DETERMINING PORK FAT QUALITY OF PIGS FED 20% DDGS AS MEASURED BY THREE METHODS WITH A STANDARD INDUSTRY MARKETING STRATEGY

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The undersigned, appointed by the Dean of the Graduate School, have examined the thesis entitled

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and hereby certify that in their opinion it is worthy of acceptance.

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DETERMINING PORK FAT QUALITY OF PIGS FED 20% DDGS AS MEASURED BY THREE METHODS WITH A STANDARD INDUSTRY MARKETING STRATEGY

ABSTRACT

Fat quality is important in meat products as it can influence further processing characteristics and pork export potential. The use of non-traditional fat sources such as dried distillers grain with solubles (DDGS) changes pork fat quality and has created challenges for end users of pork chain products. An experiment was designed to evaluate the effects of DDGS and split marketing cuts in a commercial swine facility on growth performance, fat quality and the relationship between iodine value (IV) determined by three methods in two fat depots. Pen (n = 40) was the experimental unit with 20 replications per treatment and 22 pigs per pen. Pigs were randomly allotted to dietary treatments in a 2 x 3 factorial arrangement with two levels of DDGS (0 or 20%) and chosen for one of three marketing cuts removing 4, 8 and 10 head from each pen. Fat tissue samples were removed from the anterior tip of the jowl and posterior to the sternum on the belly edge 1d postmortem. Fatty acid composition was determined via the Folch method and IVs were calculated from chemical titrations, fatty acid profile (GC IV) and in-plant Bruker® near infrared (NIR) spectroscopy. Correlations between IV determination methods were calculated. Inclusion of 20% DDGS did not change ($P > 0.05$) growth performance while marketing cut affected performance with the second cut

producing the heaviest and most efficient hogs ($P < 0.01$). Total SFA and MUFA concentrations were higher ($P < 0.01$) in belly and jowl fat from hogs fed 0% DDGS. Total PUFA and the PUFA:SFA in belly and jowl fat was higher ($P < 0.01$) when 20% DDGS was fed. DDGS inclusion increased IV in belly and jowl fat regardless of IV determination method. Regardless of dietary treatment or fat depot, Pearson Correlation Coefficients between titration and GC IV, titration and NIR, and GC IV and NIR were 0.46 ($P < 0.01$), 0.68 ($P < 0.01$) and 0.43 ($P < 0.01$), respectively. These correlations suggest methods may rank samples equally, but do not provide the same absolute IV. Belly fat had a lower IV ($P < 0.01$) compared to jowl fat using titration or GC IV methods suggesting pigs have varied degrees of physiological maturity at specific fat depots during the finishing phase. In conclusion, feeding 20% DDGS negatively affected fat quality, but not growth performance and marketing time changed growth performance.

CHAPTER 1

LITERATURE REVIEW

Introduction

The primary motivation to feed pigs is for the production of pork (Berg, 2001). A consumer's eating satisfaction, or palatability, of a meat product is often a key factor in purchase and repeat purchase decisions. Eating satisfaction relies on several factors including tenderness, juiciness and flavor (Tarrant, 1998; Bindon and Jones, 2001). Consequently, the entire meat production chain strives for continued production of high quality pork products (Berg, 2001). Similar to athletes, the type and quantity of nutrients in a swine diet ultimately influences physiochemical factors responsible for conversion of muscle to meat (Berg, 2001). Therefore, careful consideration must be given to feedstuffs included in swine diets due to their affect on the end product.

The United States agriculture industry has changed dramatically in the last century following a trend of fewer total farms, but more efficient production per swine unit. In 1910, there were 4.35 million hog farms yielding a total inventory of 58.2 million head (US Census of Agriculture, 1912). By 2007, the number of swine farms decreased to 75 thousand, but inventory totaled 67.7 million head (US Census of Agriculture, 2009). The majority (45 thousand) of hog farms in the US are small, but the largest numbers of pigs are found on large farms (US Census of Agriculture, 2009), indicating an increase in hobby farms, but also increased production on large operations.

In addition to increased hog numbers, according to a press release from the United Nations (2013), the world population is expected to reach over 9 billion people by the year 2050.

The increased hog numbers and demand for pork combined with the increased cost of production have created an opportunity for alternative feed stuffs to be utilized in swine diets. Dried distillers grains with solubles and other seed oils are popular alternatives to add highly concentrated sources of energy and fat to diets. However, the inclusion of unsaturated dietary fat sources compounded with lean biological types of the modern day pig has created issues surrounding pork fat quality.

What is Fat?

Fat often has a negative connotation from a health and physique standpoint. Yet, the constituents of fat are metabolically dynamic with adaptive and precisely regulated physiological and biochemical functions (Allen *et al*., 1976). By definition, fat is collectively adipose cells suspended in connective tissue matrix. Fat is found in nearly all anatomical locations, but fat in meat animals is predominately white fat found in four main depots as subcutaneous (backfat, SC), intermuscular (seam fat), intramuscular (marbling, IMF) and mesentery (internal) fat (Allen *et al*., 1976). The primary purpose of adipose tissue is energy storage in the form of fat and oil (Nelson and Cox, 2008; Allen *et al*., 1976). Additionally, adipose tissue is biologically active in providing structural elements of membranes, insulation, padding for organs and has a critical role in the

endocrine system as an endocrine organ producing and releasing hormones (Nelson and Cox, 2008; Allen *et al*., 1976).

Adipose tissue is a type of connective tissue comprised of lipids, water and enzymes for lipogenesis and lipolysis (Allen *et al*., 1976). The major component of fat is in fact lipids. Lipids' defining feature is their insolubility in water and the class of lipids is collectively made of three biological active groups including triglycerides (neutral fat), phospholipids and steroids (Nelson and Cox, 2008; Azain, 2001). Triglycerides, or storage lipids, are comprised of a glycerol backbone attached to three fatty acids by ester linkages, (Azain, 2001). They are the simplest lipid and the major component of adipose tissue (Nelson and Cox, 2008; Wood *et al*., 2008). Adipocytes contain lipases, which cleave fatty acids from a stored form for release to tissues needing fuel or energy. Phospholipids are structural lipids found in cell membranes. Membrane lipids have a polar, hydrophobic head and a hydrophilic tail with two fatty acids. The properties of phospholipids allow for the arrangement of a double layer membrane that acts as a barrier to polar molecules and ions (Nelson and Cox, 2008).

Fatty acids (FA) are the building blocks of triglycerides and phospholipids. Fatty acids are carboxylic acids with hydrocarbon chains containing 4 to 36 carbons. The carbon chains are unbranched and may be fully saturated (no double bonds) or unsaturated (one or more double bonds) (Nelson and Cox, 2008). There are three sources of FA for energy metabolism; synthesis of triglycerides when internal energy sources are abundant, the diet, and mobilization of triglycerides stored in adipocytes as fat droplets (Boyer, 2002). Fatty acids become incorporated into cell membranes as phospholipids or act as fuel depots in the form of triglycerides (Nelson and Cox, 2008).

Biosynthesis of Fatty Acids

De novo Synthesis

Biosynthesis of FA is a repeating sequence catalyzed in vertebrates by fatty acid synthase I. The rate-limiting step of fatty acid synthesis is the formation of malonyl-CoA from Acetyl-CoA and CO_2 prior to fatty acid synthesis. Initially, an acetyl-CoA condenses with a malonyl-CoA molecule already attached to the carrier molecule, ACP, on fatty acid synthase. The carbonyl group is reduced and the alcohol group removed. The double bond is reduced creating a saturated carbon chain. With each pass through the sequence, the carbon chain grows two carbons at a time donated from the active malonyl-CoA (Nelson and Cox, 2008). The final product of lipogenesis is the fully saturated fatty acid (SFA) 16:0 or palmitic acid (Nelson and Cox, 2008, Azain, 2001). Fatty acid elongation mechanisms add more carbons to make longer chains while mixed function oxidases desaturate chains to form mono (MUFA) and poly (PUFA) unsaturated fatty acids.

Dietary Fatty Acids

Fat from food is broken down in the stomach into triglycerides. Although FAs in the form of triglycerides are very efficient energy stores, tapping the energy is difficult due to the hydrophobic nature of FA. Ingested triglycerides must be emulsified prior to absorption in the small intestine so the FA can easily travel throughout the body. Bile salts act like detergent to break down large triglyceride molecules and convert them to micelles (Nelson and Cox, 2008). In micelles, the fatty acid tails are oriented towards the center creating a non-polar core surrounded by the salts (Boyer, 2002), which greatly

enhance the accessibility of lipases to triglycerides (Nelson and Cox, 2008). Pancreatic lipase degrades the triglycerides into FA and glycerol which easily diffuse though intestinal mucosa where they are reconverted into triglycerides and packaged as blood soluble chylomicrons (Nelson and Cox, 2008; Boyer, 2002).

Chylomicrons travel via blood to adipose and muscle tissues. In a satisfied or fed state, excess energy is stored as triglycerides in fat cells, in which case lipoprotein lipase cleaves FA from glycerol and the FA are stored in fat droplets (Nelson and Cox, 2008). These FA may be used later in an energy deficient state, such as during exercise, when the body uses up glycogen stores. The hormones epinephrine or glucagon function to activate adipocytes to release FA, which are picked up by serum albumin and transported to muscle tissue. Fatty acids may also go directly to muscle (Boyer, 2002). In either situation, FA undergo beta-oxidation, which removes two carbons in the form of acetyl co-A (Nelson and Cox, 2008) and releases CO_2 and energy in the form of ATP (Boyer, 2002).

Fatty Acids in Animal Tissues

Fat from plants and animals tend to differ greatly in terms of saturation. Oils from plants tend to be liquid and contain fatty acids with one to three double bonds while animal fats are typically more solid, being composed of more saturated fats with zero or one double bond. Sixteen and 18-carbon chain FA are the most abundant FA in porcine tissue. Palmitic acid (16:0), the end product of lipogenesis, accounts for 20 to 30% of FA in lard. Stearic acid (18:0) and oleic acid (18:1n9) from the diet or elongation and

desaturation reactions account for 10 to 15% and 40 to 50%, respectively, of the FA in pork tissue (Azain, 2001). Oleic acid is most predominant in neutral lipids and is formed from stearic acid by stearoyl Co-A desaturase. Muscle is primarily made of phospholipids, which contains large concentrations of linoleic acid, omega 3 and omega 6 fatty acids (Wood *et al*., 2008).

Differences in Fatty Acid Composition

Species. Fatty acid composition varies between species. In a study by Enser *et al*. (1996), pork had a much higher PUFA:SFA when compared to ruminant species. According to Wood *et al*. (2008), this can be attributed to the fact that compared to cattle and sheep, pigs have a much higher concentration of the major PUFA linoleic acid (18:2n6) in both muscle and fat tissue. In all species, FA composition can be altered by diet, but this is especially easy in monogastrics where FA are absorbed in the small intestine relatively unchanged and then incorporated into tissue lipids (Wood and Enser, 1997). Cattle and sheep have more saturated fat because of biohydrogenation in the rumen (Wood and Enser, 1997), therefore lowering the PUFA:SFA ratio. Yet, for the same reasons, ruminants have a more desirable n6:n3 ratio (Enser *et al*., 1996). Increased levels 18:2n6 in hogs allows for increased synthesis of 20:4n6, causing an unbalanced ratio of omega fatty acids compared to beef and lamb (Wood *et al*., 2008). Biohydrogenation in the rumen also impacts the orientation of double bonds. Double bonds from eukaryotic enzymes are in the cis orientation (Azain, 2001). Bacteria have the ability to not only form trans orientations, but also double bonds that are not separated by a methyl group (conjugated linoleic acids)(Azain, 2001).

Diet. Within species, FA composition and degree of saturation is a product of many factors including diet, genetics and breeding, sex and body composition (Martin *et al*., 1972; Wood *et al*., 2008). As briefly mentioned above, porcine are highly amenable to dietary fat concentrations. Although swine are capable of synthesizing many fatty acids in vivo, they lack the enzymes needed to make linoleic and linolenic acids. These two fatty acids are considered essential FA and must be provided in the diet. Consequently, the levels of 18:2n6 and 18:3n3, are closely linked to dietary concentrations (Wood and Enser, 1997; Wood *et al*., 2008). On the other hand, SFA and MUFAs synthesized in vivo are less readily influenced by diet (Wood and Enser, 1997). Therefore, the levels of essential FA, in particular linoleic acid, may be the most useful to use when determining fat quality from FA composition.

