EFFECTS OF ADDING MINIMALLY REFINED COTTONSEED OIL OR CRUDE GLYCEROL TO DIETS CONTAINING 40% DISTILLERS DRIED GRAINS WITH SOLUBLES (DDGS) ON GROWTH PERFORMANCE, CARCASS CHARACTERISTICS AND PORK FAT QUALITY OF GROWING-FINISHING PIGS

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CHAPTER 1: INTRODUCTION

Because of increasingly high prices of corn and soybean meal, distillers dried grains with solubles (**DDGS**) has become the most important feed co-product used in swine diets in the United States to reduce feed cost. Corn oil, present in DDGS, is highly unsaturated (NRC, 2012). Increasing dietary concentration of DDGS increases intake and deposition of unsaturated fatty acids which will increase unsaturation pork fat. Pork processors have adopted iodine value (**IV**) as a measure to describe degree of unsaturation of pork fat. Increasing IV of pork fat increases oxidation potential of pork products, and decreases belly firmness (Leick et al., 2010; Xu et al., 2010a; McClelland et al., 2012) which increases problems with processing of bellies, and reduces shelf life and consumer acceptance of retail pork products.

To maintain acceptable IV of pork fat, swine nutritionists reduce the concentration or withdraw DDGS from finishing diets (Stein and Shurson, 2009; Leick et al., 2010) which limits the potential economic benefits of feeding DDGS diets. Therefore, it is crucial to find nutritional strategies that allow maintaining use of high concentrations of DDGS in diets while securing pork fat IV acceptable to pork processors. Supplementing diets with cottonseed oil or crude glycerol may improve pork fat quality.

Cottonseed oil contains cyclopropene fatty acids (**CPFA**) which are known to inhibit desaturase enzymes responsible for synthesizing unsaturated fatty acids in the body (Nixon et al., 1977). By decreasing the endogenous synthesis of unsaturated fatty acids, we may be able to improve firmness of pork fat when diets high in DDGS are fed. To our knowledge, only two studies have been conducted to evaluate the impact of feeding cottonseed oil on pork fat quality. Ellis and Isbell (1926) and Ellis et al. (1931)

reported increased melting point and decreased unsaturation of pork fat when pigs were fed crude cottonseed oil.

Crude glycerol feeding has been reported to affect fatty acid composition of pork and fat firmness. Although diets did not include DDGS, feeding crude glycerol to pigs improved belly firmness (Schieck et al., 2010), reduced linoleic acid content and, consequently, unsaturation of pork fat (Mourot et al., 1994; Kijora et al., 1997; Lammers et al., 2008a). Therefore, feeding diets containing crude glycerol may help to mitigate soft and unsaturated pork fat caused by feeding high concentrations of DDGS to pigs.

Controlling unsaturation of pork fat has important implications for pork processors and retailers. Not only does feeding high concentrations of DDGS impacts pork fat quality, but several other factors. For instance, pork processors and producers have perceived from anecdotal observations greater unsaturation of fat from slow growing pigs compared to fast growing pigs. However, this perception has not been evaluated under controlled conditions. Knowing the influence of growth rate on fatty acid composition of pork fat is important because nutritionists may be able to adopt nutritional strategies to improve pork fat quality of pigs exhibiting growth rates that would decrease pork fat quality.

Another factor influencing management of pork fat quality is the location on the carcass where fat is sampled. Jowl fat is used commonly to predict IV of carcass fat because jowl is a low value portion of the carcass and is easily accessed in the carcass. Assessing jowl fat IV is especially important for estimating IV of belly fat because bellies are a primal cut of high value and sampling for pork fat quality assessment would

damage the belly and decrease its value. However, IV of jowl fat may not reflect fatty acid composition of the belly fat.

Poor correlation between IV of jowl and belly has been reported (Leick et al., 2010; Wiegand et al., 2011). However, Estrada (2013) reported correlations of 0.80 to 0.90 among belly fat, jowl fat and backfat IV. Adipose tissue (**AT**) is distributed widely in the body with differing fatty acid composition across depots (Wood et al., 1989; Leick et al., 2010). In addition to using IV of jowl fat to estimate IV of belly fat, prediction equations have been developed to estimate belly fat IV. Although IV product (**IVP**) is frequently used to predict IV of carcass fat from dietary parameters, studies have been inconsistent as to whether IVP is an effective predictor of IV of carcass fat (Madsen et al., 1992; Benz et al., 2011). So, understanding the relationships among fatty acid composition of jowl fat and other higher value cuts would have great utility to pork processors.

Many factors such as dietary fat, sex, fat depot, carcass fat content and growth rate of pigs can contribute to the fatty acid composition of pork fat which causes difficulty in predicting and controlling pork fat composition (Wood et al., 1989; Zhang et al., 2007; Ibrahim, 2010). A more fundamental understanding of these factors is necessary to determine how composition of pork fat can be managed to optimize quality of pork products.

Therefore, the first objective of this study was to determine the effects of adding minimally refined cottonseed oil or crude glycerol to diets containing 40% DDGS on growth performance, carcass characteristics, and carcass fat quality of growing-finishing

pigs. We hypothesized that both cottonseed oil and crude glycerol would decrease pork fat unsaturation. The second objective of this study was to evaluate the effect of growth rate on fatty acid composition, and determine the effectiveness of using jowl fat as a location for predicting belly fat and backfat IV, as well as use different variables to develop prediction equations of belly fat IV. Understanding of the effects of growth rate and depot location on pork fat IV may provide pork producers and processors essential information for managing pork fat quality more effectively.

CHAPTER 2: LITERATURE REVIEW

2.1 USE OF CORN DDGS IN SWINE DIETS

2.1.1 Ethanol and DDGS production

Fermentation of sugars to produce ethanol is an ancient biotechnology method employed by mankind. For thousands of years alcohol production has been part of the human culture to produce alcoholic beverages such as beer and wine. Before the 19th century, many internal combustion engines fueled by ethanol were developed. Henry Ford's earliest automobile designs, including the famed Model-T, were the first to use engines fueled by ethanol on a commercial basis (Thomas and Kwong, 2001). After the oil crisis of the 1970's, the federal government instituted incentives in the early 1980's to reduce reliance on foreign oil. More recently, the 2005 and 2007 Energy Acts passed by the U.S. Congress provided economic incentives for fuel ethanol production which increased from 175 million gallons in 1980 to 13.3 billion gallons in 2012 (RFA, 2013).

Sorghum, wheat, and a variety of grains can be used to produce ethanol, but corn is used most commonly because of its greater efficiency of production compared with the others mentioned. Due to the lower construction costs relative to wet-mills, today, roughly 90% of the nation's ethanol is produced using the dry-grind process (RFA, 2012). In dry-grind ethanol plants, about one-third of the corn entering the plant ends up as dried distillers grains with solubles (**DDGS**) which is an important feed ingredient for animal diets and an important source of income for ethanol plants. Feed co-products such as DDGS represented about 27% of the gross income of dry-mill ethanol plants in 2013 (RFA, 2014). In 2012, there were 209 ethanol plants in the United States consuming 121

million metric tons of corn and producing 33.3 million metric tons of distillers grains (RFA, 2013). As a consequence of this growing production, the use of DDGS as livestock feed has expanded. This expanded use has been driven by nutritional considerations and economical prices of DDGS relative to other major competing feed ingredients, such as corn and soybean meal.

2.1.2 DDGS production process

Briefly, in the dry-grind process, ground grain is mixed with water and enzymes for saccharification, and ammonia to form a slurry. Later, the aqueous slurry is cooked at 32^0C and yeast is added for fermentation, which generates carbon dioxide, ethanol, and residual solids. This mixture is distilled which separates ethanol from non-volatile components. The remaining mixture is called whole stillage which is composed of yeast and non-fermentable components of corn such as oil, fiber, protein, non-fermentable starch and minerals. The stillage is centrifuged which yields thin stillage and wet grains (Bothast and Schlicher, 2005; Ganesan et al., 2006). Final steps in processing the thin stillage and wet grains generate four major by-products: wet distillers grains (**WDG**), dried distillers grains (**DDG**), condensed distillers solubles (**CDS**) and DDGS (USDA/USDE, 2000).

Compared with corn, DDGS has a greater concentration of corn oil. Because DDGS is a combination of CDS (ether extract (**EE**) from 9% to 15%) plus DDG (EE from 8% to 10%), final fat content of DDGS can be as high as 14% (Liu and Rosentrater, 2012). This has nutritional implications that will be discussed later in this literature review. Oil content of conventional DDGS from modern Midwest ethanol plants is 11%

(Spiehs et al., 2002; Belyea et al., 2004), and ranges from 9 to 14% (Kerr et al., 2013). Ethanol biorefineries in the U.S. have implemented processes to extract oil which has decreased the oil content of DDGS. The extracted oil is a new product that is marketed for biodiesel or feed production, and increases the profit potential of ethanol biorefineries. Currently, the percentage of dry-grind plants extracting oil has increased to more than 75% (RFA, 2014). In dry-grind ethanol plants, oil extraction can occur pre- or post-fermentation using physical methods or via solvent extraction. Physical methods are more common which include heating and centrifuging thin or whole stillage. The resulting DDGS is classified by NRC (2012) as "low-fat DDGS" and contains between 5 to 8% fat. Solvent extraction of oil from DDGS typically occurs using anhydrous ethanol which is produced in ethanol plants and is highly available. The resulting DDGS is classified by the NRC (2012) as "deoiled DDGS" and contains 2 to 6% EE.

2.1.3 Factors affecting nutrient composition of DDGS

On average, about two-thirds (62.55%) of yellow dent corn is starch (NRC, 2012) which can be fermented and converted to ethanol and carbon dioxide. Consequently, the concentration of non-fermentable nutrients is expected to triple in DDGS compared with corn. According to Liu (2009), compared with nutrient composition of corn, DDGS was 3.6 times higher in protein, 3.4 times higher in oil, 3.3 times higher in ash, and 2.9 times higher in non-starch carbohydrates. However, as in any co-product that is dependent on process efficiency, variation in nutrient composition is common with DDGS. Therefore, knowing the correct nutrient composition of DDGS is an important key to formulating diets precisely. Imbalanced diets may result in poor growth of pigs and wasted nutrients excreted in feces which is costly and potentially harmful to the environment.

Factors such as processing technology, duration of fermentation, yeast residues, nutrient composition of incoming grains, and particle size of raw materials can affect nutrient composition of DDGS (Batal and Dale, 2003; Liu, 2009; Belyea et al., 2010). Even among plants with similar technology and grain supply, nutrient composition of DDGS may vary (Singh et al., 2005; Kerr et al., 2013). Spiehs et al. (2002) evaluated 118 samples of DDGS collected over 3 years from ethanol plants located in Minnesota and South Dakota. Variation within and among plants was observed for ME (3.3%), crude fat (7.8%), and crude fiber (8.7%). A notably high degree of variation was observed for Lys (17.3%), Met (13.6%), P (11.3%) and Zn (80.4%) concentration. More recently, Liu (2011) reported significant variation in nutrient composition and amino acid composition of DDGS from different plants, different years, and various grain sources.

2.1.4 Metabolizable energy of DDGS

Dried distillers grains with solubles serves as a source of energy in swine diets. Knowing the energy content is fundamental to formulate well-balanced, cost-effective diets. However, the energy content of DDGS will vary due to inherent variation in the production of DDGS. The concentration of ME among 32 sources of DDGS ranged from 3,277 to 4,336 (SD = 269) kcal/kg of DDGS on a DM basis (Pedersen et al., 2007; Stein and Shurson, 2009; Anderson et al., 2012; Kerr et al., 2013). The NRC (2012) provides ME estimates for low-, medium-, and high-oil DDGS. However, low- and medium-oil categories had low number of samples which leads to increased variation and imprecise ME value assigned when formulating diets. In addition, because of the wide range of energy density estimates, using an average ME concentration for diet formulation is imprecise and leads to under- or over-formulation of dietary energy density. This

imprecision has high economic implications for pig performance, caloric efficiency and capturing full economic value of this feed ingredient.

Methods to assess energy density of feed ingredients, such as *in vivo* studies, are costly and time consuming. Therefore, prediction equations have been developed as a rapid and inexpensive way to overcome the limitations of directly measuring concentration of ME among sources of DDGS (Pedersen et al., 2007; Cozannet et al., 2010; Anderson et al., 2012; Kerr et al., 2013; Graham, 2013).

Researchers have focused on various nutritional components that most likely influence energy density of DDGS when developing prediction equations. However, many studies do not agree on which components are most predictive. This lack of agreement leads to imprecision in diet formulation. Pedersen et al. (2007) developed prediction equations for ME based on 10 DDGS samples with EE ranging from 8.6 to 12.4% on an as-fed basis. They found that prediction of ME is possible knowing the content of GE, ash, ADF and EE. Unexpectedly, removing EE from the model still yielded a relatively precise equation ($r^2 = 0.94$). Likewise, Kerr et al. (2013) developed energy prediction models based on 11 DDGS sources (EE ranging from 8.56% to 13.23% on a DM basis). Although EE had the highest impact on prediction of GE, using EE to predict ME and DE resulted in a poor fit. But fibrous parameters such as total dietary fiber (TDF) and ADF were of primary importance. Similarly, Anderson et al. (2012) found that hemicellulose was the most important parameter for prediction of ME in 18 different corn co-products with a wide range of nutrient composition. But with addition and deletion of additional parameters, the best fit equation included GE and TDF ($r^2 = 0.72$, SD = 323).

Based on these empirical models, energy concentration in DDGS is related highly to fiber concentration. However, one would expect energy density to be related more closely to the EE concentration because lipids have much higher energy density than fiber. However, Kerr et al. (2013) speculated that fiber is more abundant (3.6 times) in DDGS than EE, and fiber exerts a negative impact on lipid digestion which would impact concentration of dietary energy. Therefore, because fiber is more abundant in DDGS than EE, and fiber is related negatively to lipid digestion, it exerts a dilutive effect on energy.

The inconsistency in parameters to better predict energy is clear among the available equations. Urriola et al. (2014) cross-validated energy prediction equations from 5 studies to find the best fit equation and consequently the nutritional components that most likely influence energy density. Urriola et al. (2014) found the best results in the work of Anderson et al. (2012) which included EE in the model (DE = – 2,161 + (1.39 × GE) – (20.7 × NDF) – (49.3 × EE); ME = – 261 + (1.05 × GE) – (7.89 × CP) + (2.47 × NDF) – (4.99 × EE)). The work of Anderson et al. (2012) was the best predictor of energy in DDGS probably because they used a high number of corn co-products with a wider range of nutrient composition compared with other authors. However, more studies are needed to cross-validate prediction equations for ME of DDGS sources differing greatly in nutritional composition (Wu et al., 2015b).

2.1.5 Growth performance of grow-finish pigs fed diets containing DDGS

The impact of DDGS feeding on growth performance is well understood and has been studied extensively (Stein and Shurson, 2009). In most studies, DDGS inclusion in diets up to 20% did not depress growth performance. In the few studies that showed

depressed pig performance, poor quality DDGS with low AA content, improper formulation methods, or low palatability of DDGS were potential causes of poor pig performance (Stein and Shurson, 2009). Growth performance of growing-finishing pigs was affected negatively by 20% dietary concentration of DDGS, when diets were formulated on a total AA basis (Whitney et al., 2006). However, when formulating diets on a standardized ileal digestible (SID) AA basis, DDGS inclusion of 30% had no detrimental effect on growth performance (Xu et al., 2010a; Lee et al., 2013; Pompeu et al, 2013) or slightly reduced both gain (Xu et al., 2010b) and gain efficiency (Asmus et al., 2014).

High dietary level of DDGS inclusion and factors such as AA digestibility and peroxidized lipids in DDGS may depress growth performance. Fewer studies have evaluated DDGS feeding at high dietary concentrations (> 40%; Cromwell et al., 2011; Hilbrands et al., 2013; Wu et al., 2014, 2015a,b). Increasing dietary levels of DDGS up to 45% linearly reduced ADG (Cromwell et al., 2011). The heating of WDG to produce DDGS in the dry milling process can oxidize oil present in DDGS and also decrease digestibility of AA. Feeding diets containing 30% peroxidized DDGS reduced growth and gain efficiency compared to pigs fed corn-soybean meal diets (Song et al., 2014). Feeding DDGS with low digestibility of AA depressed ADG and ADFI (Hilbrands et al., 2013). Therefore, when formulating diets to maximize growth performance, quality of DDGS and formulation method should be considered.

2.1.6 Carcass characteristics of pigs fed diets containing DDGS

Most studies have reported small effects of feeding DDGS to pigs on carcass characteristics such as backfat thickness and loin depth, except for dressing percentage (Stein and Shurson, 2009). The response of carcass traits to dietary DDGS is relatively constant among studies (Stein and Shurson, 2009). In their review, Stein and Shurson (2009) reported a significant reduction of dressing percentage in 8 out of 18 studies published until that time. In contrast, several researchers (Xu et al., 2010b; Cromwell et al., 2011; Lee et al., 2013; Pompeu et al., 2013) reported no effect of dietary DDGS inclusion up to 45% on dressing percentage. The decreased dressing percentage is probably due to the high fiber content of DDGS. The increased gut fill that results when high-fiber ingredients are included in diets can increase crypt depth, villi width, and intestinal mass, which reduces dressing percentage (Kass et al., 1980; Jin et al., 1994).

Several authors found backfat depth unaltered by dietary DDGS even up to inclusion rates of 45% (Whitney et al., 2006; Hilbrands et al., 2013; Pompeu et al., 2013; Lee et al., 2013). However, a few studies have reported reduced backfat thickness when dietary concentration of DDGS was 30% or more (Cromwell et al., 2011; Xu et al., 2010a; Song et al., 2014). In the work of Cromwell et al. (2011) and Song et al. (2014), final BW was reduced with DDGS inclusion in diets which could explain the decreased backfat depth. In some studies loin muscle area and depth remained unaffected by inclusion of DDGS in diets (Widmer et al., 2008; Stein and Shurson, 2009; Pompeu et al., 2013). Some studies have reported no effect of up to 45% dietary DDGS on loin muscle area (Stein and Shurson, 2009; Cromwell et al., 2011), whereas Lee et al. (2013) and Song et al. (2014) found decreased loin muscle area with increasing dietary DDGS,

probably as a result of lower final BW. Similar to other carcass characteristic measurements, increasing levels of DDGS in diets had no effect on fat-free lean percentage of pork carcasses (Whitney et al., 2006; Stein and Shurson, 2009; Cromwell et al., 2011; Hilbrands et al., 2013).

2.1.7 Effects of feeding DDGS on pork fat quality

Due to economic reasons, DDGS has been frequently added at the highest level possible to swine diets. Although oil content of DDGS constitutes a great source of energy to support the growth of pigs, swine nutritionists have been concerned about the use of DDGS in diets because of its impact on pork fat quality. Despite the recent trend of oil extraction, oil present in conventional DDGS, low-fat DDGS, and medium-fat DDGS can still modify composition of pork fat which has important implications for pork processors and retailers. Corn oil, present in DDGS, is highly unsaturated (NRC, 2012). Increasing dietary concentration of DDGS increases intake and deposition of unsaturated fatty acids which will increase unsaturation of pork fat. Increasing unsaturation of pork fat decreases pork fat quality in several aspects (see section 2.5.1). Pork processors have adopted iodine value (**IV**) as a measure to describe degree of unsaturation of pork fat (See section 2.5.2 for more discussion). Iodine value of carcass fat is increased when high (>30%) dietary concentrations of DDGS are fed. To assure acceptable pork fat quality, packers have set upper limits for IV of jowl fat of 73 g of iodine/100 g of total lipids (Benz et al., 2010). Consequently, inclusion rate of DDGS in swine diets has been limited as an approach to prevent excessive increase in IV and thus, has potentially limited the economic benefits of feeding DDGS (Leick et al., 2010).

Increasing dietary concentration of conventional DDGS (EE > 9%) linearly increases pork fat IV (Stein and Shurson, 2009; Benz et al., 2010; Leick et al., 2010; Xu et al., 2010a; Duttlinger et al., 2012). Similarly, low-oil DDGS (5.47%) included in diets at 20% increased pork fat IV, although conventional DDGS (9.6% EE) had greater impact on carcass fat IV (Graham et al., 2013). To maintain pork fat quality and ensure a packer-acceptable IV, swine nutritionists have developed strategies to improve pork fat quality of pigs fed diets containing DDGS. One strategy is to limit DDGS inclusion to 20% or less (Leick et al., 2010). Another strategy commonly used in the industry is to reduce concentration of DDGS in diets or completely withdraw it before harvest. Reducing dietary concentration of DDGS before harvest decreases dietary unsaturated fat. This allows animals to deposit less unsaturated fat and synthesize more *de novo* saturated fat which decreases pork fat unsaturation and IV. Withdrawing DDGS 3 to 4 wk before harvest from diets containing 30% DDGS results in reduction of pork fat IV to satisfactory standards (Stein and Shurson, 2009; Xu et al., 2010b). According to Madsen et al. (1992), iodine value product (**IVP**) can also be used to control quality of pork fat (IVP = (IV of dietary fat) × (crude fat %) × 0.1). Some authors have developed equations to predict carcass fat IV using IVP (Bergstrom et al., 2010; Benz et al., 2010; Paulk et al., 2015; Wu et al., 2015a). This approach allows nutritionists to maintain quality of pork fat by controlling dietary inclusion of DDGS and lipids.

Factors other than dietary fat such as sex, growth rate, and genotype may also affect fatty acid composition of pork fat (Cameron et al., 2000; Zhang et al., 2007; Correa et al., 2008). Compared to barrows, gilts and immune castrated boars have increased unsaturation of carcass fat (Zhang et al., 2007; Correa et al., 2008; Pauly et al., 2009;

Grela et al., 2013). Slow-growing pigs have increased unsaturation index compared with pigs exhibiting faster growth rates (Correa et al., 2008). Although market weight could also be considered a potential factor that affects fatty acid composition of pork fat, finishing pigs at different market weights (107, 115 and 125 kg) increased carcass fat deposition, but did not affect fatty acid composition of carcass fat (Correa et al., 2008).

