MICAH W. GREENE
The Effects of Conjugated Linoleic Acid on Carcass Composition, Meat Quality and Retail Display Characteristics in Crossbred Swine
(Under the Direction of T. DEAN PRINGLE)

Crossbred gilts were fed a corn/soybean ration containing 0, 0.5, or 2.0% conjugated linoleic acid (CLA) and humanely harvested to determine the effects of CLA on pork carcass composition, quality, and retail display characteristics. Backfat was lower in CLA-fed groups than controls. Carcass quality (marbling score, color) did not differ across treatments. Most compositional endpoints were unaffected by CLA; however, total fat of CLA-treated carcasses was reduced. Fatty acid profiles showed that CLA percentage in the subcutaneous fat increased. As expected, the percentage of saturated fatty acids increased ($P<0.05$) and monounsaturated fatty acids decreased ($P<0.01$) as CLA increased. Supplemental CLA decreased lightness and elevated redness values of ground pork. Feeding CLA to pigs tended ($P<0.17$) to depress oxidation in the 2.0% group compared to the 0.5% and control groups. Dietary CLA significantly alters fatty acid composition; however, more research is needed to document significant changes in other carcass traits.

Index Words: Pork, CLA, Backfat, Composition, Quality, Fatty Acids

THE EFFECTS OF CONJUGATED LINOLEIC ACID ON CARCASS COMPOSITION, MEAT QUALITY AND RETAIL DISPLAY CHARACTERISTICS IN CROSSBRED SWINE

by

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B.S., The University of Georgia 1999

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2001

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December 2001

ACKNOWLEDGMENT

I would like to thank all of the people who have supported me throughout my education, my friends and family. I would also like to specially thank my major professor who has constantly provided me with guidance and support as I moved to completion of my degree. Additionally, I would like to thank Gina Burden and Jeff Ford for their assistance in data collection and organization.

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CHAPTER 1

INTRODUCTION

Currently, meat animal production is based on the production of lean carcasses to appease consumer demands. One major problem associated with leaner pork carcass is an increase in the stress susceptibility of the animal. In more detail, the increase in stress susceptibility translates to a decrease in the quality of pork carcasses. The pork industry is concerned with production of a leaner carcass without altering the pork quality or compromising animal health. Much of the improvements in pork carcass leanness have occurred through genetic selection, although several compounds are available to enhance the production of lean pork products.

One such compound was introduced recently. Conjugated linoleic acid (CLA), which was first identified in fried ground beef in the early 1980's, has been reported to possess repartitioning activity. Conjugated linoleic acid is a feed additive, which has been shown to decrease lipid accretion when fed to a variety of laboratory and food animal species. Initial research conducted with CLA also indicated potential improvements to carcass quality by increasing the marbling component of carcasses. The introduction of a feed additive, which decreased the fat content of carcasses while improving the marbling excited pork producers by potentially offering a solution to decreasing carcass fat without negatively impacting pork quality. Since CLA is a feed supplement, it does not require injection, and has no withdrawal time. Many of the current repartitioning agents

used in pork production are hormones or antibiotics and require special handling and management. Additionally, consumers have negative connotations associated with hormone and antibiotic usage in animal production. Consequentially, the fact that CLA has potential health benefits and no withdrawal times should increase its use, if an economic source of the compound can be developed.

Conjugated linoleic acid has been reported to have potential human health benefits including the inhibition of cancer formation, inhibition of atherosclerotic lesions, and stimulation of an immunomodulatory response in supplemented animals. When CLA is fed to animals, it is incorporated into the tissues, and consequentially may be carried over to consumers. This incorporation of CLA and its health-related benefits should help producers market CLA-fed animals to consumers by offering a branded, nutraceutical product.

This experiment was designed to illustrate the effects of CLA-supplementation on pork carcasses. The specific purpose of the research was to determine the effects of CLA on the growth of finishing hogs, changes in carcass composition, the fatty acid profiles of pork fat, potential enhancement of pork quality, and retail display characteristics of ground pork products.

CHAPTER 2

REVIEW OF LITERATURE

Background

The term conjugated linoleic acid refers generally to mixtures of positional and geometric conjugated dienoic isomers of linoleic acid (Pariza et al., 2000). Many isomers are produced when linoleic acid is heated in the presence of base. The primary isomer found in dietary CLA is the *cis*-9, *trans*-11 isomer. The *cis*-9, *trans*-11 isomer is also produced in the rumen of ruminant animals, principally as the result of microbial dehydrogenation of linoleic acids. After the conversion of linoleic acid to *cis*-9, *trans*-11 CLA in the rumen, it may be absorbed directly or biohydrogenated into vaccenic acid (18:1 *trans*-11). Vaccenic acid may also be dehydrogenated to the *cis*-9, *trans*-11 CLA by enzymes in mammalian cells (Pariza et al., 2000). In addition to the *cis*-9, *trans*-11 isomer, a *trans*-10, *cis*-12 isomer of CLA also exists which is created by a rumen microorganism (Pariza et al., 2000). The CLA typically used for experimentation consists of the *cis*-9, *trans*-11 and the *trans*-10, *cis*-12 isomers in equal or varying amounts (Pariza et. al., 2000). Dietary sources of CLA include milk fat, meat products, and vegetable oils. Chin et al. (1994a) found CLA in a wide variety of foodstuffs, with meat and milk from ruminants having the highest content.

In a review of the physiological responses of dietary CLA supplementation, Pariza et al. (2000) reported that CLA protects cells from the catabolic effects of immune stimulation when fed to rats or chickens at 0.5% of

the diet. In addition, it has been demonstrated that CLA can provide protection from carcinogenesis when supplemented to animals and reduce blood lipids and the development of atherosclerosis in rabbits fed an atherogenic diet (Pariza et al., 2000).

Antioxidant Activity

Based on early work with CLA supplementation, it was hypothesized that CLA had antioxidant capabilities, which at least partially accounted for the health benefits associated with its feeding. Ha et al. (1990) reported that CLA was an effective antioxidant, more potent than α-tocopherol, and comparable in potency to β-hydroxytoluene (BHT). Similarly, Ip et al. (1991) showed that feeding CLA decreased lipid peroxidation in the mammary gland but not in the liver. More recently, the antioxidant potential of supplemental CLA has been questioned, and it is currently hypothesized that CLA does not possess any antioxidant properties. Banni et al. (1998) reported that CLA does not possess antioxidant properties. In agreement, Yang et al. (2000) reported that oxygen uptake was faster in CLA than linoleic acid (LA), indicating that CLA was considerably less stable than LA. Furthermore, these researchers suggested that the conjugated double bond system in CLA is more vulnerable to auto-oxidation than the nonconjugated double bonds such as those found in LA. Additional studies (Chin et al., 1997; Zhang and Chen, 1997; Yang et al., 2000) reported that CLA was very susceptible to auto-oxidation when exposed to air.

Immunomodulation

The immune system is the body's means for fighting invasions and develops a specific response against invasions from foreign microbes, toxins, or transplanted tissues. Four key features are characteristic of the immune system: specificity, diversity, memory, and self-nonself recognition. Energy is also a requirement for immune stimulation, thus animals on a maintenance plane of nutrition tend to have more severe reactions (loss of growth, loss of body condition) than animals with more available dietary energy. Conjugated linoleic acid has been hypothesized to reduce the energy required to stimulate the immune response in animals (Bassaganya-Riera et al., 2001). This would suggest that CLA acts as a nutraceutical in supplemented animals, reducing the catabolic response normally required to stimulate the immune system. Bassaganya et al. (2001) reported a trend toward increases in white blood cell counts, which included lymphocytes, neutrophils, eosinophils, basophils, and monocytes, with increased dietary CLA supplementation in pigs. Bassaganya-Riera et al. (2001) discovered that feeding CLA for approximately 42 days preceding a disease challenge could prevent the growth depression associated with immune system stimulation. This data was supported by the linear increases in the percentages of $CD8^+$ lymphocytes.

Anti-atherosclerotic Activity

Atherosclerosis is a vascular disease typically identified in individuals possessing hypercholesteremia, characterized by very high levels of blood cholesterol. One characteristic of hypercholesteremia is a defect in the low-

density lipoprotein (LDL) receptor proteins, such that the LDL particles cannot be absorbed from the bloodstream. The LDL's then accumulate in the blood where it contributes to atherosclerosis, the buildup of fat deposits on blood vessel linings.

CLA inhibits chemically-induced neoplasia at some sites in rats and mice and seems to reduce blood low-density lipoprotein (LDL) concentrations and inhibit the development of atherosclerosis in rabbits and hamsters fed atherogenic diets (Chin et al., 1994). These researchers also reported that CLA fed in conjunction with an atherogenic diet reduced the severity of atherosclerotic lesions developing in the aorta of rabbits but did not affect serum cholesterol levels. In hamsters, CLA also reduced the fatty streak area in arteries; and likewise reduced total serum cholesterol. Conjugated linoleic acid levels between 0.1% and 2% of the caloric intake resulted in significant reductions in LDL cholesterol levels and aortic atherosclerosis. In a study comparing the effects of linoleic acid or CLA supplementation, cholesterol levels were reduced by nearly 20% and symptoms of early atherosclerosis were reduced approximately three times greater with the CLA diet versus the linoleic acid diet (Haumann, 1996). There were no significant differences on the plasma LDL cholesterol until eight weeks of feeding. The LDL to HDL cholesterol ratio in the plasma was significantly lower in CLA treated animals than in controls. Conjugated linoleic acid-fed rabbits appeared to have developed less intense lesions in the abdominal aortas than control rabbits. In agreement with these findings, Lee et al. (1994) reported that CLA-fed rabbits exhibited less

histological evidence of atherogenesis in lipid deposition and in connective tissue development than non-supplemented controls.

Anticarcinogenic Activity

It has been speculated that the inhibition of carcinogenesis could result from the combined effects of CLA on tissues. This may include direct effects of one or more of the isomers on cell differentiation; the effects of CLA on vitamin A metabolism, which in turn influences cell differentiation; and the effects of the isomers on prostaglandin metabolism which may affect cancer development in mammary, forestomach, and organ tissue (Pariza et al., 2000). Some evidence indicates that the *cis*-9, *trans*-11 and the *trans*-10, *cis*-12 CLA isomers may be equally effective in inhibiting carcinogenesis (Pariza et al., 2000). Several studies with normal mammary cells and transformed cells, including MCF-7 human breast cancer cell lines, indicate that CLA may inhibit cancer growth by inducing cell cycle arrest (Evans et al., 2000). These data showed that cell number and DNA synthesis were lower in cultures treated with the crude mixture of CLA isomers. Furthermore, differentiating cultures treated with CLA for 48h had fewer cells in the S phase of the cell cycle, while cultures treated with CLA for 96h actually had a greater percentage of cells in the S phase (the phase during which DNA synthesis occurs) than control cultures. Conjugated linoleic acid appears to have its greatest impact on cell growth during the proliferative phase and the clonal expansion phase, as shown in 3T3-L1 adipocytes. Conjugated linoleic acid could also be influencing cell growth by inducing programmed cell death or apoptosis (Evans et al., 2000); by altering cellular

membrane phospholipid composition; and the response of intracellular signals due to phospholipid metabolism (Evans et al., 2000). O'Shea et al. (1999) demonstrated that treatment of MCF-7 and SW480 cancer cell cultures with a crude mixture of CLA isomers increased lipid peroxidation (based on the presence of thiobarbituric acid reactive substances) and induced the activities of several antioxidant enzymes. Shaunberg and Krokan (1995) also found that treatment of lung adenocarcinoma cultures and gleoblasoma cells with up to 40 µg of mixed CLA isomers significantly increased lipid peroxidation. Cell growth was not completely restored by α-tocopherol, suggesting that lipid peroxidation was only partially responsible for CLA suppression of cell growth. Researchers have found no effect of CLA on lipid peroxidation *in vivo* or *in vitro* (Evans et al., 2000). In general, isocaloric diets with increasing amounts of polyunsaturated fatty acids (PUFAs) are more positively associated with tumorigenesis than saturated fatty acids. Additionally, inclusion of linoleic acid (18:2, n-6)-rich, dietary corn oil usually correlates with enhanced tumorigenesis in several organs, while dietary oils rich in n-3 PUFAs, such as α-linolenate (18:3, n-3), eicosapentaenoate (20:5, n-3), docosahexaenoate (22:6, n-3) appear to be associated with inhibition of carcinogenesis. Unfortunately, the relationship between PUFAs and cancer is complicated by the fact that not all carcinogenesis models respond to n-6 and n-3 PUFAs in the prototypic model illustrated above.

