

**University of Alberta**

Nutritional Characterization of Wheat Distillers Dried Grains with Solubles in  
Grower-Finisher Pigs

by

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***Dedication***

*This thesis is dedicated to my mother Goma Devi Kandel and late father Bhanu Bhakta Kandel*

## **Abstract**

Co-product feedstuffs, if properly characterized nutritionally, are an important component to maintain the feed competitiveness of the pork industry. Hence, nutrient digestibility of distillers dried grains with solubles (DDGS) was measured using grower-finisher pigs. In Exp. 1, two methods were compared. Energy digestibility of wheat DDGS was lower when determined by difference from an N-free diet than a wheat diet, a difference explained by increased fermentation indicated by more volatile fatty acids (VFA) in feces. Standardized ileal digestibility (SID) of lysine did not differ between the methods. In Exp 2, four DDGS samples were evaluated against wheat. The nutrient digestibility was lower for DDGS than wheat. The digestible energy content was higher in corn DDGS than wheat DDGS or triticale DDGS but total feces VFA did not differ. The SID of lysine differed among DDGS. In conclusion, nutrient digestibility of DDGS differs in grower-finisher pigs depending on source and type.

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## **List of Abbreviations**

<b>AA</b>	Amino acids
<b>ADF</b>	Acid detergent fiber
<b>AID</b>	Apparent ileal digestibility
<b>ANF</b>	Anti nutritional factors
<b>BW</b>	Body weight
<b>ATTD</b>	Apparent total tract digestibility
<b>CP</b>	Crude protein
<b>DDGS</b>	Distillers dried grains with solubles
<b>DE</b>	Digestible energy
<b>DF</b>	Dietary fiber
<b>DM</b>	Dry matter
<b>EE</b>	Ether extract
<b>GC</b>	Gas chromatography
<b>GE</b>	Gross energy
<b>HPLC</b>	High performance liquid chromatography
<b>ME</b>	Metabolizable energy
<b>MT</b>	Metric tonne
<b>N</b>	Nitrogen
<b>NDF</b>	Neutral detergent fiber
<b>NE</b>	Net energy
<b>NRC</b>	National Research Council

<b>NSP</b>	Non-starch polysaccharide
<b>OM</b>	Organic matter
<b>SAS</b>	Statistical analysis system
<b>SEM</b>	Standard error of the mean
<b>SID</b>	Standardized ileal digestibility
<b>VFA</b>	Volatile fatty acids

## **Chapter 1 Literature Review**

### **1.1 Introduction**

Cost-effective diets are important for the swine industry because feed is the largest variable cost. The recent expansion of the ethanol industry in western Canada that mainly uses locally produced cereal grains has increased the price of feed grain, an increase that benefits crop producers. However, pork producers are facing more economic difficulty due to increased feed grain prices. The ethanol industry not only produces ethanol but also a co-product known as distillers dried grains with solubles (DDGS). Thus, increased availability of DDGS provides an opportunity for inclusion into swine diets. The inclusion of grain co-products such as DDGS in swine diets may help to reduce feed cost (Beltranena and Zijlstra, 2008), because DDGS can replace some grain, protein feedstuff, and inorganic phosphorus (Shurson and Dominy, 2004). However, some concerns exist about variability of the digestible nutrient content in DDGS among ethanol plants that may influence the performance of pigs. Several factors may affect nutritional value of DDGS such as quality of the grain used, overheating of distillers grain, and the amount of solubles added back to the distillers grain. Thus, nutritional characterization of wheat DDGS before its inclusion in the swine diet is important to reach a predictable swine growth performance.

Several co-products are produced by the ethanol industry in western Canada. The ethanol industry ferments grain starch into ethanol, and carbon dioxide is produced, and distillers dried grains with solubles (DDGS) remains as the main co-product for livestock feeding. Depending on the geographical

location and time of year, a range of grain feedstocks can be used for ethanol production including corn, wheat, barley, triticale, and sorghum. The nutritional value of DDGS reflects the quality of grain that was used as feedstock (Stein, 2006). The US ethanol industry alone produced 30.5 million metric tons of co-products including DDGS in 2009 (Renewal Fuel Association, 2010). Of the DDGS, 98% is produced in plants that produce ethanol for oxygenated fuels and the remaining is produced by the alcoholic beverage industry. Due to the high demand for ethanol, DDGS production continues to expand, further increasing the quantity of DDGS that is available for use in livestock diets.

The DDGS contains more protein, fat, minerals, vitamins, and fiber than the original grain (Klopfenstein et al., 2007). The high concentration of nutrients is due to the conversion of the starch from grain to ethanol and CO<sub>2</sub> during the fermentation (Weigel et al., 1997). Although the majority (77%) of DDGS is being fed to cattle, DDGS is also an acceptable feedstuff for swine and poultry (Renewal Fuel Association, 2010). Currently, DDGS has a higher nutritive value than the DDGS that was produced historically due to improved processing. The DDGS can be included cost-effectively in swine diets

The nutritional value of biofuel co-products is variable (Zijlstra and Beltranena, 2008). Therefore, an important challenge of DDGS inclusion in swine diets is to establish the digestible nutrient content of the specific sample that was procured (Stein, 2008). The nutrient composition and digestibility of DDGS differed among the DDGS sources and variation even existed within ethanol plants over time (Spiehs et al., 2002). Moreover, even using the same grain as

feedstock, the chemical composition of DDGS varied among batches (Cromwell et al., 1993; Spiels et al., 2002). The variation in quality may be due to differences in processing methods and effectiveness of fermentation, drying temperature, or the amount of solubles added back into the distillers dried grains. Because DDGS is dried using heat, digestibility of amino acids (AA), especially Lys, might be reduced due to Maillard reactions (Cromwell et al., 1993). Drying will thus increase the variation of digestible Lys further compared to the total Lys in DDGS (Stein, 2006).

Finally, DDGS contains a high amount of fiber. Fiber may modulate intestinal bacteria profile and may affect gut health in pigs (Grieshop et al., 2001); however, this hypothesis is poorly supported by research using DDGS. Pigs that were fed cereal grains that varied in chemical composition contained a markedly different intestinal microbial population (Drew et al., 2002). A beneficial microflora may reduce the susceptibility to enteric infection (Pluske et al., 2003; Wang et al., 2009). Dietary inclusion of DDGS may provide some health benefits to growing pigs subjected to a subsequent moderate ileitis challenge (Whitney et al., 2003).

## **1.2 Expansion of DDGS production**

Global production of fuel ethanol was forecasted to surpass 100 billion liters by 2010 (Shapouri, 2007). The North American ethanol production is mostly based on corn; however, wheat is the major feedstock in western Canada (Warren et al., 1994). The DDGS provide an opportunity for the livestock

industry as an alternative feedstuff to reduce feed cost. In Canada, the number of ethanol plants has risen, which in turn has increased the amount of DDGS that is available for use in the livestock feeding. Canada produced 750,000 metric tons of DDGS in 2008, while western Canada produced 460,000 metric tons in 2009 (Christensen, 2009).

In the Canadian prairies, the main substrate for ethanol production is wheat. Wheat is generally more competitive than other cereal grains grown in western Canada for ethanol production due to its high starch content, local availability, and a competitive price. The ethanol industry provides an additional market opportunity for western Canadian wheat growers. The supply of wheat DDGS is increasing, but the nutrient profile varies among plants for dairy cattle (Nuez-Ortin and Yu, 2010) that may limit the inclusion of DDGS to manage risk. Thus, measurement or prediction of the digestible nutrient content of DDGS is also important prior to inclusion in swine diets.

### **1.3 Ethanol and distillers dried grain with solubles production**

Ethanol production is a series of processing steps in which starch is first hydrolyzed to sugars by enzymes and then the sugars are fermented to ethanol and CO<sub>2</sub> by yeast. Two main production processes exist: wet milling and dry milling. These processes differ in the pre-treatment of the cereal grain that is used for ethanol production. For dry milling process, the grain is ground before fermentation. For wet-milling process, the grain is ground, and then mixed with water, followed by separation of starch that is then used for ethanol production.

Production of ethanol from 1000 kg of wheat using dry-milling produces 365 liters of ethanol, 290 kg of CO<sub>2</sub>, and 290 kg of DDGS (Vernon, 2007).

Ethanol production using dry milling contains several important steps (Figure 1). The first step is cleaning and grinding of the cereal grain. In the liquefaction step, the meal is mixed with water and passed through cookers that apply steam to denature and liquefy the starch. During saccharification, the slurry from the cookers is cooled and  $\alpha$ -amylase and glucoamylase are added to hydrolyze starch into monosaccharide and disaccharides. In the fermentation step, yeast is added to the mixture to ferment the sugars into ethanol, either as continuous or batch fermentation. In continuous fermentation, the fermenting mash flows through several vessels until the mash is fermented completely. In batch fermentation, the mash stays in 1 fermenter during the entire fermentation. Upon completion of fermentation, the liquid, i.e., the beer contains 10 to 15% alcohol.

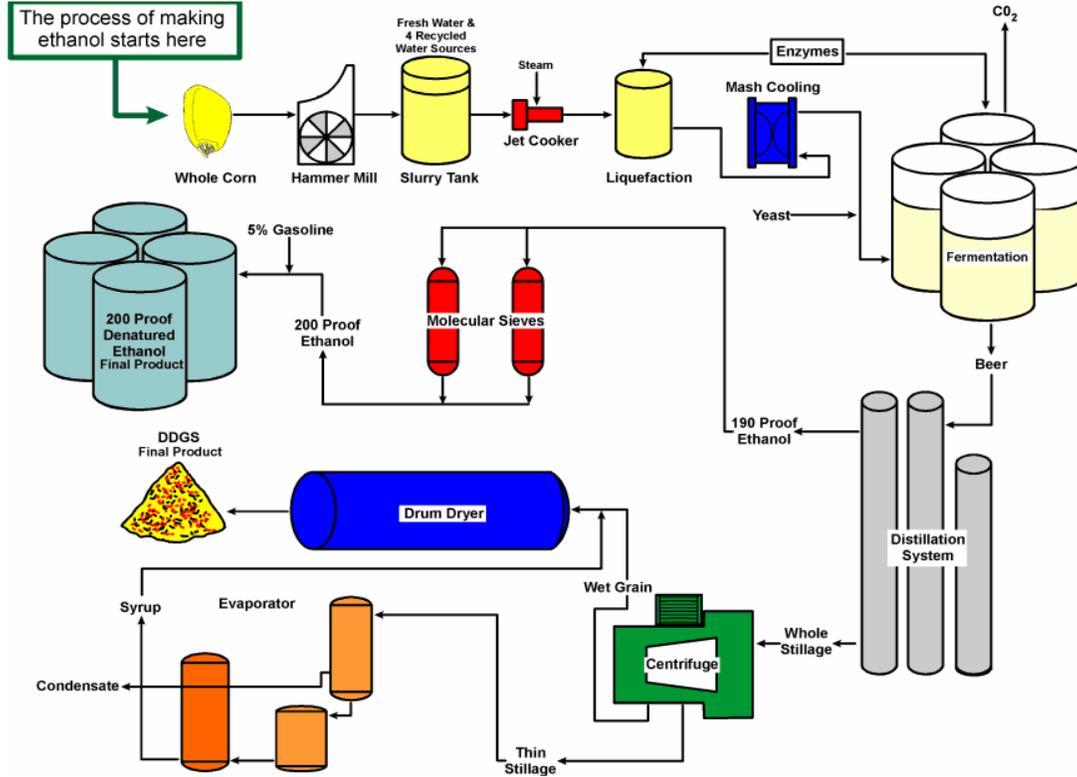


Figure 1. Dry milling ethanol production process (Delta Enterprise Network, 2009).

During distillation, alcohol is removed from the slurry. The alcohol becomes 96% pure and is named anhydrous (without water) ethanol. Finally, the ethanol used for fuel must be denatured with 2 to 5% gasoline to make it unfit for human consumption (Renewal Fuel Association, 2009). The remaining liquid, i.e., whole stillage, is centrifuged to separate water (thin stillage) from the remaining solids (wet cake). The thin stillage is then evaporated and mixed back into the wet cake and is dried together with the solids. The final product is termed DDGS.

After the Federal Government mandated an inclusion of 5% renewal fuel in gasoline by 2010, the production of ethanol and DDGS increased in Canada.

Around 40% of the wet distillers grains is used without drying in dairy and beef cattle operations. The remaining 60% are sold as DDGS for use in dairy, beef, and swine diets. The benefit of drying is the opportunity to transport DDGS for long distances including export markets. The increased availability of DDGS provides livestock producers an opportunity to reduce feed cost. However, increased DDGS production may also increase the local price of feed grains that may affect livestock producers negatively.

#### **1.4 Nutritional characteristics of DDGS**

The co-products produced from grain-based ethanol production vary in composition according to the grain source used and processing conditions during the fermentation (Spiehs et al., 2002). The DDGS may be produced in a variety of plant types that may further influence the nutritional value of DDGS (Pahm et al., 2008a).

The DDGS contains all distillers grains and at least 70% of the condensed solubles produced. If solubles are not added and only the distillers grains are dried, the final product is distillers dried grains (DDG). If the grain is de-hulled or de-germed prior to fermentation, high protein DDGS is produced that contains less fiber and fat and more protein than regular DDGS (Widmer et al., 2007).

#### **1.5 Energy availability and digestibility**

The DDGS contains a low amount of remaining starch but the concentration of most other nutrients is usually greater than in the parent grain.

Thus, most of the remaining carbohydrates are non-starch polysaccharides (NSP). The content of the different fiber fractions (analyzed as crude fiber, NDF, ADF, or total dietary fiber) is almost 3 times greater in DDGS than in the parent grain. The major portion of dietary fiber in DDGS is insoluble fiber (Urriola et al., 2010). Dietary fiber may increase the bulkiness and the water-binding capacity of the diet (Cherbut et al., 1994) and may decrease nutrient digestibility.

Corn DDGS may have similar digestible energy (DE) and metabolizable energy (ME) content (3.64 and 3.37 Mcal/kg of dry matter (DM), respectively) as corn (3.49 and 3.37 Mcal/kg of DM, respectively) for grower pigs (Spiehs et al., 1999; Stein et al., 2006). Similarly, corn DDGS had the DE and ME content 4.14 and 3.89 Mcal/kg of DM which was similar to corn 4.08 and 3.98 Mcal/kg of DM in another study for grower pigs (Pedersen et al., 2007c). In contrast, wheat DDGS has a lower energy content than wheat (Zijlstra and Beltranena, 2008). The DE content ranged from 3.00 to 4.29 Mcal/kg of DM for wheat DDGS (Beltranena and Zijlstra, 2008), but in another recent study it ranged from 2.84 to 3.91 Mcal/kg of DM for wheat DDGS (Cozannet et. al., 2010b). Triticale DDGS has an average DE content of 3.82 Mcal/kg of DM (Beltranena and Zijlstra, 2008) that is lower than the DE content of corn DDGS.

**Table 1. Energy concentration and digestibility of different DDGS in growing pigs**

Item	Grain		DDGS		
	Wheat <sup>1</sup>	Corn <sup>2</sup>	Wheat <sup>1</sup>	Corn <sup>2</sup>	Triticale <sup>3</sup>
GE, Mcal/kg of DM	4.49	4.46	5.19	5.43	-
ATTD, %	84.8	90.0	68.3	76.6	72.1
DE, Mcal/kg of DM	3.81	4.07	3.55	4.14	3.82
ME, Mcal/kg of DM	-	3.98	-	3.90	-

<sup>1</sup>Widyaratne and Zijlstra, 2008.

<sup>2</sup>Pedersen et al., 2007.

<sup>3</sup>Zijlstra and Beltranena, 2007.

The apparent total tract digestibility (ATTD) of energy and apparent ileal digestibility (AID) of corn DDGS is lower than in corn because the fiber content is higher in the co-products than in the grain (Stein et al., 2006; Pedersen et al., 2007a; Urriola et al., 2009). Fiber in DDGS has a low digestibility in the small intestine, and fermentation in the large intestine is less than 50%. These are the two reasons why the ATTD of energy in corn DDGS is lower than in corn (76.6 vs. 90.0% respectively) (Pedersen et al., 2007). But due to high fat content in DDGS compared to that in corn, the GE content of DDGS is higher than that of corn [5.43 vs. 4.50 Mcal GE/kg of dry matter (DM)]. The DE content of corn DDGS is therefore similar to corn grain (4.09 vs. 4.14 Mcal DE/kg of DM) (Stein et al., 2005). The digestibility of energy may increase as pig ages and adapts to fiber in the diet (Longland et al., 1993).

In contrast to corn DDGS, wheat DDGS contains less DE than the parent grain, wheat. Wheat DDGS contains more protein, fiber (Widyaratne and Zijlstra, 2007) and gross energy (Nyachoti et al., 2005; Cozannet et al., 2010c) but less starch than wheat. The energy digestibility of wheat DDGS is lower than wheat grain, but due to the low fat content of wheat grain and thus wheat DDGS, the low energy digestibility is not compensated with a high GE content, unlike for corn DDGS. The inclusion of 25% wheat DDGS in a diet based on wheat grain and field pea fed to pigs from 52 to 85 kg body weight did not affect ADG or G:F (Widyaratne and Zijlstra, 2007). On the other hand, increasing inclusion of wheat DDGS linearly decreased the ATTD of CP, energy and DM in diets fed to weaned pigs (Avelar et al., 2010). The nutrient content and digestibility can vary among wheat DDGS samples (Cozannet et al., 2010bc). The information on nutrient profile of new generation DDGS is limited; thus, analyses of the nutritional value of DDGS produced in modern ethanol plants are important.

