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Feeding Increasing Levels of Reduced-Oil Distillers Dried Grains with Solubles from Two Ethanol Fermentation Methods Impacts Finishing Pig Growth Performance and Belly Quality

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FEEDING INCREASING LEVELS OF REDUCED-OIL DISTILLERS DRIED GRAINS WITH SOLUBLES FROM TWO ETHANOL FERMENTATION METHODS IMPACTS FINISHING PIG GROWTH PERFORMANCE AND BELLY QUALITY

BY

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THESIS ACCEPTANCE PAGE

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

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ABSTRACT

FEEDING INCREASING LEVELS OF REDUCED-OIL DISTILLERS DRIED GRAINS WITH SOLUBLES FROM TWO ETHANOL FERMENTATION METHODS IMPACTS FINISHING PIG GROWTH PERFORMANCE AND BELLY QUALITY

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Reduced-oil distillers dried grains with solubles (DDGS) are an affordable source of energy in finishing pig diets, but differences in DDGS fermentation method on finishing pig growth performance and belly quality is relatively unknown. The objectives of this study were: 1) determine the influence of increasing dietary inclusion rate of hot fermentation (HF) and cold fermentation (CF) DDGS on growth performance, carcass characteristics and belly quality, and 2) compare the effect of HF and CF DDGS on finishing pig growth performance, carcass characteristics, and belly quality. Crossbred pigs (n = 200) were used in a randomized complete block design and assigned to one of eight treatments with varying inclusion rates of HF and CF DDGS (0, 20, 40, or 60%) and a diet with 40% inclusion of CF DDGS and withdrawal period. A 5-phase feeding program was utilized with diets formulated to be isocaloric and isolysinic. Pigs were harvested at a commercial abattoir when the average pen weight reached 122.5 kg. Two bellies per pen were collected for analyses. Orthogonal polynomial contrasts were used to determine linear and curvilinear effects. As inclusion rate of CF DDGS increased, overall average daily gain (ADG) and gain to feed ratio (G:F) decreased linearly ($P < 0.0001$), but HF DDGS did not influence ADG ($P > 0.24$) or G:F ($P > 0.25$). Pigs fed CF DDGS had decreased ADG ($P < 0.001$) and ADFI ($P = 0.03$) compared to HF DDGS. As

inclusion rate of CF DDGS increased, hot carcass weight decreased linearly ($P = 0.02$), however, as inclusion rate of HF DDGS increased a quadratic effect was observed ($P = 0.04$). Carcass yield linearly decreased ($P = 0.01$) as HF DDGS inclusion increased and tended to decrease linearly ($P = 0.08$) as CF DDGS inclusion rate increased. Carcass yield was not different between CF and HF DDGS ($P = 0.12$). As DDGS inclusion rate increased, regardless of fermentation method, belly quality decreased, evidenced by a linear decrease in belly flop scores ($P < 0.0001$) and percentage of saturated fatty acids ($P < 0.0001$). Iodine values ($P < 0.0001$) and percentage of polyunsaturated fatty acids ($P < 0.0001$) increased linearly with inclusion rate regardless of fermentation method. Increasing the inclusion rate of HF DDGS did not affect growth performance, but decreased belly quality, however, feeding increasing levels of CF DDGS decreased growth performance and belly quality. Furthermore, pigs fed HF DDGS displayed improved growth performance compared to CF DDGS.

CHAPTER 1 LITERATURE REVIEW

Ethanol and DDGS Production

Over the past 30 years, there has been an increased demand for ethanol in the United States resulting from a desired decrease in dependency on fossil fuels and foreign oil (RFA, 2019). To meet the growing demand, ethanol production increased exponentially from 175 million gallons in 1980 to approximately 16.1 billion gallons in 2019 (RFA, 2019). Ethanol is produced by extracting and fermenting starch from cereal grain, such as corn, wheat, barley, rye, and sorghum, which contain 50-70% starch on a dry matter basis (Kelsall and Piggot, 2009). In the United States, corn is the most utilized cereal grain for ethanol production and is processed into ethanol primarily through dry grinding and wet milling.

In 2019, more than 90% of ethanol fuel was produced by ethanol manufacturers utilizing the dry grind process (RFA, 2019). During dry grinding, the grain kernals ground into flour or meal before being combined with water to produce a slurry that will be fermented to produce ethanol (Rosentrater et al., 2012). The basic steps in the dry grind process are grinding, slurrying, liquefication, saccharification, fermentation, distillation, and dehydration (Figure 1A) (Cinelli et al., 2015). The initial step in the dry grind process reduces the particle size of corn by grinding it with a hammermill into a flour or meal, making it easier for water and starch hydrolyzing enzymes to break down starch during liquefication and saccharification. The flour or coarse meal is then mixed with fresh water and remaining liquid from previous batches to form a solution, referred to as mash, containing 30% solids during slurrying. After the mash is formed, liquefication occurs and enzymes are added. The mash is then heated, held, and cooked at temperatures between 120°C - 140°C (Rosentrater et al., 2012). As the mash is heated and cooked the starch granules become more accessible to enzymes that hydrolyze starch

into shorter oligosaccharides that can be broken down into individual glucose molecules for fermentation during saccharification (Kelsall and Piggot, 2009). During saccharification, the mash is cooled and oligosaccharides are broken down into individual glucose molecules (Kelsall and Piggot, 2009). Upon the completion of saccharification, mash is transported to the fermentation chamber where yeast (*Saccharomyces cerevisiae*) is added to ferment glucose to ethanol (Rosentrater et al., 2012). During fermentation, the yeast consumes glucose molecules, converting it into heat, ethanol, and carbon dioxide. The resulting liquid, known as beer, leaves the fermenter and is sent through a stripping/rectifier column to remove the ethanol (Rosentrater et al., 2012). After distillation of ethanol, remaining water and nonfermentable components, including fiber, protein, and oil, referred to as whole stillage, are centrifuged to separate the water-soluble solids (thin stillage) and coarse solids (wet cake) (Shurson, 2018). Thin stillage is processed through an evaporator to produce condensed distillers solubles (CDS). Coarse-solid products from centrifugation can be marketed as distillers wet grains (DWG), dried by themselves to create dried distillers grains (DDG), or combined with condensed distillers solubles and dried to create distillers dried grains with solubles (DDGS). The resulting distillers grain product can vary in composition based upon the method used during ethanol production.

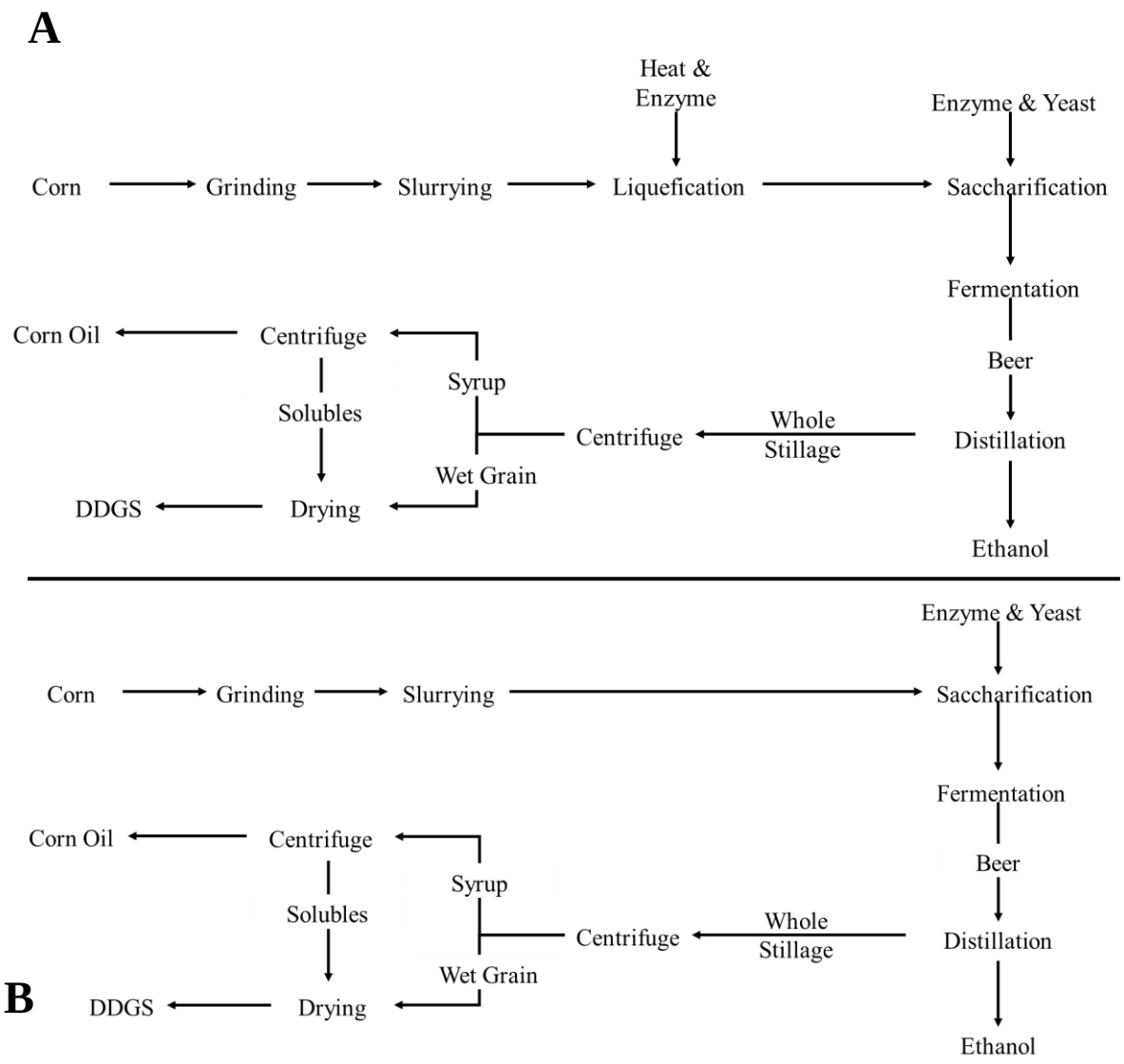

Figure 1 Diagrams of the conventional ethanol production process a) dry grind b) cold starch hydrolysis. Figure adapted from (Cinelli et al., 2015)

Within the dry grind process, there are two starch hydrolysis methods. The first is known as the dry grind process and is commonly utilized by the industry. The second has been termed granular starch hydrolysis, raw starch hydrolysis, cold hydrolysis, native starch hydrolysis, or sub-gelatinization temperature starch hydrolysis, but in this review it will be referred to as cold starch hydrolysis for simplicity (Cinelli et al., 2015). Major differences between starch hydrolysis during the dry grind process and cold starch

hydrolysis include: no high temperature liquefication and cooking steps and simultaneous saccharification and fermentation. In place of liquefication and cooking, cold starch hydrolysis utilizes a low temperature heat process below the gelatinization point of starch in combination with a raw starch hydrolyzing enzyme and other hydrolases to break down starch into glucose in preparation for the simultaneous saccharification and fermentation step (Textor et al., 1998; Robertson et al., 2006; Kelsall and Piggot, 2009; Cinelli et al., 2015). An economic benefit for ethanol producers utilizing the cold starch hydrolysis process is a reduction in the energy required for ethanol production, therefore, reducing the cost of production (Robertson et al., 2006).

In addition to cold starch hydrolysis, ethanol manufacturers have implemented oil extraction technology to further increase ethanol yields and energy efficiency of processing plants. Backend corn oil extraction technology became commercially available in 2005, and is used to produce biodiesel from extracted corn oil as a means to increase ethanol plant energy efficiency and fuel yields (Shurson, 2018). Shurson (2018) estimated approximately 90% of ethanol plants in the United States utilize a backend oil extraction technology to remove oil from whole and/or thin stillage prior to mixing and drying DDGS. Removing oil from stillage prior to DDGS manufacture has given rise to reduced oil DDGS. Historically, oil content of DDGS has been >10% but depending on the proportion of oil extracted, reduced oil DDGS may contain amounts of oil ranging from 0-9%. Before noting how these processes impact DDGS composition, it is important to understand the general nutrient composition of DDGS.

Distillers dried grains with solubles are composed of the non-fermentable portions (i.e protein, lipids, fiber and ash) of grains used in ethanol production. Dried

distillers grains with solubles are recognized as a good source of energy, protein and phosphorus for livestock (NRC, 2012; Shurson, 2018). Additionally, DDGS are a valuable feed ingredient for livestock due to cost and availability (Shurson, 2018). Studies show DDGS can be successfully utilized in beef cattle, dairy cattle, swine, and poultry rations (Klopfenstein et al., 2008; Swiatkiewicz and Koreleski, 2008; Schingoethe et al., 2009; Stein and Shurson, 2009).

DDGS Nutrient Composition

Nutrient composition of DDGS is partially characterized by the nutrient composition of the cereal grain used for ethanol production (Stein and Shurson, 2009). Corn generally contains approximately 8.2% crude protein (CP), 3.5% ether extract (EE), 1.3% ash, 62.1% starch, 9.1% neutral detergent fiber (NDF), and 2.88% acid detergent fiber (ADF) on an as-fed basis (Table 1.1) (NRC, 2012). As a result of converting most of the starch in corn to ethanol, concentration of the remaining nonfermentable nutrients in DDGS increase about three-fold (Spiehs et al., 2002; Han and Liu, 2010). Subsequently, fiber, amino acids, oil, and phosphorus content are important factors that affect utilization of DDGS in swine diets.