2.2 USE OF CRUDE GLYCEROL TO IMPROVE FAT FIRMNESS

2.2.1 Production of biodiesel and crude glycerol

Crude glycerol is a co-product of biodiesel production. Biodiesel was developed to partially or fully replace petroleum-based diesel fuel. Although the famous inventor, Rudolf Diesel, used vegetable oils as fuel in his engines in the early 1900's, it was not until the OPEC oil embargo in the 1970's that interest in alternative fuels emerged (Peterson, 1986). In 2014, the U.S. had 89 biodiesel plants with a total production capacity of 1.8 billion gallons per year (USDE, 2014). Biodiesel and glycerol are produced by the transesterification of vegetable oils or animal fats. To produce biodiesel, 20 kg of an alcohol (usually methanol due to its availability and low price) is added to 100 kg of fat with 0.5 kg of a catalytic substance (usually NaOH or KOH). This mixture yields about 10 kg of crude glycerol and 100 kg of biodiesel (Thompson and He, 2006). To achieve a complete reaction, 3 moles of alcohol are needed for every mole of triacylglycerol (Ma and Hanna, 1999; Thompson and He, 2006). However, to avoid the occurrence of a reverse reaction and to increase yields, alcohol is added at twice the required rate to produce biodiesel commercially. In addition, added catalysts increase the

yield of methyl esters (biodiesel) during the transesterification reaction (Ma and Hanna, 1999). With this approach, yield of biodiesel relative to fat input can be as high as 98%.

Crude glycerol is separated gravitationally from biodiesel and is refined to be used in the food, pharmaceutical, and cosmetic industries (Thompson and He, 2006). However with the increase in biodiesel production, crude glycerol is produced in excess of traditional uses. So, crude glycerol has been evaluated as a feed ingredient that supplies energy in diets for livestock.

2.2.2 Composition of crude glycerol

Composition of crude glycerol is based on the fat sources and reagents used to produce biodiesel and their residual content in the final product. Kerr et al. (2009) evaluated composition of crude glycerol sourced from 10 different plants that produced biodiesel from 3 different lipid sources (soybean oil, tallow, and yellow grease). They reported substantial variation in concentration of pure glycerol, moisture, ash, methanol, free fatty acids and salt among the crude glycerol sources studied. Six plants used soybean oil as a source to produce biodiesel, which yielded crude glycerol with minimal variation in components (Kerr et al., 2009). On average, crude glycerol from soybean oil sources had 84% pure glycerol, 10% moisture, 0.05% methanol, 5.6% NaCl, 7% ash, 0.14% free fatty acids, and pH of 6 (Kerr et al., 2009). In this study, a small number of plants used tallow, yellow grease, and poultry fat as a feedstock, so it was difficult to determine the variability in quality of crude glycerol produced from these lipid sources (Kerr et al., 2009).

Methanol content in crude glycerol can increase if methanol is not recovered and reused in future transesterification reactions (Ma and Hanna, 1999). In addition, if the transesterification reaction in biodiesel production is not efficient due to inferior process technology or the fat source used, fatty acids and methanol that were not transformed to methyl esters will be present in crude glycerol. Residual methanol in crude glycerol is a concern when feeding pigs because of its toxicity to pigs (Tephly, 1991). As reviewed by Kerr et al. (2011), few studies have been conducted to evaluate the toxicity of methanol in pigs. Compared to other species, pigs have low levels of folic acid and 10-formyl H_4folate dehydrogenase, which are responsible for metabolizing and eliminating methanol and its product (formic acid). Kerr et al. (2009) reported methanol content in crude glycerol ranged from 0.006% up to 14.99%. However, the Association of American Feed Control Officials (AAFCO, 2014) recommended 0.15% as a safe upper limit in crude glycerol fed to livestock.

The catalytic substances added to increase yield of biodiesel are also a concern. Residual catalysts increase the ash and salt content of crude glycerol which requires appropriate adjustments when formulating swine diets (Thompson and He, 2006). High concentrations of salt in crude glycerol may dictate that supplemental salt be reduced or eliminated from swine diets. However, dietary concentration of salt up to 3% caused no adverse effects on growth performance of swine with adequate water available (NRC, 1980).

Although there are concerns, the U.S. code of federal regulations issued by the Food and Drug Administration states that glycerol is a safe feed ingredient when used with good manufacturing and feeding practices (21CFR582.1320; AAFCO, 2014). The

AAFCO (2014) recommendation for crude glycerol use in animal feeding is as follows: “It must contain no less than 80% glycerin, not more than 15% water, not more than 0.15% methanol, and not more than 5 ppm of heavy metals. It may contain up to 8% salt and 0.1% sulfur”. In addition, the amount of crude glycerol in non-ruminants diets should not exceed 10% of the complete diet. Labels of the final product should contain the percentage of pure glycerol, water and methanol (AAFCO, 2014).

2.2.3 Metabolism of glycerol

Glycerol is a three-carbon molecule naturally occurring in the body and is the backbone of triacylglycerol. Glycerol can be found in the body as glycerol-3-phosphate during glycolysis, or it may be formed by breaking down triacylglycerol (Jungas and Ball, 1963). Metabolism of glycerol in mammals occurs mainly in the liver (Grunnet and Lundquist, 1967). Glycerol metabolism is dependent on glycerol kinase to convert glycerol into glycerol-3-phosphate which will be destined for use in glycolysis and the Krebs cycle (Lin, 1977). Although highly gluconeogenic, glycerol can be metabolized via gluconeogenesis and glycolytic pathways (Tao et al., 1983). Gluconeogenesis and glycolysis are metabolic pathways responsible for generation of energy in the body. Because of these properties, crude glycerol has been used as an effective energy source in feed for livestock (Yang et al., 2012). However, if the intake of glycerol exceeds what the liver can metabolize, glycerol will be excreted in urine which reduces the ME value of crude glycerol (Lin, 1977; Mendoza et al., 2010).

2.2.4 Energy digestibility of crude glycerol

Lammers et al. (2008b) evaluated the digestibility of energy in crude glycerol for pigs. The crude glycerol used had 86.95% total glycerol and 0.29% total fatty acids. Energy contained in crude glycerol was found to be highly digestible. Digestible energy (3,344 kcal/kg on DM basis) represented 92% of gross energy and ME (3,207 kcal/kg on DM basis) represented 96% of DE. In another study using refined glycerol, ME content was 3,584 kcal/kg of DM (Mendoza et al., 2010). Kerr et al. (2009) also found crude glycerol was very digestible in a study using 11 different sources with varying chemical composition. Metabolizable energy was 85.4% of GE, regardless of fat source used to produce biodiesel (Kerr et al., 2009). Also, GE could be predicted accurately by the equation: GE kcal/kg = -236 + (46.08 × glycerol, %) + (61.78 × methanol, %) + (103.62 × fatty acids, %), ($r^2 = 0.99$, $P < 0.01$).

2.2.5 Growth performance of pigs fed crude glycerol

Crude glycerol is recognized widely as an effective energy source in feed to support growth performance of pigs. Most studies report crude glycerol addition to diets up to 10% because of the difficulty in mixing diets if higher levels are used. In most studies, feeding up to 10% crude glycerol had no detrimental effect on growth performance. Growth performance of weaned pigs was not affected by increasing dietary concentrations of crude glycerol up to 8% (Zijlstra et al., 2009). Adding up to 5% crude glycerol to grow-finish swine diets did not affect growth performance (Mourot et al., 1994; Della Casa et al., 2009; Seneviratne et al., 2011; Duttlinger et al., 2012). Similarly, growth performance of wean-to-finish pigs was not affected by dietary inclusion of crude or refined glycerol at 10% or 15% (Lammers et al., 2008a; Mendoza et al., 2010). Pigs

fed diets containing 8% crude glycerol had greater gain and feed intake than pigs fed diets containing no crude glycerol (Schieck et al., 2010).

However, in a study conducted by Della Casa et al. (2009), pigs fed diets containing 10% crude glycerol expressed impaired gain efficiency and ADG during the finishing phase. These authors did not report the nutrient composition of the glycerol fed in their experiment, so one cannot evaluate the cause for this depressed performance. Other components in crude glycerol such as high content of moisture, salt or methanol could have caused the depressed growth performance.

2.2.6 Effect of crude glycerol feeding on carcass characteristics

Feeding crude glycerol has shown consistently no detrimental effects on carcass characteristics. Adding 5% crude glycerol to diets did not affect leanness or fat content of growing-finishing pigs (Mourot et al., 1994). Feeding up to 10% crude glycerol in diets to growing-finishing pigs did not affect HCW, fat-free lean percentage of carcasses or backfat depth (Lammers et al., 2008a; Duttlinger et al., 2012). Similarly, including up to 15% refined glycerol in diets for finishing and heavy fattening pigs did not affect any carcass characteristic measurements (Della Casa et al., 2009; Mendoza et al., 2010).

A few studies have reported some benefits of feeding crude glycerol on characteristics of pork carcasses. Lee et al. (2013) added 5% crude glycerol to diets containing 30% DDGS and found increased LM area compared to pigs fed corn-soybean meal-DDGS diets, although fat-free lean content of carcasses and other carcass characteristics were unaffected. In a study conducted by Schieck et al. (2010), pigs fed diets containing 8% crude glycerol during the finishing period had slightly greater HCW.

Most studies suggest that feeding crude glycerol has no impact on carcass characteristics. The predictable nutrient composition of crude glycerol allows nutritionists to formulate balanced diets to support growth performance and carcass composition similar to that of pigs fed diets with other traditional energy sources, such as corn.

2.2.7 Effect of crude glycerol on carcass fat quality

Effects of feeding crude glycerol on fatty acid composition of pork fat have been inconsistent across studies. Feeding diets containing crude glycerol reduced unsaturation of pork fat (Mourot et al., 1994; Kijora et al., 1997; Lammers et al., 2008a). Supplementing barley-soybean meal diets with 10% crude glycerol reduced unsaturation of backfat (Kijora et al., 1997). Feeding 5% glycerol in diets with 4% tallow or 4% rapeseed oil decreased linoleic acid content in backfat of growing-finishing pigs, and consequently reduced unsaturation (Mourot et al., 1994). Wean-to-finish pigs fed corn-soybean meal diets with 10% glycerol had decreased linoleic acid content in loin muscle compared to pigs fed diets containing no crude glycerol (Lammers et al., 2008a). Growing-finishing pigs fed diets containing 10% crude glycerol had greater concentration of monounsaturated fatty acids (MUFA) in subcutaneous fat of the ham compared to pigs fed diets containing no crude glycerol (Della Casa et al., 2009). Nonetheless, other researchers have reported no impact of crude glycerol feeding on fatty acid composition of pork fat. Diets containing 20% DDGS supplemented with up to 5% crude glycerol had no impact on fatty acid composition of belly, jowl, and backfat of growing-finishing pigs (Duttlinger et al., 2012). Glycerol supplementation of 5% had no effect on IV of belly fat and backfat from pigs fed diets containing 30% DDGS (Lee et al., 2013).

To explain this variable impact of crude glycerol feeding on fat composition, Lammers et al. (2008a) speculated that inclusion of crude glycerol in diets would reduce the inclusion of corn (and consequently corn oil), which would ultimately reduce intake of unsaturated fatty acids and decrease unsaturation of pork fat. In general, researchers did not report the nutrient composition of glycerol fed in their experiments, so one cannot evaluate the cause for this variable impact of crude glycerol feeding on fat composition. Feeding crude glycerol with high fatty acid content has a greater effect on fatty acid composition of pork fat than crude glycerol with much lower fatty acid content (Kerr et al, 2009). Ingested fatty acids are deposited directly in carcass fat depots. The amount and composition of the fatty acids ingested from crude glycerol can be reflected in fatty acid composition of carcass fat (Leick et al., 2010). Therefore, the inconsistent response of fatty acid composition in carcass fat to glycerol feeding may be due to variation in the composition and concentration of fatty acids in crude glycerol which is usually not reported in studies (Mourot et al., 1994; Kijora et al., 1997; Della Casa et al., 2009; Lee et al., 2013).

Nonetheless, feeding crude glycerol has been shown to potentially increase belly firmness. Pigs fed crude glycerol-containing diets during the finishing phase had firmer bellies than pigs fed diets containing no crude glycerol, as measured by the belly flop angle (Schieck et al., 2010). The improved belly firmness of pigs fed crude glycerol may be unrelated to decreased unsaturation of carcass fat; but rather, related to the lean content of the belly. Bellies have on average 45% fat content and 40% moisture which is an indicator of lean content (Wright et al., 2005). Feeding glycerol to pigs has been shown to increase water holding capacity (**WHC**) of loin muscle (Mourot et al., 1994;

Della Casa et al., 2009). The increased WHC of loin muscle could extend to lean tissue of bellies. Increasing WHC of the lean portion of bellies could ultimately increase tension within the muscle cells of lean tissue and increase belly firmness (Mourot et al., 1994; Della Casa et al., 2009).

Feeding glycerol-containing diets to pigs had no effect on color of belly fat (Schieck et al., 2010), backfat (Lee et al., 2013) or *Semimembranosus* muscle (Della Casa et al., 2009), and had no effect on color, cooking loss and texture of LM (Lammers et al., 2008a; Della Casa et al., 2009; Hansen et al., 2009; Mendoza et al, 2010; Schieck et al., 2010).

In conclusion, crude glycerol is a feed ingredient that supplies energy to pigs and supports acceptable growth performance. Some studies have reported improved belly firmness and decreased unsaturation of carcass fat with glycerol feeding. Consequently, it may have applications in improving poor fat quality of pigs fed DDGS-containing diets. However, impact of crude glycerol on pork fat quality is somewhat inconsistent, and therefore needs to be studied further.

2.3 USE OF COTTONSEED OIL IN SWINE DIETS

2.3.1 Cottonseed oil production and use

Commonly known as cotton, *Gossypium hirsutum* L. is the most important crop used by the textile industry to obtain natural fiber (Rashid et al., 2009). Cottonseed is also a good source of protein for human and animal nutrition (Lawhon et al., 1977; Moore et al., 1986). After the removal of the lint, oil can be solvent-extracted from cottonseeds. Cottonseed contains approximately 16% crude fat (NRC, 2012). In 2013, the U.S.

produced 363 million kg of cottonseed oil, and the domestic use was almost 272 million kg (USDA, 2014). Besides the typical use in food preparation, cottonseed oil is also an effective high energy feed ingredient for animals (Evans et al., 1961; Moore et al., 1986) and a suitable substrate to produce biodiesel (Rakopoulos et al., 2008).

2.3.2 Use of cottonseed oil in swine nutrition

Cottonseed differs from other oilseeds used for livestock feeding because it contains cyclopropene fatty acids (**CPFA**). Cyclopropene fatty acids are potent inhibitors of desaturase enzymes although the mechanism of action is unclear (Quintana et al., 1998). After ingestion and absorption, CPFA bind to desaturase enzymes and inactivate them which will reduce the formation of unsaturated fatty acids in the body (Nixon et al., 1977). For a long time, cottonseed meal has been known to increase firmness, melting point, and saturation of pork fat (Hare, 1913). However, cottonseed oil is more concentrated in CPFA than cottonseed meal due to the relatively low-oil content (<5%) of cottonseed meal (NRC, 2012). Only two studies have been conducted to evaluate the effect of feeding cottonseed oil on pork fat quality. Both studies demonstrated increased melting point temperature and decreased unsaturation of carcass fat due to supplementation with cottonseed oil (Ellis and Isbell, 1926; Ellis et al., 1931). Therefore, supplementing diets with cottonseed oil might have great potential to ameliorate the detrimental effects of feeding high concentrations of DDGS on fat quality of pigs.

2.3.3 Anti-nutritional factors of cottonseed

In livestock feeding, cottonseed oil and meal are important sources of energy and protein, respectively. However, cottonseeds differ from other plants in that they contain

gossypol which is a bioactive natural phenol capable of inhibiting several enzymes and zymogens (Tanksley et al., 1970). This will impair important biochemical reactions in the body, such as the conversion of pepsinogen to pepsin (responsible for protein digestion), and reduce the number of red blood cells and their oxygen-release capacity. Dietary gossypol can decrease growth performance and cause death of pigs at dietary concentrations of 0.019% (Hale and Lyman, 1957). In addition, free gossypol in diets may bind to AA and decrease nutrient utilization (Knabe et al., 1979).

To ameliorate these adverse effects of feeding cottonseed products, dietary supplementation of iron salts is effective. Adding ferrous sulfate in a ratio of 1:1 to free gossypol resulted in growth performance similar to pigs fed diets containing no gossypol (Clawson and Smith, 1966). In addition, genetic selection of cotton and modern processing technologies have reduced gossypol content, which makes cottonseed oil and meal safe for livestock feeding (Kuk and Tetlow, 2005).

2.3.4 Growth performance and carcass characteristics

Cottonseed oil is not used widely in animal feed because it is less available than other oils such as soybean oil or corn oil. However, cottonseed oil potentially has greater utility in swine diets, if it effectively increases pork fat firmness (Ellis and Isbell, 1926; Ellis et al., 1931). Ellis et al. (1931) are the only researchers to report effects of cottonseed oil on growth performance of pigs. These researchers reported increased body weight gain and reduced feed intake compared to pigs fed diet with no cottonseed oil. This response in growth performance to feeding cottonseed oil is similar to responses found when other fat sources where studied. Increasing energy density of diets with choice white grease decreased feed intake, and improved gain efficiency and growth rate

of growing-finishing pigs (Smith et al., 1999; De la Llata et al., 2001). Adding fat to diets to increase energy density improved gain efficiency of growing-finishing pigs (Apple et al., 2004). Although only one study evaluated growth performance of pigs fed cottonseed oil, the growth performance responses reported were similar to those of growing-finishing pigs fed isocaloric diets despite the dietary fat source used, whether vegetable or animal (Bee et al., 2002; Teye et al., 2006). The effect of dietary fat addition on growth performance is a response to energy concentration of the fat added and the subsequent increase in caloric density of the diet. Equations can predict energy concentration of fats using fatty acid composition (NRC, 2012). This enables nutritionists to predict growth performance of pigs fed diets supplemented with cottonseed oil based on fatty acid composition of the dietary lipid. Fatty acid composition of cottonseed oil is similar to corn oil, although cottonseed oil has greater concentration of palmitic acid and less linolenic acid (NRC, 2012). Therefore, we can assume that the impact of feeding cottonseed oil on growth performance would be similar to that of other oils, such as corn oil, because energy density of cottonseed oil is similar to that of other oils.

Carcass characteristics of pigs fed diets containing high fat content may vary based on fatty acid deposition rate and growth rate. However, the response of carcass characteristics to high energy density diets is not related to fat source added. Carcass characteristics were similar for growing-finishing pigs fed isocaloric diets regardless of dietary fat source used (Bee et al., 2002; Teye et al., 2006). Dietary inclusion of 5% corn oil to increase dietary energy density resulted in similar carcass characteristics compared to pigs fed diets containing no added oil (Le Dividich et al., 1987). Pigs fed increasing dietary levels of choice white grease had greater HCW, but no other carcass measurement

was affected (Smith et al., 1999; De la Llata et al., 2001). This response was likely due to greater growth rate of pigs fed diets with supplemental lipids. Many studies have reported increased backfat depth and decreased fat-free lean for pigs fed diets containing supplemental fat (Pettigrew and Moser, 1991; Apple et al., 2004). However, to maintain similar carcass characteristics among pigs fed diets with different energy density it is important to adjust diets to a similar energy:SID Lys ratio. Increasing AA density in diets with higher energy density can compensate for the lower feed intake of pigs fed diets with high energy density and result in similar rate of lean tissue deposition to pigs fed diets with lower energy density (Apple et al., 2004).

In summary, cottonseed oil is a feed ingredient that supplies energy to pigs and can support acceptable growth performance (Ellis et al., 1931). Consequently, cottonseed oil may effectively improve poor fat quality of pigs fed DDGS-containing diets. However, the impact of cottonseed oil on pork fat quality has not been studied in depth.

2.4 LIPIDS IN SWINE DIETS

2.4.1 Nutritional benefits of dietary lipids

Whether through high-oil feed ingredients such as DDGS, or supplemented oils and fats, increased dietary lipids have several benefits to swine nutrition. Oils and fats provide 2.25 times more metabolic energy than starch. In warm weather, feed intake of pigs is limited to reduce the heat increment of digestion and consequently, energy intake is reduced. Diets containing supplemental fats are more energy dense, which increases intake of energy, increases ADG, and improves G:F (Coffey et al., 1982) especially in hot weather. Fatty acids are not broken down during digestion. Therefore, the energy

dissipated during digestion is less compared with digestion of starch. This phenomenon enhances energy intake and contributes to optimal growth during heat stress (Stahly and Cromwell, 1979).

Dietary fat can slow the passage rate of digesta which can increase digestibility of other dietary components and improve growth performance (Cho and Kim, 2012). Other benefits associated with dietary fat are increased pellet quality, reduced dust in barns, increased palatability, improved transport and absorption of fat-soluble vitamins, enhanced synthesis of prostaglandins and hormones, and increased intake of essential fatty acids (Lehninger et al., 2008; NRC, 2012).

Despite these benefits, the biggest concern with fat supplementation to diets is its impact on the composition of pork fat. Fatty acid composition of the diet has a great influence on the composition of pork fat (Averette Gatlin et al., 2002a; Teye et al., 2006; Xu et al., 2010a). Although extracted lipids are more digestible than intact lipids in grains, the fermentation process of DDGS production can increase digestibility of lipids in DDGS which will affect fatty acid composition of pork fat (Davis et al., 2015). In addition, supplementation of DDGS reduces digestibility of SFA and increases digestibility of unsaturated fatty acids from extracted lipids in the diets which will cause even bigger impact in the fatty acid composition of pork fat (Davis et al., 2015). Ingested fatty acids are deposited in adipose tissue without changes in composition. Fatty acids from the diet will be oxidized to meet energy demands or deposited intact in adipose tissue. Carcass fat of pigs originates from intake of dietary fat and *in vivo* synthesis of triglycerides in adipose tissue (O'Hea and Leveille, 1969). However, fatty acid synthesis by adipose tissue was reduced when pigs were fed diets containing high fat content

(~10%), regardless of dietary fatty acid composition (Allee et al., 1972). Therefore, feeding increasing levels of dietary fat will reduce body synthesis of fat and simultaneously influence fatty acid composition of pork fat.

2.4.2 Lipid digestion, absorption, and deposition

Digestion of lipids starts in the mouth and stomach. Lipids are emulsified by bile salts which increase surface area of the lipid droplet to allow greater interaction with lipase. Fat and oils are composed of three fatty acids bound to a glycerol molecule. These structures are called triacylglycerols. Triacylglycerols are broken down enzymatically into diacylglycerol by lingual and gastric lipases (Carey et al., 1983; Singh et al, 2009).