Numerous studies have documented the influence of dietary oils on FA levels in pork tissues. Early work by Ellis and Isbell (1926), demonstrated corn and soybean oils resulted in a greater amount of unsaturation compared to peanut and rice oils. The proportion of 18:2n6 in subcutaneous backfat increased from 1.9% in low fat diets to 30.6% in pigs fed high levels of soybean oil (Ellis and Isbell, 1926). In a pair of studies by Teye *et al*. (2006a,b) hogs were fed nonconventional, inexpensive feeds stuffs, palm kernel oil (PKO) and palm oil (PO). Compared to soybean oil, PKO contains high amounts of lauric (12:0), myristic (14:0) and 18:0 and low concentrations of 18:2n6, thus yielding a significant increase in SFA, decrease in 18:2n6 concentration and overall reduction in the PUFA:SFA ratio in loin muscle. Short chain SFA and 18:2n6 are impacted the most as they are mainly derived from the diet; however, C16 and C18 from de novo synthesis have limited pull from dietary concentrations (Wood *et al*., 2008).

Interestingly, 10% added fat greatly inhibits lipogenic activity (Allee *et al*., 1971). When comparing corn diets to diets containing 10% added lard, beef tallow or coconut oil, the added dietary fat increased back fat and marbling, while de novo synthesis of fatty acids was inhibited (Allee *et al*., 1971). This is believed to result from a physiological preference to use the readily available dietary fat source, rather than deplete body resources.

For health reasons, a low omega 6:omega 3 ratio is desirable (Simopoulos, 2002). Studies have focused on improving the ratio of omega 3 and omega 6 fatty acids by incorporating high levels of linseed oil in the diet. Enser *et al*. (2000) fed boars and gilts diets with differing 18:2n6:18:3n3 ratios. Linseed oil contains increased 18:3n3 and decreased 18:2n6; a 56% increase in 18:3n3 was observed in lean tissue from pigs receiving linseed oil. Consequently, levels of eicosapentaenoic acid (EPA, 20:5n3) and docosahexaenoic acid (DHA, 22:6n3) increased in both lean and adipose tissue, thereby creating more favorable PUFA:SFA and n6:n3 ratios (Enser *et al*., 2000). As omega 6 and omega 3 FA compete for enzymes, increased dietary concentrations of 18:3n3 shifted the deposition of omega 3 fatty acids in tissue (Wood *et al*., 2008). Kouba *et al*. (2003) also found that feeding linseed oil increased omega 3 PUFAs in muscle and adipose tissue with a favorable PUFA:SFA ratio increase and n6:n3 decrease with a fast uptake occurring in neutral lipid versus phospholipid. Yet, 18:1n9 and MUFA levels were strongly depressed in pigs fed linseed oil. Kouba and Mourot (1999) attributed these lower levels of oleic and MUFA to depressed activity of stearoyl-Co-A-desaturase, which is thought to be inhibited by linolenic and linoleic (Kouba *et al*., 2003) FA activity.

Protein and energy levels also play a role in FA deposition. Wood *et al*. (2004) reported pigs fed a conventional versus low protein diet had a higher PUFA:SFA ratio. Findings by Teye *et al*. (2006a) support these data. Low protein diets lead to changes in fat deposition resulting in fatter hogs, with increased intramuscular fat (IMF) and a reduced PUFA:SFA ratio below recommended values (Teye *et al*., 2006a). According to Bee *et al*. (2002), the activity of lipogenic enzymes are decreased in low energy diets and linoleic acid is incorporated less efficiently. On the other hand, hogs fed high-energy diets are constantly in a positive energy balance and therefore do not rely on stored fat to meet energy needs (Enser, 1984).

Genetics and Breeding. The modern pig is extremely different from the pig bred 100 years ago. Prior to the 1940s, fat hogs were preferred for production of lard. During World War II, pork was an important item in rations for soldiers as well as nitroglycerin for explosives. It wasn't until the 1950s that consumer's became more health conscious and sought leaner pork products (Hollis and Curtis, 2001). Since then, modern breeding practices have focused selection on improving the lean to fat ratio by reducing backfat and looking for a "meat type" hog (Martin *et al*., 1972).

Martin *et al*. (1972) reported differences in iodine value (IV) between seven lines of pigs in chemical analysis of fat samples, with fatter carcasses having more saturated fat. Similarly, breed significantly impacted fatty acid composition of marbling in fatter, traditional pigs compared to leaner, modern breeds (Wood *et al*., 2004). Traditional Berkshire and Tamworth pigs had larger percentages of 14:0 and 16:0 and lower percentages of PUFAs 18:2n6 and 18:3n3 when compared to modern Large White and

Duroc pigs with the net result of a higher PUFA:SFA in modern pig types (Wood *et al*., 2004).

Some breeds of pigs, such as Durocs and Berkshires, are known to exhibit increased marbling in the loin eye relative to crossbred counterparts. Yet, Wood *et al.* (2004) found that even when Duroc carcasses possessed high levels of marbling, the fatty acid profile reflected one of a lean type market hog. This suggests that the FA profile follows the fattening pattern of subcutaneous fat rather than intramuscular marbling (Wood *et. al*., 2004). The differences observed between fat and lean hog breeds prefaces the discussion about FA composition and fat thickness and opens the idea of recognizing biological types versus breeds.

Body Composition and Gender. The relationship between FA composition and carcass composition has been well documented. As a pig fattens, the FA profile changes. Proportions of SFA and unsaturated FA are directly related to degree of fatness (Martin *et al*., 1972). An early study by Greer *et al*. (1965) found the leanest hogs had the highest levels of linoleic acid and Wood *et al*. (1978) saw a negative correlation between concentrations of 18:2n6 and backfat. Large White boars selected for fast growth and lean carcasses had higher concentrations of 18:2n6 compared to random mated control lines (Wood *et al*., 1978). As fat increased from 8 mm to 16 mm, levels of 18:2n6 decreased, thereby affecting the firmness of fat as well (Wood *et al*., 1986, 1989). As fatter hogs have increased levels of 18:0 and decreased levels of 18:2n6, it is not surprising that fatter hogs have firmer fat with less muscle separation (Wood *et al*., 1986). According to Wood (1984), the SFA 18:0 and 18:1 increased while 18:2n6

decreased in backfat due to an increase in de novo FA synthesis and less dietary influence.

When evaluating sexes, regardless of genetic background, Martin *et al*. (1972) found gilts to have less backfat, larger loin eyes and a higher percent fat free lean than barrows as well as lower myrstic, palmitic and higher linoleic acid contents. Additionally, boars and barrows had a higher percentage of linoleic acid compared to gilts, while barrows had greater percentages of myristic and palmitic acids than gilts or boars (Martin *et al*., 1972). When compared to intact males, gilts tended to have lower PUFA concentrations (Wood *et al*., 1978). Models developed by Sather *et al*. (1995) suggest differences in fat hardness among males and females can be attributed to the degree of difference of fatness among the sexes.

However, the difference between intact males and gilts could be attributed to leanness or immaturity of the later-maturing and leaner boars (Sather *et al*., 1995). In gilts and intact males of the same fat thickness (12 mm), boars had higher proportions of water, and lower proportions of lipid, indicating less mature tissue (Wood *et al*., 1978, 1986). Boars also have increased collagen content regardless of fat thickness compared to gilts (Wood *et al*., 1989).

Fatty Acids in the Human Diet

Health experts continue to recommend lower: total fat, saturated fat, trans FA, and cholesterol in diets to reduce the risk of health problems including cardiovascular disease, diabetes, hypertension and obesity (USDA, 2010; McGrane *et al*., 2011; Henley and Misner, 1999). Nutritionally speaking, lean meat has a relatively low fat (20 to 50 g/kg) content per recommended serving, but pork is particularly low (Enser *et al*., 1996; Henley

and Misner, 1999). This can be attributed to less marbling in pork than in beef and lamb. Research has shown that fat in general is not the cause of adverse health implications and in fact, certain FAs have been linked to decreases in coronary heart disease (CHD) (Berg, 2001). The fatty acid content of pork is approximately one half 18:1, which is considered neutral in terms of CHD, as is 18:0 found in smaller quantities in pork. The saturated fatty acid, 16:0 is considered detrimental to heart health and constitutes about one quarter of pork fatty acid profile (Pettigrew and Esnaola, 2000). Nonetheless, pork remains a healthy choice with a beneficially high PUFA:SFA ratio largely due to the high concentration of 18:2n6.

Effects of Changing Fatty Acid Composition

Altering Quality Indicators. Clearly, the fatty acid composition of pork tissue is easily altered by a variety of factors. However, this is not done without consequence. According to Wood *et al*., (2008) and Wood (1984), changing the FA composition of adipose tissue using different dietary oils changes lipid melting point and tissue firmness. Saturated fats have a much higher melting point than unsaturated fats, long chain higher than short and trans higher than cis (Wood, 1984). Strong correlations between FA concentrations and melting point and other predictors of fat quality have been documented. In Large White hogs from two genetic lines, melting points were most positively correlated and predicted by the concentration of 18:0 (Wood *et al*., 1978). Subjective and objective firmness of shoulder and loin fat was positively correlated to 18:0 and negatively correlated to 18:2n6 with the latter being a more accurate predictor of firmness (Wood *et al*., 1989).

Oxidative stability during processing and retail display depends on FA concentrations as well. Oxidation converts red muscle pigment myoglobin to brown metmyoglobin during the degradation of PUFAs in tissue membranes (Wood and Enser, 1997). The PUFAs in phospholipids are liable to oxidative breakdown and depending on the extent of oxidation, rancid odor and taste are detectable (Wood *et al*., 2008). In pork, high levels of omega 3 FA are associated with fishy flavors (Wood and Enser, 1997). However, studies have shown, high levels of vitamin E in the diet are effective in reducing oxidation (Wood *et al*., 2008) and combating fishy flavor (Wood and Enser, 1997).

Physiological Considerations. Beyond altering meat and fat characteristics that may further impact processing, there are potential effects related to biological functions. Other long chain FA (20 to 22 carbons) including arachidonic acid (20:4n6) as well as EPA (20:5n3) and DHA (22:6n3), are derived from 18:2n6 and 18:3n3 by Δ5 and Δ6 desaturase, respectively. Consequently, levels of these FA depend on the levels of their precursors (Wood *et al*., 2008). Western diets contain unbalanced ratios of omega 6 and omega 3 fatty acids largely due to the high consumption of cereal grains (Simopoulos, 1999, 2002). Although pork may have a desirable PUFA:SFA ratio, high levels of linoleic acid may have a negative impact on certain biochemical functions.

Mammalian cells do not contain the enzyme needed to convert omega 6 to omega 3 fatty acids, thus omega 3 and omega 6 FA are not interchangeable. Omega 6 and omega 3 FA have distinct and often opposing physiological functions (Simopoulos, 2002). Arachidonic acid, derived from linoleic acid, is the precursor for eicosanoids, which modulate immune response. Altering levels of eicosanoids alter cell inflammatory

and immune response (Calder and Grimble, 2002). High levels of arachidonic acid enhance immune response and create a prothrombotic and proaggregatory state (Simopoulos, 1999). Contrastingly, omega 3 FA are derived from linolenic acid and have anti-inflammatory, antithrombotic, antiarrhythmic, hypolipidemic, and vasodilatory properties (Simopoulos, 1999). Not surprisingly, lowering the intake of omega 6 FA and improving the intake ratio of omega 6:omega 3 FA is desirable to reduce the risk of many chronic diseases (Simopoulos, 1999, 2002).

Furthermore, since FA are the principle components of phospholipids, changes in fatty acids can alter the fluidity of cell membranes (Calder and Grimble, 2002; Youdim *et al*., 2000), which in turn can influence enzyme roles (Youdim *et al*., 2000). Signal molecules are generated from the membrane phospholipids that regulate proteins involved in cell signaling mechanisms within immune cells. Changing the phospholipids may change their affinity to enzymes that make the signal molecules and alter immune responsiveness (Calder and Grimble, 2002).