It is currently thought that not all of the isomers formed during food processing are biologically significant, but rather that *cis*-9, *trans*-11 and/or *trans*-9, *cis*-11 are the most biologically active (Belury, 1995). Introduction of a mixture

of CLA isomers resulted in the preferential incorporation of *cis*-9, *trans*-11 and/or *trans*-9, *cis*-11-CLA into tissue phospholipids of the forestomach, liver, and mammary tissue. In addition, *cis*-9, *trans*-11 and *trans*-9, *cis*-11-CLA were found in relatively higher proportions in uncooked animal-derived foods and human tissues (BeLury, 1995). Conjugated linoleic acid given to rat pups during the early postweaning stage, and prior to carcinogen administration, significantly decreased mammary tumorigenesis occurring after the carcinogen dose; however, it was suggested that the anticarcinogenicity of CLA may be a result of diminished mammary epithelial branching and a higher proportion of quiescent cells in the target organ. Conjugated linoleic acid administered after the carcinogen only reduced tumor numbers if it was given continuously for the experiment's duration (Doyle, 1998). Conjugated linoleic acid has received considerable attention as a cancer-fighting, chemopreventive agent in the past few years because of its ability to inhibit rat mammary tumorigenesis, mouse forestomach neoplasia, and mouse skin carcinogenesis (Belury et al., 1995).

Effects on Bone Metabolism

Bone is a multifunctional organ that consists of a structural framework of mineralized matrix and contains heterogeneous populations of chondrocytes, osteoblasts, osteocytes, osteoclasts, endothelial cells, monocytes, macrophages, lymphocytes and hemopoietic cells. This milieu of cells produces a variety of biological regulators that control local bone metabolism. Systemic calcitropic hormones [parathyroid hormone (PTH), estrogen, and 1, 25 $(OH)_2$ vitamin D_3] and autocrine and paracrine factors, including prostaglandins, cytokines and

growth factors regulate the cellular activities of bone growth to increase the length and diameter of and properly shape long bones (Watkins and Seifert, 2000). Evidence suggests that the dietary lipids influence bone modeling and remodeling (Watkins and Seifert, 2000). Epidemiological studies indicate that dietary fat intake is associated with reduced risk of vertebral and femoral fractures in adults and saturated fat intake caused an increase in bone density in children (Watkins and Seifert, 2000). Studies involving chicks and rats indicate CLA affects histomorphometric measurements of bone modeling (Watkins and Seifert, 2000). Dietary lipids (n-3 fatty acids and CLA) modulate the production of PGE_2, and alter the concentration of IGF-I in bone tissues of growing chicks and rats (Watkins and Seifert, 2000). Rats given a supplement of CLA showed a decrease in the rate of bone formation, suggesting a down-regulating effect on osteoblastic activity (Watkins and Seifert, 2000). However, studies with dietary dairy fats revealed that butter fat (source of natural CLA isomers) blended with corn oil reduced ex vivo bone PGE_2, elevated bone IGF-I concentration and increased bone formation rates in animals nearly 60% compared to those given diets higher in n-6 fatty acids (Watkins and Seifert, 2000).

Enrichment of chondrocytes (collagen secreting cells located in cartilage) with CLA and LA affected collagen synthesis in a dose dependent fashion, with CLA appearing to stimulate collagen synthesis (Watkins and Seifert, 2000). Chondrocyte production of PGE_2 was reduced by CLA. This suggests that CLA may positively influence growth plate cartilage function in the young and may reduce production of inflammatory PGE_2 in the adult. Watkins and Seifert (2000)

provided the first research to indicate that CLA (occurring naturally in milk fat) affects bone metabolism in rats. Conjugated linoleic acid was found to reduce prostaglandin E_2 (PGE_2) concentration in ex vivo bone organ culture. Bone static measurements (trabecular bone volume, trabecular thickness, trabecular separation, and trabecular number) were not affected by CLA supplementation. Bone mineral analysis of right humeri revealed that bone dry weight, ash weight, and mineral density were lower in rats fed the high (n-6) diet compared with those fed the high (n-3) diet without supplementation of CLA. Conjugated linoleic acid treatment to either of the diets lowered ex vivo PGE_2 production. Conjugated linoleic acid was shown to down regulate the circulating levels of IGF-I in the growing rat. Conjugated linoleic acid could have exerted its effect by modulating eicosanoid metabolism, which is consistent with previous work showing that CLA reduced ex vivo bone organ culture PGE_2 production. Li et al. (1999) found that dietary PUFA treatment and CLA supplementation both had a significant effect on ex vivo PGE_2 production in tibia and femur organ cultures. Rats supplemented with CLA had lowered ex vivo PGE_2 production in bone. It is believed that a lower level of PGE_2 will stimulate bone formation in animals fed diets containing moderate levels of (n-6) PUFA. In this study, rats fed CLA had decreased values for MAR and BFR which likely reflect some impact on osteoblastic function. Conjugated linoleic acid reduced the release of leukotriene B_4, a lipoxygenase product of arachidonic acid (AA), one of the most potent chemotaxins for polymorphonuclear leukocytes and monocytes and a strong bone resorption factor, from peritoneal exudates cells (Li et al., 1999).

Metabolite and Hormone Concentrations

Plasma concentrations of circulating hormones often illustrate the impact of feeding a specific diet on the metabolism and overall health of animals. Conjugated linoleic acid is a relatively new dietary supplement, that exhibits positive benefits in animal health, repartitioning of fat to lean, and immunomodulation for supplemented animals. Research conducted by Sugano et al. (1997) showed no differences in the serum concentrations of total and HDL cholesterol, triacylglycerol, phospholipids, or liver lipids of CLA treated rats compared to controls. However, when compared to linoleic acid supplementation, CLA resulted in a decreased concentration of PGE_2 in serum and spleen. When CLA was fed (1.0% of the diet) to rodents, an increase in the serum insulin levels was seen (Delany et al., 1999). In agreement, Delany and West (2000) reported increased levels of plasma insulin in CLA-fed mice, as well as lower plasma leptin levels, and no effect on plasma glucose. Turek et al. (1998) reported reduced liver PGE_2 when rats were fed a diet supplemented with modified tall oil (MTO) a dietary source of CLA. Li et al. (1999) reported that CLA lowered serum IGF-I level independent of the additional PUFAs in the diet. In addition, serum insulin-like growth factor binding protein (IGFBP) was decreased by dietary CLA supplementation although it was dependent on the dietary PUFA type in the diet.

In finishing pigs, Ostrowska et al., (1999b) reported that serum free fatty acid levels and triglyceride levels were elevated by CLA supplementation. However, this was not the case in grower pigs fed CLA (Ramsay et al., 2001).

Furthermore, Ramsay et al. (2001) reported no CLA-related changes in serum glucose, serum insulin, serum nonesterified fatty acids (NEFA), blood urea nitrogen, or IGF-I concentrations in grower pigs. In agreement, Bassaganya-Riera et al. (2001) reported no effects of CLA on plasma urea nitrogen in nursery pigs, but did report an increase in plasma α1 acyl glycoprotein (AGP) levels.

Growth and Metabolism

Researchers have reported differing results in the growth and performance of animals supplemented with dietary CLA. These differences could be attributed to feeding CLA during different phases of growth in the animals and indicate that the supplementation of CLA affects growing animals differently than animals supplemented at maturity (finishing animals). It is also important to note that different genotypes and species of animals were analyzed within different studies. One of the questions addressed by researchers is the stability of the CLA used in the research. Yang et al. (2000) reported that CLA was very unstable in the free fatty acid form as reported by Chin et al. (1997). It is unknown which form would best be resistant to oxidation prior to feeding. If oxidation of CLA occurs prior to feeding it is unknown whether CLA will lose its influence on growth performance or body composition changes.

West et al. (1998) reported a significant reduction in energy intake, growth rate, and body weight when the diets of mice were supplemented with CLA. Chin et al. (1994b), utilizing rats, reported that CLA consumption didn't affect feed intake or body weight of dams, but pups from CLA supplemented dams, during gestation and lactation, were heavier compared to controls. Sugano et al. (1997)

reported no significant differences in feed intake, weight gain, or organ weights (liver, heart, kidney, lung, spleen, brain, and perirenal adipose tissue) between rats fed linoleic acid (LA) and CLA. Delany and West (2000) reported increased total ether extract in CLA-treated mice; however, no effects of CLA were noted on the mice's respiratory quotient. Azain et al. (2000) noted no effects of CLA feeding on food intake, growth rate, and liver, spleen, heart, kidney, gastrocnemius, and soleus muscle weights in rats. There was also no reported effect of CLA on fat cell numbers in any fat pad; however, there was an overall decrease in cell diameter in the retroperitoneal pad. This reduction in cell diameter was accompanied by a significant decrease in the retroperitoneal and parametrial pad weights when rats were fed 0.5% CLA (Azain et al., 2000). There seems to be a reduced response to CLA feeding in rats versus mice. Azain et al. (2000) reported that CLA only affects cell size without producing any change in cell number, arguing against increased apoptosis as a potential fat reducing mechanism. Chin et al. (1994) and Park et al. (1997) reported improved feed efficiency for rats fed CLA, and Chin et al. (1994) speculated that if body fat were decreased, less energy would be required to maintain growth.

Ramsay et al. (2001) reported no differences in gain or feed efficiency, hot carcass weight, carcass length, dressing percentages, and liver, heart, and kidney weights in grower pigs fed supplemental CLA (0.25, 0.5, 1.0, or 2.0%). A study conducted by Bassaganya-Riera et al. (2001), utilizing grower pigs, analyzed the effects of CLA supplementation (0.67, 1.33, or 2.0%) and clean versus dirty environments on swine growth. In the first phase (weeks one and

two of the study) they reported a lower average daily gain (ADG) and average daily feed intake (ADFI) for CLA–treated pigs in the dirty environment, but no differences in the clean environment. The second phase (weeks three through five of the study) showed an increase in ADG followed by a quadratic decrease, while ADFI decreased linearly, resulting in an increase in the gain:feed ratio in a linear fashion. In the third phase (weeks six and seven of the study) no measurable differences in growth performance were found. In general, CLA-fed pigs tended to have lower percent body fat and higher percent water. In support of these findings, Thiel et al. (1998) reported an increase in ADG and Ostrowska et al. (1999a) reported enhanced feed efficiency in finishing swine supplemented with dietary CLA. Dugan et al. (1997), Dunshea et al. (1998), and Ramsay et al. (2001) did not report any differences in growth performance of CLA-supplemented pigs. Agreeing with other studies, Thiel-Cooper et al. (2001) found that at 1% dietary CLA, the ADG increased without a change in ADFI (although numerically lower with CLA supplementation), resulting in an increased gain:feed ratio in all supplemented groups. In other work, Ostrowska et al. (1999a) concluded that the gain:feed ratio was increased (+6.3%) in CLA fed pigs without significant increases in either ADG or ADFI. Fat deposition also decreased (-31%) in a linear fashion over time while the lean tissue deposition increased (+25%) in a quadratic fashion, resulting in a linear decrease in the fat:lean. Thiel-Cooper et al. (2001) working with grower pigs, reported an initial suppression of ADG during the first two week phase, followed by an increase of ADG in the second two week phase as CLA level increased, a slight depression

of feed intake during the first two weeks, and then no significant difference in feed intake during the second two weeks, resulting in an increased gain:feed ratio in CLA-treated pigs. Wiegand et al. (2001) reported that the gain:feed was higher for CLA-fed animals independent of genotype. However, Dugan et al. (1997), Dunshea et al. (1998), and Ramsay et al. (2001) did not report any differences in growth performance of CLA supplemented pigs.