Table 1.1 Chemical composition of corn (as-fed basis, corn) distillers dried grains with solubles (DDGS), and reduced-oil DDGS. Table adapted from (NRC, 2012)

		DDGS		
Item	Corn	>10% Oil	> 6 and < 9% Oil	< 4% Oil
No of Samples	37 to 163	12 to 81	4 to 13	1 to 2
Dry Matter, %	88.31 ± 2.41	89.31 ± 1.91	89.35 ± 1.55	89.25 ± 2.20
CP, %	8.24 ± 0.93	27.33 ± 1.53	27.36 ± 2.00	27.86 ± 4.73
Crude Fiber, %	1.98 ± 0.61	7.06 ± 1.24	8.92 ± 1.38	6.19
ADF, %	2.88 ± 0.83	11.75	12.02 ± 2.47	16.91
NDF, %	9.11 ± 1.97	32.5 ± 5.42	30.46 ± 5.68	33.75 ± 1.20
Either Extract, %	3.48 ± 0.78	10.43 ± 1.03	8.90 ± 0.46	3.57 ± 0.62
Starch, %	62.55 ± 4.61	6.73 ± 1.70	9.63 ± 2.95	10
Calcium, %	0.02 ± 0.01	0.12 ± 0.19	0.08 ± 0.07	0.05
Phosphorus, %	0.26 ± 0.05	0.73 ± 0.10	0.60 ± 0.20	0.76
Ash, %	1.3 ± 0.32	4.11 ± 0.91	4.04 ± 1	4.64
Essential AA %				
Arginine	0.37 ± 0.05	1.16 ± 0.17	1.23 ± 0.16	1.31
Histidine	0.24 ± 0.05	0.71 ± 0.07	0.74 ± 0.08	0.82
Isoleucine	0.28 ± 0.06	1.02 ± 0.09	1.06 ± 0.09	1.02 ± 0.28
Leucine	0.96 ± 0.15	3.13 ± 0.46	3.25 ± 0.44	3.64
Lysine	0.25 ± 0.04	0.77 ± 0.12	0.90 ± 0.13	0.68 ± 0.28
Methionine	0.18 ± 0.03	0.55 ± 0.09	0.57 ± 0.11	0.50 ± 0.12
Phenylalanine	0.39 ± 0.05	1.34 ± 0.10	1.37 ± 0.16	1.69
Threonine	0.28 ± 0.04	0.99 ± 0.08	0.99 ± 0.06	0.97 ± 0.18
Tryptophan	0.06 ± 0.01	0.21 ± 0.03	0.20 ± 0.03	0.18 ± 0.01
Valine	0.38 ± 0.05	1.35 ± 0.12	1.39 ± 0.12	1.34 ± 0.28
Swine ME, kcal/kg	3395	3434	3396	3102

Dietary fiber is about three times higher in DDGS than the whole corn grain due to starch removal during ethanol production (Table 1.1)(Liu, 2009; NRC, 2012). Dietary fiber is defined as carbohydrates (non-starch polysaccharides and lignin) that are not digested or are poorly digested in the small intestine, but are completely or partially fermented by microbes in the large intestine (Devries, 2004). Dietary fiber is composed of soluble and insoluble portions that have similar physical properties but interact with

water in the gastrointestinal tract (GIT) very differently. Soluble fiber, mainly found in small amounts in legumes, oats, barley and rye, forms a viscous gel with water in the intestinal tract. This increases the viscosity of the digesta, slowing the release and uptake of nutrients, as well as reducing digestibility of other nutrients and increasing endogenous nutrient losses (Davidson and McDonald, 1998). Insoluble fiber, mainly found in corn and wheat, has a bulking effect in the digestive tract. This increases passage rate of gastrointestinal digesta, increasing secretion and re-absorption of endogenous nutrients (Davidson and McDonald, 1998). Insoluble fiber makes up a large portion of the dietary fiber profile of corn (NRC, 2012). As both components impact digestion differently, it can be expected growth rates will be impacted based upon the amount of soluble and insoluble fiber in the diet.

Fiber is only partially digested by growing pigs compared to protein, fat, and starch (Noblet and Le Goff, 2001). Furthermore, high-fiber diets have been shown to reduce fat, protein, and energy digestibility in growing pigs (Knudsen et al., 1993; Jørgensen et al., 1996). This is due to a combination of increased endogenous nutrient losses and increased digesta passage rate (Agyekum et al., 2012b). Stein and Shurson (2009) reported corn DDGS possess six times more insoluble fiber compared to soluble fiber. Agyekum et al. (2012b) reported pigs fed diets with 30% DDGS compared to pigs fed a corn-soybean meal-based diet possessed increased weights of their portal-drained viscera (comprised of spleen, pancreas, stomach, small intestine, cecum, and colon + rectum), likely resulting in higher maintenance energy requirements, potentially influencing pig growth. Additionally, increased viscera weight also contributes to lower carcass yields (Asmus et al., 2014b).

According to the NRC (2012) fiber content of low-oil DDGS (<4% oil), medium-oil DDGS (6-9% oil), vary with no identifiable trend. However, the values for nutrient profiles for low-oil and medium-oil DDGS are based upon a small sample size and may not accurately reflect the variation in fiber content across low-and medium-oil DDGS. Nevertheless, Graham et al. (2014b) evaluated nutrient composition differences between low-, medium-, and high-oil DDGS and reported increasing oil level increased crude fiber. Several studies utilizing either sorghum or corn DDGS have shown cold starch hydrolysis DDGS have a reduced fiber content compared to DDGS from the conventional dry grind process (Robinson et al., 2008; Kelzer et al., 2010b; Nkomba et al., 2016). Feeding cold fermentation DDGS instead of conventional hot fermentation DDGS may help reduce levels of dietary fiber in swine diets. This is because cold fermentation DDGS, regardless of cereal grain, contain lower levels of ADF and NDF than conventional hot fermentation DDGS (Robinson et al., 2008; Nkomba et al., 2016). Lower fiber content suggests DDGS from cold starch hydrolysis are likely to be more digestible for pigs compared to DDGS obtained from conventional dry grind.

Crude protein and amino acid content of conventional and reduced-oil DDGS are similar (Table 1.1) (NRC, 2012; Curry et al., 2014; Curry et al., 2016; Espinosa et al., 2019). Corn DDGS have a similar proportion of amino acids compared to corn because they are not affected during fermentation. The concentration of amino acids in corn DDGS increase three-fold compared to corn before ethanol processing (Spiehs et al., 2002; Han and Liu, 2010). However, digestibility of amino acids in corn DDGS are about 10% less than corn, possibly due to heat damage via the Maillard reaction during the drying process of ethanol production (Stein and Shurson, 2009) (Pahm et al., 2008b;

Almeida et al., 2013). The Maillard reaction is a result of complex chemical reactions between amino acids and reducing sugars that form products, such as amadori compounds and premelanoidins, that are not digestible by pigs (Mauron, 1990; Pahm et al., 2008a). Lysine, the first limiting amino acid in cereal grain-based swine diets is particularly vulnerable to the Maillard reactions during the drying process because of its free ε-amine group (Mauron, 1990; Almeida et al., 2013). Previous studies (Cromwell et al., 1993; Spiehs et al., 2002; Pahm et al., 2008a) have shown greater variability of lysine content and digestibility in DDGS but, digestibility of other amino acids remain within the normal range of variation compared to other feed ingredients. As ethanol production has advanced and improved, heat damage of DDGS has decreased as evidenced by higher lysine concentrations resulting in increased lysine to crude protein ratio (Lys:CP) in DDGS from modern ethanol plants (Espinosa et al., 2019). Espinosa et al. (2019) reported that lysine concentration and Lys:CP have increased in DDGS from 0.78% to 0.99%, and 2.80 to 3.40, respectively from 2002 to 2016. This is further evidence ethanol manufacturers continue to decrease heat damage to amino acids by utilizing new processing technologies such as cold starch hydrolysis, more effective enzymes, better fractionation, and improved drying systems to enhance the nutritional value of DDGS (Espinosa et al., 2019).

It has been suggested that the extraction of oil may affect the amino acid digestibility in reduced-oil DDGS (Curry et al., 2014). Consequently, several studies (Curry et al., 2014; Gutierrez et al., 2016; Espinosa et al., 2019) have evaluated and compared the amino acid digestibility of conventional and reduced-oil DDGS, revealing conflicting and inconclusive results. In finishing pigs, Curry et al. (2014) evaluated the

standardized ileal digestibility (SID) of two reduced-oil DDGS [7.5% and 6.9% acid hydrolyzed ether extract (AEE); as-fed basis], reduced-oil DDGS with added fat in the form of corn oil, and high-oil DDGS (11.5% AEE). Pigs fed the diets containing the two reduced-oil DDGS and the two reduced-oil DDGS with added corn oil had decreased SID values for all amino acids compared to high-oil DDGS. Furthermore, Gutierrez et al. (2016) noted a similar reduction in the apparent ileal digestibility (AID) of dietary lysine as reduced-oil DDGS were fed at increasing rates to growing-finishing pigs, but was not enhanced by the addition of soybean oil to diets. In contrast, Espinosa et al. (2019) utilized eight samples of reduced-oil DDGS between 6-9% AEE and noted SID and AID values for amino acids for all eight treatments were similar with SID and AID values reported for high-oil DDGS (NRC, 2012; Curry et al., 2014; Stein et al., 2016). However, in this study there was variation in SID of amino acids (AA) observed among the sources of reduced-oil DDGS used in the study. Different results across studies may be attributed to the variation in DDGS used in the studies as it is known there is variation in DDGS nutrient profiles due to processing methods and parameters (Belyea et al., 2010).

Phosphorus (P) is an important mineral in swine diets because it plays key roles in the formation of cell membranes, bones, and teeth, in addition to being utilized in other physiological functions (Kornegay and Thomas, 1981; Kornegay, 1985; Peo Jr, 1991; Crenshaw, 2000; NRC, 2012). Corn contains approximately 0.25-0.29% P, mostly in the form of phytate, but phytate is biologically unavailable for pigs without a phytase (an enzyme that cleaves inorganic P from phytate) (NRC, 2012; Espinosa et al., 2019). As corn is processed into ethanol, the concentration of inorganic P increases, phytate P decreases, and overall concentration of the P in DDGS increases to 0.93% (Liu and Han,

2011). The shift from higher concentrations of phytate P may be the result of phytate undergoing degradation by yeast phytase during fermentation (Liu and Han, 2011). The P content in reduced-oil DDGS follows a similar trend as conventional DDGS (Espinosa et al., 2019). Increasing inorganic P and decreasing phytate P, increases the bioavailability of P in reduced-oil DDGS. Pedersen et al. (2007) reported in corn DDGS the apparent total tract digestibility (ATTD) of P in corn DDGS fed to growing pigs was greater than corn (59.1% vs 19.3%), respectively. The NRC (2012) suggests P in DDGS is more digestible relative to corn as evidenced by 65% standardized total tract digestibility (STTD) in DDGS versus 34% STTD in corn. Consequently, when formulating swine diets containing high-oil and reduced-oil DDGS there is less supplemental inorganic P needed. The concentration and bioavailability of nutrients is dependent on the grain and process utilized to manufacture ethanol. Therefore, a discussion of the variation in nutrient composition of DDGS is required to understand the value of DDGS in livestock feed.

Variation in Nutrient Composition of DDGS

The primary concern with using DDGS as a feedstuff in livestock rations is variation in nutrient composition. It is well documented DDGS composition can vary between ethanol production facilities, as well as between individual batches within plants (Cromwell et al., 1993; Belyea et al., 2004; Liu, 2009; Belyea et al., 2010). Several reasons for nutrient composition variation across the ethanol industry include the cereal grain used for ethanol production, ethanol processing methods and parameters, the amount of condensed distillers solubles (CDS) added to distillers wet grains (DWG), effect of fermentation yeast, and analytical methods (Nuez Ortín and Yu, 2009; Belyea et al., 2010; Liu, 2011; Nkomba et al., 2016).

Within the same species and variety of grain there can be variation in composition due to growing conditions such as soil conditions, fertilizer, weather, and harvesting methods (Olentine, 1986). The nutrient composition of DDGS produced from the cold fermentation process is modified compared to DDGS from the dry grinding process (Kelzer et al., 2010a; Nkomba et al., 2016). Nkomba et al. (2016) reported sorghum distillers dried grains with solubles (DDGS) produced via cold starch hydrolysis contained less crude fiber (6.84% vs 8.11%), acid detergent fiber (ADF; 29.32% vs 35.59%), and neutral detergent fiber (NDF; 32.28% vs 44.04%) than sorghum DDGS produced via the dry grinding process. Additionally, corn oil removal affects the nutrient composition of DDGS by lowering the fat/oil content. Variation in DDGS production methods influence nutrient composition (Belyea et al., 2010; Nkomba et al., 2016). Therefore, understanding the effects of utilizing DDGS in pig diets is essential.

Feeding DDGS to Growing-Finishing Pigs

Effects on growth performance

DDGS production methods have changed as technological advancements in ethanol production have been made, modifying the composition of DDGS produced resulting in improved DDGS quality (Espinosa et al., 2019). There have been inconsistent results reported when high-oil DDGS are fed in grower finishing pig diets up to and above 30% inclusion rate. Whitney et al. (2006), reported decreased average daily gain (ADG), feed efficiency (G:F) and final body weight across high-oil DDGS inclusion rates of 20 and 30%. The authors concluded 10% high-DDGS inclusion in finishing pig diets was ideal. Similarly, Linneen et al. (2008) reported a linear decrease in ADG and ADFI as high-DDGS inclusion rate increased from 0 to 20%. The authors suggested high-

DDGS should be included in finishing pig diets between 10 – 15% for ideal growth. Conversely, several studies reported high-oil DDGS could be included up to 30% and not influence overall growth performance (Xu et al., 2010b; Wang et al., 2012; Davis et al., 2015). Several studies reported inclusion rates of high-DDGS above 30% negatively impacted growth performance of growing-finishing pigs (Cromwell et al., 2011; Bergstrom et al., 2014; Hardman, 2014). Cromwell et al. (2011) revealed as DDGS inclusion level increased from 0 to 45%, ADFI and G:F were unaffected, however, ADG decreased, respectively. Furthermore, studies have shown decreased growth parameters when feeding high-oil DDGS at 60% inclusion rates (Bergstrom et al., 2009; Hardman, 2014). Several factors that could have contributed to reduction in performance include: 1) Feeding too much crude protein in diets with DDGS resulting in excess energy cost to pigs to digest excess amino acids 2) inaccurate use of DDGS nutrient and energy digestibility values in diet formulation 3) potential heat damage to some DDGS sources reducing lysine digestibility 4) decreased palatability of diets including DDGS (Hastad, 2005; Stein and Shurson, 2009; Almeida et al., 2013; Hardman, 2014).

As ethanol producers began to extract oil from thin stillage in 2005, producing reduced-oil DDGS, concern was raised about how reduced-oil DDGS would impact growth performance of grower-finisher pigs. Due to the relatively recent implementation of oil extraction technology there are fewer peer-reviewed studies that evaluated the effects of reduced-oil DDGS on growth performance of grower-finisher pigs. They have shown mixed results with regards to the impact of reduced-oil DDGS on growth performance of grower finisher pigs. Graham et al. (2014a) fed grower finisher pigs increasing levels (0%, 15%, 30%, and 45%) of medium-oil (7.63%) DDGS. The authors

reported a linear decrease in ADG and G:F as inclusion rate of medium-oil DDGS increased. The authors noted ADG decreased by approximately 2.3% for every 15% medium-oil DDGS added to the diet. In another study, Graham et al. (2014b) evaluated feeding increasing levels of low-, medium-, and high-oil DDGS on growth performance of finisher pigs in two experiments. In one experiment, finishing pigs were fed 0, 20 and 40% inclusion levels of low-oil and medium-oil DDGS. Results revealed ADG of finishing pigs fed 20 and 40% low and medium-oil DDGS were not different compared to the pigs fed the control corn-soybean meal diet. As inclusion rate of low and medium oil DDGS increased to 20 and 40% ADFI increased. Pigs fed low-oil DDGS had increased ADFI at 20 and 40% inclusion levels compared to pigs fed medium-oil DDGS. This contributed to negatively influenced G:F for pigs fed 20% and 40% low-oil DDGS compared to pigs fed medium-oil DDGS. Overall, these results suggest pigs fed low-oil DDGS needed to consume more feed to maintain the same growth rate as pigs fed medium-oil DDGS and the control diet. In the second experiment in this study the authors did not report any differences in growth performance between pigs fed increasing rates (0, 20 and 40%) of medium-oil and high-oil DDGS. In contrast, Wu et al. (2016) evaluated how low, medium, and high-oil DDGS with similar metabolizable energy values included in grower finisher diets at 40% inclusion rate effected growth performance. Wu et al. (2016) reported that pigs fed a corn-soybean meal diet had increased growth performance compared to the low, medium, and high-oil DDGS. Additionally, they did not observe a difference in growth performance across all reduced-oil and high-oil DDGS diets. Conflicting results from the 3 studies suggests additional research is needed to further investigate how reduced-oil DDGS impact growth

performance of grower finisher pigs. Due to the impact DDGS have on growth performance of finishing pigs it is important to understand how DDGS influence pork carcass characteristics.