### **1.6 Digestibility of amino acids**

The digestibility of AA in corn DDGS is about 10% units lower than in corn grain (Fastinger and Mahan, 2006) and that may be due to a greater concentration of fiber in DDGS than in the parent grain. Dietary fiber increases endogenous AA losses, increases the rate of digesta passage (Souffrant, 2001; Grieshop et al., 2001), thereby reducing apparent ileal AA digestibility. The variability in AA digestibility among corn DDGS samples is greater than among corn samples, which may be due to differences in processing technologies and

procedures among ethanol plants (Pahm et al., 2008a). The color of DDGS may indicate AA quality especially Lys damage. Corn DDGS with a light yellow color may contain more Lys while dark color DDGS may contain less digestible Lys (Pederson et al., 2005).

The variability in content and digestibility of Lys in DDGS is greater than the variability for other AA (Cozannet et al., 2010b). The digestibility of Lys in corn DDGS from 5 different sources varied from 38 to 62% indicating that Lys has the most variable digestibility among AA in DDGS (Fastinger and Mahan, 2006). The main reason is that some ethanol plant overheat the DDGS during drying, resulting in Maillard reactions between sugars and Lys (Carpenter, 1960; Moughan and Rutherford, 1996; Widyaratne and Zijlstra, 2007; Pahm et al., 2008b). Heating will reduce the Lys digestibility, but the crude protein (CP) content is not affected. Therefore, CP content is a poor indicator of Lys quality. In un-damaged DDGS, Lys content as a percentage of CP is between 3.1 and 3.3%, but this percentage can be as low as 2.1% in heat-damaged DDGS (Stein, 2007). Therefore, Lys content as percentage of CP should be measured before using DDGS in swine diets, because Lys is the first-limiting AA for pigs.

### **1.7 Availability and digestibility of phosphorus**

Phosphorus (P) is an expensive component of swine diets. Corn DDGS contains 0.79 (Pedersen et al., 2007) to 0.89% (NRC, 1998) of P on a DM basis, which is almost 3 times greater than in corn. In corn, only 14% of the total P is digestible for swine, but the ATTD of P increased to 59% for corn DDGS

(Pedersen et al., 2007). Most of the P in corn and wheat is bound in the form of phytic acid and is unavailable to the pig. The fermentation process liberates P due to the effect of added yeast. Overheating during drying also increases P availability (Batal, 2006). The ATTD of P is 52 to 58% in wheat DDGS and 50 to 68% in corn DDGS (Stein and Shurson, 2009). Wheat DDGS contains more available P than wheat so less inorganic P will be required in diets containing wheat DDGS to meet P requirements of pigs.

The digestibility of P in DDGS corresponds to availability values between 70 and 90% relative to those for dicalcium phosphate. Inclusion of microbial phytase enzyme to swine diets may increase P digestibility (Xu et al., 2006). The higher P digestibility in DDGS than in the parent grain is due to the hydrolysis of bonds that binds P to the phytate complex during the fermentation process. Therefore, with DDGS inclusion in swine diets, the available P content increases, so that the need for supplemental inorganic P is reduced (Jensen and Jorgensen, 1994) resulting in lower feed cost and P excretion by pigs. Feeding diets containing DDGS may reduce manure P content (Xu et al., 2007). Finally, digestibility of DM is reduced in diets containing DDGS because of limited fiber fermentability; thus, the mass of excreted feces will be increased.

### **1.8 Non-starch polysaccharides and dietary fiber**

The carbohydrate fraction of swine diet can be divided into 2 parts: starch and non-starch polysaccharides (NSP) of plant cell walls (cellulose, hemicelluloses, and pectins). While the pig's endogenous enzymes can hydrolyze

starch, the majority of NSP fraction cannot be digested by porcine enzymes. Instead, NSP is fermented by the gut microflora yielding volatile fatty acids, microbial biomass, lactate, and gases (Drochner, 1993). Swine diets may also contain a low amount of free sugars and oligosaccharides that may be absorbed directly or fermented, respectively (Drochner, 1993).

The NSP in cell walls varies among cereal grains. The NSP mainly consists of  $\beta$ -glucans in barley and oats, and arabinoxylan in wheat, rye and triticale (Knudsen, 1997; Zijlstra et al., 1999). The NSP content varies among samples of barley (Fairbairn et al., 1999) and wheat (Zijlstra et al., 1999). In poultry, soluble NSP increases digesta viscosity that reduces nutrient utilization and growth performance (Almirall et al., 1995). In pigs, NSP acts as a physical barrier, and the interference with digestive enzymes and digestion processes (Grieshop et al., 2001). Energy digestibility and dietary content of NSP are inversely related (Yin et al., 2002). The AID and ATTD of dietary fiber varies among sources of corn DDGS (Urriola et al., 2010) and processing methods (Belyea et al., 1989). Dietary fiber may affect fermentation in the gut by stimulating growth or metabolism of specific bacteria (Williams et al., 2001). The effects of DDGS on digestibility of energy, AA, bacterial profile and VFA production in grower pigs has not been studied thoroughly.

Dietary fiber is the sum of the polysaccharides and lignin that cannot be digested by the endogenous enzyme secretions of the gastrointestinal tract. The substrates according to this definition are the structural polysaccharides present in the plant cell wall (i.e., cellulose, hemicelluloses, pectin), structural non-

polysaccharides (i.e., lignin), and non-structural polysaccharides (gums and mucilages) secreted by the intestinal cells (Trowell and Southgate, 1976).

Most of the starch is completely digested in the small intestine (Le Goff and Noblet, 2001). In contrast, fiber is not digested by pigs (Shi and Noblet, 1993). Lignin further reduces the digestibility of other fibrous components. As a plant matures, cellulose becomes intertwined with lignin to increase the rigidity of the plant. Therefore, cellulose becomes less accessible to microbes in the hindgut, which suppress the rate and extent of fermentation.

Pectins, fructans,  $\beta$ -glucans, and other soluble dietary fiber increase digesta viscosity (Mosenthin et al., 1999). Increased viscosity in the small intestine might slow gut transit time due to suppressed intestinal contractions (Cherbut et al., 1990) that in turn leads to less mixing of digesta with digestive enzymes. Soluble dietary fiber may reduce digestion of dietary components (fibrous and non-fibrous) in the small intestine. However, the swelling associated with increased viscosity creates more surface area for microbial interaction in the hindgut. This partly explains the high ATTD of soluble fiber (Noblet and Le Goff, 2001).

Insoluble fiber is mainly fermented by microorganisms in the hindgut (Varel and Yen, 1997). Insoluble fiber can reduce the ATTD of N and ether extract (Le Goff and Noblet, 2001). Pigs fed a high fiber diet will have a heavier gastro-intestinal tract than pigs fed a low fiber diet, thereby increasing the maintenance energy requirement (Yen et al., 1991). Fermentation of NSP produces VFA and lactic acid (Bach Knudsen and Jorgensen, 2001). Hindgut

fermentation can produce 17% of the total digestible energy derived from the diet in grower pigs and 25% in sows (Shi and Noblet, 1993), because the sow has a larger hindgut and therefore more ability to digest fibrous feed. Others suggest that fermentation can supply 24 to 30% of the energy needs for grower pigs (Rérat et al., 1987).

Several factors affect NSP digestibility in pigs such as the physical and chemical characteristics of the NSP, the structure of the cell wall of the plant, and the amount of NSP present in the diet. To determine the digestibility of non-starch carbohydrates in monogastrics, we should think about the anti-nutritive effect of the NSP on nutrient digestion and absorption and the potential benefit of the fermentation products to the pig.

Total tract digestibility or fermentation of NSP increases as the pig matures (Cunningham et al., 1962) because of the more voluminous large intestine and cecum that contain a more extensive microbial population. Furthermore, larger pigs receive smaller quantity of feed relative to their body size than growing pigs. The lower relative feed intake allows a slower transit time of digesta and greater contact of endogenous enzymes and microbial populations with feed in the gut, thereby improving digestibility. Energy digestibility increases when the pig is adapted to the specific dietary fiber (Longland et al., 1993) thereby increasing VFA production and energy absorption (Castillo et al., 2007). However, not enough is known about the effect of different sources of DDGS on VFA production in grower-finisher pigs.

## **1.9 Impact of dietary fiber on microbial population in the intestine**

The stomach and small intestine of mammals do not produce enzymes capable of degrading dietary fiber. Dietary fiber that escapes the small intestine is available for bacterial fermentation in the large intestine. The fiber is degraded by the activity of microbial species that produce cellulases, hemicellulases, pectinases, and other enzymes (Varel, 1987). These microorganisms are most numerous in the cecum and colon of nonruminants. The degree of fermentation depends primarily on the source of dietary fiber and the presence of N, minerals, and vitamins that are essential for the overall nutrition of the microbial populations residing in the hindgut (Varel and Yen, 1997). The residence time of digesta is much longer in the large intestine than in the small intestine, and a considerable net absorption of water takes place (Stanogias and Percet, 1985). Thus, as the digesta flows through the gut, the DM content increases. Because fiber digestion is inherently slower than is that of non-fibrous dietary components (Demeyer and Graeve, 1991), the long residence time in the large intestine permits active bacterial fermentation of fiber.

In general, dietary fiber is fermented in the cecum and colon. Fiber initially passes through the foregut and can reduce the utilization of other components of the diet (Schulze et al., 1995). Fiber has physico-chemical properties, such as a large water-holding capacity, that exert a diverse physiological action along the entire gastrointestinal tract. The extent to which fiber exerts these effects depends on its chemical nature, the way in which fiber is physically associated with other components, its concentration in the diet, the age

and weight of the pigs or their physiological state, and the transit time in the gastrointestinal tract. Pigs fed a high-fiber diet have a higher output of gastric, biliary, and pancreatic secretions than pigs fed a low-fiber diet (Dierick et al., 1989). Some dietary fibers increase digesta viscosity, increase retention time in the small intestine, and increase endogenous N secretion in part due to the mechanical sloughing of the mucosal surface. This reduction in nutrient digestion in the small intestine as a result of feeding fiber must be considered when evaluating the energetic importance of fiber fermentation in the hindgut (Mroz et al., 1996). The decrease in nutrient digestibility may reduce the importance of the VFA contribution to the overall energy supply of pigs.

The gut microflora changes as the diet composition changes; mainly the presence of dietary fiber seems to play an important role (Awati et al., 2005). When dietary fiber is fed, more substrate enters the cecum and colon that can be fermented. In pigs fed a high fiber diet, the microbial activity was 5 times greater in the gastrointestinal tract compared to pigs fed a low fiber diet, reflected by a 5 to 9 time increase in CO<sub>2</sub> and CH<sub>4</sub> production in the gastrointestinal tract (Jensen and Jorgensen, 1994). The anaerobic bacteria concentration in the porcine gastrointestinal tract is log 7-8 colony forming units (CFU)/g in the stomach and small intestine and this concentration is up to log 10-11 CFU/g in the large intestine (Jensen and Jorgensen, 1994).

The swine gut microbiota contain cellulolytic and hemicellulolytic bacterial species such as *Ruminococcus flavefaciens*, *Butyrivibrio* spp., *Fibrobacter succinogenes*, *Ruminococcus albus*, and *Prevotella ruminicola*. A

highly active cellulolytic bacterium, *Clostridium herbivorans*, has been isolated from the large intestine of swine (Varel and Yen, 1997). The number of cellulolytic bacteria (mostly *Fibrobacter intestinalis* and *Ruminococcus flavefacien*) in adult pigs is 7 times higher than in grower pigs (Varel, 1987). The increased number of cellulolytic bacteria enhanced hindgut fermentation and VFA production and decreased the pH of digesta. A decreased pH that supports growth of beneficial bacteria, e.g., *Bifidobacteria* and *Lactobacilli*, that contributes to enhanced gut health is known as a prebiotic effect (Bouhnik et al., 2004).

Specific fiber sources may affect microbial composition in the gut. Oligofructose, galacto-oligosaccharides, and lactulose can increase *bifidobacteria* and *lactobacilli* populations in the large intestine (Macfarlane et al., 2006). Similarly, dietary guar gum or cellulose increased ileal *bifidobacteria* and *enterobacteria* in grower pigs (Owusu-Asiedu et al., 2006). However, a diet high in fermentable NSP and resistant starch increased the incidence of clinical swine dysentery in grower pigs and diarrhea in weaned pigs (Pluske et al., 2003), indicating that fiber may also cause negative effects on gut health of pigs.

### **1.10 Composition of gut bacteria**

The porcine large intestine contains both aerobic and facultative microorganism but the major species are obligate anaerobes. The fecal microflora is similar in composition to those of the colon, but may not be representative of the microflora in the cecum. The major Gram-negative species in the cecum are *Prevotella (Bacteroides) ruminicola*, *Selenomonas ruminatium*, *Butyrivibrio*

*fibrisolvens*, and *Bacteroides uniformis*. The Gram-positive species are *Lactobacillus acidophilus*, *Peptostreptococcus products*, and *Eubacterium aerofaciens* (Robinson et al., 1981). Most isolates were in the Streptococcus genus and represented 27.5% of all isolates. *Lactobacillus*, *Fusobacterium*, *Eubacterium*, *Bacteroides*, and *Peptostreptococcus* were the next most common bacteria (Moore et al., 1987). The population and activity of bacteria in the gut is affected by structure and composition, solubility, amount and type of substrate available (Macfarlane and Macfarlane, 1993). The source of fiber affects the digestion site and gut environment, thereby affecting the conditions for the proliferation of microbiota in the gut (Högberg and Lindberg, 2004).

The properties of dietary fiber can alter intestinal microbial composition in pigs. The microbial population and activity was greater in the intestine of the pigs fed soluble fiber such as field pea fiber and pectin (Jensen and Jorgensen, 1994). In weaned pigs fed the 3 dietary carbohydrates sources corn, wheat or barley, the bacterial composition was related with the dietary fiber content (Drew et al., 2002). Specifically, in the small intestine of pigs fed barley, *Lactobacilli* increased and *enterobacteria* decreased compared to pigs fed corn. Moreover, barley increased *Lactobacilli* species and *Bifidobacterium* species in the cecum, compared to corn, a change that might be due to the high  $\beta$ -glucan content in barley. Pigs fed wheat had a higher number of *Bifidobacteria* species and lower number of total anaerobes and *Clostridium* compared to pigs fed barley. Therefore, a complex relationship exists between the type and chemical composition of fermentable substrate and intestinal bacteria composition (Drew et al., 2002).

Changes in intestinal microbial composition due to different DDGS sources like wheat, corn and triticale in the intestine of grower finisher pigs have not been studied.

## **1.11 Metabolites produced by gut bacteria**

### ***1.11.1 Volatile fatty acids***

The final phase of digestion occurs in the hindgut of pig. It involves the breakdown of carbohydrates by microbes under anaerobic conditions to volatile fatty acid (VFA) and gases such as H<sub>2</sub>, CO<sub>2</sub>, and CH<sub>4</sub>. The main VFA produced in pigs are acetate, propionate, and butyrate, which account for 90 to 95% of total VFA production (Christensen et al., 1999). Several factors such as the type and chemical structure of polysaccharides fermented, activity of the colonic microbial population and gastrointestinal tract transit time can affect the composition of VFA produced in the large intestine (Englyst et al., 1987). About 95% of the VFA generated in the large intestine are absorbed from the colon during transit of digesta through the gut (Cummings, 1981). In the pig, approximately 95 mmol/d of VFA are absorbed per kg of BW (Stevens et al., 1980). The VFA are absorbed through the intestinal epithelium, and are used as an energy source for the host (Cummings, 1981). The absorbed VFA passes into the portal vein (Cummings and Macfarlane, 1991) and utilized by body tissues subsequently to meet energy requirement. A large portion of NSP is fermented by the microflora of the porcine large intestine, and can contribute up to 50% of the dietary energy (Choct and Kocher, 2000).

In pigs, fermentation of NSP and oligosaccharides is conducted by chemical degradation by the microbes. A large portion of NSP might be digested by the large intestine microflora in pigs so the NSP digestibility can be as high as 93% (Stanogias and Percet, 1985). Carbohydrate type and fermentation products are associated. For example, fermentation of soluble pectins produces 80% acetate and only small amounts of butyrate, whereas guar gum produces less acetate and more butyrate (Cummings, 1981). However, individual sugars with different fermentation rates did not cause differences in the molar ratio of VFA in different parts of the porcine large intestine (Canibe et al., 1997).

The VFA produced have unique metabolic pathways. Most absorbed acetate flows into the portal system and acts as an energy source for the local periphery. Most propionate is metabolized by the liver although some is metabolized by colonocytes. Butyrate is a major fuel for the colonocytes in humans and swine. A rapid passage of fermentable substrates into the large intestine will produce lactic and succinic acid (Sakata, 1987). The VFA play an important role in Na absorption, stimulate blood flow, and regulate absorption of nutrients (Sakata, 1987).

### ***1.11.2 Ammonia and amines***

The undigested part of protein is also fermented by bacteria and produces VFA and toxic nitrogenous substances such as ammonia and amines that can reduce growth performance and causes diarrhea in pigs (Gaskins, 2001). Gut bacteria have positive and negative effects on the host. Bacterial degradation

limits the availability of AA to the host and produces a variety of metabolites that negatively affect physiological and epithelial function. Ammonia is a toxic catabolite of microbial AA deamination and urea hydrolysis (Visek, 1984). About 40% of the urea synthesized by the liver is hydrolyzed to ammonia by bacterial urease (Drasar and Hill, 1974). In the gut, ammonia is used as an N source by anaerobic bacteria in the presence of fermentable carbohydrates (Bryant and Robinson, 1962). Thus, the addition of fermentable carbohydrates increases ammonia use by gut bacteria, thereby reducing ammonia in the lumen (Weber et al., 1987). Ammonia concentration was reduced in cecal digesta of pigs when dietary CP content was reduced (Nyachoti et al., 2006; Htoo et al., 2007). Reduced ammonia concentration benefits gut health (Gaskins, 2001).