Carcass Composition

West et al. (1998) reported decreased adipose tissue depot weights (greatest effects on the retroperitoneal and less effects on the epididymal depots) and less total carcass protein when CLA was fed to mice. Furthermore, they reported larger kidneys in the CLA supplemented mice while liver, spleen and testes weights, and carcass ash remained unchanged. Delany and West (2000) found reductions in adipose tissue weight (with the largest effect in the retroperitoneal adipose depot), body weight, and total carcass protein and increased liver and spleen weights in CLA-supplemented mice compared to controls.

In pigs, heterozygous for the stress gene, decreases were reported in last rib backfat and 10^{th} rib backfat, while increases were reported in marbling scores (also reported by Dugan et al., 1997), firmness, and *longissimus* muscle lipid percentage (Dugan et al., 1999; Wiegand et al., 1999). In addition, Thiel-Cooper et al. (2001) reported decreases in backfat at the tenth rib and increases in belly firmness with CLA supplementation. Contrary to other studies, Ramsay et al. (2001) reported that 10^{th} rib backfat depth was increased at low CLA

concentrations (0.25 and 1.0%). Although most research indicates a decrease in backfat when pigs are supplemented with CLA, the effects of CLA supplementation on loin eye area are more variable. Loin eye areas (LEA) were increased by CLA supplementation (O'Quinn et al., 2000a; Weigand et al., 1999) or remained unaffected (Eggert et al., 1999a; Wiegand et al., 2001; Theil-Cooper et al., 2001). However, there were no deleterious effects to LEA by supplementing diets with CLA.

Another indicator used to assess carcass grades is the midline backfat depth of the carcass. Conjugated linoleic acid has demonstrated an antiadipogenic nature, reducing the size of fat depots in the pork carcass. Multiple studies have demonstrated that subcutaneous fat in pigs is deposited at a decreasing rate when CLA is fed in the diet (Park et al., 1999a; O'Quinn et al., 2000a; O'Quinn et al., 2000b; Sparks et al., 1999; Eggert et al., 1999a; Eggert et al., 1999b; Ostrowska et al., 1999; Thiel-Cooper et al., 2001; Dugan et al., 1997; Dugan et al., 1999). Conjugated linoleic acid has been shown to decrease the quantity of carcass fat, and increase the percent of carcass lean in finishing pigs (Ostrowska et al., 1999a; Dunshea et al., 1998; Dugan et al., 1997). There have only been a few studies conducted to analyze the carcass compositional endpoints of carcasses treated with CLA. These studies have illustrated an increase in the fat free lean (FFL) (Eggert et al., 1999a) or no effect to the FFL (Eggert et al., 1999b), increased bone weight of the carcass and decreased carcass fat (Thiel-Cooper et al., 2001). These data indicate that there have been

no deleterious effects of CLA-supplementation on the carcass composition of pigs.

Pork Quality

CLA has been reported to significantly alter the factors utilized to assess pork quality, resulting in significant differences in the value of carcasses from CLA fed-pigs. The assessment of pork firmness is often associated with the fatty acid composition of the carcass. Carcass fat composed primarily of saturated fatty acids would be firmer than fat composed primarily of unsaturated fatty acids due to the chemical nature of saturated versus unsaturated fatty acids. In multiple studies, feeding CLA to pigs has reportedly increased the firmness of the carcass fat (Sparks et al., 1999; Eggert et al., 1997a, Eggert et al., 1997b; Theil-Cooper et al., 2001). However, in a study conducted by O'Quinn et al. (1999a) there was no difference in the firmness of pork carcasses from pigs fed CLA. Wiegand et al. (2001) reported a sharp increase in L* (lightness), increases in a* (redness) and decreases in b* (blueness) color measurements. Other studies have shown contradictory data, indicating no effects on lightness values (Dugan et al., 1999) and decreases in the redness and blueness (O'Quinn et al., 2000) of CLA-fed pigs. There were no differences in carcass shrink, visual color, or tenderness, juiciness, or flavor intensity. Similar results from sensory analyses were reported by Dugan et al. (1999), Thiel et al. (1999), Wiegand et al. (1999), and Thiel-Cooper et al. (2001). In terms of the quality, driploss is indicative of the meat's ability to retain its inherent water. Seaping cuts are not appealing to consumers and result in poor saleability. Although CLA has not been found to

increase the driploss of chops (Dugan et al., 1999; O'Quinn et al., 2000b) there has been one report of CLA decreasing the driploss in the *longissimus* muscle of supplemented pigs (O'Quinn et al., 2000b).

Currently, one of the most important factors associated with carcass quality is the marbling component of the *longissimus* muscle. As reported by many researchers, the response of marbling content to CLA feeding has varied, but most interestly, there has only been one deleterious report of CLA supplementation on the marbling component of meat animals (Eggert et al., 1999a). There have been reports of increases in marbling (Sparks et al., 1997; Eggert et al., 1997a; Dugan et al., 1999; O'Quinn et al., 2000a; Wiegand et al., 2001) and reports of no change to the marbling content (O'Quinn et al., 2000). In pH, another measure of muscle quality, Wiegand et al. (2001) found that carcasses from CLA-treated pigs had a lower three-hour postmortem pH than non-supplemented carcasses, with no difference in ultimate pH. The ultimate pH was also reported to be unaffected by CLA-supplementation (O'Quinn et al., 2000b).

Fatty Acid Profiles

Watkins and Seifert (2000) found that CLA feeding altered the fatty acid composition of rat tissues, reducing the percentage of oleic acid (18:1) in liver, skeletal muscle, heart, bone marrow, and periosteum. In rats, the *trans*-10, *cis*-12 isomer of CLA was incorporated into the phospholipid fraction of tissue lipid extracts much like that for the *cis*-9, *trans*-11 isomer. The ratio of *cis*-9, *trans*-11 : *trans*-10, *cis*-12 roughly reflected the isomeric distribution of these CLA isomers

in the diet given to rats. The *cis*-9, *trans*-11 isomer of CLA was preferentially incorporated into rat membrane phospholipids (Li and Watkins, 1998). Rat tissue fatty acid composition was significantly influenced by dietary PUFA treatment and CLA supplementation. Rats given the high (n-6) PUFA diets had higher serum levels of stearic (18:0), oleic (18:1), linoleic (18:2, (n-6)), α-linolenic (18:3, (n-3)), (18:3, (n-6)), 20:2, (n-6), 20:3, (n-6), arachidonic (20:4, (n-6)), 22:4, (n-6), total monounsaturates, and total (n-6) PUFA but lower myristic (14:0), palmitic (16:0), palmitoleic (16:1, (n-7)), eicosapentaenoic (20:5, (n-3)), docosapentaenoic (22:5, (n-3)), docosahexaenoic (22:6, (n-3)), total saturates, and total (n-3) PUFA compared with those fed diets high in (n-3) fatty acids. Dietary CLA supplementation increased the values for 16:0 and total saturates in both PUFA treatment groups while values for 22:5(n-3), 22:6(n-3) and total (n-3) PUFA were increased only in rats fed the high (n-3) diet.

Fatty acid profiles of subcutaneous fat from pigs fed dietary CLA shows an increase in the overall saturation levels. This increase in saturation is believed to be related to the inhibition of ∆-9 stearoyl-CoA desaturase activity (Lee et al., 1994; Pariza et al., 2000). Ramsay et al. (2001) reported that *latissimus* muscle and dorsal subcutaneous adipose tissue concentrations of CLA increased with increasing CLA levels in the diet of grower pigs. Dietary CLA increased the percent myristic (14:0), palmitic (16:0), and stearic acids (18:0) while decreasing percent oleic (18:1), linoleic (18:2), linolenic (18:3), and arachidonic acids (20:4). Adipose tissue appeared to be more sensitive to CLA supplementation than muscle. Conjugated linoleic acid increased the saturated:unsaturated ratio in

skeletal muscle and adipose tissue in grower pigs (Thiel-Cooper et al., 2001), while Stangl et al. (1999) reported slight reductions in n-6 polyenoic fatty acid linoleic acids and its elongation and desaturation products 18:3(n-6), 20:3(n-6), and 20:4(n-6). No significant differences were observed with total saturated fatty acid and total monounsaturated fatty acid concentrations.

Physiological Effects of Isomers

There is evidence to suggest that the different isomers of conjugated linoleic acid elicit different physiological responses. It has been postulated that the *trans*-10, *cis*-12 isomer is responsible for body composition changes in mice (Park et al., 1999). The body composition changes reported by Park et al. (1999) were reduced body fat, enhanced body water, enhanced body protein, and enhanced body ash. Some evidence illustrated an importance of the *cis*-9, *trans*-11 isomer in growth enhancement of young rodents as well as increased anticarcinogenic activity (Pariza et al., 2000). Generally speaking, the *cis*-9, *trans*-11 and the *trans*-9, *cis*-11 are the isomers thought to provide antioxidant properties and anticarcinogenic properties in animals (Azain et al., 2000).

Proposed Mechanisms of Action

There are multiple mechanisms proposed for the action of CLA on animal performance; however, all mechanisms are currently speculation. Although early research suggested that CLA had antioxidant properties, it is currently thought that CLA is actually a prooxidant. Yang et al. (2000) showed that CLA was oxidized faster than linoleic acid, suggesting that the conjugated carbon-carbon double bond is more prone to oxidation than a nonconjugated double bond.

Knowing that the CLA could be more prone to oxidation, it could be oxidized before being utilized which would reduce the effects of the compounds and may account for variations in performance illustrated between experiments. Multiple studies provide evidence suggests that free radicals and radical mediated oxidation may play a role in CLA's biological activities including cancer and atherosclerosis. Data also suggests that CLA is cytotoxic to some cancer cell lines, and inhibited proliferation in those cells (MacDonald, 2000).

Data suggests that CLA may also affect the initiation and progression of atherosclerotic lesions, by affecting lipid peroxidation. MacDonald (2000) suggested that this may be the reason fatty streaks in the aorta are reduced by almost half in atherosclerotic rabbits fed CLA and that the increase in the blood lipoproteins may be a result of the peroxidation of the fats.

It is speculated by Watkins et al. (2000) that CLA may influence bone metabolism through its effects on progesterone (PGE_2) production through the cyclooxygenase (COX) enzyme system, more likely on COX-2. Conjugated linoleic acid may alter COX-2 action/expression to influence parathyroid hormone (PTH) and growth factor progesterone dependent osteoclastic bone resorption, progesterone (PGE) receptor-mediated actions on bone cells, and cytokine-induced extracellular release of PGE_2 by osteoblasts. Other possible mechanisms of action for CLA include reduced desaturation/elongation of linoleic acid and inhibition of prostanoid biosynthesis by its isomeric analogs. It has been speculated by Li et al. (1999) that CLA could exert a regulatory effect on the production and action of insulin-like growth factor (IGF-I) and insulin like

growth factor binding protein (IGFBP) by modulating PGE_2 production to impact local bone metabolism.

In terms of potential mechanisms for fat reduction, CLA increased carnitine palmitoyltransferase, which is the rate-limiting enzyme for β-oxidation, and hormone sensitive lipase, which hydrolyzes lipids from adipocytes (Pariza et al., 1997 as reported by O'Quinn et al., 2000a). Reductions in the activity of heparin-releasable lipoprotein lipase activity in 3T3-L1 adipocytes have also been reported (Park et al., 1999). The respiratory quotient, which is typically increased at night, was lower in CLA-fed animals (Doyle, 1998). Lower respiratory quotients are typically indicators of increased fat oxidation and illustrated that mice fed CLA continued to burn fat at night. There are numerous other mechanisms for CLA action on nutrient repartitioning (Doyle, 1998).

It was suggested by Lee et al. (1994) that CLA's effect on the saturated to monounsaturated ratio was accomplished through inhibition of liver Δ-9 stearoyl-CoA desaturase acitivity. This theory was also suggested by Waters et al. (1997), and Pariza et al. (2000). Inhibition of this enzyme is probably the most plausible mechanism to explain the increases seen in the levels of fatty acid saturation.