As digesta moves into the intestine, pancreatic juices containing lipases, carboxyl ester hydrolases, and phospholipases are released into the lumen of the duodenum. These compounds are responsible for lipolysis. Carboxyl ester hydrolase and phospholipases require bile salts to function; whereas lipase activity will be enhanced by colipase and pH between 8 and 9 (Friedman and Nylund, 1980; Jensen et al., 1997). The enzyme activity of these compounds will remove fatty acids from triacylglycerol, forming monoacylglycerol and fatty acids. Monoacylglycerol and fatty acids will bind to bile salts to form micelles. Micelles are then absorbed by the enterocytes in the intestine, where the fatty acids are re-esterified into triacylglycerol. Triacylglycerols are transported in the form of chylomicrons by the lymph from enterocytes to the subclavian vein, and thereafter to the whole body. Excess triacylglycerols can also be packaged into very low-density lipoproteins (**VLDL**) in the liver and be transported through blood to adipose tissue (Lehninger et al., 2008). A portion of fatty acids can still be transported from enterocytes by the portal vein as free fatty acids (Doreau and Chilliard, 1997; Singh et al.,

2009). In capillaries of adipose tissue and muscle, lipoprotein lipase hydrolyzes chylomicrons into triacylglycerol to be absorbed and stored in adipose tissue when circulating insulin is high, or to be oxidized in muscle tissue when circulating insulin is low (Lehninger et al., 2008).

2.4.3 Enzymes related to fatty acid synthesis in adipocytes of swine adipose tissue

In pigs, the majority of lipogenesis occurs in adipocytes of adipose tissue (O'Hea and Leveille, 1969). The production of fatty acids in pigs starts by formation of malonyl-CoA from acetyl-CoA, catalyzed by acetyl-CoA carboxylase. Malonyl-CoA goes through a multienzyme system called fatty acid synthase, which adds 2-carbon units donated by other malonyl-CoA in a sequence of steps (Lehninger et al., 2008). The final product is palmitic acid (C16:0). In a similar process, enlongase enzymes will add 2-carbon units from malonyl-CoA to palmitic acid, which forms stearic acid (C18:0). Desaturase enzymes can also introduce a double bond to the fatty acid, palmitic acid, which forms palmitoleic acid (C16:1). Similarly, oleic acid (C18:1) can be formed from stearic acid (C18:0) (Lehninger et al., 2008). Mammals do not have the desaturase enzymes that are necessary to further desaturate oleic acid (C18:1) into other PUFA such as linoleic (C18:2) and linolenic (C18:3) which are essential in animal diets. If linoleic and linolenic acids are present in diets, they can be enzymatically elongated and desaturated into several PUFA required by the pig (Zhang et al., 2007; Lehninger et al., 2008).

Factor such as sex, breed, genetic line, diet, and temperature may affect the activity of desaturase, elongase, and acetyl-CoA carboxylase enzymes (Kouba et al., 1999; Zhang et al., 2007; Shirouchi et al., 2014). Dietary lipids can affect the activity of lipogenic enzymes. Increasing unsaturation of swine diets reduced desaturase enzymes in

backfat, but not in liver, loin muscle or intestine (Klingenberg et al, 1995). The lack of response of desaturase enzyme activity in liver, loin muscle, and intestine to changes in dietary fat composition may be because in pigs, the majority of lipogenesis occurs in adipose tissue (O'Hea and Leveille, 1969). Increased expression of desaturase enzymes have been related to increased content of MUFA in adipose tissue (Wang et al., 2005) which influences fatty acid composition of carcass fat. Activity of desaturase enzyme differs among fat depots which may influence composition of carcass fat. Shirouchi et al. (2014) reported differences in activity of desaturase enzymes from loin muscle and visceral fat of cattle. Kouba et al. (1999) reported greater activity in desaturase enzyme and activity of acetyl-CoA carboxylase enzyme (a marker of lipogenesis) for backfat and leaf fat compared to muscle and liver of pigs. Activity of lipogenic enzymes (glucose-6-phosphate dehydrogenase, malic enzyme and fatty acid synthase enzyme) as well as lipid deposition is not different between outer and inner layers of backfat (Bee, 2001; Bee et al., 2002). Therefore, differences in fatty acid composition of layers of backfat could be related more to activity of desaturase enzyme than lipid synthesis or deposition (Bee, 2001). In addition, selecting pigs for increased leanness reduced activity of desaturase enzyme and activity of acetyl-CoA carboxylase enzyme (Cánovas et al., 2005). Gilts have less activity of lipogenic enzymes compared to barrows (Allee et al., 1972) which increases unsaturation of carcass fat of gilts (Correa et al., 2008). This topic will be discussed more in section 2.4.5. How factors such as diet, sex, breed, and enzyme activity contribute and interact to influence final fatty acid composition of each depot is unclear. Conceptual models including a few factors (e.g. diet composition, feed intake, growth performance and fatty acid composition of backfat) may not accurately predict fatty acid

composition of pork fat (Lizardo et al., 2002). Thus, it is clear that a wide range of factors influence fatty acid composition of carcass fat and enzyme activity should not be considered alone when studying fatty acid composition.

2.4.4 Metabolism, synthesis, uptake, and use of fat

Stored lipids can provide energy to the body whenever necessary. When energy balance is positive, high insulin will increase the uptake of glucose by adipocytes. The enzyme, acetyl-CoA carboxylase, catalyzes the formation of triacylglycerols from glucose. The synthesized triacylglycerols are then stored in adipocytes. However, when blood glucose and insulin levels are low, the hormones epinephrine, glucagon, and growth hormone are released from the adrenal glands, pancreas, and anterior pituitary, respectively. These hormones activate adenylate cyclase in the adipocyte membrane which activates hormone-sensitive triacylglycerol lipase. Hormone-sensitive triacylglycerol lipase hydrolyzes triacylglycerols, which releases fatty acids into circulating blood. In blood, fatty acids bind to the protein, serum albumin. Serum albumin serves as a carrier of fatty acids to target tissues, and releases them in the cytosol of cells to serve as fuel (Jeukendrup et al., 1998; Lehninger et al., 2008).

Inside the cell, fatty acids move to the outer mitochondrial membrane, where acyl-CoA synthase catalyzes a reaction to form fatty acyl-CoA. In the inter-membrane space, carnitine acyltransferase I reacts with fatty acyl-CoA to form a fatty acyl-carnitine ester that crosses the inner mitochondrial membrane by passive diffusion into the mitochondrial matrix. Inside the matrix, fatty acids undergo β-oxidation which removes multiple 2-carbon units (acetyl-CoA) that feed into the citric acid cycle. Also, β-oxidation of fatty acids yields NADH and $FADH_2$ that supply electrons to the electron transport

chain to phosphorylate ADP into ATP. Oxidation of fatty acids in mitochondria provides energy for tissues. In liver, excess acetyl-CoA produced by β-oxidation is transformed into ketone bodies that circulate through blood to enter the citric acid cycle and replace glucose as fuel in tissues and brain cells (Doreau and Chilliard, 1997; Jeukendrup et al., 1998; Lehninger et al., 2008).

2.4.5 Characteristics and the role of adipose tissue in swine

Adipose tissue (**AT**) is distributed widely in the body. Adipose tissue can provide insulation, protect organs and tissues, serve as an endocrine organ, and store lipid (Havel, 2004; Lehninger et al., 2008). Adipose tissue is an important physiological tool to capture excess energy that is consumed by an animal. Energy not used for maintenance and growth is stored in the form of triacylglycerols and free fatty acids in cells of adipose tissue (adipocytes). Adipose tissue is composed largely of adipocytes for lipid storage, and other non-fat cells, such as vascular cells, neural cells, inflammatory cells (macrophages), immune cells, preadipocytes, and fibroblasts of the connective tissue matrix (Ibrahim, 2010). Adipocytes are composed of a cell membrane, a lipid droplet, a nucleus and a small amount of cytoplasm. Different adipose tissue depots have different lipolytic and lipogenic activity and respond differently to insulin and other hormones. This difference is prominent among visceral and subcutaneous adipose tissue, gender, and genetic predisposition (Wood et al., 1989; Zhang et al., 2007; Ibrahim, 2010). The activity of enzymes related to lipids differ among pig breeds which results in different fatty acid composition of pork fat (Zhang et al., 2007). Gilts have greater unsaturation of carcass fat compared to barrows (Table 2.1; Zhang et al., 2007; Correa et al., 2008). Part of this difference has been explained by lower activity of lipogenic enzymes in gilts

compared to barrows (Allee et al., 1972). Lower activity of lipogenic enzymes reduces production of saturated fatty acids. In addition, greater concentrations of sex hormones in gilts and immunocastrated boars compared to barrows drive dietary nutrients toward lean deposition instead of fat deposition which reduces body synthesis of fatty acids (Pauly et al., 2009; Grela et al., 2013). Fatty acids synthesized by the body are mostly saturated fatty acids. Consequently, gilts and immunocastrated boars have less production of saturated fatty acids. Therefore, unsaturated fatty acids from diets have a large influence on carcass composition of gilts and immunocastrated boars which leads to greater unsaturation of carcass fat compared with barrows (Pauly et al., 2009; Grela et al., 2013).

Table 2.1. Effects of gender on fatty acid composition, total lipid content, and fatty acid indices of loin muscle[1] (adapted from Zhang et al. 2007).

Item	Barrows	SE	Gilts	SE	Significance
Fatty acid[2]					
14:1	1.33	0.01	1.25	0.01	*
16:0	25.41	0.07	24.56	0.08	*
16:1 n-7	4.13	0.03	3.96	0.03	*
18:0	12.36	0.06	12.06	0.06	*
18:1 n-9	46.19	0.14	45.35	0.14	*
18:2 n-6	8.55	0.11	10.06	0.12	*
20:4 n-6	2.03	0.05	2.75	0.05	*
Total SFA	39.11	0.1	37.87	0.11	*
Total MUFA	50.31	0.14	49.31	0.15	*
Total PUFA	10.58	0.15	12.81	0.16	*
Total lipids[3]	3.03	0.05	2.35	0.05	*
P:S[4]	0.27	0	0.34	0	*

[1]Values are expressed as least squares means ± SE.
[2]Fatty acid values are g/100 g of total lipids.
[3]Total lipids are g/100 g of muscle.
[4]The ratio of total PUFA to total SFA.
*$P < 0.01$.

Fatty acid composition of pork fat is different among fat depots (Wood et al., 1989). Usually pork processors sample fat from jowl for analysis of fatty acid composition and to assess quality of carcass fat because jowl is a low value portion of the carcass and is easily accessed in the carcass. Wiegand et al. (2011) reported similar IV for jowl fat and belly fat from pigs fed corn-soybean meal diets. However, Leick et al. (2010) reported greater IV for belly fat compared to jowl fat for pigs fed corn-soybean meal diets. Wiegand et al. (2011) reported lower IV for backfat compared to jowl fat and belly fat. However, Weber et al. (2006) reported greater IV for backfat compared to belly fat for pigs fed corn-soybean meal diets. Monziols et al. (2007) reported the fatty acid composition of 11 adipose tissue depots including different layers of subcutaneous fat (Table 2.2). Fatty acid composition differed among sampling locations. Belly, loin and shoulder had greater concentration of PUFA in subcutaneous AT compared to intermuscular AT (Monziols et al., 2007). In contrast, subcutaneous AT from ham had less concentration of PUFA than intermuscular AT from ham. Therefore, because of the differences among AT depots, sampling fat from jowl for analysis of fatty acid composition may not accurately represent other depots of carcass fat.

Cameron and Enser (1991) reported poor correlation between fatty acids from backfat and intramuscular fat. Similarly, poor correlation (0.39) between belly and jowl IV was reported by Leick et al. (2010). Wiegand et al. (2011) found a positive correlation between IV of belly and intramuscular fat of LM (0.67). However, increasing the energy density of the diets by adding choice white grease altered the relationship, which resulted in negative correlation between belly and jowl (-0.67), and belly and intramuscular fat of

LM (-0.74). Fatty acid composition of different fat depots are relatively different and unrelated. Therefore, prediction of fatty acid composition of a fat depot based on other depots is not reliable.

Table 2.2. Lipid content and fatty acid composition of the 11 adipose tissue samples (adapted from Monziols et al., 2007)

Variables	SC[1] Ham	IM[2] Ham	SC Shou[3]	IM Shou	Outer SC loin	Inner SC loin	IM Loin	SC Belly	IMb[4] Belly	IM Belly	Flare fat	SE
Lipid content[5]	65.07^{def}	57.94^{a}	63.11^{bcd}	57.54^{a}	67.84^{fg}	66.84^{efg}	60.56^{ab}	65.72^{defg}	64.29^{cde}	61.49^{bc}	68.61^{g}	6.5
SFA[6]	40.12^{ab}	40.99^{b}	39.95^{ab}	42.74^{c}	39.55^{a}	44.26^{de}	45.12^{e}	39.84^{a}	43.58^{cd}	44.33^{de}	48.47^{f}	2.19
MUFA[6]	46.24^{g}	44.65^{def}	45.50^{fg}	45.25^{efg}	45.69^{fg}	43.04^{c}	41.93^{b}	45.77^{g}	43.70^{cd}	44.38^{de}	39.02^{a}	2.06
PUFA[6]	13.65^{d}	14.35^{e}	14.55^{e}	12.01^{b}	14.76^{e}	12.70^{bc}	12.95^{c}	14.39^{e}	12.71^{bc}	11.29^{a}	12.51^{bc}	1.42

[1]Subcutaneous adipose tissue
[2]Intermuscular adipose tissue
[3]Shoulder
[4]Intermuscular fat in the belly, in anatomical continuity with the inner layer of the subcutaneous fat in the loin
[5]Percentage by weight
[6]Percent of total fatty acids
[a,b,c,d,e,f,g]Means within a row with different superscripts differ ($P < 0.001$)

Wood et al. (1989) showed differences in composition of pork fat among different degrees of carcass fatness (Table 2.3). Pigs with increased fat deposition have greater lipid content and reduced water and collagen in adipose tissue because of the expansion of adipocytes with increased amount of lipids stored (Wood et al., 1989). Fatty acid composition of adipose tissue can have important implications to the meat industry that will be discussed later in this literature review (see section 2.5).

Table 2.3. Composition[1] of backfat in three fat-thickness groups (Wood et al., 1989).

	Average P2 backfat thickness (mm)			SE	Significance
	8	12	16		
Pigs	100	100	100		
Water	22.36	17.08	14.06	0.56	*
Lipid	69.25	77.00	81.59	0.726	*
Collagen	4.49	2.98	2.04	0.14	*
C14:0	1.49	1.51	1.49	0.021	NS[2]
C16:0	24.55	25.41	25.87	0.181	*
C16:1	2.78	2.66	2.69	0.065	NS
C18:0	13.15	13.83	13.91	0.215	*
C18:1	40.34	41.83	43.11	0.307	*
C18:2	14.94	12.38	10.65	0.368	*
C18:3	1.11	0.89	0.84	0.043	*

[1]Water, lipid and collagen as a percentage of fresh weight. Fatty acids as percentage of total weight of fatty acids.
[2]Non significant
*P <0.001

Therefore, more studies on prediction of fatty acid composition and correlation among fat depots are needed to understand the factors affecting fatty acid composition of carcass fat. This information will help the industry to monitor and predict IV of pork fat which will ultimately improve the control of pork fat quality.

2.4.6 Functional fatty acids

A group of fatty acids are known as functional lipids because they may exert a role other than energy storage. This group comprises steroid hormones (testosterone, estradiol, cortisol, and aldosterone). The affinity of receptors for their complementary steroid hormone is very high, which explains why blood concentrations of these hormones are very low. Steroid hormones activate changes in gene expression and metabolism of cells. Fatty acid derivatives known as eicosanoids have important hormone-like activities in the body. Eicosanoids are divided into three main categories: prostaglandins, leukotrienes, and thromboxanes, which exert several biological actions important to proper function of immunity, inflammation, and the central nervous system **(**Tapiero et al., 2002). Also, fatty acid derivates known as fat-soluble vitamins (A, D, E, and K) are required for the proper function of several systems in the body such as vision, calcium and phosphate metabolism, anti-oxidation, and blood clot formation (Lehninger et al., 2008).

Some fatty acids can also affect lipid synthesis and change fatty composition of adipose tissue. This function of fatty acids is important because it may have potential to ameliorate poor fat quality of pigs fed diets high in DDGS. Conjugated linoleic acids (**CLA**) are isomers of linoleic acid with double bonds in the carbon chain that are separated by a single bond. Dietary CLA down-regulates the activity of desaturase enzymes which increases saturation of pork fat (Ostrowska et al., 1999; Eggert et al., 2001). Averette Gatlin et al. (2002b) reported a reduction of 6.6% in IV of belly fat for pigs fed diets containing CLA compared to pigs fed diets without CLA. Similarly, Weber

et al. (2006) reported firmer bellies and increased saturation of belly fat from pigs fed diets containing CLA compared to pigs fed diets without CLA.

Plants like cotton and *Sterculia foetida* produce CPFA, such as sterculic and malvalic acids. Cyclopropene fatty acids can also affect the fatty acid composition of adipose tissue. They differ from other fatty acids because they contain an unsaturated ring in the carbon chain which binds to desaturase enzymes, and permanently inhibits their effects (Raju and Reiser, 1973; Quintana et al., 1998). Constant feeding of CPFA blocks newly produced desaturase enzymes in the body and reduces the formation of unsaturated fatty acids in the body (Nixon et al., 1977). *Sterculia foetida* oil contains from 50% to 60% CPFA (Raju and Reiser, 1973; Nixon et al., 1977). Nixon et al. (1977) fed diets to rats containing *Sterculia foetida* oil with total dietary CPFA content from 0.025% to 0.1% and found massive increases in saturation of body fat. Cottonseed oil contains 1% CPFA. Although CPFA content of cottonseed oil is lower than *Sterculia foetida* oil, use of cottonseed oil in swine nutrition has great potential due to its greater availability in commercial ingredient markets. Ellis and Isbell (1926) found much lower IV and firmer fat when supplementing swine diets with 4.1% cottonseed oil instead of corn oil, although CPFA content was not measured in their study. This indicates a potential effect of CPFA on decreasing unsaturation of pork carcass fat.

2.5 MEASUREMENTS OF PORK FAT QUALITY

2.5.1 Relationship between pork fat quality and fatty acid composition

Fatty acid composition of lipids plays a central role in assuring quality of bacon and processed pork (Wood et al., 2003; McClelland et al, 2012). Fats with greater degree

of unsaturation are soft at room temperature and have an oily appearance (Wood et al., 2003). Increasing dietary concentration of DDGS increases IV and decreases belly firmness (Whitney et al., 2006; Leick et al., 2010; McClelland et al., 2012). The number of double bonds present in a fatty acid is related positively to the potential for peroxidation of the fatty acid, and negatively related to fat firmness (Joo et al., 2002; Larsen et al., 2009). Double bonds are susceptible to peroxidative degeneration caused by free radicals. Peroxidized lipids have a rancid taste which is extremely undesirable to consumers. Greater pork fat peroxidation will consequently reduce shelf life and consumer acceptance. Feeding diets with increasing levels of DDGS increased peroxidation of bacon (Leick et al., 2010) and loin muscle (Wang et al., 2012). Simultaneous to lipid peroxidation, free radicals will also oxidize red oxymyoglobin to brown metmyoglobin. This will result in darker colored lean as display time increases (Wood et al., 2003).

Amount and composition of pork fat has great implications in eating quality of pig meat. Acceptability of pork can be improved by increased amounts of intramuscular fat (Fortin et al., 2004), greater proportions of MUFA, and reduced SFA and PUFA (Cameron and Enser, 1991; Wood et al., 2003). However, feeding DDGS decreases MUFA and increases PUFA concentration in pork fat (Xu et al., 2010a; Hilbrands et al., 2013). Consequently, because DDGS is commonly used in swine diets in the U.S., pork processors experience problems with processing, reduced shelf life, and poor consumer acceptance caused by soft pork fat and soft bellies (Joo et al., 2002; Trusell et al., 2011; McClelland et al., 2012).

Feeding high levels of DDGS, and the consequential high unsaturation of dietary fat has led to highly unsaturated carcass fat. Therefore, monitoring pork fat quality has become an important concern for pork processors due to increasing use of DDGS in swine diets. Several analyses are used frequently by industry and academia to evaluate pork fat quality and consumer acceptance of pork products.

2.5.2 Iodine value

The iodine value (**IV**) is an assay that measures the mass of iodine in grams that attaches to the double bonds of the fatty acids in 100 grams of a fat (AOCS, 1998). Fat samples containing unsaturated fatty acids with more double bonds will have greater IV than fats with fewer unsaturated fatty acids. Each lipid source has a unique composition of fatty acids which results in different degrees of unsaturation, and consequently, IV (Table 2.4).

Table 2.4. Iodine value of common lipid sources (adapted from NRC, 2012).

Type of lipid	Iodine Value (IV)
Choice white grease	60
Canola oil	115
Corn oil	125
Cottonseed oil	110
Soybean oil	132

Because of the differences in IV among fat sources, and the impact IV has on pork fat quality, assessment of unsaturation is extremely important to control pork fat quality. Iodine value is the most common method used to describe the degree of unsaturation of fatty acids in pork fat. Numerous methods to perform the IV test are available, including near-infrared spectroscopy, and the well-accepted chemical method, Wijs (AOAC, 1994) which is avoided due to the use of the dangerous reagent, iodine

trichloride (Kyriakidis and Katsiloulis, 2000). However, these methods are expensive and time consuming. The most common method adopted to assess IV is to use prediction equations based on fatty acid composition determined by gas chromatography. This approach is less time-consuming and relatively simple compared to other methods. The AOCS (1998) equation is the most commonly used and is as follows: IV = (0.95 × [ΣC16:1]) + (0.86 × [ΣC18:1]) + (1.732 × [ΣC18:2]) + (2.616 × [ΣC18:3]) + (0.785 × [C20:1]) + (0.723 × [C22:1]), where brackets indicate the weight percentage. However, this equation has been questioned because IV predictions do not always correspond with actual IV from chemical analysis performed using the Wijs method (Kyriakidis and Katsiloulis, 2000).

