Conjugated linoleic acid changes porcine performance, compositional and meat quality characteristics

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Conjugated linoleic acid changes porcine performance, compositional and meat quality characteristics

by

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GENERAL INTRODUCTION

Pork producers have taken strides to improve the growth, efficiency and carcass characteristics of pigs through genetic selection and more intense management schemes. Improvements in swine nutrition and facility design have also contributed to the swine industry's evolution. To maintain profitable swine farms, industry leaders and producers must continue to adapt technology and scientific findings into their swine production systems. Consumption of pork by the United States consumer has remained essentially unchanged for the last 20 years at a level of approximately 22.7 kg per capita. In comparison, chicken consumption has increased from 11.3 kg per capita in 1970 to 33.2 kg per capita in 1997 (NPPC, 1999a). This surge in chicken consumption should be an indication to pork producers that a change in the product to be marketed is needed to remain competitive with meat from other animals. Aside from creating new convenience food items from pork, it seems possible to enhance pork's market share by producing more consistent traditional retail products, which excell in meat quality attributes. Of the retail pork cuts, loin chops have historically been the most valuable, ranging from $2.77 to $3.48 per pound in the period from 1987 to 1997 (NPPC, 1999a). One goal for pork producers should be to provide pork
loin chops, with consistent flavor, tenderness and juiciness. This would afford consumers a good eating experience with each purchase. Recently, research has focused on feeding conjugated linoleic acid (CLA) to growing-finishing pigs to improve feed efficiency, decrease subcutaneous fat depth and improve meat quality attributes.

Pork meat quality can be defined as characteristics that affect the eating quality of pork products. These might include color, water holding capacity and pH (Meisinger and Miller, 1998). These quality factors can be influenced in various steps of pork production including pre-harvest handling, stunning, chilling and storage. A nutritional supplement such as CLA would certainly prove useful if it were able to accelerate growth, improve carcass composition and protect or improve meat quality attributes ultimately serving as a functional food.

On the basis of previous research within our group (Thiel et al., 1999) and other reports (Yurawecz et al., 1995), we hypothesized that CLA might function as an antioxidant or have some other chemical properties that might protect meat color and extend retail shelf-storage by decreased oxidation in fresh pork products. Thiel et al. (1998) reported redder color in fresh ground pork from CLA-fed pigs. Yurawecz et al.
(1995) suggested that the formation of furan fatty acids from CLA might protect meat products against peroxide attack. Because peroxides can act as prooxidants in meat systems and can negatively affect meat color (Kanner, 1994), this might suggest that CLA could protect color with the formation of furan fatty acids.

The first study involving CLA supplementation to pigs at Iowa State University investigated the effective dose of CLA to result in significant improvements in growth and performance (Thiel et al., 1998). The next series of studies within our research group, which are reported in this dissertation, investigated the optimal feeding duration of CLA to pigs and the impact of CLA on meat quality characteristics.

In addition to learning how long to feed CLA during the life cycle, we also wanted to study the interaction between CLA supplementation and different genetic lines of pigs. We were able to work with stress-free genotype, crossbred pigs as well as a population of stress-free, stress-carrier, and stress-positive genotype pigs. Results from these experiments might give scientists and producers valuable information on the efficacy of CLA in systems of varying genetic composition.
Dissertation Organization

This dissertation is in an alternate format, which includes a general introduction, review of the literature, three manuscripts formatted according to the style of the Journal of Animal Science, and general conclusions.
Conjugated Linoleic Acid Defined