Essential Fatty Acid Deficiency. There are visible, clinical signs of essential fatty acid deficiency. Human patients show signs including dermatitis, decreased growth, increased susceptibility to infection, and poor wound healing (Jeppesen *et al*., 1998). The brain also happens to be rich in PUFAs, particularly DHA and decline in structural and functional integrity of the brain correlates with loss of DHA (Youdim *et al*., 2000). A double blind study supplementing children with diagnosed learning difficulties found supplementing unsaturated FA reduced attention deficit hyperactivity disorder (ADHA) symptoms (Richardson and Puri, 2002). In addition to cerebral gray matter, DHA is also found in high amounts in the retina. In a study using with rhesus monkeys, mothers were

deprived of omega 3 FA during pregnancy (Neuringer *et al*., 1984). Infants from these mothers had depleted DHA levels and visual deficiencies suggesting biochemical changes in the retina and the brain are associated with omega 3 deficiencies (Neuringer *et al*., 1984).

Growth and Development

Fat Development and Accumulation

Hammond (1932) described the fattening pattern of food animals to begin at the distal ends and move toward the visceral cavity, suggesting that fat is first deposited in areas on an animal such as the jowl and shoulder before accumulating in the belly and loin regions. According to Fortin (1986), backfat measures the thickest over the shoulder and gradually decreases in depth until a point on the body over the last rib. Fat develops the slowest over the shoulder, but the most rapid rate of development occurs in the region between last rib and the fifth or sixth next to last rib (Fortin, 1986).

To take a step further, as porcine animals mature, subcutaneous fat develops in three distinct layers with the outer developing prior to the middle and inner layers (Koch *et al.* 1968; McDonald and Hamilton, 1976). The outer layer has consistently been found to contain higher levels of unsaturated FA and lower levels of SFA when compared to the middle and inner layers (Koch *et al.* 1968; McDonald and Hamilton, 1976; Irie and Sakimoto, 1992; Wiseman and Agunbiade, 1998). The middle backfat layer develops (or accumulates more depth) at a faster rate than the outer layer, which develops at the slowest rate (Fortin, 1986). Although Fortin (1986) found the inner layer to develop at an

intermediate rate, Newcom *et al*. (2005) found the inner and middle layers to develop at the same rate albeit still faster than the outer layer.

Newcom *et al.* (2005) observed as body weight increases, the growth rate of the outer layers continues to decrease and the percentage of the total backfat gets smaller. This finding agrees with Fortin (1986) who found the middle layer of fat becomes more predominant in heavier animals. The middle layer of fat (which can also be considered the inner layer if only two layers are present) is the most dynamic layer with the greatest lipogenic activity (Leymaster and Mersmann, 1991; Warnants *et al.,* 1999). This increase of lipogenic activity leads to greater de novo synthesis of SFA and MUFA in those layers (Warnants *et al.,* 1999). It has been noted that the inner layer of backfat has the greatest turnover rate, or change in FA profile, when compared to the other layers (Koch *et al.,* 1968; Warnants *et al.,* 1999) and on the same note, these changes in FA profiles can be seen within 14 days of dietary changes (Wiseman and Agunbiade, 1998; Warnants *et al*., 1999).

Apple *et al*. (2009) described iodine value differences between individual fat layers in pork SC. The highest IV occurred in the outer most backfat layer and the lowest IV was observed in the inner backfat layer (Apple *et al*., 2009). Studies have also focused on distinguishing the differences between fat depots on a carcass and even tried to find correlations between less valuable regions such as the jowl and regions that are preferred left intact such as the belly. Wiegand *et al*. (2011) reported iodine values of belly, jowl, IMF and SC were 60.97, 64.51, 55.59 and 58.26 respectively. The same study also found weak correlations between jowl and belly fat, regardless of dietary treatment (Wiegand *et al*., 2011).

Metabolic Modifiers

The process of protein and fat accretion occur simultaneously during early growth, but as the animal matures, protein accretion slows and becomes negligible while fat accumulation continues (Bergen, 1974). Thus, as an animal approaches mature size, fat content exceeds protein content (Bergen, 1974). Work by Searle *et al*., (1972) found sheep with larger "ultimate size" fatten at heavier body weights. In order to promote increased de novo protein synthesis, fat accretion must be delayed or the mechanism that turns on de novo synthesis of fat must be blocked (Bergen, 1974) creating a similar situation to late maturing hogs. In 1965, Cunningham presented the idea of changing growth by feeding agents (caffeine, theophylline, nicotine or epinephrine) that directly or indirectly change the concentration of cAMP, a major cell-signaling molecule. The β-adrenergic receptors are present on most cell type surfaces and are stimulated by the physiological agonists norepinephrine, a neurotransmitter, and epinephrine, an adrenal medullary hormone (Mersmann, 1998). The influence of β-agonists is most pronounced in ruminants. Selection preferences for increased growth in swine and poultry have lessened the effect of synthetic β-agonists in those species (Mersmann, 1998).

The two most obvious effects of administering β-agonists is the increase in muscle mass and decrease in carcass fat mass (Mersmann, 1998). Ractopamine hydrochloride (RAC; Paylean, Elanco Animal Health, Greenfield, IN) is a feed supplement in swine that repartitions nutrients away from fat deposition to increased protein synthesis and muscle mass accretion (Apple *et al*., 2007). The RAC interacts in vivo with β-adrenergic receptors of adipocytes to decrease lipogenic activity and ultimately diminish cellular response to β-adrenergic stimulation (Mills *et al*., 1990).

Adeola *et al.* (1990) reported significant interaction between RAC and dietary level of protein; as protein level increased, ADG and G:F ratio followed. However, regardless of protein level, fat decreased and leanness increased (Adeola *et al*., 1990). The review published by Apple *et al*. (2007) concluded RAC inclusion in the diet increased growth rate and growth efficiency with no detrimental effects on fresh pork color, firmness, water-holding capacity or intramuscular fat. Management changes have increased the average hog slaughter weight from 109 to 125 kg, with some hogs reaching as much as 140 kg at the time of market (Carr *et al*., 2008). Due to inclusion of RAC, pigs can be marketed at heavier weights without negatively impacting carcass leanness desired by today's consumers (Carr *et al*., 2008).

Market Timing

Two systems are typically used in commercial hog production to market animals for slaughter. All-in all-out systems are marketing strategies that market all pigs in a pen together at the same time (Conte *et al*., 2012). All-in all-out systems decrease the risk of disease transmission, reduce animal fighting and facilitate the cleaning and disinfecting of barns (Scroggs *et al*., 2002). However, due to variation in genetics, final body weights may fluctuate in a group of finishing pigs and truck-loads may suffer the economic impact of marketing non-uniform hogs outside producer's desired weight range (DeDecker *et al*., 2005a). Furthermore, floor space and feeder space may become restricted by the end of the finishing phase, thus exacerbating aggression (Conte *et al*., 2012).

Swine operations may also employ a split marketing technique, which pulls a designated proportion of the heaviest, or most physiological mature hogs from each pen, which are then marketed one to two weeks prior to the remaining animals (Scroggs *et al.*, 2002; DeDecker *et al*., 2005a). This system increases access to resources including floor and feeder space, allows time for slow maturing animals to reach target weights (DeDecker *et al*., 2005a) and simultaneous marketing of hogs with similar weights and degrees of finish. Social dynamics in pens of pigs can also change (DeDecker *et al*., 2005a). As Burman *et al*. (2008) found in rats, removing animals from a group could disrupt the established social hierarchies leading to increased aggression.

Studies (Bates and Newcomb, 1997; Woodworth *et al*., 2000) have shown growth performance is increased in the animals remaining after the heaviest individuals have been removed. Specifically, marketing strategy (one or two cuts prior to close out versus all-in all-out) improved ADG and negated sort discounts by the packer (Woodworth *et al*., 2000). When 25% of the pen (n = approximately 52) was removed, ADFI improved, but growth was similar to control pens; however, when 50% of animals were removed from pens ADFI and ADG improved (DeDecker *et al*., 2005a). Less competition from larger, dominant pigs for easy access to food is reasonably expected to help improve feed intake and ultimately growth due to the fact that pigs can fully express their potential for feed intake (Conte *et al*., 2012).

Augspurger *et al*. (2000) observed behavior differences in eating patterns as hogs were removed from pens. Pigs increased their number of visits to the feeder per day, but decreased their feed intake per visit (Augspurger *et al*., 2000). Conte *et al*. (2012) reported the number of aggressive interactions decreased during feeding events after pigs

were removed in split marketing pens, likely due to increase space and easy access to the feed trough. In contrast, Scroggs *et al*. (2002) found immune response and aggression of hogs in split-market pens following post primary removal was similar to undisturbed pens.

Carcass composition is largely unaffected by marketing strategy (Conte *et al*., 2012; DeDecker *et al*., 2005a; Woodworth *et al*., 2000; Bates and Newcomb, 1997); yet, from a practical perspective, the total pounds of pork produced in each cut is economically significant (DeDecker *et al*., 2005a). In the situation in which 25 or 50% of hogs were removed, the control and 25% removed pens produced more poundage than the 50% removed pen, and the 25% removed pen had significantly less ADFI than controls, leading to a strategic, but economically valuable finding (DeDecker *et al*., 2005a).

Further, if pigs are harvested at similar market weights, but differing maturities, it seems logical that the total fat content and fatty acid profiles would differ by marketing time. Therefore, pigs that are closer to their physiological maturity at market weight could be expected to have fat profiles that are more similar when compared to pigs that are still accumulating muscle at a rapid rate versus fat tissue accumulation (Wiegand *et al*., 2011).

Fat Quality

Fat quality affects the entire pork chain as both fresh and further processed products are subject to fat oxidation, color change, and shortened shelf-life in light of poor fat quality. The literature indicates fat quality has been important for decades, but as economic indicators encourage pork producers to use non-traditional fat sources, such as dried distiller's grain with solubles (DDGS) in swine diets, the impact of fat quality has created challenges for end users of pork chain products. Export markets for fresh pork and domestic markets of valued added pork such as sausage and bacon are especially influenced by fat quality as these are opportunities for increased profit margin and require the delivery of high quality products to meet consumer expectations.

Defining Fat Quality

The main components of fat quality include composition, titer (hardness), color, impurities, and stability (Azain, 2001). Recent the pork industry has closely associated iodine value (IV) as a fat quality threshold. The composition of a sample refers to the percentage of each individual FA and is measured through gas chromatography (Azain, 2001). The FA composition of the sample can also impact other fat quality characteristics based on the quantity of SFA and unsaturated FA present. Unsaturated FA have much lower melting points than SFA, with the *cis* double bond configuration having a decreased melting point when compared to the *trans* configuration (Gruen and Duncan, 2007). Additionally, short chain FA have lower melting points than longer chain FA, because the association between FA chains increases as chain length increases (Richards, 2007).

The firmness/hardness of fat is determined by the composition, as different FA have different melting points (Wood *et al*., 2003, 2008). The chain lengths and degree of unsaturation determine both titer and iodine value (Azain, 2001). Although fat color has no association with nutritional quality, it may indicate source and FA composition (Azain, 2001). The stability of a sample refers to the sample's ability to resist breakdown in the presence of oxygen (Azain, 2001). Oxidative rancidity is an important factor of fat quality and must be monitored during processing and retail display as it can determine the shelf-life of meats (Richards, 2007).

Importance of Fat Quality

Fat quality is important in meat products as it can influence further processing characteristics and fresh pork export potential, specifically bellies (Carr, 2005; Apple, 2010). An increased interest in belly fat quality is of particular importance in regards to the processing of bacon. According the National Daily Hog and Pork Summary, primal bellies are the most expensive primal rivaled only by primal ribs (USDA, AMS, 2013). With a market valued at $3.7 billion in 2012, bacon has become a major economic component of a pork carcass (Finkel 2013). However, soft belly fat has become a hindrance in the further processing of the most valuable pork carcass primal into bacon (Apple, 2010). Soft fat can cause bacon slices to stick together, undesirable wet and oily appearance on bacon, translucent and gray color development, separation of lean from fat and rapid oxidative rancidity development (NPPC, 1999, 2000). Furthermore, during sausage manufacturing, heating of the product associated with grinding causes unsaturated fat to melt and form a visually unattractive coating on the surface of the product (Carr *et al.*, 2005).

