

SHELF STABILITY AND QUALITY OF FRESH GROUND PORK AND PORK  
SAUSAGE FROM PIGS FED ETHANOL CO-PRODUCTS

---

A thesis presented to  
the Faculty of the Graduate School  
at the University of Missouri - Columbia

---

In Partial Fulfillment  
of the Requirements for the Degree

Master of Science

---

by

HANNAH EVANS

Dr. Bryon R. Wiegand, Thesis Supervisor

DECEMBER 2011

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

SHELF STABILITY AND QUALITY OF FRESH GROUND PORK AND PORK  
SAUSAGE FROM PIGS FED ETHANOL CO-PRODUCTS

presented by Hannah Evans

a candidate for the degree of Master of Science

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

---

Dr. Bryon R. Wiegand

---

Dr. Carol L. Lorenzen

---

Dr. Ingolf U. Gruen

## **ACKNOWLEDGENTS**

First and foremost, I would like to acknowledge my advisor, Dr. Bryon Wiegand. This thesis would not have been possible without his support, patience, encouragement, and guidance. I would like to thank Dr. Carol Lorenzen and Dr. Ingolf Gruen for serving as members of my committee, as well as Dr. Monty Kerley and Dr. Gary Allee for their assistance with my research. Furthermore, I am grateful for the knowledge and assistance I received from Rick Disselhorst, J. P., Doris Lyons, the meat lab student workers, and Dr. Wiegand's lab group throughout my time in the animal sciences department. I am forever indebted to Daniel Pompeu and Zachary Callahan as they were instrumental in the success and completion of my research project. Lastly, I would like to thank Tanner Meyer and my family for their continued support of my education and for helping me reach my goals.

## TABLE OF CONTENTS

ACKNOWLEDGMENTS.....	ii
LIST OF TABLES.....	v
LIST OF FIGURES.....	vi
Chapter	
1. LITERATURE REVIEW.....	1
Fat Quality.....	1
Lipid oxidation.....	3
Promoters of lipid oxidation.....	5
Inhibition of lipid oxidation.....	8
Dietary Modifications.....	12
Conjugated linoleic acid.....	14
Mechanisms of action for CLA.....	16
CLA inclusion in rodent diets.....	17
CLA inclusion in swine diets.....	19
CLA in health and disease.....	23
Distillers Dried Grains with Solubles (DDGS).....	25
DDGS inclusion in swine diets.....	25
Ractopamine Hydrochloride (RAC).....	30
Mechanisms of Action for RAC.....	32
RAC inclusion in swine diets.....	34
RAC interactions with fat source.....	37
2. SHELF STABILITY AND QUALITY OF FRESH GROUND PORK AND PORK SAUSAGE FROM PIGS FED A COMBINATION OF DRIED DISTILLERS GRAINS WITH SOLUBLES, RACTOPAMINE	44

## HYDROCHLORIDE, AND CONJUGATED LINOLEIC ACID

Abstract.....	44
Introduction.....	44
Materials and Methods.....	46
Sample Selection.....	46
Proximate Analysis.....	48
Lipid oxidation (TBARS).....	48
Sensory evaluation.....	49
Fatty Acid composition.....	49
Statistical Analysis.....	51
Results .....	52
Proximate Analysis.....	52
TBARS values.....	52
Sensory Evaluation.....	53
Objective Color Measurements.....	53
Fatty Acid Composition.....	55
Discussion.....	56
Proximate Analysis.....	56
TBARS values.....	57
Sensory Evaluation.....	58
Objective Color Measurements.....	59
Fatty Acid Composition.....	60
Conclusion.....	63
LITERATURE CITED.....	73

## LIST OF TABLES

Table	Page
1.1. CLA concentrations of common foods.....	40
2.1. Diet composition for pigs fed 20% DDGS with or without conjugated linoleic acid or ractopamine hydrochloride .....	64
2.2. Luta-CLA ® 60 fatty acid composition.....	65
2.3. Effects of dietary DDGS, RAC, and CLA on proximate analysis.....	66
2.4. DDGS by RAC interactions for fat %.....	66
2.5. CLA by RAC interactions for TBARS and a* (redness) values of ground pork.....	67
2.6. RAC by processing treatment interactions for TBARS and L* (lightness) values.....	67
2.7. Effects of DDGS, RAC, CLA, and processing treatment on lipid oxidation and sensory characteristics.....	68
2.8. Effects of DDGS, RAC, CLA, processing treatment, and day on objective color during retail display.....	69
2.9. CLA by process by day interactions for L* (lightness).....	70
2.10. DDGS by process interactions for a* (redness).....	70
2.11. RAC by process by day interactions for a* (redness).....	71
2.12. DDGS by RAC by process by day interactions for b* (yellowness).....	71
2.13. Effect of dietary DDGS, RAC, and CLA on fatty acid profiles (indicated as percentages) of ground pork.....	72
2.14. DDGS by RAC interactions for arachidonic acid (20:4n6).....	72

## LIST OF FIGURES

Figure		Page
1.1.	Proposed pathway of biohydrogenation of linoleic acid (18:2).....	42
1.2	Proposed pathway of biohydrogenation of linoleic acid (18:2) involving cis-9 trans-10 isomerase.....	43

## **CHAPTER 1**

### **LITERATURE REVIEW**

Pork is the most commonly consumed meat in the world, with 50 % of daily meat protein intake coming from pork. United States pork consumption has increased over the last several years and is estimated to continue increasing as the population grows. Processed pork products account for approximately 62 % of all pork consumed in the United States, with pork sausage listed as the second most consumed processed pork product (Davis and Lin, 2005).

According to Morrissey et al. (1998), lipid oxidation is a major factor influencing meat quality and acceptability. In processed meat products, lipid oxidation is one of the main causes of quality deterioration, especially in frozen products (Olsen et al. 2005). Deterioration due to lipid oxidation can occur in all meat, including refrigerated raw meat, frozen fresh meat, cooked and cured meat, freeze-dried meat, and irradiated meat products (Love and Pearson, 1970). Due to the unsaturation of pork fat, the issue of oxidative rancidity is widespread in many pork products (Larick et al., 1992).

### **FAT QUALITY**

Fat quality is important in meat products as it can influence further processing characteristics and pork export potential (Carr, 2005). The main components of fat quality include composition, titer (hardness), color, impurities, and stability (Azain, 2001). The

composition of the sample refers to the percentage of each individual fatty acid and is measured through gas chromatography. Furthermore, the fatty acid composition of the sample can impact other fat quality characteristics. The firmness/hardness of fat is determined by the composition, as different fatty acids have different melting points (Wood et al., 2003; Wood et al., 2008). Unsaturated fatty acids have much lower melting points than saturated fatty acids, with the *cis* double bond configuration having a decreased melting point when compared to the *trans* configuration (Gruen and Duncan, 2007). Additionally, short chain fatty acids have lower melting points than longer chain fatty acids, because the association between fatty acid chains increases as chain length increases (Richards, 2007). Titer is used as a measure of the hardness of the fat and is determined by the temperature (°C) where a fat sample solidifies. Another indicator of fat firmness and the degree of the unsaturation of fat is the iodine value. The iodine value is inversely related to the titer and is expressed as the grams of iodine absorbed per 100g of sample; therefore a higher iodine value indicates a higher degree of unsaturation (Azain, 2001). Wood et al. (2008) suggested that fat firmness may also be related to the degree of cohesiveness between lean and fat tissues (fat separation). According to the pork composition and quality assessment procedure (NPPC, 2000), a high quality fat is characterized by a total polyunsaturated fatty acid (PUFA) content of less than 15 %, greater than 15 % stearic acid (18:0) and an iodine value of less than 70.

Fat color can be used as an indicator of fat quality and may indicate the composition or source of the product (Azain, 2001). According to Wood et al. (2003), fat cells containing solidified fat with a high melting point appear whiter than fat with a lower melting point. Additionally, color deterioration is influenced by the degree of unsaturation of the fat, because

unsaturated fatty acids are more prone to lipid oxidation and may result in undesirable color formation. Other factors that may impact the fat color include moisture, impurities, and unsaponifiables such as sterols, vitamins, waxes, and hydrocarbons (Azain, 2001).

The stability of a fat sample indicates the resistance to oxidative rancidity and is related to the shelf-life of a product (Azain, 2001). Saturated fatty acids are more stable and less susceptible to lipid oxidation when compared to unsaturated fatty acids (Larick et al., 1992; Gruen and Duncan, 2007) due to the fact that free radicals typically attack the carbon atom adjacent to the double bond (Richards, 2007). This C-H bond is at the bis-allylic position, which is the most reactive site for hydrogen abstraction because it has a lower bond energy than other C-H bonds in fatty acids (Min and Ahn, 2005). Data from Wilson et al. (1976) indicates that turkey meat is the most susceptible to lipid oxidation, followed by chicken, pork, beef, and mutton. This is expected since poultry meat contains the highest levels of unsaturated fatty acids with beef and mutton containing predominantly saturated fatty acids. Oxidation of fats can lead to undesirable color, flavors, and odors and can be monitored to determine the end of shelf-life for some food products (Richards, 2007; Wood et al., 2003).

## **LIPID OXIDATION**

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). According to Romans et al. (2001) and Olsen et al. (2005), since bacterial spoilage is controlled at freezer temperatures, oxidative rancidity is the primary factor that determines the storage life of frozen meats. Rancidity is increased by wide temperature fluctuations and inadequate

protection from oxygen, while freezing and thawing products can also cause development of rancidity (Love and Pearson, 1971). According to Wood and Enser (1997), lipid oxidation results in the conversion of myoglobin to brown metmyoglobin and the development of rancid odors and flavors due to degradation of polyunsaturated fatty acids.

Lipid oxidation initiates when a hydrogen atom is abstracted from a fatty acid to form a fatty acid free radical ( $L\bullet$ ). Free radicals indicate any component capable of abstracting hydrogen and which contains one or more unpaired electrons in an orbital (Richards, 2007). Initiation typically occurs in the presence of trace metals, light, or heat (Frankel, 1984). In the propagation process, lipid free radicals ( $L\bullet$ ) react with  $O_2$  to form peroxy radicals ( $LOO\bullet$ ) which can react with more fatty acids to form lipid hydroperoxides (LOOH) which are the primary products of autoxidation (Frankel, 1984; Richards, 2007). Lipid hydroperoxides can also react with oxygen again to form secondary products such as epoxyhydroperoxides, ketohydroperoxides, dihydroperoxides, cyclic peroxides, and bicycloendoperoxides (Frankel, 1984; Min and Ahn, 2005). The termination of lipid oxidation occurs when free radicals reach concentrations high enough at which radicals can react with themselves or self-destruct (Richards, 2007; Min and Ahn, 2005).

Lipid hydroperoxides are colorless, tasteless, and odorless; however the decomposition of LOOH can result in the breakdown products: aldehydes, alcohols, ketones, hydrocarbons, and acids (Monahan, 2000). The presence of these compounds in foods can lead to a strong, undesirable flavor and odor known as warmed-over flavor (WOF) or rancidity (Frankel, 1984; Richards, 2007). Of the breakdown products of lipid oxidation, aldehydes are the most abundant and highly reactive and include propanal, pentenal, hexanal, and 4-hydroxynonenal (HNE).

Aldehydes increase myoglobin oxidation and encourage the pro-oxidant activity of metmyoglobin, leading to meat color deterioration. The interaction of lipid oxidation products with proteins and amino acids can lead to the development of rancidity (Frankel, 1984). Specifically, aldehydes from lipid oxidation are capable of reacting with protein to form adducts which can reduce protein stability and functionality (Min and Ahn, 2005). According to Grün et al. (2006), WOF development is not only due to the formation of lipid oxidation products, but also affected by a loss of desirable meat flavor compounds. Consequently, some researchers believe that the flavor changes due to lipid oxidation should be considered “meat flavor deterioration” (MFD) instead of the development of WOF (Monahan, 2000).

Lipid oxidation can be detected by several methods. The thiobarbituric acid (TBA) assay which utilizes spectrophotometric detection of thiobarbituric acid reactive substances (TBARS) is one of the most common methods for detecting malondialdehyde (MDA), a major oxidation product (Wong et al., 1995). Additionally, production of other volatiles, especially aldehydes can be measured to estimate lipid oxidation. According to Larick et al. (1992), hexanal is commonly used as a marker for lipid oxidation and WOF in pork products.

### ***Promoters of lipid oxidation***

According to Min and Ahn (2005), many processing factors can affect the rate of lipid oxidation in meat, including the composition of raw meat, size reduction processes such as grinding, additives such as salt, nitrite, spices, and antioxidant, cooking/heating, temperature abuse during handling, oxygen availability, and prolonged storage. The factors most pertinent to fresh pork sausage manufacturing are discussed below.

The composition of meat refers to the percent lean and percent fat of the sample and has a large impact on lipid oxidation. According to Jo et al. (1999), lipid oxidation increases as fat content increases in pork sausages. In this study, TBARS values in sausages increased with an increase in fat content from 4.7 % to 15.8 % fat. Min and Ahn (2005) agreed that the extent of lipid oxidation is directly correlated to the total lipid content of meat. Moreover, Wilson et al. (1976) indicated that as lipid levels increase in pork muscle, a corresponding increase in lipid oxidation occurred. This indicates that variation in intramuscular fat content can also influence the rate of oxidation.

According to Min and Ahn (2005), any process that disrupts the membrane, including but not limited to grinding, mincing, deboning, and flaking, allows contact between oxygen and phospholipids and increases the rate of lipid oxidation development. Additionally, grinding exposes muscle lipids to pro-oxidants due to the disruption of cellular structure (Monahan, 2000). These processes allow iron to be released from myoglobin and made available to amino acids, nucleotides, and phosphates which allows for formation of chelates, catalysts of lipid oxidation (Morrissey et al., 1998).

Sodium chloride (NaCl) is an essential ingredient in many meat products for preservation, flavor, water holding capacity, binding ability, and juiciness; however NaCl has also been implicated as a pro-oxidant in meat products (Min and Ahn, 2005). Chen et al. (1984) indicated that salt (NaCl) inclusion at 2.0 % increased lipid oxidation of ground beef during cooking and subsequent storage. According to Lee et al. (1997), sodium chloride inclusion from 0.5 to 2.0 % increased TBARS values and lipid peroxides in frozen ground pork. This acceleration in lipid oxidation was explained by the ability of sodium chloride to reduce the

activity of antioxidant enzymes found in pork: catalase (CAT), glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD). Furthermore, Monahan (2000) suggested that sodium chloride inclusion in meat products increases the release of iron from heme proteins.

Heating drastically increases the level of lipid oxidation, concurrently increasing TBARS values and volatile production. This promotion of lipid oxidation occurs by disruption of muscle cell structure, inactivation of antioxidant enzymes, and the release of oxygen and iron from myoglobin (Min and Ahn, 2005). Monahan (2000) agrees that increases in lipid oxidation following cooking may be related to disruption of cellular compartmentalization, exposure of membrane lipids to pro-oxidants, and the release of free iron from myoglobin. Mei et al. (1994) reported that the cooking of pork to temperatures higher than 60 °C led to substantial increases in TBARS values partially due to inactivation of the antioxidant enzymes: CAT, GSH-Px, and SOD in cooked pork. Monahan et al. (1992) also reported an increase in TBARS values after cooking of fresh pork chops, with rapid oxidation occurring following cooking. Kingston et al. (1998) found a higher rate of lipid oxidation in cooked pork as cooking temperatures increased, as well as accelerated lipid oxidation in pork cooked at a slower rate when compared to fast cooked pork. This may be due to a greater release of free iron from heme pigments, a catalyst of lipid oxidation, in slow cooked meat.

Prolonged frozen storage of meat may also result in an undesirable WOF through lipid oxidation product formation. Monahan et al. (1992) found that raw pork chops placed in frozen storage for four months exhibited higher TBARS values than fresh chops over an eight day retail display period. Maintaining meat products at refrigeration or freezing temperatures may retard

oxidative deterioration relative to ambient temperatures, but lipid oxidation can still occur at low storage temperatures (Monahan, 2000).

Iron is the most abundant transitional metal in muscle and has been shown to catalyze the detrimental oxidation of DNA, protein, and lipids. Iron catalyzes the initiation of oxidation by generating free radicals capable of abstracting a hydrogen atom from fatty acids (Baron and Anderson, 2002; Min and Ahn, 2005). Iron sources such as myoglobin are also catalysts for lipid oxidation (Morrissey et al., 1998). Richards et al. (2007) indicated that myoglobin and hemoglobin in muscle can promote autoxidation due to the iron and heme content. According to Baron and Andersen (2002), metmyoglobin and oxymyoglobin are major promoters of lipid oxidation at meat pH, while little evidence exists implicating deoxymyoglobin in lipid oxidation.

### ***Inhibition of lipid oxidation***

Lipid oxidation and the related development of WOF and color deterioration can be inhibited by using antioxidants and chelating agents (Love and Pearson, 1971). Antioxidants can break the chain reaction of lipid oxidation by reacting with  $LOO\bullet$  to form stable radicals which may be unreactive or form nonradical products (Frankel, 1984; Min and Ahn, 2005). Cured meats utilize the antioxidant properties of sodium nitrite; however uncured meats typically include synthetic antioxidants, butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) or natural antioxidants such as plant extracts (Sebranek et al., 2005). According to USDA regulations (USDA, 2000), BHA and BHT are permitted up to 0.01 % (based on fat content) each in fresh sausage; however consumers still raise concerns about incorporation of synthetic

antioxidants in meat products. Therefore, research analyzing the effects of natural plant antioxidants has become popular.

Many herbs exhibit antioxidant properties including ginger, clove, cinnamon, bay, thyme, savory, sage, oregano, rosemary, sweet basil, parsley, coriander, tarragon, sansho, allspice, cumin, white peppercorn, black peppercorn, nutmeg, caraway, dill seed, and fennel seed (Tanabe et al., 2002). However, when considering fresh pork sausage, sage and rosemary are the most pertinent spices to be considered including for their antioxidant properties.

Sebranek et al. (2005) conducted an experiment to determine the effectiveness of rosemary extract (FORTIUM<sup>TM</sup> R 20) on oxidation in pork sausage. Rosemary inclusion at 1500 and 2500 ppm in raw-frozen sausage had beneficial decreases in TBARS values over a 16 week storage period when compared to the control sausage which had no added antioxidants. Additionally, the sausages with rosemary extract had lower TBARS values than sausage with BHA/BHT after 42 days of storage, similar TBARS values were observed between the two antioxidants before 42 days of storage. Additionally, the rosemary extract resulted in a higher a\* reflectance value after 84 days of storage when compared to the control and BHA/BHT sausages. No differences in the sensory perception of WOF were found between treatments. When considering fresh, refrigerated sausage, rosemary extract at 2500 ppm and BHA/BHT inclusion resulted in similar TBARS values over a 14 day storage period; however both antioxidants decreased TBARS values when compared to the control. Overall, the author concluded that utilizing a rosemary extract in pork sausage resulted in a significant reduction in color deterioration and lipid oxidation with similar or superior results to synthetic antioxidants,

BHA/BHT. Utilizing rosemary as a natural antioxidant is a potential alternative to synthetic antioxidants for extending shelf-life in fresh meat products, especially raw-frozen pork sausage.

Ahn et al. (2002) compared the antioxidant properties of natural plant extracts and BHA/BHT in cooked ground beef. They discovered that rosemary inclusion resulted in lower TBARS values and hexanal content at day 0, 1, 2, and 3 of storage and lower WOF scores at day 0 and day 3 in cooked ground beef samples when compared to the control which contained no added antioxidants. Nonetheless, BHA/BHT inclusion resulted in significantly lower TBARS values, hexanal content, and WOF scores than rosemary. This indicates that while rosemary has antioxidant properties in cooked ground beef, it is not as effective as 0.01 % BHA + 0.01% BHT in controlling lipid oxidation in cooked products even when rosemary levels are increased to 0.1 % of the product. Additionally, grape seed extract (ActiVin), pine bark extract (Pycnogenol), sodium tripolyphosphate, and  $\alpha$ -tocopherol exhibited antioxidant properties in cooked ground beef.