Conjugated linoleic acid (CLA) is a derivative of the essential fatty acid, linoleic acid. Linoleic acid is an 18:2 fatty acid with double bonds in the 9 and 12 carbon positions. These bonds are both in the cis configuration. CLA isomers are a group of conjugated dienes with positional and geometric variations of linoleic acid. CLA can have double bonds in the 9,11; 10,12 and 11,13 positions and each of these positions can be cis or trans in their geometry (Chin et al., 1992). CLA is said to be conjugated because the double bonds are not methylene-separated as are most other unsaturated fatty acids. To date, 20 isomers of CLA have been reported in the literature (Sehat et al., 1998a; Lavillonniere et al., 1998). The c-9, t-11 and t-10, c-12 isomers are most abundant in plant oils and are present at 43% and 40% of the total CLA, respectively (Chin et al., 1992). Initially, the c-9, t-11 isomer was thought to be the biologically active form of CLA because it was the only isomer detected in the phospholipid fraction of tissues from animals supplemented with CLA (Ha et al., 1990). However, current reports (Park et al., 1999) show that the t-10, c-12 isomer has biological activity as well by
decreasing body fat and increasing whole body protein in rats.

Significant effort has been invested in standardizing methods for the detection of CLA isomers in various food products (Sehat et al., 1998a; Sehat et al., 1998b; Lavillonniere et al., 1998). Early methods of CLA determination used fatty acid methyl esters (FAME) of CLA isomers. The CLA isomers were methylated with BF$_3$, HCl or H$_2$SO$_4$ and were separated on a 60 m gas chromatography (GC) column. This method determined eight isomers of CLA (Sehat et al., 1998a). By extending the GC column length to 100 m, it was possible to elucidate 10 CLA isomers (Sehat et al., 1998a). Later, Lavillonniere et al. (1998) showed that by methylating the CLA isomers with NaOCH$_2$ and reducing them with hydrazine, it was possible to elute five isomers, which were previously undetectable. Sehat et al. (1998b) reported that methylation with BF$_3$, HCl or H$_2$SO$_4$ would result in the conversion of cis/trans isomers to trans/trans isomers, thus decreasing the number of geometric isomers that were detectable. Sehat et al. (1998a) also showed that by first subjecting the FAME to silver ion-HPLC and separating the FAME on a 100 m polar GC column, one is able to determine the elution order of 20 isomers of CLA.

Use of commercially produced CLA for humans and animals is typically derived from sunflower or safflower oil because
of the lower cost of production and price of raw materials compared with animal sources. Berdeaux et al. (1998) reported a method for producing the 9c, t11 and 10t, 12c isomers of linoleic acid. The first step requires the production of methyl linoleate (18:2) from safflower oil through transesterification and two successive urea crystallizations. The methyl linoleate was subjected to ethylene glycol at 180° C for 13 h. The alkalyzation resulted in 95% 9c, t11 and 10t, c12 isomers in approximately equal amounts of 44.5 and 47.17%, respectively. These isomers of CLA were further purified with a series of low-temperature (-58° C) crystallizations. The final yield of 9c, t11 and 10t, 12c were 18 and 25.7% of the original safflower oil. Isomer identity was confirmed with silver ion-thin-layer chromatography.

Sources of CLA

CLA is present in numerous food products of plant and animal origin. Several common sources of CLA are shown in Table 1. Foods derived from ruminant animals are significantly higher in CLA than foods from non-ruminant and plant sources. This is likely due to the production of CLA as an intermediate in the biohydrogenation of linoleic acid in the rumen. Dietary fats are quickly hydrolyzed to free fatty
acids (FFAs) in the rumen and unsaturated FFAs are hydrogenated by rumen microbes (Kelly et al., 1998). The result of this biohydrogenation is the absorption of more saturated fatty acids in ruminants compared with non-ruminant species (Kelly et al., 1998). Figure 1 shows the biohydrogenation of linoleic acid to stearic acid in the rumen and the formation of CLA in this pathway. CLA can escape the rumen as an incomplete biohydrogenation product and is subsequently absorbed across the intestinal wall and be deposited in muscle and fat tissue (Kelly et al., 1998). For unknown reasons, CLA content is higher in lamb meat relative to beef (Chin et al. 1992; Fritsche et al., 1999). In addition, products other than meat from ruminants tend to be high in CLA. Milk and cheese are good sources of CLA (Chin et al., 1992). Pork and chicken meat are significantly lower in CLA than ruminant sources, but turkey meat seems to have an appreciable amount of CLA (Chin et al., 1992). Reasons for the higher levels of CLA in turkey meat are yet unresolved. Plant food sources produce the lowest content of CLA. Sunflower and safflower oil contain 0.4 and 0.7 mg/g fat CLA, respectively, compared with 2.9 mg/g fat CLA in ground beef (Chin et al., 1992). Additionally, cakes, pastries and
potatoes are quite low in CLA concentration (Fritsche et al., 1999).