Conclusions

The effects of CLA on carcass composition are well documented with the most studies reporting decreased fat, and some showing increased protein contents. Fatty acid composition is consistently altered with CLA supplementation by increasing the saturation level. This has been suggested to

increase carcass and cut firmness. The effects of CLA feeding on growth performance is not as clear as the decreases in backfat depths or the fatty acid profile changes and may be due to differing responses across animal age, supplement composition, level of feeding, and supplement stability. Any one or a combination of these factors may result in increased variability in animal growth responses. It has been theorized that the *cis*-9, *trans*-11 isomer is important for CLA's anticarcinogenic properties, while the *trans*-10, *cis*-12 isomer appears responsible for the antiadipogenic properties and changes in body composition. All of these data clearly illustrate that additional research is needed to definitively and consistently demonstrate the effects of CLA on animal growth, carcass composition, and fatty acid profiles.

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CHAPTER 3

MATERIALS AND METHODS

Treatments

This experiment was conducted using two groups (Replicate 1; n = 15; Replicate 2; n= 18) of crossbred gilts fed varying levels of CLA (0, 0.5, 2.0%) for the final 47 days of the finishing period. Pigs were weaned at 25 days of age and handled through the nursery, growing, and finishing phases using standard industry practices. The replicates were conducted approximately ten months apart. As the pigs began the finishing phase, they were randomly assigned to groups receiving a corn-soybean meal diet (Table 1) containing 0, 0.5, or 2.0 % CLA supplemented in the diet balanced by soy oil addition in varying amounts. The CLA utilized in the first replicate was the free fatty acid form (CLA-60 produced by Conlinco; Detroit Lakes, MN), however, due to availability problems, a methyl ester form of CLA (CLA-65 produced by BASF; Mount Olive, NJ) was used for the second replicate. The CLA content of the diet is shown in Table 2. Animals in both replicates were raised according to IACUC standards. Growth data and feed intake was measured across the feeding trials. The average daily gain (ADG) average daily feed intake (ADFI) and feed to gain ratio (F:G) were determined for the feeding trials. Animals in replicate one were individually housed, thus ADFI and F:G were measured per animal. The animals used in the second replicate were penned according to treatment, and thus ADFI and F:G were measured as averages per pen. Ultrasound data was collected across

replicates, beginning at the onset of CLA feeding. The initial backfat (10th rib) and loin eye area of pigs were determined using ultrasound. One day prior to harvest, final ultrasound backfat and loin eye area were determined.

Slaughter

Upon completion of the feeding trial, the gilts were weighed and harvested, under federal inspection at the University of Georgia Meat Science Technology Center in Athens, Georgia. The gilts were electrically stunned using 275 volts and 6 amps for a duration of approximately ten seconds. Standard industry slaughter techniques were used to produce a hairless, eviscerated carcass, which was split down the vertebral column. Following splitting, fat samples were taken from the subcutaneous depot (at approximately the 10th rib) on the left side of the carcass. Each carcass half was weighed and placed into the carcass chill cooler (~-2ºC).

Carcass Yield and Quality Traits

Following a 24-hour chill (-2ºC), carcasses were moved into the carcass holding cooler (2ºC), and 24 h later, the carcasses were graded. The carcasses were split between the 10th and 11th ribs and backfat thickness was measured at the 1st rib, last rib, the last lumbar vertebrae and the 10th rib. Loin eye areas were traced and measured using a digitizer pad (Jandel Scientific, Corte Madera, CA). Evaluation of meat quality attributes included instrumental measurements of ultimate pH at the 10th rib cut surface (Cole Parmer pH-20 handheld pH meter; Cole Parmer Instrument Company, Vernon Hills, IL) and Hunter L*a*b* reflectance (CIE,1978) of the loin eye muscle measured once in the proximal and

once in the distal half of the muscle. In addition, subjective meat quality attributes were collected following a 30-min bloom time using the five-point NPPC standards for color, marbling, and wateriness/firmness (NPPC, 1999). Muscle color (L*, a*, b*) was also measured on the *semimembranosus* muscle during fabrication.

Fabrication

After grading, carcasses were fabricated into bone-in primals and subsequently to boneless, trimmed subprimals according to NAMP specifications (NAMP, 1996). Weights of the primals and subprimals were recorded. To initiate fabrication, the pillar and wing of the diaphragm were removed, weighed, and discarded. The weight of the remaining carcass was defined as the adjusted carcass side weight. The tail was then removed by cutting between the first and second coccygeal vertebrate, weighed and discarded. The rear and forefeet were removed by cutting immediately dorsal to the hock and knee joints. Each foot was weighed and recorded separately, and then discarded. The shoulder was then separated from the carcass by cutting 2.54 cm posterior to the elbow and perpendicular to the long axis of the carcass. The neck bones were removed, and the associated lean trim remained with the Boston Butt (BB), the neckbones were then weighed, recorded, and discarded. The jowl was removed by measuring 2.54 cm anterior to the ear dip and cut parallel to the posterior cut surface of the shoulder. The jowl was skinned, and the jowl weight and jowl skin weight was recorded.

The BB was separated from the Picnic Shoulder (PS) by cutting 1.27 cm ventral to the base of the scapula, and parallel to the posterior cut surface. A strip of skin (2.54 cm) was removed from the dorsal edge of the PS and the fat was beveled to the cut surface. The breast flap was removed at the crease, and separated into skin and fat. The PS weight was recorded as the 405, and the skin and fat weights were recorded. The remaining skin and bone was removed from the PS, without destroying the cushion. The PS was then trimmed to 0.64 cm fat thickness. Finally, the PS was trimmed to 0 cm fat (denuded) and the weights were recorded. The lean trim, bone, and skin associated with the PS was weighed and recorded. The skin was removed from the BB removing as little fat as possible with the skin. Then the BB was trimmed to 0.64 cm fat, weighed and recorded as the 406. The blade bone was removed and the BB, weighed and recorded as the 406A. The fat and lean overlying the blade bone was removed and the weight was recorded as a 407. The blade bone was trimmed and the bone and lean trim weights were recorded. The 407 was trimmed to 0 cm fat trim, weighed and recorded as the denuded BB.

The loin and the belly were removed from the ham by cutting 3.81 cm anterior to the aitch bone and perpendicular to the shank bone. A cut 2.54 cm below the attachment of the ribs to the vertebral column and 1.27 cm ventral to the tenderloin on the posterior end was made to separate the belly from the loin. The spare ribs were then separated from the belly. The weight of the belly (408) and the spare ribs (416) were recorded. The belly was trimmed such that the thickness of the smaller end was within 3.08 cm of the larger end. The skin was

then removed from the belly, and the weight of the 409 skinless belly, the skin, the fat trim, and the lean trim was recorded.

The loin was skinned and the fat trimmed to 0.64 cm fat thickness. The weight of the skin, the fat trim, and the 410 loin were recorded. The blade was removed and separated into lean and bone and the weight of the 411 loin, associated bone and lean trim was recorded. A cut between the last two lumbar vertebrae separated the loin from the sirloin. The blade portion was removed such that eleven ribs remained on the loin, producing the 412C. The blade, sirloin, and 412C loin were weighed and recorded. The tenderloin was then separated from the sirloin weighed and recorded. The blade and sirloin portions of the loin were deboned, weighed and recorded as boneless sirloin and boneless blade. Each portion was then trimmed to 0 cm fat, weighed, and recorded. The tenderloin was then separated from the loin and weighed. The chine bone was then removed and weighed and recorded. The loin was also weighed upon removal of the chine and recorded as 412D. All bones were then removed, the back ribs were weighed and recorded. The remaining bone and the 412E loin was weighed and recorded. The fat was then removed to 0 cm on the 412E loin weighed and recorded as 0 cm fat 412E.

The flank muscle was removed from the ham. The skin, fat, cutaneous muscle, and lymph nodes over the knuckle were removed. The pelvic fat was then trimmed flush with the surface. The weight of the ham was recorded as ham 401. The fat trim, lean trim, and skin associated with the ham was also weighed and recorded. The skin was removed such that it did not exceed one

quarter of the distance from the stifle joint to the ham face. Fat was removed from the skin and weighed with the ham providing the 402. The skin was also weighed and recorded. The ham was then completely skinned, and the pelvic bone and femur were removed. The outer shank was removed, weighed, and recorded as the heel. The cushion, the inner shank, the knuckle, the lite butt, and the outside were also removed, weighed, and recorded. The outside, the knuckle, and the inside cushion was trimmed to 0.64 cm fat, weighed and recorded, then trimmed to 0 cm fat weighed and recorded. Ham bone, lean trim, and skin were weighed and recorded.

Fatty Acid Profiles

The fatty acid profile of adipose tissue was determined by the FAME procedure of Azain (1993) in replicate 1 using gas chromatography with a flame ionization detector (Shimadzu GC System Shimadzu Scientific Instruments Inc, Columbia, MD, Replicate 1; Agilent 6850 Series GC System Agilent Technologies Palo Alto, CA, Replicate 2). Tissue samples were saponified by adding 50-100 mg of backfat in 1 mL of 4N NaOH and 1 mL of methanol (MeOH). Heptadecanoic acid (1mg/mL methanol) was added as an internal standard, prior to saponification. Samples were placed into tightly capped screw top vials (20 mL) and heated at 75ºC for 6 h. The content of the vials was cooled on ice for 5 min and was acidified by the addition of 2 mL of 3N HCl. The fatty acids were extracted into 3 mL of hexane and transferred to a second vial for methylation. Once into the second vial, 3 mL of MeOH and 1 mL of 14% boron trifluoride in methanol were added and samples were heated at 95ºC for 1 h.

Samples were then cooled to room temperature and 4 mL of water and 2 mL of the hexane phase was transferred to small Teflon-capped vials for gas chromatography analysis. In the first replicate, the fatty acid methyl esters were separated on a Supelcowax-10 fused capillary column (Supelco, Bellefont, PA) under isothermal conditions. The column temperature was 240°C, injector temperature was 250°C, and detector temperature was 260°C. The sample size used was 0.5 µL and the carrier gas was helium.

In the second replicate the FAME procedure of Park and Goins (1994) was conducted to determine the fatty acid profile of the adipose tissue. Twenty-five mg of lipid was weighed into screw top centrifuge tubes. Then, 200 µL of methylene chloride and 1 mL of internal standard in methanol. The tube was flushed with nitrogen and capped. The samples were then heated at 90°C for 30 min. Following heating, the tubes were allowed to cool to room temperature for approximately 10 min. Following cooling, 2 mL of 14% boron triflouride in methanol was added, the tubes were flushed with nitrogen and capped. The tubes were once again heated at 90°C for 20 min and allowed to cool to room temperature. One mL of hexane was added and the samples were vortexed for 1 min. The upper layer was transferred to a tube containing anhydrous sodium sulfate, and 1 mL of hexane was added to complete the transesterification. The fatty acid methyl esters in the second replicate were separated on an Supelco 2560 (Supelco, Bellefont, PA) capillary column. The oven temperature was programmed from 150 to 165°C at 1.0°C/min, then to 167°C at 0.2°C/min and then to 225°C at 1.5°C/min. The carrier gas used was hydrogen and the make-

up gas was nitrogen. The injector was maintained at 250°C and the detector at 250°C. The sample size was 1.0 µL. In both replicates peak identification was based on known standards, which included pure samples of *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA. Under these measurement conditions, the *cis*-9, *trans*-11 isomer elutes after linoleic acid and is followed by the *trans*-10, *cis*-12 isomer (See Appendix 1 for the specific retention times of the fatty acids).

Lipid Analysis

In the first replicate, the *longissimus* from each carcass was ground once through a coarse plate (1.27 cm), hand mixed and ground through a fine plate (0.07 cm). In addition, the remainder of the carcass lean was ground through a coarse plate, hand mixed and coarse ground again. Samples were obtained from the ground *longissimus* (0.4 kg) and the carcass grind (1 kg) vacuum packaged, and frozen for subsequent analysis. In the second replicate, all muscles and lean trim were coarse ground (1.27 cm) and 2 kg of sample was removed. The samples were ground through a fine plate (0.07 cm) and sampled for lipid analysis.