Another study conducted by Chen et al. (1999), evaluated the effectiveness of synthetic and natural antioxidants on lipid oxidation in ground pork patties. Rosemary oleoresin at 0.02 % reduced TBARS values in raw and cooked pork patties after three days of storage, with TBARS values similar to BHT; however, no differences were observed for TBARS values between the control and rosemary-added at day 0 or day 7. Hexanal, propanal, and higher boiling point components such as 1-butanol, 1-pentanol, and nonanal were measured in cooked pork patties over 7 days of storage. Rosemary inclusion decreased the propanal content of cooked patties at day 3 and 7, but had no other antioxidant effects on the changes of volatiles. Additionally, no improvements were seen in L\* (lightness) or b\* (yellowness) values for raw patties stored over a

7 day period. On the other hand, at day 0 and 7,  $a^*$  (redness) was higher for pork patties with rosemary when compared to the control patties which had no added antioxidants. Overall, rosemary was responsible for reductions in lipid oxidation, volatiles, and color deterioration in raw and cooked pork patties; however, rosemary oleoresin was not as effective in terms of its antioxidant properties as has been reported in other studies.

Wong et al. (1995) discovered that rosemary and sage provided protection against lipid oxidation in ground beef samples. Both plant extracts resulted in a decrease in MDA content and TBARS values. Commercial rosemary and sage extracts contain carnosol, rosmanol, isorosmanol, and epirosmanol which are phenolic antioxidants that react with lipid radicals to stabilize them. Tanabe et al. (2002) also indicated that sage and rosemary extracts effectively inhibited lipid oxidation in pork frankfurters. Furthermore, McCarthy et al. (2001) found that rosemary was an effective antioxidant in raw and cooked pork with TBARS values similar to BHA/BHT. Rosemary inclusion also resulted in higher  $a^*$  (redness) reflectance values when compared to the control in raw pork patties after nine days of storage; however it did not prevent color deterioration as effectively as BHA/BHT. Cooked pork color did not differ due to rosemary inclusion. One concern with rosemary inclusion is the development of an associated musty/herby flavor (St. Angelo et al., 1991); however this might be avoided by utilizing rosemary extract at low concentrations.

While antioxidant inclusion is effective in reducing lipid oxidation in pork products, Kingston et al. (1998) suggested that combining approaches to decrease lipid oxidation and WOF development may be the most effective method to minimize this problem in pork. Elimination of oxygen from packaging is an effective way to reduce lipid oxidation. This can be achieved by

vacuum packaging or modified atmosphere packaging utilizing CO<sub>2</sub> or N<sub>2</sub>. Jo et al. (1999) also indicated that packaging type influenced the production of volatiles, the extent of lipid oxidation, and color deterioration in cooked pork sausages during storage.

### **DIETARY MODIFICATIONS**

In addition to minimizing lipid oxidation through processing techniques, research shows that dietary manipulation in swine can influence the rate of oxidative deterioration in pork products. Since the rate of lipid oxidation is greatly influenced by the degree of unsaturation of the fat (Larick et al., 1992), dietary changes can be made pre-slaughter to increase the saturation of pork fat and consequently influence the oxidative stability of the fat. There are many other factors that can influence the fatty acid composition of pork fat, including animal age, sex, genetics, and muscle location (Wood, 1984); however nutrition is the main factor through which fatty acid composition can be modified in non-ruminant animals (Kloareg et al., 2007).

According to Wilson et al. (1976), the PUFA content of meat from non-ruminant animals is directly correlated to the level of PUFAs in the diet. Moreover, Gatlin et al. (2002a) reported that the fatty acid profile of carcass lipids in pigs varies directly with the dietary fat composition. Specifically, as the level of dietary PUFA decreases, a linear decrease occurs in the linoleic acid (18:2) content of carcass fat, as well as an increase in MUFAs, especially palmitoleic (16:1) and oleic (18:1) acid. Larick et al. (1992) also indicated that as the unsaturated fatty acid content of the diet increased, a subsequent increase occurred in the concentration of unsaturated fatty acids in pork fat and a decrease in the concentration of saturated fatty acids. This increase in unsaturated fatty acids in the diet led to an increase in the production of volatile compounds in

LM samples including pentanal and hexanal, two major decomposition products of lipid oxidation.

Fat from non-ruminant animals is easily manipulated through nutrition since dietary fatty acids are absorbed unchanged from the intestines and incorporated into tissue lipids (Wood and Enser, 1997). Therefore, diet can significantly impact the fatty acid composition of pork fat (Wilson et al., 1976; Gatlin et al., 2002b). However, deposited fatty acids can originate from dietary fatty acids or be synthesized endogenously via *de novo* fatty acid synthesis (Kloareg et al., 2007). *De novo* fatty acid synthesis occurs in the cytosol and begins with the conversion of acetyl-coA to malonyl co-A via the acetyl-coA carboxylase (ACC) enzyme. Next, elongation and desaturation toward the methyl end take place by way of fatty acid synthase (FAS) to eventually form palmitic acid (16:0), the main product of *de novo* fat synthesis (Nelson and Cox, 2008). In the endoplasmic reticulum, palmitic acid can then be elongated via fatty acid elongase and desaturated via stearyl-coA desaturase, which inserts a double bond at carbon 9 from the carboxyl terminal end, to form other fatty acids. According to Kloareg et al. (2007), of the palmitic acid from *de novo* lipogenesis, approximately 29 % will be deposited unchanged as palmitic acid, while 67 % will be elongated to stearic acid (18:0), then 68 % of the stearic acid will be desaturated to oleic acid (18:1).

The proportion of deposited fat coming from *de novo* fatty acid synthesis varies between animals and can be affected by diet and genetics. According to Allee et al. (1971), inclusion of dietary fat leads to a decrease in *de novo* fat synthesis in swine. Ractopamine inclusion has also been implicated in decreasing *de novo* fat synthesis in pigs (Wiegand et al., 2011). Additionally, selection for leaner pigs with less backfat has resulted in pigs with decreased *de novo*

lipogenesis, consequently leading to an increase in the unsaturation of pork fat (Wood and Enser, 1997). Correa et al. (2008) also indicated that an increase in carcass leanness was associated with an increase in the unsaturation of pork bellies and subsequent susceptibility to rancidity. As previously mentioned, the primary products of *de novo* fatty acid synthesis include palmitic acid (16:0), stearic acid (18:0), and oleic acid (18:1). On the other hand, the fatty acid composition of swine diets contains more unsaturated fatty acids. Therefore, by decreasing *de novo* fatty acid synthesis through diet and genetics, a higher proportion of carcass fat will be derived from dietary sources. As a result, carcasses will exhibit a lower proportion of saturated fatty acids from *de novo* synthesis and a higher proportion of the unsaturated fatty acids coming from the diet (Gatlin et al., 2002b).

### ***Conjugated Linoleic Acid***

Conjugated linoleic acid (CLA) is a group of positional and geometric isomers of linoleic acid (18:2) that contains conjugated double bonds at varying positions along the carbon chain (Ostrowska, 1999; Christie, 2003). The main dietary sources of CLA are displayed in Table 1; Chin et al. (1992) established ruminant meat and dairy products as the primary contributors of CLA in the diet. Research interest for the use of CLA in animal models began after the discovery of CLA as an anticarcinogen in ground beef (Ha et al., 1987). The use of CLA as a fat source in swine diets was approved by the FDA on October, 29, 2008 and has since been marketed in the U.S. under the trade name of Luta-60 (BASF Corporation, Florham Park, NJ). Many studies have been conducted to examine the effects of dietary CLA inclusion on animal performance, body composition, and meat quality characteristics in rodents and swine.

One primary hypothesis for high concentrations of CLA in ruminant meat and dairy products is the fact that CLA is an intermediate in the ruminal biohydrogenation of linoleic acid (Kelly et al., 1998). The cis-9, trans-11 CLA isomer is the predominant isomer in meat from ruminants, while the trans-10, cis-12 isomer can also be found in meat products (Bauman et al., 1999). According to Griinari and Bauman (1999), there are two primary sources for CLA in milk and meat from ruminants. One source results from the cis-9, trans-11 CLA isomer originating from incomplete biohydrogenation of linoleic acid to stearic acid in the rumen (Figure 1). This process includes the isomerization of linoleic acid to cis-9, trans-11 octadecadienoic acid utilizing the common cis-12, trans-11 isomerase, followed by the hydrogenation of the cis-double bond to form a trans monoenoic acid (trans vaccenic acid), and completed with the hydrogenation of the trans double bond to achieve stearic acid. The bacterium in the rumen responsible for producing the cis-12, trans-11 isomerase enzyme is *B. fibrisolvens* (Stanton, 1997). Another proposed pathway describes the formation of the trans-10, cis-12 CLA isomer from linoleic acid involving the cis-9, trans-10 isomerase (Figure 2). In this pathway, linoleic acid is isomerized to trans-10, cis-12 octadecadienoic acid, then hydrogenated to trans-10 octadecenoic acid and further hydrogenated to stearic acid (Griinari and Bauman, 1999). Linolenic acid pathways do not involve CLA as an intermediate (Kelly et al. 1998).

The second source of CLA in milk and meat products is synthesized in animal tissues from trans vaccenic acid (trans-11 C18:1; Bauman et al., 1999). CLA can be produced endogenously from trans-11 octadecenoic acid via the  $\Delta 9$  desaturase enzyme and incorporated into milk and tissues (Griinari and Bauman, 1999). Griinari et al. (2000) confirmed that endogenous synthesis of CLA from trans-11 octadecenoic acid does occur in lactating cows,

shown by an increase in milk fat CLAs when trans-11 octadecenoic acid was abomasally infused.

### ***Mechanism of Action for CLA***

There are two isomers of CLA which are considered biologically active, c9, t11 CLA and t10, c12 CLA. The t10, c12 CLA isomer seems to bring about the majority of changes in fat storage and metabolism (Pariza et al., 2001; Pariza et al., 2003), while the overall effects of CLA on growth and feed efficiency may be due to the interaction between the two biologically active isomers (Pariza et al., 2001).

Although the complete biological basis for the effects of CLA are unknown, the t10, c12 CLA isomer has been shown to reduce lipid uptake by adipocytes by inhibiting stearyl-coA desaturase (SCD; Smith et al., 2002) and lipoprotein lipase (LPL; Pariza et al., 2001). LPL is the predominant enzyme in fat uptake for storage; therefore by directly inhibiting LPL, CLA may result in reduced fat uptake by adipocytes (Park and Pariza, 2007). SCD is the rate-limiting enzyme in fatty acid desaturation, converting saturated fatty acids to monounsaturated fatty acids (Smith et al., 2002). According to Park and Pariza (2007), monounsaturated fatty acids are the main substrate for fat deposition in adipose tissue; hence by inhibiting SCD, CLA may reduce fat mass. Smith et al. (2002) also speculated that in order to achieve larger adipocyte volumes, maximal desaturase activity may be required. The inhibition of SCD by CLA also causes a shift in the fatty acid profile of tissues from unsaturation towards saturation, specifically by influencing the ratio of palmitoleic acid/palmitic acid and oleic acid/stearic acid, also referred to as the  $\Delta^9$  desaturation index (Ntambi, 2002). As mentioned earlier, the fatty acid composition of

tissues may have a large impact on fat quality including firmness and oxidative stability (Wood et al, 2003).

Another mechanism by which CLA may elicit body composition changes is by increasing apoptosis of pre-adipocytes and adipocytes, thereby reducing adipose tissue mass and cell numbers (Pariza et al., 2001; Park and Pariza, 2007). House et al. (2005) suggested that an increase in tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) is responsible for apoptosis of adipocytes in mice; however this hasn't been demonstrated in other species. Additionally, CLA may decrease *de novo* lipogenesis due to reductions in acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS; House, 2005). Ostrowska et al. (1999) suggested that CLA reduced *de novo* synthesis and use of preformed fatty acids. CLA may also reduce sterol regulatory element-binding protein-1 (SREBP-1) expression. SREBPs are responsible for transcriptional activity of genes involved in cholesterol, fatty acid, triglyceride, and phospholipid synthesis (House et al., 2005).

The effects of CLA in skeletal muscle are not well understood; however several authors indicate that CLA increases  $\beta$ -oxidation of fatty acids in skeletal muscle (Pariza et al., 2001; Ntambi, 2002; Park and Pariza, 2007; House, 2005). CLA has been shown to enhance carnitine palmitoyl transferase (CPT) activity in muscle (Park et al., 1997). CPT is the rate limiting enzyme in fatty acid  $\beta$ -oxidation, indicating that CLA feeding causes fat to be preferentially used as an energy source which helps to reduce body fat (Ntambi, 2002; Park and Pariza, 2007).

### ***CLA inclusion in rodent diets***

Chin et al. (1994) observed the effects of supplementing CLA to rats during gestation. The author discovered that pups from dams fed CLA during gestation and lactation were

significantly heavier when compared to pups from control dams. Additionally, tissues from dams and fetuses fed the diet containing CLA had higher CLA concentrations when compared to control animals. In the second experiment, dams were fed a control diet or a CLA supplemented diet during gestation and lactation and pups at weaning were fed the same diet as their mother for 8-10 weeks. Results indicated that animals receiving a 0.5% CLA supplemented diet had higher body weights when compared to control animals. Food intake did not differ due to treatment, however a significant improvement in feed efficiency was seen when CLA was fed.

Azain et al. (2000) conducted an experiment to determine the effect of 0.5% CLA inclusion in diets for growing female rats fed for 35 d. No differences were found in food intake or growth rate of rats when CLA was included in the diets. Retroperitoneal and parametrial fat pad weights were lower in rats fed CLA when compared to the control. Additionally, there was a 44% reduction in the accretion rate of the fat pad for rats fed CLA when compared to rats receiving a diet with no CLA. There was no effect of treatment on the number of adipocytes per fat pad; however there was a decrease in average cell diameter with CLA inclusion.

In a follow-up experiment, Azain et al. (2000) fed 0.5% CLA for 7d or 49d to growing female rats. A reduction in growth rate was observed for rats fed CLA for 7 d when compared to a control diet without CLA, but food intake was not affected by dietary treatment. CLA inclusion for 7 d and 49 d led to a 28% and 27% decrease in parametrial pad weights, respectively. The retroperitoneal pad was also decreased in rats fed CLA for 7 d and 49 d. Furthermore, rats fed CLA for 49 d had a lower proportion of MUFA in adipose tissue when compared to the control diet. There was a time dependent increase in CLA content of tissues when CLA was included in the diet.

### *CLA inclusion in swine diets*

Eggert et al. (2001) showed no effect of CLA feeding at 1.0% of the diet from 75 to 120 kg of body weight on feed efficiency, ADG, or average daily feed intake (ADFI) when compared to diets containing 1.0 % sunflower oil (SFO). Additionally, the author observed no effect on back fat thickness at the first rib, last rib, last lumbar, or 10<sup>th</sup> rib or LMA of genetically lean gilts when compared to diets containing 1.0% SFO. However, the author speculated that the ability of CLA to reduce back fat thickness may be limited to fatter populations of pigs. Moreover, no differences in dressing percentage, longissimus muscle color, firmness, or marbling scores, ultimate pH, or drip loss percentage were present in this study. Feeding CLA at 1.0% of the diet to gilts led to increased levels of total SFA, decreased UFA, and higher levels of total CLA in longissimus muscle samples when compared to diets containing 1.0% SFO. Additionally, the author reported similar results in belly fat samples, with an increase in SFA, decrease in MUFA and UFA, and higher level of CLA after 1.0% CLA feeding. These changes in fatty acid composition led to lower iodine values of longissimus muscle (57.31 vs. 61.55) and belly fat samples (57.69 vs. 66.37), and a higher belly firmness scores for pigs consuming 1.0% CLA than 1.0% SFO. However, it should be noted that CLA had a more significant impact on belly fat than longissimus muscle fat, which can be seen by the differences in iodine value.

Wiegand et al. (2001) fed CLA to barrows at 0.75% of the diet from 40 kg to 106 kg of body weight and reported an increase in feed efficiency when CLA was fed compared to non-CLA fed animals. The explanation provided for this improvement was CLA's capacity to regulate energy metabolism and nutrient partitioning. CLA supplementation has the ability to decrease body fat; therefore animals fed CLA require less energy to sustain animal growth,

increasing efficiency. No differences were seen for ADG, carcass shrink at 24 h postmortem, HCW, or longissimus muscle area were observed when pigs were fed 0.75% CLA in this study. Back fat thickness at the 10<sup>th</sup> rib and last rib were lower for CLA fed pigs compared to the control. The author observed no differences for subjective color scores when CLA was fed, but an increase in marbling score and a tendency toward an increase in firmness scores were reported in CLA fed pigs. The correlation between marbling score and firmness was 0.89, indicating a strong relationship between the two quality traits. Hexane extractable lipids were also higher for CLA fed pigs when compared to the control, confirming the differences seen in marbling scores. Additionally, loin samples from CLA fed pigs had a lower percentage of moisture than the control, which would be anticipated due to the increase seen in lipid percentage.

In a study conducted by Dugan et al. (1997, 1999), pigs were fed either 2 % CLA oil (50% total CLA) or 2% sunflower oil from 60 kg to 106 kg of body weight. No difference was seen in ADG between treatments, but pigs fed CLA tended to have decreased feed intake and improved feed efficiency when compared to pigs fed sunflower oil. In this study, CLA feeding increased lean and decreased subcutaneous fat in total commercial cuts when compared to cuts from animals fed sunflower oil. Specifically, the lean percentage of the loin was increased by 3.6% with CLA feeding. The author concluded that CLA may have the ability to repartition nutrients from fat accretion to lean deposition, consequently improving feed efficiency. *Longissimus thoracis (LT)* glycogen utilization and lactate accumulation were not affected by diet, consequently postmortem LT pH did not differ between treatments. However at 3 hr post mortem, CLA-fed pigs were 1.15 °C warmer than sunflower oil fed pigs. The author attributed these differences to the larger LT mass of CLA-fed pigs. Additionally, CLA-fed pigs had higher

marbling scores and intramuscular fat levels, and decreased LT moisture when compared to pigs receiving sunflower oil; however, these differences did not affect sensory evaluation scores for tenderness, juiciness, flavor, or overall palatability. There was no difference in LT shear force, drip loss, or incidence of PSE pork due to treatment. Therefore, from this study it was concluded that feeding CLA had no adverse effects on meat quality parameters and may have the potential to improve pork quality by increasing intramuscular fat content.

Larsen et al. (2009) reported no differences in back fat thickness at the first rib, last rib, last lumbar, or 10<sup>th</sup> rib or longissimus muscle area between pigs fed CLA at 0.75% of the diet for 56 d and the control group which did not receive CLA. Also, the author found no differences in carcass weight with CLA inclusion in the diet. A decrease in lipid oxidation of bacon after 2 months of vacuumed and refrigerated storage was observed in this study, and TBARS values were significantly lower for CLA fed pigs when compared to control (0.1498 vs. 0.1638, respectively). However, such a small difference has little biological significance as this difference would most likely not be detected by consumers and both values are below the oxidative rancidity threshold of 0.5 to 1.0 indicated by Tarladgis et al. (1960). The sensory scores further confirmed this conclusion as no differences were reported for any sensory attributes, including aroma, lean color, flavor, off-flavor, and brittleness. Moreover, the author reported an increase in SFA and decrease in PUFA of belly fat when pigs were fed 0.75% CLA. Consequently, iodine values decreased from 67.44 to 57.25 when CLA was added to the diets. Additionally, palmitic acid, stearic acid, and palmitoleic acid were higher, while oleic acid, linoleic acid, and arachidonic acid were lower in belly fat samples from pigs fed CLA when compared to the control. The author also reported higher concentrations of CLA isomers in

bacon from CLA fed pigs. The shift in saturation of the fatty acid profile led to an increase in belly bar firmness as well as greater bacon slab firmness, as measured by compression test, when CLA was included in the diet. However this change in firmness did not affect bacon production as the author reported no differences in belly processing yields or sliceability, as measured by the number of acceptable/unacceptable slices.

