to drying could have been due to greater concentration of simple sugars in pretreated WS than in untreated WS as evidenced by reduction in NSP concentration in WS due to pretreatment of WS that was used in the second study.

Based on results from the studies, it is apparent that the poor digestibility of DDGS by pigs and limited effect of fiber-degrading enzymes on the digestibility of DDGS by pigs is mainly because of recalcitrance of fiber in corn to enzymatic hydrolysis and not because of drying of Wet DG into DDGS because, pretreatment technologies that increase susceptibility of fiber to enzymatic degradation improved the digestibility of DDGS. Heat and CA pretreatment technologies can be attractive methods of improving the digestibility DDGS. However, future research should be conducted to:

1. Identify optimal conditions for pretreating WS with heat a lone or CA. In this thesis research, WS was pretreated at 160°C for 20 min, and hence there is need to determine whether greater or lower pretreatment temperature and time would be optimal.
2. Identify optimal time for predigesting WS with fiber-degrading enzymes. In this thesis research, WS was predigested for 24 h, and hence there is need to determine whether longer or shorter predigestion time would be optimal.
3. Identify best enzyme complex to pre-digest WS. In this thesis research, only one enzyme product was used to predigest the WS. There is need to determine the effect of predigesting WS with other products that have enzymes that target NSP that is present in WS on the nutritive value of the resulting DDGS for pigs.
4. Determine the effects of pretreating WS with organic acids other than CA on nutritive value of the resulting DDGS. There are several other organic acids such as acetic acid and maleic acid that could be used for the pretreatment.
5. Identify optimal conditions for drying pretreated WS. The pretreated WS fed in the third was dried at conditions under which DDGS is dried in ethanol plants. It

appears that pretreated WS should be dried at lower temperatures than the ones that are currently used to dry the DDGS because pretreated WS has a higher concentration of reducing sugars than DDGS.

6. Effects of including pretreated or predigested DDGS in diets for pigs on growth performance and health of the pigs with goal of identifying optimal dietary levels of the pretreated or predigested DDGS.

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