Lipid analysis was conducted in triplicate using the procedure of Bligh and Dyer (1959). Samples were weighed (2.5 g $\pm$ 0.1 g) into 50 mL conical centrifuge tubes. Aluminum pans were numbered, placed into a drying oven, then into a dessicator, and weighed to determine dry pan weights. After weighing out the samples, methanol (10 mL) and chloroform (5 mL) was added to each tube. The mixture of chloroform-methanol (1:2) and meat was homogenized at mid-speed using a Polytron homogenizer. Between samples, the homogenizer was cleaned

with a chloroform-methanol mixture (1:2). After homogenizing, the samples were allowed to stand for approximately 1 h at room temperature to allow solvent to penetrate the samples. Following the extraction, 5 mL of chloroform, and 5 mL of potassium chloride (1M) were added to the meat homogenate. The tubes were capped and vortexed. After vortexing, the tubes were placed into an ice bath for 5 min, and then transferred to a tabletop centrifuge. Samples were centrifuged 10 min at 0ºC and 2000 rpms. Following centrifugation, the top (aqueous) layer was aspirated off without disturbing the pellet. The tube was gently thumped to dislodge the pellet, and the organic layer was poured into a preweighed aluminum pan. The aluminum pans were left under a hood overnight to allow evaporation. On the following day, the pans were placed into a drying oven for 15 min at 90ºC. After drying in the oven, the pans were placed into a dessicator for 5 min and weighed. Lipid content was calculated using the following formula: (wt of lipid and pan - wt of pan) / wt of sample

Retail Display and Thiobarbituric Acid Reactive Substance Levels

Retail display characteristics were only monitored in the second replicate. Patties were hand-formed from the finely ground samples mentioned previously. Four patties per carcass side were made and placed into styrofoam trays. Each tray included two patties and was wrapped with an oxygen permeable overwrap. Samples were placed into a cooler at 4ºC and allowed to remain for 1, 4, 7, or 11 d. At the designated time, one patty from each animal was removed from the simulated display and Hunter L*, a*, and b* was measured. The remaining patty on each tray was overwrapped again to maintain retail conditions.

After completion of the appropriate display time and instrumental color assessment, samples were weighed (approximately 5 g) in triplicate into 50 mL disposable conical centrifuge tubes for determination of malonaldehyde formation (TBAR; Ahn et al., 1998). The tubes were homogenized (setting 7) in 15 mL of deionized water for 15 sec using a Polytron homogenizer. Following homogenization, the volume of the homogenate was measured. From each tube, 1 mL of the meat homogenate was transferred to centrifuge tubes. Tetraethoxypropane (TEP) standards were created containing the following concentrations of TEP: 0, $4X10^{-10}$, $6X10^{-10}$, $8X10^{-10}$, $1X10^{-9}$, $2X10^{-9}$, $4X10^{-9}$, $6X10^{-9}$, $8X10^{-9}$, and $1X10^{-8}$ moles/mL. After completing the TEP standards, 50 µL of a betahydroxytoluene solution (7.2%) was added to all of the tubes, including the TEP standards. Next, 2 mL of a 20 mM thiobarbituric acid in 15% trichloroacetic acid solution (TBA/TCA) were added to all of the tubes. Upon the addition of the TBA/TCA solution the tubes were vortexed. The tubes were then placed in a boiling water bath (>90ºC) for 15 min. After heating, the samples and standards were cooled in a tap water bath for 10 min. The samples were then centrifuged for 15 min (Jouan CR 312 Jouan Inc., Winchester, VA) at 3000 X g. Absorbance (531nm) of the standards and samples was recorded (Milton Roy Spectronic 1201 Thermo Spectronic, Rochester, NY), with water being the blank. The standard curve was used to estimate TBAR values reported as mg of malonaldehyde per kg of meat.

Statistical Analysis

Data were analyzed using the General Linear Model procedure of SAS. Results are presented as least squared means ± pooled SEM. P-values of ≤ 0.05 were considered to be significant. The model used to analyze carcass yield and quality traits, compositional endpoints, and fatty acid profiles included the main effects of diet (0, 0.5, 2.0) and replicate and their interaction. Due to differences in handling of animals in the finishing phase across the replicates, growth traits were analyzed separately across replicates. For the retail display data, single degree of freedom contrast were used to determine differences between treatment groups, contrasting the control group with the treated groups, and contrasting treated groups. Additionally, regression (linear and quadratic) analysis was used to determine the response over time in TBARS and instrumental color measurements.

CHAPTER 4

RESULTS AND DISCUSSION

Ultrasound Data

The ultrasound data (Table 3) indicated a reduction in final backfat in treated animals, with the 0.5% treatment group being lower ($P<0.05$) than controls. When corrections for animal live weight were incorporated, final backfat measures were lower ($p<0.05$) in the CLA-treated versus control pigs. There was a diet by replicate interaction ($P<0.05$) for ultrasound final loin muscle area (LMA). In replicate one, there was no effect of CLA on LMA; however, in replicate two, the 0.5% CLA group had lower ($P<0.5$) LMA than the 2.0% group while controls were intermediate. These differences appear related to differences in live weight since final LMA per kg of live weight was not different ($P>0.05$) across treatments. The decrease in fat depth of ultrasound data agrees with Thiel-Cooper et al. (2001); however, the findings for ultrasound loin muscle area (LMA) are contrary to the increased muscling reported by Sparks et al. (1999) for CLA-treated pigs.

Growth Traits

Multiple papers have reported changes (more information) in the body weight (West et al., 1998), average daily gain (Bee, 2000; O'Quinn et al., 2000a; O'Quinn et al., 2000b; Thiel-Cooper et al., 2001), average daily feed intake (Eggert et al., 1999b; Dugan et al., 1999; Thiel-Cooper et al., 2001; O'Quinn et al., 2000a; Bee, 2000), and feed : gain (Sparks et al., 1997; Dugan et al., 1997;

Ostrowska et al., 1999; O'Quinn et al., 2000b; Thiel-Cooper et al., 2001) of CLA-fed animals. Our data showed that feeding CLA did not alter (P>0.05) final body weight, average daily gain (ADG), or average daily feed intake (ADFI) (Table 3) in replicate one. These results are in agreement with the rodent papers of Chin et al. (1994), Park et al. (1997), Sugano et al. (1997), and Azain et al. (2000) that reported no significant difference in growth traits across CLA level. In the first replicate of our study, there were no changes (P>0.05) in ADG, ADFI, or feed:gain indicating that CLA-supplementation did not alter growth traits of these individually-housed pigs. However, in the second replicate, a reduction (P<0.05) in final body weight, ADG, and feed:gain of 0.5% CLA-pigs compared to controls and 2.0% groups was found. The difference in response across the replicates was due to the performance of the pigs in the 0.5% CLA-fed group. Pigs in the 0.5% CLA, replicate 2 group had lower ADFI and lower live weights and ADG than other groups in replicate 1 and 2. This may be due to a problem with pigs in this group urinating in the feeders, which resulted in depressed feed intake.

Carcass Yield Traits

Conjugated linoleic acid has been identified as a potent repartitioning agent, increasing lean and decreasing fat. In the present study, CLA supplementation reduced (P<0.05) carcass fat measured at the first rib (Table 5). The 0.5% CLA-supplemented group had lower (P<0.05) tenth rib fat than control with the 2.0% CLA-supplemented group being intermediate. Conjugated linoleic acid supplementation did not affect (P>0.05) the carcass weight, loin eye area or loin area expressed as a percentage of side weight. Numerical reductions in

backfat at the last lumbar and last rib were present. These reductions in backfat agree with most of the literature findings (Dugan et al., 1997; Thiel-Cooper et al., 2001). In agreement with other findings (O'Quinn et al., 2000a; Ramsay et al., 2001; Thiel-Cooper et al., 2001), the loin eye area was numerically decreased across treatment groups; however, when expressed as a percentage of side, there was no difference in loin eye area. Our data agreed with Ramsay et al. (2001) in terms of the hot carcass weight, and the dressing percentage across treatment groups illustrating no differences.

Carcass Quality Traits

Changes in carcass quality with CLA treatment are shown in Table 6. Neither ultimate pH, nor carcass marbling, wateriness-firmness, or visual color scores (NPPC, 1999) were affected ($P>0.05$) by CLA treatment. Contrary to these findings, Dugan et al. (1999) and Eggert et al. (1999b) reported improvements in *longissimus* color, firmness, and marbling content of CLA supplemented carcasses. The increases in carcass marbling (Dugan et al., 1997; Ostrowska et al., 1999) are particularly interesting because these authors also reported reductions in carcass fat. The findings of these authors suggest that CLA supplementation caused a reduction of fat deposition in the subcutaneous depots and an increased fat deposition in the intramuscular depots. However, the findings of this study do not substantiate this altered deposition theory. No differences ($P>0.05$) were found in the lightness (L*), redness (a*), or yellowness (b*) of the *longissimus* muscle in CLA-fed animals. Feeding the 2.0% CLA diet increased ($P<0.05$) the redness (Hunter a*) in the

semimembranosus and tended to increase redness (P<0.19) in the *longissimus dorsi* compared to controls with 0.5% CLA being intermediate. Other researchers have reported increases in the L* (O'Quinn et al., 2000b; Wiegand et al., 2001) and reductions in the b* (O'Quinn et al., 2000a) values in CLA supplemented animals.

Carcass Compositional Endpoints

The analysis of compositional endpoints involving percentage of lean (four lean cuts, boneless denuded cuts, and fat free lean) was not altered (P>0.05) by CLA supplementation. Numerical increases in lean were minute and largest in the 0.5% treatment group (Table 7). This is in contrast to the findings of Dugan et al. (1997) and Ostrowska et al. (1999) who reported that carcass composition endpoints are directly impacted by changes in lean growth rates. Bone weights were also numerically increased in the 0.5% CLA treatment group (Table 7). The report of increased bone weight agrees with the study conducted by Watkins and Seifert (2000) and is in agreement with Li et al. (1999). In agreement with Dugan et al. (1997) and Ostrowska et al. (1999) the total fat percentage was lower in the 0.5% CLA group than the controls.

Fatty Acid Profiles

Fatty acid composition of subcutaneous fat across the dietary treatments is shown in Table 8. Total lipid content of the subcutaneous fat was reduced linearly (P<0.05) with increasing level of CLA. The total lipid content of subcutaneous fat was lower (P<0.05) for 0.5% CLA treatment compared to controls. Conjugated linoleic acid feeding at the 2.0% level increased (P<0.05)

the saturated fatty acid content (C 16:0, stearic acids) in the fatty acids of subcutaneous fat (Table 8). In terms of individual fatty acids (Table 8), palmitic acid (16:0) was higher ($P<0.05$) in the 2.0% CLA-fed group compared to 0.5% and control groups. In addition, stearic acid increased ($P<0.05$) incrementally as CLA level increased. In contrast, the unsaturated fatty acids (palmitoleic, oleic, linoleic, and α-linolenic) generally decreased as the level of CLA supplementation increased. As expected, the CLA isomers *cis*-9, *trans*-11 and *trans*-10, *cis*-12 increased ($P<0.05$) across treatment groups. Again, these changes in fatty acid composition are consistent with the theory that CLA causes an inhibition of the Δ-9 stearoyl-CoA desaturase enzyme resulting in increased saturation levels overall (Lee et al., 1994; Pariza et al., 2000). Similarly, Lee et al., 1994, Stangl et al., 1999, and Thiel-Cooper et al., 2001 have shown increased saturation of fat from CLA-supplemented pigs. Feeding CLA incrementally decreased ($P<0.05$) the percentage of monounsaturated fatty acids (Table 9) in the subcutaneous fat. In addition, the percentage of polyunsaturated fatty acids (without including CLA) was lower ($P<0.05$) in the 2.0% group than either the control or the 0.5% groups.