White et al. (2009) reported that feeding CLA at 0.6% of the diet 10 days prior to slaughter had no effect on ADG, ADFI, or feed efficiency when compared to pigs not fed CLA. Additionally, no differences were observed for longissimus muscle area, back fat thickness at the 10<sup>th</sup> and last rib, subjective scores for loin color, marbling, and firmness and loin drip loss. When considering the fatty acid profiles, the author reported increases in myristic acid, stearic acid, and CLA content and decreases in oleic and vaccenic acid in subcutaneous fat and belly fat after feeding 0.6% CLA. Also, CLA fed pigs had higher SFA: UFA ratio and lower IV when compared to non CLA fed pigs in the outer (68.31 vs. 71.11) and middle (65.25 vs. 68.36) layer of subcutaneous fat and belly fat (65.66 vs. 67.70). Although the fatty acid composition of the belly fat shifted towards saturation, there was no effect on the belly bend percentage, an indicator of belly firmness.

Joo et al. (2002) conducted an experiment in which CLA was fed at increasing levels (0%, 1.0%, 2.5%, or 5.0%) of the diet to gilts for four weeks and found no differences in ultimate pH, CIE L\*, a\*, b\* reflectance values or WHC of pork loin at d0. Additionally, the author reported no effect on the incidences of PSE or DFD after CLA feeding. However after seven days of storage, loins from pigs fed 5.0% CLA presented significantly lower purge loss and lower L\* and b\* values when compared to the control. The differences in L\* value may be

attributed to the differences in WHC, while the differences in b\* values may be due to decreased oxidation of the loin from CLA fed pigs. This was hypothesized since TBARS values after 7 days of storage from pigs fed 5.0% CLA were significantly lower than control pigs. This is most likely due to the increase in saturation seen in the fatty acid profile following CLA feeding. However the author speculated that it could also be because CLA is more stable than other PUFA as the conjugated structure allows it to withstand attack by free radicals. When considering the fatty acid profiles, CLA feeding led to increased CLA content in pork loins when compared to the control. Furthermore, SFA content of pork loin increased while Oleic acid, Linoleic acid, and UFA decreased with increasing CLA levels in the diet.

### ***CLA in health and disease***

Research interest utilizing CLA also includes the effects of CLA on health and disease in animals and humans. CLA has been shown to prevent atherosclerosis, different types of cancer, and improve immune function in many animal models (Bhattacharya et al. 2006).

Atherosclerosis is the development of abnormal fat deposits in the wall of an artery and in humans, atherosclerosis of the coronary arteries is a major cause of coronary heart disease (CHD; Rudel, 1999). In a study conducted by Kritchevsky et al. (2000) utilizing rabbits, CLA feeding led to a decrease in maximal plaque thickness in the abdominal aorta and less severe atherosclerosis of the arch and thoracic aorta, indicating that CLA can inhibit atherogenesis and cause regression of established lesions. Toomey et al. (2006) saw a reduction in the development of atherosclerosis and resolution of aortic lesions with CLA feeding in mice. The author attributed these results to a decrease in inflammation due to a decrease in TNF- $\alpha$ , altered

formation of prostaglandins (PGs), and a decrease in inflammatory related genes by activation of Peroxisome proliferator-activated receptors (PPARs; Toomey et al., 2006; Bhattacharya et al., 2006). This is critical as atherosclerosis begins with an inflammatory condition (Kritchevsky, 2003). Bhattacharya et al. (2006) discussed some of the proposed mechanisms for CLA's action on atherosclerosis including a down-regulation of SREBPs and SCD. Overall, CLA feeding can inhibit atherosclerosis in rabbits, hamsters, and mice and may have the potential to resolve atherosclerotic lesions (Kritchevsky, 2003).

CLAs are potent anticarcinogens (McGuire and McGuire, 2000). According to Kelley et al. (2007) and Banni et al. (2003), feeding a mixture of CLA isomers can inhibit chemically induced tumors of the mammary gland, skin, colon, and fore-stomach in animal models. Ip et al., (1994) exhibited that feeding CLA resulted in a significant reduction in mammary tumor yield in rats. Mixtures of CLA isomers can inhibit carcinogenesis of malignant tumors at each of the major stages of cancer development: initiation, promotion, progression and metastasis (Pariza et al., 1999; Kelley et al., 2007). Additionally, Pariza et al. (1999) stated that CLA may affect cancer development either through directly affecting carcinogenesis, by reducing excessive body fat accumulation, or by reducing cachexia which is associated with advanced cancer and some cancer treatment strategies. Speculated mechanisms for CLA's effect on cancer development and growth include modified eicosanoid metabolism (Bhattacharya et al., 2006), specifically tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ; Pariza et al., 1999), alteration of tissue fatty acid compositions, cell cycle regulation including apoptosis, and lipid peroxidation (Kelley et al., 2007; Banni et al., 2003). McGuire and McGuire (2000) added that CLA competes with linoleic acid in the synthesis of Arachidonic acid which is a precursor for eicosanoids linked to tumor promotion.

Additionally, Bhattacharya et al., (2006) stated that a decrease in insulin-like growth factor (IGF) II synthesis may play a part in the inhibition of cancer cell growth. Overall, it is well known that CLA reduces cancer development and growth; however the mechanisms contributing to CLAs effect on carcinogenesis is unclear and elicits further research.

### ***Dried distillers grains with solubles (DDGS)***

Dried distillers grains with solubles (DDGS) are a byproduct of ethanol production (Dooley, 2008). The DDGS produced by ethanol plants is characterized by the grain that was used to produce the ethanol, but variability still exists in chemical composition when the same grain is used at different ethanol plants (Stein, 2007; Stein and Shurson, 2009). While DDGS contains a higher gross energy (GE) than corn (5,434 kcal/kg and 4,496 kcal/kg respectively), the values for metabolizable energy (ME) and digestible energy (DE) of DDGS are similar to corn (4,140 vs. 4,088 kcal/kg and 3,897 vs. 3,989 kcal/kg respectively). This is due to the decreased digestibility of DDGS because of the high dietary fiber content (Stein, 2007). Additionally, DDGS typically contains approximately 10 % oil, made up of a high percentage of unsaturated fatty acids, approximately 81 % (54 % linoleic acid), and a low percentage of saturated fatty acids, 13 % (Xu et al., 2010). The inclusion of DDGS in swine diets has increased over the past 10 years, due to a rise in ethanol production and greater availability of byproducts for incorporation in livestock diets (Stein and Shurson, 2009). Several studies have been designed to examine the use of DDGS in swine diets.

### ***DDGS inclusion in swine diets***

Leick et al. (2010) fed increasing levels of DDGS (0, 15, 30, 45, 60%) to growing pigs for 14 weeks prior to slaughter. Final body weight (BW) of pigs decreased linearly with increasing DDGS levels in the diet, with the less DDGS (0 and 15%) having higher BW than the greater DDGS (30, 45, and 60%) diets. The author speculated that this could be a result of a decrease in palatability of diets containing high levels of DDGS; however ADFI was not measured in this study. DP and HCW also decreased linearly with increasing levels of DDGS, but HCW was not different between the pigs fed 0 and 15% DDGS. LM depth, back fat depth, and calculated percent lean were not affected by the level of DDGS included in the diet. DDGS inclusion also had no effect on loin subjective color scores or objective color values for L\*, a\*, or b\*, while subjective marbling scores decreased with increasing DDGS. Subjective firmness scores were lower for pigs fed the 30% DDGS diet when compared to all other treatment groups, but all other levels of DDGS inclusion did not differ for firmness. There was a trend towards a decrease in 48 h pH and a significant linear increase in drip loss with increasing DDGS level. However, drip loss was not different between the less DDGS (0 and 15%) diets and only increased with the greater DDGS levels (30, 45, and 60%). DDGS inclusion did not affect percent moisture or fat of the loin. When considering belly characteristics, linear decreases in belly length, average thickness, trimmed weight, and belly firmness were seen as DDGS level increased. It was concluded that DDGS levels higher than 15% of the diet negatively impacted belly quality since belly thickness of the less DDGS diets (0 and 15%) were higher than the greater DDGS diets (30, 45, and 60%). Therefore, it was determined that between 15 and 30% dietary DDGS inclusion was the separation point for belly quality characteristics. Belly fat objective color values of L\*, a\*, and b\*, percent pump uptake, belly cook loss, and sliced bacon

cook loss were not affected by DDGS level. TBARS values for d 21 of storage were higher for greater DDGS diets (30, 45, and 60%) than less DDGS diets (0 and 15%). Additionally, TBARS values for the 30, 45, and 60% DDGS diets were at or above the threshold for rancidity detection of 0.5 mg of TBARS/kg of tissue (Tarladgis et al., 1960). On the other hand, bacon TBARS values were not affected by DDGS inclusion, which may have been due to the ability of nitrite to delay lipid oxidation. The fatty acid composition of jowl fat samples was greatly impacted by the inclusion of DDGS in the diet. As DDGS level increased, linear decreases were seen for C16:0, C18:0, C18:1t, C18:1c, and C18:3n6, while a linear increase was observed for C18:2c. Additionally, MUFA: PUFA and SFA: UFA decreased linearly with increasing levels of DDGS, which led to a linear increase in Iodine value. Belly fat samples exhibited a linear decrease in C18:1c and a linear increase in C18:2c with increasing DDGS levels. These shifts led to a linear decrease in MUFA: PUFA and a linear increase in IV with increasing DDGS. Overall, the author concluded that feeding DDGS levels higher than 15% of the diet led to a major shift in the fatty acid profiles and a decline in belly quality which may cause problems with lipid oxidation and belly processing characteristics.

In a study conducted by Benz et al. (2010), pigs were fed increasing levels of DDGS (0, 5, 10, 15, or 20%) for 57 or 78 days prior to slaughter. For pigs fed DDGS for 57 d, increasing levels of dietary DDGS led to a linear increase in C18:2n-6, C20:2, total PUFA, PUFA:SFA, and IV and a linear decrease in C16:0, C18:1n-7, and total MUFA for belly fat, back fat, and jowl fat samples. For belly fat and back fat samples from pigs fed for 57 d, increasing DDGS levels led to a linear decrease in C16:1 and C18:1n-9 cis and a linear increase in C18:3n-3. Total SFA was decreased in belly fat samples and C18:0 was decreased in jowl samples with increasing levels of

dietary DDGS for 57 d. When the feeding duration was increased to 78 d, a linear decrease in C16:0, C18:1n-9 cis, and total MUFA was observed and a linear increase in C18:2n-6, total PUFA, PUFA: SFA, and IV was seen for belly fat, back fat, and jowl fat samples. For jowl and belly fat samples from pigs fed 78 d, an increase was seen in C20:2. For belly fat and back fat samples, C18:3n-3 increased linearly and C14:0 and C16:1 decreased linearly with increasing DDGS inclusion after 78 d. Belly fat samples after 78 d of feeding also exhibited an increase in C20:4n-6 and a decrease in C18:1n-7, C20:0, and total SFA with increasing levels of dietary DDGS. Additionally, pigs fed 20% DDGS for 78 d reached a jowl IV of greater than 73, which the author reported is a threshold set by some packing plants for acceptable fat quality. Overall, it can be concluded that feeding increasing levels of DDGS in swine diets increases the Iodine value of belly fat, back fat, and jowl fat samples due to a shift in the fatty acid profile from saturation towards unsaturation.

Whitney et al. (2006) conducted a study in which pigs were fed increasing levels of DDGS (0, 10, 20, or 30%) for 91 d. The results indicated that with increasing levels of DDGS in the diet, ADG decreased, feed efficiency declined, and final BW decreased. Pigs receiving 20 or 30 % DDGS in the diet had lower ADG and final BW than pigs fed 0 or 10 % DDGS, while only pigs fed 30 % DDGS revealed lower feed efficiency than pigs fed 0 or 10 % DDGS. A linear decrease was observed for HCW, DP, back fat depth, and loin depth as dietary DDGS levels increased. A linear increase in belly fat IV, as well as a decrease in belly thickness and belly firmness was seen with increasing DDGS. The author selected at IV of 70 to indicate acceptable fat firmness and pigs fed 20 or 30% DDGS exhibited an IV of greater than 70, while those receiving lower levels of DDGS (0 or 10%) had acceptable IV. Overall, this study revealed that

feeding greater than 20 % DDGS can lead to reductions in animal performance and decrease the saturation of fatty acids, leading to softer bellies and potentially impact further processing traits.

In an experiment conducted by Xu et al. (2010), pigs were fed increasing levels of DDGS (0, 10, 20, or 30%). No differences were seen for ADG; however a linear reduction in ADFI and a consequent linear increase in feed efficiency was seen with increasing levels of DDGS. Additionally, HCW and ultimate pH were not affected by DDGS inclusion, while DP decreased linearly with increasing dietary DDGS. This change in DP may be due to an increased dietary NDF content in DDGS leading to an increase in gut fill at slaughter (Kennelly and Aherne, 1980). Last rib back fat depth was decreased and carcass fat-free lean percentage was increased linearly with increasing levels of DDGS. Marbling and firmness scores of LM decreased linearly with increased dietary DDGS, where pigs fed the diet containing 20 or 30 % DDGS had lower marbling scores when compared to the control diet and pigs fed the 30% DDGS diet had lower firmness scores when compared to the control. Feeding diets containing 10% DDGS did not affect marbling or firmness of the LM. In regards to the fatty acid profile, feeding increasing levels of DDGS linearly increased PUFA, C18:2, C18:3, C20:2 and C20:3 and linearly decreased SFA, C14:0, C16:0, C18:0, MUFA, C16:1, C18:1, and C20:1 in back fat samples. Results for belly fat samples were similar to back fat samples, where increasing levels of DDGS led to linear increases in PUFA, C18:2, C18:3, C20:2, and C20:3 and linear decreases in SFA and MUFA. Intramuscular fatty acid profiles from the LM were not altered as drastically as the other depots, but a linear increase in C18:2 and linear decreases in SFA and MUFA were observed with increasing DDGS levels in the diet. DDGS inclusion led to a linear increase in IV of back fat, belly fat, and IM fat which led to a reduction in belly firmness with increasing levels of DDGS,

with no difference in belly thickness. No differences were observed for LM TBARS values at 1-28 d of storage due to dietary treatment, despite the changes in the fatty acid profiles. LM subjective color was not affected by DDGS, however objective color values for a\* and b\* were decreased with increasing DDGS inclusion, indicating a less red and less yellow color of the loin. Belly fat color measurements did not differ due to treatment; however back fat color measurements indicated a decrease in L\* for pigs fed 20 or 30 % DDGS when compared to the control and a linear increase in b\* with increasing levels of dietary DDGS. DDGS inclusion did not affect LM cooking loss or sensory characteristics of flavor, off-flavor, tenderness, juiciness, and overall acceptability. Furthermore, bacon cooking yield and sensory characteristics of flavor, off-flavor, crispiness, and overall acceptability did not differ due to DDGS inclusion. Bacon fattiness and tenderness were decreased linearly with increasing levels of dietary DDGS. Overall, DDGS inclusion up to 30% had no detrimental effects on lipid oxidation or pork sensory characteristics, despite decreased saturation and increased IV of fatty acid profiles of belly fat, back fat, and IM fat. However, belly firmness was lower at higher levels of DDGS inclusion and DP was decreased for pigs consuming increasing levels of DDGS.

In summary, DDGS can typically be fed up to 20 or 30 % of the diet without detrimental effects of animal performance. However, due to the high concentration of polyunsaturated fatty acids in DDGS, high dietary levels can lead to increased unsaturation of pork fat. This may increase the susceptibility to lipid oxidation, WOF development, color deterioration, and reduce the shelf life.

### ***Ractopamine Hydrochloride (RAC)***

RAC is a phenethanolamine that can function as a beta-adrenergic agonist ( $\beta$ AA) by binding to beta-adrenergic receptors ( $\beta$ -AR). The endogenous ligands for  $\beta$ -AR are norepinephrine and epinephrine (Mersmann, 2001). In 1999, RAC was approved for inclusion in finishing swine diets from 68 to 109 kg in the United States by the Food and Drug Administration (FDA). In May 2006, the FDA approved RAC feeding at 5 to 10 ppm to finishing swine for the last 20.4 to 40.8 kg of body weight gain prior to slaughter (Carr et al., 2009). Marketed as Paylean<sup>®</sup> (Elanco Animal Health, Greenfield, IN), ractopamine is commonly utilized in swine diets in the United States. Phenethanolamines are considered repartitioning agents due to their ability to repartition nutrients away from adipose tissue accretion and towards muscle (Moody et al., 2000). They function in swine, sheep, beef cattle, and chickens; however the effects tend to vary between species with cattle and sheep having the largest response, followed by pigs and then chickens with the smallest response. This difference in response between species may be due to intense genetic selection for growth rate in certain species such as chickens, which leaves less potential for improvements in growth or it may be because different species vary in beta-adrenergic receptor ( $\beta$ AR) selectivity and signaling pathways (Mersmann, 1998; Moody et al., 2000).

RAC in swine is a phenethanolamine that binds to  $\beta$ -1AR and is fed to swine in order to improve animal performance. Factors that influence the response in animal performance to RAC include dietary protein, duration of feeding, dosage, animal age or weight, and genetics (Moody et al., 2000). According to Mills (2002), down-regulation of  $\beta$ -AR receptors limits the effectiveness of RAC and creates a plateau in animal performance after extended feeding

periods. Additionally, Moody et al. (2000) reported that the largest improvements in animal performance and carcass characteristics occur when RAC is fed to heavier weight pigs.

### ***Mechanism of Action for RAC***

Beta-adrenergic agonists ( $\beta$ -AA) function by binding to  $\beta$ -AR and activating the  $G_s$  protein. The  $\beta$ -AR is a receptor with seven hydrophobic regions that snake back and forth across the plasma membrane seven times with the ligand binding site in the center of the seven hydrophobic transmembrane domains (Mills and Mersmann, 1995, Nelson and Cox, 2008). After a ligand binds to the receptor, the GDP bound to the  $G_s$  protein is replaced by GTP and activation of the  $G_s$  protein occurs. After activation of the G protein by the  $\beta$ -AR, the  $\alpha$ -subunit of the G protein binds to and activates adenylate cyclase which catalyzes synthesis of the second messenger, cyclic adenosine monophosphate (cAMP), from ATP leading to an increase in the cytosolic cAMP (Nelson and Cox, 2008). cAMP is responsible for binding the regulatory subunit of protein kinase A in order to release the catalytic subunit which phosphorylates several intracellular proteins. Protein kinase A phosphorylates the cAMP response element binding protein (CREB). The phosphorylation increases the transcriptional activity of the CREB, which can then bind to a cAMP response element in the regulatory part of a gene and stimulate transcription (Mersmann, 1998). Additionally, protein kinase A phosphorylates some proteins which are enzymes activated by phosphorylation such as hormone sensitive lipase (Mersmann, 1998; Mills and Mersmann, 1995)

In adipose tissue,  $\beta$ -AAs stimulate hormone sensitive lipase which is responsible for adipocyte triacylglycerol degradation to free fatty acids and glycerol. Consequently, Mills and

Mersmann (1995) and Mersmann (1998) stated that increased blood concentrations of nonesterified fatty acids (NEFA) may occur after  $\beta$ -AA feeding and indicate activation of the adipocyte lipolytic system. Additionally, phosphorylation of serine of the insulin receptor, insulin-regulated glucose transport protein (GLUT-4) and ACC leads to inhibition of insulin signaling, glucose transport and fatty acid synthesis (Mills and Mersmann, 1995). Mersmann (1998; 2002) also indicated that fatty acid and triacylglycerol synthesis are inhibited by  $\beta$ -AA leading to a reduction in fat deposition in adipocytes.