Cadaverine, histamine, putrescine, and tyramine are active amines produced in the gut. High putrescine and cadaverine concentrations were associated with diarrhea at weaning in pigs (Porter and Kenworthy, 1969). Reduction of dietary CP content from 24 to 18% decreased cadaverine concentration in the large intestine of pig by 49% (Schneider et al., 1989). The concentration of one of the major amines, putrescine, in the cecum was decreased by reducing dietary CP while balancing for AA using supplemental crystalline AA (Htoo et al., 2007).

### **1.12 Diet formulation to study energy and amino acid digestibility**

In swine nutrition, digestible nutrient content is critical for accurate feed formulation; thus a requirement to determine digestibility of energy and AA in

feedstuffs exists. For energy, the ATTD of GE is determined and forms the basis for both DE and ME values for feedstuffs. For AA, the situation is more complex.

For AA, apparent ileal digestibility (AID) as opposed to ATTD is used to determine AA digestibility (Lin et al., 1987; Knabe et al., 1989; Mosenthin et al., 2000). Using AID avoids the impact of fermentation of AA by microbial populations in the large intestine (Sauer et al., 1977), because residual AA or ammonia can be used for microbial protein synthesis. The AID of AA varies widely among and within ingredients, due to processing method and environmental factors that impact the chemical composition of feedstuffs (Sauer and Ozimek, 1986; Yin et al., 2008).

The ileal digestibility values can be expressed as AID, standardized ileal digestibility (SID) or true ileal digestibility (TID). These terminologies are used to indicate the correction for ileal endogenous AA losses. In AID, endogenous AA loss is not corrected while in SID basal endogenous losses are subtracted. This correction can either be completed via a standard correction or by including an N-free diet in the experiment (Stein et al., 2007). In TID, the specific endogenous losses caused by feedstuff composition such as fiber and anti-nutritional factors are also considered (Stein et al., 2007). The lack of correction for endogenous AA losses that resulted in lack of additivity of feedstuffs values in a complete feed and a discount of the AA value of cereal grains.

During determinations of AA digestibility, not only endogenous AA losses but also high dietary protein content should be avoided, because the levels of AA in test diets has an important effect on AID determinations (Fan et al., 1995). For

feedstuffs such as wheat DDGS, feed intake might also become a problem to use the direct method with the test feedstuff being the sole AA ingredient in the diet (Adeola, 2001). For high protein and high fiber feedstuffs such as wheat DDGS, these problems can be avoided by using the difference method in which a control diet without the test feedstuff is compared to a diet that contain the test feedstuff (Fan and Sauer, 1995). For example, wheat or corn starch can be used as the main feedstuff in the control diet (Fan et al., 1994). Especially for high fiber feedstuffs, AA digestibility should be determined using difference method (Adeola, 2001).

In the difference method for digestibility measurements of a nutrient or energy, the formulation of both the test and the basal diet are involved. The basal diet will have only the basal ingredient or ingredients and that will be the sole source of nutrient in the diet. The test diet contains a mixture of basal feed ingredient and the test feed ingredient. For example, wheat or N-free as a basal diet and fed to pigs to determine the digestibility of the basal diet. Simultaneously, other pigs can be fed the basal diet (wheat or N-Free) plus a known quantity of test feedstuff (e.g. wheat DDGS) and determine the digestibility of the mixture. Then the digestibility of the component of test feedstuff (wheat DDGS) can be determined by the difference method using the formula provided previously (Adeola, 2001). After that, the nutrient digestibility in the test diet can be determined by the difference method. The assumption is that the test and basal feed ingredient do not interact; however, this interaction had not been tested. In other words, the basal diet may impact the values obtained

for the test feedstuff, because endogenous losses are not measured for each test diet and thus not corrected for.

### **1.13 Summary**

Ethanol can be produced from corn, barley, wheat, rye, triticale, and sorghum, and the co-product DDGS is produced. The DDGS can be used in swine diet to reduce feed cost. One of the biggest challenges for DDGS is dealing with the variability in digestible nutrient content among DDGS sources. The nutritional value of DDGS is characterized by the grain and production processes used. Color, particularly lightness and yellowness, may predict AA digestibility reasonably, especially for Lys. Particle size, bulk density, color, smell, and flowability can vary among DDGS sources and thus are part of the quality characteristics. Ethanol plants use a wide range of production processes that create a range in DDGS quality.

Because DDGS can be overheated during drying, a risk exists that the Lys content and digestibility could be reduced due to Maillard reactions (Cromwell et al., 1993). The chemical composition, digestibility and nutritional value of the DDGS should be known before incorporation into swine diet formulation. Much research has been conducted with corn DDGS but little with wheat DDGS. Thus, information is lacking about western Canadian wheat and triticale DDGS and its comparison with corn DDGS.

Due to lack of this information, two studies were conducted to characterize and determine the effect of wheat DDGS and different sources of

DDGS on energy and AA digestibility and their effects on VFA profile in ileal digesta and feces of grower-finisher pigs. For study 1, the main objective was to determine the digestibility of energy, crude protein and AA of wheat DDGS using the difference methods using two basal diets in finisher pigs. For study 2, objective 1 was to test the hypothesis that DDGS from different sources will have similar digestibility of energy, CP, and AA in grower pigs. Objective 2 was to test the hypothesis that DDGS from different sources do not affect VFA profile in ileal digesta and feces of grower pigs.

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## Chapter 2

Influence of control diet on energy, crude protein and amino acid digestibility of wheat distillers dried grains with solubles in finisher pigs

### 2.1 Abstract

Wheat distillers dried grains with solubles (DDGS) is the main co-product from the bio-ethanol industry in western Canada, but little is known about its nutrient digestibility and resulting volatile fatty acid (VFA) production in the pig intestine. The objective of this experiment was to compare the digestible energy (DE) and standardized ileal digestibility (SID) of CP and AA of wheat DDGS using 2 types of control diets based on either wheat grain or corn starch (N-free) using the difference method and resulting VFA profile in ileal digesta and feces of growing pigs. In a double  $4 \times 4$  Latin square design, 8 ileal cannulated finishing pigs (initial BW  $80 \pm 2.5$  kg) were fed one of 4 diets (wheat grain, wheat grain plus wheat DDGS, corn starch plus wheat DDGS or N-free diet). In 9-d periods consisting sequentially of a 5 d of adaptation and 2 d of feces and 2 d of digesta collection, 8 observations per diet were obtained. The wheat DDGS contained 5.09 Mcal GE/kg DM, 41.7% CP and 0.79% Lys. The apparent total tract digestibility of energy in wheat DDGS was higher ( $P < 0.007$ ) when digestibility was calculated by difference from the wheat instead of the corn starch diet (68.8 vs. 66.0%) and the DE content followed an identical pattern (3.49 vs. 3.35 Mcal/kg of DM). The apparent ileal digestibility (AID) and SID of most of AA

including Lys, Thr and Arg did not differ ( $P > 0.05$ ) between the two methods. The AID and SID of CP and AA was lower ( $P < 0.05$ ) for wheat DDGS than wheat grain. The concentration of acetate, butyrate and total VFA was higher ( $P < 0.001$ ) in ileal digesta of pigs fed the wheat grain diet than any other diet. The VFA concentration in digesta did not differ ( $P > 0.05$ ) between pigs fed wheat grain plus wheat DDGS diet and corn starch plus wheat DDGS. The concentrations of VFA, except acetate, were higher ( $P < 0.001$ ) in feces of pigs fed wheat grain diet. Propionate, butyrate, valerate, caproate and total VFA did not differ ( $P > 0.05$ ) between the feces of pigs fed wheat grain and wheat plus wheat DDGS diets. In conclusion, energy digestibility of wheat DDGS differed when determined by the difference from wheat grain or corn starch-based diets. However, digestibility of CP and most of the AA did not differ between the two methods.

## **2.2 Introduction**

Feed is the highest variable cost in swine production. With increasing feed grain prices, wheat distillers dried grains with soluble (DDGS) that is produced by the western Canadian ethanol industry provides an opportunity for swine producers to mitigate feed costs. Wheat DDGS is a source of energy, AA, and P (Zijlstra et al., 2007) and can be included in swine diets (Stein and Shurson, 2008). The nutrient composition of DDGS depends on the grain and its quality that was used to produce ethanol (Cromwell et al., 1993; Fastinger and Mahan, 2006). However, even if the same grain is used, the chemical composition of

DDGS may vary (Fabiosa, 2008). Currently, little information exists about the digestible nutrient value of wheat DDGS, especially from modern ethanol plants.

Wheat DDGS also contains fiber. Dietary NSP may modulate the cellulolytic bacteria and VFA production in the swine gut (Ehle et al., 1982; Varel et al., 1982; Varel et al., 1984; Yang et al., 2010). However, the extent of fermentation of wheat DDGS and its influence on VFA production in pig intestine is not clear.

The nutrient digestibility of high fiber feedstuffs can be determined by difference from cereal-based (Widyaratne and Zijlstra, 2007) or corn starch-based diets (Stein et al., 2006). The use of these 2 control diets has not been compared. In the present study, we prepared two sets of diet; one pair containing wheat grain (Method 1) and one pair containing corn starch (Method 2), and applied the difference method to determine the nutrient digestibility of wheat DDGS. Furthermore, the VFA concentration in ileal digesta and feces were used as an indicator of fermentation.

We hypothesized that wheat DDGS is an important source of energy, protein and AA and that the digestible nutrient profile would not differ between the 2 methods. The objectives of the study were to assess energy, protein, AA digestibility and VFA concentration in ileal digesta and feces of finisher pigs fed corn starch-or wheat based diet.

## **2.3 Materials and methods**

The experimental procedures were reviewed by the University Animal Policy and Welfare Committee, University of Alberta and followed guidelines established by the Canadian Council on Animal Care (2009). The study was conducted at the Swine Research and Technology Centre, University of Alberta (Edmonton, AB, Canada).

### ***Experimental Diets and Design***

The wheat DDGS used for study was sourced from Terra Grain Fuels, Belle Plain, Saskatchewan, Canada. Four diets were prepared: wheat grain, wheat grain plus wheat DDGS, corn starch plus wheat DDGS, and corn-starch-based N-free diet (Table 2.1). The N-free diet was used to calculate basal endogenous AA and CP losses for the other 3 diets and energy digestibility of wheat DDGS. The 3 other diets were formulated to provide 16% CP and exceed the requirement (NRC, 1998) for most nutrients. Chromic oxide (0.4% of diet) was used as an indigestible marker. Diets were mixed for 15 min using a horizontal paddle mixer (SPC-2784, Marion Mixers, Marion, IA).

The study was a double  $4 \times 4$  Latin square design including an N-free diet. The 4 diets were initially allotted to 8 pigs randomly, so that each pig consumes each diet over 4 experimental periods. Therefore, 2 observations per diet per period were obtained. Each 9 d experiment period consisted of a 5-d diet adaptation followed sequentially by 2-d feces collection and a 2-d digesta collection.

### ***Experimental Procedures***

Eight cross-bred barrows (initial BW,  $80 \pm 2.5$  kg: Duroc  $\times$  Large white/Landrace F<sub>1</sub>; Genex Hybrid; Hypor, Regina, Saskatchewan) were housed individually in metabolism pens made of PVC planking sides with a plexi-glass windows and plastic flooring. The pens ( $1.2 \times 1.5 \times 0.95$  m) were equipped with a stainless steel, dry self-feeder attached to the front of the pen. A nipple drinker was attached near the feeder. The pen provided freedom of movement for the pig. Pigs were surgically fitted with a T-cannula at the distal ileum (Sauer et al., 2000) and were housed in an environmentally-controlled room. Lights were on from 0800 to 2200 hr. The room temperature was set to 22°C. After surgery, pigs recovered for 7 d with a gradual increase in feed allowance. Pigs were then switched to the first assigned experimental diet. Pigs were weighed at the beginning of each period and offered the assigned experimental diet at  $2.8 \times$  maintenance ( $110 \text{ kcal DE/kg BW}^{0.75}$ , NRC, 1998) divided equally into morning (0800 h) and afternoon (1500 h) meals. Pigs had a free access of water.

In the morning of the 1<sup>st</sup> and 2<sup>nd</sup> day of the collection period, plastic bags were snapped between the inner Velcro and the leather rings fixed around the anus and attached for the collection of feces. Feces were collected continuously for 48 hr. Fresh feces and digesta were collected and stored immediately at -80°C for VFA analyses. After digesta collection for VFA analyses, a plastic bag containing approximately 15 mL of 5% formic acid was attached to the open T-cannula for the collection of digesta for digestibility measurements.

Digesta was collected continuously from 0800 until 1800h. Feces voided during digesta collection were promptly cleaned to prevent coprophagy and recycling of the Cr<sub>2</sub>O<sub>3</sub> marker. Collected digesta and feces were pooled by pigs within period and then thawed, homogenized, sub sampled and frozen at -20°C for digestibility measurements.

### ***Chemical Analyses***

Feces and digesta were freeze-dried. Ingredients, feed samples and lyophilized feces and digesta were ground through a 1-mm screen using a centrifugal mill (model ZM 200, Retsch Co., Newtown, PA).

Ingredients, diets, digesta, and feces were analyzed for dry matter using a hot air oven at 135°C for 2 hr (method 930.15; AOAC, 1990). Gross energy of ingredient, diets, digesta and feces were determined using a bomb calorimeter (model C 5003, IKA GmbH and Co., Germany). Ingredients, diets, feces and digesta were analyzed for N by combustion (method 990.03; AOAC, 2006) using a Leco protein/N analyzer (model FP-582, Leco Co., St. Joseph, MI). Diets, feces and digesta were analyzed for chromic oxide using a spectrophotometer at 440 nm after ashing at 450°C (Fenton and Fenton, 1979).

Ingredient, diets, and digesta were analyzed for AA using high performance liquid chromatography (Model 210; Varian Instruments Inc. Walnut Creek, CA) (method 982.30E; AOAC, 2006) at the University of Alberta, AB. Samples were hydrolyzed with 6 N HCl for 24 h at 110°C (method 982.30 E; AOAC, 2006) using b-amino-n-butyric acid as an internal standard. Cysteine and Methionine were

determined as methionine sulphone and cysteic acid after cold performic acid oxidation overnight before hydrolysis (method 982.30 E; AOAC, 2006). Available lysine and complete AA profile in ingredients were analyzed at the University of Missouri (Columbia, MO) using method 982.30E (AOAC, 2006). Ingredient were analyzed for crude fat (method 920.39(A); AOAC, 2006), crude fiber (method 978.10; AOAC, 2006), ADF (method 973.18 A-D; AOAC, 2006), NDF (Holst, 1973), and total dietary fiber (method 985.29; AOAC, 2006).

For VFA analyses, digesta and feces were thawed and stirred. One g of sample was mixed with 3 g of distilled water. Then 300 µl of internal standard (isocaproic acid) and 200 µL of 25% phosphoric acid were added followed by vortexing until the sample was completely dissolved in the liquid. Then the tube was centrifuged at  $17,000 \times g$  for 30 min. The supernatant was then analyzed for VFA using gas chromatography, (model 3400, Varian Instruments Inc., Walnut Creek, CA) (method 996.06; AOAC, 2006).

### ***Calculations***

Digestibility calculations were conducted on a DM basis using the indicator method (Adeola, 2001; Stein et al., 2006; Stein et al., 2007). The apparent digestibility (AD) of nutrients in diets was calculated as follows:

Apparent digestibility, % =  $100 - [100 \times (\text{concentration of Cr}_2\text{O}_3 \text{ in feed} \times \text{concentration of component in feces or digesta} / \text{concentration of Cr}_2\text{O}_3 \text{ in feces or digesta} \times \text{concentration of component in feed})]$

The basal ileal endogenous loss ( $I_{\text{end}}$ ) of an AA or CP protein (g/kg of DM intake) was calculated by using the equation for the N-free diet (Stein et al., 2007):

$$I_{\text{end}} = \{ \text{AA or CP in digesta} \times (\text{Cr}_2\text{O}_3 \text{ in feed} / \text{Cr}_2\text{O}_3 \text{ in digesta}) \}$$

Standardized ileal digestibility (SID) values for amino acid were calculated by correcting the AID for basal endogenous losses by the equation (Stein et al., 2007):

$$\text{SID} = \{ \text{AID} + (\text{IAA}_{\text{end}} / \text{AA in feed}) \}$$

The apparent total tract digestibility (ATTD) of energy in diets was also calculated using the first equation above. Then, the analyzed concentration of GE in the diet was multiplied by the ATTD of GE to obtain DE values.

Digestibility of the component in the DDGS was calculated using

$$A, \% = 100 \times [(T \times T_p) - (B \times B_p) / A_p]$$

where: T is the digestibility, %, of the component in the total diet (basal plus test article); B is the digestibility, %, of the component in the basal diet; Bp is the proportion, %, of the component in the total diet contributed by the basal diet; Ap is the proportion, %, of the component in the total diet contributed by the test article (Adeola, 2001).

$$T_p = B_p + A_p = 100\%$$

The content of SID CP and AA was calculated by multiplying SID measured in the digesta sample with total CP and AA content of DDGS.

### ***Statistical Analyses***

The nutrient digestibility and VFA data were analyzed as a Latin square using the PROC MIXED procedure (SAS Inst. Inc., Cary, NC) using the individual pig as the experimental unit. The model included diet as a fixed effect and period and pig as random effects. The N-free diet was not incorporated in the contrast to compare ingredients. Instead the N-free diet was used for calculating the endogenous nitrogen loss to calculate standardized ileal digestibility of protein and AA. Digestibility values of wheat DDGS based on wheat grain and corn starch diets were compared using a preplanned contrast. A probability of  $P < 0.05$  was accepted as statistically significant.