Measuring Fat Quality

Several techniques and methods have been developed and utilized to measure fat quality and to classify pork bellies based on those measurements. Apple (2010) provides a list of subjective appraisals used by various researchers measuring subcutaneous and belly fat quality. Visual fat and belly firmness has been ranked on a five point scale with one being the softest and five the firmest fat (Skelley *et al*., 1975; Miller *et al*., 1990, Myer *et al*., 1992). Visual oiliness can be ranked on a similar scale with one being extremely oily and five being no oil (Miller *et al*., 1990). Mimicking butchers and consumers, Maw *et al*. (2003) used a thumb and finger squeezing technique to rank fat hardness on a numeric one to five scale with one being the hardest and five the softest. Fat quality indicators such as melting points and firmness have also been correlated to individual FA and FA ratios (Wood *et al*., 1989; Enser, 1984). Fat puncture tests (Dransfield and Jones, 1984), and compression tests of fat and bellies (Nishioka and Irie, 2005; Engel *et al*., 2001; Apple *et al*., 2007) are mechanical methods that measure the force needed to puncture or compress sample cores at a specified level.

In recent years, the belly flop and belly flex tests have become popular methods to determine belly firmness. In a belly flop test, according to Thiel-Cooper *et al*. (2001), bellies are skinned, squared and spareribs are removed prior to suspending the belly along the longitudinal midline on a stainless steel rod and measuring the distance between the ham and shoulder ends both lean side up and down. Whitney *et al*. (2006) developed an equation to determine the upper angle of the triangle created by hanging a belly over a bar. Rentfrow *et al*. (2003) measured the lateral and vertical belly flex over a pipe with skin side down in front of a grid. However, belly temperature, belly thickness, bar

diameter and shape and measurement locations all cause variation and should be considered when using the belly flop or flex methods (Apple, 2010).

Iodine value is also widely used to measure the chemical unsaturation of fat and can be found via titrations (AOAC, 1984), a calculation based on the AOCS (1998) equation or by advancing spectroscopy methods (Herrero, 2008; AOCS 2009c). The NPPC (2000) recommended a maximum IV of 70 for acceptable fat and some plants have moved towards using IV as their sole fat quality indicator. However, the use of iodine value on a large scale is limited by fundamental discrepancies. Whitney *et al*. (2006) reported IV accounts for only 14% of the variation in belly firmness while belly thickness accounted for 33%. Apple *et al*. (2009) found IV differed between individual fat layers and Wiegand *et al*. (2011) reported IV differed within fat depot. Moreover, Trusell *et al*. (2011) found considerable variation exists within fresh bellies in terms of composition and firmness. Iodine value also does not have the ability to distinguish the location of double bonds nor the degree of isomerization (Averette-Gatlin *et al*., 2005). Thus two fats with the same IV could be structurally different; for example, a triglyceride with two saturated fatty acids and one PUFA could have the same IV as a triglyceride with three MUFAs (Averette-Gatlin *et al*., 2005). Standardizing the location for collecting samples and measuring fatty acid composition would help strengthen the use of IV to sort bellies into categories based on belly firmness (Trusell *et al*., 2011).

Dried Distillers Grain with Solubles in Swine Diets

Global production of ethanol animal feed co-products has steadily increased since 2006. The United States is the world leader in grain-ethanol production so not surprisingly the majority of the world's co-products, such as dried distillers grain with solubles (DDGS), originate in the US (FAO, 2012a). In just the last decade, the US production of ethanol and DDGS has boomed from 2.7 million tons in 2000 to 32.5 million tons in 2010 (FAO, 2012b). Dried distillers grain is the primary co-product produced from ethanol production and is simply all that remains of a corn kernel that is not used in fermentation (FAO, 2012b). By the late 2000s, knowledge and research had created a new market for DDGS in the swine industry (FAO, 2012c).

Feed accounts for two thirds of the cost of producing hogs and consequently effort has been made to optimize feed efficiency and lower operating costs (Ewan, 2001; Hollis and Curtis, 2001). Historically, the majority of DDGS produced have been fed to cattle (Shurson *et al*., 2003). However, growth of the ethanol industry in the last decade has greatly influenced the availability and incorporation of co-products in swine and other livestock industries as feedstuffs (Stein and Shurson, 2009). Modern plants have made the use of DDGS more appealing to livestock producers. "New generation" DDGS from modern ethanol plants contain a highly concentrated source of protein and energy in the form of oil and contain higher levels of digestible phosphorus, amino acids and energy compared to older, traditional plants (Shurson *et al.*, 2003). Typically, DDGS contain 6 to 12% oil, consisting of a high percentage of unsaturated FA, (approximately

81% with 54% of that being linoleic acid), and a low percentage (13%) of SFA (Xu *et al*., 2010).

DDGS and Growth Performance

Dried distillers grain with solubles may be incorporated into swine diets at any phase of production. In grower-finisher rations, acceptable growth can be accomplished with inclusion levels up to 30% (Stein and Shurson, 2009). However, inconsistent data has been reported on the effects of feeding high levels of DDGS to finishing hogs on growth performance. In a recent study by Dahlen *et al.* (2011), inclusion of 20% DDGS in the diet did not negatively impact initial or final BW, ADG or ADFI, and gain efficiency was similar compared to a control corn and soybean meal diet. Similarly, Widmer *et al*. (2008, 2007) found 10 or 20% DDGS did not affect ADG, ADFI or G:F. These studies concur with Drescher *et al.* (2008) and Duttlinger *et al.* (2008) who also found swine diets with up to 20% DDGS did not alter growth performance.

Some studies revealed inclusion of DDGS levels up to 30% did not affect overall growth performance (Cook *et al.*, 2005; DeDecker *et al.*, 2005b; Gaines *et al.*, 2007a,b), but in some cases 30% DDGS inclusion lowered G:F compared to controls (Gaines *et al.*, 2007a,b). Fu *et al.* (2004) reported a linear decrease in ADFI, but no difference in G:F when DDGS was included at increasing levels of 0, 10, 20, and 30%.

There are studies that also report high inclusion levels of DDGS negatively impact growth parameters during the growing and finishing phase. Although Cromwell *et al.* (2011) found ADFI and G:F were not affected, ADG was linearly reduced as hogs were fed increasing levels of DDGS (0 to 45%). In hogs fed increasing levels of DDGS (0 to 30%) for 56 d, there was a tendency for linear decrease in ADG and ADFI; while in

the same paper, hogs fed increasing levels of DDGS up to 20% for 78 d, ADG and ADFI decreased linearly (Linneen *et al.,* 2008). Whitney *et al.* (2006) found pigs fed 20 or 30% DDGS had reduced ADG compared to 0 or 10% DDGS; moreover, G:F was decreased in pigs fed 30% DDGS compared to 0, 10 or 20% DDGS. Hinson *et al.* (2007) reported inclusion of DDGS at 10 or 20% in the diet reduced ADG, ADFI and final BW.

From the above-mentioned studies and many others not reported here, there is substantial disagreement on the net effect of high inclusion levels of DDGS in swine grower-finisher diets. Nutrient composition and digestibility varies among DDGS sources (Shurson *et al.*, 2003) and according to Stein and Shurson (2009), discrepancies in pig performance could be attributed to differences in the quality of DDGS. For example, if DDGS contain a low concentration of digestible lysine, pig performance would be expected to decline (Stein and Shurson, 2009).

DDGS and Carcass Composition

Current literature indicates controversial findings on the inclusion of DDGS in swine diets at high levels and the impact on carcass composition and carcass quality. Whitney *et al.* (2006) reported slaughter weight, hot carcass weight (HCW) and dressing percent (DP) decreased linearly with increasing levels (0, 10, 20 or 30%) of DDGS. These data agree with Xu *et al.* (2010), who also observed DP declined linearly at the same inclusion levels, as did last rib back. However, in the same study, due to decreased backfat, pigs fed DDGS had higher percent fat free lean (Xu *et al.*, 2010). Several other studies also reported HCW (Hinson *et al*., 2007; Gaines *et al.*, 2007a), and DP (Dahlen *et al.*, 2011; Gaines 2007a,b) decreased with DDGS in the diet.

Fu *et al.* (2004) examined the effect of 0, 10, 20 or 30% DDGS on carcasses characteristics. No differences were seen in backfat, loin depth, percentage lean or yield in any diet containing DDGS. These results agree with studies by Widmer *et al.* (2007) and Dahlen *et al.* (2011) who found no difference in percent lean, backfat, HCW, DP in DDGS diets compared to controls. Xu *et al.* (2010) analyzed the effects of increasing the content (0, 10, 20 or 30%) of DDGS to pigs in the grow-finish phase on carcass composition and found HCW and ultimate pH were also unaffected by DDGS in the diet. In two experiments Gaines *et al.* (2007a,b) found no difference in loin depth and percent lean or HCW and fat depth, respectively, in DDGS diets compared to controls.

DDGS and Carcass Quality

Conflicting results on the effects of DDGS on carcass quality attributes have also been reported. Whitney *et al*. (2006) saw no meaningful impact on carcass quality when DDGS were including in swine diets; color measurements, visual appraisal of color, firmness, marbling, ultimate pH, water holding capacity, drip loss, cooking loss and WBSF were not affected. This is in agreement with Widmer *et al.* (2008) who found DDGS in the diet had no effect on subjective color, L* or a* values, marbling, drip loss or purge loss. Contrastingly, Xu *et al.* (2010) found loin muscle marbling and firmness decreased with inclusion of 20 and 30% DDGS, but 10 % DDGS had no effect. However, loin muscle color and subjective color were not significantly different (Xu *et al*., 2010).

DDGS and Fat Quality

During fermentation, the starch in corn is converted to ethanol leaving behind three nutrient fractions, protein, fiber and oil. Dried distillers grains with solubles

contain nutrient fractions that are two to three times more concentrated than corn. Consequently, DDGS contain a very high concentration of unsaturated fat in the form of oil (Shurson *et al.*, 2003). Dietary unsaturated FA may impact fat deposition and have a negative impact on pork fat quality.

Several studies have reported belly thickness is not affected by 20% DDGS inclusion in the diet (Dahlen *et al.*, 2011; Xu *et al.*, 2010; Weimer *et al.*, 2008; Whitney *et al.*, 2006). However, it has been published that DDGS negatively impacts belly firmness. Dahlen *et al.* (2011) reported bellies from pigs fed 20% DDGS were softer than pigs fed a control diet. This finding agrees with Cromwell *et al.* (2011), Weimer *et al.* (2008) and Widmer *et al.* (2008) who found belly firmness decreased when DDGS levels increased from 10% to 20% to 30%.

Increased belly softness can be explained by an increase in PUFA in fat tissue. A cooperative study reported PUFA levels increased while SAT and MUFA levels decreased in subcutaneous fat with increasing (15, 30 and 45%) levels DDGS in the diet (Cromwell *et al.*, 2011). Xu *et al.* (2010) demonstrated PUFAs increased and SAT decreased in belly and subcutaneous fat with increased (10, 20 and 30%) levels of DDGS. In a study by McClelland *et al.* (2012), when DDGS increased up to 45% in the diet, PUFA levels in subcutaneous and belly fat increased, while SFA and MUFA deceased.

It has been reported that inclusion of DDGS in the diets of growing/finishing swine increases fat IV. Whitney *et al.* (2006) reported IV of belly fat increased from 66.8 to 72.0 from the control diet to 30% inclusion of DDGS. Xu *et al.* (2010) reported lower initial IVs and also included loin fat IV. The IV of backfat, belly fat and loin fat increased linearly from 58.4 to 72.4, 61.4 to 72.3 and 54.8 to 57.7 respectively when 0 to

30% DDGS were included in the diet (Xu *et al.*, 2010). Benz *et al.* (2010) saw similar trends when only 20% DDGS was fed. The IV of backfat increased from 68.3 to 72.8, jowl fat 70.7 to 73.8 and belly fat 70.2 to 74.5 (Benz *et al.,* 2010). McClelland *et al.* (2012) also found IV of inner and outer backfat and belly fat to increase up to 80.8, 79.1 and 79.5 when diets contained 45% when compared to control diets that produced IVs of 65.1, 63.4 and 65.4, respectively.