In muscle tissue,  $\beta$ -AAs promote muscle growth through hypertrophy (Yang et al., 1989), seemingly by promoting amino acid transport and protein synthesis and decreasing protein degradation (Mills and Mersmann, 1995). Mersmann (1998) stated that animals treated with  $\beta$ -AA have an increased mRNA transcript for myosin,  $\alpha$ -actin, and calpastatin. Calpastatin inhibits the activity of the protease, calpain, leading to a reduction in protein degradation.  $\beta$ -AAs do not cause an increase in DNA (Mills, 2002) but do increase nitrogen retention in muscle (Mills and Mersmann, 1995)

Another potential mechanism for  $\beta$ -AA action is an increase in blood flow to certain regions of the body. An increase in blood flow to skeletal muscle can promote hypertrophy by providing increased amounts of substrates and energy for protein synthesis (Mersmann, 1998). Yang et al. (1989) indicated that peripheral vasodilation is a response to  $\beta$ -AA and may increase blood flow to skeletal muscles. Furthermore, increased blood flow to adipose tissue may allow for removal of NEFAs from the tissue and enhance lipolysis (Mersmann, 1998). According to Yang et al. (1989),  $\beta$ -AAs alter the contractile properties of tension development and speed of contraction of skeletal muscle which are important for maintaining healthy muscle.

Over time, an animal's physiological response to  $\beta$ -AA is reduced due to a desensitization of the  $\beta$ -AR to the agonist (Dunshea, 1993). Moreover, acute  $\beta$ -AR desensitization results from phosphorylation of the receptor which uncouples the receptor from the G protein. This leads to a decrease in the sensitivity and maximal response to the agonist and causes the  $\beta$ -AR to be internalized away from the cell surface and receptors may also be degraded over time (Mills and Mersmann, 1995). However, withdrawal of the  $\beta$ -AA typically results in a return of the response (Dunshea, 1993).

### ***RAC inclusion in Swine Diets***

Due to the potential for increasing carcass leanness and animal performance, many experiments have been conducted to analyze the effects of RAC inclusion in swine diets.

Gu et al. (1991a, b) conducted a study in which barrows were fed 0 or 20 ppm RAC from 59 to 100 kg, 73 to 114 kg, or 86 to 127 kg of body weight. RAC increased ADG and total lean gain, but decreased ADFI, resulting in improved feed efficiency for barrows regardless of weight group. RAC increased dressing percentage and LEA regardless of weight group. RAC also increased percentage of fat standardized lean which was calculated by taking the weight of dissected lean standardized to contain 10% fat. Additionally, RAC decreased dissected fat (DF), DF as a percentage of total dissected weight, and fat depth at the 10<sup>th</sup> rib. RAC decreased percentage of lean containing fat (PLF), which was calculated as the intramuscular and dissectable fat within lean as a percentage of the dissected lean. Overall, RAC inclusion increased lean quantity and reduced fat, specifically at the 10<sup>th</sup> rib, intramuscular fat, and

undissectable fat contained within the dissected lean, of barrow carcasses regardless of body weight.

In a study conducted by Uttaro et al. (1993), pigs were fed 0 or 20 ppm RAC from 64 to 100 kg of body weight. The author demonstrated that feeding RAC increased ADG, improved feed efficiency and decreased the days to market weight by 6 days. The final live weight of the pigs did not differ in this study due to protocol of slaughtering animals at a weight end point. Results indicated that RAC inclusion in the diets did not affect drip loss, WHC, L\* value, or the occurrence of PSE pork. It was observed that RAC feeding led to a decrease in cooking loss of pork loins when compared to the control. Also, WBSF values were higher for RAC pork loins when compared to the control. Fresh loins and hams from pigs fed RAC had lower a\* and b\* values when compared to the control. The authors suggested that this may indicate a decrease in oxymyoglobin with RAC feeding or a shift in the number of red, white, and intermediate muscle fibers. Overall, this study reported no detrimental effects on pork quality when RAC was fed at 20 ppm. Furthermore, feeding RAC decreased 10<sup>th</sup> rib fat depth and increased lean depth, resulting in an increased predicted lean yield. Chemical analysis of the loin showed a higher lean content and a lower fat content of raw loins from pigs fed RAC when compared to control pigs. Chemical analysis of the ham only revealed a decrease in fat content of RAC hams. Furthermore, bellies from pigs fed RAC were heavier in weight, had greater lean thickness, and decreased chemical fat content when compared to control animals. Trimmed shoulder weights were similar between treatment groups, while loin, belly, and ham weights were increased in RAC fed pigs. This observation led the authors to believe that the distribution of lean from pigs fed RAC was altered from the control pigs. The authors discussed that this difference may be attributed to the

rapidly developing muscle fibers in the posterior of the body being more responsive to RAC than the slower-developing muscle in the anterior of the body.

In a study conducted by Fernández-Dueñas et al. (2008), pigs were fed 3 concentrations of RAC (0, 5, or 7.4 ppm) for two durations (final 21 or 28 d prior to slaughter). The results indicated that HCW was increased for RAC-fed pigs when compared to the control, but no differences in back fat depth or muscle depth were observed. Additionally, RAC feeding had no effect on loin pH, ham pH, Japanese color score of the loin, L\* and a\* of the loin and ham, or loin subjective color, firmness, and marbling. However, b\* values of the loin and SM were significantly lower for pigs fed 7.4 ppm RAC when compared to the control. The percentage of boneless shoulder, boneless cuts, and total lean cuts of the carcass were increased for pigs fed 5 ppm RAC when compared to the control. RAC inclusion had no effect on cook loss, shear force, percent moisture, percent fat, or sensory traits of juiciness, tenderness, and flavor of loin samples. Overall, this study revealed that RAC inclusion in swine diets may lead to increased carcass merit as shown by increased lean cut yield, without negatively impacted pork quality.

In a study conducted by Carr et al. (2005), pigs were fed 0 or 10 ppm RAC for 28 days prior to slaughter. The results indicate that ADG was not affected by RAC inclusion; however ADFI was decreased for RAC-fed pigs. Consequently, RAC inclusion improved feed efficiency over the duration of the trial. Carcasses from pigs fed RAC exhibited lower leaf fat weights, 10<sup>th</sup> rib back fat, and last rib back fat when compared to non RAC-fed pigs. Additionally, DP and LM area were increased with RAC inclusion. Objective color measurements of the LM showed that feeding RAC decreased a\* and b\* values, indicating a less red and less yellow color of the loin. RAC inclusion had no effect on subjective color, firmness, marbling, objective fat color, 45 min

pH, ultimate pH, or drip loss of loins when compared to controls. Belly firmness was decreased in RAC-fed pigs indicating a lower-quality belly. In terms of the sensory characteristics, RAC inclusion had no effect on pork flavor, juiciness, off flavor, and cooking loss; however loin chops from pigs fed RAC had higher shear force values and lower tenderness scores from a trained sensory panel. The author attributed this to the increase in muscle fiber diameter with RAC inclusion. No differences were seen among treatments for proximate analysis of loin or fat samples. When analyzing the fatty acid profiles, pigs fed RAC had higher C18:2 when compared to the control, but no differences were observed for other individual fatty acids or calculated iodine values of back fat. Overall, it was concluded that feeding RAC at 10 ppm for the final 28 days prior to slaughter improved feed efficiency, increased LMA and DP, and decreased subcutaneous fat without detrimental effects on pork quality, sensory characteristics, and fatty acid profiles.

### ***RAC interactions with fat source***

Leick et al. (2010) fed RAC at 0 or 5 ppm with 0, 15, 30, 45, or 60 % DDGS for four weeks prior to slaughter. RAC inclusion did not affect final BW, HCW, DP, back fat depth, loin muscle depth, or calculated percent lean, and no RAC x DDGS interactions were observed for these parameters. A decrease in marbling and color scores was seen with the inclusion of RAC, but subjective firmness scores were not affected by RAC. Objective color scores validated the subjective scores as RAC loins had greater L\* values and decreased a\* values than non-RAC fed pigs, indicating a slightly lighter and less red loin color. RAC did not affect b\* values of loins. Loin pH, drip loss, percent moisture, and percent fat were not affected by RAC inclusion. Additionally, bellies from RAC fed pigs showed no differences in belly length, width, average

thickness, trimmed weight, firmness, or L\*, a\*, b\* reflectance values of the belly fat. RAC did not affect percent pump of the bellies, belly cook loss, or sliced bacon cook loss. Lipid oxidation, as measured by TBARS, was not different in loin samples or bacon samples from pigs fed RAC. When considering the fatty acid profile of jowl samples, RAC did not affect concentrations of any fatty acid except RAC inclusion led to a decrease in C12:0 of jowl samples. In belly samples, RAC did not affect concentrations of any individual fatty acids except 20:4, where RAC feeding led to a decrease in C20:4 when compared to non-RAC fed pigs. Furthermore, RAC feeding did not affect total MUFA, total PUFA, MUFA: PUFA, SFA: UFA, or IV of jowl or belly samples. Overall, no RAC x DDGS interactions were seen for any meat quality parameters, and feeding RAC failed to elicit the improvements in carcass characteristics seen in previous studies, but had no detrimental effects on pork quality.

In a study conducted by Apple et al. (2007), crossbred pigs were fed either 0 or 10 ppm RAC with 5% of dietary fat from either beef tallow (BT) or soybean oil (SBO) for 35 days prior to slaughter. RAC inclusion did not affect L\*, a\*, or b\* for the rectus abdominus or belly fat, belly thickness, or belly compression values. Bellies from pigs fed RAC did have lower subjective firmness scores when bellies were measured skin-side down, however no differences was observed when bellies were measured skin-side up or when parallel to the bar. In terms of the fatty acid profile of belly fat samples, feeding RAC did not affect percentages of most saturated fatty acids; however significant RAC x fat source interactions were seen for C16:0 and C18:0. C16:0 concentrations were higher in belly fat from pigs fed BT with RAC when compared to pigs fed SBO without RAC, while pigs fed SBO with RAC had lower concentrations of C16:0 than all other dietary treatments. C18:0 concentrations were lower in

belly samples from pigs fed SBO with RAC when compared to all other treatment groups. Within SBO-fed pigs, total SFA of belly fat was decreased with RAC inclusion. RAC inclusion did not alter total MUFA or the individual percentages of MUFA in belly fat samples, except for an RAC x fat source interaction on C14:1. Belly fat from pigs fed BT with RAC had decreased C14:1 when compared to pigs fed BT without RAC. Total PUFA tended to increase in belly fat when RAC was included in the diet, most likely due to a tendency for higher levels of C18:2n6 and C18:3n3 when RAC was fed. Additionally, an RAC x fat source interaction was observed for C20:3n6, where belly fat from pigs fed SBO with RAC had higher levels of C20:3n6 when compared to all other treatment groups. The main effect of RAC had no effect on PUFA: SFA or IV, however a significant RAC x fat source interaction existed. Belly fat from pigs fed SBO with RAC had higher PUFA: SFA and IV when compared to pigs fed SBO without RAC and BT-fed pigs, but RAC had no effect on these values within BT-fed pigs. These results indicate that feeding RAC exacerbates the effect of dietary fat source on the fatty acid composition of pork bellies and interacts differently with different fat sources.

Apple et al. (2008) reported the loin quality and shelf-life characteristics of the study previously described by Apple et al. (2007). No RAC x dietary fat source interactions were observed for ADG, ADFI, or G: F in this study. Across the entire 35 d feeding trial, RAC-fed pigs had greater ADG and heavier BW than control pigs. ADFI was not affected by RAC inclusion, which led to an improvement in feed efficiency for RAC-fed pigs across the 35 d feeding period. This improvement in feed efficiency was likely due to RAC repartitioning energy from fat deposition to protein synthesis, because lean tissue deposition is more efficient than fat deposition in terms of energy utilization. Additionally, no RAC x dietary fat source interactions

were seen for carcass cutability traits, LM pH, drip loss percentage, TBARS values, WBSF values, cook loss, or retail display characteristics. The main effect of RAC did increase HCW, LM depth, fat-free lean yield, and decrease 10<sup>th</sup> rib fat depth when compared to control pigs. Ultimate pH of the LM was higher from RAC-fed carcasses when compared to control, but LM drip loss percentage did not differ due to RAC inclusion. When considering the fatty acid composition of the back fat, RAC inclusion led to a decrease in total SFA and C16:0 when compared to non RAC-fed pigs. There was a significant RAC x dietary fat source interaction for C18:0, where back fat from pigs fed SBO with RAC had lower C18:0 than pigs fed SBO without RAC. Total MUFA and Oleic acid (C18:1n9c) was decreased in RAC-fed pigs when compared to the control. Conversely, total PUFA was increased in back fat with dietary RAC inclusion, mainly due to increases in linoleic acid (C18:2n6),  $\alpha$ -linolenic acid (C18:3n3), eicosadienoic acid (C20:2), and Arachidonic acid (C20:4n6) with dietary RAC inclusion. Overall, the PUFA: SFA ratio and IV of back fat from pigs fed RAC were higher than pigs not receiving RAC. The data from five days of retail display indicate that loin chops from RAC fed pigs had higher subjective color and marbling scores across all five days of display when compared to chops from non RAC-fed pigs. Objective color measurements indicated that loin chops from RAC-fed pigs had lower L\*, a\*, b\*, and lower chroma values than chops from control pigs; this indicates a darker, less red, less yellow, and less vivid color for the chops with RAC inclusion. There was no effect of RAC inclusion on TBARS values of LM during the 5 d of retail display, LM cooking loss, or WBSF values of LM. Additionally, no RAC x fat source interactions were observed for these parameters. Overall, it can be concluded from this study that RAC inclusion in the last 35 d prior to slaughter can be beneficial to animal performance and carcass leanness without detrimental

effects on pork quality. However, RAC inclusion may increase the degree of unsaturation of pork fat, consequently increasing IV.

Overall, RAC is an effective repartitioning agent and consistently increases carcass leanness and improves animal performance in swine. However, current research indicates that RAC may increase the unsaturation of pork fat, and exacerbate the impact of unsaturated dietary fat sources such as DDGS.

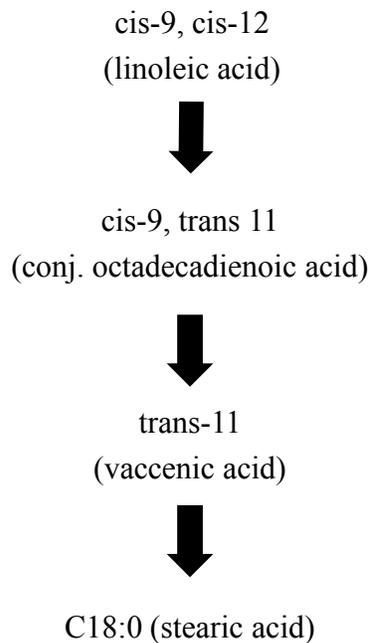
In summary, DDGS inclusion in swine diets is becoming more common due to economic concerns, but may lead to an increase in the unsaturated fatty acid content of carcass fat. RAC is commonly used in the swine industry to promote lean gain, but may further increase the unsaturation of fat, especially when fed in combination with PUFAs. On the other hand, CLA has shown to increase the saturated fatty acid content of pork fat without detrimental effects on animal performance or meat quality. Therefore, the question arises, what are the interactive effects of dietary DDGS, RAC, and CLA on fatty acid composition and subsequent lipid oxidation in swine. Additionally, how does the incorporation of a natural antioxidant, rosemary, mitigate the issue of oxidative deterioration in highly unsaturated fat.

**Table 1.1 CLA concentrations of common foods**

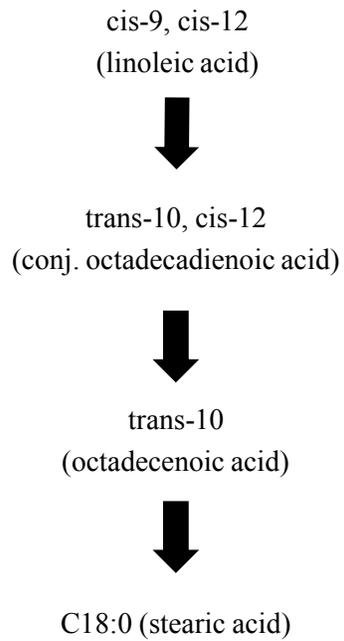
Foodstuff	Total CLA (mg/g fat)
Ground Beef	4.3
Lamb	5.6
Pork	0.6
Chicken	0.9
Egg Yolk	0.6
Turkey	2.4
Medium Cheddar Cheese	4.1
Mozzarella Cheese	4.9
Homogenized milk	5.5
Butter	4.7
Safflower oil	0.7
Sunflower oil	0.4

Adapted from Chin et al. (1992).

**Figure 1.1 Predominant pathway of biohydrogenation of linoleic acid (C18:2). Adapted from Griinari and Bauman (1999).**



**Figure 1.2 Proposed pathway of biohydrogenation of linoleic acid (C18:2) involving cis-9, trans-10 isomerase. Adapted from Griinari and Bauman (1999).**



## **CHAPTER 2**

# **SHELF STABILITY AND QUALITY OF FRESH GROUND PORK AND PORK SAUSAGE FROM PIGS FED A COMBINATION OF DRIED DISTILLERS GRAINS WITH SOLUBLES, RACTOPAMINE HYDROCHLORIDE, AND CONJUGATED LINOLEIC ACID**

### **ABSTRACT**

An experiment was performed to evaluate the effects of ractopamine (RAC), conjugated linoleic acid (CLA) and distillers dried grains with solubles (DDGS) on fatty quality and shelf stability of ground pork. Diets were arranged in a 2 x 2 x 2 factorial design within a completely randomized design. Picnic shoulders (n=72) were selected from pigs fed one of eight dietary treatments, consisting of two levels of DDGS inclusion (0 or 20% DDGS), two levels of RAC (0 and 7.4 mg/kg), and two levels of CLA (0 and 0.6%). Picnic shoulder trim from each animal was divided into three sections and each was assigned to one of three processing treatments: A) ground pork, B) fresh pork sausage, or C) fresh pork sausage + rosemary extract. Samples were analyzed for fatty acid profiles, TBARS, and color during retail display. Higher IV were seen with DDGS ( $P < 0.0001$ ) and RAC ( $P = 0.004$ ) inclusion and lower IV with CLA ( $P < 0.0001$ ).

### **INTRODUCTION**

Fat quality and fatty acid composition are important factors in pork products as they can influence further processing characteristics, shelf-life due to lipid oxidation, and pork export

potential (Carr, 2005). Fatty acid composition of tissues greatly impacts the susceptibility to lipid oxidation and the subsequent development of undesirable colors, flavors, and odors (Wood et al., 2003). Diet plays a large role in determining the fatty acid composition of pork fat since dietary fatty acids are absorbed unchanged from the intestine and incorporated into tissue lipids in non-ruminant animals (Wood and Enser, 1997).

The use of polyunsaturated fat sources in swine diets such as distillers dried grains with solubles (DDGS) has increased over the last several years due to a rise in ethanol production and a greater availability of byproducts for incorporation into livestock feeds (Stein and Shurson, 2009). Several studies have shown that DDGS inclusion up to 20% in swine diets maintains animal performance but increases iodine values (IV) and unsaturation of pork fat (Whitney et al., 2006; Leick et al., 2010; Benz et al., 2010; Xu et al., 2010).

Furthermore, recent studies have shown that ractopamine hydrochloride (RAC) inclusion in swine diets in order to improve animal performance and carcass leanness may have detrimental effects on pork fat quality (Carr et al., 2005; Apple et al., 2007; Apple et al., 2008). Conjugated linoleic acid (CLA) is a group of positional and geometric isomers of linoleic acid naturally found in ruminant meat and milk products as a result of rumen biohydrogenation of fatty acids (Christie, 2003; Griinari and Bauman, 1999). Studies have shown that CLA inclusion in swine diets could mitigate some of the negative effects caused by diets high in unsaturated fatty acids. Eggert et al. (2001), Wiegand et al. (2001), and Larsen et al. (2009) demonstrated CLA's ability to reduce IV by increasing the saturation of pork fat.