### **2.4 Results**

Pigs consumed their daily meals and feed refusals were not observed. One pig was removed from the experiment during the fourth period due to peritonitis. The DDGS contained 43.4% CP, 4.6% ether extract, and 30.3% NDF (Table 2.2). In wheat grain, the available Lys was 97.8% but only 80% of total Lys was available in the wheat DDGS (Table 2.3). In DDGS, the insoluble NSP was 78.3% of the total NSP and the total arabinose was 22.3% of the total NSP (Table 2.4). Diet nutrient profile indicated that the diets were mixed properly (Table 2.5).

### ***Diet Digestibility***

The AID and ATTD of GE was higher ( $P < 0.001$ ) for the corn starch-based DDGS diet than the wheat-based DDGS diet (Table 2.6). The AID and the

ATTD of GE was higher ( $P < 0.01$ ) in the wheat grain diet than wheat DDGS diets, resulting in a higher ( $P < 0.001$ ) DE content for the wheat grain than wheat DDGS diets.

The AID of CP and AA did not differ between methods (Table 2.7), except for phenylalanine. The AID of CP, Lys and Thr was lower ( $P < 0.01$ ) for the wheat DDGS diets than the wheat diet.

### ***Ingredient Digestibility***

The ATTD of energy for wheat DDGS was lower ( $P < 0.01$ ) in the corn starch-based diet than in the wheat-based DDGS diet (Table 2.8). The SID of CP and AA did not differ between the 2 methods, except for phenylalanine. The ATTD of energy and SID of CP and AA was higher ( $P < 0.05$ ) in the wheat grain than wheat DDGS.

### ***Digestible Nutrient Content of Ingredients***

The DE content for wheat DDGS was lower ( $P < 0.01$ ) in corn starch-based than wheat-based diet (Table 2.9). The SID CP and AA content did not differ between the two methods. The SID CP and AA content was higher ( $P < 0.001$ ) in wheat DDGS than wheat grain.

### ***VFA in Ileal Digesta and Feces***

Acetate, butyrate, and total VFA in ileal digesta was higher ( $P < 0.001$ ) in pigs fed the wheat grain diet than pigs fed the DDGS diets (Table 2.10) and did

not differ between pigs fed the wheat- or corn starch- based DDGS diets except butyrate. The isobutyrate, isovalerate, valerate and branched-chain fatty acids (BCFA) in ileal digesta did not differ among diets.

The VFA profile in feces differed ( $P < 0.001$ ) among diets (Table 2.11). Between pigs fed the DDGS diets, propionate, butyrate, and total VFA in feces was higher for wheat-based than corn-starch based diets. Feces BCFA was higher ( $P = 0.001$ ) in pigs fed the wheat grain diet than wheat DDGS diets.

## **2.5 Discussion**

The results of this study demonstrated that the dietary energy but not AA digestibility differed between the 2 control diets (wheat-based and cornstarch-based) using the difference method to determine the digestible nutrient profile of wheat DDGS in swine. Therefore, the type of control diet selected to determine the nutritional value of feedstuffs using the difference method may impact the energy value of test ingredients.

The AA digestibility in high protein feedstuffs are usually determined using the direct method (Lin et al., 1987). The AID of AA in feedstuffs is dependent on dietary composition (Fan et al., 1994). Especially for high fiber feedstuffs, energy digestibility should be determined using the difference method (Adeola, 2001). For the high fiber feedstuffs such as DDGS, wheat grain (Widyaratne and Zijlstra, 2007) or corn starch (Stein et al., 2006) can be used as a control diet. In the present study, diets containing wheat DDGS were based on wheat grain or corn starch to allow determination of energy digestibility by

difference method and protein and AA digestibility, using either the difference or direct method, respectively. Furthermore, the VFA content in ileal digesta and feces was compared.

The composition of wheat DGDS varies among experiments. The CP content of the wheat DDGS in the present study was 43.4% of DM, which is higher than previous studies (34.4 and 36.1%; Emiola et al., 2009b; Cozannet et al., 2010b; respectively) but lower than others (44.5%; Widyaratne and Zijlstra, 2007). The gross energy content of wheat DDGS in the present study (5.07 Mcal/kg) was similar to previous studies (5.19, 4.77, and 4.98 Mcal/kg of DM; Widyaratne and Zijlstra, 2007; Emiola et al., 2009a; Cozannet et al., 2010b; respectively). The total Lys content was 0.79% in the present study, a content higher than reported previously (0.72, 0.67, 0.63%; Widyaratne and Zijlstra, 2007; Nyachoti et al., 2005; Lan et al., 2008; respectively). Findings reported thus depend on the quality of wheat DDGS used in a specific study.

### ***Digestibility of energy***

Several factors can influence energy digestibility of feedstuffs fed to pigs. The addition of wheat DDGS in swine diets increased the fiber content of these diets, and reduced energy digestibility (Bach Knudsen et al., 1993). The effect of dietary fiber on energy digestibility depends on its physicochemical properties (Jha et al., 2010). In DDGS, a major portion of fiber is insoluble fiber (Urriola et al., 2010). Furthermore, mean particle size can affect energy digestibility of DDGS (Yanez et al., 2011). The digestible energy (DE) content of the wheat

DDGS in the present study was 3.35 Mcal/kg DM, a value higher than reported by some (3.22 Mcal/kg of DM; Nyachoti et al., 2005) but less than others (3.55 and 3.42 Mcal/kg of DM; Widyaratne and Zijlstra, 2008; Sauvant et al., 2004).

The calculated ATTD of energy differed between the two diets used using the difference method. The higher ATTD of energy of wheat DDGS calculated using the wheat grain based-diet compared with corn starch based-diet is explained by the higher VFA concentration in feces in pigs fed the wheat DDGS diet based on wheat grain. Similarly, the energy as VFA available to the pig from the hindgut fermentation increased from 7 to 18% of available energy when dietary NSP increased from 77 to 240 g/kg of feed DM (Anguita et al., 2006).

In diet formulation, the diet DE value is assumed to be cumulative or additive, so that the combined total DE provided by individual feedstuffs equals to total DE content of the diet. Furthermore, diet DE content is assumed to be independent of other dietary components (Whittemore and Moffat, 1976; Young et al., 1977). These assumptions are reasonable for ingredients of similar chemical composition, but might be questionable for dissimilar feedstuffs containing atypical levels of protein, fat, fiber and non-carbohydrate energy sources. Under the latter conditions, the DE departed from linearity of substitution in a compound diet (Frape et al., 1976). Finally, DE is lower for young pigs with a developing digestive system than for older pigs with a matured digestive system with more active microflora (Morgan and Whittemore, 1981).

The lower ATTD of energy in wheat DDGS diet than wheat grain might be due to the higher fiber content in wheat DDGS than wheat grain. During

fermentation in the ethanol plant, wheat starch is fermented into ethanol and CO<sub>2</sub>. Protein, fat and fiber therefore concentrated nearly 2 to 3 times. In the present study, the NSP content, especially arabinoxylans, was higher in DDGS than in wheat grain. The swine digestive system does not produce xylanase; the energy digestibility was likely reduced because pig's intestine cannot hydrolyze the arabinoxylan present in DDGS (Widyaratne and Zijlstra, 2008).

### ***Digestibility of protein and AA***

The AID and SID of CP was lower for wheat DDGS than wheat grain. The difference might be due to the higher content of ADF and NDF in wheat DDGS. Furthermore, wheat DDGS contains more insoluble NSP that can reduce CP digestibility (Shi and Noblet, 1993 and Le Goff and Noblet, 2001). Finally, dietary fiber in the large intestine of pigs may shift N excretion from urine to feces (Zervas and Zijlstra, 2002).

The AID coefficients of AA did not differ between the difference and direct method, indicating that for AA method might be less important. The AID of AA was less for wheat DDGS than wheat grain. The AID of AA including Lys and Lys availability may be reduced due to heat damage during drying (Stein and Shurson, 2009) and extent of addition of solubles to the distillers grains in the DDGS production process (Cromwell et al., 1993). High temperature during DDGS production mainly affects Lys, which is the first limiting AA for pigs. The high temperature promotes the Maillard reaction between AA especially Lys and sugar moieties. Heating may thus reduce digestibility and availability of AA and

change the color of the final product to dark. The reduced AA availability and reduced digestibility is due to the formation of amadori products that reduce the rate of AA digestion by blocking the sites of enzyme binding (Papadopoulos, 1989).

The SID of AA was higher in wheat grain than wheat DDGS. The SID of Lys did not differ between wheat DDGS based on wheat grain (Method 1) or based on corn starch (Method 2). Dietary fiber can also reduce AA digestibility (Schulze et al., 1994) by reducing true digestibility of the feedstuff or by increasing endogenous losses that are ingredient-specific; the SID of AA does not correct for these losses (Stein and Shurson, 2008). The wheat DDGS used in the present study contained twice the total dietary fiber than wheat grain.

### ***VFA concentration***

Fermentability of feedstuffs affects dietary VFA production in the gut. Digesta total VFA, acetate, and butyrate were higher in pigs fed wheat grain diet than pigs fed wheat DDGS diets. Feces but not digesta VFA differed between pigs fed the DDGS diet based on wheat grain (Method 1) and based on corn starch (Method 2). Less cellulolytic bacteria are present in the swine small intestine. Feeding cereal grain instead of merely starch made more substrate available for fiber-fermenting bacteria in the large intestine, thereby increasing fecal VFA. However, the VFA profile is also affected by the substrate fermented. The in vitro fermentation of starch produced more butyrate but xylans and pectins promoted the production of more acetate (Englist and Macfarlane, 1987). Similarly, pigs fed

a diet having sugar beet pulp produced more acetic acid compared with other diets (Wang et al., 2004). The inclusion of DDGS in the diet decreased the concentration of acetate, butyrate and total VFA in the digesta and feces, because DDGS is less fermentable than wheat grain due to the higher insoluble fiber content in wheat DDGS.

In conclusion, energy digestibility of wheat DDGS differed when determined from a wheat grain-based or a corn starch-based diets. However, digestibility of CP and most of the AA did not differ between the two methods.

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**Table 2.1.** Ingredient composition of experimental diets

Item	Wheat DDGS <sup>1</sup>			
	Wheat	Wheat (Method 1)	Cornstarch (Method 2)	Corn starch
Ingredient, %				
Cornstarch <sup>2</sup>	-	-	52.37	85.33
Wheat DDGS <sup>3</sup>	-	40.00	40.00	-
Wheat grain	96.30	56.30	-	-
Sugar	-	-	3.04	5.00
Solka floc <sup>4</sup>	-	-	-	3.00
Canola oil	-	-	1.22	2.00
Limestone	1.10	1.10	1.50	1.00
Dicalcium phosphate	0.80	0.80	-	1.20
Salt	0.40	0.40	0.50	0.50
Vitamin premix <sup>5</sup>	0.50	0.50	0.50	0.50
Mineral premix <sup>6</sup>	0.50	0.50	0.50	0.50
KCO <sub>3</sub>	-	-	-	0.50
MgO	-	-	-	0.10
Cr <sub>2</sub> O <sub>3</sub>	0.40	0.40	0.40	0.40

<sup>1</sup>DDGS, distillers dried grain with solubles.

<sup>2</sup>Cornstarch, Melojel (National Starch and Chemical Co., Bridgewater, NJ).

<sup>3</sup>Wheat DDGS (Terra Grain Fuels, Belle Plain, Saskatchewan, Canada).

<sup>4</sup>Solka floc (International Fiber Corporation, North Tonawanda, NY).

<sup>5</sup>Provided the following per kg of diet: vitamin A, 82,500 IU; vitamin D, 8,250 IU; vitamin E, 400 IU; menadeone, 40 mg; thiamine, 10 mg; riboflavin, 50 mg; niacin, 350 mg; D-pantothenic acid, 150 mg; vitamin B<sub>12</sub>, 0.25 mg; biotin, 2 mg; and folic acid, 20 mg.

<sup>6</sup>Provided the following per kg of diet: copper, 500 mg; iron, 800 mg; manganese, 250 mg; zinc, 1000 mg; iodine, 5 mg; and selenium, 1 mg.

**Table 2.2.** Analyzed chemical composition of wheat grain and wheat distillers dried grains with solubles (DM basis)

Item,%	Wheat grain	Wheat DDGS <sup>1</sup>
Moisture	12.7	9.1
Ether extract	1.5	4.6
Crude fiber	2.7	7.3
CP	14.6	43.4
Ash	2.0	5.5
Soluble NSP	1.3	4.0
Insoluble NSP	11.2	16.7
Total NSP	12.5	20.7
ADF	3.6	15.2
NDF	15.6	30.3
Total dietary fiber	12.1	28.2
Ca	0.14	0.12
P	0.40	1.01

<sup>1</sup>DDGS, distillers dried grain with solubles.

**Table 2.3.** Analyzed AA concentration of wheat grain and wheat distillers dried grains with solubles (DM basis)

Item, %	Wheat grain	Wheat DDGS <sup>1</sup>
Indispensable AA		
Arginine	0.66	1.76
Histidine	0.32	0.80
Isoleucine	0.53	1.47
Leucine	0.97	2.61
Lysine	0.44	0.97
Methionine	0.23	0.64
Phenylalanine	0.65	1.77
Threonine	0.39	1.17
Tryptophan	0.19	0.35
Valine	0.64	1.18
Dispensable AA		
Alanine	0.50	1.48
Aspartate	0.72	1.94
Cysteine	0.31	0.76
Glutamate	4.09	8.94
Glycine	0.59	1.61
Proline	1.34	3.33
Serine	0.54	1.52
Tyrosine	0.38	1.13

Lysine as % of CP	3.01	2.23
Available lysine	0.43	0.78
Lysine availability	97.7	80.4

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<sup>1</sup>DDGS, distillers dried grain with solubles.

**Table 2.4.** Analyzed NSP composition in wheat grain and wheat distillers dried grains with solubles (DM basis)

Item, %	Wheat grain	Wheat DDGS <sup>1</sup>
Arabinose		
Total	2.06	4.80
Insoluble	1.73	4.03
Soluble	0.33	0.77
Xylose		
Total	3.33	8.11
Insoluble	2.95	6.12
Soluble	0.38	1.99
Mannose		
Total	0.21	0.80
Insoluble	0.17	0.50
Soluble	0.04	0.30
Glucose		
Total	6.10	6.86
Insoluble	5.98	5.78
Soluble	0.12	1.08
Galactose		
Total	0.43	0.93
Insoluble	0.16	0.42
Soluble	0.27	0.51
Total NSP	12.08	21.52
Insoluble	10.99	16.86
Soluble	1.09	4.66

<sup>1</sup>DDGS, distillers dried grain with solubles; NSP, non-starch polysaccharide.

**Table 2.5.** Analyzed chemical and AA composition of experimental diets (DM basis)

Item, %	Wheat DDGS <sup>1</sup>			
	Wheat	Wheat (Method 1)	Cornstarch (Method 2)	Cornstarch
Moisture	12.5	10.8	8.9	9.2
Crude protein	14.5	25.7	17.8	0.56
Ether extract	1.4	2.7	2.8	0.89
Crude fiber	2.8	4.7	3.5	1.7
Ash	4.7	6.4	4.8	3.7
GE, Mcal/kg	3.79	4.05	4.06	3.77
Indispensable AA				
Arginine	0.62	1.00	0.63	-
Histidine	0.23	0.36	0.23	-
Isoleucine	0.55	0.99	0.66	0.01
Leucine	0.91	1.63	1.08	0.03
Lysine	0.37	0.55	0.35	0.01
Phenylalanine	0.62	0.68	0.72	-
Threonine	0.37	0.65	0.45	-
Valine	0.64	1.12	0.79	0.01
Dispensable AA				
Alanine	0.50	0.95	0.65	0.01
Aspartate	0.73	1.19	0.80	0.03

Glutamate	4.31	7.08	4.51	0.05
Glycine	0.60	1.05	0.73	-
Serine	0.43	0.76	0.53	-
Tyrosine	0.27	0.50	0.34	-

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<sup>1</sup>DDGS, distillers dried grain with solubles.

**Table 2.6.** Digestibility of gross energy and DE content of the diets (DM basis)<sup>1</sup>

Item	Wheat DDGS <sup>2</sup>				P-value			
	Wheat	Wheat (Method 1) <sup>3</sup>	Cornstarch (Method 2) <sup>4</sup>	Cornstarch	SEM	Diet	Method 1 vs. 2	Wheat DDGS vs. Wheat
AID of diet								
GE, %	75.5 <sup>b</sup>	59.8 <sup>c</sup>	74.7 <sup>b</sup>	90.2 <sup>a</sup>	2.52	< 0.001	< 0.001	0.004
DE, Mcal/kg	3.22 <sup>b</sup>	2.70 <sup>c</sup>	3.31 <sup>b</sup>	3.70 <sup>a</sup>	0.11	< 0.001	< 0.001	0.063
ATTD of diet								
GE, %	88.3 <sup>b</sup>	77.3 <sup>d</sup>	84.7 <sup>c</sup>	95.6 <sup>a</sup>	0.41	< 0.001	< 0.001	< 0.001
DE, Mcal/kg	3.77 <sup>b</sup>	3.48 <sup>c</sup>	3.75 <sup>b</sup>	3.92 <sup>a</sup>	0.02	< 0.001	< 0.001	< 0.001

<sup>a, b, c</sup>Means within a row lacking a common superscript letter differ ( $P < 0.05$ ).

<sup>1</sup>Means are least squares means of 7 observations for wheat + wheat DDGS diet and 8 observations for other diets.