Retail Display Characteristics

The final segment of this study involved the monitoring of color changes and the accumulation of lipid oxidation products in ground patties after 1, 4, 7, or 11 days of simulated retail display. Control patties were lighter (higher L*; $P<0.05$) than patties from CLA-supplemented pigs (0.5% and 2.0% CLA) after 1, 4, and 7 d of retail display (Figure 1). However by day 11, Hunter L* values were similar ($P>0.05$) between treatments. Patty redness (a*; Figure 2) decreased

during retail display, with patties from CLA-treated (0.5% and 2.0%) pigs being redder ($P<0.05$) than controls through 7 d of retail display. However, as seen with L* values, no differences ($P>0.05$) were found by d 11. The lower redness values observed with CLA supplementation agree with those reported for intact *longissimus* and *semimembranosus* data. Hunter b* (Figure 3) values (yellowness) remained relatively constant through 7 days of retail display and then decreased ($P<0.05$) from d 7 to 11. Hunter b* values were lower ($P<0.05$) for the 0.5 than control or 2.0% treatments at d 7. As expected, malonaldehyde formation (TBAR values), an indicator of lipid oxidation, (TBARS) increased over time with the greatest changes occurring after 4 d of display. In addition, there was a tendency for the 2.0% CLA patties to have lower TBAR values (Figure 4) than the control or 0.5% CLA-supplemented groups after 1 ($P<0.09$), 4 ($P<0.10$), and 7 d ($P<0.17$). This is consistent with the CLA treatment effects on fatty acid profiles in that the fat from the 2.0% treated pigs was more saturated than the fat from control and 0.5% CLA-treated pigs, providing fewer potential sites for oxidation.

CHAPTER 5

CONCLUSIONS

Conjugated linoleic acid supplementation reduces carcass fatness without negatively impacting carcass quality traits. Conjugated linoleic acid supplementation may enhance consumer acceptability by producing darker and redder pork products and extending shelf life. Conjugated linoleic acid content of adipose tissue was increased with supplementation. Due to the incorporation of CLA into carcass lipids and its potential health benefits for humans, CLA supplementation may offer a method for producing animal-based nutraceutical products.

Future Projects

Although no current research exists to accurately determine the effects of each individual isomer, researchers have speculated that the *trans*-10, *cis*-12 isomer is the active isomer, resulting in body composition changes. To be certain, various pure isomers should be used to determine the actual agent responsible for the changes in body composition. Studies containing pure isomers would potentially provide answers to multiple questions, including the causative agent(s) for changes in composition, antioxidant/prooxidant properties, impacts to growth traits, and quality traits. Although the changes are typically associated with the major isomers, the minor isomers produced by the base catalyzed isomerization of linoleic acid would also have to be analyzed to determine the activities in animal tissues. Additionally, multiple forms of CLA are

used in experimental procedures (ie. free fatty acids, methylated fatty acids, etc.). There has been no research conducted to determine which form of CLA is most biologically active. Although some research has been conducted to analyze the effects of natural sources and industrial sources, no research has focused on the actual form of the fatty acid used. This may be the reason for much of the variability in the research conducted. Through a better understanding of the impact of different forms of CLA, we may better understand the most active and the most stable forms of the fatty acid.

The identification of the most biologically active isomer and the most biologically active form may provide the results required to stimulate utilization of CLA for animal feeding and optimal output in industry. Additionally, it may be possible to make CLA cost effective if an easily synthesized method were determined. Currently, the feed supplement is not cost effective to commercial swine producers, and this must be one of the first issues to address before progressing to actual isomer and active-form testing. With implications on human health emerging, a potential target market may be opening for a “healthy pork” or “CLA enhanced” product.

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Table 1: Composition of control and conjugated linoleic acid (CLA) supplemented diets

Diet	Control	0.5% CLA	2.0% CLA
Corn (% of diet)	70.68	70.68	70.68
Soybean Meal (% of diet)	21.0	21.0	21.0
Feed Fat (% of diet)	1.52	1.52	1.52
Soy Oil (% of diet)	3.33	2.50	0
CLA (% of diet)	0	0.85	3.33
Dicalcium Phosphate (% of diet)	1.85	1.85	1.85
Limestone (% of diet)	0.75	0.75	0.75
Salt (% of diet)	0.35	0.35	0.35
Lysine (% of diet)	0.2	0.2	0.2
Methionine (% of diet)	0.02	0.02	0.02
Vitamin Mix (% of diet)	0.15	0.15	0.15
Mineral Mix (% of diet)	0.15	0.15	0.15
Diet Fatty Acid Composition			
16:0 (% of fatty acids in diet)	13.7	13.7	13.7
16:1 (% of fatty acids in diet)	1.8	1.7	1.9
18:0 (% of fatty acids in diet)	3.5	3.5	3.6
18:1 (% of fatty acids in diet)	26.4	26.2	25.9
18:2 (% of fatty acids in diet)	50.6	46.2	32.6
18:3 (% of fatty acids in diet)	3.8	3.3	1.3
CLA t9, c11 (% of fatty acids in diet)	0	1.95	7.65
CLA c10, t12 (% of fatty acids in diet)	0	2.43	9.50

Table 2: Least squares means of ultrasound data for conjugated linoleic acid (CLA) supplemented pigs

	Control	0.5% CLA	2.0% CLA	SEM	Replicate
N	11	11	11		
Initial Backfat (cm)	1.63	1.56	1.67	0.064	<0.01
Final Backfat (cm)	2.07[a]	1.76[b]	1.89[ab]	0.103	0.01
Initial Loin Eye Area (cm^2)	29.01	27.44	28.05	1.038	<0.01
Final Loin Eye Area (cm^2)[*]	39.47	38.43	41.16	1.057	<0.01
Adjusted Initial Backfat (cm/kg)	0.024	0.022	0.024	0.0009	<0.01
Adjusted Final Backfat (cm/kg)	0.021[a]	0.017[b]	0.018[b]	0.0009	0.02
Adjusted Initial Loin Eye Area (cm^2/kg)	0.437[a]	0.395[b]	0.403[ab]	0.1268	<0.01
Adjusted Final Loin Eye Area ($cm^{2/}$/kg)	0.398	0.375	0.391	0.1249	<0.01
Diet * Replicate					
Final Loin Eye Area (cm^2) Rep 1	32.96[a]	34.58[a]	33.10[a]	1.562	
Final Loin Eye Area (cm^2) Rep 2	45.98[bc]	42.28[b]	49.22[c]	1.426	

[ab] Means within a row with different superscripts differ (P<0.05)
[*] Significant diet * replicate interaction (P<0.03)

Table 3: Least squares means for growth traits of conjugated linoleic acid (CLA) supplemented pigs

	Control	0.5% CLA	2.0% CLA	SEM
Replicate 1				
N	5	5	5	
Initial Weight (kg)	59.20	59.31	59.29	1.926
Final Weight (kg)	102.7	105.6	102.4	3.64
Gain (kg/d)	0.925	0.984	0.917	0.0673
Feed Intake (kg/d)	2.05	2.01	2.00	0.108
Feed : Gain	2.24	2.08	2.19	0.116
Replicate 2				
N	6	6	6	
Initial Weight (kg)	67.04	67.04	67.20	2.592
Final Weight (kg)	105.1[a]	94.9[b]	106.4[a]	3.14
Gain (kg/d)	0.809[a]	0.592[b]	0.835[a]	0.0393
Feed Intake (kg/d)	2.07	1.85	2.05	0.0
Feed : Gain	2.58[a]	3.17[b]	2.51[a]	0.142

[ab] Means within a row with different superscripts differ (P<0.05)

Table 4: Least squares means for carcass yield traits and compositional endpoints of conjugated linoleic

	Control	0.5% CLA	2.0% CLA	SEM	Replicate
N	11	11	11		
Slaughter Weight (kg)	102.1	99.7	103.2	5.49	0.63
Hot Carcass Weight (kg)	77.4	76.1	78.5	4.52	0.995
Chilled Side Weight (kg)	37.1	36.5	37.3	1.59	0.30
Dressing Percentage (%)	75.83	76.35	75.99	0.495	0.08
Backfat 1st Rib (cm)	4.17^{a}	3.76^{b}	3.68^{b}	0.059	0.11
Backfat 10th Rib (cm)	1.80^{a}	1.47^{b}	1.68^{ab}	0.033	0.74
Backfat Last Lumbar (cm)	2.08	1.75	2.01	0.068	0.07
Backfat Last Rib (cm)	2.03	1.85	2.01	0.039	0.03
Loin Eye Area (cm^2)	44.32	43.51	42.84	1.347	<0.01
Loin Eye Area (cm^2/kg of side weight)	1.20	1.20	1.13	1.319	<0.01
Four Lean Cuts (% of side weight)	69.87	71.07	70.07	0.495	0.64
Boneless Denuded Cuts (% of side weight)	60.94	61.26	60.80	0.530	0.11
Fat Free Lean (% of side weight)	56.86	58.53	57.54	0.705	<0.01
Total Fat (% of side weight)	23.80^{a}	21.27^{b}	22.96^{ab}	0.901	<0.01
Bone Weight (% of side weight)	17.86	18.41	18.29	0.349	0.03

^{ab} Means within a row with different superscripts differ (P<0.05)

Table 5: Least squares means for carcass quality traits of conjugated linoleic acid (CLA) supplemented pigs

	Control	0.5% CLA	2.0% CLA	SEM	Replicate
N	11	11	11		
Marbling**	1.7	1.5	1.7	0.21	0.52
Color**	2.6	2.6	2.7	0.11	<0.01
Wateriness-Firmness #	7.4	8.5	7.6	0.449	0.02
pH 24 Hour	5.48	5.52	5.51	0.048	0.12
Hunter L* LD	52.3	51.9	52.9	0.99	<0.01
Hunter a* LD	10.4	10.2	11.2	0.38	0.12
Hunter b* LD	5.8	5.5	6.3	0.45	<0.01
Hunter L* SM	50.6	49.8	50.1	1.22	<0.01
Hunter a* SM	11.22[a]	11.75[ab]	13.14[b]	0.516	0.02
Hunter b* SM	5.7	5.3	6.2	0.56	<0.01

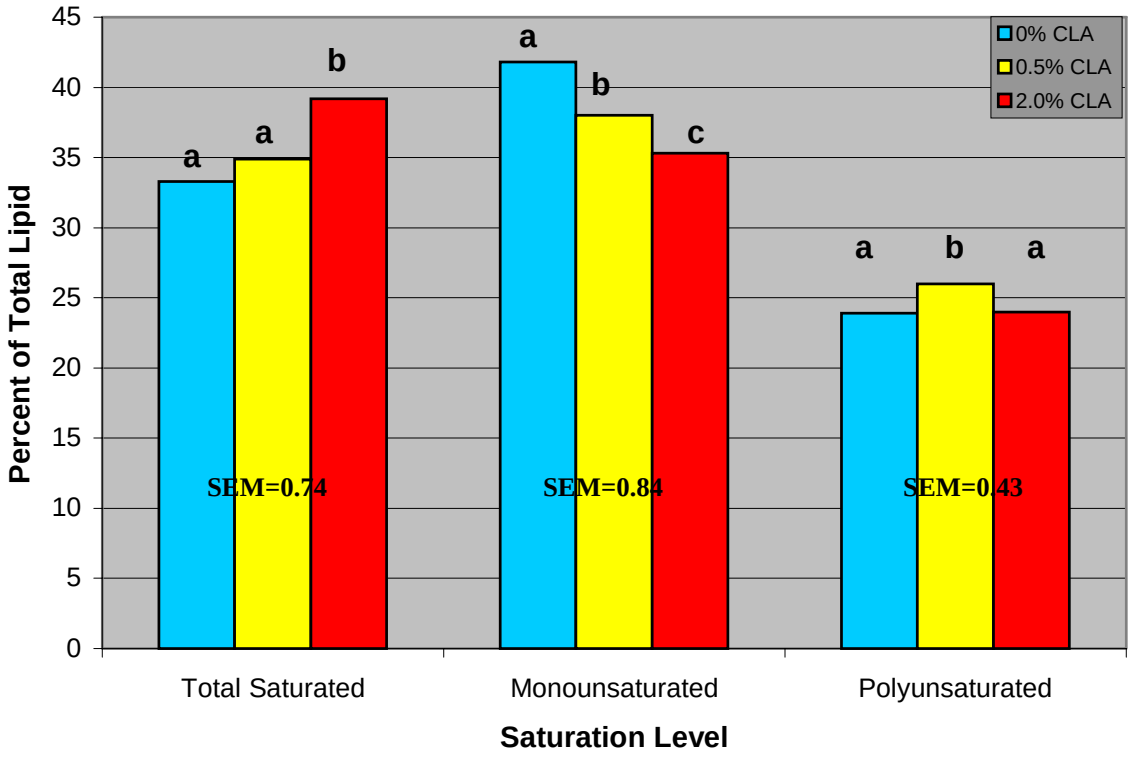
[ab] Means within a row with different superscripts differ (P<0.05)
** NPPC 5-point standards
#Converted to a 15-point standard from the NPPC 5-point scale to compensate for +/-
LD *Longissimus dorsi*
SM *Semimembranosus*