Effects on carcass characteristics

There are controversial findings in the literature regarding the influence of DDGS on carcass characteristics, specifically hot carcass weight and carcass yield (Stein and Shurson, 2009). Leick et al. (2010) reported hot carcass weight (HCW) and carcass yield decreased with increasing levels (0, 15, 30, 45, and 60%) of high-oil DDGS. These data agree with several other studies, as inclusion level of high-oil DDGS increases, HCW and carcass yield decrease (Whitney et al., 2006; Linneen et al., 2008; Overholt et al., 2016). However, other studies have reported no differences in HCW or carcass yield as high-oil DDGS inclusion rate increases (Widmer et al., 2008; Xu et al., 2010a; Davis et al., 2015). In comparison Wu (2015) reported 40% inclusion of reduced-oil DDGS decreased HCW and carcass yield. These data agree with several other studies confirming that as inclusion of reduced-oil DDGS increases in finishing pig diets HCW and carcass yield are linearly decreased (Graham et al., 2014a; Graham et al., 2014b). A decrease in carcass yield in pigs fed increasing levels of DDGS is thought to be linked to the approximately 3 times increased amount of NDF content in DDGS causing an increase in gut fill, intestinal and visceral organ mass (Kass et al., 1980; Agyekum et al., 2012a; Asmus et al., 2014b). Several studies reported DDGS did not influence loin depth, back fat depth or percentage of fat free lean (Widmer et al., 2008; Leick et al., 2010; Davis et al., 2015). Conversely, Xu et al. (2010b) reported as high-oil DDGS inclusion levels increased fat depth

decreased. The reduction in backfat depth may be due to decreased energy available for fat deposition resulting from low digestibility of lipid in DDGS. Additionally, studies (Graham et al., 2014a; Graham et al., 2014b; Wu et al., 2016) evaluating reduced-oil DDGS found that percentage fat-free lean was not affected by reduced-oil DDGS. This indicates that swine diets including high and reduced-oil DDGS can provide adequate amounts of digestible amino acids for lean tissue growth and development. Aside from understanding how high-oil and reduced-oil DDGS influence carcass characteristics it is important to understand how pork fat quality, an indicator of consumer satisfaction, is inflenced by DDGS.

Effects on pork fat quality

Pork fat quality is determined by the fatty acid composition that in turn affects fat firmness, color, and shelf-life (Azain, 2001). Pork fat firmness is a primary concern for pork processors because soft fat creates challenges when further processing bellies into bacon due to slicing difficulties, oily appearance, and increased vulnerability to oxidation causing rancidity (NPPC, 2000; Benz et al., 2010). Both high-oil (Whitney et al., 2006; Benz et al., 2010) and reduced-oil (Graham et al., 2014a; Graham et al., 2014b; Wu et al., 2016) DDGS fed to growing-finishing pigs have negatively influenced pork fat quality. This is caused by the concentration and fatty acid composition of corn oil in DDGS. However, (Graham et al., 2014b; Wu et al., 2016) observed improved pork fat quality as reduced-oil DDGS were fed at the same levels to growing-finishing pigs as high-oil DDGS.

The fatty acid profile of pork fat, specifically the relationship between saturated fatty acids (SFA) and unsaturated fatty acids (UFA) can impact other pork fat

characteristics including firmness, color, and oxidative stability (Azain, 2001). Unsaturated fatty acids have lower melting points compared to saturated fatty acids. Naturally, the fatty acid profile of pork has a higher polyunsaturated fatty acid (PUFA) content, specifically linoleic acid (C18:2) in relation to SFA (Wood and Enser, 1997). This is because dietary fatty acids are digested unmodified and deposited directly in adipose tissue (Farnworth and Kramer, 1987; Wood et al., 2008). Corn DDGS have historically contained >10% corn oil which contains increased proportion of UFA (81%), especially linoleic acid (54%) and a decreased concentration of SFA (13%). It has been well documented that the fatty acid composition of pork fat will reflect the composition of the dietary fat consumed (Averette Gatlin et al., 2002). Accordingly, studies have shown that the fatty acid profile of pigs fed both high-oil (Whitney et al., 2006; Benz et al., 2010) and reduced-oil (Graham et al., 2014a; Graham et al., 2014b; Wu et al., 2016) DDGS increased in PUFA, particularly linoleic acid, and decreased in SFA. However, when pigs were fed reduced-oil DDGS with lower concentrations of corn oil compared to high-oil DDGS the concentration of PUFA was decreased, particularly linoleic acid, indicating as more corn oil is extracted the negative effects of feeding DDGS on pork fat quality may be reduced (Graham et al., 2014b; Wu et al., 2016). Iodine value (IV) is most commonly used to measure the degree of unsaturation in pork fat. Feeding increasing levels of high-oil and reduced-oil DDGS to growing-finishing pigs results in elevated IV values (Whitney et al., 2006; Benz et al., 2010; Graham et al., 2014a; Graham et al., 2014b; Wu et al., 2016). However, the extent of reduction of negative effects on fat quality due to lower oil content in reduced-oil DDGS is unknown. A potential factor effecting the magnitude of the reduced negative effect on fat quality of reduced-oil

DDGS may relate to the variability of digestibility of the non-extracted oil remaining in the reduced-oil DDGS (Kerr et al., 2013).

Consequently, further research is needed to determine the effects of reduced-oil DDGS on pork fat quality. Due to limited number of published studies available on the impact of reduced-oil DDGS and cold starch hydrolysis DDGS on finishing pig growth performance, carcass characteristics, and belly quality further research is also warranted. Thus, the objectives of this study were: 1) determine the influence of increasing dietary inclusion rate of hot fermentation and cold fermentation DDGS on growth performance, carcass characteristics and belly quality, and 2) compare the effect of hot fermentation and cold fermentation DDGS on finishing pig growth performance, carcass characteristics, and belly quality.

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CHAPTER 2 FEEDING INCREASING LEVELS OF REDUCED-OIL DISTILLERS DRIED GRAINS WITH SOLUBLES FROM TWO ETHANOL FERMENTATION METHODS IMPACTS FINISHING PIG GROWTH PERFORMANCE AND BELLY QUALITY

Introduction

Distillers dried grains with solubles (DDGS) is a coproduct of the ethanol industry, commonly used to replace corn and soybean meal in swine diets due to nutritional content, affordability, and availability (Shurson, 2018). Historically, DDGS has contained greater than 10% oil, providing a feeding value similar to corn when fed to pigs (Stein and Shurson, 2009). Since 2005, the economic value of corn oil has increased, resulting in over 90% of ethanol plants installing oil extraction technology to recover a greater proportion of corn oil, therefore, producing reduced oil DDGS ranging from 4-10% oil content (Shurson, 2018). Several studies have shown feeding reduced oil DDGS to finishing pigs has similar effects as conventional DDGS on finishing pig growth performance, carcass characteristics, and fat quality (Graham et al., 2014a; Graham et al., 2014b; Wu et al., 2016).

Ethanol manufacturers have typically used high temperature fermentation systems to produce ethanol and DDGS. However, new technological advances in ethanol production systems have led to the development of cold fermentation methods, which use starch hydrolyzing enzymes at low temperatures in lieu of high temperatures to produce ethanol and DDGS (Cinelli et al., 2015). Cold fermentation systems have become more popular amongst ethanol manufactures due to the reduction in energy and water usage, increasing energy efficiency (Robertson et al., 2006). A benefit of cold fermentation is that it minimizes the probability of maillard reactions occurring which reduces DDGS amino acid digestibility. Also, cold fermentation (CF) DDGS contain less fiber compared

to hot fermentation (HF) DDGS, indicating digestibility of CF DDGS may be easier for monogastric animals such as pigs (Nkomba et al., 2016). Yet, there is limited research available on how reduced oil DDGS from cold fermentation and hot fermentation systems influence finishing pig growth performance, carcass characteristics, and belly quality.

Therefore, there were two objectives of this study. The first objective was to determine the influence of increasing dietary inclusion rate of HF and CF DDGS on growth performance, carcass characteristics and belly quality. The second objective was to compare the effect of HF and CF DDGS on finishing pig growth performance, carcass characteristics, and belly quality. We hypothesized increasing levels of HF and CF DDGS will have a negative effect on growth performance, carcass characteristics and belly quality. Furthermore, we hypothesized CF DDGS will improve growth performance compared to HF DDGS.

Materials and Methods

General

The Institutional Animal Care and Use Committee at South Dakota State University (Brookings, SD) approved all experimental protocols (#18-084E). In the experiment, pigs were housed in an environmentally controlled, mechanically ventilated grower-finisher barn containing 49 pens at the South Dakota State University Swine Extension and Research Facility in Brookings, SD. Pigs were housed in 1.75 m x 2.36 m pens with concrete slatted floors, a 2-hole wean-to-finish dry feeder, and one cup water to

allow ad libitum access to feed and water. Feed was delivered to pigs using a digital scale system (Feed Logic Corp, Wilmar, MN).

Animals and Diets

A total of 200 crossbred [Compart Duroc males X (Large White X Landrace females)] ; PIC, Hendersonville, TN; initially 44.37 kg BW), mixed sex pigs were used in a 79-day growth study to determine the effects of feeding increasing dietary inclusion rates of reduced-oil HF and CF DDGS in finishing pig diets on growth performance, carcass characteristics, and belly quality. Pigs were weighed 2 weeks prior to the start of the trial and were assigned to one of 40 pens with 5 pigs (3 gilts and 2 barrows, or 3 barrows and 2 gilts) per pen. Pigs were assigned to pens based on initial weight and sex, with initial weight being the blocking factor. At the beginning of the trial, pens were randomly assigned to 1 of 8 dietary treatments with 5 replications per treatment. Diets were fed in meal form with the control diet containing no DDGS. Treatment groups were fed a corn-soybean meal diet with 20, 40, or 60% inclusion rate of HF or CF DDGS. An additional treatment group was fed the 40% CF DDGS diet and allowed a withdrawal period during the last 14 d or 21 d on feed. During the withdrawal period, pigs initially fed the 40% CF DDGS diet were switched to the control diet. A five phase feeding program was utilized: phase 1 from 40.8 to 54.4 kg (Table 2.1), phase 2 from 54.4 to 77.1 kg (Table 2.2), phase 3 from 77.1 to 96.2 kg (Table 2.3), phase 4 from 96.2 to 109.8 kg (Table 2.4), and phase 5 from 109.8 to 122.5 kg (Table 2.5).

Diets were formulated to contain the same metabolized energy (ME), lysine to calorie ratio for each phase, and to meet or exceed all nutritional requirements (NRC, 2012). Soybean oil was added to diets containing increased DDGS inclusion rates to

maintain equal ME across all diets. Ractopamine hydrochloride (Elanco Animal Health, Greenfield IN) was included in all diets during phase 4 at 0.03% and phase 5 at 0.04%.

Two sources of DDGS were utilized in this study. Due to logistical issues CF DDGS were obtained from two batches from an ethanol production plant in Emmetsburg, Iowa (Poet, LLC; Sioux Falls, SD). A single batch of HF DDGS were obtained from an ethanol plant in Lamberton, Minnesota (Highwater Ethanol, LLC; Lamberton, MN). Feed samples were collected from every feeder randomly during each phase and pooled for analysis. The DDGS samples were taken randomly from each batch at one time point and mixed together. The DDGS and diet samples from each phase were analyzed for crude protein, fat, crude fiber, acid detergent fiber (ADF), neutral detergent fiber (NDF) and amino acid content at two laboratories; 1) a commercial laboratory (Minnesota Valley Testing Laboratory; New Ulm, Minnesota) and 2) the University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MI). Diet nutrient composition results from both labs and the average are shown by phase (tables 2.6, 2.7, 2.8, 2.9, 2.10). The DDGS composition is shown in Table 2.11. The HF and CF DDGS as well as soybean oil fatty acid profile (AOCS method Ce-1b-89 modified; (AOCS, 1998)) are reported in Table 2.17. Table 2.12 contains all analysis methods utilized by each laboratory.

Individual pig weight and weight of feed in feeders were obtained when phase changes occurred on days 0, 13, 23, 31, 46, 58, 71, and 78 to calculate ADG, ADFI, and G:F. Pigs were marketed in two separate groups (d 72 & 79) to achieve an average off-test body weight of 122.5kg, and then transported to a commercial packing plant and harvested. Prior to transportation, pigs were individually tattooed to allow for individual

carcass data collection. There were no pigs from either of the 60% DDGS diets marketed in the first group due to lighter body weights. All treatments except for 60% HF and CF DDGS were represented in both harvest dates. Pens from the 40% CF DDGS treatment were split across the marketing groups resulting in a 14 d and 21 d withdrawal periods. Immediately after evisceration, a sequential identification number was written on the shoulder of each carcass and respective individual number tattoo was recorded.

Hot carcass weight (HCW) was measured and carcass yield was calculated by dividing HCW by live weight. Fat depth and loin depth data were collected by a Fat-O-Meater probe (SFK Technology A/S; Herlev, Denmark) that was inserted between the third and fourth rib from the posterior end of the carcass. Percent fat free lean was calculated according to the National Pork Producers Council (NPPC, 2000) procedure using the equation, %FFL = ((15.31 – (31.277 x backfat depth (in)) + (3.813 x loin depth (in)) + (0.51 x HCW (kg))) / HCW) x 100. Carcasses were sent through a blast chill for approximately 120 minutes and held in equilibration bay until fabrication at approximately 18 hr postmortem. Once in equilibrium bays, carcasses were given a crayon identification on the belly and lumbar vertebrae of the right side of the carcass for tracking through fabrication to collect bellies and boneless loins. Fat samples, approximately 50 grams, were taken at the clear plate from the left side of the carcass for iodine value (IV) analysis. Carcasses were fabricated and labeled bone-in loins and skin-on bellies were collected.

Loins

Loins were cut into boneless Canadian back loins (IMPS #413), and an identifier button was placed in the boneless center-cut of each loin to maintain identification. Boneless loins were collected and weighed. Visual muscle color (6-point visual scale; (NPPC, 1999)), marbling (10-point visual score; (NPPC, 1999)), subjective firmness (5-point subjective scale; (NPPC, 1991)), and objective color measurements (L*, a*, b*; Konica Minolta CR-400 colorimeter with a D65 light source, 2" observer, 8 mm aperture) were evaluated on the ventral surface of the boneless loin.

Bellies

At the plant, skin-on bellies were measured for weight, length, width, scribe line width, belly depth at 25%, 50%, and 75% the length of the belly from the shoulder end, and a subjective belly flop score (on a scale 1-5 ; 1 = soft, 5 = firm). Average belly depth was calculated as an average of belly depth at 25, 50, and 75% the length of the belly from the shoulder end. A subsample of 2 bellies per pen (from a barrow and gilt closest to the average pen weight) were transported back to the South Dakota State University Meat Laboratory and frozen for further analysis.