To obtain more accurate results for IV of pork fat, Meadus et al. (2010) proposed an addition to the existing AOCS (1998) equation based on generalizations of AOCS (1998) equations of Knothe (2002): (1.57 × [C20:2]) + (2.38 × [C20:3]) + (3.19 × [C20:4]) + (4.01 × [C20:5]) + (2.93 × [C22:4]) + (3.68 × [C22:5]) + (4.64 × [C22:6]). The proposed amendment has been used to calculate IV of pork fat (Meadus et al., 2010) and could generate more accurate predictions because it includes more unsaturated long-chain fatty acids than the AOCS (1998) equation which are highly susceptible to peroxidation and would influence highly the pork fat quality. However, the AOCS (1998) equation to predict IV still is the most commonly used equation.

Iodine value of belly fat is around 67 when feeding corn-soybean meal diets (Duttlinger et al., 2012). Feeding corn-soybean meal diets containing 30% DDGS resulted in belly fat IV of 72 (Xu et al., 2010a). However, when feeding diets with high concentrations of DDGS (greater than 45%), dietary content of unsaturated fat increases

which leads to bellies with IV as high as 97 (Leick et al., 2010). To control pork fat quality, some packers have set their upper limit for jowl IV around 73 assuming belly fat IV will be similar (Benz et al., 2010). Jowl is used as a reference depot because of its low economic value compared to belly.

2.5.3 Thiobarbituric acid reactive substances assay

Thiobarbituric acid reactive substances (**TBARS**) are lipid hydroperoxides and aldehydes naturally occurring as a result of lipid peroxidation. Briefly, in this assay, 2-thiobarbituric acid reacts with saturated aldehydes which are oxidation products of unsaturated fatty acids. This reaction generates red pigments that can be quantified with a spectrophotometer (Tarladgis et al., 1960). The TBARS assay is usually reported as malondialdehyde (**MDA**) equivalents. This assay is well-accepted and a commonly used method to assess lipid peroxides because of its simplicity and quickness to obtain results. However, compounds other than oxidation products can react with 2-thiobarbituric acid and generate red pigments (Draper et al., 1993). This interference leads to a high MDA equivalent result and a falsely high measure of peroxidation. In addition, results of TBARS assays may also be biased because aldehydes might have volatilized as a very pungent odor in a process called autoxidation. Autoxidation that occurs before the TBARS analysis will generate a low MDA equivalent result and a falsely low measure of peroxidation. Although lipid peroxidation may occur, consumer acceptance of pork decreases only at MDA equivalents greater than 0.5 mg/kg of meat (Wood et al., 2008).

2.5.4 Pork fat firmness

Fat firmness is important for consumer acceptance of pork products, especially in export markets. Fatty acid composition is related directly to fat firmness. Fats that are more saturated are firmer at room temperature than unsaturated fats. Soft fat has a lower melting point than hard fat and an undesired oily appearance in retail cuts with greater fat content, such as bacon. In addition, a soft and oily fat compromises processing of bacon because slices stick together.

There are several methods to assess firmness of fat and meat cuts. Belly firmness is assessed often with the belly flop test. Briefly, the fresh belly is suspended perpendicularly on a bar and distance between the drooping belly ends is measured. Greater distances are a result of firmer bellies (Leick et al., 2010). In addition, length of the bellies can also be measured to assess belly firmness angle calculated by the isosceles triangle formed when bellies are suspended on a horizontal bar. Greater upper angle of the triangle results from firmer bellies (Schieck et al., 2010; Trusell et al., 2011; McClelland et al., 2012). Bellies contain high proportions of both lean tissue and adipose tissue. Consequently, belly firmness angle cannot be attributed entirely to fat firmness characteristics. Belly firmness angle can give a general assessment of belly firmness, but is not sensitive enough to distinguish small differences in firmness among bellies.

Subjective fat firmness methods are also a quick and inexpensive way to assess fat firmness. The fat sample is compressed between the index finger and thumb and a subjective firmness score is assigned (Dransfield and Jones, 1984; Maw et al., 2003). More accurate and objective ways to assess firmness are available by uniaxial compression using texture analyzer machines, such as the Shimadzu Texture Analyzer or

the Instron Universal Testing Machine. Small cores of fat samples are removed and compressed between lower and upper flat plates of the machine. The compression force response in N or kg is reported (Trusell et al., 2011; Browne et al., 2013b). Similarly, the durometer has been used to assess fat hardness of pork fat (Juarez et al., 2011).

The slip test, or melting point test, is also a method to assess fat firmness quantitatively. With small variations in the method, it has been employed widely to assess fat firmness (Deman et al., 1983; Hrdinka et al., 1996). Briefly, in this method fat samples are melted in an oven and capillary tubes are filled with the melted fats. Filled capillary tubes are frozen until fat is hardened. Afterwards, capillary tubes are placed in a water bath (17ºC) with increasing temperature (2ºC/min) to determine the temperature when fat samples melt and slip in capillary tubes (Deman et al., 1983; Hrdinka et al., 1996).

2.5.5 Bacon quality assessment

Bacon is a valuable retail cut of the pork belly. Modern bellies have fat content from 40% to 50%. Although Larsen et al. (2009) found that fatty acid composition of bacon does not affect cooking characteristics of bacon, increasing dietary DDGS and consequently dietary fat reduced bacon fractures, cooking loss, and distortion scores (Widmer et al., 2008; McClelland et al., 2012). Therefore, fatty acid composition might have a central role in bacon quality. Assessment of bacon quality can be performed with raw or cooked bacon. Bacon shatter scores can be evaluated subjectively by rolling perpendicularly the slices over the index finger and counting the fractures (Rentfrow et al., 2003; McClelland et al., 2012). Raw and cooked bacon slices may have length and weight measured to determine cook loss and cook shrinkage (Rentfrow et al., 2003;

Widmer et al., 2008; Leick et al., 2010; McClelland et al., 2012). Cooked bacon slices may also be scored based on distortion (Rentfrow et al., 2003; Widmer et al., 2008; McClelland et al., 2012). Because bacon has roughly same content of lean and fat tissue, the quality assessments mentioned here cannot be attributed to characteristics of either fat or lean tissue alone. So, bacon quality assessments mentioned here provide a general assessment of bacon quality, which is important for consumer acceptance.

2.6 SUMMARY

Utilizing DDGS in swine diets to support acceptable growth performance of pigs and reduce costs of diets is very popular in the U.S. However, fat content in DDGS increases unsaturation of pork fat which results in reduced shelf life of retail cuts and processing problems due to soft fat. Cottonseed oil or crude glycerol may influence pork fat quality by decreasing unsaturation. Diets containing cottonseed oil contains cyclopropene fatty acids (CPFA) which are known to inhibit desaturase enzymes. Feeding cottonseed oil dramatically increased melting point, firmness, and decreased unsaturation of pork fat in studies conducted over 50 years ago. Feeding CPFA inhibits desaturase enzymes which decreases unsaturation of carcass fat. Feeding crude glycerol to pigs improved belly firmness, and reduced unsaturation of pork fat in previous studies when DDGS was not being fed. Potentially, feeding cottonseed oil or crude glycerol may decrease unsaturation of pork fat caused by feeding DDGS which ultimately may improve pork fat quality.

CHAPTER 3: EFFECTS OF ADDING MINIMALLY REFINED COTTONSEED OIL OR CRUDE GLYCEROL TO DIETS CONTAINING 40% DISTILLERS DRIED GRAINS WITH SOLUBLES (DDGS) ON GROWTH PERFORMANCE, CARCASS CHARACTERISTICS AND PORK FAT QUALITY OF GROWING-FINISHING PIGS

SUMMARY

Feeding diets containing more than 20% DDGS reduces fat firmness in pork carcasses, but supplementing diets with cottonseed oil or crude glycerol may improve pork fat firmness. The objective of this experiment was to assess the effect of feeding minimally-refined cottonseed oil or crude glycerol on growth performance, carcass composition and fat quality of growing-finishing pigs. Mixed sex pigs (n = 216; initial BW = 24 ± 4 kg) were blocked by BW and allotted to 1 of 3 dietary treatments: 1) a basal corn-soybean meal diet with 40% DDGS (CON); 2) CON plus 5% minimally-refined cottonseed oil (COT); or 3) CON plus 8% crude glycerol for the last 6 wk before harvest (GLY). Although diets were not isocaloric, total AA to ME ratios were equal among diets. Carcass composition was estimated using real-time ultrasound 2 d before harvest. Belly fat was sampled and fatty acid composition was analyzed. Gilts (16/diet) closest to the mean BW of each pen were harvested (BW = 115 ± 8 kg) and bellies were retrieved for in-depth analysis of pork fat quality. Overall, ADFI of pigs fed COT (2.30 kg/d) was less ($P < 0.01$) than pigs fed CON and GLY (2.47 and 2.49 kg/d, respectively). Pigs fed COT (0.93 kg/d) had greater ($P < 0.01$) ADG compared with pigs fed CON and GLY (0.88 and 0.87 kg/d, respectively). Greater ($P < 0.01$) G:F was observed for pigs fed COT (0.41) than pigs fed CON and GLY diets (0.36 and 0.35, respectively). Final BW of pigs fed COT (124.3 kg) was greater ($P < 0.01$) than pigs fed CON and GLY (118.9 and 118.6

kg, respectively). Consequently, pigs fed COT had greater ($P < 0.01$) HCW (94.9 kg) compared with CON and GLY (89.9 and 89.2 kg, respectively). No differences were observed for dressing percentage (75.7, 76.3, and 75.3%), fat-free carcass lean percentage (50.5, 49.7, and 50.0%) and belly flop angle (6.21, 8.57 and 6.06°) for CON, COT and GLY, respectively. On d 7 post-mortem, TBARS concentration of belly fat was greater (P=0.01) for pigs fed COT (1.44 ng MDA/mg fat) than for pigs fed GLY (1.04 ng MDA/mg fat; $P = 0.01$), but similar to pigs fed CON (1.25 ng MDA/mg fat). Belly fat of pigs fed COT had greater ($P < 0.01$) IV (78.6) compared with pigs fed CON or GLY (71.2 and 70.6, respectively). Pigs assigned to COT had greater ($P < 0.01$) melting point of belly fat compared with pigs fed CON and GLY (30.4 vs. 26.3 and 25.3°C, respectively). In conclusion, pigs fed COT had improved growth performance which was due to greater energy density of diets, but carcass composition was not affected by dietary treatments. Feeding neither COT nor GLY improved carcass fat quality of pigs fed diets containing 40% DDGS.

Key words: Carcass fat, cottonseed oil, crude glycerol, DDGS, firmness, pigs

INTRODUCTION

Because of increasingly high prices of corn and soybean meal, DDGS has become a very important feed co-product used in swine diets to reduce cost. However, increasing dietary concentration of DDGS increases unsaturation of pork fat as measured by iodine value (**IV**). Increasing IV of pork fat increases oxidation potential of pork products, and decreases belly firmness (Leick et al., 2010; Xu et al., 2010a; McClelland et al., 2012) which increases problems with processing of bellies, and reduces shelf-life and consumer

acceptance of retail cuts. To maintain low pork fat IV, swine nutritionists reduce concentration or withdraw DDGS from finishing diets (Stein and Shurson, 2009; Leick et al., 2010) which limits the potential economic benefits of DDGS.

Cottonseed oil contains cyclopropene fatty acids (**CPFA**) which are known to inhibit desaturase enzymes responsible for synthesizing unsaturated fatty acids in the body (Nixon et al., 1977). To our knowledge, only two studies have been conducted to evaluate the impact of feeding cottonseed oil on pork fat quality. Ellis and Isbell (1926) and Ellis et al. (1931) reported increased melting point and decreased unsaturation of pork fat when pigs were fed crude cottonseed oil. Feeding crude glycerol to pigs improved belly firmness (Schieck et al., 2010), and reduced linoleic acid content and, consequently, unsaturation of pork fat (Mourot et al., 1994; Kijora et al., 1997; Lammers et al., 2008a).

We hypothesized that dietary cottonseed oil or crude glycerol would decrease unsaturation of pork fat. Therefore, the objectives of this study were to determine the effects of adding minimally refined cottonseed oil or crude glycerol to diets containing 40% DDGS on growth performance, carcass characteristics, and fat quality of growing-finishing pigs.

MATERIAL AND METHODS

Animal care and use procedures of this experiment were approved (protocol number 1104B98715) by the Institutional Animal Care and Use Committee of the University of Minnesota.

Animals, housing and diets

This experiment was conducted at the University of Minnesota, West Central Research and Outreach Center in Morris, MN.

Mixed-sex Duroc x (Yorkshire x Landrace) pigs (n = 216; initial BW = 24 ± 4 kg) were housed in the grower-finisher swine unit (9 pigs/pen) with initial BW as the blocking factor. The facility was a totally enclosed, environmentally-controlled finishing barn containing 24 pens. Pens (1.6 × 4.5 m) had totally slotted, concrete floors, 1 stainless steel feeder with 4 feeding spaces, and 1 nipple drinker. Pens were assigned randomly within block to 1 of the 3 dietary treatments in a randomized complete block design resulting in 8 pens per treatment. Single lots of cottonseed oil, crude glycerol and 2 lots of DDGS from a single source were used in this experiment (Table 3.1). Experimental diets (Table 3.2, 3.3, 3.4) consisted of: 1) a basal corn-soybean meal diet with 40% DDGS (**CON**); 2) CON diet plus 5% minimally-refined cottonseed oil added throughout the experiment (**COT**); or 3) CON plus 8% crude glycerol added for the last 6 wk before harvest (**GLY**). Diets were formulated based on standardized ileal digestible (SID) AA and available P, and met or exceed NRC (2012) nutrient recommendations for pigs gaining 715, 877 and 901g of live weight per day in phase 1, phase 2 and phase 3, respectively. Throughout the 108-d growing-finishing period, a three-phase feeding program was adopted to match dietary nutrient concentration to the pigs' stage of growth. The targeted BW for each phase was 25 to 55 kg, 55 to 90 kg and 90 to 120 kg, for phases 1, 2 and 3, respectively. Pigs had *ad libitum* access to treatment diets and water throughout the experiment. Metabolizable energy concentration of diets was not similar across treatments, however, the ratio of SID Lys to ME was kept constant across dietary treatments within phase.

Feed and ingredient analyses

Single lots of crude glycerol, cottonseed oil, and two lots of DDGS from the same source were used in the experiment. Samples were obtained as ingredients were added into the mixer during feed manufacturing. Feed samples were collected from the discharge auger as diets were manufactured. Feed samples for each phase and feed ingredient samples were selected randomly for analysis of nutrient composition. Nutrient composition of feed and feed ingredients was analyzed using the following procedures: crude protein (Kjeldahl method 984.13, AOAC, 2006), crude fat (method 920.39, AOAC, 2006), crude fiber (method 978.10, AOAC, 2006), complete AA profile (method 982.30, AOAC, 2006), moisture (feed and DDGS, method 934.01, AOAC, 2006; cottonseed oil, method Ca 2c-25, AOCS, 2009), ash (method 942.05, AOAC, 2006), total Ca (method 968.08, AOAC, 2006), total P (method 968.08, AOAC, 2006), cyclopropene fatty acids (high performance liquid chromatography; Wood, 1986), gossypol (method Ba 7-58, AOCS, 2009), free fatty acids in crude glycerol (method Ca 5a-40, AOCS, 1998), sodium chloride in glycerol (method 937.09, AOAC, 2006), and methanol (gas chromatography/flame ionization detector, Minnesota Valley Testing Laboratory, New Ulm, MN, USA).

Growth performance

Pigs were weighed individually when dietary treatments were applied and subsequently, every 2 wk during the study, as well as when diet changes occurred. Individual BW of pigs within a pen was averaged to calculate ADG. Feed disappearance

was measured on each day pigs were weighed to calculate ADFI on a pen basis. Gain efficiency (G:F) was calculated as ADG/ADFI on a pen basis.

Table 3.1. Analyzed composition of minimally refined cottonseed oil, crude glycerol and DDGS[1] used in experimental diets (as-fed basis)

Item	Percentage
Cottonseed oil, minimally refined (DM basis)[2]	
Moisture	0.08
Total gossypol	< 0.02
Dihydrosterculic acid	0.265
Malvalic acid	0.469
Sterculic acid	0.283
Total cyclopropene fatty acids	1.017
Glycerol, crude[3]	
Free fatty acid	0.04
Salt, as sodium chloride	6.16
Glycerol	80.4
Methanol	0.16
DDGS[4]	
Moisture	14.98
CP	27.47
Crude fat	10.99
Ash	4.12
Total P	0.74
Total Ca	0.04

[1] Distillers dried grains with solubles.
[2]Cottonseed oil, minimally refined (PBSY; ADM, Decatur, IL).
[3]Glycerol, crude (SoyMor Biodiesel LLC, Albert Lea, MN).
[4]DDGS (DENCO LLC, Morris, MN).

Table 3.2. Composition of experimental diets (as-fed basis)[1]

	Phase I (25 to 55 kg BW)			Phase II (55 to 90 kg BW)			Phase III (90 to 120 kg BW)		
Ingredient, %	CON	COT	GLY	CON	COT	GLY	CON	COT	GLY
Corn, yellow dent	37.02	28.41	37.02	46.03	37.83	37.78	51.56	44.81	43.24
Corn DDGS	40.00	40.00	40.00	40.00	40.00	40.00	40.00	40.00	40.00
Soybean meal, 46.5% CP	20.12	23.76	20.12	11.52	14.75	12.10	6.06	7.81	6.76
Cottonseed oil	-	5.00	-	-	5.00	-	-	5.00	-
Crude glycerol	-	-	-	-	-	8.00	-	-	8.00
Limestone	1.55	1.52	1.55	1.44	1.42	1.43	1.43	1.41	1.40
Salt	0.35	0.35	0.35	0.35	0.35	-	0.35	0.35	-
Monocalcium phosphate	0.28	0.28	0.28	0.04	0.03	0.07	-	-	-
VTM premix[2]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
L-Lys hydrochloride	0.30	0.30	0.30	0.30	0.30	0.30	0.28	0.30	0.28
Tylan 40[3]	0.125	0.125	0.125	0.05	0.05	0.05	0.05	0.05	0.05
Agrado Plus[4]	-	-	-	0.02	0.02	0.02	0.02	0.02	0.02

[1]CON = corn-soybean meal with 40% DDGS basal diet; COT = CON + 5% minimally refined cottonseed oil (PBSY; ADM, Decatur, IL) fed throughout the experiment; GLY = CON fed during the first 8 weeks, CON + 8% crude glycerol (SoyMor Biodiesel LLC, Albert Lea, MN) fed during the last 6 weeks of the experiment.

[2]Vitamin and trace mineral premix supplied the following per kilogram of diet: vitamin A, 8,818 IU; vitamin D_3, 1,653 IU; vitamin E, 33 IU; vitamin K, 3.3 mg; riboflavin, 5.5 mg; niacin, 33 mg; pantothenic acid, 22 mg; vitamin B_{12}, 0.03 mg; iodine as ethylenediamine dihydroiodide, 0.30 mg; selenium as sodium selenite, 0.30 mg; zinc as polysaccharide complex of zinc, 55 mg; iron as polysaccharide complex of iron, 33 mg; manganese as polysaccharide complex of manganese, 5.5 mg; and copper as polysaccharide complex of copper, 3.9 mg.

[3]Tylan 40, tylosin phosphate, 88.2 g/kg (Elanco Animal Health, Indianapolis, IN).

[4]Agrado Plus, ethoxyquin (Novus International Inc., St. Charles, MO).

Table 3.3. Calculated nutrient content of experimental diets (as-fed basis)[1]

	Phase I (25 to 55 kg BW)			Phase II (55 to 90 kg BW)			Phase III (90 to 120 kg BW)		
Item	CON	COT	GLY	CON	COT	GLY	CON	COT	GLY
Dry matter, %	87.58	88.22	87.58	87.45	88.09	87.64	87.35	87.97	87.54
ME, kcal/kg[2]	3,262	3,513	3,262	3,280	3,533	3,258	3,284	3,537	3,262
CP, %	23.73	24.72	23.73	20.48	21.3	20.07	18.37	18.65	18.01
Crude fat %	5.20	10.01	5.20	5.26	10.07	4.99	5.28	10.10	5.02
Total Ca, %	0.73	0.73	0.73	0.62	0.62	0.62	0.59	0.59	0.58
Total P, %	0.63	0.63	0.63	0.55	0.54	0.53	0.51	0.51	0.50
SID Lys, %	1.09	1.17	1.09	0.88	0.95	0.88	0.73	0.78	0.73
Linoleic acid (C18:2), %	2.57	5.03	2.57	2.66	5.12	2.53	2.71	5.19	2.59
Gossypol, %	ND[3]	0.001	ND	ND	0.001	ND	ND	0.001	ND
Total CPFA[4], %	ND	0.051	ND	ND	0.051	ND	ND	0.051	ND
Total Lys:ME, g/Mcal[5]	4.0	3.9	4.0	3.3	3.3	3.3	2.8	2.7	2.8

[1]CON = corn-soybean meal with 40% DDGS basal diet; COT = CON + 5% minimally refined cottonseed oil fed throughout the experiment; GLY = CON fed during the first 8 weeks, CON + 8% crude glycerol fed during the last 6 weeks of the experiment.
[2]Calculated ME from corn, cottonseed oil (NRC, 2012), soybean meal (NSNG, 2010), crude glycerol (Kerr et al., 2009), and DDGS (Shurson and Kerr, 2012).
[3]Not determined.
[4]Total cyclopropene fatty acids = dihydrosterculic acid + malvalic acid + sterculic acid.
[5]Calculated standardized ileal digestible SID Lys: ME. Tabular Lys values for corn (NRC, 2012) and soybean meal (NSNG, 2010) were used.