Table 6: Least squares means for the fatty acid profile of conjugated linoleic acid (CLA) supplemented pigs

	Control	0.5% CLA	2.0% CLA	SEM	Replicate
N	11	11	11		
Total Lipid (mg/100mg Tissue)	68.9	65.7	67.1	1.55	<0.01
16:0 (% of total lipid)	21.8[a]	21.7[a]	23.6[b]	0.40	<0.01
16:1 (% of total lipid)	2.86[a]	2.52[b]	2.30[b]	0.115	<0.01
18:0 (% of total lipid)	10.9[a]	12.5[b]	14.7[c]	0.45	0.05
18:1 (% of total lipid)	39.0[a]	35.5[b]	33.0[c]	0.65	0.02
18:2 (% of total lipid)	22.4[a]	23.6[a]	19.0[b]	0.47	0.10
18:3 (% of total lipid)*	1.32[a]	1.35[a]	0.83[b]	0.029	<0.01
CLA 9/11 (% of total lipid)*	0.16[a]	0.67[b]	2.31[c]	0.063	<0.01
CLA 10/12 (% of total lipid)	0.04[a]	0.34[b]	1.51[c]	0.058	0.40
Diet * Replicate					
18:3 (% of total lipid) Rep 1	1.13[a]	1.14[a]	0.51[b]	0.045	
18:3 (% of total lipid) Rep 2	1.51[c]	1.56[c]	1.15[a]	0.037	
CLA 9/11 (% of total lipid) Rep 1	0.22[a]	0.57[b]	1.64[c]	0.097	
CLA 9/11 (% of total lipid) Rep 2	0.10[a]	0.77[b]	2.99[d]	0.079	

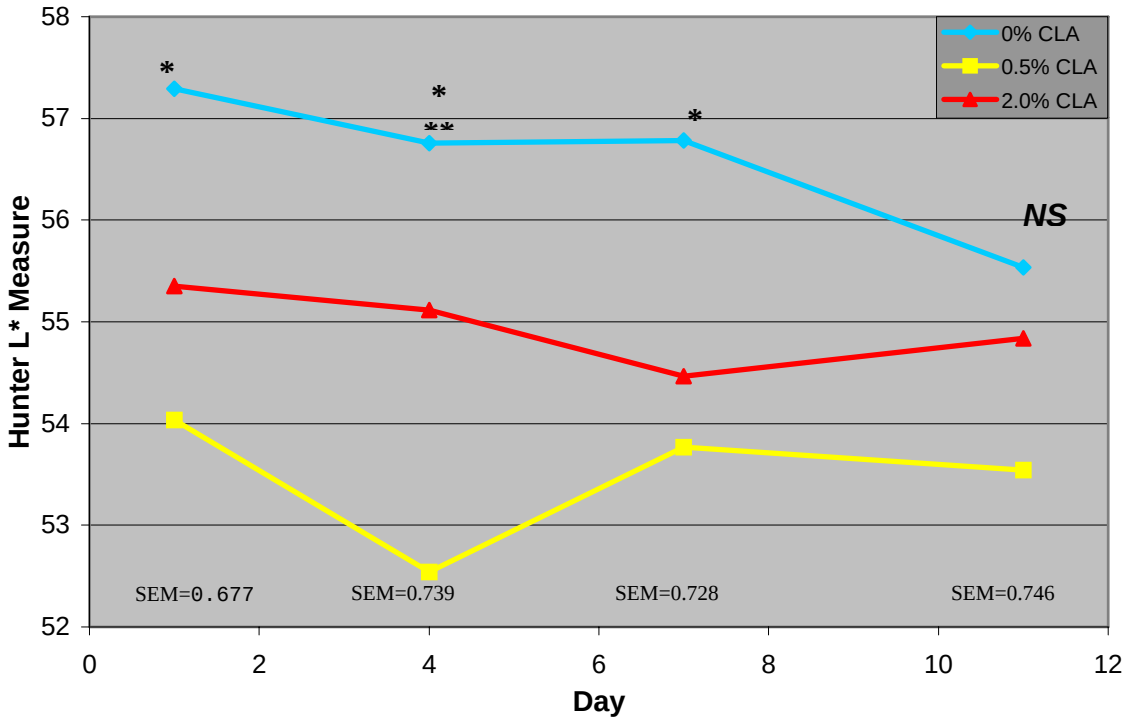
*Significant diet*replicate interaction (P<0.01)
[abcd] Means within a row with different superscripts differ (P<0.05)

Figure 1: Effects of CLA Feeding on Fatty Acid Profiles of Subcutaneous Fat



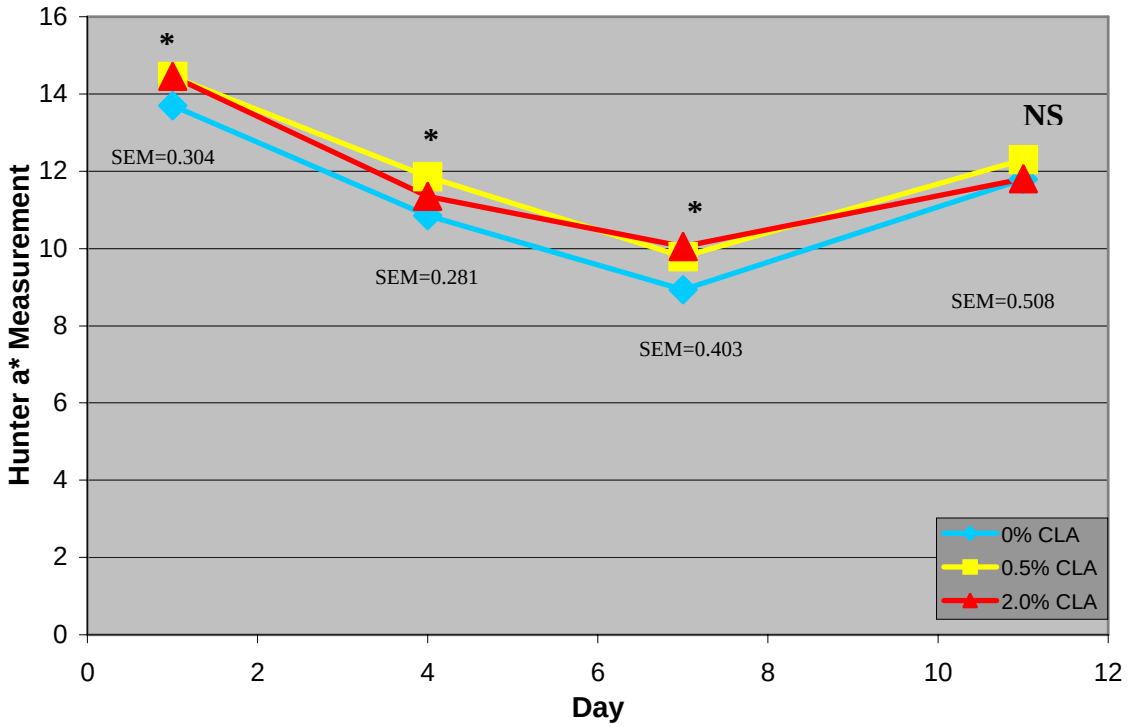
[abc] Means within a row with different superscripts differ (P<0.05)
Significant replicate effects for Total Saturated, Monounsaturated, and Polyunsaturated (P<0.05)

Figure 2: Effects of CLA Feeding on Hunter L* Color



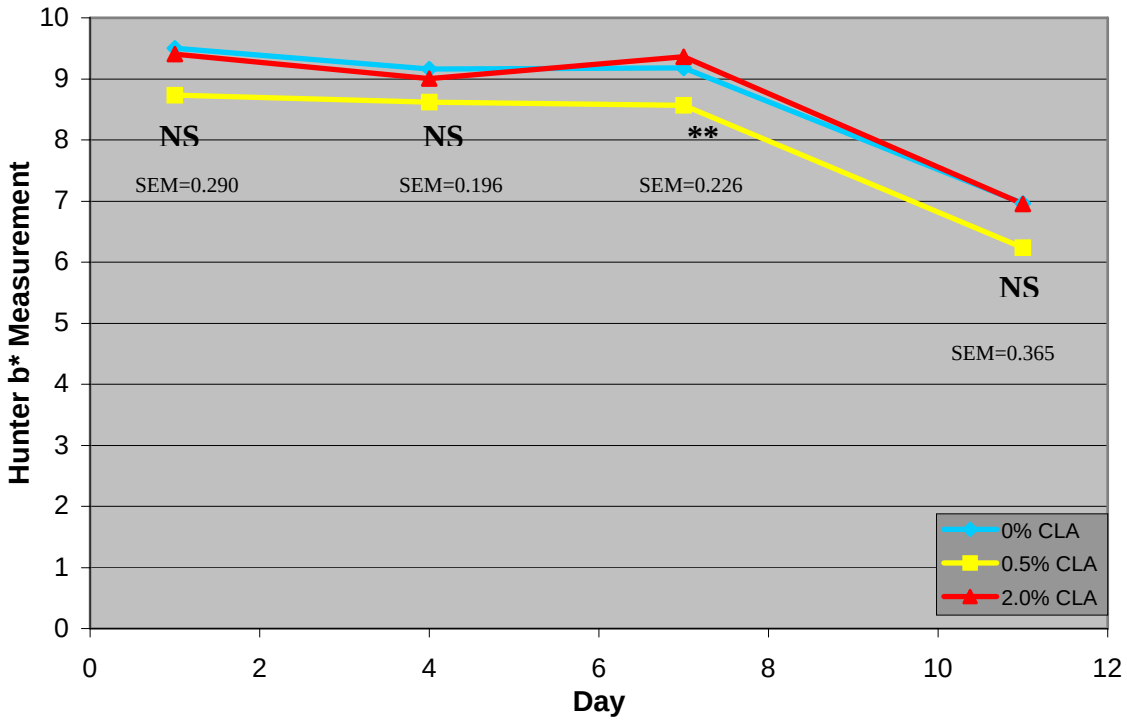
* Significant contrast comparing 0 vs. 0.5 and 2.0% (P<0.05)
** Significant contrast comparing 0.5 vs. 2.0% (P<0.05)