Fat Quality Analysis

Fat samples collected at the plant from the clear plate region of the shoulder were measured using Bruker near-infrared technology. Belly samples for fatty acid profile and iodine value (IV) analysis were taken by cutting a 3-inch slice off of on the anterior end of the belly from a region on the navel edge posterior to the sternum and anterior to any

mammary tissue. The 3-inch slice was cut in half and the most dorsal portion was used for fatty acid profile and IV analysis. Fatty acid profile (AOCS method Ce-1b-89 modified; (AOCS, 1998)) and analyzed IV by iodine titration (AOCS method Cd1-25 modified; (AOCS, 1998)) was conducted at a commercial laboratory (Diversified Labs, LLC, Chantilly, VA).

Statistical Analysis

Growth data was analyzed as a randomized complete block design utilizing PROC MIXED procedure of SAS (SAS Institute Inc., Cary, NC) with pen as the experimental unit. The 14 d and 21 d 40% CF DDGS withdrawal groups were analyzed for statistical differences in all data. Finding no statistical differences between 14 d and 21 d 40% CF DDGS withdrawals, all data were combined as a single group for analysis. Treatment and block were used as main effects in the model for growth performance data analysis for each individual phase except for phase 5. In the analysis for phase 5 and cumulative growth data, treatment and block were used as main effects and days on feed included as a covariate. Days on feed was utilized as a covariate to account for additional days on feed for the second group of pigs. The model used for the analysis of loin, belly, and carcass measurements (except for HCW), included treatment and slaughter group as main effects with HCW as a covariate in the model. Hot carcass weight was used as a covariate for the model to account for variation due to different hot carcass weights. For all growth performance, carcass, loin, and belly data, orthogonal polynomial contrasts were used to determine linear and quadratic effects of increasing HF and CF DDGS. The 40% CF DDGS withdrawal group was not included in the orthogonal polynomial

contrasts. A single degree of freedom contrast was used to partition the sums of squares to compare inclusion rates of HF versus CF DDGS. The 40% CF DDGS withdrawal group was included in the single degree of freedom contrasts to compare inclusion rates of HF versus CF DDGS. Significance was determined when $P \leq 0.05$ and tendencies were determined when $0.05 < P \leq 0.10$.

Results and Discussion

Chemical Analysis

Chemical analysis of HF and CF DDGS samples are reported in table 2.11. Analyzed samples of HF DDGS and CF DDGS showed variability in nutrient composition. CF DDGS analyzed samples contained lower concentrations of CP, fat, ADF, NDF, and the majority of essential amino acids compared to HF DDGS. Variation in CP, fat, ADF, NDF, and essential amino acid concentration between HF and CF DDGS may be due to differences in ethanol production methods (Belyea et al., 2004; Belyea et al., 2010). Nkomba et al. (2016) investigated how cold fermentation and hot fermentation ethanol processes impacted the nutrient profile of sorghum DDGS and found cold fermentation DDGS contained less ADF and NDF than hot fermentation DDGS. Graham et al. (2014b), utilized hot fermentation reduced-oil DDGS with a similar 5.4% fat concentration as the CF DDGS and observed higher CP concentration, but similar concentrations of all other essential amino acids. In addition analyzed samples of CF DDGS contained less ADF but slightly more NDF compared to 5.4% reduced-oil DDGS used by Graham et al. (2014b). Nutrient content variability between studies could be attributed to differences in corn used as raw materials or ethanol production methods.

Compared to the NRC (2012), nutrient profile for medium-oil DDGS, HF DDGS used in this study contained numerically greater amounts of CP and essential amino acids but similar levels of ADF and NDF. According to NRC (2012), the lysine content of medium-oil DDGS is 0.90%. HF and CF DDGS contained 0.99% and 1.00% lysine, respectively.

Growth Performance

Growth performance data are reported in Table 2.13. Cumulatively, pigs fed diets containing increased levels of HF DDGS showed no negative impact ($P > 0.24$) on growth performance as there were no linear or quadratic effects on ADG, ADFI, and G:F as percent HF DDGS increased. Comparatively, as CF DDGS inclusion rate increased, cumulative ADG and G:F decreased linearly from 0% to 60% inclusion rate($P < 0.001$). This was evident through phase II and phase III of the study, pigs fed increasing levels of CF DDGS grew slower through phase II ($P < 0.01$) and III ($P < 0.0001$). This was the result of linearly decreased ADG ($P < 0.01$) and ADFI ($P < 0.02$) during the respective phases. Unlike cumulative results from HF DDGS, but similar to CF DDGS, Hardman (2014) and Graham et al. (2014a) showed as reduced-oil DDGS inclusion rate increased from 0% to 60% and 0% to 45%, respectively, ADG decreased linearly. Cumulatively, pigs fed HF DDGS had increased ADG ($P < 0.001$) compared with pigs fed CF DDGS diets. Cumulative G:F results from pigs fed CF DDGS agreed with Graham et al. (2014a), showing as reduced-oil DDGS inclusion rate increase from 0% to the highest inclusion rate, feed efficiency decreased linearly. Another study conducted by Graham et al. (2014b) utilized reduced-oil DDGs with the same oil concentration as CF DDGS in

the current study and observed a similar G:F decrease, but also reported a linear decrease in ADFI. However, in the present study a linear or quadratic decrease ($P > 0.53$) were not observed for ADFI. Differences in ADFI may be attributed to how diets for each respective study were formulated. Diets in the study conducted by Graham et al. (2014b) were formulated using total AA and SID AA coefficients resulting in 2-6% higher concentrations of crude protein in the diet as DDGS inclusion rate increased from 0% to 20% and 20% to 40% through finishing phases. Increased levels of crude protein in pig diets result in decreased feed intake (Chen et al., 1999). The diets in the current study were formulated to be isocaloric and isoenergetic to control for feed intake between HF and CF DDGS. Interestingly, pigs fed HF DDGS had increased ADFI compared to CF DDGS ($P = 0.03$). Decreased ADFI for pigs fed CF DDGS could be due to pigs eating to satiety, limiting adequate nutrient supply to fulfill nutritional requirements for maximum growth as a result of palatability, fiber content and/or nutrient availability (Bach Knudsen, 2001; Hastad, 2005). Numerically cumulative the G:F data range between HF and CF DDGS were within 0.03, but statistically CF DDGS revealed a linear decrease ($P < 0.0001$) and HF DDGS did not ($P = 0.25$). There was not a statistical difference ($P = 0.66$) between feed efficiency of HF and CF DDGS. To the author's knowledge there is no previous research comparing growth performance of growing finishing pigs fed reduced-oil HF and CF DDGS. Therefore, this study provides evidence suggesting growth performance of finishing pigs differs between reduced-oil HF and CF DDGS when fed at increasing levels.

Carcass Characteristics

Carcass data are reported in table 2.14. Hot carcass weights (HCW) of pigs fed increasing levels of CF DDGS decreased linearly ($P = 0.02$). In contrast, HCW of pigs fed HF DDGS followed a quadratic effect, with the heaviest pigs in the 0% treatment and decreasing across 20 and 40%, then increasing at 60% ($P = 0.04$). Also, HCW of pigs fed HF DDGS displayed a tendency to be greater than pigs fed CF DDGS ($P = 0.07$). Different effects of HF and CF DDGS on HCW may be related to differences in final body weight prior to harvest. Carcass yield linearly decreased ($P = 0.01$) as HF DDGS inclusion increased and tended to decrease linearly ($P = 0.08$) as CF DDGS inclusion rate increased. These findings are consistent with previous research that observed a similar decrease in HCW and carcass yield as reduced-oil (Graham et al., 2014a; Graham et al., 2014b) and conventional DDGS inclusion rate increased (Whitney et al., 2006; Linneen et al., 2008; Cromwell et al., 2011). The decrease in HCW and carcass yield is thought to be caused by increases in intestinal and organ weights that may be caused by variable amounts of insoluble fiber found in corn DDGS (Agyekum et al., 2012a; Asmus et al., 2014a). Loin depth, percent lean and backfat were not influenced by dietary treatment ($P > 0.39$). Backfat and percent lean results were similar to other studies evaluating reduced-oil DDGS (Graham et al., 2014a; Graham et al., 2014b). Comparatively, Graham et al. (2014a) reported as reduced-oil DDGS inclusion rate in the diet increased, loin depth decreased linearly. A linear decrease in loin depth may have been the result of a combination of factors including: decreased energy intake, increased maintenance energy requirements due to higher fiber content increasing organ weights, and an excess crude

protein intake requiring additional energy to deaminate and excrete (Agyekum et al., 2012a; Graham et al., 2014a).

Boneless Loin

Boneless loin data are reported in Table 2.15. All quality measurements for this study were recorded on the ventral side of the whole boneless Canadian back loin (IMPS 413). In comparison, other studies (Whitney et al., 2006; Leick et al., 2010; Xu et al., 2010b) evaluated the effects of conventional DDGS dietary inclusion on pork loin quality on the cut anterior surface of the pork loin between the 10^{th} and 11^{th} rib. DDGS treatment did not have an effect on loin weight or subjective firmness ($P > 0.05$). These results for subjective firmness conflict observations made by Leick et al. (2010) that showed as DDGS inclusion rate increased from 0% to 30% firmness decreased and then increased from 30% to 60%. Although there is a statistical difference in subjective firmness values observed by Leick et al. (2010), they most likely would not be noticed by a consumer. Subjective loin color scores from pigs fed HF and CF DDGS diets decreased linearly ($P \leq 0.05$) as inclusion rate increased. These results are in contrast to previous research (Whitney et al., 2006; Leick et al., 2010) that showed increased DDGS did not influence subjective color score. Values for subjective color scores in the current study were within a 3 color score on the NPPC (1999) 10 point color scale. Consequently, visual differences may not be noticed by a consumer. Boneless loin subjective marbling score decreased linearly as both HF and CF DDGS inclusion rates increased ($P < 0.02$). However, there was no difference between HF and CF boneless loin subjective marbling scores ($P = 0.43$). Previous research (Whitney et al., 2006; Leick et al., 2010; Xu et al., 2010b) shows

conflicting subjective marbling results, indicating increasing DDGS has inconsistent impacts on marbling. As HF and CF DDGS inclusion rate increased redness or a* values linearly decreased for HF (P = 0.02) and had tendency to decrease for CF DDGS (P = 0.05), respectively. This indicates that has HF and CF DDGS increased boneless pork loins displayed a paler, less desirable red color that is potentially unappealing to consumers. As HF DDGS increased b* decreased from 0.18 at 20% to 0 at 40% and 60% (P < 0.01). However, the changes in b* value would not be able to be detected by the human eye. The current study showed increasing inclusion rate of HF and CF reduced-oil DDGS had minimal effects on boneless pork loin color, firmness, and marbling, suggesting that pork loin quality is impacted similarly by HF and CF DDGS.

Belly Dimension, Quality, and Fatty Acid Profile

Belly dimensions and quality data are reported in Table 2.16. Belly dimensions are important to pork producers and processors because pork belly is manufactured into bacon, a very popular and important item in the food industry. Due to the popularity of bacon, pork belly is highly valued. The current study revealed HF and CF DDGS inclusion rate did not affect belly dimensions, including belly weight, length or scribeline width. In this study belly weights were similar (P > 0.38) across all treatments, implying pigs fed up to 60% reduced-oil DDGS may not compromise belly size. The results from this study show that reduced-oil DDGS do not negatively impact belly size contrary to a previous study by Leick et al. (2010) that reported as inclusion rate of conventional HF DDGS increased belly weight, length and width decreased linearly. Average belly depth decreased linearly as HF DDGS inclusion rate increased (P < 0.0001) but CF DDGS

followed a quadratic trend ($P < 0.04$), decreasing from 0% to 20% and then increasing at 40% and slightly decreasing at 60%. The results of HF DDGS bellies agree with previous research (Whitney et al., 2006; Leick et al., 2010) that showed as inclusion of conventional DDGS increased from 0 to 60% average belly depth decreased linearly. However, average belly depth results from pigs fed CF DDGS do not agree with previous research. Belly depth results from pigs fed CF DDGS may be due to variation in pigs between previous research and finishing pigs in this study. Arkfeld et al. (2017) reported that 83.5% of variation in belly depth can be attributed to the pig.

Belly quality is determined by the fat content and fatty acid profile that impacts firmness, shelf-life (lipid oxidation and color stability), and flavor (Wood et al., 2004). In the current study belly firmness was measured by assigning belly flop scores on a scale from 1 to 5 with 1 indicating soft, 5 indicating firm, and remaining numbers between indicating degrees of softness or firmness, respectively. Results revealed belly flop scores followed a similar linear decline ($P < 0.0001$) as both HF and CF DDGS increased, implying as increasing levels of HF and CF reduced-oil DDGS are incorporated into growing-finishing pig diets bellies become softer. Previous research (Whitney et al., 2006; Leick et al., 2010; Cromwell et al., 2011; McClelland et al., 2012) came to the same conclusion but utilized slightly different variations of the belly flop test to measure firmness. Instead of assigning subjective flop scores, bellies were hung over a metal rod and the distance between the caudal and cranial ends of the belly measured to determine a flop or flex value. A higher value meant the belly was firmer and lower values softer. Softer bellies are more difficult to process into bacon due to difficulty handling and slicing (Leick et al., 2010).

Increased belly softness as HF and CF reduced-oil DDGS increased are supported by iodine value and fatty acid profile results. Iodine value (IV) is the measurement of the degree unsaturation of fatty acids in fat and is used as an indicator of fat firmness. It is defined by the number of grams of iodine absorbed per 100 grams of fat (Azain, 2001). As HF and CF DDGS inclusion rate increased, IV increased linearly ($P < 0.0001$) regardless of location, analysis, or calculation, indicating that unsaturated fatty acid levels increased as DDGS levels increased. Increasing IV in relation to increasing levels of HF and CF reduced-oil DDGS show similar trends as previous research feeding conventional (Whitney et al., 2006; Leick et al., 2010; Cromwell et al., 2011; McClelland et al., 2012) or reduced-oil DDGS (Graham et al., 2014a; Graham et al., 2014b). Belly fat IV from iodine titration and shoulder fat IV were different between ethanol production method with HF DDGS having lower values compared to CF DDGS ($P < 0.04$). These results were unexpected because previous research by Graham et al. (2014b) investigated the effect of low, medium, and high-oil DDGS on IV reported that belly, jowl, and backfat IV from pigs fed increasing amounts of reduced-oil DDGS with different oil content (9.6% vs 5.4%) increased linearly, but, belly and backfat IV were increased by a greater magnitude in pigs fed 9.6% oil DDGS compared to 5.4% oil DDGS. Corn DDGS were used in both studies, implying they contain corn oil that is high in unsaturated fatty acids, specifically polyunsaturated fatty acids C18:2 and C18:3. Therefore, DDGS with different oil concentrations would contain slightly different proportions of unsaturated and saturated fatty acids that would be deposited into swine adipose tissue and influence IV. Fatty acid profile data for the HF and CF DDGS are reported in Table 2.17 support this idea, as CF DDGS contain a lower concentration of polyunsaturated fatty acids

(PUFA) such as essential fatty acids C18:2 and C18:3 than HF DDGS that would be directly deposited into adipose tissue. In the current study HF DDGS and CF DDGS contained 6.85% and 5.4% oil, respectively and showed that IV from pigs fed CF DDGS with less oil content were higher than pigs fed HF DDGS at every inclusion rate. A potential reason that may explain part of the difference amongst studies may be due to the addition of supplemental soybean oil to maintain isocaloric diets across all treatments in the current study. The soybean oil utilized in the study diets contained 5-6% increased concentration of PUFA compared to HF and CF DDGS (Table 2.17). In pigs it is known that fatty acid composition of fat depots closely reflect fatty acid composition of the diet (Averette Gatlin et al., 2002). Therefore, the higher levels of soybean oil added to CF DDGS than HF DDGS could have contributed increased amounts of unsaturated fatty acids, increasing IV in carcasses from pigs fed CF DDGS. The limited research indicates acceptable IV ranges between 70 (Barton-Gade, 1987; Madsen et al., 1992; NPPC, 2000) and 74 (Boyd et al., 1997) in the pork industry. However, there is not a consensus range of acceptable IV among pork processors. Putting the IV results from this study into perspective using the 70-74 range as acceptable, only the IV values from the control would be considered acceptable. Previous research on conventional DDGS (Whitney et al., 2006) reduced-oil DDGS (Graham et al., 2014b) has shown that bellies from pigs fed 30% and 40%, respectively can produce IV within the acceptable range.