CLA in Animal Health and Immune Function

Anticarcinogenesis

CLA is by no means a new compound. As early as the 1960s, nutritionists had identified the production of CLA by studying biohydrogenation of linoleic acid in the rumen (Kemp et al., 1975). Pariza et al. (1979) showed that CLA was a molecule which exhibited antimutagenic properties in fried ground beef patties. This discovery led to a large body of evidence that suggested CLA had potent anticarcinogenic potential (Ip, 1994; Belury, 1995). The vast majority of CLA and cancer research has been carried out in rat and mouse models. Early trials utilized a topical dressing containing a mixture of CLA isomers which was applied to 7,12 dimethylbenz(a)anthracene (DMBA) induced skin tumors in mice. The incidence of tumors was decreased by 20% (Belury, 1995). Researchers have studied both the initiation and promotion of skin cancer tumors. Ha et al. (1987) stated that CLA will actually inhibit the initiation of cancer cell growth in skin cells, while Belury et al. (1995) reported the inhibition of cancer cell promotion because of CLA action in mouse skin
tumors. Reports suggested that CLA activated and increased the action of PPARγ, a steroid hormone receptor in mouse skin, which has chemoprotective action in mouse keratinocytes (Kavanaugh et al. 1998; Vanden Heuvel et al., 1998). CLA also activated PPAR-α and -β with an increasing linear response with increasing concentrations of CLA (Moya-Camarena et al., 1998). Additionally, DMBA-induced mammary tumors in rats were reduced in total numbers, multiplicity and weight by 32, 56 and 60% with 0.5, 1 and 1.5% CLA in the diet, respectively (Ip et al., 1991). Park et al. (1998) investigated CLA effects on in vitro growth of MCF-7 human breast cells. Linoleic acid promoted (5-9%) cancer growth while CLA inhibited (11-20%) cell growth in a dose-dependent response. One hypothesis for the mechanistic function of CLA in cancer suppression is the fact that the eicosanoid prostaglandin E₂ (PGE₂) suppresses the immune system in animals (Goodwin and Webb, 1980). Arachadonic acid is the precursor for PGE₂ and has been shown to be significantly decreased in animals fed CLA (Ha et al., 1987; Cook et al., 1993; Li et al., 1998). Thus, overall production of PGE₂ is decreased and the immune system may not be suppressed to the same extent as control diet animals. Park et al. (1998) reported no change in PGE₂ secretion, suggesting that PGE₂ modulation is not the mechanism by which
CLA reduces cancer cell growth. This result was in contrast to reports by Belury et al. (1998) who showed that 1.5% dietary CLA decreased (P < .05) PGE$_2$ production and was proposed to be the mechanism by which CLA depresses cancer cell growth. Other reports showed that PGE$_2$ production was significantly decreased in guinea pigs supplemented with 0.25% CLA for two weeks (Whigham et al., 1998; Whigham et al., 1999). Another study demonstrated that CLA decreased arachadonic acid levels in human prostate cancer cells, but no decrease in cancer cell proliferation was observed (Gallagher et al., 1998). Given these mixed results, it seems that the mechanism by which CLA inhibits cancer cell growth is yet unresolved and may be species dependent.