Table 3.4. Analyzed nutrient content of experimental diets (as-fed basis)[1]

	Phase I (25 to 55 kg)			Phase II (55 to 90 kg)			Phase III (90 to 120 kg)		
	CON	COT	GLY	CON	COT	GLY	CON	COT	GLY
Dry matter, %	87.56	87.95	87.56	86.58	87.47	84.90	86.91	87.92	85.23
ME, kcal/kg[2]	3,262	3,513	3,262	3,280	3,533	3,258	3,284	3,537	3,262
CP, %	22.97	24.87	22.97	20.65	21.26	19.82	18.24	18.04	19.01
Crude fat %	4.12	8.76	4.12	4.53	9.24	6.65	4.83	10.37	6.65
Crude fiber %	4.48	4.99	4.48	4.75	4.74	4.41	4.45	4.11	4.05
Total Ca, %	0.78	0.81	0.78	0.55	0.44	0.84	0.45	0.54	0.50
Total P, %	0.52	0.55	0.52	0.46	0.48	0.45	0.46	0.46	0.41
Total Lys, %	1.34	1.40	1.34	1.07	1.18	1.19	0.97	0.97	1.12
Total Met + Cys, %	0.80	0.82	0.80	0.69	0.71	0.84	0.68	0.72	0.67
Total Thr, %	0.88	0.93	0.88	0.75	0.81	0.84	0.68	0.69	0.77
Total Trp, %	0.23	0.25	0.23	0.21	0.20	0.20	0.18	0.17	0.19
Total Lys:ME, g/Mcal	4.1	4.0	4.1	3.3	3.3	3.6	2.9	2.7	3.4

[1]CON = corn-soybean meal with 40% DDGS basal diet; COT = CON + 5% minimally refined cottonseed oil fed throughout the experiment; GLY = CON fed during the first 8 weeks, CON + 8% crude glycerol fed during the last 6 weeks of the experiment.
[2]Calculated ME from corn, cottonseed oil (NRC, 2012), soybean meal (NSNG, 2010), crude glycerol (Kerr et al., 2009), and DDGS (Shurson and Kerr, 2012).

Carcass characteristics

Loin muscle (**LM**) area and backfat thickness at the 10th rib were measured using real-time ultrasound 2 d before harvest. A trained technician using an ALOKA 500V (Corometrics Medical Systems, Wallingford, CT) machine fitted with a 12.5 cm long, 3.5 MHz linear array transducer recorded all measurements. The transducer was placed at the 10th rib perpendicular to the dorsal midline for scanning. The computer software package, Quality Evaluation and Prediction (Iowa State University, Ames, IA), was used to measure linear depth of backfat and LM area.

Pigs were harvested at Natural Food Holdings, Sioux City, IA (n = 165) and at the Andrew Boss Laboratory of Meat Science (**ABLMS**), University of Minnesota, St. Paul, MN (n = 47). Final BW was measured 2 d before harvest for pigs harvested in St. Paul, and 3 d before harvest for pigs harvested in Iowa. Hot carcass weight (HCW) was determined at each harvest location. Fat-free lean percent was calculated according to the following equation (NPPC, 2000): Fat-free lean % = [2.620 + (0.401 × HCW, kg) - (3.358 × ultrasound 10th rib backfat depth, cm) + (0.306 × ultrasound 10th rib LM area, cm^2) + (0.456 × sex) (barrow = 1, gilt = 2)] / HCW, kg × 100. Carcass dressing percentage was calculated as: dressing, % = (HCW, kg / final BW, kg) × 100.

Pork fat firmness and color

Two gilts (n = 47) closest to the mean final BW of each pen were selected for in-depth evaluation of fat quality at the ABLMS. Bellies from the right side of each carcass were evaluated for belly width, weight, and length. Bellies were delimited into 2 rows (dorsal and ventral) and 4 columns (cranial to caudal), and belly thickness, without accounting for skin, was determined by inserting a steel probe. Bellies were subjected to

the belly flop angle firmness test (Rentfrow et al., 2003). To conduct this test, bellies were placed perpendicularly on a smoke stick with the skin side down. Distance between the two drooping ends of the belly was determined. Belly length and distance between the ends of the bellies were used to calculate the firmness score. Belly flop angle was determined by the upper angle of the isosceles triangle formed by placing the belly on the smoke stick. The belly flop angle firmness score was calculated as: $\cos^{-1}$ {[0.5 × (length^2) – (distance^2)] / [0.5 × (length^2)]}.

From each of the 47 carcasses, fat samples were retrieved from the tip of the jowl, backfat at the 10th rib, and belly from the caudal end at the ventral midline, frozen, and stored at – 20º C until further analyses. As needed for each analysis, fat samples were allowed to thaw at room temperature. To assess subjective fat firmness of jowl, backfat, and belly, a trained panelist blinded to treatments ranked (1 = extremely soft to 5 = extremely firm) each fresh fat sample by compressing it between the thumb and the index finger. The belly fat samples were also evaluated by 8 trained panelists to assess fat color according to the NPPC (2000) Japanese fat color scale (1 = white to 4 = yellow).

To assess objective fat firmness, cores (1 cm^3) of jowl, backfat and belly fat samples were compressed (skin side down) to 80% of the core thickness between upper and lower flat plates of a Shimadzu texture analyzer (model EZ-SX, Shimadzu, Corp., Jiangsu, China). For each compression analysis, the compression/tension load cell was set to 500 N, and the cross head speed was set at 100 mm/min. Belly fat samples were used to determine melting point temperature. Fat samples were melted in an oven and glass capillary tubes with internal diameter of 1.0 mm were filled with fat by placing the tube in contact with the melted fats. Filled capillary tubes were placed in ice to freeze the fat.

After fat samples were hardened, capillary tubes were removed from ice and placed in a water bath. Cold water was poured in the water bath with the capillary tubes and a thermometer. Water temperature was increased slowly to determine the temperature when fat samples melted and slipped in the capillary tubes.

Pork fat quality

Fresh belly fat samples were frozen on dry ice and shipped to be analyzed at the Agricultural Utilization Research Institute in Marshall, MN. Fat samples were stored at 4º C from day 0 to day 7 to simulate a retail display. Lipid peroxidation of belly fat was assessed by thiobarbituric acid reactive substances (**TBARS**) using a distillation method according to Tarladgis et al. (1960). Briefly, fat tissue was blended with distilled water and HCl in Kjeldahl flasks, heated, and distillated. Five ml of the distillate was mixed with 5 ml of thiobarbituric acid reagent and heated in a water bath. After cooling, optical density of the samples was determined against a blank sample (thiobarbituric acid reagent mixed with distilled water) with a spectrophotometer at a wavelength of 538 μm. Optical density measurements were used to calculate concentration of TBARS. Results were expressed in micrograms of malondialdehyde per gram of belly fat tissue.

Fatty acid composition of jowl, backfat, and belly fat was determined according to the procedures of Browne et al. (2013a) at the University of Arkansas, Fayetteville, AR. Briefly, fat samples were placed into flasks maintained under vacuum and freeze-dried. Fat samples were subjected to transesterification by addition of methanolic KOH. Samples were centrifuged to separate phases and to obtain fatty acid methyl esters. Gas chromatography was used to separate methyl esters. Iodine value was calculated according

to the following equation (AOCS, 1998): (0.95 × [ΣC16:1]) + (0.86 × [ΣC18:1]) + (1.732 × [ΣC18:2]) + (2.616 × [ΣC18:3]) + (0.785 × [C20:1]) + (0.723 × [C22:1]) where brackets indicate the weighted percentage of each fatty acid.

Bacon quality

Bellies were processed commercially into bacon. Briefly, bellies were injected with standard cure solution to target 110% of the green weight and cooked in a smokehouse. After cooking, bellies were transported to ABLMS and weighed to determine cooked weight. Cook yield was calculated as: (cook weight/green weight) x 100. Bellies were trimmed, peeled, and sliced. Two slices were selected from 5 equal longitudinal sections of the belly for bacon quality evaluation. Length and weight of each bacon slice was recorded. Uncooked bacon slices received shatter scores (1 = no visual cracks to 6 = severe cracks) from 4 longitudinal locations by rolling the slices over the index finger, as described by Rentfrow et al. (2003). Subsequently, bacon slices from each section were cooked on a flat griddle at 157° C for 3 min. Cooked bacon slices were weighed and length was measured to determine cooking loss and cooking shrink, respectively. Cooked bacon slices received distortion scores (1 = flat slab to 5 = completely curled slab) as described by Rentfrow et al. (2003).

Statistical analysis

Data were analyzed using PROC MIXED (SAS Inst. Inc., Cary, NC) in a randomized complete block design. The statistical model included dietary treatment as the fixed effect, and block and pen as random effects. Repeated measures in time were used to analyze growth performance and TBARS data. When repeated measures were used, the

statistical model included dietary treatments and time as fixed effects, and block and pen as random effects. Belly firmness was analyzed with belly thickness as a covariate. Pen was considered the experimental unit. All reported means are least squares means. Means were separated using the PDIFF option of SAS with the Tukey-Kramer adjustment for multiple comparisons. The SE for each mean calculated by PROC MIXED was averaged to calculate the pooled SE for each variable.

RESULTS AND DISCUSSION

For reasons unrelated to dietary treatments, 4 pigs died during the experimental period. During the feeding phase, 2 pigs assigned to GLY and 1 pig assigned to CON died with signs of *Streptococcus suis* infection. During transportation of pigs to be harvested in St. Paul, 1 pig assigned to COT died suddenly.

Experimental diets

Metabolizable energy concentration across dietary treatments was different, but the ratio of total Lys to ME was constant across dietary treatments which met the formulation targets (Table 3.3, 3.4). The experimental design dictated the dietary concentration of DDGS across dietary treatments of 40% to simulate maximum dietary levels fed on commercial swine farms. The main objective of the experiment was to determine if the simple addition of crude glycerol or minimally refined cottonseed oil could improve fat firmness of pigs fed diets typical of commercial conditions. Consequently, it was not possible to formulate diets with similar metabolizable energy concentration across treatments. The consistent ratio of total Lys to ME across dietary treatments partially compensated for the inherent differences in ME concentration of diets.

Growth Performance

Growth performance results for each phase are presented in Table 3.5. Overall, ADFI of pigs fed COT (2.30 kg/d) was less ($P < 0.01$) than pigs fed CON and GLY (2.47 and 2.49 kg/d, respectively). There was a significant diet × phase interaction for ADFI, indicating that dietary treatment affected feed intake differently over time. The lack of dietary effect on ADFI in phase 1 compared to phases 2 and 3 is probably because grower pigs still have limited feed intake compared to finisher pigs which could have reduced the effect of dietary treatments on early phases. Over the entire experiment, pigs fed COT (0.93 kg/d) had greater ($P < 0.01$) ADG compared with pigs fed CON and GLY (0.88 and 0.87 kg/d, respectively). Similarly, greater ($P < 0.01$) G:F was observed for pigs fed COT (0.41) than for pigs fed CON and GLY diets (0.36 and 0.35, respectively). Consequently, final BW of pigs fed COT was greater ($P < 0.01$) than pigs fed CON or GLY (Table 3.6).

We are not aware of any recent studies that investigated the effects of feeding cottonseed oil on pig performance and carcass quality. Pigs fed COT had greater ADG and G:F than did pigs fed CON or GLY diets. This improved growth performance of pigs fed COT can be explained by the greater energy density of the COT diets compared with CON or GLY. Diets with elevated energy density decreased feed intake and improved ADG and G:F of pigs (Smith et al., 1999; Apple et al., 2004; Cho and Kim, 2012). Although cottonseed products contain gossypol which may decrease growth performance, gossypol levels in COT diets were below levels known to cause depressed growth performance (Hale and Lyman, 1957). These results indicate that cottonseed oil with low gossypol concentration is a useful feed ingredient in swine diets to support rapid and efficient growth performance.

Table 3.5. Effect of dietary treatments on growth performance

	Dietary treatments[1]												
	Phase I			Phase II			Phase III				P-value		
Item	CON	COT	GLY	CON	COT	GLY	CON	COT	GLY	Pooled SE	Diet[2]	Phase	Diet x Phase
No. of pens	8	8	8	8	8	8	8	8	8				
No. of pigs	71	72	70	71	72	70	71	72	70				
ADFI, kg	1.78	1.71	1.81	2.81^{a}	2.56^{b}	2.76^{ab}	3.05^{a}	2.81^{b}	3.14^{a}	0.05	<0.01	<0.01	0.01
ADG, kg	0.86	0.91	0.85	0.97	1.02	0.95	0.87^{xy}	0.92^{x}	0.85^{y}	0.02	<0.01	<0.01	0.99
G:F	0.48^{a}	0.53^{b}	0.47^{a}	0.34^{a}	0.40^{b}	0.35^{a}	0.28^{a}	0.33^{b}	0.27^{a}	0.01	<0.01	<0.01	0.55

[1]CON = corn-soybean meal with 40% DDGS basal diet; COT = CON + 5% minimally refined cottonseed oil fed throughout the experiment; GLY = CON fed during the first 8 weeks, CON + 8% crude glycerol fed during the last 6 weeks of the experiment.
[2]Diet= dietary treatment.
[a,b]Means within a row and phase with different superscripts differ ($P < 0.05$).
[x,y]Means within a row and phase with different superscripts differ ($P < 0.10$).

In our study, glycerol feeding had no effect on growth performance. However, there are conflicting reports in the scientific literature concerning effects of glycerol on swine growth performance. Adding 10% pure glycerol to finishing pig diets can reduce ADG and efficiency of gain (Della Casa et al., 2009). However, in a study conducted by Schieck et al. (2010), dietary crude glycerol inclusion of 8% throughout the growing-finishing period increased ADG and ADFI, but there was no effect on performance when pigs were fed crude glycerol-containing diets only for 8 wk before harvest. Other authors have reported no effect of glycerol feeding on growth performance of growing-finishing pigs (Lammers et al., 2008a; Duttlinger et al., 2012; Lee et al., 2013; Lammers et al., 2015). The variable response of pigs to dietary crude glycerol may be explained by the variable concentration of glycerol and other components in the crude glycerol used in these experiments (Kerr et al., 2009). Inclusion level of glycerol in diets may also influence growth performance. Della Casa et al. (2009) reported similar gain efficiency for pigs supplemented with 5% pure glycerol compared to pigs not supplemented with pure glycerol. However, increasing pure glycerol supplementation to 10% decreased gain efficiency for grow-finish pigs compared with pigs not supplemented with pure glycerol (Della Casa et al., 2009).

Most of these authors did not report the chemical composition of crude glycerol fed in their experiment. So, one cannot evaluate precisely the effects of dietary glycerol on growth performance. Other than glycerol itself, other factors in crude glycerol could affect growth performance, such as low content of glycerol, high content of salt and methanol, or high dietary inclusion level (> 10%) which may decrease feed flow (Kerr et al., 2011).

Carcass characteristics

Pigs were harvested in St. Paul after 108 days on feed or in Iowa after 109 days on the experimental treatments. Each dietary treatment was represented equally on each harvest day. Therefore, because of the improved growth performance, greater final BW and similar dressing percentage, COT pigs had greater ($P < 0.01$) HCW (Table 3.6) than pigs fed CON or GLY. However, no differences were observed among dietary treatments for carcass dressing percentage, fat-free lean percentage, backfat depth and LM area. Neither COT nor GLY diets improved belly firmness as measured by the belly flop angle (Table 3.6).

Table 3.6. Effect of dietary treatments on carcass characteristics and belly firmness

	Dietary treatments[1]				
Item	CON	COT	GLY	Pooled SE	*P*-value
No. of pens	8	8	8		
No. of pigs	71	71	70		
Initial body weight, kg	23.62	23.58	23.61	1.51	0.96
Final body weight, kg	118.9[a]	124.3[b]	118.6[a]	2.35	<0.01
HCW, kg	89.9[a]	94.9[b]	89.2[a]	1.81	<0.01
Dressing percent	75.70	76.30	75.30	0.43	0.28
Fat-free lean, %	50.50	49.70	50.00	0.44	0.47
10th-rib backfat depth, cm	2.11	2.26	2.22	0.09	0.28
LM area, cm^2	42.90	44.18	42.51	1.02	0.29
No. of pigs	16	16	16		
Belly thickness, cm	2.75[x]	3.18[y]	3.01[xy]	0.13	0.07
Belly firmness, degrees[2]	6.21	8.57	6.06	0.95	0.16

[1]CON = corn-soybean meal with 40% DDGS basal diet; COT = CON + 5% minimally refined cottonseed oil fed throughout the experiment; GLY = CON fed during the first 8 weeks, CON + 8% crude glycerol fed during the last 6 weeks of the experiment.
[2]Belly firmness angle adjusted for belly thickness.
[a,b]Means within a row with different superscripts differ ($P < 0.05$).
[x,y]Means within a row with different superscripts differ ($P < 0.10$).

The carcass characteristic results for COT-fed pigs are in agreement with others who fed diets with increased energy density. When pigs were harvested at a standardized time endpoint, feeding diets with different energy density but adjusted to a similar AA:energy ratio, resulted in similar carcass characteristics (Smith et al., 1999). Others studied the impact of increasing dietary energy density on carcass characteristics when pigs were harvested at a standardized time endpoint and found greater HCW and increased fatness in carcasses compared with pigs fed low energy density diets (Le Dividich et al., 1987; Apple et al., 2004). The lack of response of carcass traits to glycerol feeding is in agreement with results from other studies. Increasing crude glycerol concentration in growing-finishing pig diets up to 10% did not affect carcass characteristics (Lammers et al., 2008a; Schieck et al., 2010; Duttlinger et al., 2012; Lee et al., 2013; Lammers et al., 2015).

High inclusion level (more than 20%) of DDGS in growing-finishing pig diets decreases belly firmness (Whitney et al., 2006; Xu et al., 2010a; Cromwell et al., 2011). Decreased belly firmness causes difficulties in processing bellies into sliced bacon and reduces consumer acceptance of bacon (Trusell et al., 2011). Belly firmness is related highly to fatty acid composition of belly fat. Firmness of bellies from pigs fed cottonseed oil has not been reported before the present study; but, Ellis and Isbell (1926) found a dramatic increase in backfat firmness for pigs fed diets containing 4.1% cottonseed oil. However, results from our study did not mimic this result. Few studies have assessed belly firmness of pigs fed crude glycerol. Supplementation of 5% crude glycerol in diets containing 30% DDGS did not improve belly firmness of pigs (Lee et al., 2013). However, Schieck et al. (2010) found improved belly firmness in pigs fed corn-soybean

meal basal diets supplemented with 8% crude glycerol for 8 wk before harvest, but not when pigs received crude glycerol-supplemented diets throughout the growing-finishing period. The improved belly firmness of pigs fed crude glycerol observed by Schieck et al. (2010) may be related to the lean content of the belly. Feeding glycerol to pigs can increase water holding capacity of lean tissue which could increase tension within muscle cells and increase firmness of the lean tissue in bellies (Mourot et al., 1994; Della Casa et al., 2009). Consumers' demands in the past decades have led to genetic improvements to increase lean in bellies to 50% and increase PUFA concentration in bellies which consequently have decreased belly firmness (Wright et al., 2005; Trusell et al., 2011). The lack of response of belly firmness to COT or GLY feeding may be because bellies were quite soft due to high unsaturation of belly fat and high lean content in bellies which is softer than fat tissue.

Table 3.7. Effect of dietary treatments on firmness, subjective color scores, and melting point of pork fat

	Dietary treatments[1]				
Item	CON	COT	GLY	Pooled SE	*P*-value
No. of pigs	16	15	16		
Subjective firmness scores[2]					
Belly fat	4.67	4.22	4.45	0.17	0.22
Jowl fat	3.93	4.15	4.12	0.21	0.74
Backfat	3.25[ab]	2.64[a]	3.38[b]	0.19	0.05
Compression, N[3]					
Belly fat	0.0110[x]	0.0108[xy]	0.0106[y]	0.0001	0.08
Jowl fat	0.0107	0.0110	0.0107	0.0001	0.31
Back fat	0.0109	0.0109	0.0109	0.0002	0.99
Belly fat subjective color scores[4]	2.48	2.48	2.42	0.13	0.92
Belly fat melting point, ^{0}C	26.3[a]	30.4[b]	25.3[a]	0.86	<0.01

[1]CON = corn-soybean meal with 40% DDGS basal diet; COT = CON + 5% minimally refined cottonseed oil fed throughout the experiment; GLY = CON fed during the first 8 weeks, CON + 8% crude glycerol fed during the last 6 wk of the experiment.
[2]Subjective firmness scores from 1 = very soft to 5 = very firm.
[3]Newtons of force
[4]Color scores from 1 = pinkish white to 4 = dark red.
[a,b]Means within a row with different superscripts differ ($P < 0.05$).
[x,y]Means within a row with different superscripts differ ($P < 0.10$).

Pork fat firmness, color, and melting point

Subjective firmness scores of belly, jowl, and backfat were not different when comparing COT or GLY with CON (Table 3.7). The force required to compress belly fat cores to 80% of their original height tended to be less ($P = 0.07$) for GLY than for CON indicating somewhat softer fat. No differences were observed in compression force of jowl fat and backfat for pigs fed CON, COT and GLY. No differences were observed in subjective color scores of belly fat for pigs fed CON, COT, and GLY. Pigs assigned to COT had greater ($P < 0.01$) melting point of belly fat compared with pigs fed CON and GLY.

Fat quality can be assessed by firmness and melting point tests of pork fat. Soft pork fat has a higher degree of unsaturation and lower melting point compared to firmer fat. Soft fat impairs meat processing and consumer acceptance of retail products (Wood et al., 2003; Trusell et al., 2011). Neither COT nor GLY diets decreased fat softness caused by the high DDGS concentration in treatment diets. However, in the work of Ellis and Isbell (1926), feeding diets containing cottonseed oil improved backfat firmness. Glycerol feeding to swine can decrease the degree of unsaturation which should ultimately increase fat firmness and melting point (Mourot et al., 1994; Kijora et al., 1997; Lammers et al., 2008a). However, our study indicated no changes in firmness or melting point. This is in agreement with other authors who reported no changes in fatty acid composition of pigs fed crude glycerol which would result in no changes in fat firmness (Duttlinger et al., 2012; Lee et al., 2013). The lack of response to cottonseed oil and crude glycerol feeding on subjective color scores of belly fat indicates that both feed ingredients can deliver acceptable pork fat color to consumers. There is no previous study

that reported the effect of cottonseed oil on color of pork fat. However, our results for GLY feeding are in agreement with others who found no effect of crude glycerol feeding on pork fat color (Schieck et al., 2010; Lee et al., 2013).

For a long time, cottonseed meal has been known to increase firmness, melting point, and saturation of pork fat (Hare, 1913) due to the presence of CPFA. Feeding CPFA can inhibit desaturase enzyme in pork fat and consequently the synthesis of unsaturated fatty acids (Nixon et al., 1977; Quintana et al., 1998). Feeding COT diets increased melting point of belly fat compared to CON and GLY, indicating a slightly higher degree of saturation in these fat samples. This is in agreement with Ellis and Isbell (1926) who found greater backfat melting point for pigs fed diets containing cottonseed oil. However, melting point was the only indicator measured in this study that suggested cottonseed oil improved firmness of pork fat.