<sup>2</sup>DDGS, distillers dried grain with solubles; AID, apparent ileal digestibility; ATTD, apparent total tract digestibility.

<sup>3</sup>Method 1: Wheat grain diet was used as the basal diet to calculate the nutrient digestibility of wheat DDGS.

<sup>4</sup>Method 2: Cornstarch/N-free diet was used as the basal diet to calculate nutrient digestibility of wheat DDGS.

**Table 2.7.** Apparent ileal digestibility of CP and AA in the experimental diets fed to finisher pigs (DM basis)<sup>1</sup>

AID, %	Wheat DDGS <sup>2</sup>			SEM	P-value		
	Wheat	Wheat (Method 1) <sup>3</sup>	Cornstarch (Method 2) <sup>4</sup>		Diet	Method 1 vs. 2	Wheat DDGS vs. Wheat
CP	78.4 <sup>a</sup>	68.5 <sup>b</sup>	69.9 <sup>b</sup>	2.69	0.007	0.621	0.002
Indispensable AA							
Arginine	80.1	75.3	74.1	2.70	0.118	0.586	0.051
Histidine	86.6 <sup>a</sup>	80.2 <sup>ab</sup>	67.6 <sup>b</sup>	4.57	< 0.001	0.065	0.031
Isoleucine	80.8 <sup>a</sup>	70.3 <sup>b</sup>	66.1 <sup>b</sup>	2.38	< 0.001	0.116	< 0.001
Leucine	85.8 <sup>a</sup>	79.8 <sup>b</sup>	77.7 <sup>b</sup>	1.68	0.006	0.256	0.002
Lysine	84.1 <sup>a</sup>	76.0 <sup>b</sup>	74.7 <sup>b</sup>	2.21	0.004	0.551	< 0.001
Phenylalanine	87.4 <sup>a</sup>	74.7 <sup>b</sup>	84.4 <sup>a</sup>	1.63	< 0.001	<0.001	0.001
Threonine	75.0 <sup>a</sup>	67.3 <sup>b</sup>	68.0 <sup>ab</sup>	3.31	0.024	0.809	0.007
Dispensable AA							

Alanine	72.9 <sup>a</sup>	67.1 <sup>ab</sup>	66.0 <sup>b</sup>	3.19	0.041	0.702	0.014
Aspartate	73.3 <sup>a</sup>	55.6 <sup>b</sup>	50.8 <sup>b</sup>	3.94	< 0.001	0.172	< 0.001
Glutamate	90.0 <sup>a</sup>	81.3 <sup>b</sup>	79.8 <sup>b</sup>	1.46	< 0.001	0.386	< 0.001
Glycine	71.2	63.3	63.1	3.85	0.794	0.942	0.029
Serine	80.7 <sup>a</sup>	72.3 <sup>b</sup>	70.5 <sup>b</sup>	2.27	0.001	0.474	0.003
Tyrosine	72.8	74.9	74.2	2.75	0.741	0.832	0.457

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<sup>a, b, c</sup>Means within a row lacking a common superscript letter differ ( $P < 0.05$ ).

<sup>1</sup>Means are least squares means of 7 observations for wheat grain + wheat DDGS diet and 8 observations for the other diets.

<sup>2</sup>DDGS, distillers dried grain with solubles; AID, apparent ileal digestibility.

<sup>3</sup>Method 1: Wheat grain diet was used as the basal diet to calculate the nutrient digestibility of wheat DDGS.

<sup>4</sup>Method 2: Cornstarch/N-free diet was used as the basal diet to calculate nutrient digestibility of wheat DDGS.

**Table 2.8.** Digestibility of gross energy and standardized ileal digestibility of CP and AA of wheat grain and wheat distillers dried grains with solubles in finisher pigs (DM basis)<sup>1</sup>

Item, %	Wheat DDGS <sup>2</sup>			SEM	P-value		
	Wheat	Wheat (Method 1) <sup>3</sup>	Cornstarch (Method 2) <sup>4</sup>		Diet	Method 1 vs. 2	Wheat DDGS vs. Wheat
ATTD of GE	88.3 <sup>a</sup>	68.8 <sup>b</sup>	66.0 <sup>c</sup>	0.88	< 0.001	0.007	< 0.001
CP	85.7 <sup>a</sup>	73.2 <sup>b</sup>	76.2 <sup>b</sup>	2.02	0.008	0.831	0.010
Indispensable AA							
Arginine	86.5	80.0	80.0	2.94	0.082	0.989	0.029
Histidine	88.1 <sup>a</sup>	81.2 <sup>ab</sup>	69.0 <sup>b</sup>	4.45	0.014	0.060	0.021
Isoleucine	86.0 <sup>a</sup>	73.7 <sup>b</sup>	70.9 <sup>b</sup>	2.80	0.003	0.326	0.001
Leucine	92.0 <sup>a</sup>	83.8 <sup>b</sup>	83.3 <sup>b</sup>	2.39	0.002	0.826	0.007
Lysine	87.0 <sup>a</sup>	77.9 <sup>b</sup>	77.3 <sup>b</sup>	2.33	0.009	0.800	0.003
Phenylalanine	90.8 <sup>a</sup>	76.9 <sup>b</sup>	87.5 <sup>a</sup>	1.91	0.001	0.003	0.005

Threonine	80.4 <sup>a</sup>	70.9 <sup>b</sup>	73.0 <sup>ab</sup>	3.46	0.021	0.522	0.007
Dispensable AA							
Alanine	79.8 <sup>a</sup>	71.7 <sup>b</sup>	72.4 <sup>ab</sup>	3.43	0.031	0.831	0.010
Aspartate	84.8 <sup>a</sup>	63.2 <sup>b</sup>	61.3 <sup>b</sup>	5.15	< 0.001	0.602	< 0.001
Glutamate	108.3 <sup>a</sup>	92.1 <sup>b</sup>	96.4 <sup>b</sup>	7.03	0.002	0.155	< 0.001
Glycine	84.3 <sup>a</sup>	72.0 <sup>b</sup>	75.0 <sup>b</sup>	4.20	0.012	0.423	0.004
Serine	85.5 <sup>a</sup>	75.6 <sup>b</sup>	74.9 <sup>b</sup>	2.61	0.002	0.796	0.006
Tyrosine	75.3	76.6	76.6.0	2.75	0.870	0.986	0.604

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<sup>a, b, c</sup>Means within a row lacking a common superscript letter differ ( $P < 0.05$ ).

<sup>1</sup>Means are least squares means of 7 observations for wheat grain + wheat DDGS diet and 8 observations for the other diets.

<sup>2</sup>DDGS, distillers dried grain with solubles.

<sup>3</sup>Method 1: Wheat grain diet was used as the basal diet to calculate nutrient digestibility of wheat DDGS.

<sup>4</sup>Method 2: Cornstarch/N-free diet was used as the basal diet to calculate nutrient digestibility of wheat DDGS.

**Table 2.9.** Content of DE, standardized ileal digestible CP and AA of wheat grain and wheat distillers dried grains with solubles in finisher pigs (DM basis)<sup>1</sup>

Item,	Wheat DDGS <sup>2</sup>			SEM	P-value		
	Wheat	Wheat (Method 1) <sup>3</sup>	Cornstarch (Method 2) <sup>4</sup>		Diet	Method 1 vs. 2	Wheat DDGS vs. Wheat
DE, Mcal/kg	3.77 <sup>a</sup>	3.49 <sup>b</sup>	3.35 <sup>c</sup>	0.04	< 0.001	0.007	< 0.001
CP, %	15.74 <sup>b</sup>	34.03 <sup>a</sup>	35.43 <sup>a</sup>	0.81	< 0.001	0.075	< 0.001
Indispensable AA, %							
Arginine	0.52 <sup>b</sup>	1.29 <sup>a</sup>	1.29 <sup>a</sup>	0.03	< 0.001	0.878	< 0.001
Histidine	0.23 <sup>b</sup>	0.49 <sup>a</sup>	0.41 <sup>a</sup>	0.24	< 0.001	0.042	< 0.001
Isoleucine	0.49 <sup>b</sup>	0.90 <sup>a</sup>	0.87 <sup>a</sup>	0.02	< 0.001	0.197	< 0.001
Leucine	0.87 <sup>b</sup>	2.06 <sup>a</sup>	2.05 <sup>a</sup>	0.04	< 0.001	0.740	< 0.001
Lysine	0.32 <sup>b</sup>	0.68 <sup>a</sup>	0.68 <sup>a</sup>	0.01	< 0.001	0.734	< 0.001
Phenylalanine	0.57 <sup>b</sup>	1.10 <sup>c</sup>	1.25 <sup>a</sup>	0.02	< 0.001	< 0.001	< 0.001

Threonine	0.30 <sup>b</sup>	0.59 <sup>a</sup>	0.60 <sup>a</sup>	0.02	< 0.001	0.435	< 0.001
Dispensable AA, %							
Alanine	0.40 <sup>b</sup>	1.12 <sup>a</sup>	1.13 <sup>a</sup>	0.04	< 0.001	0.815	< 0.001
Aspartate	0.60 <sup>b</sup>	0.90 <sup>a</sup>	0.87 <sup>a</sup>	0.06	< 0.001	0.469	< 0.001
Glutamate	4.66 <sup>b</sup>	8.57 <sup>a</sup>	8.87 <sup>a</sup>	0.37	< 0.001	0.134	< 0.001
Glycine	0.50 <sup>b</sup>	1.73 <sup>a</sup>	1.79 <sup>a</sup>	0.07	< 0.001	0.296	< 0.001
Serine	0.39 <sup>b</sup>	0.99 <sup>a</sup>	0.98 <sup>a</sup>	0.23	< 0.001	0.693	< 0.001
Tyrosine	0.18 <sup>b</sup>	0.61 <sup>a</sup>	0.61 <sup>a</sup>	0.01	< 0.001	0.941	< 0.001

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<sup>a, b, c</sup>Means within a row lacking a common superscript letter differ ( $P < 0.05$ ).

<sup>1</sup>Means are least squares means of 7 observations for wheat grain + wheat DDGS diet and 8 observations for the other diets.

<sup>2</sup>DDGS, distillers dried grain with solubles.

<sup>3</sup>Method 1: Wheat grain diet was used as the basal diet to calculate nutrient digestibility of wheat DDGS.

<sup>4</sup>Method 2: Cornstarch/N-free diet was used as the basal diet to calculate nutrient digestibility of wheat DDGS.

**Table 2.10.** Effects of diets on volatile fatty acid concentration (mmol/kg of ileal digesta) in finisher pigs (as is basis)<sup>1</sup>

Item	Wheat DDGS <sup>2</sup>				SEM	<i>P</i> -value		
	Wheat	Wheat (Method 1)	Cornstarch (Method 2)	Cornstarch		Diet	Method 1 vs. 2	Wheat DDGS vs. Wheat
Acetate	35.89 <sup>a</sup>	22.36 <sup>b</sup>	18.37 <sup>b</sup>	17.94 <sup>b</sup>	3.50	< 0.001	0.124	< 0.001
Butyrate	7.47 <sup>a</sup>	3.79 <sup>b</sup>	1.49 <sup>c</sup>	1.17 <sup>c</sup>	0.77	< 0.001	0.004	< 0.001
Caproate	0.027 <sup>b</sup>	0.046 <sup>a</sup>	0.047 <sup>a</sup>	0.037 <sup>ab</sup>	0.0047	0.016	0.955	0.002
Isobutyrate	0.32	0.64	0.30	0.49	0.24	0.631	0.260	0.563
Isovalerate	0.17	0.14	0.20	0.13	0.04	0.477	0.269	0.941
Propionate	2.14 <sup>a</sup>	1.33 <sup>ab</sup>	0.73 <sup>b</sup>	1.52 <sup>ab</sup>	0.43	0.045	0.204	0.012
Valerate	0.04	0.12	0.05	0.08	0.03	0.365	0.168	0.265
BCFA <sup>3</sup>	0.50	0.79	0.50	0.62	0.22	0.731	0.329	0.565
Total VFA	42.08 <sup>a</sup>	26.77 <sup>b</sup>	22.21 <sup>b</sup>	19.20 <sup>b</sup>	4.5	< 0.001	0.226	< 0.001

<sup>a, b, c</sup>Within a row, means without a common superscript differ ( $P < 0.05$ ).

<sup>1</sup>Means are least squares means of 7 observations for wheat grain + wheat DDGS diet and 8 observations for the other diets.

<sup>2</sup>DDGS, distillers dried grain with soluble; BCFA, branched chain fatty acids.

<sup>3</sup>Isobutyrate + isovalerate.

**Table 2.11.** Effects of diets on volatile fatty acid concentration (mmol/kg of feces) in finisher pigs (as is basis)<sup>1</sup>

Item	Wheat DDGS <sup>2</sup>				P-value			
	Wheat	Wheat	Cornstarch	Cornstarch	SEM	Diet	Method	Wheat DDGS
		(Method 1)	(Method 2)				1 vs. 2	vs. Wheat
Acetate	78.88	70.24	62.03	80.65	7.62	0.142	0.872	0.319
Butyrate	31.85 <sup>a</sup>	34.77 <sup>a</sup>	14.53 <sup>b</sup>	9.38 <sup>b</sup>	3.58	< 0.001	0.002	0.006
Caproate	0.53 <sup>a</sup>	0.36 <sup>ab</sup>	0.11 <sup>b</sup>	0.61 <sup>a</sup>	0.09	0.011	0.107	0.029
Isobutyrate	4.80 <sup>a</sup>	3.60 <sup>b</sup>	3.60 <sup>b</sup>	2.13 <sup>c</sup>	0.35	< 0.001	0.992	0.002
Isovalerate	7.01 <sup>a</sup>	5.01 <sup>b</sup>	4.71 <sup>b</sup>	2.15 <sup>c</sup>	0.52	< 0.001	0.655	0.001
Propionate	41.29 <sup>a</sup>	43.08 <sup>a</sup>	29.77 <sup>b</sup>	15.60 <sup>c</sup>	3.70	< 0.001	0.008	0.083
Valerate	4.15 <sup>a</sup>	4.05 <sup>a</sup>	3.37 <sup>a</sup>	1.51 <sup>b</sup>	0.32	< 0.001	0.085	0.171
BCFA <sup>3</sup>	11.82 <sup>a</sup>	8.61 <sup>b</sup>	8.32 <sup>b</sup>	4.28 <sup>c</sup>	0.86	< 0.001	0.779	0.001
Total VFA	168.55 <sup>a</sup>	160.87 <sup>ab</sup>	118.15 <sup>b</sup>	112.05 <sup>b</sup>	15.18	0.006	0.010	0.093

<sup>a, b, c</sup>Within a row, means without a common superscript differ ( $P < 0.05$ ).

<sup>1</sup>Means are least squares means of 7 observations for wheat grain + wheat DDGS diet and 8 observations for the other diets.

<sup>2</sup>DDGS, distillers dried grain with soluble; BCFA, branched chain fatty acids.

<sup>3</sup>Isobutyrate + isovalerate.

## **Chapter 3**

Effect of different sources and types of distillers dried grains with solubles on energy and amino acid digestibility, and volatile fatty acid concentration in ileal digesta and feces of growing pigs

### **3.1 Abstract**

Distillers dried grains with solubles (DDGS) is a co-product produced from the bio-ethanol industry in western Canada using different cereal grains, but little is known about their variability in energy, AA and CP digestibility and volatile fatty acid (VFA) production in the pig intestine. With grower pigs, the hypothesis that energy and nutrient digestibility and VFA concentration in ileal digesta and feces would not differ among sources of DDGS was tested. Five ileal cannulated growing pigs (initial BW  $29.0 \pm 0.5$  kg) were fed with one of 5 diets (wheat grain or wheat grain containing wheat DDGS-Terra, wheat DDGS-Husky, triticale DDGS or corn DDGS) in  $5 \times 5$  Latin square design. The ATTD and AID of energy and AID of CP was lower ( $P < 0.001$ ) for DDGS than wheat grain. The DE value was higher ( $P < 0.001$ ) for corn DDGS than wheat DDGS or triticale DDGS. The SID of lysine was higher ( $P < 0.001$ ) in wheat grain than DDGS. It differed between the two wheat DDGS samples; it was higher ( $P < 0.001$ ) in wheat DDGS Terra than the wheat DDGS Husky. The SID of Thr was higher ( $P < 0.001$ ) in wheat grain than DDGS. Among DDGS sources, Thr. was highest for wheat DDGS-Husky, intermediate for corn DDGS and wheat DDGS-Terra, and lowest for triticale DDGS. The total VFA concentration in ileal digesta and feces

was highest ( $P < 0.007$ ) in wheat grain diet and did not differ among the DDGS sources. In conclusion, DDGS differs in nutrient digestibility depending on cereal of origin. The low VFA concentration in digesta and feces indicates that DDGS of all sources did not contain much fermentable fiber.

### **3.2 Introduction**

Prices of feed grains continue to rise long-term; hence, the need for alternative feedstuffs to mitigate feed cost for swine. Distillers dried grains with solubles (DDGS) is a co-product of the bio-ethanol industry in western Canada using cereal grains such as wheat and corn (Widyaratne and Zijlstra, 2007). The DDGS contains more CP, fat, vitamins and minerals than the parent cereal grain due to conversion of starch into ethanol during the ethanol production process (Stein and Shurson, 2009). However, the digestible nutrient profile of western Canadian DDGS is relatively unknown compared to corn DDGS for swine.