Belly fatty acid profile data are reported in Table 2.18. Belly fatty acid profiles were mainly comprised of C16:0, C18:0, C18:1, and C18:2. As HF and CF DDGS inclusion rate increased, total saturated fatty acid (SFA), total monounsaturated fatty acid (MUFA), C16:0, C18:0, and C18:1 content decreased linearly ($P < 0.0001$). Results from

this study agree with previous studies (Benz et al., 2010; Leick et al., 2010; Xu et al., 2010b). Also, other SFA (C10:0, C14:0, C20:0) and C16:1 also showed a linear decrease as HF and CF DDGS inclusion rate increased ($P < 0.02$). Total polyunsaturated fatty acid (PUFA) content, C18:2, C18:3, C20:2, C20:4 content increased linearly as HF and CF DDGS rate increased ($P < 0.0001$). Additionally, total PUFA content, C18:2, C18:3, C20:2, C20:4 content was increased in bellies from pigs fed CF DDGS ($P < 0.02$). Results from this study agree with previous studies (Benz et al., 2010; Leick et al., 2010; Xu et al., 2010b). The ratio of unsaturated fatty acids (UFA) to SFA increased linearly as inclusion rate of HF and CF DDGS increased ($P <0.0001$) indicating as DDGS inclusion rate increased UFA levels increased in relation to SFA. Bellies from pigs fed CF DDGS had a higher UFA:SFA ratio compared to HF DDGS ($P <0.01$). The PUFA:SFA ratio increased linearly ($P <0.0001$) as inclusion rate of HF and CF DDGS increased showing as DDGS inclusion rate increased PUFA content increased in relation to decreasing SFA levels. Additionally, CF DDGS bellies had higher PUFA:SFA ratio compared to HF DDGS ($P <0.0001$). Higher UFA, PUFA and corresponding lower SFA levels indicate as DDGS inclusion rates increased belly fat became more unsaturated, therefore softer, decreasing belly quality. These results in combination with the IV results show as inclusion rate of reduced-oil DDGS increased bellies became softer. Softer bellies can cause issues when further processing into bacon including, difficulty slicing, oily appearance in the package, and increased susceptibility to lipid oxidation (Leick et al., 2010).

Conclusion

Increasing inclusion of HF DDGS in diets of finishing pigs did not affect overall growth performance. Conversely, increasing inclusion of CF DDGS negatively affected overall growth performance by decreasing ADG and worsening feed efficiency. Pigs fed HF DDGS had improved growth performance compared to pigs fed CF DDGS. Overall, as HF & CF DDGS inclusion increased, carcass yield decreased. Additionally, as HF and CF DDGS inclusion increased, belly quality decreased as evidenced by a linear increase in iodine value, polyunsaturated fatty acid content and decreased flop scores. Further research is needed to investigate the mechanism influencing the divergent effect of cold fermentation and hot fermentation DDGS on finishing pig growth performance and belly quality.

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Table 2.1 Phase 1 reduced-oil DDGS diet composition (as-fed basis)

	Distillers dried grains with solubles inclusion rate, %						
	Control[3]	Hot			Cold		
	0	20	40	60	20	40[4]	60
Ingredient, %							
Corn	65.69	60.02	53.46	46.64	59.78	53.07	46.06
Soybean meal 46.5%	28.71	24.17	20.57	17.24	24.41	20.95	17.80
Hot Fermentation DDGS	0.00	10.00	20.00	30.00	0.00	0.00	0.00
Cold Fermentation DDGS	0.00	0.00	0.00	0.00	10.00	20.00	30.00
Soy oil	2.05	2.28	2.53	2.79	2.31	2.60	2.89
Lysine	0.31	0.40	0.46	0.51	0.39	0.45	0.50
Methionine	0.11	0.10	0.08	0.05	0.10	0.07	0.05
Threonine	0.28	0.28	0.27	0.26	0.28	0.26	0.25
Tryptophan	0.02	0.03	0.04	0.05	0.03	0.04	0.05
Limestone	1.02	1.17	1.32	1.46	1.14	1.25	1.37
21% Monocalcium phosphate	1.07	0.80	0.53	0.26	0.81	0.55	0.29
Salt	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin, trace mineral Premix[2]	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated analysis							
Amino Acids[1], %							
Lysine	1.10	1.10	1.10	1.10	1.10	1.10	1.10
TSAA	0.64	0.64	0.64	0.64	0.64	0.64	0.64
Thr	0.86	0.86	0.86	0.86	0.86	0.86	0.86
Trp	0.22	0.22	0.22	0.22	0.22	0.22	0.22
Ile	0.68	0.68	0.68	0.68	0.68	0.68	0.68
Val	0.75	0.75	0.76	0.78	0.75	0.76	0.78
Leu	1.44	1.51	1.61	1.71	1.50	1.58	1.67
TSAA:Lys	58.00	58.00	58.00	58.00	58.00	58.00	58.00
Thr:Lys	78.00	78.00	78.00	78.00	78.00	78.00	78.00
Trp:Lys	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Ile:Lys	62.00	60.00	60.00	60.00	60.00	60.00	60.00
Val:Lys	68.00	68.00	69.00	71.00	68.00	69.00	71.00
Leu:Lys	131.00	138.00	146.00	155.00	136.00	144.00	152.00
Total Lysine, %	1.23	1.25	1.27	1.29	1.25	1.27	1.29
ME, kcal/kg	1534	1534	1534	1534	1534	1534	1534
SID Lys/ME, g/Mcal	3.25	3.25	3.25	3.25	3.25	3.25	3.25
CP,%	19.34	19.51	20.01	20.59	19.71	20.36	21.12
Ca, total, %	0.68	0.68	0.68	0.68	0.68	0.68	0.68
P, total, %	0.60	0.59	0.58	0.57	0.59	0.58	0.56
Available P, %	0.30	0.30	0.30	0.30	0.30	0.30	0.30

[1]Standardized Ileal Digestibility

[2] Provided per kg of complete diet: 11,002 IU vitamin A supplement, 1651 IU vitamin D_3 supplement, 55.1 IU vitamin E supplement, 0.044 mg vitamin B_{12} supplement, 4.4 mg menadione as menadione dimethylpyrimidinol bisulfite, 9.91 mg riboflavin supplement, 60.6 mg D-pantohenic acid as D-calcium, 55.1 mg niacin supplement, 1.1 mg folic acid, 3.3 mg pyridoxine as pyridoxine hydrochloride, 3.3 mg thiamine as thiamine mononitrate and 0.171 mg biotin, 165 mg Zn as zinc sulfate, 165 mg Fe as ferrous sulfate, 43.5 mg Mn as manganese sulfate, 16.5 mg Cu as basic copper chloride, 0.36 mg I as ethylenediamine dihydriodide and 0.3 mg of Se as sodium selenite.

[3]Control = corn and soybean meal based diet with no DDGS added; Hot = Hot fermentation ethanol production method; Cold = Cold fermentation ethanol production method

[4]40% Cold Fermentation withdrawal treatment fed 40% cold diet during phase 1

Table 2.2 Phase 2 reduced-oil DDGS diet composition (as-fed basis)

	Distillers dried grains with solubles inclusion rate, %						
	Control[3]	Hot			Cold		
	0	20	40	60	20	40[4]	60
Ingredient, %							
Corn	72.92	59.72	45.94	31.41	59.34	45.21	30.39
Soybean meal 46.5%	21.67	14.53	7.88	1.33	14.91	8.63	2.44
Hot Fermentation DDGS	0.00	20.00	40.00	60.00	0.00	0.00	0.00
Cold Fermentation DDGS	0.00	0.00	0.00	0.00	20.00	40.00	60.00
Soy oil	2.05	2.56	3.11	3.91	2.62	3.23	4.06
Lysine	0.31	0.43	0.54	0.64	0.43	0.53	0.63
Methionine	0.07	0.03	0.00	0.00	0.03	0.00	0.00
Threonine	0.24	0.22	0.19	0.17	0.22	0.18	0.15
Tryptophan	0.03	0.04	0.05	0.07	0.04	0.06	0.07
Limestone	0.99	1.28	1.52	1.72	1.22	1.41	1.51
21% Monocalcium phosphate	0.97	0.42	0.00	0.00	0.44	0.00	0.00
Salt	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin, trace mineral Premix[2]	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated analysis							
Amino Acids[1], %							
Lysine	0.94	0.94	0.94	0.94	0.94	0.94	0.94
TSAA	0.54	0.54	0.55	0.60	0.54	0.56	0.61
Thr	0.73	0.73	0.73	0.73	0.73	0.73	0.73
Trp	0.19	0.19	0.19	0.19	0.19	0.19	0.19
Ile	0.57	0.56	0.56	0.56	0.56	0.56	0.56
Val	0.64	0.67	0.71	0.74	0.67	0.70	0.74
Leu	1.28	1.47	1.67	1.87	1.44	1.62	1.79
TSAA:Lys	58.00	58.00	59.00	64.00	58.00	60.00	65.00
Thr:Lys	78.00	78.00	78.00	78.00	78.00	78.00	78.00
Trp:Lys	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Ile:Lys	61.00	60.00	60.00	60.00	60.00	60.00	60.00
Val:Lys	68.00	71.00	75.00	79.00	71.00	75.00	79.00
Leu:Lys	137.00	157.00	178.00	199.00	154.00	173.00	191.00
Total Lysine, %	1.05	1.09	1.13	1.18	1.09	1.13	1.17
ME, kcal/kg	1539	1539	1539	1539	1539	1539	1539
SID Lys/ME, g/Mcal	2.76	2.76	2.76	2.76	2.76	2.76	2.76
CP,%	16.60	17.60	18.80	20.00	18.00	19.50	21.00
Ca, total, %	0.63	0.63	0.63	0.69	0.63	0.63	0.67
P, total, %	0.55	0.53	0.53	0.62	0.53	0.52	0.61
Available P, %	0.27	0.27	0.30	0.41	0.27	0.29	0.40

[1]Standardized Ileal Digestibility

[2] Provided per kg of complete diet: 11,002 IU vitamin A supplement, 1651 IU vitamin D_3 supplement, 55.1 IU vitamin E supplement, 0.044 mg vitamin B_{12} supplement, 4.4 mg menadione as menadione dimethylpyrimidinol bisulfite, 9.91 mg riboflavin supplement, 60.6 mg D-pantohenic acid as D-calcium, 55.1 mg niacin supplement, 1.1 mg folic acid, 3.3 mg pyridoxine as pyridoxine hydrochloride, 3.3 mg thiamine as thiamine mononitrate and 0.171 mg biotin, 165 mg Zn as zinc sulfate, 165 mg Fe as ferrous sulfate, 43.5 mg Mn as manganese sulfate, 16.5 mg Cu as basic copper chloride, 0.36 mg I as ethylenediamine dihydriodide and 0.3 mg of Se as sodium selenite.

[3]Control = corn and soybean meal based diet with no DDGS added; Hot = Hot fermentation ethanol production method; Cold = Cold fermentation ethanol production method

[4]40% Cold Fermentation withdrawal treatment fed 40% cold diet during phase 2

Table 2.3 Phase 3 reduced-oil DDGS diet composition (as-fed basis)

	Distillers dried grains with solubles inclusion rate, %						
	Control[3]	Hot			Cold		
	0	20	40	60	20	40	60
Ingredient, %							
Corn	77.56	63.92	50.05	32.76	63.54	49.32	32.83
Soybean meal 46.5%	17.24	10.57	3.93	0.00	10.95	4.68	0.00
Hot Fermentation DDGS	0.00	20.00	40.00	60.00	0.00	0.00	0.00
Cold Fermentation DDGS	0.00	0.00	0.00	0.00	20.00	40.00	60.00
Soy oil	2.04	2.56	3.13	4.08	2.62	3.25	4.19
Lysine	0.31	0.42	0.52	0.55	0.41	0.52	0.57
Methionine	0.05	0.00	0.00	0.00	0.00	0.00	0.00
Threonine	0.22	0.19	0.16	0.10	0.18	0.15	0.10
Tryptophan	0.03	0.04	0.05	0.05	0.04	0.06	0.06
Limestone	0.90	1.19	1.39	1.71	1.12	1.28	1.50
21% Monocalcium phosphate	0.90	0.35	0.00	0.00	0.37	0.00	0.00
Salt	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin, trace mineral Premix[2]	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated analysis							
Amino Acids[1], %							
Lysine	0.83	0.83	0.83	0.83	0.83	0.83	0.83
TSAA	0.48	0.48	0.52	0.59	0.48	0.53	0.59
Thr	0.65	0.65	0.65	0.65	0.65	0.65	0.65
Trp	0.17	0.17	0.17	0.17	0.17	0.17	0.17
Ile	0.50	0.50	0.50	0.54	0.50	0.50	0.52
Val	0.57	0.61	0.64	0.72	0.60	0.64	0.70
Leu	1.18	1.38	1.58	1.84	1.36	1.53	1.73
TSAA:Lys	58.00	58.00	63.00	71.00	58.00	64.00	71.00
Thr:Lys	78.00	78.00	78.00	78.00	78.00	78.00	78.00
Trp:Lys	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Ile:Lys	60.00	60.00	60.00	65.00	60.00	60.00	63.00
Val:Lys	68.00	73.00	78.00	87.00	73.00	77.00	84.00
Leu:Lys	143.00	167.00	191.00	221.00	163.00	184.00	209.00
Total Lysine, %	0.94	0.98	1.02	1.07	0.97	1.01	1.05
ME, kcal/kg	1543	1543	1543	1543	1543	1543	1543
SID Lys/ME, g/Mcal	2.44	2.44	2.44	2.44	2.44	2.44	2.44
CP,%	14.90	16.10	17.30	19.30	16.40	18.00	20.00
Ca, total, %	0.57	0.57	0.57	0.68	0.57	0.57	0.66
P, total, %	0.52	0.50	0.51	0.62	0.49	0.50	0.60
Available P, %	0.25	0.25	0.29	0.41	0.25	0.28	0.40

[1]Standardized Ileal Digestibility

[2] Provided per kg of complete diet: 11,002 IU vitamin A supplement, 1651 IU vitamin D_3 supplement, 55.1 IU vitamin E supplement, 0.044 mg vitamin B_{12} supplement, 4.4 mg menadione as menadione dimethylpyrimidinol bisulfite, 9.91 mg riboflavin supplement, 60.6 mg $_D$-pantohenic acid as $_D$-calcium, 55.1 mg niacin supplement, 1.1 mg folic acid, 3.3 mg pyridoxine as pyridoxine hydrochloride, 3.3 mg thiamine as thiamine mononitrate and 0.171 mg biotin, 165 mg Zn as zinc sulfate, 165 mg Fe as ferrous sulfate, 43.5 mg Mn as manganese sulfate, 16.5 mg Cu as basic copper chloride, 0.36 mg I as ethylenediamine dihydriodide and 0.3 mg of Se as sodium selenite.