Fatty acid composition of pork fat

Belly fat of pigs assigned to COT had greater ($P < 0.01$) concentration of SFA and PUFA and lower concentration of MUFA compared with pigs fed CON or GLY (Table 3.8). These shifts in fatty acid concentration of belly fat yielded increased ($P < 0.01$) IV of belly fat for pigs fed COT. Similar changes in fatty acid composition of jowl fat and back fat were observed (Tables 3.9 and 3.10).

Table 3.8. Effect of dietary treatments on fatty acid composition (as a weight percentage) of belly fat

	Dietary treatments[1]				
Item	CON	COT	GLY	Pooled SE	*P*-value
No. of pigs	71	71	70		
Fatty acid, wt %					
C10:0	0.07[a]	0.05[b]	0.06[ab]	0.01	0.04
C12:0	0.08	0.08	0.08	0.003	0.83
C14:0	1.46	1.44	1.46	0.03	0.86
C15:0	0.06	0.06	0.06	0.001	0.17
C16:0	23.55[a]	22.31[b]	23.74[a]	0.17	<0.01
C16:1*c*	2.04[a]	0.83[b]	1.98[a]	0.06	<0.01
C17:0	0.35[a]	0.32[b]	0.34[ab]	0.01	0.05
C18:0	11.44[a]	15.90[b]	11.67[a]	0.20	<0.01
C18:1*t*	0.27[a]	0.37[b]	0.27[a]	0.01	<0.01
C18:1*c*9	33.00[a]	20.78[b]	33.51[a]	0.45	<0.01
C18:1*c*11	2.82[a]	1.46[b]	2.72[a]	0.06	<0.01
C18:2*n*-6	20.83[a]	32.63[b]	20.17[a]	0.63	<0.01
C18:2*c*9*t*11	0.11[a]	0.06[b]	0.11[a]	0.004	<0.01
C18:3*n*-6	0.05	0.05	0.05	0.002	0.91
C18:3*n*-3	0.70[a]	0.64[b]	0.69[a]	0.01	<0.01
C20:0	0.18[a]	0.20[b]	0.18[a]	0.003	<0.01
C20:1*c*11	0.60[a]	0.40[b]	0.60[a]	0.01	<0.01
C20:2	0.77[a]	1.12[b]	0.74[a]	0.02	<0.01
C20:3*n*-6	0.11	0.11	0.11	0.003	0.60
C20:3*n*-3	0.08[a]	0.07[b]	0.08[a]	0.001	<0.01
C20:4*n*-6	0.30	0.29	0.28	0.01	0.08
C22:5*n*-3	0.07[a]	0.05[b]	0.06[a]	0.002	<0.01
SFA	37.49[a]	40.71[b]	37.77[a]	0.41	<0.01
MUFA	38.81[a]	23.65[b]	39.08[a]	0.54	<0.01
PUFA	22.68[a]	34.89[b]	22.14[a]	0.66	<0.01
IV[2]	71.15[a]	78.57[b]	70.57[a]	0.72	<0.01

[1]CON = corn-soybean meal with 40% DDGS basal diet; COT = CON + 5% minimally refined cottonseed oil fed throughout the experiment; GLY = CON fed during the first 8 weeks, CON + 8% crude glycerol fed during the last 6 weeks of the experiment.
[2]IV = iodine value.
[a,b]Means within a row with different superscripts differ ($P < 0.05$).

Table 3.9. Effect of dietary treatments on fatty acid composition (as a weight percentage) of jowl fat

	Dietary treatments[1]				
Item	CON	COT	GLY	Pooled SE	*P*-value
No. of pigs	16	15	16		
Fatty acid, wt %					
C10:0	0.05[a]	0.03[b]	0.05[a]	0.003	<0.01
C12:0	0.07	0.06	0.06	0.002	0.37
C14:0	1.31	1.24	1.28	0.03	0.39
C15:0	0.07	0.06	0.06	0.003	0.11
C16:0	21.21[a]	19.74[b]	20.93[a]	0.23	<0.01
C16:1*c*	2.15[a]	1.10[b]	2.26[a]	0.11	<0.01
C17:0	0.38	0.34	0.34	0.02	0.14
C18:0	9.73[a]	14.94[b]	9.07[a]	0.34	<0.01
C18:1*t*	0.30[a]	0.41[b]	0.28[a]	0.01	<0.01
C18:1*c*9	35.00[a]	22.40[b]	37.16[a]	0.68	<0.01
C18:1*c*11	2.84[a]	1.63[b]	3.09[a]	0.09	<0.01
C18:2*n*-6	22.08[a]	33.61[b]	20.65[a]	0.76	<0.01
C18:2*c*9*t*11	0.12[a]	0.06[b]	0.13[a]	0.01	<0.01
C18:3*n*-6	0.05	0.05	0.05	0.002	0.51
C18:3*n*-3	0.78[a]	0.71[b]	0.77[a]	0.02	0.01
C20:0	0.17[a]	0.20[b]	0.16[a]	0.01	<0.01
C20:1*c*11	0.70[a]	0.46[b]	0.75[a]	0.02	<0.01
C20:2	0.98[a]	1.42[b]	0.98[a]	0.02	<0.01
C20:3*n*-6	0.14	0.14	0.14	0.01	0.50
C20:3*n*-3	0.11[a]	0.09[b]	0.12[a]	0.002	<0.01
C20:4*n*-6	0.33	0.33	0.31	0.01	0.36
C22:5*n*-3	0.08[a]	0.06[b]	0.08[a]	0.002	<0.01
SFA	32.98[a]	36.60[b]	31.97[a]	0.48	<0.01
MUFA	41.00[a]	25.99[b]	43.54[a]	0.82	<0.01
PUFA	24.74[a]	36.50[b]	23.28[a]	0.79	<0.01
IV[2]	76.09[a]	82.75[b]	75.80[a]	0.85	<0.01

[1]CON = corn-soybean meal with 40% DDGS basal diet; COT = CON + 5% minimally refined cottonseed oil fed throughout the experiment; GLY = CON fed during the first 8 weeks, CON + 8% crude glycerol fed during the last 6 weeks of the experiment.
[2]IV = iodine value.
[a,b]Means within a row with different superscripts differ ($P < 0.05$).

Table 3.10. Effect of dietary treatments on fatty acid composition (as a weight percentage) of backfat

	Dietary treatments[1]				
Item	CON	COT	GLY	Pooled SE	*P*-value
No. of pigs	16	15	16		
Fatty acid, wt %					
C10:0	0.05[a]	0.03[b]	0.05[a]	0.003	<0.01
C12:0	0.07[a]	0.05[b]	0.06[ab]	0.002	0.03
C14:0	1.24[x]	1.10[y]	1.21[x]	0.04	0.07
C15:0	0.06	0.06	0.06	0.003	0.45
C16:0	21.77[a]	19.44[b]	21.51[a]	0.30	<0.01
C16:1*c*	1.36[a]	0.62[b]	1.51[a]	0.08	<0.01
C17:0	0.39	0.33	0.38	0.02	0.13
C18:0	11.70[a]	14.87[b]	10.94[a]	0.41	<0.01
C18:1*t*	0.28[a]	0.42[b]	0.29[a]	0.01	<0.01
C18:1*c*9	32.10[a]	20.14[b]	33.53[a]	0.66	<0.01
C18:1*c*11	2.22[a]	1.30[b]	2.39[a]	0.07	<0.01
C18:2*n*-6	24.27[a]	37.43[b]	23.54[a]	0.83	<0.01
C18:2*c*9*t*11	0.11[a]	0.06[b]	0.12[a]	0.01	<0.01
C18:3*n*-6	0.05	0.05	0.05	0.004	0.92
C18:3*n*-3	0.76[ab]	0.69[a]	0.79[b]	0.02	0.02
C20:0	0.21	0.20	0.19	0.01	0.32
C20:1*c*11	0.65[a]	0.40[b]	0.66[a]	0.02	<0.01
C20:2	0.98[a]	1.40[b]	0.96[a]	0.03	<0.01
C20:3*n*-6	0.13	0.13	0.13	0.01	0.79
C20:3*n*-3	0.10[a]	0.08[b]	0.10[a]	0.002	<0.01
C20:4*n*-6	0.30	0.31	0.29	0.01	0.54
C22:5*n*-3	0.07[a]	0.05[b]	0.06[a]	0.002	<0.01
SFA	35.49	36.08	34.41	0.63	0.21
MUFA	36.62[a]	22.90[b]	38.40[a]	0.76	<0.01
PUFA	26.78[a]	40.21[b]	26.07[a]	0.87	<0.01
IV[2]	75.92[a]	86.60[b]	76.33[a]	1.08	<0.01

[1]CON = corn-soybean meal with 40% DDGS basal diet; COT = CON + 5% minimally refined cottonseed oil fed throughout the experiment; GLY = CON fed during the first 8 weeks, CON + 8% crude glycerol fed during the last 6 weeks of the experiment.
[2]IV = iodine value.
[a,b]Means within a row with different superscripts differ ($P < 0.05$).
[x,y]Means within a row with different superscripts differ ($P < 0.10$).

Corn oil present in DDGS has a high concentration of PUFA and linoleic acid that are deposited in pork fat. In addition, dietary fat also blocks *de novo* fatty acid synthesis (Chilliard, 1993). Consequently, unsaturation of pork fat, as measured by IV, will increase as a result of feeding high concentration of DDGS, which ultimately decreases firmness of pork fat and bellies (Xu et al., 2010a). Cottonseed oil has the potential to decrease unsaturation of pork fat caused by feeding high dietary levels of DDGS. Cottonseed oil contains CPFA which are known to inhibit the desaturase enzyme responsible for creating unsaturated fatty acids in the body (Raju and Reiser, 1973). Supplementing diets with cottonseed oil can lower IV of pork fat (Ellis and Isbell, 1926). Although belly fat of pigs fed COT diets had a greater melting point, indicating decreased unsaturation, surprisingly, fatty acid composition of pigs fed COT diets were actually more unsaturated than pigs fed CON and GLY. One would expect pork fat with a greater melting point to have a lower degree of unsaturation. Despite the higher PUFA concentration, pigs fed COT did have a greater proportion of SFA, and less MUFA compared to pigs fed CON or GLY which suggests that CPFA may have reduced body synthesis of unsaturated fatty acids. Zoeller and Wood (1984) added CPFA to an *in vitro* cell culture and reported massive increase in concentration of SFA, and decrease in concentration of MUFA, but no changes in concentration of PUFA, which suggests that CPFA may be more effective in inhibiting formation of MUFA than of PUFA.

Two factors may explain the inability of cottonseed oil to increase fat firmness. First, raw cottonseed oil is not available commercially; but, a minimally-processed source was available and used to assure the highest concentration of CPFA possible. Cyclopropene fatty acids are removed during commercial refinement of raw cottonseed

oil. Nixon et al. (1977) fed diets containing 0.25% CPFA to rats and found significant increases in saturation of body fat. However, in the present study, total CPFA content in COT diets was only 0.05% which may not have been high enough to elicit results similar to Nixon et al. (1977). Second, studies that reported effective decreases in unsaturation of carcass fat with CPFA feeding used diets with similar crude fat content and fatty acid composition across treatments which was not repeated in the present study (Ellis and Isbell, 1926; Raju and Reiser, 1973; Nixon et al., 1977). In the present study, crude fat content of control diets ranged from about 4.1 to 4.8% largely due to the inclusion of 40% DDGS. However, addition of 5% minimally – refined cottonseed oil increased crude fat content of COT diets to 8.75 to 10%. The objective of the dietary treatments of our study was to mimic commercial diets with high inclusion level of DDGS. Supplementing oil to CON and GLY diets to achieve similar crude fat content of COT diets would misrepresent commercial diets and limit the application of results commercially. Although cottonseed oil has CPFA, cottonseed oil also contains high levels of unsaturated fatty acids similar to corn oil supplied by DDGS (cottonseed oil IV = 110 vs. corn oil IV = 125; NRC, 2012). The experimental design dictated that COT diets would have roughly twice as much oil content compared to CON and GLY to evaluate if the simple addition of cottonseed oil could mitigate pork fat issues caused by high DDGS feeding. Feeding diets containing high inclusion of lipids with high IV will have significant impact on fatty acid composition of carcass fat. In addition, COT diets had supplemental extracted lipids (cottonseed oil) which are more digestible than intact lipids (grains and DDGS) and can have greater impact on fatty acid composition of carcass fat (Davis et al., 2015). Furthermore, supplementation of DDGS reduces digestibility of SFA

and increases digestibility of unsaturated fatty acids from extracted lipids in the diets (cottonseed oil) which will cause an even larger impact on the fatty acid composition of pork fat (Davis et al., 2015). Consequently, COT fed pigs would have even greater lipid deposition compared to GLY and CON fed pigs. Therefore, the greater PUFA intake of pigs assigned to COT diets may have overpowered the effects of CPFA, resulting in increased unsaturation of carcass fat.

Effects of feeding crude glycerol on fatty acid composition of pork fat have been inconsistent across studies. Some studies have reported decreased unsaturation of carcass fat from pigs fed diets containing glycerol. Feeding diets containing 5% and 10% glycerol increased oleic (C18:1) and decreased linoleic (C18:2) acids in pork fat, which decreased the unsaturation index of backfat (Mourot et al., 1994; Kijora et al., 1997). Lammers et al. (2008a) found decreased linoleic acid in LM of pigs fed corn-soybean meal diets containing 10% crude glycerol compared to pigs not supplemented with crude glycerol. Similarly, Della Casa et al. (2009) found greater content of oleic (C18:1) and monounsaturated fatty acids (MUFA) in subcutaneous fat of hams from growing-finishing pigs fed diets containing 10% crude glycerol compared to pigs fed diets without crude glycerol. Lammers et al. (2015) reported lower concentrations of PUFA and greater concentrations of MUFA for pigs fed diets containing 10% crude glycerol compared to pigs not supplemented with crude glycerol. However, in our study, glycerol feeding in diets with an inherently elevated concentration of unsaturated fatty acids did not reduce unsaturation of pork fat. Our results are in agreement with other authors who showed that feeding diets containing 20% and 30% DDGS with crude glycerol up to 5% had no effect on fatty acid composition of pork fat (Duttlinger et al., 2012; Lee et al., 2013).

The disparity in response to dietary glycerol among studies is difficult to explain. Lammers et al. (2008a) speculated that the inclusion of crude glycerol in diets reduces the inclusion of corn (and consequently corn oil), which would ultimately reduce intake of unsaturated fatty acids and reduce unsaturation of pork fat. In addition, chemical composition of crude glycerol usually is not reported in studies, so the residual fatty acid content of crude glycerol used in these studies is not known. Feeding crude glycerol with high residual concentration of fatty acids could influence the composition of pork fat. Kerr et al. (2009) reported the residual fatty acid concentration of crude glycerol can vary widely among sources. However, Schieck et al. (2010) showed that even feeding crude glycerol with low residual concentration of fatty acids (0.1%) is capable of increasing belly firmness, which could indicate a higher degree of saturation. However, fatty acid composition of pork fat was not analyzed in the work of Schieck et al. (2010). Therefore, the inconsistent response to crude glycerol feeding on fatty acid composition may be due to dietary changes in crude fat content and IVP, or variation in composition of crude glycerol.

Belly fat oxidation and bacon characteristics

No difference was observed in belly fat oxidation on d 0 post-mortem, as measured by TBARS. On d 7 post-mortem, TBARS concentration of belly fat was greater ($P < 0.01$; Table 3.11) for pigs fed COT compared with pigs fed GLY, but both GLY and COT were similar to CON. No significant differences were observed for bacon cooking shrinkage, bacon cooking loss, bacon shatter scores or bacon distortion scores across dietary treatments (Table 3.12).

On the day of harvest, TBARS concentration of belly fat from all treatments were below 0.5 ng MDA/mg of fat which is considered the threshold that can be detected by consumers (Wood et al., 2008). However, on d 7 post-mortem, TBARS concentration of belly fat from all treatments was greater than the threshold that can be detected by consumers, indicating neither COT nor GLY diets were able to reduce oxidation of pork fat. Nonetheless, there was a significant treatment × day interaction, indicating that treatment diets affected pork fat differently over display time. Pork fat oxidation is related directly to the fatty acid composition. Greater degree of unsaturation in pork fat makes it more prone to peroxidation (Wood et al., 2003). Our study showed that feeding COT or GLY diets to pigs did not decrease pork fat unsaturation. Consequently, no reduction in peroxidation would be expected.

Table 3.11. Effect of dietary treatments on thiobarbituric acid-reactive substance (TBARS) concentration in belly fat tissue

	Dietary treatments[1]									
	Day 0[2]			Day 7				*P*-value		
	CON	COT	GLY	CON	COT	GLY	Pooled SE	Diet[3]	Day	Diet x Day
No. of pigs	16	15	16	16	15	16				
TBARS, ng/mg	0.09	0.09	0.10	1.25[ab]	1.44[a]	1.04[b]	0.06	0.01	<0.01	0.01

[1]CON = corn-soybean meal with 40% DDGS basal diet; COT = CON + 5% minimally refined cottonseed oil fed throughout the experiment; GLY = CON fed during the first 8 weeks, CON + 8% crude glycerol fed during the last 6 weeks of the experiment.

[2]Belly fat was analyzed in samples collected at harvest (day 0) and after 7 days of storage at 4º C.

[3]Diet=dietary treatment effect

[a,b]Means within a row with different superscripts differ ($P < 0.05$).

Table 3.12. Effect of dietary treatments on bacon characteristics

	Dietary treatments[1]				
Item	CON	COT	GLY	Pooled SE	*P*-value
No. of pigs	16	15	16		
Cook shrink, %	13.48^{xy}	11.01^{x}	14.89^{y}	1.22	0.07
Cook loss, %	37.83^{xy}	36.09^{x}	39.72^{y}	1.45	0.08
Shatter scores[2]	2.22	2.22	2.22	0.11	0.99
Distortion scores[3]	1.43	1.59	1.49	0.11	0.57

[1]CON = corn-soybean meal with 40% DDGS basal diet; COT = CON + 5% minimally refined cottonseed oil fed throughout the experiment; GLY = CON fed during the first 8 weeks, CON + 8% crude glycerol fed during the last 6 weeks of the experiment.
[2]Shatter scores from 1 = no visual cracks to 6 = severe cracks.
[3]Distortion scores from 1 = flat slice to 5 = completely curled slice.
[x,y]Means within a row with different superscripts differ ($P < 0.10$).

The increase in dietary DDGS, and the consequent increase in belly PUFA concentration increases potential for rancidity and causes poor bacon slicing and handling while processing at room temperature (Wood et al., 2003; Leick et al., 2010; McClelland et al., 2012). In our study, feeding COT or GLY diets resulted in similar bacon characteristics to feeding CON diets. The lack of differences in bacon quality may be because fatty acid composition of bellies was rather unsaturated. Treatment diets were very high in unsaturated fat content which resulted in high unsaturation of belly fat and similar bacon quality among dietary treatments.

In conclusion, feeding GLY diets had no impact on growth performance of pigs. However, COT diets improved growth performance probably because of the greater energy density of the COT diets compared with CON or GLY. Neither COT nor GLY supplementation of diets increased belly firmness or reduced IV of carcass fat compared with pigs fed diets containing 40% DDGS. Dietary GLY had no impact on fatty acid

composition of pork fat. Pigs assigned to COT actually had increased IV of carcass fat, probably due to the added unsaturated fatty acids contributed by cottonseed oil which could not be overcome by the CPFA content of the cottonseed oil. Neither COT nor GLY supplementation of diets reduced oxidation of pork fat based on the TBARS measurements. Therefore, supplementation of swine diets with high-oil DDGS concentration and cottonseed oil or crude glycerol had no potential to reduce DDGS-induced soft carcass fat.

CHAPTER 4: EFFECT OF GROWTH RATE AND FAT DEPOTS ON FATTY ACID COMPOSITION AND PREDICTION OF FATTY ACID COMPOSITION

SUMMARY

Fatty acid composition of pork is critical in assuring quality of pork fat and pork products and has important implications to pork processors and retailers. However, how factors like growth rate, gender, and fat depot contribute to fatty acid composition of pork fat is unclear. Mixed sex pigs (n = 216; initial BW = 24 ± 4 kg) were blocked by BW and allotted to one of three dietary treatments. At slaughter, belly fat was sampled from all pigs. Additional fat samples were collected from jowl, back, and belly from 47 gilts (about 16/treatment). Fatty acid composition of fat samples was analyzed and iodine value (IV) was calculated according to AOCS (1998). Two methods were used to compare IV among pigs with different growth rates. The pen-based grouping method was based on pigs within a pen and considered fatty acid composition of the two fastest and the two slowest growing barrows or gilts (based on ADG) within each pen. The barn-based grouping method was based on the entire barn where slow-growing pigs were defined as pigs with ADG ≥ 1 SD below the mean ADG of all pigs in the barn regardless of pen. Pigs exhibiting average growth rate were defined as pigs that expressed ADG up to 0.25 SD above or below the mean ADG of all pigs in the barn. Fast growing pigs were defined as pigs with ADG ≥ 1 SD above the mean ADG of all pigs in the barn. In the pen-based method, growth rate had no effect on IV of bellies collected from barrows ($P > 0.7$; SE= 0.86; 71 vs. 70.3 for fast and slow-growing pigs, respectively) or gilts ($P > 0.8$; SE=1.13; 76.9 vs. 77.2 for fast and slow-growing pigs, respectively). Results from the barn-based method indicated no differences ($P > 0.3$) in IV of belly fat (72.4, 73.6, and

74.1, SE = 0.8) for fast, average, and slow-growing pigs, respectively. Gilts exhibited belly fat IV greater than barrows (76.8 vs. 70.0; SE = 0.65; $P < 0.01$). Iodine values of jowl and belly (r = 0.60), jowl and backfat (r = 0.84), and belly and backfat (r = 0.63) were correlated ($P < 0.01$). Although all correlations were highly significant, IV of jowl only explained about 40% of the variation in IV of belly fat. Using data from all pigs in the experiment (n = 213), the best fit equation to predict belly fat IV was: belly fat IV = 75.95 + (3.324 × concentration of dietary C18:2) – (6.327 × backfat depth, cm) (R^2 = 0.61). In conclusion, growth rate had no important effects on fatty acid composition of carcass fat. Jowl and backfat had low correlations with fatty acid concentrations of belly fat making them poor predictors of belly fat quality.