Simultaneous Feeding of RAC and DDGS

According to Leick *et al*. (2010) no significant interactions between RAC and DDGS were reported on belly measurements, loin thiobarbituric acid reactive substances (TBARS), FA profiles or iodine value. Findings from Pompeu et al. (2013) concur that inclusion of RAC does not interact with diet source to affect growth performance and carcass measurements. Thus, inclusion of DDGS with RAC follows similar trends previously described. Similar to Widmer *et al*. (2008) and Whitney *et al*. (2006), Leick *et al*. (2010) found DDGS with RAC decreased DP and HCW, yet loin depth, back fat and percent lean were not affected. Furthermore, in accordance with Benz *et al.* (2010), increasing DDGS content increased proportions of PUFAs and consequently increased belly and jowl IV (Leick *et al*. 2010).

Further Processing of Carcasses from Pigs Fed DDGS.

The quality of bacon and other processed pork products largely depends on the fat in those products (McClelland *et al*., 2012), and it has been determined that inclusion of DDGS in swine diets alters the FA profile of pork and pork fat resulting in unsaturated starting materials (Wood et al., 2008). In agreement with Whitney *et al*. (2006), Weimer et al. (2008), Widmer *et al*. (2008) and Leick *et al*. (2010) reported increasing DDGS

negatively impacted belly characteristics by decreasing belly weight, length, thickness and firmness. However, in terms of bacon processing, it is not safe to assume soft bellies are inferior in quality (McClelland *et al*., 2012). Belly weight prior to and after pumping with brine for bacon curing was not different between control and treatment diets containing DDGS (McClelland *et al*., 2012). Although Leick *et al*. (2010) did not find any differences in brine uptake from DDGS or control bellies, McClelland *et al*. (2012) found percent uptake of brine decreases linearly with increasing DDGS.

Belly weights after smoking (Leick *et al*., 2010; McClelland *et al*., 2012) as well as weight after slicing (McClelland *et al*., 2012) did not differ by DDGS inclusion. Slicing yield varied between diets, but no significant differences were found in average slicing yield amongst DDGS and control diets, thereby revealing no apparent issues with slicing high IV bellies on high-speed commercial slicers in this particular study (McClelland *et al*., 2012). These findings agree with an experiment conducted at the same research station by Ulery *et al*. (2010), who found IV of pork bellies did not affect slicing yield. In a pair of studies, LaBerge *et al*. (2011 a,b), feeding 30% DDGS with and without beef tallow had no effect on slicing yield nor did 40% inclusion of DDGS affect bacon smokehouse, slicing or cooking yields.

Shattering scores were decreased with increased DDGS inclusion in the study by McClelland *et al*. (2012), yet LaBerge *et al*. (2011b) saw no differences in bacon shattering from pigs fed 40% DDGS. The same two studies found no differences in shrink or distortion of bacon during cooking (McClelland *et al*., 2012; LaBerge *et al*., 2011b). This agrees with Widmer *et al*. (2008) and Leick *et al*. (2010) who also reported no differences in sliced bacon cooking loss and bacon distortion as DDGS was added to

the diet. Further, bacon TBARS were not affected by inclusion of DDGS in the diet (Leick *et al*., 2010).

Sensory characteristics of bacon are often not impacted by inclusion of DDGS in the diet. Panelists did not detect off flavors or tenderness differences in bacon from bellies of pigs fed increasing DDGS (McClelland *et al*., 2012). These findings concur with Widmer *et al*. (2008) and Xu *et al*. (2010) who also found no differences in bacon flavor or crispiness either. Panelists did however find bacon fattiness and tenderness reduced linearly with increased DDGS levels in the study by Xu *et al*. (2010).

At the current time, very little data has been published on sensory evaluation of sausage type products from pigs fed DDGS. McClelland *et al*. (2012) found TBARS of bratwurst sausage from pigs fed 30 or 45% DDGS increased to a greater extent than control or 15% DDGS from days 0 to 7 in a shelf life study. Sensory panelists detected increased juiciness and scored sausage from DDGS pigs as having a softer texture, but no off flavors were reported (McClelland *et al*., 2012). A study was performed with sows fed 30% DDGS during gestation and 15% DDGS during lactation and then used to make sausage; results collected from a taste panel found fresh and cooked bratwurst from these animals had a less desirable appearance, but DDGS did not affect texture, taste or overall acceptability (Wert *et al.*, 2009).

Quality characteristics of fresh whole muscle products were not significantly impacted by inclusion of DDGS. Widmer *et al*. (2008), Xu *et al*. (2010) and McClelland *et al*. (2012) reported no differences in tenderness, juiciness or off flavor in fresh pork chops. Xu *et al*. (2010) found LM fat TBARs were not affected by inclusion of DDGS

in diet. Not until day 21 did loin TBARS from 30, 45 and 60% DDGS differ significantly with higher values from 0 or 15% (Leick *et al*. 2010).

Laboratory Methods For Determining Fat Quality

Fat Extraction

Water insoluble lipids easily dissolve in chemicals such as benzene, chloroform, diethyl ether, hexanes or methanol (Shahidi and Wanasundara, 2002). There are three fat extraction methods widely used in current laboratory analysis. Ether extraction of fat according to AOAC Method 960.39 (AOAC, 2007) utilizes dried fat samples and allows ether to drip through them. After six hours, samples are dried and fat percentage is determined by weight loss (Dow *et al*., 2011). Rapid determination of moisture and fat in meat by microwave and nuclear magnetic resonance is described by Keeton *et al.* (2003). A CEM SMART Trac rapid fat analysis system can be used to determine fat percentage on a dry basis using nuclear magnetic resonance and converted to a wet basis (Dow *et al*., 2011).

Fatty acids can also be extracted using the Folch Method according to Folch et al. (1957) and Morrison and Smith (1964). Fat percentage can be determined directly by weight of extracted fat (Dow *et al*., 2011) and FA profiles can be determined using gas chromatography. In comparison, the Folch and ether methods extract similar amounts of fat, greater than what CEM can detect (Dow *et al*., 2011). Numerically speaking, the Folch method extracts the greatest amount of fat, but this is possibly an overestimation due to the extraction of phospholipids in membranes as well as the triglycerides of neutral

fat (Dow *et al*., 2011). Yet, the Folch method holds significant relevance as the FA composition can be determined and reported quantitatively by column gas chromatography from extracted methyl esters of fatty acids (AOCS, 2009a).

Extracting Fatty Acids via Folch Method

The Folch solvent, 2:1 chloroform:methanol is most efficient at extracting lipids because it has both polar and non-polar components (Shahidi and Wanasundara, 2002). During extraction, samples are initially homogenized in the chloroform:methanol mixture. Homogenizing samples reduces particle size and increases surface area allowing increased contact of the solvent (Shahidi and Wanasundara, 2002). Potassium chloride (KCl) is added to a filtered sample and two distinct layers form. The upper aqueous phase is removed while the lower phase containing tissue lipids (Folch *et al*., 1957) is evaporated with nitrogen.

Potassium hydroxide (KOH) is added to the dried sample to initiate the saponification reaction, which hydrolyzes FA from a triglyceride molecule. During, the saponification reaction, a strong base such as KOH attacks the carbon of the ester joining glycerol to a FA. The FA is cleaved from the glycerol backbone with the donated OH^- ion forming a carboxylic acid. The hydrogen of the acid is taken by the remaining negative ion to form glycerol leaving the free FA with a net negative charge. At this point, free FAs seek something to bind the net negative charge and inhibit reformation of triglycerides. Boron triflouride in methanol is therefore added to act as an acid catalyst in the transesterification reaction that methylates the acid group on the free FA removing the net negative charge. The remaining molecule is known as a fatty acid methyl ester (FAME).

Following FAME formation, hexane and saturated sodium chloride (NaCl) are added which forms two layers. The upper hexane layer is removed and added to sodium sulfate (Na_2SO_4) to remove any moisture in the sample. The dehydrated hexane containing FAMEs is evaporated to dryness once more. Drying stabilizes the FAMEs for storage. The sample is reconstituted with hexane and transferred to chromatograph vials. The stable FAMEs are loaded into a gas chromatograph to determine fatty acid profiles.

Iodine Value Determination

Iodine value (IV) is a measure of the unsaturation of FA and is expressed in terms of the number of grams of iodine absorbed per 100 g of sample (AOCS, 2009b). Notably, IV can be measured from several locations or fat depots on a carcass including but not limited to the anterior tip of the jowl, the belly from a location on the midline posterior to the sternum and anterior to mammary tissue, subcutaneous backfat from the 10^{th} rib or intermuscular fat. Several methods explained below are available to determine iodine value.

IV Titrations. Chemical IV titrations can be performed according to the WIJS method (AOAC, 1984). Fat samples must be melted to aid dissolving in 1:1 cyclohexane:acetic acid. Excess iodine is added in the form of WIJS solution (iodine monochloride, ICl). Samples are incubated to provide iodine and chloride molecules the opportunity to bind the carbons of double bonds in unsaturated fats. After the incubation period, the reaction is stopped with 150 ml of water and potassium iodide (KI) is added to bind with any iodine from the WIJS solution that did not bind to double bonds in the adipose tissue. The reaction produces potassium chloride (KCl) and iodine (I_2) molecules. Samples are titrated with sodium thiosulfate ($Na_2S_2O_3$), which reacts with I_2

to form colorless iodide ions (I^-). Starch solution is used as an indicator and once all the iodine has reacted, the solution is colorless. The volume needed to induce complete color changes can be used to calculate the iodine value via the amount of iodine present. If a large volume of $Na_2S_2O_3$ is used, there was a large amount of iodine left in the solution, meaning the iodine did not have many carbons of double bonds to bind, thus yielding a lower iodine value. On the other hand, if only a small volume of $Na_2S_2O_3$ is used, there was very little iodine left after binding carbons of double bonds and the iodine value would be higher.

Gas-Liquid Chromatography (GC). Gas-liquid chromatography separates mixtures of volatile compounds based on their ability to dissolve in inert material or volatilize and move through a separation column in a current of inert gas. To determine the FA profile of a tissue via GC, the FA must be extracted and converted into FAMEs. When the FAMEs are loaded into a GC column, the column is heated to volatilize the compounds. The order of elution depends on the adsorbent in the column and boiling point of the FA. Thus, mixtures of FA with varying chain lengths and degrees of saturation can be separated and quantified (Nelson and Cox, 2008) into a FA profile. The IV from FA profiles can then be calculated according to the equation described by AOCS (1998).

Fourier Transform Near Infrared (FT-NIR). According to the AOCS (2009c), FT-NIR determines IV using near infrared spectra in the C-H 2nd overtone stretching region. When molecules absorb near infrared radiation, the energy transitions the molecule from a ground to excited state. This jump creates vibrations read as overtone bands. Different types of bonds create different bands. The ratio of band intensities

correlates with IV (AOCS, 2009c). The Bruker Corporation manufactures and sells FT-NIR products for lab and production line purposes. According to the Bruker Corporation (2013), FT-NIR "offers a practical alternative to time consuming, wet chemical methods and chromatographic techniques." The equipment requires no sample preparation and provides timely quantitative and qualitative data (Bruker Corporation, 2013).

Raman Spectroscopy. Raman spectroscopy offers advantages over traditional methods as it is a direct, non-invasive technique that requires small sample portions (Herrero, 2008). Raman spectroscopy involves a light scattering technique where a molecule scatters light from a high intensity laser light source (Horiba Scientific, 2013). There is a small amount of light that is scattered at different wavelengths known as Raman scatter that depends on the chemical structure of the molecule. The resulting spectrum provides peaks that correspond to specific molecular bond vibrations from individual bonds such as C-C and C=C, thereby providing information pertaining to the chemical structure and identity of the molecules in a sample (Horiba Scientific, 2013). The Raman spectra is also very sensitive to the configuration of double bonds and distinguishes differences in stretching vibrations of cis and trans configurations (Afseth and Larat, 2013). Iodine value can be estimated directly by measuring the Raman spectra of the sample (Herrero, 2008). The relative intensity of stretching vibrations can estimate the number of single and double bonds as well as overall chain length. A mathematical model can then be developed to predict iodine value based on number of double bonds and chain length.