In addition to manipulating the fatty acid composition of pork fat through dietary modifications, lipid oxidation in ground pork products can also be reduced through the utilization of antioxidants. Recent studies have been designed to investigate the effectiveness of natural antioxidants such as rosemary due to consumer concerns about synthetic antioxidants (Sebranek et al., 2005).

Therefore, the objective of this study was to evaluate the interactive effects of DDGS, RAC, and CLA on fatty acid composition and oxidation in ground pork and to determine if the antioxidant properties of rosemary extract could retard lipid oxidation in highly unsaturated pork fat.

## **MATERIALS AND METHODS**

### ***Sample Selection and Preparation***

Picnic shoulders (n=72) (IMPS #405; NAMP, 2007) were selected from carcasses of barrows fed one of eight diets (Table). Dietary treatments were set up in a 2 x 2 x 2 factorial arrangement in a completely randomized design 9 replications per treatment. The main effects were DDGS inclusion (0 or 20%), Ractopamine Hydrochloride (RAC; Paylean, Elanco Animal Health, Greenfield, IN, U.S.A.) inclusion (0 or 7.4 mg/kg), and CLA (Luta-60, BASF Corporation, Florham Park, NJ, U.S.A.) inclusion (0 or 0.6%). Barrows were individually penned and had ad libitum access to feed and water for 28 days prior to slaughter.

Picnic shoulders were skinned and deboned and boneless picnic shoulder trim (IMPS #405A; NAMP, 2007) was weighed, boxed, and placed in freezer storage. Boneless trim was ground through a kidney plate and then a 10 mm plate using an LEM 0.35 HP meat grinder

(Harrison, OH, U.S.A.). Picnic shoulder trim from each animal was then divided into three sections and each section was assigned to one of three processing treatments: A) ground pork, B) fresh pork sausage, or C) fresh pork sausage + rosemary extract, resulting in a 2 by 2 by 2 by 3 factorial arrangement in a split plot design. The main plot contained the effect of DDGS, RAC, CLA, and all possible interactions and the subplot contained the effect of processing treatment and all interactions with the main plot effects.

Fresh pork sausage was produced utilizing AC Legg 10 sausage seasoning (Calera, AL, U.S.A.) at 2.0% of the product and the rosemary extract utilized was Fortium R10 Dry (Kemin Food Technologies, Des Moines, IA, U.S.A.) at 0.18% of the product. All products were then fine ground in the LEM meat grinder using a 4.5 mm plate. Two 113.5 gram patties were made for each product using an LEM non-stick adjustable burger press (LEM 534, Harrison, OH, U.S.A.). The remaining product from each picnic shoulder was placed in a whirl-pak and frozen at -10 °C for later fatty acid and proximate analysis. The patties were then placed on a foam tray with an absorbent pad, overwrapped in oxygen-permeable polyvinylchloride film, and placed in a coffin-style retail cooler for 7 days maintained at an average temperature of 5°C when at loaded level. Patties were displayed under continuous 968 lux of fluorescent lighting. Pork patty surface objective color measurements ( $L^*$ ,  $a^*$ ,  $b^*$ ) were taken at d 1, 3, 5, and 7 of retail display utilizing a Minolta Chroma Meter CR-410 (Minolta Camera Co., Osaka, Japan) with a D65 light source and 10 degree observer. The instrument was calibrated before each analysis with an identical wrap as used for the samples placed over a white tile standard. Samples were evaluated while wrapped in packaging and in duplicate. After three days of retail display, one of the two patties was removed from the display cooler, vacuum packaged, and frozen at -10 °C for trained

sensory panel analysis. After seven days of retail display, the other patty was removed from the display cooler and a 20 g sample was collected from a subset of six patties per treatment group for lipid oxidation determination using the TBARS procedure described by Pegg (2001). This sample was placed in a 50 mL conical centrifuge tube and flash frozen using liquid nitrogen, then stored in a -20 °C freezer until analysis.

### ***Proximate Analysis***

Proximate analysis was performed in duplicate on all meat products according to Keeton, Hafley, and Eddy (2003) using a CEM Moisture/Solids Analyzer and Smart Trac Rapid Fat Analysis system (CEM Corp., Matthews, NC, U.S.A.). Two square sample pads were placed into the moisture/solids analyzer, dried, and tared. Approximately 3g of meat sample was then smeared across one of the pads, the second pad was placed on top of the sample, and moisture percentage was determined by weight using the CEM moisture/solids analyzer. Following moisture analysis, the dried sample and pads were rolled in TRAC paper and placed into the CEM TRAC tube and packed tightly at the bottom. The tube was then placed in the CEM rapid fat analyzer where fat percentage was determined on dry basis using nuclear magnetic resonance and later converted to wet basis.

### ***Lipid Oxidation Determination***

Lipid oxidation was determined using the thiobarbituric acid reactive substances (TBARS) extraction method described by Pegg (2001). Briefly, 5 g of meat sample was added to 2.5 mL of an antioxidant solution, 50 mL of ice-cold TCA reagent, and 50 mL of distilled water, then homogenized using a Hamilton Beach 2-speed Handheld blender (Model # 59760, Southern Pines, NC, U.S.A.). The sample was then filtered and a 5 mL aliquot of sample solution was

added along with 5 mL of TBA to a 50 mL conical centrifuge tube. The tubes were capped, vortexed, and heated in a boiling water bath for 35 minutes. Tubes were removed from the water bath, cooled in ice for 5 minutes, and then the sample was transferred to a glass cuvette. Absorbance was measured at 532 nm using a spectrophotometer (Thermo spectronic Genesys 20 4001/4). TBARS values were obtained from the absorbance as described by the authors.

### ***Sensory Evaluation***

Sensory evaluation was conducted at Iowa State University. Before the trained sensory evaluation test sessions began, 5 panelists were trained in each of two, 1 hour sessions for the evaluation of the attributes being tested, using samples representing all treatments. Samples for training and testing were prepared and evaluated as described. Samples were thawed under refrigeration for approximately 24 hours. After thawing, samples were removed from the vacuum package and placed on individual trays labeled with a three digit blinding code. A clam-shell grill (George Forman, Model #GRP99) was preheated to 350 °F. Both sausage patty samples/ID number were placed on the grill and the top closed. Samples were cooked for three minutes without turning. Samples was removed from the grill and cut into fourths. After cutting both sausage patties into fourths, one ¼ piece of sausage pattie was placed in each of five Styrofoam cups (prelabeled with the corresponding three digit code number), covered and served immediately to the five sensory panelists. Panelists scored each sample for intensity of pork sausage flavor and oxidized/rancid flavor on a 15-point unstructured line scale with 0=none and 15=intense.

### ***Fatty Acid Determination***

The methodology utilized for fatty acid determination was an adaptation of the methods used by Folch et al. (1957) and Morrison and Smith (1964). At the moment of the analysis, approximately 1 g of sample was placed in a glass tube and 5 mL of a solution of chloroform:methanol (CHCL<sub>3</sub>:CH<sub>3</sub>OH, 2:1, v/v) was added to the tube in order to extract lipids. The sample was homogenized using an Omni International 2000 homogenizer (Waterbury, CT, U.S.A.) for 30 seconds. Next, the sample was filtered through a sintered glass filter funnel fitted with a Whatman 2.4 cm GF/C filter and a solution of 0.74% KCl was added to the tube at a volume of 8 mL. The sample was allowed to sit for 2 h to separate the phases and then the upper phase was carefully removed and discarded. The lower phase was then transferred to a glass tube and evaporated to dryness with nitrogen gas in a heated water bath at 70 °C using a Meyer N-Evap Analytical Evaporator (Organomation Associates Inc., Berlin, MA, U.S.A.).

A 1 mL solution of 0.5 N KOH in MeOH was added to the sample and the tube was placed in a water bath at 70 °C for 10 min. Then, a 1 mL solution of 14% boron trifluoride (BF<sub>3</sub>) in MeOH was added to the tube which was flushed with nitrogen, loosely capped and placed in a water bath at 70 °C for 30 min. After 30 min, the sample was cooled to room temperature and 2 mL of HPLC grade hexane and 2 mL of saturated NaCl was added to the tube. Next, the upper layer was removed and placed in a glass tube with approximately 800 mg of Na<sub>2</sub>SO<sub>4</sub> in order to remove moisture from the sample. Following this, 2 mL of hexane was added to the tube with saturated NaCl and once more, the upper layer was removed and placed in the same tube with Na<sub>2</sub>SO<sub>4</sub>.

The liquid portion was then transferred to a scintillation vial which was placed in a water bath at 70 °C and the sample was evaporated with nitrogen. A Varian 420 gas chromatograph

(Varian, Pala Alto, CA, U.S.A.) was used to analyze fatty acid methyl esters; samples were injected onto a fused silica capillary column (SPTM – 2,560; 100 m x 0.25 mm x 0.2 µm film thickness; Supelco, Bellefonte, PA, U.S.A.). The temperature of the injector and of the flame-ionization detector was held constant at 240°C and 260°C, respectively. Helium was used as the carrier gas at a constant pressure of 37 psi and the oven was operated at 140°C for 5 min (temperature programmed 2.5°C/min to 240°C and held for 16 min). Fatty acids were normalized which means that the area of each peak was represented as a percentage of the total area. Iodine value (IV) was determined based on the equation described by AOCS (1998):  $IV = (0.95 \times C16:1) + (0.86 \times C18:1n9) + (1.732 \times C18:2n6) + (2.616 \times C18:3n3) + (0.785 \times C20:1)$ . The ratio between polyunsaturated fatty acids (PUFA) and saturated fatty acids (SFA) was calculated using the equation:  $[(C18:2n6c) + (C18:3n3)]/[(C14:0) + (C16:0) + (C18:0)]$ .

### ***Statistical Analysis***

Statistical Analysis for fatty acid profiles and proximate analysis was analyzed using the MIXED procedure of SAS (SAS Institute, Inc., Cary, NC, U.S.A.) to obtain LSmeans and SE estimates. The model included the fixed effects of DDGS, RAC, and CLA and all possible interactions; samples were pooled by pig across process. Furthermore, statistical analysis for TBARS values and sensory panel scores was analyzed using the MIXED procedure of SAS and the model included the fixed effects of DDGS, RAC, CLA, processing treatment (A=ground pork, B=pork sausage, or C=pork sausage with rosemary), and all possible interactions. Pig nested within DDGS, RAC, and CLA was used as the denominator of F to test the effects. Color during retail display was analyzed using the repeated measures option in the MIXED procedure. The model included the fixed effects of DDGS, RAC, CLA, processing treatment, day (1, 3, 5, or

7), and all possible interactions. Means were separated using the PDIFF option. Significance was determined at  $\alpha < 0.05$ .

## **RESULTS**

### ***Proximate Analysis***

While the main effects of DDGS and CLA had no effect on moisture percentage ( $P = 0.072$  and  $P = 0.425$  respectively), RAC inclusion significantly increased moisture in ground picnic shoulders (Table 2) when compared to non-RAC fed pigs (67.77 vs. 66.00 %;  $P = 0.0003$ ). CLA did not affect fat percentage ( $P = 0.282$ ), however a significant DDGS by RAC interaction was observed ( $P = 0.046$ ; Table 3). Picnic shoulders from pigs fed the control diet lacking DDGS and RAC had the highest fat percentage (17.71 %), while DDGS inclusion and RAC inclusion separately decreased fat content when compared to the control (12.54 % and 14.18 % respectively); however no additive effect was observed when pigs received both DDGS and RAC (12.59 %).

### ***TBARS values***

A significant interaction between RAC and CLA was observed for TBARS values ( $P = 0.033$ ; Table 4). In non-RAC fed pigs, CLA decreased TBARS values when compared to non-CLA fed pigs (0.654 vs. 0.831). Moreover, in non-CLA fed pigs, RAC decreased TBARS values from 0.831 to 0.559, indicating that RAC was more effective at lowering TBARS values than CLA, but no additive effect was seen on TBARS values when both RAC and CLA were fed ( $p > 0.05$ ). Additionally, a RAC by process interaction existed for TBARS values ( $p < 0.05$ ; Table 5). Within each processing treatment, RAC inclusion led to decreased TBARS values. In non-

RAC fed pigs, processing treatment B (pork sausage) resulted in lower TBARS values than A (ground pork); however processing treatment C (sausage with rosemary extract) had lower TBARS values than both A and B. In RAC-fed pigs, no difference existed between process A and B or process B and C ( $p > 0.05$ ); however TBARS values for ground pork (process A) were significantly higher than for pork sausage (process B). Dietary DDGS inclusion did not affect TBARS values ( $P = 0.78$ ; Table 6).

### ***Sensory Panel***

Sensory panel scores are presented in Table 6. Although differences were detected in TBARS values, there were no differences ( $P > 0.05$ ) in subjective flavor or rancidity scores of pork patties due to the main effects of DDGS, CLA, RAC, or processing treatment and no interactions were observed.

### ***Objective Color Measurements***

Objective color measurements collected from pork patties over seven days of retail display are presented in Table 7. Minolta L\* (lightness) reflectance values were significantly lower for DDGS-fed pigs over the entire display period (52.97 vs. 53.87;  $P = 0.024$ ), indicating a darker pork patty. Additionally, a significant interaction was observed between RAC and processing treatment for L\* values ( $P = 0.017$ ; Table 5). Within processing treatments B and C, RAC inclusion led to lower L\* reflectance values when compared to non-RAC fed pigs. No difference was observed in processing treatment A due to RAC inclusion ( $P > 0.05$ ). Also, in RAC-fed pigs, patties in processing treatment A had higher L\* values than both B and C, whereas in non-RAC fed pigs, no difference occurred in L\* values due to processing treatment.

CLA by process by day interactions for L\* values are presented in table 8. Dietary CLA inclusion resulted in lower L\* reflectance values ( $P = 0.002$ ) on days 1, 3, and 7 for processing treatments A and B, and on day 5 for processing treatment C when compared to pigs not fed CLA.

A significant interaction between CLA and RAC was observed for a\* (redness) values ( $P = 0.042$ ; Table 4). Patties from RAC-fed pigs had higher a\* values than non-RAC fed pigs regardless of CLA inclusion. In non-RAC fed pigs, CLA increased a\* values (14.10 vs. 14.76), but had no effect in RAC-fed pigs (15.45 vs. 15.41). Additionally, a DDGS by process interaction was detected for a\* values ( $P = 0.045$ ; Table 9). For patties in processing treatment A, DDGS inclusion decreased a\* values (16.11 vs. 15.68), but no difference occurred in processing treatments B or C. Regardless of DDGS inclusion, patties from processing treatment A exhibited higher a\* values than B. Furthermore, a\* values of patties from processing treatment C were lower than A and B for pigs receiving DDGS and non DDGS-fed pigs. Table 10 contains the RAC by process by day interactions for a\* values. Regardless of RAC or processing treatment, a\* values decreased from day 1 to day 7 of storage ( $P = 0.018$ ). Dietary RAC inclusion led to higher a\* values for all processing treatments and days of storage except for patties from processing treatment B on day 1. On days 1, 3, and 7, patties from B and C had lower a\* values than patties from processing treatment A, regardless of RAC inclusion. Finally, patties from processing treatment C had lower a\* values than patties from B on days 1 and 5 for non RAC-fed pigs and days 3, 5, and 7 for RAC-fed pigs.

The main effect of CLA inclusion did not affect b\* (yellowness) values of patties during retail display ( $P = 0.943$ ); however, a significant interaction between DDGS, RAC, processing

treatment, and day was observed for b\* values ( $P = 0.004$ , Table 11). The b\* values of patties decreased from day 1 to day 7 for all treatments, except for patties from DDGS fed pigs in processing treatment A that did not receive RAC. Patties from processing treatments B and C had higher b\* values on days 1 and 3 when compared to patties from A, regardless of DDGS or RAC inclusion. Also, patties from processing treatment A had higher b\* values than B on day 5 for RAC-fed pigs, regardless of DDGS inclusion. Patties from pigs fed RAC with no DDGS had lower b\* values on days 1, 3, 5 and 7 for processing treatment B and day 5 for C. Patties from pigs fed RAC with DDGS had higher b\* values on day 7 for processing treatment B. Dietary DDGS inclusion in non-RAC fed pigs resulted in lower b\* values on day 5 for patties from processing treatments A and C and on days 1, 3, and 7 for processing treatment B.

### ***Fatty acid composition***

The main effects of DDGS, CLA, and RAC are presented in table 12. Dietary DDGS inclusion resulted in higher concentrations of linoleic acid (14.41 vs. 16.95 %;  $P < 0.0001$ ), total PUFA (17.6 vs 20.38;  $P < 0.0001$ ) and PUFA:SFA, but lower concentrations of palmitic acid (23.32 vs. 22.22 %;  $P < 0.0001$ ), palmitoleic acid (2.33 vs. 1.99 %;  $P < 0.0001$ ), and oleic acid (40.42 vs. 39.39 %;  $P = 0.003$ ) which led to a decrease in total SFA (37.36 vs. 36.01 %;  $P = 0.003$ ) and total monounsaturated fatty acids (MUFA; 45.04 vs. 43.62 %;  $P = 0.0004$ ). Furthermore, these changes in the fatty acid composition due to DDGS inclusion increased the IV from 64.38 to 67.60 in pork patties ( $P < 0.0001$ ). Dietary RAC inclusion resulted in higher percentages of linoleic acid (15.19 vs. 16.17 %;  $P = 0.002$ ), total PUFA (18.40 vs. 19.57 %;  $P = 0.002$ ), and PUFA:SFA, as well as a decrease in palmitic acid (23.08 vs. 22.46 %;  $P = 0.008$ ) and total SFA (37.28 vs. 36.09 %;  $P = 0.009$ ). Total MUFA content did not differ due to RAC

inclusion ( $P = 0.965$ ). Subsequently, RAC inclusion led to an increase in IV from 65.16 to 66.82. ( $P = 0.004$ ).

On the other hand, CLA inclusion increased concentrations of palmitic acid (22.2 vs. 23.34 %;  $P < 0.0001$ ), palmitoleic acid (2.09 vs. 2.22 %;  $P = 0.043$ ), and stearic acid (11.12 vs. 12.68 %;  $P < 0.0001$ ), resulting in an increase in total SFA (35.17 vs. 38.19 %;  $< 0.0001$ ). In addition, CLA inclusion decreased oleic acid (41.66 vs. 38.15 %;  $< 0.0001$ ), linolenic acid (1.02 vs. 0.88 %;  $P = 0.004$ ), total MUFA (46.08 vs. 42.58;  $P < 0.0001$ ), and PUFA:SFA. The inclusion of dietary CLA also resulted in an increase in the concentration of the 9 *cis* 11 *trans* isomer and the 10 *trans* 12 *cis* isomer of CLA, as well as total CLAs (0.19 vs. 1.04 %;  $P < 0.0001$ ). Overall, these changes in fatty acid composition due to CLA inclusion led to a reduction in IV from 67.70 to 64.28 ( $P < 0.0001$ ). An interaction between DDGS and RAC occurred for arachidonic acid (20:4n6) and is presented in table 13 ( $P = 0.045$ ). Both DDGS inclusion and RAC inclusion increased arachidonic acid when compared to the control, but no additive effect was observed when both DDGS and RAC were fed.

## DISCUSSION

### *Proximate Analysis*

The impact of RAC inclusion on moisture percentage of pork carcasses is inconclusive. In this experiment, RAC inclusion increased moisture percentage, this is supported by Yen et al. (1990) who also observed an increase in percentage moisture in hams following RAC inclusion. However Carr et al. (2005) and Adeola et al. (1990) found no difference in moisture content with RAC feeding and Stites et al. (1994) observed a decrease in moisture

content of loin muscle with RAC inclusion. The inclusion of RAC in swine diets has consistently been shown to reduce fat content as seen in this study (Yen et al., 1990; Uttaro et al., 1993; Carr et al., 2005).