The nutrient composition of DDGS depends on the quality and sources of cereal grain used to produce ethanol, but even if the same grain is used, variability in the chemical composition of DDGS may be observed in different production batches within plants. Digestibility of energy and amino acids (AA) in corn DDGS varies among sources (Fastinger and Mahan, 2006; Stein et al., 2006). Nutrient digestibility of corn DDGS is lower from old generation ethanol plants than new ethanol plants (Spiehs et al., 2002). Therefore, the nutritional information about DDGS produced in old-style ethanol plants (Nyachoti et al., 2005; Widyaratne and Zijlstra, 2007; Lan et al., 2008) is not expected to be valid

anymore. Furthermore, different climatic condition also influences the composition of corn DDGS (Belyea et al., 2004). Currently, limited information exists about value such as crude protein, amino acids and energy content and digestibility of DDGS produced from wheat, corn and triticale in western Canada. Similarly, the information regarding how DDGS affects the volatile fatty acids production in swine is also scarce.

The null hypothesis for this experiment was that energy and nutrient digestibility and VFA profile in ileal digesta and feces would not differ in pigs due to source of DDGS. The objectives were to determine energy, protein and AA digestibility and VFA concentration in ileal digesta and feces in growing pigs.

### **3.3 Materials and methods**

The experimental protocol were reviewed and approved by the University Animal Policy and Welfare Committee, University of Alberta and followed guidelines established by the Canadian Council on Animal Care (2009). The experiment was conducted at the Swine Research and Technology Centre, University of Alberta, Edmonton, AB, Canada.

#### ***Experimental diets and design***

For this study, 4 DDGS samples were obtained. Wheat DDGS was obtained from two plants: Terra Grain (Belle Plain, SK, Canada) and Husky Energy (Lloydminster, SK) while the corn DDGS was from US origin and was sourced from a local feed mill (Wetaskiwin Co-op, Wetaskiwin, AB, Canada).

The triticale DDGS was custom-produced by the Alberta Distillers Ltd. (Calgary, AB). The test diets were formulated to provide approximately 16% CP and exceed NRC requirements for most nutrients. Salt, vitamin and trace mineral premixes, and Cr<sub>2</sub>O<sub>3</sub> marker were added to the diets. Diets were mixed for 15 min in a horizontal, paddle mixer.

Dietary treatments included 4 diets containing 1 of the 4 DDGS samples and a wheat grain basal diet. The study was 5 × 5 Latin-square design with 5 dietary treatments allotted to 5 pigs in 5 periods, so that each pig consumed each diet. Each 16-d experimental period consisted of a 12 d diet adaptation followed sequentially by a 2 d feces collection and 2 d digesta collection.

### *Experimental procedures*

The study involved five 30 kg cross-breed barrows (Hypor, Regina, SK, Canada). Pigs were surgically fitted with a T-cannula at the distal ileum (Sauer et al., 2000). After surgery, pigs recovered for 7 d.

Pigs were housed in metabolism pens in an environmentally controlled room. Lights were on from 0800 to 2000 h. The rectangular, individual metabolism pens (1.2 × 1.5 × 0.95 m) were equipped with a stainless steel, dry self-feeder and a bowl drinker attached to the front of the pen. The pen provided full freedom of movement for the pig. The room temperature was set to 22°C. Pigs were weighed at the beginning of each period and offered the assigned experimental diet at 2.8 times maintenance (110 kcal DE/kg BW<sup>0.75</sup>, (NRC, 1998) divided into 2 meals at 0800 and 1500 h. Pigs had free access to water.

The morning of feces collection, colostomy bags were snapped between the inner Velcro and the leather ring and attached for the collection of feces. Feces were collected continuously for approximately 48 h each period. Fresh feces were collected and stored immediately at -80°C for VFA analyses. Fresh digesta for VFA analyses were collected in the morning of digesta collections and stored immediately at -80°C. After samples were collected for VFA, a plastic bag containing approximately 15 mL of 5% formic acid was attached to the open T-cannula for the collection of digesta. Digesta was collected continuously from approximately 0800 until 1800 h. Feces lying on the pen floor during digesta collection were promptly cleaned to prevent coprophagy and recycling of the Cr<sub>2</sub>O<sub>3</sub> marker. Collected digesta and feces were pooled by pigs within period and frozen. Thereafter, these were thawed, homogenized, sub sampled and frozen at -20°C for the digestibility study.

### *Chemical analysis*

Feces and digesta subsamples were freeze-dried. Lyophilized feces and digesta, test ingredients and feed samples were ground through a 1-mm sieve using a centrifugal mill (model ZM 200; Retsch Co., Newton, PA).

Samples were analyzed for DM content using a hot air oven at 135°C for 2 hr (method 930.15; AOAC, 1990). Gross energy of ingredient, diets, digesta and feces were analyzed using an oxygen bomb calorimeter (model C 5003, IKA GmbH and Co., Germany). Ingredients, diets, feces and digesta were analyzed for N content by combustion (method 990.03; (AOAC, 2006) using a Leco protein/N analyzer

(model FP-582, Leco Co., St. Joseph, MI). Diets, feces and digesta were analyzed for chromic oxide by spectrophotometry at 440 nm using spectrophotometer after ashing at 450°C (Fenton and Fenton, 1979).

Test ingredients, diets, and digesta were analyzed for AA content by high performance liquid chromatography (Prostar Model 210; Varian Instruments Inc. Walnut Creek, CA) using method 982.30E (AOAC, 2006) at the University of Alberta (Edmonton, AB). Briefly, samples were hydrolyzed with 6 N HCl for 24 h at 110°C (method 982.30 E; (AOAC, 2006) using b-amino-n-butyric acid as an internal standard. Cysteine and Methionine were determined as methionine sulphone and cysteic acid after cold performic acid oxidation overnight before hydrolysis (method 982.30E; AOAC, 2006). Available lysine in ingredients was analyzed using method 982.30E (AOAC, 2006). Ether extract was determined using method 920.39(A) (AOAC, 2006). Crude fiber was determined using method 978.10 (AOAC, 2006), ADF was analyzed using method 973.18(A-D) (AOAC, 2006), NDF (Holst, 1973), and total dietary fiber was analyzed using method 985.29 (AOAC, 2006).

For VFA analysis, digesta and feces samples were thawed and stirred. One g of sample was mixed with 3 g of distilled water. Then, 300 µl of internal standard (isocaproic acid) and 200 µL of 25% phosphoric acid was added followed by vortexing until the sample was completely dissolved, followed by centrifugation at 17,000 × g for 30 min. The clear supernatant was then transferred to the GC vial and analyzed for VFA using gas chromatography (model 3400; Varian Instruments Inc. Walnut Creek, CA) using method 996.06 (AOAC, 2006).

### *Calculations*

Digestibility values were calculated on a DM basis using chromic oxide as an indicator. The apparent ileal digestibility (AID) and apparent total tract digestibility (ATTD) of the component in the test diet was calculated using the following equation (Adeola, 2001):

$$\text{ATTD or AID, \%} = 100 - [100 \times (\text{concentration of Cr}_2\text{O}_3 \text{ in feed} \times \text{concentration of component in feces or digesta} / \text{concentration of Cr}_2\text{O}_3 \text{ in feces or digesta} \times \text{concentration of component in feed})]$$

The basal ileal endogenous loss ( $I_{\text{end}}$ ) of an amino acid or crude protein (g/kg of dry matter intake) was based on historical averages (Jansman et al., 2002). Standardized ileal digestibility (SID) values for each indispensable amino acid were calculated by the equation (Stein et al., 2007):

$$\text{SID} = [\text{AID} + (\text{IAA}_{\text{end}} / \text{AA in feed})]$$

The content of SID CP and AA was calculated by multiplying SID measured in the digesta sample with total CP and AA content of DDGS. The analyzed concentration of gross energy (GE) in the diet was multiplied by the ATTD to get the DE of the diet. The DE in the DDGS samples was calculated by subtracting the proportion of DE in the wheat diet from the DE in each of the DDGS containing diets according to the difference method (Adeola, 2001):

$$\text{Digestibility of the component in the test article, A, \%} = 100 \times [(T \times T_p) - (B \times B_p) / A_p]$$

Where: T is the digestibility, %, of the component in the total diet (basal plus test article); B is the digestibility, %, of the component in the basal diet; Bp is the proportion, %, of the component in the total diet contributed by the basal diet; Ap is the proportion, %, of the component in the total diet contributed by the test article.

$$T_p = B_p + A_p = 100\%$$

### *Statistical analyses*

The nutrient digestibility and VFA data were analyzed as a Latin square using the PROC MIXED procedure (SAS Inst. Inc., Cary, NC), using the individual pig as the experimental unit. The model included diet as a fixed effect and period and pen were the random terms. A probability of  $P < 0.05$  was considered significant.

### **3.4 Results**

The BW at the start of period 1 was  $37.0 \pm 2$  kg and the final BW at the end of period 5 was  $80.0 \pm 4$  kg. Pigs completed their meal; however, pigs fed the triticale DDGS diets took longer to finish their feed allowance.

### *Chemical composition of feedstuffs*

The content of CP, ether extract, crude fiber, ash, and GE value was higher in DDGS than the wheat grain (Table 3.2). The content of CP was highest in wheat DDGS-Terra, intermediate for triticale and wheat DDGS-Husky, and

lowest in corn DDGS. The content of ether extract was highest in corn DDGS, intermediate for wheat DDGS-Husky, and lowest for triticale and wheat DDGS-Terra.

The content of most indispensable AA was higher in wheat DDGS samples than in triticale or corn DDGS samples (Table 3.3). The content of lysine in wheat was less than that of wheat DDGS. The Lys content was higher in wheat DDGS and corn DDGS than triticale DDGS. The available lysine content was highest in corn DDGS, intermediate for both wheat DDGS, and lowest for triticale DDGS. But the Lys/CP ratio was highest in corn DDGS and wheat, medium in wheat DDGS-Husky and lowest in wheat DDGS-Terra and triticale DDGS.

The NSP content was similar among DDGS samples, but the total NSP was almost double in DDGS than in wheat grain (Table 3.4). The dietary nutrient composition reflected the changes in nutrient composition of DDGS, indicating that diets were mixed properly (Table 3.5).

### ***Nutrient digestibility***

The AID and ATTD of gross energy and AID of CP was greater ( $P < 0.001$ ) for the wheat grain diet than the DDGS diets (Table 3.6). Within DDGS diets, the AID and ATTD of energy was highest ( $P < 0.05$ ) for the corn DDGS diet and lowest for the triticale DDGS and wheat DDGS-Terra diet. The DE value of the wheat diet was higher ( $P < 0.05$ ) than that of the DDGS diets. The AID of CP was lowest ( $P < 0.05$ ) for the triticale DDGS diet. The AID of lysine was lower ( $P < 0.001$ ) in DDGS diets than in the wheat grain diet. Among DDGS, the

AID of lysine was lowest ( $P < 0.05$ ) in the wheat DDGS-Husky diet and highest for the wheat DDGS-Terra and corn DDGS diets. The AID of threonine did not differ among DDGS diets.

In case of ingredients, the ATTD of energy of wheat grain was higher ( $P < 0.05$ ) than for DDGS (Table 3.7). Among DDGS, the ATTD of energy of wheat-Husky and corn was higher ( $P < 0.05$ ) than that of wheat-Terra and triticale. The SID of CP between the 2 wheat DDGS samples did not differ ( $P > 0.05$ ), but both were lower ( $P < 0.05$ ) than that of wheat grain. The SID of CP was lowest ( $P < 0.05$ ) for triticale DDGS. The SID of AA was higher ( $P < 0.001$ ) in wheat grain than any other DDGS samples. The SID of Lys was higher ( $P < 0.05$ ) in wheat grain than any DDGS, and was higher ( $P < 0.05$ ) in wheat DDGS-Terra than wheat DDGS-Husky. The SID of Thr was higher ( $P < 0.05$ ) in wheat DDGS than triticale and corn DDGS, but did not differ ( $P > 0.05$ ) between the 2 wheat DDGS samples.

Among DDGS, the DE value of corn was higher ( $P < 0.05$ ) than for wheat-Terra and triticale, and intermediate for wheat-Husky (Table 3.8). The SID Lys content was lower ( $P < 0.05$ ) in DDGS samples than wheat, and did not differ among DDGS sources ( $P > 0.05$ ). The SID content of Leu, Met, Thr and Arg was higher ( $P < 0.05$ ) in wheat DDGS Husky than other DDGS.

#### ***VFA concentration in digesta and feces***

In ileal digesta, total VFA and butyrate concentration was higher ( $P < 0.05$ ) in pigs fed the wheat grain diet than the DDGS diets, and did not differ

among DDGS diets (Table 3.9). The concentration of other VFA did not differ among diets.

In feces, total VFA concentration did not differ ( $P > 0.05$ ) among diets (Table 3.10). The concentration of acetate was higher ( $P < 0.05$ ) in pigs fed the wheat grain diet than in pigs fed triticale, corn, and wheat DDGS-Terra diets.

### **3.5 Discussion**

The results of the present study indicate that DDGS, the main co-product of the bio-ethanol industry, has a variable nutritional value for grower-finisher pigs. The digestibility of energy, protein, and AA differed among sources of DDGS resulting in differing VFA profile in digesta and feces in grower pigs fed these DDGS sources.

#### ***Change in chemical composition of DDGS***

In the present study, the chemical characteristics of DDGS sources varied. The purpose of ethanol production is to maximize the conversion of sugars in starch to ethanol so the resulting DDGS should contain a low amount of starch. The chemical composition and nutritional value of DDGS depends on the type of cereal grain used for ethanol production, drying method, completeness and duration of fermentation and the amount of solubles added to the distillers grain (Spiehs et al., 2002; Zijlstra and Beltranena, 2008). The CP content of wheat DDGS Husky in the present study was similar to previous research (Thacker, 2006; Cozannet, 2010), but it was higher in wheat DDGS-Terra. The CP and NDF

content of corn DDGS and wheat DDGS was similar but the GE value and ADF content was lower than reported previously (Widyaratne and Zijlstra, 2007).

The inclusion of DDGS in a wheat-based diet increased the content of total dietary fiber, which is mostly insoluble. Fiber reduces energy and nutrient digestibility of DDGS (Urriola et al., 2010), although the extent of the effect depends on the physical and chemical properties of fiber.

Available lysine and lysine as a ratio to CP are indicators of lysine damage. In the present study, the Lys/CP ratio was higher in both wheat DDGS than measured previously for wheat DDGS (1.62 and 2.17%; Widyaratne and Zijlstra, 2007; Widyaratne et al., 2009; respectively) indicating that modern wheat-based ethanol plants produce higher quality wheat DDGS than historically. The laboratory-defined available Lys was much higher in corn DDGS than wheat or triticale DDGS indicating that operators of wheat-based ethanol plant can improve the protein quality in wheat DDGS further. Likely, too high temperatures during drying caused overheating that converted some lysine to an undigestible or unavailable Maillard product. In the present study, the physicochemical properties of DDGS sources differed not only due to the type of cereal grains, but also processing plant used for ethanol production (Weigel, et al., (1997; Stein and Shurson, 2009). Specifically, the methods and duration of fermentation and drying temperature and amount of solubles added with distillers grains likely affected these physicochemical properties of DDGS (Spiehs et al., 2002; Nyachoti et al., 2005).

### ***Change in energy digestibility***

The DE value of all 4 sources of DDGS was lower than wheat grain. The higher arabinoxylan content in DDGS than wheat grain was likely a main cause. Pigs do not produce enzymes to digest NSP. Instead, NSP or fiber is fermented in the distal small or large intestine by intestinal microflora. The average ATTD of GE of all samples was also lower than determined previously for corn DDGS and old-type wheat DDGS (Widyaratne and Zijlstra, 2008). The lower value of ATTD of wheat DDGS compared with wheat grain is due to the higher ADF and NDF content in DDGS. The ATTD of triticale DDGS was even lower than reported previously (Beltranena and Zijlstra, 2008).

In the present study, corn DDGS had the highest DE value among DDGS. Corn DDGS also had the lowest ADF content and highest ether extract content. Therefore, the DE value of DDGS is inversely related to ADF content (Cozannet et al., 2010) and positively to ether extract content. Indeed, fiber or NSP content decreases DM and AA digestibility (Eggum et al., 1982). The NSP content in all sources of DDGS was almost double that of wheat, a change that might have contributed to the lower energy digestibility.

### ***Changes in CP and AA digestibility***

The AID and SID of CP and AA differed among DDGS samples. The AID values of CP in wheat and corn DDGS did not differ, similar to previous research (Widyaratne and Zijlstra, 2007; Yang et al., 2010); however, the AID value of CP was lower in triticale DDGS. Triticale DDGS contained more total dietary fiber

than the other DDGS sources. The NSP can reduce efficiency of N utilization by increasing endogenous N losses and decreasing N absorption (Grieshop et al., 2001).

In the present study, basal endogenous protein and AA losses were based on historical averages (Jansman et al., 2002). The SID value for CP for wheat DDGS and corn DDGS was less than reported previously (Yang et al., 2010), but it was similar to recent reports (Cozannet et al., 2010) for the wheat DDGS. In the present study, lysine was the least digestible AA in DDGS regardless of origin, similar to previous research (Lan et al., 2008; Widyaratne and Zijlstra, 2007). As discussed, heat damage of lysine occurred during drying process that caused Maillard reactions and decreased lysine available to the pig. The biologically determined lysine quality therefore corresponded to the chemically-defined lysine quality for the DDGS.

### *Changes in VFA profile*

As expected, the VFA concentration was lower in ileal digesta than feces. In the present study, the source of DDGS did not affect digesta VFA concentration. Furthermore, fecal acetate and propionate did not differ among DDGS sources, indicating that fermentability of DDGS did not differ among sources. However, digesta butyrate increased with DDGS in the diet, indicating that feeding more DDGS fiber increased fermentation by small intestine bacteria, similar to previous studies (Wang et al., 2004). Branched-chain amino acids are partly fermented into branched-chain fatty acids (Macfarlane et al., 1992); their

concentration was higher in feces than ileal digesta. Therefore, some undigested protein or remaining endogenous AA losses were fermented in the large intestine (Htoo et al., 2007). The concentration of total VFA in feces did not differ among the DDGS sources; therefore, DDGS sources were similar in fermentable fiber content.