[3]Control = corn and soybean meal based diet with no DDGS added; Hot = Hot fermentation ethanol production method; Cold = Cold fermentation ethanol production method

[4]40% Cold Fermentation withdrawal treatment fed 40% cold diet during phase 3

Table 2.4 Phase 4 reduced-oil DDGS diet composition (as-fed basis)

	Distillers dried grains with solubles inclusion rate, %						
	Control[3]	Hot			Cold		
	0	20	40	60	20	40[4]	60
Ingredient, %							
Corn	69.44	56.71	42.97	28.38	56.33	42.25	27.36
Soybean meal 46.5%	25.12	17.50	10.84	4.29	17.87	11.59	5.40
Hot Fermentation DDGS	0.00	20.00	40.00	60.00	0.00	0.00	0.00
Cold Fermentation DDGS	0.00	0.00	0.00	0.00	20.00	40.00	60.00
Soy oil	2.04	2.53	3.08	3.91	2.60	3.20	4.05
Lysine	0.31	0.44	0.55	0.66	0.44	0.54	0.65
Methionine	0.09	0.05	0.01	0.00	0.05	0.00	0.00
Threonine	0.26	0.25	0.22	0.19	0.24	0.21	0.17
Tryptophan	0.02	0.04	0.05	0.07	0.04	0.06	0.07
Limestone	0.95	1.24	1.49	1.73	1.18	1.38	1.53
21% Monocalcium phosphate	0.99	0.45	0.00	0.00	0.47	0.00	0.00
Salt	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin, trace mineral Premix	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Ractopamine	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated analysis							
Amino Acids[1], %							
Lysine	1.02	1.02	1.02	1.02	1.02	1.02	1.02
TSAA	0.59	0.59	0.59	0.62	0.59	0.59	0.63
Thr	0.79	0.79	0.79	0.79	0.79	0.79	0.79
Trp	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Ile	0.63	0.61	0.61	0.61	0.61	0.61	0.61
Val	0.69	0.71	0.75	0.79	0.71	0.75	0.79
Leu	1.36	1.54	1.74	1.93	1.51	1.68	1.85
TSAA:Lys	58.00	58.00	58.00	61.00	58.00	58.00	62.00
Thr:Lys	78.00	78.00	78.00	78.00	78.00	78.00	78.00
Trp:Lys	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Ile:Lys	62.00	60.00	60.00	60.00	60.00	60.00	60.00
Val:Lys	68.00	70.00	74.00	78.00	70.00	74.00	77.00
Leu:Lys	134.00	151.00	171.00	190.00	149.00	166.00	182.00
Total Lysine, %	1.14	1.18	1.22	1.26	1.18	1.22	1.26
ME, kcal/kg	1537	1537	1537	1537	1537	1537	1537
SID Lys/ME, g/Mcal	3.00	3.00	3.00	3.00	3.00	3.00	3.00
CP,%	18.00	18.80	20.00	21.10	19.20	20.70	22.20
Ca, total, %	0.63	0.63	0.63	0.70	0.63	0.63	0.69
P, total, %	0.57	0.55	0.54	0.64	0.55	0.54	0.62
Available P, %	0.28	0.28	0.30	0.42	0.28	0.29	0.40

[1]Standardized Ileal Digestibility

[2] Provided per kg of complete diet: 11,002 IU vitamin A supplement, 1651 IU vitamin D_3 supplement, 55.1 IU vitamin E supplement, 0.044 mg vitamin B_{12} supplement, 4.4 mg menadione as menadione dimethylpyrimidinol bisulfite, 9.91 mg riboflavin supplement, 60.6 mg $_D$-pantohenic acid as $_D$-calcium, 55.1 mg niacin supplement, 1.1 mg folic acid, 3.3 mg pyridoxine as pyridoxine hydrochloride, 3.3 mg thiamine as thiamine mononitrate and 0.171 mg biotin, 165 mg Zn as zinc sulfate, 165 mg Fe as ferrous sulfate, 43.5 mg Mn as manganese sulfate, 16.5 mg Cu as basic copper chloride, 0.36 mg I as ethylenediamine dihydriodide and 0.3 mg of Se as sodium selenite.

[3]Control = corn and soybean meal based diet with no DDGS added; Hot = Hot fermentation ethanol production method; Cold = Cold fermentation ethanol production method
[4]40% Cold Fermentation withdrawal treatment fed 40% cold diet during phase 4

Table 2.5 Phase 5 reduced-oil DDGS diet composition (as-fed basis)

	Distillers dried grains with solubles inclusion rate, %						
	Control[3]	Hot			Cold		
	0	20	40	60	20	40[4]	60
Ingredient, %							
Corn	69.49	56.76	43.02	28.43	56.38	42.30	27.41
Soybean meal 46.5%	25.09	17.47	10.81	4.26	17.85	11.56	5.37
Hot Fermentation DDGS	0.00	20.00	40.00	60.00	0.00	0.00	0.00
Cold Fermentation	0.00	0.00	0.00	0.00	20.00	40.00	60.00
Soy oil	2.00	2.50	3.05	3.87	2.56	3.16	4.02
Lysine	0.31	0.44	0.55	0.66	0.44	0.54	0.65
Methionine	0.09	0.05	0.01	0.00	0.05	0.00	0.00
Threonine	0.26	0.25	0.22	0.19	0.24	0.21	0.17
Tryptophan	0.02	0.04	0.05	0.07	0.04	0.06	0.07
Limestone	0.95	1.24	1.50	1.73	1.18	1.38	1.53
21% Monocalcium phosphate	0.99	0.45	0.00	0.00	0.47	0.00	0.00
Salt	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin, trace mineral Premix	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Ractopamine	0.04	0.04	0.04	0.04	0.04	0.04	0.04
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated analysis							
Amino Acids[1], %							
Lysine	1.02	1.02	1.02	1.02	1.02	1.02	1.02
TSAA	0.59	0.59	0.59	0.62	0.59	0.59	0.63
Thr	0.79	0.79	0.79	0.79	0.79	0.79	0.79
Trp	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Ile	0.63	0.61	0.61	0.61	0.61	0.61	0.61
Val	0.69	0.71	0.75	0.79	0.71	0.75	0.79
Leu	1.36	1.54	1.74	1.93	1.51	1.68	1.85
TSAA:Lys	58.00	58.00	58.00	61.00	58.00	58.00	62.00
Thr:Lys	78.00	78.00	78.00	78.00	78.00	78.00	78.00
Trp:Lys	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Ile:Lys	62.00	60.00	60.00	60.00	60.00	60.00	60.00
Val:Lys	68.00	70.00	74.00	78.00	70.00	74.00	77.00
Leu:Lys	134.00	151.00	171.00	190.00	149.00	166.00	182.00
Total Lysine, %	1.14	1.18	1.22	1.26	1.18	1.22	1.25
ME, kcal/kg	1536	1536	1536	1536	1536	1536	1536
SID Lys/ME, g/Mcal	3.00	3.00	3.00	3.00	3.00	3.00	3.00
CP,%	17.90	18.80	20.00	21.10	19.10	20.70	22.20
Ca, total, %	0.63	0.63	0.63	0.70	0.63	0.63	0.69
P, total, %	0.57	0.55	0.54	0.64	0.55	0.54	0.62
Available P, %	0.28	0.28	0.30	0.42	0.28	0.29	0.40

[1]Standardized Ileal Digestibility

[2] Provided per kg of complete diet: 11,002 IU vitamin A supplement, 1651 IU vitamin D_3 supplement, 55.1 IU vitamin E supplement, 0.044 mg vitamin B_{12} supplement, 4.4 mg menadione as menadione dimethylpyrimidinol bisulfite, 9.91 mg riboflavin supplement, 60.6 mg $_D$-pantohenic acid as $_D$-calcium, 55.1 mg niacin supplement, 1.1 mg folic acid, 3.3 mg pyridoxine as pyridoxine hydrochloride, 3.3 mg thiamine as thiamine mononitrate and 0.171 mg biotin, 165 mg Zn as zinc sulfate, 165 mg Fe as ferrous sulfate, 43.5 mg Mn as manganese sulfate, 16.5 mg Cu as basic copper chloride, 0.36 mg I as ethylenediamine dihydriodide and 0.3 mg of Se as sodium selenite.

[3]Control = corn and soybean meal based diet with no DDGS added; Hot = Hot fermentation ethanol production method; Cold = Cold fermentation ethanol production method

[4]40% Cold Fermentation withdrawal treatment fed control diet during final 14 or 21 days on feed

Table 2.6 Phase 1 reduced-oil DDGS diet analyzed nutrient composition (as-fed basis)

	Distillers dried grains with solubles inclusion rate, %							
	Control[3]	Hot			Cold			
	0	20	40	60	20	40	40W[4]	60
Crude Protein								
Calculated	19.34	19.51	20.01	20.59	19.71	20.36	20.36	21.12
Lab 1[1]	18.07	20.31	19.45	21.71	18.79	20.60	20.68	21.06
Lab 2[2]	14.70	19.30	18.80	21.10	18.20	20.70	20.80	20.00
Average	16.39	19.81	19.13	21.41	18.50	20.65	20.74	20.53
Fat								
Calculated	4.74	5.53	6.33	7.13	5.35	5.96	5.96	6.57
Lab 1[1]	2.07	2.11	3.06	4.18	2.26	2.84	2.89	3.50
Lab 2[2]	4.39	4.66	5.60	6.13	4.47	5.23	5.49	6.25
Average	3.23	3.39	4.33	5.16	3.37	4.04	4.19	4.88
Crude Fiber								
Lab 1[1]	2.11	2.73	3.23	3.63	2.56	2.90	3.02	3.66
ADF								
Calculated	3.41	3.95	4.51	5.09	3.96	4.54	4.54	5.12
Lab 2[2]	2.54	2.97	3.67	3.84	3.19	3.08	3.23	3.33
NDF								
Calculated	8.34	10.51	12.67	14.83	10.14	11.93	11.93	13.72
Lab 2[2]	5.69	6.61	10.80	12.40	7.99	8.68	10.30	12.10
Lysine								
Calculated	1.23	1.25	1.27	1.29	1.25	1.27	1.27	1.29
Lab 1[1]	1.36	1.34	1.24	1.36	1.35	1.41	1.24	1.34
Lab 2[2]	1.21	1.34	1.24	1.37	1.23	1.33	1.42	1.35
Average	1.28	1.34	1.24	1.36	1.29	1.37	1.33	1.34
Histidine[1]	0.52	0.52	0.50	0.60	0.53	0.58	0.53	0.57
Isoleucine[1]	0.84	0.83	0.75	0.90	0.83	0.87	0.78	0.83
Leucine[1]	1.66	1.76	1.79	2.14	1.70	1.92	1.80	1.97
Methionine[1]	0.37	0.33	0.47	0.37	0.39	0.41	0.36	0.39
Phenylalanine[1]	0.95	0.94	0.94	1.03	0.92	1.00	0.91	0.96
Threonine[1]	1.01	1.04	0.91	0.96	0.93	0.96	0.96	1.01
Tryptophan[1]	0.22	0.23	0.23	0.23	0.24	0.24	0.26	0.25
Valine[1]	0.93	0.94	0.88	1.08	0.94	1.01	0.91	1.00

[1] University of Missouri Agricultural Experiment Station Chemical Laboratories

[2] Minnesota Valley Testing Laboratories

[3] Control = corn and soybean meal based diet with no DDGS added; Hot = Hot fermentation ethanol production method; Cold = Cold fermentation ethanol production method

[4] 40 W = 40% cold fermentation withdrawal during final 14 or 21 days on feed

Table 2.7 Phase 2 reduced-oil DDGS diet analyzed nutrient composition (as-fed basis)

	Distillers dried grains with solubles inclusion rate, %							
	Control[3]		Hot		Cold			
	0	20	40	60	20	40	40W[4]	60
Crude Protein								
Calculated	16.62	17.63	18.80	19.97	17.99	19.51	19.51	21.04
Lab 1[1]	16.34	17.97	18.52	21.10	17.18	18.97	18.68	20.49
Lab 2[2]	15.90	16.70	18.90	21.10	16.30	18.60	18.80	19.60
Average	16.12	17.34	18.71	21.10	16.74	18.79	18.74	20.05
Fat								
Calculated	4.89	6.49	8.12	9.97	6.11	7.36	7.36	8.80
Lab 1[1]	2.23	2.95	4.69	8.30	3.28	5.05	4.39	5.92
Lab 2[2]	4.30	5.68	6.87	8.42	5.70	7.16	6.61	8.13
Average	3.27	4.32	5.78	8.36	4.49	6.11	5.50	7.03
Crude Fiber								
Lab 1[1]	2.11	3.48	4.12	5.53	3.19	3.84	5.28	5.61
ADF								
Calculated	3.24	4.38	5.52	6.64	4.40	5.56	5.56	6.71
Lab 2[2]	1.86	3.88	4.85	7.19	4.51	3.64	4.06	5.00
NDF								
Calculated	8.42	12.74	17.05	21.29	12.00	15.57	15.57	19.08
Lab 2[2]	7.64	8.80	15.10	21.70	11.60	13.60	13.60	17.00
Lysine								
Calculated	1.05	1.09	1.13	1.18	1.09	1.13	1.13	1.17
Lab 1[1]	1.14	1.13	1.23	1.19	1.21	1.22	1.23	1.16
Lab 2[2]	1.06	1.19	1.20	1.26	1.06	1.21	1.27	1.32
Average	1.10	1.16	1.22	1.23	1.13	1.22	1.25	1.24
Histidine[1]	0.47	0.49	0.54	0.59	0.46	0.52	0.54	0.54
Isoleucine[1]	0.74	0.73	0.77	0.79	0.68	0.71	0.74	0.72
Leucine[1]	1.51	1.72	2.09	2.31	1.63	1.85	1.90	2.05
Methionine[1]	0.30	0.34	0.36	0.37	0.33	0.34	0.34	0.37
Phenylalanine[1]	0.85	0.85	1.05	0.95	0.85	0.84	0.87	0.86
Threonine[1]	0.77	0.80	0.91	0.87	0.91	0.81	0.88	0.80
Tryptophan[1]	0.20	0.20	0.22	0.23	0.20	0.22	0.23	0.22
Valine[1]	0.83	0.88	0.96	1.04	0.80	0.89	0.92	0.94