Varying results exist for feeding DDGS on fat content of pork carcasses. Whitney et al. (2006), Leick et al. (2010), and Cook et al. (2005) found no difference in carcass fat when DDGS were fed, however Xu et al. (2010) agrees with the results found in this experiment and observed a decrease in fat thickness of pork carcasses from pigs fed DDGS, potentially due to a decrease in ADFI. In this study, pigs fed DDGS also had lower ADFI than the control (unpublished data). Linneen et al. (2008) discovered a tendency ( $P < 0.1$ ) for pigs fed DDGS to have less backfat than the control. This could be attributed to a higher level of crude protein in DDGS which has been shown to decrease fat accretion (Chen et al., 1999).

### ***TBARS values***

It is well accepted that unsaturated fatty acids are more susceptible to lipid oxidation than saturated fatty acids (Larick et al., 1992; Min and Ahn, 2005). The dietary inclusion of CLA results in a shift of the fatty acid profile from unsaturation towards saturation (Eggert et al., 2001; Larsen et al., 2009; White et al., 2009); thereby decreasing the potential for lipid oxidation. Similar to the results found in this study, Joo et al. (2002) and Larsen et al. (2009) discovered decreased TBARS values when CLA was fed. Dietary RAC inclusion led to a decrease in TBARS values in this experiment most likely due to a decrease in the total fat content. Jo et al., (1999) reported an increase in lipid oxidation as the fat content of sausage increased. Min and Ahn (2005) also indicated that oxidation was directly linked to the fat composition of a meat

sample. Dietary DDGS inclusion did not affect TBARS values in this study, which is similar to the findings of Xu et al. (2010). On the other hand, Leick et al. (2010) found DDGS inclusion to increase TBARS after 21 days of storage and DDGS inclusion resulted in TBARS above the threshold of 0.5 indicated by Tarladgis et al. (1960). The reason TBARS values did not differ due to DDGS inclusion in this study may be due to two opposing factors. The inclusion of DDGS led to a lower fat content which reduces the potential for lipid oxidation, while the high PUFA content of fat from DDGS fed pigs may increase the susceptibility to oxidation (Min and Ahn, 2005).

The TBARS values for pork sausage were less than fresh ground pork in patties from non-RAC fed pigs, most likely due to the fact that the pork sausage contained sage. Sage has been shown to exhibit antioxidant properties (Wong et al., 1995; Tanabe et al., 2002). However, the pork sausage also contained sodium chloride which is a known pro-oxidant (Chen et al., 1984; Min and Ahn, 2005). Patties from processing treatment C had even lower TBARS values due to the addition of rosemary extract, an effective antioxidant (Chen et al., 1999; McCarthy et al., 2001; Sebranek et al., 2005). Both rosemary and sage contain phenolic antioxidants that react with lipid radicals to stabilize them (Tanabe et al., 2002). In RAC fed pigs, there was less separation of TBARS values due to processing treatment which may be the result of less opportunity for oxidation to occur due to the lower fat content of RAC pork patties.

### ***Sensory Panel***

Although no differences occurred in sensory panel scores for flavor or rancidity due to treatment, panelists detected some level of oxidation in all samples. According to Tarladgis et al.,

(1960), the threshold for detection of oxidation in pork is 0.5 to 1.0 TBARS values for trained panelists. This agrees with the data from the current study which showed panelist detection of oxidation at TBARS values of 0.5 to 0.9. This indicates that although all samples exhibited some level of oxidation and development of warmed over flavor (WOF), panelists could not separate the differences between treatments that were observed for TBARS values.

### ***Objective Color Measurements***

Even though statistical differences were detected for L\*, a\*, and b\* reflectance values, the practical significance of this is limited due to the small numerical changes. According to the NPPC pork quality standards (1999), a difference of approximately six points for L\* value separates the subjective color scores that can be detected visually. Nonetheless, as expected, a\* (redness) values decreased as display time increased, which is similar to findings reported by Houben et al. (1998) and Frederick et al. (2004), due to an increase in metmyoglobin during retail display. RAC inclusion led to higher a\* values due to a lower fat content. Additionally, CLA inclusion led to higher a\* values in non-RAC fed pigs possibly due to the reduction of lipid oxidation and the formation of metmyoglobin as a result of an increase in the saturation of the fat. Furthermore, the autooxidation of CLA has been reported to form furan fatty acids that can protect cells against peroxide attack and consequent oxidative deterioration (Yurawecz et al., 1995). In ground pork patties, DDGS inclusion may have resulted in lower a\* values due to the formation of metmyoglobin. Lipid oxidation results in the loss of red color because myoglobin becomes oxidized to metmyoglobin (Houben, 1998; Wood and Enser, 1997) and the high PUFA content of fat from DDGS-fed pigs may have accelerated oxidation (Leick et al., 2010).

Higher L\* values can indicate a greater amount of light reflection as a result of protein denaturation (Hansen et al., 2004). Aldehydes from lipid oxidation are capable of reacting with protein and can reduce protein stability and functionality (Min and Ahn, 2005); therefore lipid oxidation might result in higher L\* values. Patties from CLA-fed pigs had lower L\* values in ground pork and pork sausage, potentially due to the inhibition of lipid oxidation because of a shift in the fatty acid composition towards saturation. Dietary DDGS and RAC inclusion led to lower L\* values only as a result of a lower fat content.

### ***Fatty acid composition***

It is well known that in non-ruminant animals, dietary fatty acids are absorbed unchanged and incorporated into tissue lipids (Wood and Enser, 1997; Gatlin et al., 2002a; Kloareg et al., 2007). Furthermore, Allee et al. (1971) stated that dietary fat inclusion in swine leads to a decrease in *de novo* fatty acid synthesis, consequently resulting in a higher proportion of tissue lipids being derived from dietary sources. Therefore, when feedstuffs such as DDGS which are high in PUFA are fed to swine, increases in the PUFA content of the carcass are expected. According to Xu et al. (2010), DDGS are comprised of approximately 10 % oil and a high level of unsaturated fatty acids (81%) with 54% of that contributed by linoleic acid (18:2n6). Benz et al. (2010) observed an increase in linoleic acid, PUFA, and IV and a decrease in palmitic acid and MUFA in belly, back, and jowl samples from pigs fed DDGS for 78 d. Furthermore, feeding DDGS at 20 % of the diet led to IV over 73 in jowl samples. Whitney et al. (2006) reported increases in IV of belly fat for pigs fed 20 % DDGS inclusion for 91 d with a calculated IV over 70. Xu et al. (2010) observed increases in PUFA, specifically linoleic acid, and decreases in SFA and MUFA of belly fat and back fat samples with DDGS inclusion. Additionally, increases in IV

for belly fat, back fat, and intramuscular fat (IMF) samples were reported with DDGS inclusion. Similar findings were reported in the current study with DDGS inclusion leading to an increase in the unsaturation and IV of ground pork. However, although DDGS inclusion negatively impacted IV, the values calculated in this study were below most thresholds of unacceptability, except for Eggert et al. (2001) who reported the threshold of acceptability at an IV of less than 65. The Danish meat research institute (Goodband et al., 2006) and (Boyd et al., 1997) described an acceptable iodine value as less than 70 or 74, respectively. Moreover, according to the NPPC pork composition and quality assessment procedures, a high quality fat has an IV of less than 70, while DDGS fed pigs in this study had a mean IV of 67.60. This is in contrast to previous studies which report IV greater than 70, this is most likely because of the shorter duration of this feeding trial (28 days).

Previous studies have reported a tendency for dietary RAC inclusion to lead to an increase in the unsaturation of pork fat; however these results are not always consistent and are more pronounced when RAC is fed with an unsaturated fat source. Leick et al. (2010) observed no effect of RAC inclusion on MUFA, PUFA, or IV of belly or jowl samples. Moreover, Carr et al. (2005) reported an increase in linoleic acid with RAC inclusion, but no difference in SFA, MUFA, PUFA, or IV of subcutaneous fat samples in RAC-fed pigs. On the other hand, Apple et al. (2007) reported a decrease in the SFA content, mostly driven by a decrease in stearic acid, of belly fat samples when RAC was fed in conjunction with soybean oil (SBO). A tendency ( $P < 0.1$ ) for an increase in PUFA, specifically linoleic acid, was also reported. Apple et al. (2008) observed decreases in MUFA, especially oleic acid, and an increase in PUFA, linoleic acid, and arachidonic acid for back fat samples with RAC inclusion, leading to an increase in IV for RAC-

fed pigs. Dietary RAC inclusion results in leaner carcasses which have less *de novo* synthesis of SFA (Wiegand et al., 2011; Kloareg et al., 2007). Wood and Enser (1997), Gatlin et al. (2002a) and Correa et al. (2008) have also reported decreases in *de novo* synthesis in leaner pigs, resulting in a higher proportion of carcass fat being derived from dietary sources; consequently increasing the unsaturation of pork fat. Therefore, it isn't surprising that RAC inclusion resulted in higher IV and PUFA and a lower proportion of SFA of ground pork in this study. The increase in linoleic acid specifically is also of concern as it is the fatty acid that provides the best prediction of fat firmness and quality characteristics (Wood et al., 2008). Larick et al. (1992) also indicated that high dietary levels of linoleic acid result in rapid oxidation and production of volatile compounds including pentanal and hexanal, common aldehydes implicated in the development of warmed-over flavor (Frankel, 1984).

According to Smith et al. (2002), dietary CLA inclusion inhibits the activity of stearyl co-A desaturase (SCD) which is the rate limiting enzyme in fatty acid desaturation. This inhibition leads to a shift in the fatty acid profile from palmitoleic acid (16:1) to palmitic acid (16:0) and oleic acid (18:1) to stearic acid (18:0) which is also referred to as a decrease in the  $\Delta 9$  desaturase index (Ntambi et al., 2002). The t10 c12 isomer of CLA is thought to be responsible for the decrease in SCD activity (Smith et al., 2002). Larsen et al. (2009) reported an increase in SFA, specifically palmitic acid and stearic acid, an increase in palmitoleic acid, and a decrease in PUFA and IV driven by a decrease in oleic acid, linoleic acid, and arachidonic acid of belly fat samples when CLA was fed. White et al. (2009) also reported increases in SFA, specifically stearic acid, and a decrease in oleic acid and IV in back fat and belly fat samples of pigs fed CLA. Joo et al. (2002) observed an increase in SFA and a decrease in oleic acid, linoleic acid

and total unsaturated fatty acids (UFA) of loin samples after CLA feeding. These studies are all in agreement with the current results which demonstrated a shift in the fatty acid profile towards a higher proportion of SFA and lower IV. Although health concerns arise with high levels of SFA, the main increase due to CLA occurs in stearic acid which is considered neutral, meaning it does not have a cholesterol raising effect when consumed by humans, this is unlike other SFA (Hu et al., 1999; Connor, 1999; Hu et al., 2001) Larsen et al. (2009), White et al. (2009), and Joo et al. (2002) all reported increases in CLA content of tissues for CLA-fed pigs, similar to the results previously described in this experiment.

## **CONCLUSIONS**

Utilizing a combination of DDGS and RAC in diets is currently economically advantageous in the U.S. swine industry. In this study, feeding 7.4 mg/kg RAC and 20% DDGS led to increases in IV specifically due to a shift in the fatty acid profile from saturation towards unsaturation; however this was mitigated with the inclusion of 0.6 % CLA. Highly unsaturated pork fat increases susceptibility to lipid oxidation and raises concerns over product shelf-life; however in this study due to the lower fat content of pork shoulder trim from DDGS and RAC fed pigs, no increases were seen in TBARS. Additionally, the IV calculated in this study were acceptable according to the literature, the sensory panel did not detect differences in rancidity due to treatment, and limited changes in Minolta color values were observed. Furthermore, rosemary can be utilized as an effective antioxidant in pork to extend shelf life and decrease TBARS. Consequently, DDGS and RAC feeding in combination for 28 days prior to slaughter can result in acceptable ground pork and pork sausage products for up to seven days of refrigerated retail display.

Table 2.1: Diet composition for pigs fed 20% DDGS with or without conjugated linoleic acid or ractopamine hydrochloride

	Corn - Soybean Meal				20% DDGS <sup>1</sup>			
	-	-	+	+	-	-	+	+
Ractopamine	-	-	+	+	-	-	+	+
Conjugated Linoleic Acid	-	+	-	+	-	+	-	+
Corn	82.67	79.80	73.20	70.32	67.03	64.13	55.72	52.87
SBM 48%	13.00	15.30	22.25	24.55	8.75	11.05	20.00	22.30
DDGS	0.00	0.00	0.00	0.00	20.00	20.00	20.00	20.00
Fat, Choice White Grease	2.00	1.40	2.00	1.40	2.00	1.40	2.00	1.40
Monocal	0.60	0.55	0.65	0.60	0.20	0.20	0.25	0.15
Limestone	0.90	0.93	0.80	0.83	1.13	1.13	1.00	1.05
Salt	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
L-Lysine	0.17	0.17	0.25	0.25	0.25	0.25	0.26	0.26
MHA <sup>2</sup>	0.00	0.00	0.05	0.05	0.00	0.00	0.01	0.01
L-Threonine	0.01	0.01	0.12	0.12	0.00	0.00	0.07	0.07
Paylean <sup>®*</sup>	0.00	0.00	0.04	0.04	0.00	0.00	0.04	0.04
Conjugated Linoleic Acid	0.00	1.20	0.00	1.20	0.00	1.20	0.00	1.20
Vitamin Premix <sup>3</sup>	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Trace Mineral Premix <sup>4</sup>	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Total (%)	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
NRC ME (Mcal/kg)	0.706	0.690	0.705	0.690	0.699	0.683	0.698	0.682
Crude Protein (%)	13.20	13.33	16.96	17.09	15.31	15.44	19.78	19.91
TID <sup>5</sup> Lysine (%)	0.65	0.66	0.95	0.96	0.65	0.66	0.95	0.96
TID Methionine+Cysteine:Lysine	65.29	65.19	57.99	57.97	66.75	66.63	58.09	58.07
TID Threonine:Lysine	64.23	64.19	68.19	68.14	64.51	64.46	68.12	68.07
TID Tryptophan:Lysine	18.08	18.39	17.58	17.76	17.50	17.76	18.36	18.54
Total Phosphorus (%)	0.45	0.45	0.50	0.50	0.45	0.46	0.50	0.50
Available Phosphorus (%)	0.18	0.18	0.20	0.20	0.22	0.23	0.25	0.24
Ca (%)	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Na (%)	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17

<sup>1</sup>corn dried distiller's grains with solubles

<sup>2</sup>L-Met precursor HMTBA, an 88% aqueous solution of 2-hydroxy-4-(methylthio) butanoic acid, Novus International Inc., St. Louis, MO.

<sup>3</sup>Provided per kilogram of final diet: vitamin A, 6,614 IU; vitamin D3, 661 IU; vitamin E, 13.2 IU; riboflavin, 4.96 mg; vitamin B12, 0.02 mg; Menadione, 2.4 mg; D-pantothenic acid, 16.9 mg; niacin, 19.8 mg.

<sup>4</sup>Provided per kilogram of final diet: Iron, 110 mg; Zinc, 110 mg; Manganese, 22 mg; copper, 11 mg; iodine, 0.2 mg; selenium 0.198 mg.

<sup>5</sup> True ileal digestible

\* Elanco Animal Health, Greenfield, IN.

Table 2.2 Luta-CLA ® 60 fatty acid composition

Chemical name	content (%)
methyl oleate	45.00
9, 11 octadecadienoic acid, methyl ester, (9c, 11t)	35.00
10, 12 Octadecadienoic acid, methyl ester, (10t, 12c)	35.00
Octadecanoic acid, methyl ester	15.00
hexadecanoic acid, methyl ester	15.00

Table 2.3 Effects of dietary DDGS<sup>1</sup>, RAC<sup>2</sup>, and CLA<sup>3</sup> on proximate analysis

	DDGS		RAC		CLA		SEM	p-value		
	0%	20%	0	7.4 mg/kg	0%	0.60%		DDGS	RAC	CLA
moisture %	66.5	67.3	66.0	67.8	67.1	66.7	0.330	0.072	0.0003	0.425
fat % <sup>4</sup>	15.1	13.4	16.0	12.6	13.8	14.7	0.624	0.053	0.0003	0.282

<sup>1</sup> Distillers Dried Grains with solubles

<sup>2</sup> Ractopamine HCl

<sup>3</sup> Conjugated linoleic acid

<sup>4</sup> DDGS x RAC interaction; (P = 0.046)

Table 2.4 DDGS<sup>1</sup> x RAC<sup>2</sup> interactions for fat %

DDGS	0%	20.0%	0%	20.0%	SEM	P-value
RAC	0	0	7.4 mg/kg	7.4 mg/kg		
	17.7 <sup>b</sup>	12.5 <sup>a</sup>	14.2 <sup>a</sup>	12.6 <sup>a</sup>	0.882	0.046

<sup>1</sup> Distillers Dried Grains with solubles

<sup>2</sup> Ractopamine HCl

<sup>a</sup> Means within a row lacking a common superscript differ (p < 0.05)

Table 2.5 CLA<sup>1</sup> x RAC<sup>2</sup> interactions for TBARS<sup>3</sup> and a\* (redness) values of ground pork

CLA	0%	0.6%	0%	0.6%		
RAC	0	0	7.4 mg/kg	7.4 mg/kg	SEM	P-value
TBARS value	0.831 <sup>c</sup>	0.654 <sup>b</sup>	0.559 <sup>a</sup>	0.572 <sup>ab</sup>	0.043	0.033
a* (redness)	14.10 <sup>a</sup>	14.76 <sup>b</sup>	15.45 <sup>c</sup>	15.41 <sup>c</sup>	0.172	0.042

<sup>1</sup> Conjugated linoleic acid

<sup>2</sup> Ractopamine HCl

<sup>3</sup> Thiobarbituric acid reactive substances

<sup>a</sup> Means within a row lacking a common superscript differ (p < 0.05)

Table 2.6 RAC<sup>1</sup> x processing treatment interactions for TBARS<sup>2</sup> and L\* (lightness) values

RAC	0	0	0	7.4 mg/kg	7.4 mg/kg	7.4 mg/kg		
process	a <sup>3</sup>	b <sup>4</sup>	c <sup>5</sup>	a	b	c	SEM	P-value
TBARS value	0.922 <sup>d</sup>	0.705 <sup>c</sup>	0.602 <sup>b</sup>	0.622 <sup>bc</sup>	0.562 <sup>ab</sup>	0.514 <sup>a</sup>	0.043	0.014
L* (lightness)	53.31 <sup>bc</sup>	53.27 <sup>bc</sup>	53.21 <sup>b</sup>	53.81 <sup>c</sup>	52.43 <sup>a</sup>	52.48 <sup>a</sup>	0.030	0.017

<sup>1</sup> Ractopamine HCl

<sup>2</sup> Thiobarbituric acid reactive substances

<sup>3</sup> fresh ground pork (no additives)

<sup>4</sup> fresh pork sausage

<sup>5</sup> fresh pork sausage with rosemary extract

<sup>a</sup> Means within a row lacking a common superscript differ (p < 0.05)

Table 2.7 Effects of DDGS<sup>1</sup>, RAC<sup>2</sup>, CLA<sup>3</sup>, and processing treatment on lipid oxidation and sensory characteristics

	DDGS		RAC		CLA		SEM	process			SEM	p-value			
	0%	20%	0	7.4 mg/kg	0%	0.60%		a <sup>4</sup>	b <sup>5</sup>	c <sup>6</sup>		DDGS	RAC	CLA	process
TBARS <sup>7</sup> value <sup>8,9</sup>	0.648	0.660	0.743	0.566	0.696	0.613	0.030	0.772	0.633	0.558	0.030	0.780	0.0002	0.062	<0.0001
Sensory evaluation <sup>10</sup>															
Sausage flavor	4.78	4.86	4.93	4.70	4.75	4.88	0.277	n/a	4.88	4.76	0.269	0.835	0.562	0.738	0.736
Rancidity	2.28	1.67	1.84	2.11	1.87	2.08	0.275	n/a	2.17	1.78	0.267	0.117	0.495	0.583	0.288