CHAPTER 2

DETERMINING PORK FAT QUALITY OF PIGS FED 20% DDGS AS MEASURED BY THREE METHODS WITH A STANDARD INDUSTRY MARKETING STRATEGY

ABSTRACT

Fat quality is important in meat products as it can influence further processing characteristics and pork export potential. The use of non-traditional fat sources such as dried distillers grain with solubles (DDGS) changes pork fat quality and has created challenges for end users of pork chain products. An experiment was designed to evaluate the effects of DDGS and split marketing cuts in a commercial swine facility on growth performance, fat quality and the relationship between iodine value (IV) determined by three methods in two fat depots. Pen (n = 40) was the experimental unit with 20 replications per treatment and 22 pigs per pen. Pigs were randomly allotted to dietary treatments in a 2 x 3 factorial arrangement with two levels of DDGS (0 or 20%) and chosen for one of three marketing cuts removing 4, 8 and 10 head from each pen. Fat tissue samples were removed from the anterior tip of the jowl and posterior to the

sternum on the belly edge 1d postmortem. Fatty acid composition was determined via the Folch method and IVs were calculated from chemical titrations, fatty acid profile (GC IV) and in-plant Bruker® near infrared (NIR) spectroscopy. Correlations between IV determination methods were calculated. Inclusion of 20% DDGS did not change ($P > 0.05$) growth performance while marketing cut affected performance with the second cut producing the heaviest and most efficient hogs ($P < 0.01$). Total SFA and MUFA concentrations were higher ($P < 0.01$) in belly and jowl fat from hogs fed 0% DDGS. Total PUFA and the PUFA:SFA in belly and jowl fat was higher ($P < 0.01$) when 20% DDGS was fed. DDGS inclusion increased IV in belly and jowl fat regardless of IV determination method. Regardless of dietary treatment or fat depot, Pearson Correlation Coefficients between titration and GC IV, titration and NIR, and GC IV and NIR were 0.46 ($P < 0.01$), 0.68 ($P < 0.01$) and 0.43 ($P < 0.01$), respectively. These correlations suggest methods may rank samples equally, but do not provide the same absolute IV. Belly fat had a lower IV ($P < 0.01$) compared to jowl fat using titration or GC IV methods suggesting pigs have varied degrees of physiological maturity at specific fat depots during the finishing phase. In conclusion, feeding 20% DDGS negatively affected fat quality, but not growth performance and marketing time changed growth performance.

Introduction

The inclusion of unsaturated dietary fat sources compounded with lean biological types of the modern day pig has created issues surrounding pork fat quality. Fat quality

is important in meat products as it can influence further processing characteristics and pork export potential (Carr, 2005). The inclusion of dried distiller's grains with solubles (DDGS) in swine diets has increased over the past 10 years due to a rise in ethanol production and greater availability of co-products for incorporation in livestock diets (Stein and Shurson, 2009). Previous studies have indicated the inclusion of unsaturated oils in swine diets increases PUFA and decreases SFA and MUFA content in various fat tissues (Ellis and Isbell, 1926, Xu *et al*., 2010, Benz *et al*., 2010).

Furthermore, proportions of fatty acids are directly related to degree of fatness (Martin *et al*., 1972). As animals reach maturity and backfat depth increases, proportions of linoleic acid decreases (Greer *et al*., 1965; Wood *et al*., 1978, 1986). According to Wood *et al*. (1984), saturated fatty acids increase and proportions of unsaturated fatty acids such as linoleic acid decrease with increased fat accumulation due to increased de novo fat synthesis and less dietary influence.

Fattening patterns of food animals appears to be from the distal ends and toward the visceral cavity. These patterns would indicate that finishing pigs would likely deposit fat earlier in the jowl and over the front shoulder prior to deposition of fat in the loin and belly region (Hammond, 1932). If pigs are harvested at similar market weights, but differing maturities, it seems logical that the total fat content and fatty acid profiles would differ by fat depot. Therefore, pigs that are closer to their physiological maturity at market weight could be expected to have fat profiles that are more similar when compared to pigs that are still accumulating muscle at a rapid rate versus fat tissue accumulation (Wiegand *et al*., 2011).

Iodine value (IV) measures the degree of unsaturation via presence of double bonds (AOCS, 2009c) and has been widely used an indicator of fat quality (NPPC, 2000). The IV can be determined by a number of methods including chemical titrations, calculated from fatty acid concentrations and rapidly evolving spectroscopy methods. However, the relationship between methods must be determined before less expensive techniques can replace the gold standard wet lab methods.

Standardizing location for collecting samples and measuring FA composition and/or IV would strengthen the ability to sort carcasses into categories based on belly firmness. Thus, the objective of this experiment was to determine pork fat quality as indicated by iodine value of belly and jowl fat determined by three methods from commercially raised pigs fed 20% DDGS and slaughtered using an standard industry split marketing strategy.

Materials and Methods

Animals

The University of Missouri Animal Care and Use Committee approved animal care and experimental protocols prior to initiation of this experiment. A total number of 40 pens containing 22 crossbred barrows (initial body weight = 43.17 kg; PIC 1050 X PIC 337 genetics) were housed in a typical commercial grow-finish facility, with fully slatted concrete floors, stainless cup waters and four hole stainless feeders with 127 cm of linear feeder space. Feed and water was offered *ad libitum* throughout the study. The

pens were 2.44 m wide and 5.80 m long providing a total of 14.12 m^2 of floor space. Pens provided 0.64 m^2 of floor space at initial stocking.

Diets

Twenty pens were randomly assigned to receive a control corn-soy diet (Table 1) and 20 pens were fed a diet containing 20% DDGS (Table 2). All phases of feeds were mixed at a central feed mill prior to delivery to the facility. Ractopamine was included during phase IV (100 kg to market weight) of both control and DDGS diets. Feed was augured in from outside feed tanks (separate tanks were used for each treatment feed) into the equipment room where it was pre-weighed in batch form via the Howema mixer prior to its distribution to individual pens. Feed was issued three times per day and feed issue amount at each feeding was recorded via the computerized feed system. Feed weigh backs were taken each time pigs were weighed in order to calculate interim performance.

Growth Performance and Marketing Cuts

Body weight (BW), average daily gain (ADG), average daily feed intake (ADFI) and gain to feed ratio (G:F) were calculated for each pen on days 20, 38, 70, 89, 95, 109 and 116. Finished hogs were marketed in three cuts on days 95, 109 and 116. The heaviest hogs, with appropriate finish, were removed first taking 4, 8 and 10 head per pen in the first, second and third marketing cuts, respectively. For block one, pen density decreased from 0.64 m^2 per pig to 0.78 m^2 after first cut and to 1.01 m^2 after the second cut. For block two, pen density decreased from 0.64 m^2 per pig to 0.74 m^2 after first cut and to 0.94 m^2 after the second cut.

Harvest and Sample Collection

At each marketing cut, hogs were tattooed and transported to Farmland Foods, Inc. in Milan, Missouri where hogs were humanely slaughtered following standard U.S. pork industry practices and USDA/FSIS inspection criteria. Belly and jowl tissue samples were collected 24 h post mortem from chilled carcasses. Belly samples were taken from a region on the midline posterior to the sternum and anterior to mammary tissue and jowl samples were taken from the anterior tip of the jowl region at the site of head removal. Samples were sealed in Ziplock® bags and transported to the University of Missouri Meat Science Laboratory where samples were labeled and frozen at -20.0°C until analysis.

Sample Analysis

Fatty Acids and GC IV Determination. All samples were separated from any muscle, skin and/or lymph gland tissue and ground prior to fatty acid analysis. Fatty acid profiles were determined according to an adaptation of the methodologies described by Folch *et al*. (1957) and Morrison and Smith (1964). At the moment of analysis, approximately 100 mg of adipose tissue was homogenized in 5 mL of chloroform:methanol ($CHCl_3$:CH_3OH, 2:1, v/v) in a glass tube to extract lipids and samples were filtered through a sintered glass funnel fitted with a Whatman 2.4 cm GF/C filter.

A volume of 8 mL of 0.74% KCl was added to each sample and after two hours of rest, two distinct phases formed. The upper phase was carefully removed and discarded while the lower phase was evaporated to dryness with nitrogen gas in a heated water bath at 70°C. At the point of dryness, 1 mL of 0.5 N KOH in MeOH was added to each tube

and heated for 10 minutes in a 70°C water bath. Following this, 1 mL of 14% boron trifluoride (BF_3) in MeOH was added and samples were flushed with nitrogen and heated in the 70°C water bath for an additional 30 minutes in order to form fatty acid methyl esters (FAME).

After cooling to room temperature, FAMEs were extracted by adding 2 mL of HPLC grade hexane and 2 mL of saturated NaCl. Two distinct layers formed; the upper layer was removed and added to approximately 800 mg of Na_2SO_4. At this point, an additional 2 mL of hexane were added to the tube containing NaCl and once more, the upper layer was removed and added to the tube containing Na_2SO_4. The hexane portion was removed from the salt and added to a labeled scintillation vial. The salt was rinsed a final time with 1 mL of hexane and the liquid was added to the vial. Samples were evaporated to dryness in a water bath at 70°C under nitrogen flow. Lastly, samples were reconstituted with 1 ml HPLC grade hexane and transferred to gas chromatograph vials.

The stable FAMEs were loaded into a Varian 3800 gas chromatograph (Varian, Palo Alto, CA) to determine fatty acid profiles. The GC column utilized was a fused silica capillary column (SPTM – 2560; 100 m x 0.25 mm x 0.2 μm film thickness; Supelco, Bellefonte, PA). Temperature of the injector was held constant at 240°C and temperature of the flame-ionization detector was held at 260°C. The oven operated at 140°C for 5 min, then temperature programmed at 2.5°C/min to 240°C and held for 16 min. Helium, the carrier gas, was maintained at a constant pressure of 255.11 kPa. Individual fatty acid areas were normalized and so that the area under each peak represents a percentage of the total area.

Total saturated fatty acid (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acid (PUFA) contents were calculated according to the following equations: SFA = (C10:0 + C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C22:0 + C23:0); MUFA = (C14:1 + C15:1 + C16:1 + C17:1 + C18:1n9t + C18:1n9c + C18:1n7 + C20:1 + C22:1n9 + C24:1); PUFA = (C18:2n6t + C18:2n6c + C18:3n6 + C18:3n3 + C18:9c11t + C18:10t12c + C18:9c11c + C18:9t11t + C20:2 + C20:3n6 + C20:3n3 + C20:4n6 + C22:5n3 + C22:6n3). The ratio between PUFAs and SFAs was calculated using the equation: [(C18:2n6c) + (C18:3n3)]/[(C14:0 + C16:0 + C18:0)]. The following equations were used to calculated total omega 3 and omega 6 fatty acid content: total omega 3 = C18:3n3 + C20:3n3 + C22:5n3 + C22:6n3); total omega 6 = (C18:3n6 + C20:3n6 + C20:4n6). Finally, IV from fatty acid profiles were determined according to the equation described by AOCS (1998): IV = (0.95 x C16:1) + [0.86 x (C18:1n9t + C18:1n9c)] + [1.732 x (C18:2n6t + C18:2n6c)] + (2.616 x C18:3n3) + (0.785 x C20:1).

***IV Titrations*.** Iodine value titrations were performed according to a modified WIJS method (AOAC, 1984). Adipose tissue was melted and approximately 0.6 to 0.8 g were place in a 500 ml Erlenmeyer flask and dissolved in 15 ml of 1:1 cyclohexane:acetic acid. Samples were incubated for 30 min in 25 ml of WIJS (iodine) and the reaction was stopped with 150 ml of water. Fifteen mL of 15% KI was added and samples were titrated within 30 min of stopping the reaction with sodium thiosulfate. The volume needed to produce colorless ions was recorded and used to calculate iodine value using the following equation: [(titration volume of blank – titration volume of sample) × 0.1 ×

12.69] / weight of sample, where volumes are in mL, 0.1 is the normality of sodium thiosulfate, 12.69 is a constant and weight is in grams .

IV by near infrared imaging. Rapid NIR determination of IV was performed using a Bruker® NIR system currently utilized by Farmland Foods, Inc., for in-plant measurement of the belly fat depot. The NIR equipment was used according to manufacturers' recommended operating procedures for this application.

Statistical Analysis

The experiment was defined as a 2 x 3 factorial arrangement. Growth performance data was analyzed using the PROC MIXED procedure of SAS (SAS Inst., Cary, NC) with pen serving as the experimental unit. Iodine values were analyzed using the PROC GLM procedure of SAS and correlations between iodine determination methods were calculated with PROC CORR procedure of SAS. The statistical model included the fixed effects of marketing cuts (95, 109 or 116 days on feed) and dietary treatment (control corn and soy diet or corn and soy with 20% DDGS). Fixed effects were arranged as factorials within a completely randomized design. Least squares means and standard errors were estimated. Level of significance was predetermined at $P < 0.05$.