[1] University of Missouri Agricultural Experiment Station Chemical Laboratories
[2] Minnesota Valley Testing Laboratories

[3] Control = corn and soybean meal based diet with no DDGS added; Hot = Hot fermentation ethanol production method; Cold = Cold fermentation ethanol production method
[4]40 W = 40% cold fermentation withdrawal during final 14 or 21 days on feed

Table 2.8 Phase 3 reduced-oil DDGS diet analyzed nutrient composition (as-fed basis)

	Distillers dried grains with solubles inclusion rate, %							
	Control[3]	Hot			Cold			
	0	20	40	60	20	40	40W[4]	60
Crude Protein								
Calculated	14.92	16.08	17.27	19.32	16.44	17.97	17.97	20.00
Lab 1[1]	12.61	15.56	17.60	20.11	14.65	15.88	16.49	18.60
Lab 2[2]	11.90	13.20	17.60	20.00	14.70	16.30	17.20	18.60
Average	12.26	14.38	17.60	20.06	14.68	16.09	16.85	18.60
Fat								
Calculated	4.97	6.57	8.22	10.16	6.20	7.46	7.46	8.98
Lab 1[1]	1.50	3.59	5.25	7.96	3.14	6.74	3.89	5.05
Lab 2[2]	4.00	5.90	6.93	8.51	5.76	6.48	6.55	8.07
Average	2.75	4.75	6.09	8.24	4.45	6.61	5.22	6.56
Crude Fiber								
Lab 1[1]	1.74	3.04	4.11	5.45	3.03	3.82	5.25	5.38
ADF								
Calculated	3.14	4.29	5.43	6.61	4.31	5.47	5.47	6.65
Lab 2[2]	1.43	2.88	4.36	7.46	3.71	2.91	5.54	4.07
NDF								
Calculated	8.48	12.80	17.10	21.31	12.06	15.62	15.62	19.11
Lab 2[2]	6.42	11.10	15.50	19.30	11.50	15.40	14.10	17.00
Lysine								
Calculated	0.94	0.98	1.02	1.07	0.97	1.01	1.02	1.05
Lab 1[1]	0.85	0.96	1.00	1.19	1.08	1.11	1.00	1.25
Lab 2[2]	1.02	1.03	1.04	1.11	1.00	1.02	1.15	1.19
Average	0.93	0.99	1.02	1.15	1.04	1.07	1.07	1.22
Histidine[1]	0.34	0.41	0.48	0.58	0.43	0.48	0.48	0.53
Isoleucine[1]	0.50	0.56	0.65	0.78	0.60	0.64	0.64	0.68
Leucine[1]	1.07	1.45	1.82	2.32	1.43	1.71	1.72	1.93
Methionine[1]	0.23	0.23	0.33	0.39	0.28	0.32	0.32	0.37
Phenylalanine[1]	0.58	0.67	0.78	0.94	0.70	0.75	0.74	0.80
Threonine[1]	0.62	0.69	0.76	0.84	0.72	0.76	0.76	0.77
Tryptophan[1]	0.17	0.17	0.21	0.21	0.20	0.18	0.20	0.20
Valine[1]	0.58	0.70	0.84	1.03	0.73	0.82	0.82	0.90

[1] University of Missouri Agricultural Experiment Station Chemical Laboratories
[2] Minnesota Valley Testing Laboratories
[3] Control = corn and soybean meal based diet with no DDGS added; Hot = Hot fermentation ethanol production method; Cold = Cold fermentation ethanol production method
[4] 40 W = 40% cold fermentation withdrawal during final 14 or 21 days on feed

Table 2.9 Phase 4 reduced-oil diet analyzed nutrient composition (as-fed basis)

	Distillers dried grains with solubles inclusion rate, %							
	Control[3]	Hot			Cold			
	0	20	40	60	20	40	40W[4]	60
Crude Protein								
Calculated	17.96	18.80	19.97	21.13	19.16	20.67	20.67	22.19
Lab 1[1]	16.21	17.53	20.30	22.23	19.25	19.41	19.35	21.70
Lab 2[2]	14.90	17.20	20.20	21.90	19.00	18.90	19.60	20.10
Average	15.56	17.37	20.25	22.07	19.13	19.16	19.48	20.90
Fat								
Calculated	4.80	6.40	8.03	9.91	6.02	7.27	7.27	8.74
Lab 1[1]	1.63	3.34	4.92	7.56	3.43	4.85	5.52	6.01
Lab 2[2]	4.14	5.35	6.41	7.90	5.84	6.88	6.68	7.69
Average	2.89	4.35	5.67	7.73	4.64	5.87	6.10	6.85
Crude Fiber								
Lab 1[1]	2.39	3.27	4.80	5.55	3.53	4.01	5.60	5.32
ADF								
Calculated	3.33	4.45	5.59	6.71	4.47	5.63	5.63	6.78
Lab 2[2]	2.40	2.78	5.10	7.73	3.75	4.28	4.79	4.42
NDF								
Calculated	8.39	12.71	17.02	21.26	11.97	15.54	15.54	19.05
Lab 2[2]	8.45	11.40	17.30	18.60	11.70	14.30	14.50	16.70
Lysine								
Calculated	1.14	1.18	1.22	1.26	1.18	1.22	1.22	1.26
Lab 1[1]	1.21	0.97	1.28	1.43	1.37	1.26	1.28	1.42
Lab 2[2]	1.12	0.90	1.23	1.29	1.32	1.24	1.33	1.46
Average	1.16	0.94	1.26	1.36	1.35	1.25	1.31	1.44
Histidine[1]	0.46	0.50	0.58	0.62	0.51	0.55	0.54	0.61
Isoleucine[1]	0.74	0.73	0.84	0.86	0.76	0.77	0.75	0.82
Leucine[1]	1.42	1.70	2.14	2.48	1.71	1.90	1.90	2.21
Methionine[1]	0.32	0.30	0.38	0.39	0.37	0.34	0.32	0.41
Phenylalanine[1]	0.82	0.84	0.97	1.03	0.94	0.89	0.87	0.96
Threonine[1]	0.86	0.75	0.94	0.96	0.97	0.86	0.85	0.97
Tryptophan[1]	0.20	0.21	0.21	0.25	0.25	0.21	0.22	0.25
Valine[1]	0.82	0.87	1.03	1.11	0.89	0.94	0.94	1.06

[1] University of Missouri Agricultural Experiment Station Chemical Laboratories

[2] Minnesota Valley Testing Laboratories

[3] Control = corn and soybean meal based diet with no DDGS added; Hot = Hot fermentation ethanol production method; Cold = Cold fermentation ethanol production method

[4] 40 W = 40% cold fermentation withdrawal during final 14 or 21 days on feed

Table 2.10 Phase 5 diet analyzed nutrient composition (as-fed basis)

	Distillers dried grains with solubles inclusion rate, %							
	Control[3]		Hot		Cold			
	0	20	40	60	20	40	40W[4]	60
Crude Protein								
Calculated	17.95	18.80	19.96	21.12	19.15	20.66	17.95	22.19
Lab 1[1]	15.72	22.30	19.84	21.77	17.67	19.40	15.89	20.65
Lab 2[2]	16.00	22.05	20.25	22.00	17.15	17.55	15.90	20.35
Average	15.86	22.18	20.05	21.89	17.41	18.48	15.90	20.50
Fat								
Calculated	4.77	6.37	8.00	9.87	5.99	7.23	4.77	8.70
Lab 1[1]	1.45	4.46	4.38	7.35	2.94	4.08	3.67	5.80
Lab 2[2]	4.01	5.77	6.71	7.90	5.39	6.09	4.63	7.84
Average	2.73	5.11	5.54	7.63	4.17	5.08	4.15	6.82
Crude Fiber								
Lab 1[1]	2.27	4.37	4.44	5.64	3.21	4.13	2.27	5.39
ADF								
Calculated	3.33	4.45	5.59	6.71	4.47	5.63	3.33	6.78
Lab 2[2]	2.32	6.18	5.49	7.42	3.08	5.26	3.17	5.69
NDF								
Calculated	8.39	12.71	17.02	21.26	11.97	15.55	8.39	19.05
Lab 2[2]	7.64	15.95	15.30	21.10	10.80	14.15	7.84	18.30
Lysine								
Calculated	1.14	1.18	1.22	1.26	1.18	1.22	1.14	1.25
Lab 1[1]	1.18	1.41	1.28	1.29	1.19	1.29	1.18	1.41
Lab 2[2]	1.10	1.28	1.21	1.32	1.19	1.26	1.15	1.38
Average	1.14	1.34	1.24	1.30	1.19	1.28	1.16	1.39
Histidine[1]	0.40	0.59	0.56	0.60	0.49	0.54	0.46	0.60
Isoleucine[1]	0.64	0.91	0.81	0.83	0.71	0.77	0.72	0.81
Leucine[1]	1.29	2.02	2.04	2.34	1.60	1.83	1.42	2.13
Methionine[1]	0.30	0.38	0.36	0.39	0.32	0.34	0.31	0.38
Phenylalanine[1]	0.75	1.03	0.93	0.99	0.82	0.87	0.81	0.94
Threonine[1]	0.74	1.06	0.92	0.96	0.83	0.94	0.82	0.92
Tryptophan[1]	0.21	0.25	0.21	0.22	0.23	0.21	0.23	0.22
Valine[1]	0.70	1.06	0.99	1.06	0.84	0.94	0.81	1.03

[1] University of Missouri Agricultural Experiment Station Chemical Laboratories

[2] Minnesota Valley Testing Laboratories

[3] Control = corn and soybean meal based diet with no DDGS added; Hot = Hot fermentation ethanol production method; Cold = Cold fermentation ethanol production method

[4] 40 W = 40% cold fermentation withdrawal during final 14 or 21 days on feed

Table 2.11 DDGS composition, % (as-fed basis)

DDGS Composition

	Hot Fermentation			Cold Fermentation		
	Lab 1[1]	Lab 2[2]	Average	Lab 1[1]	Lab 2[2]	Average
Moisture	12.30	12.87	12.59	11.32	12.13	11.73
Protein	28.84	28.60	28.72	26.49	26.00	26.25
Fat	7.43	6.26	6.85	5.55	5.40	5.48
Crude Fiber	8.34	-	8.34	8.58	-	8.58
ADF	-	10.15	10.15	-	7.49	7.49
NDF	-	31.30	31.30	-	26.60	26.60
Ash	3.89	-	3.89	5.42	-	5.42
AA, %						
Alanine	2.05	-	2.05	1.88	-	1.88
Arginine	1.41	-	1.41	1.19	-	1.19
Aspartic Acid	1.93	-	1.93	1.80	-	1.80
Cysteine	0.62	-	0.62	0.60	-	0.60
Glutamic Acid	4.00	-	4.00	4.05	-	4.05
Glycine	1.14	-	1.14	1.09	-	1.09
Histidine	0.89	-	0.89	0.79	-	0.79
Hydroxylysine	0.01	-	0.01	0.04	-	0.04
Hydroxyproline	0.41	-	0.41	0.24	-	0.24
Isoleucine	1.20	-	1.20	1.03	-	1.03
Lanthionine	0.14	-	0.14	0.06	-	0.06
Leucine	3.45	-	3.45	2.90	-	2.90
Lysine	1.09	0.89	0.99	1.09	0.91	1.00
Methionine	0.60	-	0.60	0.55	-	0.55
Ornithine	0.04	-	0.04	0.07	-	0.07
Phenylalanine	1.40	-	1.40	1.21	-	1.21
Proline	2.23	-	2.23	2.12	-	2.12
Serine	1.34	-	1.34	1.23	-	1.23
Taurine	0.07	-	0.07	0.08	-	0.08
Threonine	1.16	-	1.16	1.05	-	1.05
Tryptophan	0.20	-	0.20	0.22	-	0.22
Tyrosine	1.06	-	1.06	0.96	-	0.96
Valine	1.57	-	1.57	1.35	-	1.35

[1] University of Missouri Agricultural Experiment Station Chemical Laboratories

[2] Minnesota Valley Testing Laboratories

Table 2.12 Analytical methods for feed analysis

	Analytical Methods	
	Lab 1[1]	Lab 2[2]
Moisture	AOAC 934.1	AOAC 930.15
Crude Protein	AOAC 984.13	AOAC 990.03
Fat	AOAC 920.39	AOAC 2003.05
Crude Fiber	AOAC 978.10	-
Neutral Detergent Fiber	-	AOAC 2002.04
Acid Detergent Fiber	-	AOAC 973.18
Ash	AOAC 942.05	-
Complete Amino Acid Profile	AOAC 982.30	-
Lysine	-	AOAC 994.12

[1] University of Missouri Agricultural Experiment Station Chemical Laboratories

[2] Minnesota Valley Testing Laboratories

Table 2.13 The influence of ethanol production method and treatment (0, 20, 40, 40W, 60) of reduced-oil distillers dried grains with solubles (DDGS) on growth performance (n = 40) of finishing pigs.