<sup>1</sup> Distillers Dried Grains with solubles

<sup>2</sup> Ractopamine HCl

<sup>3</sup> Conjugated linoleic acid

<sup>4</sup> fresh ground pork (no additives)

<sup>5</sup> fresh pork sausage

<sup>6</sup> fresh pork sausage with rosemary extract

<sup>7</sup> Thiobarbituric acid reactive substances

<sup>8</sup> CLA x RAC interaction; (P = 0.033)

<sup>9</sup> RAC x process interaction; (P = 0.014)

<sup>10</sup> 15-point unstructured line scale with 0=none and 15=intense

Table 2.8 Effects of DDGS<sup>1</sup>, RAC<sup>2</sup>, CLA<sup>3</sup>, and processing treatment on objective color during retail display

	DDGS		RAC		CLA		process					day				p-value					
	0%	20%	0	7.4 mg/kg	0%	0.60%	SEM	a <sup>4</sup>	b <sup>5</sup>	c <sup>6</sup>	SEM	1	3	5	7	SEM	DDGS	RAC	CLA	process	day
L* (lightness) <sup>7,8</sup>	53.87	52.97	53.93	52.91	53.64	53.20	0.27	54.56	52.85	52.85	0.21	53.54	53.66	53.40	53.08	0.201	0.024	0.010	0.261	<0.0001	<0.0001
a* (redness) <sup>9,10,11</sup>	15.01	14.85	14.43	15.43	14.78	15.09	0.12	15.90	14.77	14.13	0.11	16.54	15.43	14.47	13.28	0.101	0.347	<0.0001	0.076	<0.0001	<0.0001
b* (yellowness) <sup>12</sup>	10.57	10.35	10.55	10.38	10.47	10.46	0.11	10.10	10.51	10.77	0.09	10.91	10.56	10.34	10.04	0.079	0.150	0.269	0.943	<0.0001	<0.0001

<sup>1</sup> Distillers Dried Grains with solubles  
<sup>2</sup> Ractopamine HCl  
<sup>3</sup> Conjugated linoleic acid  
<sup>4</sup> fresh ground pork (no additives)  
<sup>5</sup> fresh pork sausage  
<sup>6</sup> fresh pork sausage with rosemary extract  
<sup>7</sup> CL  $\otimes$  process x day interaction; (P = 0.002)  
<sup>8</sup> RAC x process interaction; (P = 0.017)  
<sup>9</sup> CLA x RAC interaction (P = 0.042)  
<sup>10</sup> DDGS x process interaction; (P = 0.045)  
<sup>11</sup> RAC x process x day interaction; (P = 0.018)  
<sup>12</sup> DDGS x RAC x process x day interaction; (P = 0.004)

Table 2.9 CLA<sup>1</sup> x process x day interactions for L\* (lightness)

process	A <sup>2</sup>	A	A	A	B <sup>3</sup>	B	B	B	C <sup>4</sup>	C	C	C
day	1	3	5	7	1	3	5	7	1	3	5	7
CLA												
0%	55.12	55.33	53.17	55.00	52.94	53.12	54.34	52.48	53.16	53.38	53.09	52.55
0.6%	54.75	54.90	53.69	54.56	52.14	52.13	53.93	51.72	53.10	53.10	52.19	52.19

SEM=0.327; (P = 0.002)

<sup>1</sup> Conjugated linoleic acid

<sup>2</sup> fresh ground pork (no additives)

<sup>3</sup> fresh pork sausage

<sup>4</sup> fresh pork sausage with rosemary extract

Table 2.10 DDGS<sup>1</sup> x process interactions for a\* (redness)

process	A <sup>2</sup>	B <sup>3</sup>	C <sup>4</sup>
DDGS			
0%	16.11 <sup>d</sup>	14.69 <sup>b</sup>	14.23 <sup>a</sup>
20%	15.68 <sup>c</sup>	14.84 <sup>b</sup>	14.02 <sup>a</sup>

SEM = 0.153; (P = 0.045)

<sup>1</sup> Distillers dried grains with solubles

<sup>2</sup> fresh ground pork (no additives)

<sup>3</sup> fresh pork sausage

<sup>4</sup> fresh pork sausage with rosemary extract

<sup>a</sup> Means within a row lacking a common superscript differ (p < 0.05)

Table 2.11 RAC x process x day interactions for a\* (redness)

process	A	A	A	A	B	B	B	B	C	C	C	C
day	1	3	5	7	1	3	5	7	1	3	5	7
RAC												
0	17.68	16.23	13.66	13.30	15.74	14.58	14.81	12.28	15.27	14.26	13.47	11.92
7.4 mg/kg	18.59	17.46	14.59	15.69	16.11	15.23	15.83	13.59	15.87	14.82	14.48	12.90

SEM = 0.20; (P = 0.018)

<sup>1</sup> Ractopamine HCl

<sup>2</sup> fresh ground pork (no additives)

<sup>3</sup> fresh pork sausage

<sup>4</sup> fresh pork sausage with rosemary extract

Table 2.12 DDGS<sup>1</sup> x RAC<sup>2</sup> x process x day interactions for b\* (yellowness)

process	A <sup>3</sup>	A	A	A	B <sup>4</sup>	B	B	B	C <sup>5</sup>	C	C	C	
day	1	3	5	7	1	3	5	7	1	3	5	7	
DDGS													
RAC													
0%	0	10.48	10.16	10.63	9.95	11.59	11.32	10.26	10.50	11.56	11.13	11.10	10.36
0%	7.4 mg/kg	10.24	9.89	10.43	9.68	10.86	10.49	9.82	9.90	11.46	11.03	10.61	10.25
20%	0	10.17	9.93	10.11	9.85	11.14	10.61	10.11	9.79	11.16	10.87	10.28	10.05
20%	7.4 mg/kg	10.18	9.87	10.33	9.72	10.98	10.71	9.85	10.27	11.13	10.70	10.51	10.17

SEM = 0.203; (P = 0.004)

<sup>1</sup> Distillers dried grains with solubles

<sup>2</sup> Ractopamine HCl

<sup>3</sup> fresh ground pork (no additives)

<sup>4</sup> fresh pork sausage

<sup>5</sup> fresh pork sausage with rosemary extract

Table 2.13 Effect of dietary DDGS<sup>1</sup>, RAC<sup>2</sup>, and CLA<sup>3</sup> on fatty acid profiles of ground pork

	DDGS		RAC		CLA		SEM	p-value		
	0%	20%	0	7.4 mg/kg	0%	0.60%		DDGS	RAC	CLA
palmitic acid (16:0)	23.32	22.22	23.08	22.46	22.20	23.34	0.16	<0.0001	0.008	<0.0001
palmitoleic acid (16:1)	2.33	1.99	2.20	2.11	2.09	2.22	0.04	<0.0001	0.155	0.043
stearic acid (18:0)	11.97	11.82	12.15	11.65	11.12	12.68	0.19	0.568	0.062	<0.0001
oleic acid (18:1n9)	40.42	39.39	39.93	39.88	41.66	38.15	0.24	0.003	0.893	<0.0001
linoleic acid (18:2n6)	14.41	16.95	15.19	16.17	15.73	15.63	0.22	<0.0001	0.002	0.751
linolenic acid (18:3n3)	0.94	0.95	0.93	0.96	1.02	0.88	0.03	0.790	0.492	0.004
arachidonic acid (20:4n6) <sup>4</sup>	0.57	0.64	0.58	0.63	0.63	0.58	0.02	0.028	0.120	0.116
CLA (18:2 9c11t)	0.32	0.32	0.32	0.32	0.15	0.50	0.02	0.944	0.793	<0.0001
CLA (18:2 10t12c)	0.19	0.19	0.18	0.20	0.04	0.35	0.01	0.632	0.370	<0.0001
Total CLA	0.60	0.63	0.61	0.62	0.19	1.04	0.03	0.618	0.742	<0.0001
SFA	37.36	36.01	37.28	36.09	35.17	38.19	0.31	0.003	0.009	<0.0001
MUFA	45.04	43.62	44.32	44.34	46.08	42.58	0.27	0.0004	0.965	<0.0001
PUFA	17.60	20.38	18.40	19.57	18.74	19.23	0.24	<0.0001	0.001	0.162
PUFA:SFA	0.42	0.51	0.44	0.49	0.49	0.44	0.01	<0.0001	0.001	0.001
IV	64.38	67.60	65.16	66.82	67.70	64.28	0.39	<0.0001	0.004	<0.0001

<sup>1</sup> Distillers Dried Grains with solubles

<sup>2</sup> Ractopamine HCl

<sup>3</sup> Conjugated linoleic acid

<sup>4</sup> DDGS x RAC interaction (P = 0.045)

Table 2.14 DDGS<sup>1</sup> x RAC<sup>2</sup> interactions for arachidonic acid (20:4n6)

DDGS	0%	20.0%	0%	20.0%	SEM	P-value
RAC	0	0	7.4 mg/kg	7.4 mg/kg		
	0.510 <sup>a</sup>	0.651 <sup>b</sup>	0.629 <sup>b</sup>	0.636 <sup>b</sup>	0.033	0.045

<sup>1</sup> Distillers Dried Grains with solubles

<sup>2</sup> Ractopamine HCl

<sup>a</sup> Means within a row lacking a common superscript differ (p < 0.05)

## LITERATURE CITED

- Adeola, O., Darko, E. A., He, P., and Young, L. G. (1990). Manipulation of porcine carcass composition by Ractopamine. *Journal of animal science*, 68, 3633.
- Ahn, J., Gruen, I. U., & Fernando, L. N. (2002). Antioxidant properties of natural plant extracts containing polyphenolic compounds in cooked ground beef. *Journal of Food Science*, 67, 1364-1369.
- Allee, G. K., O’Hea, E. K., Leveille, G. A., & Baker, D. H. (1971). Influence on dietary protein and fat on lipogenesis and enzymatic activity in pig adipose tissue. *The Journal of Nutrition*, 101, 869-878.
- AOCS (1998). Recommended practice Cd 1c-85. In Official Methods and Recommended Practices of the AOCS, 5<sup>th</sup> ed. American Oil Chemists soceity, Champaign, Il.
- Apple, J. K., Maxwell, C. V. , Sawyer, J. T., Kutz, B. R., Rakes, L. K., Davis, M. E., Johnson, Z. B., Carr, S. N. , & Armstrong, T. A. (2007). Interactive effect of Ractopamine and dietary fat source on quality characteristics of fresh pork bellies. *Journal of Animal Science*, 85, 2682-2690.
- Apple, J. K., Maxwell, C. V., Kutz, B. R., Rakes, L. K., Sawyer, J. T., Johnson, Z. B., Armstrong, T. A., Carr, S. N., & Matzat, P. D. (2008). Interactive effect of Ractopamine and dietary fat source on pork quality characteristics of fresh pork chops during simulated retail display. *Journal of Animal Science*, 86, 2711-2722.
- Azain, M. J., Hausman, D. B., Sisk, M. B., Flatt, W. P., & Jewell, D. E. (2000). Dietary Conjugated linoleic acid reduces rat adipose tissue cell size rather than number. *The Journal of Nutrition*, 130, 1548-1554.
- Azain, M. J. (2001). Fat in Swine Nutrition. In. A. J. Lewis and L. L. Southern (Eds.), Swine Nutrition, 2<sup>nd</sup> ed. (pp 95-106). Boca Raton, FL: CRC Press.
- Banni, S., Heys, S. D., & Wahle, K. W. J. (2003). Conjugated linoleic acids as anticancer nutrients:studies in vivo and cellular mechanisms. In. J. Sébédio, W. W. Christie, and T. Adlof (Eds.), *Advances in Conjugated Linoleic Acid Research, volume 2* (pp. 267-278). Champaign, IL: AOCS press.
- Baron, C. P. & Andersen, H. J. (2002). Myoglobin-induced lipid oxidation. A Review. *Journal of Agricultural and Food Chemistry*, 50, 3887-3897.
- Bauman, D. E., Baumgard, L. H., Corl, B. A., & Griinari, J. M. (1999). Biosynthesis of conjugated linoleic acid in ruminants. Proceedings of the American society of animal sciences, 1999. Available at <http://www.asas.org/jas/sympsia/proceedings/0937.pdf>. Accessed July 15, 2011.

- Benz, J. M., Linneen, S. K., Tokach, M. D., Dritz, S. S., Nelssen, J. L., DeRouchey, J. M., Goodband, R. D., Sulabo, R. C., & Prusa, K. J. (2010). Effects of dried distillers grains with solubles on carcass fat quality of finishing pigs. *Journal of Animal Science*, 88, 3666-3682.
- Bhattacharya, A., Banu, J., Rahman, M., Causey, J., & Fernandes, G. (2006). Biological effects of conjugated linoleic acids in health and disease. *Journal of Nutritional Biochemistry*, 17, 789-810.
- Boyd, R. D., Johnston, M. E., Scheller, K., Sosnicki, A.A., & Wilson, E. R. (1997). Relationship between dietary fatty acid profile and body fat composition in growing pigs. PIC USA R&D Technical Memo 153. Pig Improvement Company USA, Franklin, KY.
- Carr, S. N., Rincker, P. J., Killefer, J., Baker, D. H., Ellis, M., & McKeith, F. K. (2005). Effects of different cereal grains and Ractopamine hydrochloride on performance, carcass characteristics, and fat quality in late-finishing pigs. *Journal of Animal Science*, 83, 223-230.
- Carr, S. N., Hamilton, D. N., Miller, K. D., Schroader, A. L., Fernández-Dueñas, D., Killefer, J., Ellis, M., & McKeith, F. K. (2009). The effect of Ractopamine hydrochloride (Paylean) on lean carcass yields and pork quality characteristics of heavy pigs fed normal and amino acid fortified diets. *Meat Science*, 81, 533-539.
- Chen, C. C., Pearson, A. M., Gray, J. I., & Merkel, R. A. (1984). Effects of salt and some antioxidants upon the TBA numbers of meat. *Food Chemistry*, 14, 167-172.
- Chen, H. Y., Lewis, A. J., Miller, P.S., & Yen, J. T. (1999). The effect of excess protein on growth performance and protein metabolism of finishing barrows and gilts. *Journal of animal science*, 77, 3238-3247.
- Chen, X., Jo, C., Lee, J. I., & Ahn, D. U. (1999). Lipid oxidation, volatiles, and color changes of irradiated pork patties as affected by antioxidants. *Journal of Food Science*, 64, 16-19.
- Chin, S. F., Storkson, J. M., Albright, K. J., Cook, M. E., & Pariza, M. W. (1994). Conjugated Linoleic Acid is a Growth Factor for rats as shown by enhanced weight gain and improved feed efficiency. *The Journal of Nutrition*, 124, 2344-2349.
- Christie, W. W. (2003). Analysis of Conjugated linoleic acid: An Overview. In J. Sébédio, W. W. Christie, and T. Adlof (Eds.), *Advances in Conjugated Linoleic Acid Research*, Volume 2. (pp. 1-5). Champaign, IL: AOCS press.
- Connor, W. E. (1999). Harbingers of coronary heart disease: dietary saturated fatty acids and cholesterol. Is chocolate benign because of its stearic acid content. *American Journal of clinical nutrition*, 70, 951-952.
- Cook, D., Paton, N., & Gibson, M. (2005). Effect of dietary level of distillers dried grains with solubles (DDGS) on growth performance, mortality, and carcass characteristics of grow-finish barrows and gilts. *Journal of animal science*, 83 (suppl 1), 335 (Abstract).

- Correa, J. A., Garipey, C., Marcoux, M., & Faucitano, L. (2008). Effects of growth rate, sex, and slaughter weight on fat characteristics of pork bellies. *Meat Science*, 80, 550-554.
- Davis, C. G. & Lin, B. (2005). Factors affecting U. S. pork consumption. Electronic outlook report from the economic research service, Report No. LDP-M-130-01. Available online at the USDA-ERS web site, accessed September 10, 2011:  
<http://www.ers.usda.gov/publications/LDP/may05/ldpm13001/ldpm13001.pdf>.
- Dooley, F. J. (2008). U.S. Market potential for dried distillers grain with solubles. Working paper # 08-12, Purdue University, Department of Agricultural Economics.
- Dugan, M. E. R., Aalhus, J. L., Schaefer, A. L., & Kramer, J. K. G. (1997). The effect of conjugated linoleic acid on fat to lean repartitioning and feed conversion in pigs. *Canadian Journal of Animal Science*, 77, 723-725.
- Dugan, M. E. R., Aalhus, J. L., Jeremiah, L. E., Kramer, J. K. G., & Schaefer, A. L. (1999). The effects of feeding conjugated linoleic acid on subsequent pork quality. *Canadian Journal of Animal Science*, 79, 45-51.
- Dunshea, F. R. (1993). Effect of metabolism modifiers on lipid metabolism in the pig. *Journal of Animal Science*, 71, 1966-1977.
- Eggert, J. M., Belury, M. A., Kempa-Steczko, A., Mills, S. E., Schinckel, A. P. (2001). Effects of conjugated linoleic acid on the belly firmness and fatty acid composition of genetically lean pigs. *Journal of Animal Science*, 79, 2866-2872.
- Fernández-Dueñas, D. M., Myers, A. J., Scramlin, S. M., Parks, C. W., Carr, S. N., Killefer, J., & McKeith, F. K. (2008). Carcass, meat quality, and sensory characteristics of heavy body weight pigs fed Ractopamine hydrochloride (Paylean). *Journal of Animal Science*, 86, 3544-3550.
- Folch, J., Lees, M., & Sloan-Stanley, G.N. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, 226, 497-509.
- Frankel, E. N. (1984) Lipid Oxidation: Mechanisms, products, and biological significance. *Journal of the American Oil Chemist's Society*, 61, 1908-1917.
- Frankel, E. N. (1991). Recent Advances in Lipid Oxidation. *J Sci Food Agric*, 54, 495-511.
- Frederick, B. R., van Heugten, E., & See, M. T. (2004). Timing of magnesium supplementation administered through drinking water to improve fresh and stored pork quality. *Journal of animal science*, 82, 1454-1460.
- Gatlin, L. A., See, M. T., Hansen, J. A., Sutton, D., & Odle, J. (2002a). The effects of dietary fat sources, levels, and feeding intervals on pork fatty acid composition. *Journal of Animal Science*, 80, 1606-1615.

- Gatlin, L. A., See, M. T., Larick, D. K., Lin, X., & Odle, J. (2002b). Conjugated linoleic acid in combination with supplemental dietary fat alters pork quality. *The Journal of Nutrition*, 132, 3105-3112.
- Goodband, B., DeRouchey, J., Tokach, M., Dritz, S., & Nelssen, J. (2006). A practical look at nutritional attempts to improve pork quality. London Swine Conference Proc. (pp. 125–139). London, UK.
- Griinari, J. M., & Bauman, D. E. (1999). Biosynthesis of conjugated linoleic acid and its incorporation into meat and milk in ruminants. In M. P. Yurawecz, M. M. Mossoba, J. K. G. Kramer, M. W. Pariza, & G. Nelson (Eds.), *Advances in conjugated linoleic acid research* (pp. 180–200). Champaign, IL: American Oil Chemists Society Press.
- Griinari, J. M., Corl, B. A., Lacy, S. H., Chouinard, P. Y., Nurmela, K. V. V., & Bauman, D. E. (2000). Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by  $\Delta 9$ -desaturase. *Journal of Nutrition*, 130, 2285-2291.
- Gruen, I. & Duncan, S. (2007). Lipids: Basic Concepts. In Y. H. Hui (Ed.), *Food Chemistry: Principles and Applications*. 2<sup>nd</sup> edition. (pp. 6.9-6.12) West Sacramento, CA: Science Technology System.
- Grün, I. U., Ahn, J., Clarke, A. D., & Lorenzen, C. L. (2006). Reducing oxidation of meat. *Food Technology*, 60, 37-43.
- Gu, Y., Schinckel, A. P., Forrest, J. C., Kuei, C. H., & Watkins, L. E. (1991a). Effects of Ractopamine, genotype, and growth phase on finishing performance and carcass value in swine: I. Growth performance and carcass merit. *Journal of Animal Science*, 69, 2685-2693.
- Gu, Y., Schinckel, A. P., Forrest, J. C., Kuei, C. H., & Watkins, L. E. (1991b). Effects of Ractopamine, genotype, and growth phase on finishing performance and carcass value in swine: II. Estimation of lean growth rate and lean feed efficiency. *Journal of Animal Science*, 69, 2694-2702.
- Ha, Y. L., Grimm, N. K., & Pariza, M. W. (1987). Anticarcinogens from fried ground beef: heat-altered derivatives of linoleic acid. *Carcinogenesis*, 8, 1881-1887.
- Hansen, E., Juncher, D., Henckel, P., Karlsson, A., Bertelsen, G., & Skibsted, L. H. (2004). Oxidative stability of chilled pork chops following long term freeze storage. *Meat science*, 68, 479-484.
- Houben, J. H., Eikelenboom, G., & Hoving-Bolink, A. H. (1998). Effect of the dietary supplementation with vitamin E on colour stability and lipid oxidation in packaged, minced pork. *Meat Science*, 48, 265-273.
- House, R. L., Cassady, J. P., Eisen, E. J., McIntosh, M. K., & Odle, J. (2005). Conjugated linoleic acid evokes de-lipidation through the regulation of genes controlling lipid metabolism in adipose and liver tissues. *Obesity Review*, 6, 247-258.