Key words: fatty acid composition, growth rate, iodine value, pigs

INTRODUCTION

Due to the increasing prices of traditional feed ingredients, DDGS has become a cost effective feed ingredient used widely in swine diets in the United States. High amounts of PUFA from DDGS are deposited in pork fat which increases unsaturation and decreases shelf life of pork fat, and overall quality of bacon and other primal cuts (Leick et al., 2010; Xu et al., 2010a; McClelland et al., 2012). Therefore, controlling unsaturation of pork fat has important implications for pork processors and retailers. In addition to DDGS use in swine diets, other factors can influence pork fat composition which may influence negatively pork fat quality. For instance, pork processors and producers have perceived from anecdotal observations greater unsaturation of fat from slow growing pigs compared to fast growing pigs. However, this perception has not been

evaluated under controlled conditions. Knowing the influence of growth rate on fatty acid composition of pork fat is important because nutritionists may be able to adopt nutritional strategies to improve pork fat quality of pigs exhibiting growth rates that would decrease pork fat quality.

Another factor influencing management of pork fat quality is the location on the carcass where fat is sampled. Jowl fat is used commonly to predict iodine value (IV) of carcass fat because jowl is a low value portion of the carcass. Assessing jowl fat IV is especially important for estimating IV of belly fat because bellies are a primal cut of high value, and damage from sampling would reduce value. However, IV of jowl fat may not reflect fatty acid composition of belly. Poor correlation between IV of jowl and belly has been reported (Leick et al., 2010; Wiegand et al., 2011). However, Estrada (2013) reported correlations of 0.80 to 0.90 among belly fat, jowl fat and backfat IV. Adipose tissue (**AT**) is distributed widely in the body with differing fatty acid composition across depots (Wood et al., 1989; Leick et al., 2010). In addition to using IV of jowl fat to estimate IV of carcass fat, prediction equations have been developed to estimate carcass fat IV. Meadus et al. (2010) proposed an addition to the existing AOCS (1998) equation to obtain more accurate results for calculation of IV of pork fat. However, the AOCS (1998) equation to predict IV still is the most commonly used equation. Although IV product (**IVP**) is frequently used to predict IV of carcass fat from dietary parameters, studies have been inconsistent as to whether IVP is an effective predictor of IV of carcass fat (Madsen et al., 1992; Benz et al., 2011). So, understanding the relationships between fatty acid composition of jowl and other higher value cuts would have great utility for pork processors.

Many factors such as dietary concentration and composition of fat, gender, fat depot, and growth rate of pigs can contribute to fatty acid composition of pork fat which causes difficulty in predicting and controlling pork fat composition (Wood et al., 1989; Zhang et al., 2007; Ibrahim, 2010). A more fundamental understanding of these factors is necessary to determine how composition of pork fat can be managed to optimize quality of pork products. Therefore, the objective of this study was to clarify the effect of growth rate on fatty acid composition, evaluate the effectiveness of using jowl fat as a location for predicting belly fat and backfat IV, as well as use different variables to develop prediction equations for belly fat IV. Understanding of the effects of growth rate and depot location on pork fat IV will support pork producers and processors in managing pork fat quality more effectively.

MATERIAL AND METHODS

Animal care and use procedures for this experiment were approved (protocol number 1104B98715) by the Institutional Animal Care and Use Committee of the University of Minnesota.

Animals, housing and diets

This experiment was conducted at the University of Minnesota's West Central Research and Outreach Center in Morris, MN. Descriptions of animals, housing, diets and analysis of feed and ingredients can be found in Chapter 3 of this thesis. Briefly, mixed-sex pigs (n = 216; initial wt.=24 ± 4 kg) were housed in the grower-finisher swine unit (9 pigs/pen) and blocked based on initial BW. The facility was an environmentally-controlled finishing barn containing 24 pens. Pens (1.6 × 4.5 m) had 1 stainless steel

feeder with 4 feeding spaces, and 1 nipple drinker. Pens were assigned randomly within block to 1 of the 3 diets resulting in 8 pens per diet. Experimental diets (Tables 3.2, 3.3, 3.4) consisted of: 1) a basal corn-soybean meal diet with 40% DDGS (dietary fat = 4.8%; CON); 2) basal diet plus 5% minimally-refined cottonseed oil added throughout the experiment (dietary fat = 10.3%; COT); or 3) basal diet plus 8% crude glycerol added for the last 6 wk before harvest (dietary fat = 6.6%; GLY). Diets were formulated to meet or exceed NRC (2012) nutrient recommendations for pigs. Pigs had *ad libitum* access to diets and water throughout the experiment. Metabolizable energy concentration of diets was different across treatment diets; however, the ratio of total Lys to ME was kept constant across dietary treatments (Table 3.4).

Growth performance and carcass traits

Pigs were weighed individually when dietary treatments were applied, every 2 wk during the study, and when diet changes occurred. Feed disappearance was measured on each day pigs were weighed to calculate ADFI on a pen basis. Gain efficiency (G:F) was calculated as ADG/ADFI on a pen basis. Pigs were harvested at Natural Food Holdings, Sioux City, IA (n = 165) and at the Andrew Boss Laboratory of Meat Science, University of Minnesota, St. Paul, MN (n = 47). Final BW was measured 2 d before harvest for pigs harvested in St. Paul, and 3 d before harvest for pigs harvested in Iowa. Hot carcass weight (HCW) was determined at each harvest location.

Loin muscle area and backfat thickness at the 10^{th} rib was measured using real-time ultrasound 2 d before harvest. A trained technician using an ALOKA 500V (Corometrics Medical Systems, Wallingford, CT) machine fitted with a 12.5 cm long, 3.5

MHz linear array transducer recorded all measurements. The transducer was placed at the 10^{th} rib perpendicular to the dorsal midline for scanning. The computer software package, Quality Evaluation and Prediction (Iowa State University, Ames, IA), was used to measure linear depth of backfat and LM area. Fat free lean percentage was calculated according to the following equation (NPPC, 2000): Fat-free lean % = [2.620 + (0.401 × HCW, kg) - (3.358 × ultrasound 10th rib backfat depth, cm) + (0.306 × ultrasound 10th rib LM area, cm^2) + (0.456 × sex)] / HCW, kg × 100 where sex equals barrow (1) or gilt (2). Dressing percentage was calculated as: dressing, % = (HCW, kg / final BW, kg) × 100.

Fatty acid composition of pork fat

All layers of adipose tissue from the tip of the jowl, backfat at the 10th rib, and from belly on the caudal end at the ventral midline were collected from gilts (n = 47) harvested in St. Paul. Adipose tissue from the caudal end of bellies at the ventral midline were collected from all pigs (n = 165) harvested in Sioux City, IA. Fatty acid composition of all adipose tissue samples was determined according to the procedures of Browne et al. (2013a) at the University of Arkansas, Fayetteville. Briefly, adipose tissue samples were placed into flasks maintained under vacuum and freeze-dried. Samples were subjected to transesterification by addition of methanolic KOH. Samples were centrifuged to separate phases and to obtain fatty acid methyl esters. Gas chromatography was used to separate methyl esters. Iodine value was calculated according to the following equation (AOCS, 1998): IV= (0.95 × [ΣC16:1]) + (0.86 × [ΣC18:1]) + (1.732 × [ΣC18:2]) + (2.616 × [ΣC18:3]) + (0.785 × [C20:1]) + (0.723 × [C22:1]) where brackets indicate the weight percentage of each fatty acid. The activity index of Δ^9 desaturase

enzyme (Smith et al., 2002) was calculated as: Activity index= (C16:1 + C18:1 *cis*-9 + C18:1 *cis*-11)/(C14:0 + C16:0 + C18:0 + C16:1 + C18:1 *cis*-9 + C18:1 *cis*-11).

Statistical analysis

Two grouping methods were used to compare fatty acid composition among pigs with different growth rates. In the pen based method, fatty acid composition of bellies from the two fastest compared with the two slowest growing (based on ADG) barrows (4 pigs/pen) or gilts (4 pigs/pen) within each pen were compared (pen method). In a second method, fatty acid composition of 3 groups was compared (barn based method). The slow-growing pigs (n=37) were defined as pigs with ADG equal to or greater than 1 SD below the mean ADG of the entire barn independent of pen groups. Pigs considered "average" (n=46) expressed ADG that was up to 0.25 SD above or below the mean ADG of the entire barn. The fastest growing pigs were defined as pigs with ADG greater than or equal to 1 SD above the mean ADG (n=35) of the entire barn.

Data were analyzed using PROC MIXED (SAS Inst. Inc., Cary, NC) in a randomized complete block design. The statistical model included growth rate as the fixed effect, and block and pens as random effects. Gender was considered as a covariate for analyses comparing growth rate. Pigs were considered the experimental unit. Means were separated with the PDIFF option of SAS with Tukey-Kramer adjustment. The SE calculated by PROC MIXED was averaged to calculate the pooled SE for each trait.

Data to predict belly fat IV were analyzed using a stepwise regression with PROC STEPWISE of SAS with a forward selection approach. The predictor variables were: initial BW, final BW, percentage of BW gain during the growing-finishing period, ADG

of pigs, ADG on a pen basis, average ADFI on a pen basis, gain to feed on a pen basis, dietary C18:2 concentration, intake of C18:2 on a pen basis, dressing percentage, backfat depth, loin muscle area, HCW, fat-free carcass lean percentage, and fatty acid composition of jowl and backfat. The predictor variables were used in the regression with variable significance set at $P < 0.15$.

RESULTS AND DISCUSSION

For reasons unrelated to dietary treatments, 4 pigs died during the experimental period. During the feeding phase, 1 gilt and 1 barrow assigned to GLY and 1 gilt assigned to CON died with signs of *Streptococcus suis* infection. During transportation of pigs to be harvested in St. Paul, 1 gilt assigned to COT died suddenly.

Experimental diets

Metabolizable energy concentration across dietary treatments was different, but the ratio of total Lys to ME was constant across dietary treatments and met the formulation targets (Chapter 3, Tables 3.3 and 3.4). The experimental design dictated that all diets contain 40% DDGS to simulate a maximum DDGS inclusion rate of diets fed on commercial swine farms. The addition of cottonseed oil made it impossible to formulate diets with similar metabolizable energy concentration across treatments. So, the consistent ratio of total Lys to ME across dietary treatments partially compensated for the inherent differences in ME concentration of these diets. Analyzed nutrient content of manufactured diets showed lower EE concentration compared to formulation target. This discrepancy is probably because diets were formulated based on published nutrient composition of ingredients, and not analyzed nutrient compositions of ingredients used.

Grouping according to growth rate

Both pen and barn based methods adopted to categorize different growth rates effectively divided pigs into distinct groups with significantly different growth performance (Tables 4.1, 4.2, 4.3). Growth rate distribution of pigs assigned to group categories on the barn-based grouping method is presented at Figure 4.1. Regardless of grouping method used for growth rate analysis, initial BW was similar for slow, average and fast growing pigs. Pigs in groups exhibiting fast growth rates had greater ADG compared to pigs exhibiting average or slow growth rates. Because pigs were slaughtered at about the same time, final BW and HCW were affected largely by growth rate.

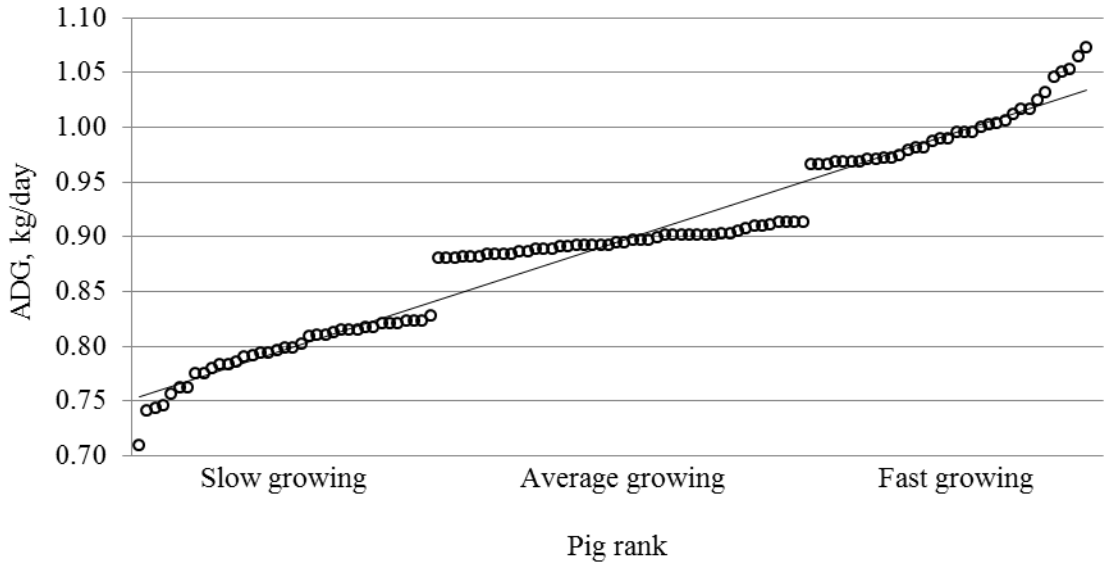

Figure 4.1. Growth rate distribution of pigs assigned to growth rate group categories (barn-based method)

Table 4.1. Growth rate[1] of pigs grouped according to pens (pen grouping method)

Trait	Fast	Slow	SE	*P*-value
Gilts				
No.	46	46		
Initial BW, kg	23.4	23.4	1.39	0.72
Final BW, kg	121.5	115.0	2.62	<0.0001
ADG, kg	0.91	0.85	0.01	<0.0001
HCW, kg	92.7	88.2	1.86	<0.0001
Barrows				
No.	48	48		
Initial BW, kg	24.0	23.7	1.57	0.11
Final BW, kg	128.0	116.6	1.95	<0.0001
ADG, kg	0.96	0.86	0.009	<0.0001
HCW, kg	95.2	88.6	1.88	<0.0001

[1]Growth rate categorization was 2 fastest versus 2 slowest growing (based on ADG) gilts (4 pigs/pen) or barrows (4 pigs/pen) within each pen.

Table 4.2. Main effects of growth rate[1] of pigs grouped across the entire barn (barn grouping method)

Trait	Fast	Average	Slow	SE	*P*-grw[2]	Barrow	Gilt	SE	*P*-sex[3]
No. of pigs	35	46	37			61	57		
Initial BW, kg	23.5	23.5	23.6	1.51	0.97	23.7	23.3	1.50	0.06
Final BW, kg	130.5[a]	120.4[b]	109.2[c]	1.61	<0.01	120.5	119.6	1.58	0.12
ADG, kg	0.99[a]	0.90[b]	0.79[c]	<0.01	<0.01	0.89	0.89	<0.01	0.46
HCW, kg	96.0[a]	92.5[b]	83.2[c]	1.44	<0.01	90.7	90.4	1.31	0.73

[1]The slow-growing pigs were defined as pigs with ADG equal or greater than 1 SD below the mean ADG of the entire barn independent of pen groups. Average growth rate pigs expressed ADG that was up to 0.25 SD above or below the mean ADG of the entire barn. The fastest growing pigs were defined as pigs with ADG greater than or equal to 1 SD above the mean ADG of the entire barn.

[2]*P*-value for growth rate effect.

[3]*P*-value for sex effect.

[a,b,c]Means within a row with different superscripts within main effect differ ($P < 0.05$).

Table 4.3. Interactive effects of growth rate and gender for pigs grouped across the entire barn (barn grouping method)

	Fast[1]		Average[1]		Slow[1]			*P*-sex*growth
Trait	Barrows	Gilts	Barrows	Gilts	Barrows	Gilts	SE	rate[2]
No. of pigs	28	7	20	26	13	24		
Initial BW, kg	23.8[a]	23.2	23.9[a]	23.2[b]	23.5	23.7	1.52	0.08
Final BW, kg	132.2[a]	128.9[b]	120.6[c]	120.1[c]	108.6[d]	109.7[d]	1.68	0.02
ADG, kg	1.00[a]	0.98[b]	0.89[c]	0.90[c]	0.79[d]	0.80[d]	0.006	0.03
HCW, kg	97.7[a]	94.2[ab]	92.9[b]	92.2[b]	81.6[c]	84.7[c]	1.69	0.06

[1]The fastest growing pigs were defined as pigs with ADG greater than or equal to 1 SD above the mean ADG of the entire barn. Average growth rate pigs expressed ADG that was up to 0.25 SD above or below the mean ADG of the entire barn. The slow-growing pigs were defined as pigs with ADG equal or greater than 1 SD below the mean ADG of the entire barn independent of pen groups.

[2]*P*-value for interaction between sex and growth rate.

[a,b,c,d]Means within a row with different superscripts differ ($P < 0.05$).

Effect of growth rate on fatty acid composition

In the pen-based grouping method, growth rate of gilts and barrows had no effect on concentrations of SFA, MUFA and PUFA or Δ^9 desaturase enzyme activity index for belly fat (Tables 4.4 and 4.5). Although the concentration of a few fatty acids (C20:0, C20:1, C20:2, C22:5) were affected ($P < 0.05$) by growth rate in gilts, the concentration of these same fatty acids were too small to cause any significant changes as measured by SFA, MUFA, PUFA and IV of belly fat in both gilts and barrows. The Δ^9 desaturase enzyme activity calculated from fatty acid composition has been used to estimate the activity of Δ^9 desaturase enzyme (Smith et al., 2002). Higher index numbers mean higher activity of Δ^9 desaturase enzyme, and consequently higher production of unsaturated fatty acids. In this study, the Δ^9 desaturase enzyme activity was not affected by growth rate which suggests that pigs with different growth rates have similar synthesis of unsaturated fatty acids. Therefore, the hypothesis that slow growing pigs have higher synthesis of unsaturated fatty acids was not confirmed in this analysis.

Table 4.4. Effect of growth rate[1] of gilts within pens on fatty acid composition (as a weight percentage) and enzyme activity of belly fat (pen grouping method)

Trait	Fast	Slow	SE	*P*-value
No. of gilts	46	46		
Fatty acids[2], wt %:				
C14:0	1.34	1.37	0.03	0.32
C16:0	21.82	22.33	0.26	0.12
C16:1*c*	1.66	1.68	0.12	0.88
C18:0	12.11	11.78	0.43	0.43
C18:1*t*	0.31	0.32	0.01	0.60
C18:1*c*9	29.74	29.16	1.25	0.46
C18:1*c*11	2.35	2.36	0.13	0.91
C18:2*c*6	25.86	26.26	1.34	0.67
C18:2*c*9*t*11	0.09	0.10	0.01	0.17
C18:3*n*6	0.05	0.05	<0.01	0.60
C18:3*n*3	0.72	0.75	0.01	0.23
C20:0	0.19	0.18	<0.01	0.02
C20:1	0.57	0.53	0.02	0.09
C20:2	1.00	0.94	0.04	0.09
C20:3*n*6	0.13	0.13	<0.01	0.50
C20:3*n*3	0.09	0.09	<0.01	0.33
C20:4*n*6	0.31	0.33	0.01	0.02
C22:5	0.07	0.07	<0.01	0.07
SFA	35.98	36.22	0.45	0.68
MUFA	34.63	34.05	1.49	0.54
PUFA	28.34	28.73	1.38	0.69
Iodine value	76.90	77.15	1.13	0.81
Desaturase index[3]	0.48	0.48	0.01	0.58

[1]Growth rate categorization was 2 fastest versus 2 slowest growing (based on ADG) gilts (4 pigs/pen) within each pen.

[2]Fatty acid as weight percentage of belly fat.

[3]Δ^9 desaturase enzyme activity (Smith et al., 2002): (C16:1 + C18:1 *cis*-9 + C18:1 *cis*-11)/(C14:0 + C16:0 + C18:0 + C16:1 + C18:1 *cis*-9 + C18:1 *cis*-11).

Table 4.5. Effect of growth rate[1] of barrows within pens on fatty acid composition and enzyme activity of belly fat (pen grouping method)

Trait	Fast	Slow	SE	*P*-value
No. of barrows	48	48		
Fatty acids[2], wt %:				
C14:0	1.53	1.59	0.03	0.05
C16:0	24.15	24.46	0.19	0.15
C16:1*c*	1.54	1.56	0.14	0.75
C18:0	14.08	14.14	0.58	0.82
C18:1*t*	0.29	0.30	0.01	0.18
C18:1*c*9	28.76	28.63	1.43	0.83
C18:1*c*11	2.29	2.29	0.16	0.99
C18:2*c*6	23.24	22.92	1.32	0.62
C18:2*c*9*t*11	0.08	0.09	0.01	0.56
C18:3*n*6	0.04	0.04	<0.01	0.66
C18:3*n*3	0.62	0.62	0.01	0.98
C20:0	0.19	0.19	<0.01	0.59
C20:1	0.52	0.52	0.02	0.98
C20:2	0.80	0.78	0.04	0.18
C20:3*n*6	0.10	0.10	<0.01	0.64
C20:3*n*3	0.07	0.07	<0.01	0.38
C20:4*n*6	0.26	0.25	0.01	0.32
C22:5	0.06	0.06	<0.01	0.65
SFA	40.47	40.92	0.54	0.32
MUFA	33.40	33.31	1.73	0.90
PUFA	25.27	24.93	1.35	0.60
Iodine value	70.96	70.32	0.86	0.39
Desaturase index[3]	0.44	0.44	0.02	0.60

[1]Growth rate categorization was 2 fastest versus 2 slowest growing (based on ADG) barrows (4 pigs/pen) within each pen.

[2]Fatty acid as weight percentage of belly fat.

[3]Δ^9 desaturase enzyme activity (Smith et al., 2002): (C16:1 + C18:1 *cis*-9 + C18:1 *cis*-11)/(C14:0 + C16:0 + C18:0 + C16:1 + C18:1 *cis*-9 + C18:1 *cis*-11).

Despite small shifts in SFA and MUFA with the barn based grouping method, growth rate had no effect on concentration of PUFA and overall fatty acid composition as measured by IV (Table 4.6). Although small shifts in fatty acid concentrations among growth rate groups were observed, bellies of slow growing pigs had similar fatty acid composition to bellies of average and fast growing pigs. Slow growing pigs tended to have lower concentrations of SFA than fast growing pigs and lower concentrations of MUFA than pigs with average growth rate. The estimated Δ^9 desaturase enzyme activity index for fast growing pigs was lower than for pigs expressing average growth rates, but both were similar ($P > 0.05$) to slow growing pigs. This means that slow growing pigs have similar synthesis of unsaturated fatty acids compared with pigs that displayed average or fast growth. The small effect of growth rate in SFA and MUFA (although not large enough to affect IV) obtained with the barn based grouping method may be because fast growing pigs grew 10% faster than average growing pigs which grew 14% faster than slow growing pigs. The complete lack of a growth rate effect on fatty acid composition obtained in the pen based grouping method may be because the difference in growth rate was only about 8% between fast and slow growing pigs.