**Immune modulation**

The ability of CLA to decrease carcinogenesis has an obvious relationship to an animal's immune system response. Several studies have investigated the mechanisms by which CLA modulates the immune system in order for the body to mount a response to physiological challenges (Hayek et al., 1999; Turek et al., 1998 and De Voney et al., 1998). One study utilized 40 young (4 mo) and 40 old (22 mo) mice. The CLA treated animals received 1% CLA in the diet for 8 wk. Immune response was measured by changes in natural killer (NK) cell
proliferation, PGE$_2$ levels and interleukin (IL)-2 production. CLA-fed young mice exhibited significantly higher IL-2 production compared with control diet young mice (P < .05). CLA showed no effect on NK cell activity or PGE$_2$ production in young or old mice (Hayek et al., 1999). In addition, CLA increased T-cell responsiveness in the presence of concanavalin A and phytohemagglutinin, T-cell mitogens, in vitro (Hayek et al., 1999). However, in vivo T-cell function was not enhanced by CLA supplementation. A study using weanling rats supplemented with 1% CLA in the diet resulted in significant reduction of interleukin-6 (IL-6) and basal levels of tumor necrosis factor (TNF) (Turek et al., 1998). In this study, no differences were observed for interleukin-1 production with CLA supplementation. DeVoney et al. (1999) reported that supplementation of 0.5% of a 90% pure CLA oil which was high in the t-10, c-12 isomer, increased lymphocyte proliferation whereas the c-9, t-11 and t-9, c-11 isomers counteracted this increase. A human study with resistance-trained, male subjects showed a trend (P < .07) for CLA reducing the neutrophil/lymphocyte ratio which is a general indication of decreased immune stress (Krieder et al., 1998).
Antiatherogenesis

Other research has focused on the role of CLA in reducing atherogenesis. Fifty male hamsters, approximately eight weeks old, were fed varying levels (0, 0.06, 0.11 and 1.1%) of CLA for 11 weeks (Nicolosi et al., 1997). Plasma lipid analysis from blood collected at 4, 8 and 11 weeks exhibited lower plasma total cholesterol, low density lipoprotein and triglycerides for the CLA diet animals, collectively. Additionally, the CLA-fed hamsters showed less early atherosclerosis in aortic tissue as determined by morphometric analysis (Nicolosi et al., 1997). Another study used 12 rabbits fed a diet containing 14% fat and 0.1% cholesterol. Six of the rabbits were also randomly assigned to a CLA supplementation of 0.5 g/rabbit per day (Lee et al., 1994). By 12 weeks of feeding, the CLA-fed rabbits exhibited markedly lower blood serum LDL cholesterol and triglycerides. CLA-fed rabbits also showed less atherosclerosis upon analysis of their aortas (Lee et al., 1994). Gavino et al. (1998) reported that 1% CLA in the diet decreased (P < .001) total serum cholesterol in Syrian hamsters as early as 2 weeks into the feeding trial. Analysis of aortas at 4 weeks of feeding yielded no differences in fatty streak formation between CLA and control diet animals (Gavino et al., 1998). Using a pig
model, no differences were exhibited for fatty streaks in the aortas of market pigs fed a control or 0.75% CLA diet (Wiegand, Parrish, and Kritchevsky, unpublished data). One possible explanation for the lack of differences because of CLA may be that the pigs were 4-6 months of age and likely were too young to form measurable fatty streaks in their aortic tissue (Kritchevsky, 1999).

If the previous data regarding decreased carcinogenesis, decreased atherogenesis and enhanced immune function with CLA in animal models can be extrapolated to human health, there are obvious reasons for increasing the concentration of CLA in foods for human consumption. Further research on CLA in animal products, such as pork chops and bacon, might result in foods that can protect against life threatening diseases.