- Hu, F. B., Stampfer, M. J., Manson, J. E., Ascherio, A., Colditz, G. A., Speizer, F. E., Hennekens, C. H., & Willett, W. C. (1999). Dietary saturated fats and their food sources in relation to the risk of coronary heart disease in women. *American Journal of Clinical Nutrition*, 70, 1001-1008.
- Hu, F. B., Manson, J. E., & Willett, W. C. (2001). Types of dietary fat and risk of coronary heart disease: a critical review. *Journal of the American college of nutrition*, 20, 5-19.
- Ip, C., Singh, M., Thompson, H.J., & Scimeca, J.A. (1994). Conjugated linoleic acid suppresses mammary carcinogenesis and proliferative activity of the mammary gland in the rat. *Cancer Research*, 54, 1212-1215.
- Jo, C., Lee, J. I., & Ahn, D. U. (1999). Lipid oxidation, color changes, and volatiles production in irradiated pork sausage with different fat content and packaging during storage. *Meat Science*, 51, 355-361.
- Joo, S. T., Lee, J. I., Ha, Y. L., & Park, G. B. (2002). Effects of dietary conjugated linoleic acid on fatty acid composition, lipid oxidation, color, and water-holding capacity of pork loin. *Journal of Animal Science*, 80, 108-112.
- Keeton J. T., Hafley, B. S., Eddy, S. M., Moser, C. R., McManus, B. J., & Leffler, T. P. (2003). Rapid determination of moisture and fat in meats by microwave and nuclear magnetic resonance analysis. *Journal of AOAC International*, 86, 1193-1202.
- Kelley, N. S., Hubbard, N. E., & Erickson, K. L. (2007). Conjugated linoleic acid isomers and cancer. *Journal of Nutrition*, 137, 2599-2607.
- Kelly, M. L., Berry, J. R., Dwyer, D. A., Griinari, J. M., Chouinard, P. Y., Van Amburgh, M. E., & Bauman, D. E. (1998). Dietary fatty acid sources affect conjugated linoleic acid concentrations in milk from lactating dairy cows. *Journal of Nutrition*, 128, 881-885.
- Kennelly, J. J. & Aherne, F. X. (1980). The effect of fiver addition to diets formulated to contain different levels of energy and protein on growth and carcass quality of swine. *Canadian Journal of Animal Science*, 60, 385-393.
- Kingston, E. R., Monahan, F. J., Buckley, D. J., & Lynch, P. B. (1998). Lipid oxidation in cooked pork as affected by Vitamin E, Cooking and storage conditions. *Journal of Food Science*, 63, 386-389.
- Kloareg, M., Noblet, J., & Milgen, J. V. (2007). Deposition of dietary fatty acids, de novo synthesis and anatomical partitioning of fatty acids in finishing pigs. *British Journal of Nutrition*, 97, 35-44.
- Kritchevsky, D. (2003). Conjugated linoleic acid in experimental atherosclerosis. In J. Sébédio, W. W. Christie, and T. Adlof (Eds.), *Advances in Conjugated Linoleic Acid Research*, Volume 2. (pp. 292-300). Champaign, IL: AOCS press

- Kritchevsky, D., Tepper, S. A., Wright, S., Tso, P., & Czarnecki, S. K. (2000). Influence on conjugated linoleic acid (CLA) on establishment and progression of atherosclerosis in rabbits. *Journal of the American College of Nutrition*, 19, 472S-477S.
- Larick, D. K., Turner, B. E., Schoenherr, W. D., Coffey, M. T., & Pilkington, D. H. (1992). Volatile compound content and fatty acid composition of pork as influenced by linoleic acid content of the diet. *Journal of Animal Science*, 70, 1397-1403.
- Larsen, S. T., Wiegand, B. R., Parrish Jr., F. C., Swan, J. E., & Sparks, J. C. (2009). Dietary conjugated linoleic acid changes belly and bacon quality from pigs fed varied lipid sources. *Journal of Animal Science*, 87, 285-295.
- Lee, S. K., Mei, L., & Decker, E. A. (1997). Influence of sodium chloride on antioxidant enzyme activity and lipid oxidation in frozen ground pork. *Meat Science*, 46, 349-355.
- Leick, C. M., Puls, C. L., Ellis, M., Killefer, J., Carr, T. R., Scramlin, S. M., England, M. B., Gaines, A. M., Wolter, B. F., Carr, S. N., & McKeith, F. K. (2010). Effect of distillers dried grains with solubles and Ractopamine (Paylean) on quality and shelf-life of fresh pork and bacon. *Journal of Animal Science*, 88, 2751-2766.
- Linneen, S. K., Derouchey, J. M., Dritz, S. S., Goodband, R. D., Tokach, M. D., & Nielson, J. L. (2008). Effects of dried distillers grains with solubles on growing and finishing pig performance in a commercial environment. *Journal of animal science*, 86, 1579-1587.
- Love, J. D. & Pearson, A. M. (1971). Lipid oxidation in meat and meat products: A review. *Journal of the American Oil Chemist's society*, 48, 547-549.
- McCarthy, T. L., Kerry, J. P., Kerry, J. F., Lynch, P. B., & Buckley, D. J. (2001). Evaluation of the antioxidant potential of natural food/plant extracts as compared with synthetic antioxidants and vitamin E in raw and cooked pork patties. *Meat Science*, 57, 45-52.
- McGuire, M. A. & McGuire, M. K. (2000). Conjugated linoleic acid (CLA): A ruminant fatty acid with beneficial effects on human health. *Journal of Animal Science*, 77, 1-8.
- Mei, L., Crum, A. D., & Decker, E. A. (1994). Development of lipid oxidation and inactivation of antioxidant enzymes in cooked pork and beef. *Journal of Food Lipids*, 1, 273-283.
- Mersmann, H. J. (1998). Overview of the Effects of  $\beta$ -Adrenergic Receptor Agonists on Animal Growth including Mechanisms of Action. *Journal of Animal Science*, 76, 160-172.
- Mersmann, H. J. (2002). Beta-Adrenergic receptor modulation of adipocyte metabolism and growth. *Journal of Animal Science*, 80, E24-E29.
- Mills, S. & Mersmann, H. J. (1995). Beta-Adrenergic Agonists, Their Receptors, and growth: Special Reference to the peculiarities in pigs. In S. B. Smith and D. R. Smith (Eds.), *The Biology of Fat in Meat Animals, Current Advances*. (pp. 2-29). Champaign, IL: American Society of Animal Science
- Mills, S. (2002). Biological basis of the Ractopamine response. *Journal of Animal Science*, 80, E28-E32.

- Min, B. & Ahn, D. U. (2005). Mechanism of lipid peroxidation in meat and meat products-a review. *Food Science and Biotechnology*, 14, 152-163.
- Monahan, F. J., Gray, J. I., Booren, A. M., Miller, E. R., Buckley, D. J., Morrissey, P. A., & Gomaa, E. A. (1992). Influence of Dietary treatment on lipid and cholesterol oxidation in pork. *Journal of Agricultural and Food Chemistry*, 40, 1310-1315.
- Monahan, F. J. (2000). Oxidation of lipids in muscle foods: fundamental and applied concerns. In E. Decker, C. Faustman, and C. J. Lopez-Bote (Eds.), *Antioxidants in muscle foods: nutritional strategies to improve quality*. (pp. 3-18). New York, NY: John Wiley & Sons
- Moody, D. E., Hancock, D. L., & Anderson, D. B. (2000). Phenethanolamine repartitioning agents. In: J. P. F. D'Mello (ed.) *Farm Animal Metabolism and nutrition*. (pp. 65-96). CAB international, Wallingford, Oxun, U.K.
- Morrison, W. R. & Smith, L. M. (1964). Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. *Journal of lipid research*, 5, 600-608.
- Morrissey, P. A., Sheehy, P. J. A., Galvin, K., Kerry, J. P., & Buckley, D. J. (1998). Lipid stability in meat and meat products. *Meat Science*, 49, S73-S86.
- NAMP (2007). *Meat Buyer's Guide*. North American Meat Processors Association, Reston, VA
- Nelson, D. L. & Cox, M. M. (2008). *Lehninger: Principles of Biochemistry* 5<sup>th</sup> Ed. New York, N.Y.: W. H. Freeman and Company.
- NPPC (1999). *Pork quality standards*. National pork producers council. Des Moines, IA.
- NPPC (2000). *Pork fat quality*. In *Pork Carcass Composition and Quality Assessment Procedures*. Berg, E. P., Ed., National Pork Producers Council, Des Moines, IA, 32.
- Ntambi, J. M., Choi, Y., Park, Y., Peters, J. M., & Pariza, M. W. (2002). Effects of conjugated linoleic acid (CLA) on immune responses, body composition and stearyl-coA desaturase. *Canadian Journal of Applied Physiology*, 27(6), 617-627.
- Olsen, E., Vogt, G., Veberg, A., Ekeberg, D., & Nilsson, A. (2005). Analysis of early lipid oxidation in smoked, comminuted pork or poultry sausage with spices. *Journal of Agricultural and food chemistry*, 53, 7448-7457.
- Ostrowska, E., Muralitharan, M., Cross, R. F., Bauman, D. E., Dunshea, F. R. (1999). Dietary conjugated linoleic acids increase lean tissue and decrease fat deposition in growing pigs. *Journal of Nutrition*, 129, 2037-2042.
- Pariza, M. W., Park, Y., & Cook, M. E. (1999). Conjugated linoleic acid and the control of cancer and obesity. *Toxicological sciences*, 52, 107-110.

- Pariza, M. W., Park, Y., & Cook, M. E. (2001). The biologically active isomers of conjugated linoleic acid. *Progress in Lipid Research*, 40, 283-298.
- Pariza, M. W., Park, Y., Xu, X., Ntambi, J., & Kang, K. (2003) Speculation on the mechanisms of action of conjugated linoleic acid. In J. Sébédio, W. W. Christie, and T. Adlof (Eds.), *Advances in Conjugated Linoleic Acid Research*, Volume 2. (pp. 251-256). Champaign, IL: AOCS press.
- Park, Y., Albright, K. J., Liu, W., Storkson, J. M., Cook, M. E., & Pariza, M. W. (1997). Effect of conjugated linoleic acid on body composition in mice. *Lipids*, 32, 853-858.
- Park, Y. & Pariza, M. W. (2007). Mechanisms of body fat modulation by conjugated linoleic acid (CLA). *Food Research International*, 40, 311-323.
- Pegg, R. B. (2001) Spectrophotometric measurement of secondary lipid oxidation products. In R. Wrolstad (Ed.) *Current protocols in food analytical chemistry* (pp. D 2.4.1-D2.4.18). New York, NY: John Wiley & Sons.
- Richards, M. P. (2007). Lipid: Functional properties. In Y. H. Hui (Ed.), *Food Chemistry: Principles and Applications*. 2<sup>nd</sup> edition. (pp. 7.1-7.11) West Sacramento, CA: Science Technology System.
- Romans, J. R., Costello, W. J., Carlson, C. W., Greaser, M. L., & Jones, K. W. (2001). The meat we eat. 14<sup>th</sup> edition. Danville, IL: Interstate Publishers, Inc.
- Rudel, L.L. (1999). Invited Commentary: Atherosclerosis and conjugated linoleic acid. *British Journal of Nutrition*, 81, 177-179.
- Sebranek, J. G., Sewalt, V. J. H., Robbins, K. L., & Houser, T. A. (2005). Comparison of a natural rosemary extract and BHA/BHT for relative antioxidant effectiveness in pork sausage. *Meat Science*, 69, 289-296.
- Smith, S.B., Hively, T. S., Cortese, G. M., Han, J. J., Chung, K. Y., Casteñada, P., Gilbert, C. D., Adams, V. L., & Mersmann, H. J. (2002). Conjugated linoleic acid depresses the  $\Delta^9$  desaturase index and stearoyl coenzyme A desaturase enzyme activity in porcine subcutaneous adipose tissue. *Journal of Animal Science*, 80, 2110-2115.
- Stanton, C., Lawless, F., Kjellmer, G., Harrington, D., Devery, R., Connolly, J. F., & Murphy, J. (1997). Dietary influences on bovine milk cis-9, trans-11 conjugated linoleic acid content. *Journal of food science*, 62, 1083-1086.
- Stein, H. H. (2007). Distillers dried grains with solubles (DDGS) in diets fed to swine. Swine Focus No. 001. University of Illinois, Urbana-Champaign.
- Stein, H. H. & Shurson, G. C. (2009). The use and application of distillers dried grains with solubles in swine diets. *Journal of Animal Science*, 87, 1292-1303.
- Stites, C. R., McKeith, F. K., Singh, S. D., Bechtel, P. J., Jones, D. J., & Mowrey, D. H. (1994). Palatability and visual characteristics of hams and loin chops from swine treated with Ractopamine hydrochloride. *Journal of muscle foods*, 5, 367-376.

- Tanabe, H., Yoshida, M., & Tomita, N. (2002). Comparison of the antioxidant activities of 22 commonly used culinary herbs and spices on the lipid oxidation of pork meat. *Animal Science Journal*, 73, 389-393.
- Tarladgis, B. G., Watts, B. M., & Younathan, M. T. A. (1960). A distillation method for the quantitative determination of malonaldehyde in rancid foods. *Journal of the American Oil Chemists Society*, 37, 44-48.
- Toomey, S., Harhen, B., Roche, H. M., Fitzgerald, D., & Belton, O. (2006). Profound resolution of early atherosclerosis with conjugated linoleic acid. *Atherosclerosis*, 187, 40-49.
- USDA. (2000). Code of federal regulations, title 9, chapter III, part 318.7. Approval of substances for use in the preparation of products, Washington, DC, USA: Government printing office.
- Uttaro, B. E., Ball, R. O., Dick, P., Rae, W., Vessie, G., & Jeremiah, L. E. (1993). Effect of Ractopamine and sex on growth, carcass characteristics, processing yield, and meat quality characteristics of crossbred swine. *Journal of Animal Science*, 71, 2439-2449.
- Weber, T. E., Richert, B. T., Belury, M. A., Gu, Y., Enright, K., & Schinckel, A. P. (2006). Evaluation of the effects of dietary fat, conjugated linoleic acid, and Ractopamine on growth performance, pork quality, and fatty acid profiles in genetically lean gilts. *Journal of Animal Science*, 84, 720-732.
- White, H. M., Richert, B. T., Radcliffe, J. S., Schinckel, A. P., Burgess, J. R., Koser, S. L., Donkin, S. S., & Latour, M. A. (2009). Feeding conjugated linoleic acid partially recovers carcass quality in pigs fed dried corn distillers grains with solubles. *Journal of Animal Science*, 87, 157-166.
- Whitney, M. H., Shurson, G. C., Johnston, L. J., Wulf, D. M., & Shanks, B. C. (2006). Growth performance and carcass characteristics of grower-finisher pigs fed high-quality corn distillers dried grain with solubles originating from a modern Midwestern ethanol plant. *Journal of Animal Science*, 84, 3356-3363.
- Wiegand, B. R., Parrish Jr., F. C., Swan, J. E., Larsen, S. T., & Bass, T. J. (2001). Conjugated linoleic acid improves feed efficiency, decreases subcutaneous fat, and improves certain aspects of meat quality in Stress-Genotype pigs. *Journal of Animal Science*, 79, 2187-2195.
- Wiegand, B. R., Sparks, J. C., Parrish Jr., F. C., & Zimmerman, D. R. (2002). Duration of feeding conjugated linoleic acid influences growth performance, carcass traits, and meat quality of finishing barrows. *Journal of Animal Science*, 80, 637-643.
- Wiegand, B. R., Hinson, R. B., Ritter, M. J., Carr, S. N., & Allee, G. L. (2011). Fatty acid profiles and iodine value correlations between four carcass fat depots from pigs fed varied combinations of Ractopamine (Paylean) and energy. *Journal of Animal Science* (In Press).

- Wilson, B. R., Pearson, A. M., & Shorland, F. B. (1976). Effect of total lipids and phospholipids on warmed-over flavor in red and white muscle from several species as measured by thiobarbituric acid analysis. *Journal of Agricultural and Food Chemistry*, 24, 7-11.
- Wood, J. D. (1984). Fat deposition and the quality of fat tissue in meat animals. In J. Wiseman (Ed). *Fats in animal nutrition* (pp. 407-435). London, U.K.: Butterworths.
- Wood, J. D. & Enser, M. (1997). Factors influencing fatty acids in meat and the role of antioxidants in improving meat quality. *British Journal of Nutrition*, 78, S49-S60.
- Wood, J. D., Richardson, R. I., Nute, G. R., Fisher, A. V., Campo, M. M., Kasapidou, E., Sheard, P. R., & Enser, M. (2003). Effects of fatty acids on meat quality: A review. *Meat Science*, 66, 21-32.
- Wood, J. D., Enser, M., Fisher, A. V., Nute, G. R., Sheard, P. R., Richardson, R. I., Hughes, S. I., & Whittington, F. M. (2007). Fat deposition, fatty acid composition and meat quality: A review. *Meat Science*, 78, 345-358.
- Wood, J. D., Enser, M., Fisher, A. V., Nute, G. R., Sheard, P. R., Richardson, R. I., Hughes, S. I., & Whittington, F. M. (2008). Fat deposition, fatty acid composition and meat quality: A review. *Meat Science*, 78, 343-358.
- Wong, J. W., Hashimoto, K., & Shibamoto, T. (1995). Antioxidant activities of rosemary and sage extracts and vitamin E in a model meat system. *Journal of Agricultural and Food Chemistry*, 43, 2707-2712.
- Xu, G., Baidoo, S. K., Johnston, L. J., Bibus, D., Cannon, J. E., & Shurson, G. C. (2010). Effects of feeding diets containing increasing levels of corn distillers dried grains with solubles (DDGS) to grower-finisher pigs on growth performance, carcass composition, and pork fat quality. *Journal of Animal Science*, 88, 1398-1410.
- Yang, Y. T. & McElligott, M. A. (1989). Multiple actions of  $\beta$ -adrenergic agonists on skeletal muscle and adipose tissue. *Biochemistry Journal*, 261, 1-10.
- Yen, J. T., Mersmann, H. J., Hill, D. A., & Pond, W. G. (1990). Effects of Ractopamine on genetically obese and lean pigs. *Journal of Animal Science*, 68, 3705-3712.
- Yurawecz, M.P., Hood, J. K., Mossoba, M. M., Roach, J. A. G., & Ku, Y. (1995). Furan fatty acids determined as oxidation products of conjugated octadecadienoic acid. *Lipids*, 30, 595-598.