Iodine value is the primary measurement of fatty acid composition monitored by pork processors. Although small changes were observed in SFA and MUFA, the lack of difference in IV of belly fat between slow growing and fast growing pigs in the present study does not confirm the perception of pork processors. As commonly practiced by pork producers, the heaviest pigs in pens are removed and marketed before slower growing pigs which is known as “topping” pens. Pork processors have perceived that pigs that are marketed later because of slower growth rates have greater unsaturation in

carcass fat than faster growing pigs. This perception was not confirmed in this study. However, one must interpret this finding with caution. According to Stalder (2015), the average growing-finishing pig on commercial farms gained 0.79 to 0.84 kg/day. This growth rate is very similar to that of the "slow" growing pigs in our study (0.79 kg/day). So, "slow" growing pigs in our study did not represent well slow growing pigs under commercial conditions. Our inability to find differences in fat quality between slow and fast growing pigs as described by pork processors may have been because our population of pigs did not adequately reflect the conditions and range of growth rates observed in commercial settings. Furthermore, it is important to note that all pigs in this study were slaughtered at about the same time which meant that slow growing pigs were lighter at harvest than faster growing pigs. Commonly on commercial farms, pigs remain in pens until reaching the target market BW. Therefore, the small effects of growth rate on SFA and MUFA in the barn based grouping method, although not sufficient to affect IV, could be partially attributed to differences in final body weight in addition to effects of growth rate, because slaughter weight has been shown to influence fatty acid composition of carcass fat (Garcia-Macias et al., 1996; Lo Fiego et al., 2005; Apple et al., 2009).

Table 4.6. Effect of growth rate[1] of pigs from the entire barn on fatty acid composition of belly fat (barn grouping method)

	Fast	Average	Slow	SE	*P*-grw[2]	Barrow	Gilt	SE	*P*-sex[3]	*P*-S*G[4]
Fatty acids[5], wt.%:										
C16	23.76[a]	22.95[b]	23.02[b]	0.22	0.04	24.29	22.20	0.18	<0.01	0.14
C16:1*c*	1.57[ab]	1.73[a]	1.54[b]	0.07	0.10	1.60	1.63	0.06	0.67	0.25
C18	13.69[a]	12.59[b]	12.99[ab]	0.32	0.07	14.02	12.17	0.26	<0.01	0.63
C18:1*c*9	28.58[x]	30.26[y]	29.01[x]	0.59	0.09	29.42	29.14	0.47	0.68	0.23
C18:2*c*6	24.06	23.74	24.87	0.69	0.42	22.33	26.12	0.55	<0.0001	0.29
C18:3*n*3	0.64	0.67	0.67	0.01	0.20	0.60	0.72	0.01	<0.0001	0.09
C20	0.19	0.19	0.18	<0.01	0.32	0.19	0.19	<0.01	0.20	0.64
C20:1	0.53[a]	0.58[b]	0.52[a]	0.02	<0.01	0.54	0.54	0.01	0.81	0.45
C20:2	0.85	0.90	0.88	0.03	0.38	0.78	0.98	0.02	<0.0001	0.38
C20:3*n*6	0.11	0.11	0.12	<0.01	0.36	0.10	0.13	<0.01	<0.0001	0.16
C20:3*n*3	0.07[a]	0.08[b]	0.08[a]	<0.01	<0.01	0.07	0.09	<0.01	<0.0001	0.02
C20:4*n*6	0.28[ab]	0.27[a]	0.30[b]	0.01	0.06	0.25	0.31	0.01	<0.0001	0.06
C22:5	0.06	0.06	0.06	<0.01	0.69	0.05	0.07	<0.01	<0.0001	0.92
SFA	39.63[ax]	37.76[b]	38.24[aby]	0.46	0.02	40.63	36.46	0.42	<0.0001	0.91
MUFA	33.23[a]	35.37[bx]	33.72[aby]	0.72	0.07	34.26	33.96	0.57	0.72	0.27
PUFA	26.21	26.00	27.12	0.72	0.44	24.31	28.57	0.58	<0.0001	0.27
Iodine value	72.42	73.62	74.08	0.80	0.39	70.00	76.75	0.65	<0.0001	0.47
Desaturase index[6]	0.447[a]	0.477[bx]	0.461[aby]	0.007	0.02	0.449	0.474	0.006	<0.01	0.44

[1]The slow-growing were defined as pigs with ADG ≤ 1 SD below the mean ADG of the barn independent of pens. Average growth rate pigs expressed ADG that was up to 0.25 SD above or below the mean ADG of the barn. The fastest growing pigs were defined as pigs with ADG ≥ 1 SD above the mean ADG of the barn.
[2]*P*-value for effect of growth rate.
[3]*P*-value for effect of growth sex.
[4]*P*-value for interaction between sex and growth.
[5]Fatty acid as weight percentage of belly fat.
[6]Δ^9 desaturase activity (Smith et al., 2002): (C16:1 + C18:1 *cis*-9 + C18:1 *cis*-11)/(C14:0 + C16:0 + C18:0 + C16:1 + C18:1 *cis*-9 + C18:1 *cis*-11)
[a,b]Means within a row within main effects with different superscripts differ ($P < 0.05$).
[x,y]Means within a row within main effects with different superscripts differ ($P < 0.10$).

Not many studies have been published relating growth rate of pigs and fatty acid composition. The reported effects of growth rate on saturation of fatty acids in pork fat are variable. Hardman et al. (2014) evaluated the effect of a topping program on fatty acid composition by removing pigs from test every 7 d when reaching target final BW of 112 kg. In agreement with the present study, no effect of marketing day was observed on IV of jowl fat, backfat or belly fat of pigs (Hardman et al., 2014). Correa et al. (2008) evaluated the effect of growth rate on fatty acid composition based on age at target slaughter weight. Two groups of pigs were selected based on estimated breeding values for growth rate of the sire-line. Pigs that were younger (-10 days) based on the sire-line calendar at 100 kg of live BW were considered the fast growing pigs. Pigs that were older (+2 days) based on the sire-line calendar at 100 kg of live BW were considered the slow growing pigs. Contrary to the present study, pigs that were older at market weight (slow growers) pigs had lower concentrations of SFA and greater PUFA and IV for bellies compared to pigs younger at market weight (fast growers). The greater saturation of fast-growing pigs in the work of Correa et al. (2008) was attributed to the greater fat deposition (36% vs 29% loin fat percentage) compared with slow-growing pigs and consequently greater *de-novo* fatty acid synthesis which is more saturated (Correa et al., 2006; Correa et al., 2008; Lehninger et al., 2008). Lo Fiego et al. (2005) evaluated the effect of final BW of pigs harvested on the same day and reported lower concentrations of SFA and greater PUFA and IV in raw ham for pigs with lower final BW compared to pigs heavier at market, which would indicate greater saturation of fast-growing pigs. However, caution is required when relating the effects of final BW reported by Lo Fiego et al. (2005) with growth rate effects because, unlike the present study, initial BW was

not reported, one cannot assume heavier pigs at harvest day actually grew faster than lighter pigs at harvest day, and growth rates were not specified. In the study of Lo Fiego et al. (2005), heavier pigs at harvest day exhibited greater backfat depth (37 mm vs 33 mm) compared to lighter pigs at harvest. Commonly, older pigs have greater fat deposition and have greater *de-novo* fatty acid synthesis which is more saturated (Correa et al., 2008; Lehninger et al., 2008). Therefore, one cannot assume the greater saturation of pigs heavier at market reported by Lo Fiego et al. (2005) is in fact, an effect of faster growth rate or higher fat deposition caused by higher final bodyweight. The inconsistency of the few studies on effects of growth rate on fatty acid unsaturation indicates multiple factors may influence saturation of pork fat. In the present study, no important differences in fatty acid composition of belly fat were observed among pigs with differing growth rates.

Effect of gender on fatty acid composition

Fatty acid composition of belly fat differed between barrows and gilts (Table 4.6). Compared to barrows, gilts had lower concentration of SFA, greater ($P < 0.01$) concentration of PUFA and greater IV, but similar ($P > 0.05$) concentration of MUFA. Similarly, the estimated activity index of Δ^9 desaturase enzyme was greater for gilts than for barrows, which suggests greater deposition of unsaturated fat in gilts. No interactions between growth rate and gender were observed for SFA, MUFA, PUFA and IV.

The greater unsaturation observed for gilts was expected as others have reported similar results. Correa et al. (2008) reported lower concentration of SFA and greater concentrations of PUFA and IV in belly fat of gilts compared to barrows. Zhang et al.

(2007) reported greater unsaturation of fat in loin muscle of gilts compared to barrows, although no differences were observed in Δ^9 desaturase enzyme activity index. In contrast, Benz et al (2010) reported no differences between gilts and barrows in IV, SFA, MUFA, and IV of backfat, although gilts had greater concentration of PUFA in backfat.

The greater unsaturation of carcass fat in gilts has been explained by lower activity of lipogenic enzymes in gilts compared with barrows (Allee et al., 1972). Lower activity of lipogenic enzymes reduces production of saturated fatty acids. In addition, the greater levels of the sex hormone (estradiol) in gilts compared with barrows drives dietary nutrients to lean deposition instead of fat deposition which reduces body synthesis of saturated fatty acids (Grela et al., 2013). Fatty acids synthesized by the body are mostly saturated fatty acids (Lehninger et al., 2008). Consequently, gilts have less production of saturated fatty acids than barrows. In addition, swine diets have a greater ratio of unsaturated fatty acids (**UFA**) to SFA. Unsaturated fatty acids from diets have greater digestibility than SFA which will cause UFA to have greater impact in fatty acid composition of pork fat than SFA (Davis et al., 2015). Fatty acids from diets will be deposited in carcass fat with little modification and will directly influence fatty acid composition of carcass fat. Therefore, because gilts have less fatty acid synthesis than barrows, the fatty acid composition from diets has a larger influence on fatty acid composition of carcass fat in gilts compared with barrows.

Relationship of fatty acid composition among fat depots

Fatty acid composition of belly fat was correlated positively with jowl and backfat for all traits analyzed (Table 4.7). Similarly, fatty acid composition of jowl fat was

correlated positively with backfat. Although all correlations were highly significant ($P < 0.01$) statistically, IV, SFA, MUFA, PUFA and C18:2 composition of jowl fat only explained about 40% of the variation in these same traits for belly fat because of the low correlation coefficients. Jowl fat was a more reliable predictor of IV, SFA, MUFA, PUFA and C18:2 composition of backfat than belly fat. Belly is a high-value cut which is processed into bacon. Sampling belly fat for IV analysis damages bellies which reduces yield of bacon. Ideally, accurate prediction of belly fat IV from other low-value parts of the carcass such as jowl would avoid the loss of belly parts for IV analysis. However, this experiment showed that both jowl fat and backfat were poor predictors of fatty acid composition of belly fat which means that an accurate prediction of belly fat IV from fatty acid composition of jowl fat or backfat is less likely.

Different adipose tissue depots have different lipolytic and lipogenic activity and response differently to insulin and other hormones which influences fatty acid composition (Kouba et al., 1999; Ibrahim, 2010; Shirouchi et al., 2014). Kouba et al. (1999) reported greater activity in desaturase enzyme and activity of acetyl-CoA carboxylase enzyme (a marker of lipogenesis) for backfat and leaf fat compared to muscle and liver of pigs. Similarly, Shirouchi et al. (2014) reported differences in activity of desaturase enzymes from loin muscle and visceral fat of cattle. These differences in enzymatic activity among depots cause differences in fatty acid composition among depots. Differences in IV among jowl fat, backfat and belly fat are frequently reported (Weber et al., 2006; Leick et al., 2010; Wiegand et al., 2011). Monziols et al. (2007) reported highly significant differences in fatty acid composition among 11 adipose tissue depots (Table 2.2). In agreement with the results of the present study, most studies have

reported poor or no correlation in fatty acid composition among fat depots (Cameron and Enser, 1991; Leick et al., 2010; Wiegand et al., 2011). Poor correlation (r = 0.39) between belly and jowl IV was reported by Leick et al. (2010). Wiegand et al. (2011) reported no correlation between IV of belly and jowl fat. However, increasing the energy density of the diets by adding choice white grease resulted in a negative correlation between IV of belly fat and jowl fat (r = -0.67; Wiegand et al., 2011). Cameron and Enser (1991) reported poor correlation between fatty acids from backfat and intramuscular fat. Most studies reported poor correlation between belly, jowl and backfat IV. In contrast, Estrada (2013) reported correlations of 0.80 to 0.90 among belly fat, jowl fat and backfat IV. Estrada (2013) speculated that the possible reason for this difference between studies is that in his study, unlike other studies, a wide range of IV for belly fat (59 to 91 g/100g of fat) was obtained with several inclusion rates of DDGS in diets which could be leading to the higher correlation among fat depots compared to other studies. In conclusion, fatty acid composition of different fat depots is relatively different and unrelated which causes imprecision when using carcass fat depots to predict fatty acid composition of belly fat. Therefore, prediction of fatty acid composition of a fat depot based on other fat depots is not very reliable.

Table 4.7. Pearson correlation coefficients for fatty acid composition of belly fat, jowl fat and backfat[1]

Composition	r	*P* Value
IV		
Belly fat vs jowl fat	0.60	<0.0001
Belly fat vs backfat	0.63	<0.0001
Jowl fat vs backfat	0.84	<0.0001
SFA		
Belly fat vs jowl fat	0.37	0.013
Belly fat vs backfat	0.25	0.0957
Jowl fat vs backfat	0.64	<0.0001
MUFA		
Belly fat vs jowl fat	0.63	<0.0001
Belly fat vs backfat	0.62	<0.0001
Jowl fat vs backfat	0.98	<0.0001
PUFA		
Belly fat vs jowl fat	0.66	<0.0001
Belly fat vs backfat	0.67	<0.0001
Jowl fat vs backfat	0.94	<0.0001
C18:2		
Belly fat vs jowl fat	0.66	<0.0001
Belly fat vs backfat	0.67	<0.0001
Jowl fat vs backfat	0.94	<0.0001

[1]Data collected from 47 gilts harvested at Andrew Boss Laboratory of Meat Science, St. Paul, MN.

Prediction of fatty acid composition

Considering the high economic value of pork bellies, a regression analysis was used to create prediction equations of belly fat IV using variables such as diet composition, growth performance, and carcass characteristics. Using data from all pigs in the experiment (n = 213) the best fit equation to predict belly fat IV was: belly fat IV = 75.95 + (3.324 × concentration of dietary C18:2) – (6.327 × backfat depth, cm) (R^2 =

0.61). To predict belly fat IV with data from other fat depots, a selected group of 47 gilts were used. These gilts were selected because their BW at harvest was closest to the mean BW of each pen. Fatty acid composition of belly, jowl, and back fat were determined. A regression analysis using variables such as dietary composition (C18:2 concentration and intake of C18:2 on a pen basis), growth rate, carcass characteristics and fatty acid composition (jowl fat and backfat) was used to create prediction equations for belly fat IV. The best fit equation to predict belly fat IV was: belly IV = (7.16938 × concentration of backfat C14:0) + (0.63232 × backfat IV) (R^2 = 0.43). Removing fatty acid composition of backfat from the model, estimation of belly IV could be calculated as: belly IV = 92.701 – (22.84 × concentration of jowl C20:1) (R^2 = 0.38). The low R^2 prediction of belly fat IV shows the difficulty in predicting belly fat IV in this experiment.

Predicting fatty acid composition of pork fat can be an important tool to pork producers because of limits on IV of carcasses imposed by pork processors (Benz et al., 2011). Prediction equations for pork fat IV may allow nutritionists to maintain quality of pork fat by controlling dietary inclusion of DDGS and fats. To obtain more accurate results for calculation of IV of pork fat, Meadus et al. (2010) proposed an addition to the existing AOCS (1998) equation based on generalizations of AOCS (1998) equations of Knothe (2002). The proposed amendment has been used to calculate IV of pork fat (Meadus et al., 2010) and could generate more accurate predictions because it includes more unsaturated long-chain fatty acids than the AOCS (1998) equation which are highly susceptible to peroxidation and would influence highly the pork fat quality. However, the AOCS (1998) equation to predict IV still is the most commonly used equation. According to Madsen et al. (1992), iodine value product (**IVP**) can be used to control quality of pork

fat (IVP = (IV of dietary fat) × (crude fat, %) × 0.1). Some authors have developed equations to predict carcass fat IV using IVP (Bergstrom et al., 2010; Benz et al., 2011). Benz et al. (2011) fed diets with different fatty acid composition to growing-finishing pigs and predicted IV for jowl fat and backfat. Similar to the results of this experiment, better prediction of jowl fat IV was observed from dietary C18:2 concentration (R^2 = 0.90, $P < 0.01$) compared with IVP (R^2 = 0.32, P = 0.24). Prediction of backfat IV from dietary C18:2 concentration had lower precision (R^2 = 0.73, $P < 0.03$); (Benz et al., 2011). The lower R^2 from the present study compared to the results reported by Benz et al. (2011) might be because Benz et al. (2011) predicted jowl fat and backfat IV, but not belly fat IV as in the present study. However, Bergstrom et al. (2010) reported high precision in predicting belly fat IV from diet IVP (R^2 = 0.90). Although many equations with relatively high precision have been developed, most were not validated in subsequent experiments. Conceptual models including a few factors may not accurately predict fatty acid composition of pork fat because many factors such as diet, gender, genetic line, and lipogenic enzyme activity contribute to final fatty acid composition of each fat depot (Lizardo et al., 2002). Thus, it is clear that a wide range of factors influence fatty acid composition of carcass fat. Paulk et al. (2015) used existing literature to develop prediction equations for IV of belly fat, jowl fat, and backfat with several predictor variables which were further validated in an experiment. Best fit equation (R^2 = 0.94) to predict belly fat IV included predictors such as net energy, fatty acid composition of diets and carcass composition data (Paulk et al., 2015). However, limitations for the prediction equations were observed when a fat blend was fed or when IV observed of pork fat was below 65 or higher than 74. In summary, the equations to predict belly fat

IV in the present study had lower R^2 compared to what other authors have reported probably due to the different variables used among the various models. Other studies that reported higher R^2 included more predictor variables; however, the equations have not been successfully validated under diverse conditions.

In conclusion, growth rate had no important influence on fatty acid composition of carcass fat as measured by IV. This result does not confirm the perception from anecdotal observations of pork processors and producers. Fatty acid composition of jowl fat was a poor predictor of fatty acid composition of belly fat. Fatty acid composition of belly fat had low correlation with jowl fat and backfat. Prediction equations for pork fat IV may allow nutritionists to maintain quality of pork fat. However, in this study, prediction equations for belly fat IV had low R^2 and are unreliable.

CHAPTER 5: SUMMARY AND CONCLUSIONS

Utilizing corn distillers dried grains with solubles (**DDGS**) in swine diets to support growth performance of pigs and reduce costs of diets is very popular in the United States. However, fat content in DDGS increases unsaturation of pork fat which results in reduced shelf life of retail cuts and processing problems (Leick et al., 2010; Xu et al., 2010a; McClelland et al., 2012). Feeding cottonseed oil or crude glycerol may influence pork fat quality by decreasing unsaturation. Cottonseed oil contains cyclopropene fatty acids (**CPFA**) which are known to inhibit desaturase enzymes responsible for synthesizing unsaturated fatty acids in the body (Nixon et al., 1977). By decreasing the endogenous synthesis of unsaturated fatty acids, we may be able to improve firmness of pork fat when diets high in DDGS are fed. Crude glycerol feeding has been reported to affect fatty acid composition of pork and fat firmness (Mourot et al., 1994; Kijora et al., 1997; Lammers et al., 2008; Schieck et al., 2010). Potentially, feeding cottonseed oil or crude glycerol may decrease unsaturation of pork fat caused by feeding DDGS which ultimately may improve pork fat quality.

In addition, pork processors and producers have perceived from anecdotal observations greater unsaturation of fat from slow growing pigs compared to fast growing pigs. Knowing the influence of growth rate on fatty acid composition of pork fat is important because nutritionists may be able to adopt nutritional strategies to improve pork fat quality of pigs exhibiting growth rates that would decrease pork fat quality.

The first objective of this study was to determine the effects of adding minimally refined cottonseed oil or crude glycerol to diets containing 40% DDGS on growth performance, carcass characteristics, and fat quality of growing-finishing pigs. We

hypothesized that both cottonseed oil and crude glycerol would decrease pork fat unsaturation.

In conclusion of chapter 3, feeding diets containing crude glycerol (**GLY**) had no impact on growth performance of pigs. However, diets supplemented with cottonseed oil (**COT**) improved growth performance probably because of the greater energy density of the COT diets compared with CON (control) or GLY. Neither COT nor GLY supplementation of diets increased belly firmness or reduced IV of carcass fat compared with pigs fed CON diets. Dietary GLY had no impact on fatty acid composition of pork fat. Pigs assigned to COT actually had increased IV of carcass fat, probably due to the added unsaturated fatty acids contributed by cottonseed oil which could not be overcome by the CPFA content of the cottonseed oil. Neither COT nor GLY supplementation of diets reduced oxidation of pork fat based on the TBARS measurements. Therefore, supplementation of swine diets high in conventional DDGS concentration with cottonseed oil or crude glycerol appears to have no potential to reduce DDGS-induced soft carcass fat.

The second objective of this study was to clarify the effect of growth rate on fatty acid composition, evaluate the effectiveness of using jowl fat as a location for predicting belly fat and backfat IV, as well as use different variables to develop prediction equations of belly fat iodine value (**IV**). Understanding of the effects of growth rate and depot location on pork fat IV will support pork producers and processors in managing pork fat quality more effectively.

In conclusion of chapter 4, growth rate had no important influence on fatty acid composition of carcass fat as measured by IV. This result does not confirm the perception

from anecdotal observations of pork processors and producers. Fatty acid composition of jowl fat was a poor predictor of fatty acid composition of belly fat. Fatty acid composition of belly fat had low correlation with jowl fat and backfat. Prediction equations for pork fat IV may allow nutritionists to maintain quality of pork fat. However, in this study prediction equations for belly fat IV had low R^2 and are unreliable.

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