The result of the present study indicated that the energy, CP and AA digestibility and VFA concentration in ileal digesta and feces differed among grower pigs fed different sources of DDGS, thereby rejecting the null hypothesis. This research demonstrated that the variability in the digestibility of nutrients and VFA profile in ileal digesta and feces exists in DDGS produced from same or different cereal origin and ethanol production plants. The implication of this study is that the variability in digestibility of energy, protein and AA and VFA concentration in ileal digesta and feces cannot be minimized by sourcing DDGS from the same or different botanical origin or ethanol plants. Therefore, the nutrient value of DDGS should be analyzed or predicted accurately prior to formulating swine diets. The higher nutrient content in DDGS than expected can further reduce feed cost, increasing profit to the producer.

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**Table 3.1** Composition of the experimental diets (as fed basis)

Ingredient, %	Wheat diet	DDGS diets <sup>1</sup>
Wheat grain	96.30	56.30
DDGS		40.00
Limestone	1.10	1.10
Di-calcium phosphate	0.80	0.80
Salt	0.40	0.40
Vitamin premix <sup>2</sup>	0.50	0.50
Mineral premix <sup>3</sup>	0.50	0.50
Cr <sub>2</sub> O <sub>3</sub>	0.40	0.40

<sup>1</sup>DDGS, distillers dried grains with soluble. Diets contained 40% of 1 of 4 DDGS sources: wheat DDGS (Terra Grains Fuels, Belle Plain, SK, Canada or DDGS Husky Energy, Lloydminster, SK), triticale DDGS, or corn DDGS.

<sup>2</sup>Provided the following per kg of diet; vitamin A, 82,500 IU; vitamin D, 8,250 IU; vitamin E, 400 IU; menadeone, 40 mg; thiamine, 10 mg; riboflavin, 50 mg; niacin, 350 mg; d-pantothenic acid, 150 mg; vitamin B<sub>12</sub>, 0.25 mg; biotin, 2 mg; and folic acid, 20 mg.

<sup>3</sup>Provided the following per kg of diet: copper, 500 mg; iron, 800 mg; manganese, 250 mg; zinc, 1000 mg; iodine, 5 mg; and selenium, 1 mg.

**Table 3.2** Analyzed nutrient composition of wheat grain, wheat DDGS-Terra, wheat DDGS-Husky, triticale DDGS, and corn DDGS (DM basis)

Item, %	DDGS <sup>1</sup>				
	Wheat	Wheat-Terra	Wheat-Husky	Triticale	Corn
Moisture	12.28	8.58	6.32	8.66	7.72
Ether extract	1.7	4.57	8.56	5.12	12.04
Crude fiber	3.21	7.12	7.84	7.76	6.69
CP	14.72	43.42	35.45	34.07	29.03
Crude ash	2.04	5.52	4.94	4.19	4.24
ADF	3.90	12.50	10.48	13.43	8.92
NDF	16.44	27.89	39.90	35.61	32.43
Total dietary fiber	13.20	26.59	30.81	31.72	30.87
Total NSP	12.18	22.48	24.76	23.23	23.04
Insoluble NSP	11.61	16.03	20.38	19.46	21.96
Soluble NSP	0.57	6.45	4.38	3.77	1.08
Ca	0.18	0.12	0.15	0.14	0.07
P	0.43	1.03	0.95	0.90	0.82
GE, Mcal/kg	3.91	4.55	4.81	4.54	4.89

<sup>1</sup>DDGS, distillers dried grains with solubles; NSP, non-starch polysaccharide.

**Table 3.3.** Analyzed amino acid composition of wheat grain, wheat DDGS-Terra, wheat DDGS-Husky, triticale DDGS, and corn DDGS (DM basis)

Item, %	DDGS <sup>1</sup>				
	Wheat	Wheat-Terra	Wheat-Husky	Triticale	Corn
Indispensable AA					
Arginine	0.68	1.76	1.59	1.52	1.38
Histidine	0.34	0.81	0.75	0.67	0.75
Isoleucine	0.56	1.54	1.33	1.27	1.08
Leucine	1.01	2.63	2.73	2.26	3.33
Lysine	0.48	0.96	0.97	0.80	0.98
Methionine	0.24	0.63	0.59	0.54	0.57
Phenylalanine	0.68	1.77	1.58	1.54	1.27
Threonine	0.41	1.18	1.13	1.08	1.15
Tryptophan	0.18	0.34	0.32	0.25	0.21
Valine	0.68	1.83	1.65	1.63	1.42
Dispensable AA					
Alanine	0.52	1.50	1.52	1.39	1.96
Aspartate	0.75	1.94	1.86	1.82	1.92
Cysteine	0.33	0.75	0.65	0.65	0.55
Glutamate	4.47	8.86	6.79	6.84	3.90
Glycine	0.60	1.6	1.38	1.37	1.14
Proline	1.37	3.35	2.82	2.85	2.07
Serine	0.55	1.54	1.42	1.29	1.35

Tyrosine	0.40	1.14	1.10	0.94	1.14
Available Lysine	0.47	0.80	0.82	0.63	0.89
Lys as % of CP	3.3	2.2	2.7	2.3	3.4

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<sup>1</sup>DDGS, distillers dried grains with solubles.

**Table 3.4.** Non-starch polysaccharides concentration of wheat grain, wheat DDGS-Terra, wheat DDGS-Husky, triticale DDGS, and corn DDGS (DM basis)

Item, %	DDGS <sup>1</sup>				
	Wheat	Wheat-Terra	Wheat-Husky	Triticale	Corn
<b>NSP</b>					
Total	12.18	22.48	24.76	23.23	23.04
Total insoluble	11.61	16.03	20.38	19.46	21.96
Total soluble	0.57	6.45	4.38	3.77	1.08
<b>Arabinose</b>					
Total NSP	2.28	5.28	5.57	5.9	5.46
Insoluble	1.94	3.97	4.55	4.92	5.41
Soluble	0.34	1.31	1.02	0.94	0.05
<b>Xylose</b>					
Total NSP	3.36	8.42	7.96	7.84	7.58
Insoluble	3.05	5.84	6.85	6.59	7.52
Soluble	0.31	2.58	1.1	1.25	0.06
<b>Mannose</b>					
Total NSP	0.32	0.97	1.64	1.45	1.19
Insoluble	0.2	0.57	1.08	0.96	0.86
Soluble	0.12	0.4	0.56	0.49	0.33
<b>Glucose</b>					
Total NSP	6.54	7.94	8.38	7.15	7.34
Insoluble	6.25	5.35	7.15	6.39	6.85

Soluble	0.29	1.59	1.23	0.76	0.49
Galactose					
Total NSP	0.26	0.9	1.22	0.93	1.49
Insoluble	0.16	0.31	0.75	0.6	1.32
Soluble	0.1	0.59	0.47	0.33	0.17

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<sup>1</sup>DDGS, distillers dried grains with solubles; NSP, non-starch polysaccharide.

**Table 3.5.** Analyzed nutrient composition of experimental diets (DM basis)

Item, %	Wheat	DDGS <sup>1</sup>			
		Wheat-Terra	Wheat-Husky	Triticale	Corn
Moisture	12.81	10.76	10.41	11.14	9.85
Ether extract	1.34	2.69	4.01	2.78	5.55
Crude fiber	2.40	4.25	4.61	4.69	4.16
CP	15.10	26.56	23.50	22.74	20.27
Crude ash	4.65	6.11	5.58	5.66	5.60
GE, Mcal/kg	3.77	4.03	4.13	4.00	4.17
Indispensable					
AA					
Arginine	0.51	0.99	0.79	0.73	0.60
Histidine	0.21	0.3	0.29	0.25	0.24
Isoleucine	0.47	0.84	0.77	0.73	0.55
Leucine	0.79	1.43	1.38	1.24	1.25
Lysine	0.35	0.43	0.36	0.45	0.42
Methionine	0.80	0.17	0.14	0.14	0.12
Phenylalanine	0.56	0.98	0.89	0.86	0.67
Threonine	0.32	0.56	0.51	0.46	0.43
Valine	0.55	1.10	0.92	0.87	0.70
Dispensable AA					
Alanine	0.44	0.91	0.84	0.74	0.83

Aspartate	0.64	1.07	1.00	0.91	0.84
Glutamate	3.82	6.43	5.35	4.6	3.14
Glycine	0.59	1.23	0.99	0.84	0.67
Serine	0.38	0.78	0.62	0.58	0.47
Tyrosine	0.19	0.44	0.34	0.31	0.32

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<sup>1</sup>DDGS, distillers dried grains with solubles.

**Table 3.6.** Apparent ileal digestibility (AID) and apparent total tract digestibility (ATTD) of energy, DE content, and apparent ileal digestibility (AID) of CP and AA of diets fed to grower pigs (DM basis)

Item, %	DDGS <sup>1</sup>					SEM	P-value
	Wheat	Wheat-Terra	Wheat-Husky	Triticale	Corn		
Energy							
AID	70.0 <sup>a</sup>	54.1 <sup>bc</sup>	56.3 <sup>bc</sup>	50.8 <sup>c</sup>	58.1 <sup>b</sup>	1.7	< 0.001
ATTD	84.2 <sup>a</sup>	72.7 <sup>d</sup>	75.1 <sup>bc</sup>	73.2 <sup>cd</sup>	75.4 <sup>b</sup>	0.55	< 0.001
DE Mcal/kg	3.59 <sup>a</sup>	3.26 <sup>c</sup>	3.41 <sup>b</sup>	3.28 <sup>c</sup>	3.46 <sup>b</sup>	0.03	< 0.001
CP	73.8 <sup>a</sup>	64.4 <sup>bc</sup>	68.5 <sup>ab</sup>	61.1 <sup>c</sup>	66.7 <sup>b</sup>	1.5	< 0.001
Indispensable AA							
Arginine	71.7 <sup>ab</sup>	74.5 <sup>a</sup>	73.0 <sup>a</sup>	62.9 <sup>c</sup>	65.3 <sup>bc</sup>	1.8	< 0.007
Histidine	83.5 <sup>a</sup>	74.2 <sup>ab</sup>	78.3 <sup>ab</sup>	63.4 <sup>b</sup>	71.8 <sup>ab</sup>	3.6	< 0.001
Isoleucine	78.5 <sup>a</sup>	64.9 <sup>b</sup>	75.2 <sup>a</sup>	65.2 <sup>b</sup>	65.4 <sup>b</sup>	1.8	< 0.001
Leucine	80.7 <sup>a</sup>	71.3 <sup>c</sup>	79.3 <sup>ab</sup>	71.1 <sup>c</sup>	75.2 <sup>bc</sup>	1.4	< 0.001

Lysine	68.0 <sup>a</sup>	47.2 <sup>b</sup>	37.1 <sup>c</sup>	42.0 <sup>bc</sup>	46.3 <sup>bc</sup>	2.5	< 0.001
Methionine	83.0 <sup>ab</sup>	75.8 <sup>b</sup>	83.8 <sup>a</sup>	75.0 <sup>b</sup>	81.6 <sup>ab</sup>	1.9	< 0.011
Phenylalanine	82.1 <sup>a</sup>	78.4 <sup>ab</sup>	78.0 <sup>ab</sup>	76.4 <sup>bc</sup>	72.4 <sup>c</sup>	1.2	< 0.001
Threonine	67.9 <sup>a</sup>	62.7 <sup>a</sup>	62.6 <sup>a</sup>	50.9 <sup>b</sup>	52.1 <sup>b</sup>	2.3	< 0.001
Valine	74.6 <sup>a</sup>	65.8 <sup>b</sup>	71.4 <sup>a</sup>	62.5 <sup>b</sup>	62.9 <sup>b</sup>	1.8	< 0.001
Dispensable AA							
Alanine	65.7 <sup>a</sup>	63.9 <sup>a</sup>	66.8 <sup>a</sup>	54.1 <sup>b</sup>	65.9 <sup>a</sup>	1.9	< 0.006
Aspartate	69.6 <sup>a</sup>	50.1 <sup>bc</sup>	56.1 <sup>b</sup>	45.4 <sup>c</sup>	53.8 <sup>bc</sup>	2.2	< 0.001
Glutamate	89.3 <sup>a</sup>	79.1 <sup>c</sup>	84.8 <sup>b</sup>	74.2 <sup>d</sup>	78.0 <sup>cd</sup>	1.2	< 0.001
Glycine	66.5 <sup>a</sup>	66.0 <sup>a</sup>	64.9 <sup>a</sup>	48.4 <sup>b</sup>	49.8 <sup>b</sup>	2.5	< 0.001
Serine	76.5 <sup>a</sup>	71.2 <sup>a</sup>	70.8 <sup>a</sup>	59.9 <sup>b</sup>	61.3 <sup>b</sup>	1.6	< 0.001
Tyrosine	69.0 <sup>a</sup>	68.8 <sup>a</sup>	67.1 <sup>a</sup>	57.1 <sup>b</sup>	64.7 <sup>ab</sup>	2.3	< 0.013

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<sup>a, b, c</sup>Within a row, means without a common superscript differ ( $P < 0.05$ ).

<sup>1</sup>DDGS, distillers dried grains with solubles; AID, apparent ileal digestibility; ATTD, apparent total tract digestibility.

**Table 3.7.** Apparent total tract digestibility of energy, and standardized ileal digestibility of CP and AA of wheat grain, wheat DDGS-Terra, wheat DDGS-Husky, Triticale DDGS, and corn DDGS in grower pigs (DM basis)

Item, %	DDGS <sup>1</sup>					SEM	P-value
	Wheat	Wheat-Terra	Wheat-Husky	Triticale	Corn		
Energy							
ATTD	84.2 <sup>a</sup>	58.8 <sup>c</sup>	64.7 <sup>b</sup>	60.0 <sup>c</sup>	65.5 <sup>b</sup>	1.3	< 0.001
CP	80.2 <sup>a</sup>	68.3 <sup>bc</sup>	73.0 <sup>b</sup>	65.7 <sup>c</sup>	71.8 <sup>bc</sup>	1.5	< 0.001
Indispensable AA							
Arginine	78.4 <sup>a</sup>	78.1 <sup>a</sup>	77.4 <sup>ab</sup>	67.7 <sup>c</sup>	71.2 <sup>bc</sup>	1.8	0.001
Histidine	91.5 <sup>a</sup>	79.9 <sup>ab</sup>	84.2 <sup>ab</sup>	70.1 <sup>b</sup>	78.9 <sup>ab</sup>	3.6	0.011
Isoleucine	85.6 <sup>a</sup>	69.0 <sup>c</sup>	79.6 <sup>b</sup>	69.8 <sup>c</sup>	71.6 <sup>c</sup>	1.7	< 0.001
Leucine	86.1 <sup>a</sup>	74.4 <sup>d</sup>	82.5 <sup>ac</sup>	74.6 <sup>cd</sup>	78.8 <sup>bc</sup>	1.4	< 0.001
Lysine	78.1 <sup>a</sup>	55.5 <sup>b</sup>	47.2 <sup>c</sup>	50.0 <sup>b</sup>	54.8 <sup>b</sup>	2.5	< 0.001
Methionine	95.5 <sup>a</sup>	81.7 <sup>c</sup>	91.0 <sup>a</sup>	81.82 <sup>bc</sup>	90.0 <sup>ab</sup>	1.9	0.003

Phenylalanine	87.5 <sup>a</sup>	81.5 <sup>b</sup>	83.5 <sup>b</sup>	79.9 <sup>bc</sup>	77.0 <sup>c</sup>	1.2	< 0.001
Threonine	84.9 <sup>a</sup>	72.5 <sup>bc</sup>	73.4 <sup>b</sup>	62.8 <sup>d</sup>	64.8 <sup>cd</sup>	2.3	< 0.001
Valine	83.3 <sup>a</sup>	70.6 <sup>c</sup>	76.7 <sup>b</sup>	67.9 <sup>c</sup>	69.9 <sup>c</sup>	1.8	< 0.001
Dispensable AA							
Alanine	75.8 <sup>a</sup>	68.8 <sup>b</sup>	72.2 <sup>ab</sup>	60.1 <sup>c</sup>	71.4 <sup>ab</sup>	1.9	0.002
Aspartate	75.2 <sup>a</sup>	54.0 <sup>bc</sup>	59.7 <sup>b</sup>	49.3 <sup>c</sup>	58.1 <sup>b</sup>	2.2	< 0.001
Glutamate	92.3 <sup>a</sup>	80.8 <sup>cd</sup>	86.9 <sup>b</sup>	76.7 <sup>d</sup>	81.7 <sup>c</sup>	1.2	< 0.001
Glycine	80.4 <sup>a</sup>	72.7 <sup>a</sup>	73.3 <sup>a</sup>	58.2 <sup>b</sup>	62.3 <sup>b</sup>	2.5	< 0.001
Serine	92.5 <sup>a</sup>	78.9 <sup>b</sup>	80.8 <sup>b</sup>	70.4 <sup>c</sup>	74.4 <sup>bc</sup>	1.6	< 0.001
Tyrosine	82.9 <sup>a</sup>	74.9 <sup>ab</sup>	75.3 <sup>ab</sup>	65.7 <sup>b</sup>	73.1 <sup>b</sup>	2.3	0.002

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<sup>a, b, c</sup>Within a row, means without a common superscript differ ( $P < 0.05$ ).

<sup>1</sup>DDGS, distillers dried grains with solubles; ATTD, apparent total tract digestibility.