Results

Growth Performance

Growth performance, shown in Table 3, was not significantly impacted by the inclusion of 20% DDGS in the diet. No difference was detected in final BW, ADG, ADFI or G:F between pens fed control and pens fed 20% DDGS. The final BW of the

first, second and third marking cuts increased over time and all groups differed significantly ($P < 0.0001$) from each other. The ADG of pens from each marketing cut differed ($P < 0.0001$) with the second marketing cut having the greatest ADG. Pen ADFI also increased over time and each marketing group differed significantly ($P < 0.0001$) from each other. The G:F ratio of pigs in the third marketing cut was significantly lower ($P < 0.0001$) from pigs in the first and second marketing cuts.

Fatty Acid Composition

Marketing time did not affect total SFA, MUFA, PUFA or PUFA:SFA in belly or jowl fat. Belly fat (Table 4) of pigs fed the control diet had significantly ($P < 0.0001$) higher levels of C16:0, C16:1, C18:0, C18:1n9c and total SFAs and MUFAs. The levels of C18:2n6c, C18:3n3, C20:4n6 and total PUFAs were significantly ($P < 0.0001$) higher in the belly fat of pigs fed 20% DDGS (Table 4). Belly fat from pigs fed 20% DDGS also had significantly higher levels of total n3 FA ($P = 0.02$) and total n6 FA ($P = 0.01$) as well as a higher PUFA:SFA ($P < 0.0001$).

Similar trends in the fatty acid composition were observed in jowl fat. Jowl fat (Table 5) from pigs fed the control diet had significantly ($P < 0.0001$) higher levels of C16:0, C16:1, C18:0, C18:1n9c, total SFAs and total MUFAs. The levels of C18:2n6c, C18:3n3, C20:4n6, total MUFAs and the PUFA:SFA were significantly ($P < 0.0001$) higher in the jowl fat of pigs fed 20% DDGS. Feeding DDGS also significantly increased total n3 FA ($P = 0.02$) and total n6 FA ($P = 0.01$) in jowl fat.

IV of Belly and Jowl

Inclusion of 20% DDGS significantly increased iodine value of belly fat (Table 6) when determined by titration ($P = 0.0004$), GC calculation ($P < 0.0001$) or NIR

spectroscopy ($P < 0.0001$). Similar to the belly depot, the iodine value of jowl fat (Table 7) significantly increased with 20% DDGS in the diet when iodine value was determined via titration ($P = 0.0004$) or GC calculation ($P < 0.0001$). Iodine value of belly fat was significantly ($P = 0.01$) different between marketing cuts when determined by NIR spectroscopy. Regardless of diet or marketing cut the iodine value of belly fat was significantly ($P < 0.0001$) lower according to both the titration and GC determination methods (Table 8).

Iodine Value Determination Methods

Regardless of dietary treatment, marketing cut or fat depot, Pearson Correlation Coefficients between titration and GC IV, titration and NIR, and GC IV and NIR were 0.46 **(**$P < 0.0001$), 0.68 **(**$P < 0.0001$) and 0.43 **(**$P < 0.0001$), respectively (Table 9). Pearson Correlation Coefficients between titration and GC IV, titration and NIR, and GC IV and NIR of belly fat from pigs fed the control diet were 0.19 **(**$P = 0.07$), 0.45 **(**$P < 0.0001$) and 0.46 **(**$P < 0.0001$), respectively (Table 10). Pearson Correlation Coefficients between titration and GC IV, titration and NIR, and GC IV and NIR of belly fat from pigs fed the DDGS were 0.36 **(**$P < 0.0005$), 0.53 **(**$P < 0.0001$) and 0.32 **(**$P = 0.0027$), respectively (Table 11). Pearson Correlation Coefficients between titration and GC IV for jowl fat from pigs fed a control diet (Table 12) and DDGS (Table 13) were 0.32 ($P = 0.002$) and 0.20 ($P = 0.06$), respectively.

Discussion

In agreement with several studies, our results show inclusion of 20% DDGS in the diet did not negatively impact final BW, ADG, ADFI or gain efficiency (Dahlen *et al*., 2011; Widmar *et al*., 2008, 2007; Drescher *et al*., 2008; Duttlinger *et al*., 2008). However, marketing time significantly influenced growth parameters, specifically ADG, in the pigs remaining after the first cut, but removed prior to close-out of the barn. By removing the fast growing, early maturing hogs in the first cut, feeder and floor space expanded allowing the remaining hogs to more closely meet their genetic potential. The genetically superior animals grew faster and gained more thereby surpassing the slow growing, late maturing hogs left in the third cut.

Inclusion of DDGS in the diet decreases SFA and MUFA while increasing PUFA regardless of fat depot (McClelland *et al*., 2012; Benz *et al*., 2010; Xu *et al*., 2010; Apple *et al*., 2009; Whitney *et al*., 2006). Xu *et al*. (2010a), Ulery *et al*. (2010) and McClelland *et al* (2012) further reported that with increasing dietary DDGS content, amounts of SFA and MUFAs were reduced and PUFAs were increased in belly fat. These findings agree with Benz *et al.* (2010) who found increasing levels of dietary DDGS led to a linear increase in C18:2n6, C20:2, total PUFA and PUFA:SFA, and a linear decrease in C16:0, C18:1n7, and total MUFA for belly fat, back fat, and jowl fat samples.

As early as 1926, research demonstrated feeding corn and soybean oils resulted in a greater amount of unsaturated pork fat compared to peanut and rice oils (Ellis and Isbell, 1926)). The fatty acid profile of monogastrics is easily manipulated as dietary fat passes through the digestive system relatively unchanged and the changes can occur

within 14 days of altering the diet (Warnants *et al*., 1999; Wiseman and Agunbiade, 1998). In particular, essential long chain PUFA are closely linked to dietary concentrations (Wood *et al*., 2008; Wood and Enser, 1997). Although swine are capable of synthesizing many fatty acids in vivo, they lack the desaturase enzymes needed to make the essential linoleic and linolenic acids. In contrast, SFA and MUFAs synthesized in vivo are less readily influenced by diet (Wood and Enser, 1997). According to Allee *et al.* (1971) increasing dietary fat levels in pigs diets also inhibits de novo synthesis. Pigs show preferential deposition of dietary fat rather than utilizing valuable energy and extracting fat from internal sources (Allee *et al*., 1971). Belly and jowl fat from pigs fed DDGS have increased unsaturation indicating the large dietary influence on the fatty acid profile.

Iodine values, which measure the degree of unsaturation, directly reflect the fatty acid levels from fatty acid analysis. Pigs fed 20% DDGS had significantly higher (more unsaturated) IV when compared to control diet diets. Whitney *et al*. (2006) and Benz *et al*. (2008) also reported GC calculated iodine value of belly fat increased with increasing levels of DDGS in the diet. In our study, regardless of determination method or depot, IV was significantly impacted by the inclusion of DDGS in the swine diet. Dried distillers grain with solubles is a very highly concentrated source of protein and energy in the form of oil (Stein and Shurson, 2009) and is the likely contributor to increased IV in fat.

As pigs develop, fat accumulates first at the distal ends prior to deposition in the visceral cavity (Hammond, 1932). Subcutaneous fat also develops in three distinct layers, with each layer developing independently at differing rates (Koch *et al*., 1968;

McDonald and Hamilton, 1976). Studies have provided evidence that IV differs by fat layer (Apple *et al*., 2009) as well as by depot (Wiegand *et al*., 2011) suggesting the fatty acid profile of pigs will change with maturation from early in life to time of slaughter (Wood *et al*., 2008). As pigs accumulate fat mass and protein accretion slows, the influence of de novo synthesis becomes increasingly prevalent (Wood *et al*., 2008). Wood *et al*. (1984) reported that as a pig fattens, 18:0 and 18:1 proportions increase while 18:2n6 levels decrease. Therefore, pigs and individual tissues at differing maturities have different levels of saturation. Jowl fat consistently yields a higher iodine value, suggesting jowl fat is physiologically more mature.

Although significant, iodine value methods are not strongly correlated. The three methods are able to rank samples equally, but do not provide the same absolute IV. Wiegand *et al*., (2011) also reported weak correlations between jowl and belly fat regardless of dietary treatment. Each method used in the current study quantified different substrates and there may be unaccounted for variation between methods as well as unknown error in the methods. Standardizing location and method for collecting data would strengthen the use of iodine value to sort bellies into categories based on firmness (Trusell et al., 2011).

The current industry perception is soft bellies are inferior quality and hinder further processing. However, several studies have reported bacon processing and yield are unaffected by inclusion of DDGS in the diet (McClelland *et al*., 2012; Leick *et al*., 2010; Ulery *et al*., 2010; LaBerge *et al*., 2011 a,b). Further, extensive variation in IV exists within bellies (Trussel *et al*., 2011) complicating the ability to use a single sample from a raw belly to accurately predict the quality of an entire belly.

Implications

Inclusion of 20% DDGS did not negatively impact overall growth performance of hogs raised in a commercial setting, but increased iodine value. Differences in iodine value between fat depots and marketing times suggest differences in rate of lipid tissue accumulation. Our data suggests using IV as a range versus a threshold would be more beneficial as an industry application, however, there is a need to understand processing characteristics and shelf life of products with elevated IV prior to standardizing IV in a commercial setting. In conclusion, these results suggest feeding 20% DDGS negatively affects fat quality but not growth performance and decreasing pen density with marketing cuts impacts growth.

Table 1. Composition of the grower-finisher control diets

	Phase I (32-55 kg)	Phase II (55-77 kg)	Phase III (77-100 kg)	Phase IV (100 kg-market)
Corn	683.62	725.71	754.76	671.51
SBM 48	193.18	152.27	125.00	209.09
DDGS	0.00	0.00	0.00	0.00
CWG	9.09	9.09	9.09	9.09
Monocal	2.95	2.27	1.82	1.14
Limestone	10.91	10.80	10.00	9.55
Salt	4.55	4.55	4.55	4.55
L-Lysine	2.32	2.23	2.00	2.00
Alimet	0.39	0.09	0.00	0.30
L-Threonine	0.64	0.64	0.43	0.92
Vitamin premix + phystase	0.45	0.45	0.45	0.27
Trace Mineral Premix	0.91	0.91	0.91	0.36
Optiphos	0.08	0.08	0.08	0.09
Paylean	0.00	0.00	0.00	0.23
	909.09	909.09	909.09	909.09
NRC ME (Mcal/lb)	1.53	1.54	1.54	1.54
SID, Lysine %	0.93	0.81	0.72	0.95
Available P, %	0.28	0.26	0.25	0.20
Ca, %	0.58	0.55	0.50	0.50
SID M + C:Lys	58.25	58.24	60.88	58.13
SID Thr:Lys	17.42	65.21	65.07	68.10
SID Ile:Lys	63.55	63.74	65.21	65.60
SID Val:Lys	73.00	74.73	77.76	74.88

Table 2. Composition of the grower-finisher diets containing 20% DDGS

	Phase I (32-55 kg)	Phase II (55-77 kg)	Phase III (77-100 kg)	Phase IV (100 kg-market)
Corn	539.58	579.76	607.74	528.37
SBM 48	159.09	117.27	90.91	170.45
DDGS	181.82	181.82	181.82	181.82
CWG	9.09	9.09	9.09	9.09
Monocal	0.00	0.00	0.00	0.00
Limestone	10.23	12.05	10.91	10.23
Salt	4.55	4.55	4.55	4.55
L-Lysine	2.77	2.73	2.45	2.73
Alimet	0.16	0.00	0.00	0.20
L-Threonine	0.36	0.39	0.18	0.80
Vitamin premix + phystase	0.45	0.45	0.45	0.27
Trace Mineral Premix	0.91	0.91	0.91	0.36
Optiphos	0.08	0.08	0.08	0.00
Paylean	0.00	0.00	0.00	0.23
	909.09	909.09	909.09	909.09
NRC ME (Mcal/lb)	1.52	1.52	1.52	1.52
SID, Lysine %	0.92	0.80	0.71	0.95
Available P, %	0.34	0.34	0.33	0.27
Ca, %	0.49	0.55	0.50	0.50
SID M + C:Lys	58.18	59.51	63.85	58.10
SID Thr:Lys	17.51	65.06	65.22	68.12
SID Ile:Lys	66.56	66.83	69.11	66.84
SID Val:Lys	78.52	80.68	84.95	78.43