**CLA Effects on Growth and Body Composition**

Extensive research has investigated the effects of CLA supplementation on the growth and body composition of laboratory animals, and more recently livestock species. CLA has consistently decreased body fat percentages in rat and mouse models. Eight-week-old mice supplemented with 0.5% CLA in the diet exhibited a 7% decrease in whole body fat and a 1.5% increase in whole body protein accompanied by a 6%
increase in whole body water (Park et al., 1999). The CLA-fed mice showed slightly reduced body weight gain and significantly lower feed intake (P < .001) compared with control-diet mice (Park et al., 1999). In a similar study, weanling mice and 6-wk-old mice were fed a 0.5% CLA diet and exhibited 57% and 60% decreases in body fat and 5% and 14% increases in lean body mass, respectively (Park et al., 1997). Mice fed varying levels (0.25%, 0.50%, 0.75% and 1.0%) of CLA by weight showed no declines in feed intake compared with controls and the 0.75% and 1.0% diet groups exhibited decreased adiposity index scores based on fat pad weights (De Lany et al., 1998). Other researchers have shown similar decreases in body fat percentages in rabbits, rats and chicks (Chin et al., 1994; Cook et al., 1993). Research to investigate the mechanism by which CLA decreases fat percentages in animals is currently underway. Preadipocyte (3T3-L1) cells were radiolabeled and exposed to 0.18, 0.36, 1.78 and 3.57 μmol/L of CLA. CLA treated cells showed a decrease in the proliferation of preadipocytes by 8, 17, 31 and 36%, respectively. However, CLA actually promoted the hypertrophy of mature adipocytes. These data suggest that CLA doesn't change the size of adipocytes, but might prevent the creation of new cells. Brodie et al. (1999) reported that CLA
(25–100 μmol/L) inhibits differentiation and proliferation of 3T3-L1 preadipocytes in a dose-dependent manner, when preadipocyte cells were cultured for 2 to 8 d in vitro.

Dairy cattle have also been used as a model to determine the effects of CLA supplementation (Corl et al., 1999; Griinari et al., 1999 and Baumgard et al., 1999). A study was designed to determine which rumen constituents were responsible for the biohydrogenation of CLA in the rumen (Corl et al., 1999). This study showed that not only was linoleic acid converted to CLA in the rumen, but trans-11, oleic acid was also converted to CLA by way of delta-9 desaturase activity. When delta-9 desaturase was inhibited, the stearic acid to oleic acid ratio increased by 181% and CLA production decreased by 40% (Corl et al., 1999). Another study using forage-fed dairy cows supplemented with 5.3% sunflower oil (Griinari et al., 1999) demonstrated that after 11 days of feeding, the sunflower oil-fed cows produced milk with 2.5% fatty acid content contributed by cis-9, trans-11 CLA. By day 18 of feeding, however, the milk fat percentage had decreased from 3.49% to 1.95% in the high CLA producing cows. Those cows exhibiting the greatest milk fat depression also exhibited the highest levels of trans-10, cis-12 CLA concentration in their milk samples. This relationship has
implicated the trans-10, cis-12 CLA isomer as being responsible for milk fat depression in dairy cows (Griinari et al., 1999). Furthermore, Baumgard et al. (1999) reported that ruminal infusion of trans-10, cis-12 CLA depressed milk fat percentage and milk fat yield compared with cis-9, trans-11 CLA and control diet animals. These authors also hypothesized that the decreases in body fat of animals fed CLA is probably due to this same mechanism (inhibition of delta-9 desaturase) as milk fat reduction in dairy cows.