	DDGS Inclusion, %									P-value				
	Control[1]	Hot			Cold					Hot		Cold		Hot vs Cold
	0	20	40	60	20	40	40W[2]	60	SEM	Linear	Quadratic	Linear	Quadratic	
Phase I														
Initial BW, kg	44.09	44.19	44.07	44.74	44.48	44.56	43.67	45.14	0.74	0.68	0.77	0.36	0.95	0.82
ADG, kg	1.08	1.05	1.03	1.06	1.03	1.04	1.01	1.03	0.04	0.70	0.39	0.49	0.76	0.58
ADFI, kg	2.39	2.34	2.28	2.30	2.25	2.26	2.17	2.22	0.07	0.36	0.70	0.18	0.46	0.13
G:F	0.45	0.45	0.46	0.45	0.46	0.47	0.47	0.47	0.01	0.48	0.54	0.82	0.75	0.45
Phase II														
ADG, kg	1.08	1.08	1.00	0.99	1.03	0.98	0.95	0.84	0.03	<.0001	0.89	<.0001	0.28	<0.01
ADFI, kg	2.75	2.83	2.68	2.70	2.73	2.53	2.58	2.48	0.07	0.44	0.71	0.02	0.88	0.01
G:F	0.40	0.38	0.38	0.37	0.39	0.37	0.37	0.34	0.02	0.21	0.77	0.11	0.54	0.64
Phase III														
ADG, kg	1.22	1.22	1.15	1.15	1.14	1.03	1.04	0.96	0.04	0.10	0.91	<.0001	0.96	<.0001
ADFI, kg	3.45	3.48	3.40	3.36	3.28	3.10	3.23	3.03	0.07	0.32	0.59	<.0001	0.45	<.0001
G:F	0.36	0.35	0.35	0.34	0.33	0.32	0.34	0.31	0.01	0.16	0.54	0.01	0.59	0.04
Phase IV														
ADG, kg	1.25	1.25	1.18	1.20	1.25	1.14	1.16	1.14	0.05	0.42	0.85	0.03	0.90	0.36
ADFI, kg	3.43	3.37	3.26	3.33	3.25	3.07	3.25	3.21	0.09	0.42	0.56	0.10	0.17	0.09
G:F	0.37	0.37	0.39	0.36	0.37	0.36	0.36	0.35	0.01	0.65	0.88	0.36	0.14	0.72
Phase V[3]														
ADG, kg	1.14	1.04	1.19	1.13	1.09	1.05	1.36	1.04	0.03	0.47	0.69	0.02	0.46	0.58
ADFI, kg	3.36	3.22	3.57	3.44	3.22	3.45	3.66	3.33	0.08	0.19	0.98	0.74	0.94	0.70
G:F	0.34	0.32	0.34	0.33	0.31	0.37	0.33	0.31	0.01	0.44	0.56	0.03	0.55	0.69
Cumulative[3]														
Final BW, kg	130.98	129.13	127.76	130.42	129.38	124.72	127.17	121.98	1.65	0.61	0.22	0.02	0.78	0.01
ADG, kg	1.14	1.11	1.09	1.12	1.11	1.05	1.09	1.01	0.02	0.47	0.24	<.0001	0.70	<.001
ADFI, kg	3.04	3.03	3.02	3.09	2.98	2.93	2.96	2.92	0.06	0.92	0.78	0.53	0.66	0.03
G:F	0.38	0.37	0.37	0.36	0.36	0.37	0.36	0.35	0.01	0.25	0.46	<.0001	0.42	0.66

[1]Control = corn-soybean meal-based diet with no DDGS added; Hot fermentation ethanol production method; Cold Hydrolysis ethanol production method

[2]40W = 40% Cold fermentation DDGS withdrawal during final 14 or 21 days on feed

[3] Model Adjusted for days on feed as a covariate

Table 2.14 The influence of ethanol production method and treatment (0, 20, 40, 40W, 60) of reduced-oil distillers dried grains with solubles (DDGS) treatment on carcass characteristics (n = 40) of finishing pigs

	DDGS % Inclusion									P-value					
	Control[1]		Hot		Cold						Hot		Cold		
	0	20	40	60	20	40	40W[4]	60	SEM	Treatment	Linear	Quadratic	Linear	Quadratic	Hot vs Cold
HCW, kg	103.07	99.50	98.01	101.31	100.32	97.31	99.06	93.01	4.18	0.01	0.84	0.04	0.02	0.88	0.07
Carcass Yield[2,5], %	75.65	74.57	74.13	73.95	74.95	74.72	74.48	74.27	0.34	0.04	0.01	0.12	0.08	0.80	0.12
Back fat[3,5], mm	15.56	15.49	16.19	14.64	15.76	15.46	16.80	15.22	0.60	0.39	0.32	0.06	0.67	0.75	0.40
Loin Depth[3,5], mm	57.58	61.35	59.93	58.16	59.46	59.03	60.10	58.10	1.37	0.56	0.88	0.13	0.66	0.11	0.53
Percent Lean[5], %	53.36	53.65	53.14	53.92	53.35	53.54	52.77	53.56	0.40	0.62	0.42	0.26	0.61	0.97	0.37

[1]Control = corn and soybean meal based diet with no DDGS added; Hot = Hot fermentation ethanol production method; Cold = Cold fermentation ethanol production method
[2]Carcass Yield = Hot Carcass Weight / Live Weight
[3]Loin depth and backfat measured between 3rd and 4th from last rib
[4]40W = 40% Cold fermentation DDGS withdrawal during 14 or 21 days on feed
[5]Hot carcass weight used as covariate

Table 2.15 The influence of ethanol production method and treatment (0, 20, 40, 40W, 60) of reduced-oil distillers dried grains with solubles (DDGS) on boneless loin weight and quality (n = 40) of finishing pigs

	DDGS % Inclusion									P-value					
	Control[1]	Hot			Cold						Hot		Cold		
	0	20	40	60	20	40	40W[2]	60	SEM	Treatment	Linear	Quadratic	Linear	Quadratic	Hot vs Cold
Boneless Loin Weight, kg[5]	3.91	4.10	4.07	4.03	4.11	4.08	3.94	4.08	0.08	0.57	0.28	0.43	0.13	0.33	0.79
Subjective Color[3,5]	3.38	3.34	3.11	3.13	3.07	3.27	3.00	3.02	0.11	0.04	0.05	0.78	0.01	0.76	0.15
Subjective Firmness[5]	2.28	2.28	2.02	1.75	2.20	2.29	2.19	2.08	0.25	0.52	0.06	0.71	0.54	0.83	0.26
Subjective Marbling[4,5]	2.41	1.95	2.00	1.57	1.79	1.58	1.99	1.57	0.18	0.07	0.01	0.66	0.02	0.14	0.43
L*[5]	48.30	48.88	47.70	48.51	48.88	46.70	48.29	47.25	0.90	0.67	0.68	0.80	0.72	0.95	0.38
a*[5]	9.07	8.44	8.24	8.17	8.29	7.72	8.08	7.45	0.30	0.10	0.05	0.28	0.02	0.50	0.08
b*[5]	0.51	0.18	0.00	0.00	0.02	0.00	0.00	0.00	0.22	0.46	0.01	0.36	0.22	0.63	0.43

[1]Control = corn and soybean meal based diet with no DDGS added; Hot = Hot fermentation ethanol production method; Cold = Cold fermentation ethanol production method

[2]40W = 40% Cold fermentation DDGS withdrawal during 14 or 21 days on feed

[3]Color scale 1-6, 1 = Pale pinkish-grey to white, 6 = Dark purplish-red

[4]Marbling scale 1-10

[5]Hot carcass weight used as covariate

Table 2.16 The influence of ethanol production method and treatment (0, 20, 40, 40W, 60) of reduced-oil distillers dried grains with solubles (DDGS) belly dimensions and quality measurements (n = 40) of finishing pigs

	DDGS Inclusion, %														
	Control[1]	Hot			Cold						Hot		Cold		
	0	20	40	60	20	40	40W[4]	60	SEM	Treatment	Linear	Quadratic	Linear	Quadratic	Hot vs Cold
Weight, kg[7]	7.41	7.57	7.39	7.52	7.51	7.67	7.68	7.83	0.13	0.38	0.76	0.91	0.20	0.73	0.07
Length, cm[7]	63.27	63.41	62.51	63.00	62.81	61.60	63.50	62.32	0.70	0.58	0.60	0.48	0.44	0.42	0.42
Width, cm[7]	35.47	36.50	37.00	37.51	36.74	36.77	36.49	37.50	0.49	0.19	<.0001	0.90	0.08	0.78	0.72
Scribe line Width, cm[7]	8.12	7.99	8.08	8.06	8.23	7.73	7.99	8.18	0.21	0.54	0.86	0.44	0.97	0.35	0.92
25% Depth, cm[7]	2.59	2.40	2.41	2.26	2.37	2.46	2.59	2.46	0.10	0.14	0.04	1.00	0.54	0.21	0.08
50% Depth, cm[7]	2.12	1.94	1.80	1.73	1.78	1.84	1.98	1.79	0.07	0.01	0.01	0.48	<.0001	0.01	0.63
75% Depth, cm[7]	2.08	1.91	1.81	1.69	1.88	1.91	2.02	1.92	0.06	<.0001	<.0001	0.75	0.09	0.05	<0.01
Average Depth, cm[7]	2.28	2.10	2.02	1.90	2.02	2.08	2.21	2.06	0.06	0.01	<.0001	0.71	0.07	0.04	0.07
Belly Iodine Value from Titration[6, 7]	73.07	81.54	83.43	89.76	83.03	87.50	82.16	95.67	1.25	<.0001	<.0001	0.38	<.0001	0.41	0.02
Calculated Iodine Value[3, 7]	70.99	78.61	80.98	86.57	79.47	84.23	79.07	92.09	1.33	<.0001	<.0001	0.34	<.0001	0.70	0.10
Shoulder Iodine Value[2, 7]	72.54	77.86	81.95	89.08	80.54	85.77	80.13	89.79	0.69	<.0001	<.0001	0.30	<.0001	<.0001	0.04
Flop Score[5, 7]	3.12	2.41	1.93	1.55	2.23	1.91	2.58	1.69	0.18	<.0001	<.0001	0.78	<.0001	0.08	0.30

[1] Control = corn-soybean meal-based diet with no DDGS added; Hot fermentation ethanol production method; [2]Cold Hydrolysis ethanol production method;

[2] Fat sample taken from clear plate and analyzed with Bruker near infrared

[3]Calculated Iodine value = (C16:1*0.95)+(SUM(C18:1)*0.86)+(SUM(C18:2)*1.732)+(SUM(C18:3)*2.616)+(C20:1*0.785)+(C22:1*0.723) (1998 AOAC)

[4]40W = 40% Cold Fermentation DDGS withdrawal during 14 or 21 days on feed

[5]Scale 1-5; 1 = soft, 5 = firm

[6]AOCS method Cd1-25 modified

[7]Hot carcass weight used as covariate

Table 2.17 Reduced-oil DDGS and soybean oil Fatty Acid Profile (as-fed basis), % of total

	CF DDGS[1]	HF DDGS	Soybean Oil
C14	0.07	0.05	0.07
C16	13.68	13.78	10.85
C16:1	0.09	0.09	0.08
C17	0.07	0.06	0.12
C18	2.18	1.92	3.89
C18:1	26.52	25.39	21.43
C18:2	54.88	56.45	53.95
C18:3	1.51	1.32	8.83
C20	0.42	0.4	0.29
C20:1	0.32	0.3	0.19
C20:2	0.04	0.04	0.03
C22	0.15	0.14	0.22
Other	0.07	0.06	0.05
Total SFA[2]	2.89	2.57	4.59
Total MUFA[3]	26.93	25.78	21.7
Total PUFA[4]	56.43	57.81	62.81
Total UFA[5]	83.36	83.59	84.51
UFA:SFA[6]	28.84	32.53	18.41
PUFA:SFA[7]	19.53	22.49	13.68

[1]Hot fermentation ethanol production method; Cold Fermentation ethanol production method; Soybean Oil
[2]Total SFA = C14:0 + C16:0 + C17:0 + C18:0 + C20:0 + C22:0
[3]Total MUFA = C16:1 + C18:1 + C20:1
[4]Total PUFA = C18:2 + C18:3 + C20:2
[5]Total UFA = C16:1 + C18:1 + C18:2 + C18:3+ C20:2
[6]UFA : SFA = Total UFA / Total SFA
[7]PUFA : SFA = Total PUFA / Total SFA

Table 2.18 The influence of ethanol production method and treatment (0, 20, 40, 40W, 60) of reduced-oil distillers dried grains with solubles (DDGS) treatment on fatty acid profile of belly adipose tissue, % of total (n = 40) of finishing pigs

	DDGS Inclusion, %									P-value					
	Control[1]	Hot			Cold						Hot		Cold		
	0	20	40	60	20	40	40W[2]	60	SEM	Treatment	Linear	Quadratic	Linear	Quadratic	Hot vs Cold
C10:0[8]	0.07	0.07	0.06	0.06	0.06	0.06	0.06	0.04	0.00	0.03	<.0001	0.82	0.03	0.37	0.21
C12:0[8]	0.07	0.06	0.06	0.06	0.06	0.06	0.06	0.05	0.00	0.16	0.02	0.64	0.06	0.31	0.76
C14:0[8]	1.17	1.05	1.02	0.97	1.09	1.09	1.04	0.92	0.04	0.01	<.0001	0.43	0.01	0.42	0.50
C15:0[8]	0.02	0.03	0.04	0.05	0.02	0.04	0.03	0.03	0.01	0.11	0.03	0.64	0.12	0.63	0.06
C16:0[8]	22.40	20.39	19.94	19.02	20.40	19.61	20.33	17.47	0.42	<.0001	<.0001	0.19	<.0001	0.97	0.29
C16:1[8]	2.04	1.81	1.55	1.51	1.79	1.68	1.47	1.21	0.11	<.0001	<.0001	0.27	<.0001	0.29	0.21
C17:0[8]	0.19	0.26	0.26	0.28	0.23	0.23	0.24	0.29	0.02	<.0001	<.0001	0.18	0.01	0.68	0.07
C18:0[8]	12.77	11.00	11.33	10.09	10.91	10.22	12.03	9.38	0.39	<.0001	<.0001	0.42	<.0001	0.16	0.50
C18:1[8]	40.34	39.01	37.08	35.42	38.40	35.85	37.25	33.22	0.55	<.0001	<.0001	0.93	<.0001	0.55	0.02
C18:2[8]	17.33	22.06	24.28	27.88	22.76	26.53	23.23	32.00	0.82	<.0001	<.0001	0.34	<.0001	0.89	0.02
C18:3[8]	1.50	1.79	1.98	2.28	1.85	2.07	1.90	2.47	0.07	<.0001	<.0001	0.84	<.0001	0.76	0.23
C20:0[8]	0.27	0.23	0.24	0.22	0.25	0.25	0.27	0.22	0.01	<.0001	<.0001	0.42	<.0001	0.56	0.02
C20:1[8]	0.77	0.78	0.69	0.72	0.76	0.70	0.75	0.67	0.03	0.26	0.19	0.66	0.02	0.83	0.74
C20:2[8]	0.78	0.99	1.02	1.12	1.03	1.14	1.01	1.31	0.05	<.0001	<.0001	0.14	<.0001	0.31	0.01
C20:3[8]	0.24	0.27	0.28	0.24	0.26	0.29	0.26	0.30	0.02	0.39	0.94	0.13	0.04	0.63	0.17
C20:4[8]	0.22	0.26	0.25	0.28	0.27	0.28	0.26	0.32	0.01	<.0001	0.01	0.74	<.0001	0.46	0.01
C21:0[8]	0.03	0.06	0.08	0.06	0.06	0.06	0.05	0.05	0.03	0.95	0.32	0.50	0.81	0.62	0.56
Total SFA[3,8]	36.74	32.96	32.87	30.60	32.90	31.44	33.89	28.38	0.74	<.0001	<.0001	0.27	<.0001	0.56	0.37
Total MUFA[4,8]	42.97	41.45	39.19	37.52	40.85	38.12	39.32	35.08	0.62	<.0001	<.0001	0.78	<.0001	0.43	0.02
Total PUFA[5,8]	20.12	25.42	27.86	31.85	26.21	30.33	26.73	36.44	0.92	<.0001	<.0001	0.33	<.0001	0.87	0.02
UFA:SFA[6,8]	1.73	2.03	2.04	2.29	2.04	2.19	1.95	2.56	0.30	<.0001	0.08	<.0001	0.64	<.0001	0.25
PUFA:SFA[7,8]	0.56	0.78	0.85	1.05	0.80	0.97	0.79	1.31	0.05	<.0001	<.0001	0.69	<.0001	0.38	0.04

[1]Control = corn-soybean meal-based diet with no DDGS added; Hot fermentation ethanol production method; [2]Cold Hydrolysis ethanol production method;
[2]40W = 40% Cold Fermentation DDGS withdrawn during final 14 or 21 days on feed
[3]Total SFA = C10:0 + C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C21:0 + C24:0
[4]Total MUFA = C16:1 + C18:1 + C20:1
[5]Total PUFA = C18:2 + C18:3 + C18:3 Gamma + C20:2 + C20:3 + C20:4
[6]UFA : SFA = Total UFA / Total SFA
[7]PUFA : SFA = Total PUFA / Total SFA
[8]Hot carcass weight used as covariate