Table 3. Growth performance means of hogs fed 20% DDGS and marketed in three cuts

	Control			20 % DDGS				P-value		
Item	First Cut	Second Cut	Third Cut	First Cut	Second Cut	Third Cut	SEM	Diet	Cut	D x C[1]
Final BW, kg	115.03[c]	128.94[b]	131.11[a]	114.56[c]	128.94[b]	131.40[a]	0.89	0.87	<0.0001	0.93
ADG, kg	0.79[b]	1.11[a]	0.88[c]	0.78[b]	1.16[a]	0.87[c]	0.03	0.83	<0.0001	0.73
ADFI, kg	2.01[c]	2.80[d]	3.03[a,b]	2.02[c]	2.97[b]	3.12[a]	0.05	0.08	<0.0001	0.22
G:F	0.39[a]	0.39[a]	0.29[b]	0.39[a]	0.39[a]	0.28[b]	0.12	0.12	<0.0001	0.99

[a-d] LS means with in a row lacking common superscripts differ at P < 0.05.
[1] D x C = Diet by cut interaction

Table 4. Interactive effects of 20% DDGS in the diet and marketing time on fatty acid (FA) composition of belly fat

Item	Control			20 % DDGS			SEM	P-value		
	First Cut	Second Cut	Third Cut	First Cut	Second Cut	Third Cut		Diet	Cut	D x C[1]
Palmitic Acid (16:0)	23.53	24.17	23.52	22.60	23.30	22.50	0.28	<.0001	0.18	0.76
Palmitoleic Acid (16:1)	2.25	2.34	2.67	2.05	2.07	1.98	0.06	<.0001	0.66	0.62
Stearic Acid (18:0)	11.84	12.00	11.86	10.74	11.05	10.83	0.23	<.0001	0.14	0.98
Oleic Acid (18:1n9t)	0.12	0.20	0.39	0.11	0.13	0.09	0.09	0.02	0.15	0.04
Oleic Acid (18:1n9c)	40.14	41.61	40.56	38.22	40.70	38.03	0.40	<.0001	<.0001	0.06
Linoleic Acid (18:2n6t)	0.10	0.08	0.08	0.08	0.08	0.07	0.01	0.003	0.007	0.40
Linoleic Acid (18:2n6c)	14.06	13.78	14.09	18.41	18.58	19.35	0.52	<.0001	0.78	0.75
Linolenic Acid (18:3n3)	0.57	0.60	0.71	0.63	0.60	0.67	0.04	0.05	<.0001	0.30
Arachidonic Acid (20:4n6)	0.07	0.23	0.24	0.07	0.25	0.28	0.01	<.0001	<.0001	0.13
Total SFA	37.81	38.25	37.43	35.73	36.57	35.35	0.48	<.0001	0.62	0.89
Total MUFA	46.10	46.75	45.9	43.67	44.52	42.73	0.46	<.0001	0.44	0.56
Total PUFA	15.48	14.23	15.98	20.00	17.72	21.32	0.61	<.0001	0.23	0.76
PUFA:SFA	0.40	0.37	0.40	0.55	0.50	0.59	0.02	<.0001	0.40	0.86
Total n-3 FA	0.59	0.65	0.75	0.65	0.68	0.72	0.02	0.02	<.0001	0.20
Total n-6 FA	0.08	0.54	0.61	0.08	0.83	0.72	0.07	0.01	<.0001	0.09

[1] D x C = Diet by cut interaction

Table 5. Interactive effects of 20% DDGS in the diet and marketing time on fatty acid (FA) composition of jowl fat

Item	Control			20 % DDGS			SEM	P-value		
	First Cut	Second Cut	Third Cut	First Cut	Second Cut	Third Cut		Diet	Cut	D x C[1]
Palmitic Acid (16:0)	21.91	21.95	22.30	20.89	20.72	21.64	0.28	<.0001	0.18	0.76
Palmitoleic Acid (16:1)	2.25	2.21	2.19	2.02	1.94	2.03	0.06	<.0001	0.66	0.62
Stearic Acid (18:0)	10.38	10.41	10.84	9.62	9.48	10.09	0.23	<.0001	0.14	0.98
Oleic Acid (18:1n9t)	0.13	0.13	N/D[2]	0.16	0.12	N/D[2]	0.09	0.02	0.15	0.04
Oleic Acid (18:1n9c)	41.20	41.44	42.20	39.24	39.58	40.01	0.40	<.0001	<.0001	0.06
Linoleic Acid (18:2n6t)	0.06	0.06	0.08	0.07	0.05	0.07	0.01	0.00	0.01	0.40
Linoleic Acid (18:2n6c)	15.90	15.67	15.97	20.02	20.26	19.60	0.52	<.0001	0.78	0.75
Linolenic Acid (18:3n3)	0.65	0.96	0.71	0.71	1.04	0.74	0.04	0.05	<.0001	0.30
Arachidonic Acid (20:4n6)	0.10	0.26	0.23	0.11	0.28	0.25	0.01	<.0001	<.0001	0.13
Total SFA	34.61	34.41	35.34	32.76	32.11	33.87	0.48	<.0001	0.62	0.89
Total MUFA	47.02	46.75	47.53	44.56	44.25	45.01	0.46	<.0001	0.44	0.56
Total PUFA	17.63	17.97	16.48	21.99	22.86	20.44	0.61	<.0001	0.23	0.76
PUFA:SFA	0.50	0.50	0.45	0.66	0.68	0.60	0.02	<.0001	0.40	0.86
Total n-3 FA	0.67	1.08	0.84	0.74	1.20	0.87	0.02	0.02	<.0001	0.20
Total n-6 FA	0.16	0.45	0.41	0.17	0.50	0.44	0.07	0.01	<.0001	0.09

[1] D x C = Diet by cut interaction
[2] Limits undetectable

Table 6. Mean iodine values of belly fat determined by three different methods.

Item	Control			20 % DDGS			SEM	P-value		
	First Cut	Second Cut	Third Cut	First Cut	Second Cut	Third Cut		Diet	Cut	D x C[4]
Titration[1]	66.96[c]	66.91[c]	67.05[c,d]	73.47[a]	70.62[a,b,d]	68.93[b,c]	1.55	0.0004	0.11	0.50
GC[2]	63.01b,[b,c,d]	60.82[c,b]	66.33[a,d]	68.85[a]	61.33[b]	67.72[a]	1.52	<0.0001	0.14	0.13
NIR[3]	65.64[b,c,d]	67.89[b,c]	67.50[a,d]	71.10[a]	70.91[b]	73.16[a]	0.67	<0.0001	0.01	0.08

[a-d] LS means with in a row lacking common superscripts differ at P<0.05.

[1] Iodine value determined by chemical titration; AOAC (1984)

[2] Iodine Value = (0.95 x C16:1) + [0.86 x (C18:1n9t + C18:1n9c)] + [1.732 x (C18:2n6t + C18:2n6c)] + (2.616 x C18:3n3) + (0.785 x C20:1); AOCS (1998)

[3] Iodine value determined by NIR spectroscopy

[4] D x C = Diet by cut interaction

Table 7. Mean iodine values of jowl fat determined by two different methods.

Item	Control			20 % DDGS			SEM	P-value		
	First Cut	Second Cut	Third Cut	First Cut	Second Cut	Third Cut		Diet	Cut	D x C[3]
Titration[1]	74.04[a]	70.47[b,c]	71.96[a,b,c]	74.49[a]	75.14[a]	73.44[a,c]	1.55	0.0004	0.11	0.50
GC[2]	67.29[d]	69.21[c,d]	69.59[c,d]	72.54[a,c]	75.31[a]	71.50[b,c]	1.52	<0.0001	0.14	0.13

[a-d] LS means with in a row lacking common superscripts differ at P<0.05.

[1] Iodine value determined by chemical titration; AOAC (1984)

[2] Iodine Value = (0.95 x C16:1) + [0.86 x (C18:1n9t + C18:1n9c)] + [1.732 x (C18:2n6t + C18:2n6c)] + (2.616 x C18:3n3) + (0.785 x C20:1); AOCS (1998)

[3] D x C = Diet by cut interaction

Table 8. Iodine values of belly and jowl fat independent of diet

Item	Belly	Jowl	SEM	P-Value
Titration[1]	68.99	73.26	0.55	<0.0001
GC[2]	64.67	70.91	0.54	<0.0001

[1] Iodine value determined by chemical titration; AOAC (1984)
[2] Iodine Value = (0.95 x C16:1) + [0.86 x (C18:1n9t + C18:1n9c)] + [1.732 x (C18:2n6t + C18:2n6c)] + (2.616 x C18:3n3) + (0.785 x C20:1); AOCS (1998)

Table 9. Pearson Correlation Coefficients and P-values for three measures of iodine value

	Titration[1]	GC[2]	NIR[3]
Titration[1]	1.00	0.462	0.681
	-	<.0001	<.0001
GC[2]	-	1.00	0.431
	-	-	<.0001
NIR[3]	-	-	1.00
	-	-	-

[1] Iodine value determined by chemical titration; AOAC (1984)
[2] Iodine Value = (0.95 x C16:1) + [0.86 x (C18:1n9t + C18:1n9c)] + [1.732 x (C18:2n6t + C18:2n6c)] + (2.616 x C18:3n3) + (0.785 x C20:1); AOCS (1998)
[3] Iodine value determined by NIR spectroscopy

Table 10. Pearson Correlation Coefficients and P-values for three measures of iodine value in belly fat of pigs fed a control diet

	Titration[1]	GC[2]	NIR[3]
Titration[1]	1.00	0.193	0.448
	-	0.065	<.0001
GC[2]	-	1.00	0.457
	-	-	<.0001
NIR[3]	-	-	1.00
	-	-	-

[1] Iodine value determined by chemical titration; AOAC (1984)
[2] Iodine Value = (0.95 x C16:1) + [0.86 x (C18:1n9t + C18:1n9c)] + [1.732 x (C18:2n6t + C18:2n6c)] + (2.616 x C18:3n3) + (0.785 x C20:1); AOCS (1998)
[3] Iodine value determined by NIR spectroscopy

Table 11. Pearson Correlation Coefficients and P-values for three measures of iodine value of belly fat fed 20% DDGS

	Titration[1]	GC[2]	NIR[3]
Titration[1]	1.00	0.355	0.533
	-	0.0005	<.0001
GC[2]	-	1.00	0.321
	-	-	0.0027
NIR[3]	-	-	1.00
	-	-	-

[1] Iodine value determined by chemical titration; AOAC (1984)
[2] Iodine Value = (0.95 x C16:1) + [0.86 x (C18:1n9t + C18:1n9c)] + [1.732 x (C18:2n6t + C18:2n6c)] + (2.616 x C18:3n3) + (0.785 x C20:1); AOCS (1998)
[3] Iodine value determined by NIR spectroscopy

Table 12. **Pearson Correlation Coefficients and P-values for two measures of iodine value in jowl fat of pigs fed a control diet**

	Titration[1]	GC[2]
Titration[1]	1.00	0.320
	-	0.002
GC[2]	-	1.00
	-	-

[1] Iodine value determined by chemical titration; AOAC (1984)
[2] Iodine Value = (0.95 x C16:1) + [0.86 x (C18:1n9t + C18:1n9c)] + [1.732 x (C18:2n6t + C18:2n6c)] + (2.616 x C18:3n3) + (0.785 x C20:1); AOCS (1998)

Table 13. Pearson Correlation Coefficients and P-values for two measures of measures iodine value of jowl fat from pigs fed 20% DDGS

	Titration[1]	GC[2]
Titration[1]	1.00	0.195
	-	0.062
GC[2]	-	1.00
	-	-

[1] Iodine value determined by chemical titration; AOAC (1984)
[2] Iodine Value = (0.95 x C16:1) + [0.86 x (C18:1n9t + C18:1n9c)] + [1.732 x (C18:2n6t + C18:2n6c)] + (2.616 x C18:3n3) + (0.785 x C20:1); AOCS (1998)

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