Figure 3: Effects of CLA Feeding on Hunter a* Color



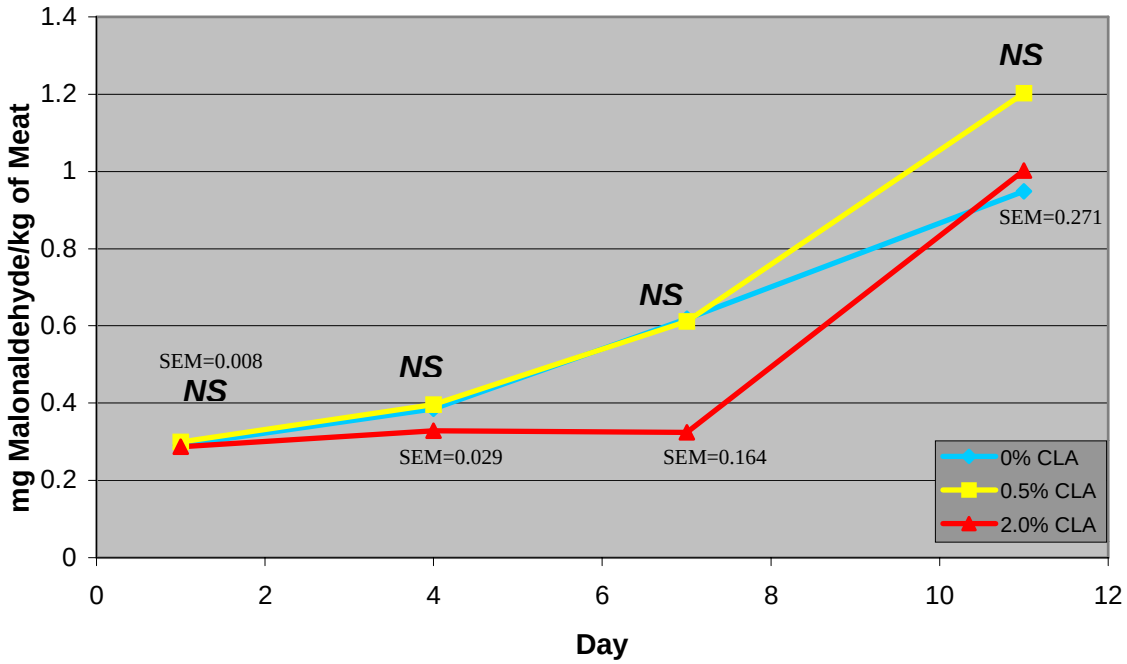
* Significant contrast comparing 0 vs. 0.5 and 2.0% (P<0.05)

Figure 4: Effects of CLA Feeding on Hunter b* Color



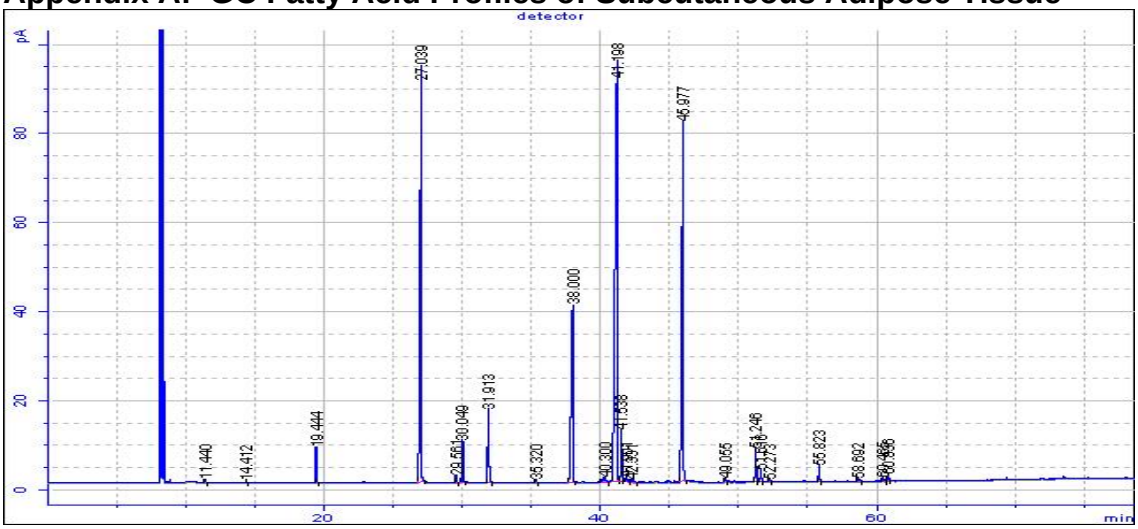
** Significant contrast comparing 0.5 vs. 2.0% (P<0.05)

Figure 5: Effects of CLA Feeding on Malonaldehyde Formation

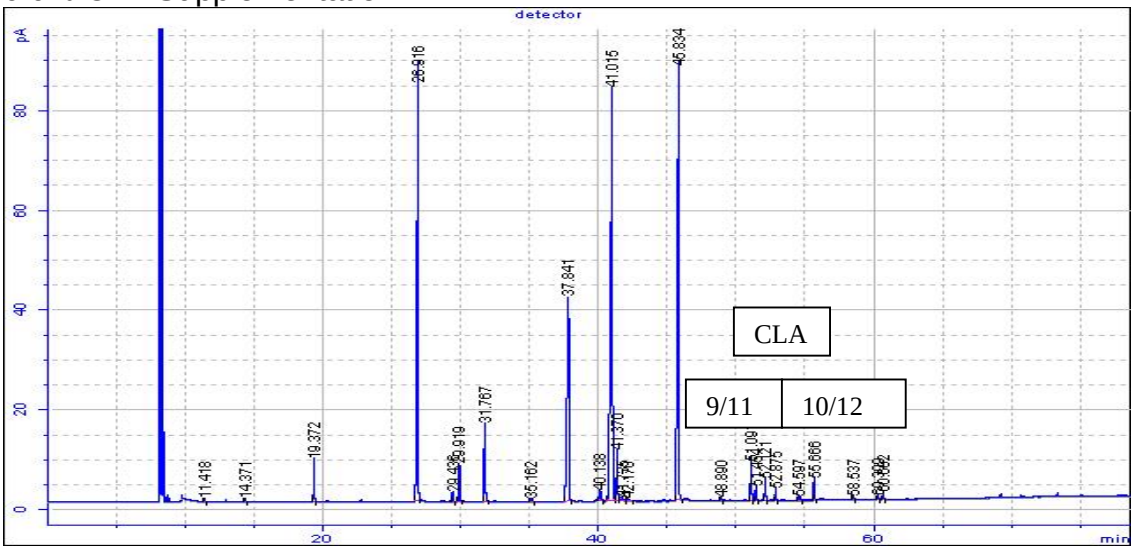


APPENDICES

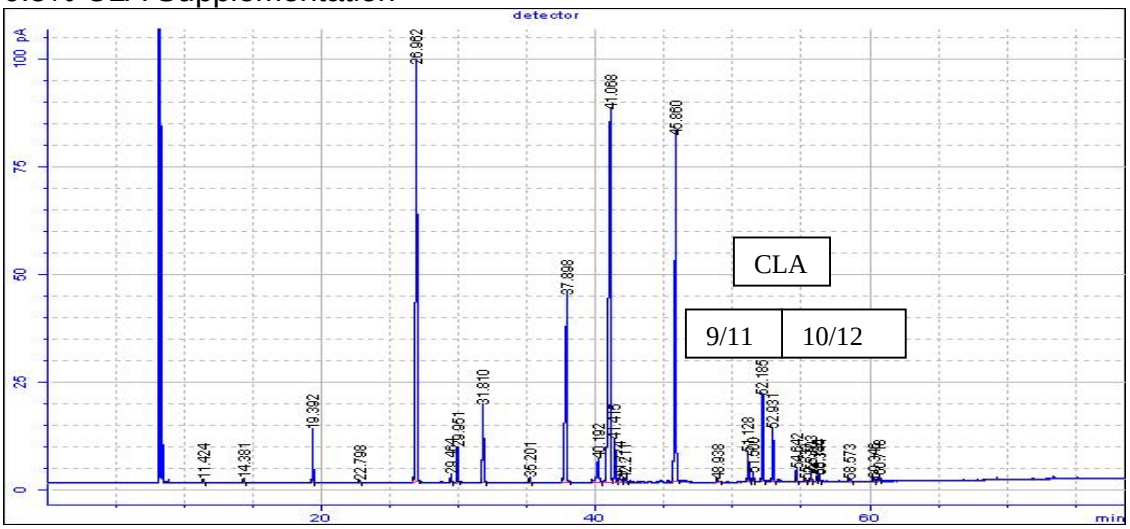
Appendix A: GC Fatty Acid Profiles of Subcutaneous Adipose Tissue



0.0% CLA Supplementation



0.5% CLA Supplementation



2.0% CLA Supplementation

The retention time of the *cis*-9, *trans*-11 isomer of CLA is 51.091-51.128; The retention time of the *trans*-10, *cis*-12 isomer of CLA is 52.121-52.185

Appendix B: Summary of conjugated linoleic acid (CLA) supplementation trials in pigs

Study	CLA Level (%)	Length of Feeding	Gain	Feed Efficiency	Effects of CLA on Carcass Composition	Effects of CLA on Carcass Quality	Subjective
Dugan et al., 1997	0 or 2.0	61.5-106 kg	↔	↑ FCE	↑ lean ↓ intermuscular fat ↑increased loin	NR	NR
Dunshea et al., 1998	0, 0.125, 0.25, 0.50, 0.75, 1.00	8 weeks	↑	↑ FCE	↑final weight, protein, ↓ fat weight ↓ water content	NR	NR
Bassaganya et al., 1999	0, 0.67, 1.33, or 2.0	42 days	↑Phase 2 ADG	↑G:F	↓ fat, and ↑ protein	NR	NR
Dugan et al., 1999	0 or 2.0	61.5-106 kg; 45 days	NR	NR	↑marbling	Obj. color: ↑ chroma values, ↔ b*, ↔ LT shear force, drip loss, or soluble protein	↔ pH decline, ↑ marbling, ↔ measured palatability characteristics
Eggert et al., 1999a	0 or 1.0	23-136 kg	↔	↔ FCE	↓10th rib backfat, ↑ percent fat free lean, ↔ LEA	↔ L*a* b*, ↑loin color	↑ loin marbling, firmer bellies
Eggert et al., 1999b	0 or 1.0	90-115 kg or 65-115 kg	↔	↔ FCE	↑ LEA, ↓ 10th rib backfat, ↔ % fat free lean	↑color, ↔ L*a*b*	↑ belly firmness, loin firmness, and marbling
Ostrowska et al., 1999	0, 0.07, 0.14, 0.275, 0.41, 0.55	8 weeks	NR	↑G:F during 1st 4 weeks	↓carcass fat, ↑backfat ↑carcass protein	NR	NR
Sparks et al., 1999	0 or 1.25	Last 0, 29, 58, or 87 kg	↔	↑G:F	↓backfat, ↓ backfat at 1st, 10th rib, and last lumbar, and a linear↑ LEA	↔ color	↑marbling and firmness
Bee, 200	0 or 2	35 days	NR	NR	↑ saturation level, and ↓monounsaturation level	NR	NR
O'Quinn et al., 2000a	(1) 0, 0.5 CLA, or 0.5 MTO (2) 0, 0.25, 0.5, or 1.0 MTO	(1) 37.6-106.4 kg (2)	(1) ↑ (2) ↔	(1) ↑ G:F (2) ↔ FCE	MTO ↓ backfat, ↑ LEA, % lean	↑ b*	(1) ↔ driploss or shear force (2) ↓muscle drip loss ↑increased belly firmness
O'Quinn et al., 2000b	0 or 0.5 MTO	45.4-78.9 kg and 78.9-117.5 kg	↑	↑G:F during 45.4-78.9, and tended ↑ during 45.4-117.5	↓ average backfat, and 10th rib backfat	↑ L* values	NR
Bassaganya-Riera et al., 2001	0, 0.67, 1.33, or 2.0	7 weeks	↑ in the clean room	NR	NR	NR	NR
Ramsay et al., 2001	0, 0.25, 0.5, 1.0, or 2.0	20-55 kg	↔	↔ FCE	↑ % of palmitic, stearic, and ↓ % of oleic, linoleic, linolenic, and arachidonic acids	NR	NR
Theil-Cooper et al., 2001	0, 0.12, 0.25, 0.5, or 1.0	26-114 kg	↑	↑G:F	↓10th rib fat depth, ↓ subcutaneous fat, and↓ intermuscular fat, and a ↑ in bone	NR	↑belly firmness, finished loin weight tended
Weigand et al., 2001	0 or 0.75	40-106 kg	NR	↑G:F	NR	↔ subjective color	↓ postmortem pH, loin muscle area and sensory characteristics unaffected, ↑marbling, firmness

↑-Increasing; ↓-Decreasing; ↔-No Change; NR-Not Reported; FCE-Feed Conversion Efficiency; MTO-Modified Tall Oil; CLA-Conjugated Linoleic Acid