**Table 3.8.** DE content and standardized ileal digestible content of CP and AA of wheat grain, wheat DDGS-Terra, wheat DDGS-Husky, Triticale DDGS, and corn DDGS fed to grower pigs (DM basis)

Item, %	DDGS <sup>1</sup>					SE	P-value
	Wheat	Wheat-Terra	Wheat-Husky	Triticale	Corn		
						M	
DE, Mcal/kg	3.59 <sup>a</sup>	2.95 <sup>c</sup>	3.32 <sup>b</sup>	3.01 <sup>c</sup>	3.43 <sup>ab</sup>	0.07	< 0.001
CP	14.6 <sup>c</sup>	32.3 <sup>a</sup>	28.2 <sup>b</sup>	21.4 <sup>d</sup>	26.3 <sup>c</sup>	0.31	< 0.001
Indispensable AA							
Arginine	0.93 <sup>ab</sup>	0.98 <sup>a</sup>	1.08 <sup>a</sup>	0.69 <sup>c</sup>	0.82 <sup>bc</sup>	0.03	< 0.001
Histidine	0.44 <sup>a</sup>	0.28 <sup>b</sup>	0.43 <sup>a</sup>	0.46 <sup>a</sup>	0.33 <sup>b</sup>	0.02	< 0.001
Isoleucine	0.98 <sup>a</sup>	0.81 <sup>b</sup>	0.99 <sup>a</sup>	0.77 <sup>b</sup>	0.79 <sup>b</sup>	0.03	< 0.001
Leucine	2.00 <sup>a</sup>	1.59 <sup>b</sup>	2.03 <sup>a</sup>	2.16 <sup>a</sup>	1.43 <sup>b</sup>	0.07	< 0.001
Lysine	0.55 <sup>a</sup>	0.40 <sup>b</sup>	0.35 <sup>b</sup>	0.39 <sup>b</sup>	0.34 <sup>b</sup>	0.02	< 0.001
Methionine	0.19 <sup>ab</sup>	0.18 <sup>bc</sup>	0.23 <sup>a</sup>	0.15 <sup>c</sup>	0.17 <sup>bc</sup>	0.01	< 0.001

Phenylalanine	1.20 <sup>ab</sup>	1.10 <sup>bc</sup>	1.27 <sup>a</sup>	1.06 <sup>cd</sup>	0.99 <sup>d</sup>	0.03	< 0.001
Threonine	0.67 <sup>a</sup>	0.46 <sup>c</sup>	0.64 <sup>ab</sup>	0.55 <sup>bc</sup>	0.51 <sup>c</sup>	0.02	< 0.001
Valine	1.19 <sup>a</sup>	0.98 <sup>b</sup>	1.21 <sup>a</sup>	0.95 <sup>b</sup>	0.97 <sup>b</sup>	0.03	< 0.001
Dispensable AA							
Alanine	1.15 <sup>a</sup>	0.94 <sup>bc</sup>	1.22 <sup>c</sup>	1.13 <sup>ab</sup>	0.92 <sup>c</sup>	0.04	< 0.001
Aspartate	0.45 <sup>c</sup>	0.84 <sup>b</sup>	1.07 <sup>a</sup>	0.85 <sup>b</sup>	0.89 <sup>b</sup>	0.03	< 0.001
Glutamate	6.94 <sup>ab</sup>	7.97 <sup>a</sup>	7.36 <sup>ab</sup>	3.53 <sup>c</sup>	5.83 <sup>b</sup>	0.42	< 0.001
Glycine	1.40 <sup>a</sup>	1.59 <sup>a</sup>	1.46 <sup>a</sup>	0.80 <sup>b</sup>	0.91 <sup>b</sup>	0.07	< 0.001
Serine	0.93 <sup>ab</sup>	0.83 <sup>b</sup>	0.95 <sup>a</sup>	0.63 <sup>c</sup>	0.68 <sup>c</sup>	0.03	< 0.001
Tyrosine	0.48 <sup>a</sup>	0.44 <sup>ab</sup>	0.51 <sup>a</sup>	0.35 <sup>c</sup>	0.38 <sup>bc</sup>	0.02	< 0.001

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<sup>a, b, c</sup>Within a row, means without a common superscript differ ( $P < 0.05$ ).

<sup>1</sup>DDGS, distillers dried grains with solubles.

**Table 3.9.** Effects of diet on VFA concentration in ileal digesta (mmol/kg as is)

VFA	DDGS <sup>1</sup>					SEM	<i>P</i> -value
	Wheat	Wheat-Terra	Wheat-Husky	Triticale	Corn		
Acetate	23.38	17.85	18.58	24.31	22.58	2.11	0.116
Butyrate	4.11 <sup>a</sup>	2.15 <sup>b</sup>	2.19 <sup>b</sup>	2.40 <sup>b</sup>	2.28 <sup>b</sup>	0.53	0.037
Caproate	0.06	0.07	0.07	0.07	0.05	0.02	0.695
Isobutyrate	0.13	0.07	0.09	0.11	0.53	0.17	0.351
Isovalerate	0.22	0.15	0.18	0.20	0.25	0.06	0.720
Propionate	4.55	3.53	2.98	4.55	3.71	1.16	0.410
Valerate	0.22	0.28	0.27	0.28	0.19	0.08	0.340
BCFA	0.36	0.22	0.27	0.31	0.78	0.18	0.238
Total VFA	40.1 <sup>a</sup>	24.1 <sup>b</sup>	22.7 <sup>b</sup>	33.2 <sup>ab</sup>	30.5 <sup>ab</sup>	3.21	0.007

<sup>a, b, c</sup>Within a row, means without a common superscript differ ( $P < 0.05$ ).

<sup>1</sup>DDGS, distillers dried grains with solubles; BCFA, branched-chain fatty acid.

**Table 3.10.** Effects of diet on VFA concentration in feces (mmol/kg as is)

VFA	DDGS <sup>1</sup>					SEM	P-value
	Wheat	Wheat-Terra	Wheat-Husky	Triticale	Corn		
Acetate	64.62 <sup>a</sup>	49.00 <sup>b</sup>	62.32 <sup>ab</sup>	56.38 <sup>b</sup>	54.51 <sup>b</sup>	2.24	0.002
Butyrate	21.37	27.03	24.98	20.96	27.97	2.56	0.124
Caproate	0.50	0.62	1.49	0.91	0.84	0.42	0.460
Isobutyrate	5.13	3.87	4.39	4.43	4.46	0.45	0.118
Isovalerate	7.80 <sup>a</sup>	5.58 <sup>b</sup>	6.31 <sup>ab</sup>	6.49 <sup>ab</sup>	6.74 <sup>ab</sup>	0.67	0.081
Propionate	32.36 <sup>b</sup>	34.61 <sup>a</sup>	38.44 <sup>a</sup>	36.62 <sup>a</sup>	41.58 <sup>a</sup>	2.45	0.045
Valerate	4.21	4.99	5.93	4.76	5.70	0.85	0.403
BCFA	12.93	9.45	10.71	10.93	11.20	1.12	0.091
Total VFA	132.1	130.8	156.0	134.8	140.5	7.22	0.157

<sup>a, b, c</sup>Within a row, means without a common superscript differ ( $P < 0.05$ ).

<sup>1</sup>DDGS, distillers dried grains with solubles; BCFA, branched chain fatty acid.

## **Chapter 4 General Discussion**

### **4.1 Research Summary**

Two studies were conducted to determine the effect of wheat, corn and triticale DDGS on energy and AA digestibility and their effects on VFA profile in ileal digesta and feces of grower-finisher pigs. In experiment 1, the null hypothesis was that the determined nutrient digestibility coefficient and digestible nutrient profile of wheat DDGS using the difference method in finisher pigs would not differ if either a wheat or N-free diet was used as control diet. Based on the results, the null hypothesis was supported for protein and AA digestibility, but rejected for digestibility of energy and DE value of wheat DDGS. Thus, the control diet fed impacted the energy digestibility coefficient and DE value of wheat DDGS.

Wheat DDGS contains fiber that may impact energy digestibility. During ethanol production, the fermentation process converts the sugars in grain starch to ethanol and CO<sub>2</sub>. Therefore protein, fat and fiber concentrate 3 times in DDGS compared to the parent wheat grain. Energy digestibility is inversely related to the ADF and NDF content in wheat (Zijlstra et al., 1999). The same relation exists for wheat co-products that contain more wheat fiber. The increased content of ADF (4 times) and NDF (2 times) in wheat DDGS compared to wheat grain is likely a reason for the lower ATTD of GE in wheat DDGS than wheat.

Several other factors may affect energy digestibility of feedstuffs fed to pigs. Apart from chemical characteristics such as fiber content, physical characteristics such as particle size affect energy digestibility of DDGS (Yanez et

al., 2011). Finally, feedstuffs fermentability might have affected the obtained energy digestibility values. The higher ATTD of energy of wheat DDGS in the wheat-based diet than in the corn starch-based/N-free diet coincided with a higher VFA concentration in feces of pigs fed the wheat-based wheat DDGS diet. Dietary energy fermented into VFA from the hindgut fermentation increased with the concentration of dietary NSP (Anguita et al., 2006), but might also have affected by N content, because N is a limiting nutrient for bacteria.

In feed formulation, the assumption is made that DE provided by each feedstuff is additive, meaning that the energy contribution per unit of feed is independent of other diet components (Whittemore and Moffat, 1976; Young et al., 1977). This approach is reasonable for feedstuffs of similar chemical composition. However, this assumption might not be correct for feedstuffs included in diets that contain atypical levels of protein, fat, fiber and non-carbohydrate energy sources, creating a condition for a departure in DE from the linearity of substitution (Frape et al., 1976). Furthermore, achieved DE will be lower for young pigs with less mature gut to digest feed than older pigs with a mature gut and more active bacteria population in the large intestine (Morgan and Whittemore, 1981).

In case of protein, the AID and SID of AA were lower in wheat DDGS than in the wheat grain. The increased amount of ADF and NDF in wheat DDGS may reduce N digestibility or increased fermentability of fiber in the large intestine may shift N excretion from urine to feces in pigs (Zervas and Zijlstra, 2002). However, wheat DDGS contains more insoluble NSP and the negative

effect of NSP on ATTD of N is a more likely scenario (Shi and Noblet, 1993; Le Goff and Noblet, 2001). Fiber may also increase microbial growth in the large intestine (Bach Knudsen and Hansen, 1991; Bach Knudsen et al., 1991), because fiber provides energy for microbes to synthesize microbial protein. Microbes in gut may use urea as a source of ammonia N for the microbial protein synthesis. Synthesis of urea is triggered by AA in excess of the requirement and ammonia resulting from fermentation of undigested protein. Combined, undigested protein flowing through the ileum is excreted into feces as microbial protein instead of being absorbed as ammonia (Galassi et al., 2010).

The AID of Lys may be reduced during the DDG drying process (Stein and Shurson, 2009) and perhaps during the addition of solubles to the dried distillers grains (Cromwell et al., 1993). High temperature changes the nutritional profile of DDGS in particular for Lys, which is the first limiting AA for pigs. The high temperature favors the Maillard reaction between AA, especially Lys, and sugar moieties that reduces digestibility and availability of AA and changes the color of the final product into dark brown. The reduced digestibility and availability of AA is due to the formation of Amadori products that reduce the rate of AA digestion by blocking the sites of enzymatic attack (Papadopoulos, 1989).

The ileal digesta of pigs fed wheat grain diet contained more acetate, butyrate and total VFA than ileal digesta of pigs fed the wheat DDGS diet. Similar trends were found in feces. The inclusion of DDGS in the diet decreased the concentration of acetate, butyrate and total VFA concentration in the digesta

and feces that might be due to the less amount of soluble NSP present in the DDGS.

In experiment 2, the hypothesis tested was that energy and nutrient digestibility and VFA concentration in pig intestine does not differ among DDGS samples of 3 feedstocks of origin: corn, wheat, and triticale. Wheat DDGS samples were obtained from two ethanol plants.

The CP content in wheat DDGS from Husky-Lloydminster used in this experiment was similar to previous research (Thacker, 2006), but the CP content was higher in wheat DDGS from Terra. The CP and NDF of corn DDGS and wheat DDGS were similar but the GE and ADF were lower than the wheat DDGS studied previously (Widyaratne and Zijlstra, 2007). The inclusion of DDGS in a wheat-based diet increased the total dietary fiber in the diet and this fiber was mostly insoluble. The physical and chemical properties of fiber can affect energy and nutrient digestibility (Urriola et al., 2010).

The physicochemical properties of DDGS samples varied and that might be due to the type of cereal grain used for ethanol production (Weigel et al., 1997). Processing may also affect the nutritional value of DDGS, because the duration of fermentation, drying temperature, and amount of solubles added to distillers grains are some of the factors that affect the physicochemical properties of DDGS (Spiels et al., 2002; Nyachoti et al., 2005). The lower ATTD of energy and DE content of wheat DDGS as compared with the parent grain is due the higher fiber and lower starch content in DDGS compared to wheat grain. The ATTD of GE in triticale DDGS was 5% units lower than that reported in previous

work (Beltranena and Zijlstra, 2008). The high NSP content in DDGS also decreased DM and AA digestibility (Eggum et al., 1982). The NSP can lower the efficiency of N utilization by increasing endogenous N losses and decreasing the absorption of essential AA (Grieshop et al., 2001). Finally, physicochemical properties of dietary fiber such as solubility, viscosity, and water-holding capacity may influence the ileal digestibility of nutrients and endogenous loss in pigs (Souffrant, 2001).

In experiment 2, the value for basal endogenous protein and AA losses were based on historical averages (Jansman et al., 2002). The AID values of CP in wheat and corn DDGS did not differ, similar to previous research (Widyaratne and Zijlstra, 2007; Yang et al., 2010) but the AID of CP was lower for triticale DDGS. Triticale DDGS contained more TDF than the other DDGS that may have increased endogenous N loss. Endogenous N loss is mainly affected by the dry matter intake, physical and chemical composition of diet (Boisen and Moughan, 1996), and level of protein in the diet (Souffrant, 2001). The SID value for CP for wheat DDGS and corn DDGS was less than some studies (Yang et al., 2010) and similar to others (Cozannet et al., 2010). In this thesis work, Lys was the least digestible AA in DDGS ingredient regardless of the origin of feedstock used, similar to other findings (Lan et al., 2008; Widyaratne and Zijlstra, 2007) likely due to heat damage during drying process that caused Maillard reactions and decreased Lys availability for the pig.

The DDGS was not fermented well by intestinal microbes in pigs. As expected, the VFA concentration was lower in ileal digesta than feces. The source

of DDGS did not affect ileal VFA concentration; however, the VFA concentration differed among DDGS sources that might be due to different levels of fermentable NSP among the DDGS samples tested. Branched chain AA after fermentation provides branched chain fatty acids (Macfarlane et al., 1992). The branch chain fatty acid content in feces was higher than in ileal digesta supporting that undigested protein was fermented in the large intestine (Htoo et al., 2007).

#### **4.2 Limitations of the studies**

The work described in this thesis work has some limitations. For example, I did not measure the color of the DDGS sample that is one of the visual indicators to predict the protein quality of DDGS. Feed processing variables of DDGS were not included, and the DDGS samples that I used were unground. A recent study indicated that the particle size greatly affects the nutrient digestibility in pigs (Yanez et al., 2011); this is one variable that was not standardized in this thesis. We also did not determine the level of mycotoxins in the DDGS sample that might have affected nutrient digestibility. We did not balance the energy and AA content in the diet, a factor that might have affected the VFA profile. In experiment 2, pigs fed the triticale DDGS diet took more time to finish their daily diet allowance than pigs fed the other DDGS diets, indicating that the taste or smell of diet might have been an issue. We evaluated a limited number of samples in this study. Finally, the microbial characterization for both experiment were not completed due to limitations in the lab of a collaborator.

### **4.3 Future research**

This thesis did not include a growth performance study that would have allowed us to determine if growth performance of pigs differed among different sources of DDGS. Some findings suggest that the fiber present in DDGS may aid to maintain the gut health of pigs. The relationship between feedstuffs and gut health is a novel area and further research regarding the effect of DDGS produced in western Canada on gut health is warranted. Another important area of research is to evaluate the digestible nutrient profile of all the DDGS produced in Canada. It is also important to evaluate the effect of the amount of soluble added back to the distillers grain on digestible nutrient profile in pigs. The digestible nutrient profile of feedstuffs cannot be analyzed for each batch using animal models so a rapid method to predict the chemical and digestible nutrient profile of DDGS is recommended. Such a prediction could be achieved via *in vitro* digestibility techniques, near infrared reflectance spectroscopy (NIRS), or combinations thereof.

### **4.4 Conclusions and implications**

In conclusion, wheat DDGS is an important source of dietary energy and AA but less favorable regarding lysine and fermentable fiber. The energy digestibility of wheat DDGS is higher when measured by the difference from wheat grain as compared to cornstarch. The nutrient content of DDGS varied with the source and cereal of origin. The reduced lysine and high fiber content in DDGS are the main obstacles for the digestible nutrient content in grower finisher

pigs. This can be resolved by reducing the drying temperature and time as well as by adopting fractionation technology to reduce the fiber portion of the grain before fermentation. It is recommended to formulate the swine diet based on digestible nutrient content rather than total nutrient content for predictable performance. It is advised that the swine diet should be formulated based on digestible nutrient content if DDGS is to be included as a partial replacement for grain or soybean meal. The analysis or prediction of the digestible nutrient content of DDGS prior to formulating the diet is strongly recommended so that the pork industry can minimize the feed cost without affecting growth performance.

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**Wheat DDGS (Husky)**



**Wheat DDGS (Terra)**



**Triticale DDGS**



**Corn DDGS**

**Figure 2. DDGS samples used in the study**