The results of decreased fat percentage in laboratory animals have been encouraging to the application of CLA in large, livestock-species models. Several experiments have been conducted with growing-finishing pigs (Thiel et al., 1998; Eggert et al., 1999 and Cook et al., 1998). Thiel et al. (1998) assigned diets containing 0, .12, .25, .5 and 1.0% CLA from 26 kg to 116 kg of body weight to forty growing pigs. CLA-diet pigs exhibited 17% less (P < .03) 10th rib fat compared with control-diet pigs. Another study used a 1% CLA diet versus a 1% sunflower oil diet (Eggert et al., 1999a). One hundred sixty crossbred gilts (23 kg) from two genetic populations were fed their respective diets until an average serial harvest weight of 46, 58, 91, 114 or 136 kg was reached. Carcass data were reported for the 91, 114 and 136
kg groups and showed a significant decrease ($P < .05$) in tenth rib fat (1.63 cm vs. 1.85 cm) for CLA and control diet pigs, respectively. Also, the CLA diet pigs exhibited higher ($P < .05$) percent fat free lean (54.8% vs. 53.3%) compared with control diet pigs. Eggert et al. (1999b) also evaluated the supplementation of CLA (1%) with high oil corn for various durations in the finishing phase (90 kg to 115 kg and 65 kg to 115 kg). CLA was thought to be related to decreased 10th rib fat, but did not affect percent fat free lean when fed in conjunction with high oil corn (Eggert et al., 1999b). Cook et al. (1998) reported data from 24 crossbred pigs fed 0, 4.8 or 9.5 g CLA-60/kg diet for 84 days. CLA supplementation resulted in a 24% reduction in backfat depth and increased lean percentage. Data summarized by Pettigrew, (1999) from seven experiments with CLA supplementation to finishing pigs showed a trend toward decreased backfat depth and increased lean percentage.

Reports for lab animals showed increased lean body mass with CLA supplementation. Thus, it has been hypothesized that CLA would increase longissimus muscle area in growing-finishing pigs. Results have been mixed from various studies. Eggert et al. (1999b) reported that CLA seemed to be related to increased loineye area, but three other studies, (Eggert et
al., 1999a; Cook et al., 1998 and Thiel et al., 1998) reported no differences for loineye area with CLA supplementation. If whole body protein is increased in pigs with CLA supplementation, it seems that the increase is not expressed as additional longissimus muscle growth. Thiel et al. (1998) however showed that percent closely trimmed retail yield was increased with CLA supplementation in a pig model.

A few reports show changes in bone metabolism and deposition in rats and pigs (Li et al., 1998; Thiel et al., 1998). Marks et al. (1993) showed that PGE$_2$ is a mediator of bone metabolism and resorption. Raisz et al. (1974) reported that PGE$_2$ at high levels would inhibit bone matrix formation while Monin et al. (1990) showed that a decrease in PGE$_2$ levels would actually stimulate bone formation. Li et al. (1998) showed decreased PGE$_2$ levels in rats with a subsequent increase in bone formation as well as a change in the fatty acid profile of the bone periosteum. Sebidio et al. (1998) have suggested that PGE$_2$ decreases are caused by competitive inhibition of CLA on n-6 fatty acid formation that might lower substrate for cyclooxygenase. Furthermore, Li et al. (1998) speculated that 18:1 and 18:2 fatty acids were decreased in bone tissue due to an inhibition of liver, delta-9 desaturase activity by CLA. CLA supplementation resulted in an increase
in 12:0, 14:0 and 16:0 fatty acid levels in bone periosteum supporting the hypothesis that delta-9 desaturase activity is inhibited by CLA.

The impact of CLA on growth and carcass characteristics is becoming relatively well defined, barring a few mixed results. These mixed results may be explained by genetic differences in pig populations with various genetic lines expressing carcass traits at different levels. These previous data show that CLA has the ability to increase growth rate and change the body composition of pigs.

Meat quality, which is ultimately important to the consumer, might also be affected by CLA supplementation and is yet to be explored in any great detail in swine. A basic review of factors affecting meat quality is likely a reasonable place to start to understand how feeding CLA may benefit pork meat quality. Meat quality can be defined as a combination of traits that include appearance, taste, nutritional value and wholesomeness of meat (NPPC, 1992). Meat color, water holding capacity, and lipid oxidation are three factors, that have direct impact on meat quality and consumer acceptance of meat products. It is also important to understand how postmortem pH decline and ultimate pH in the muscle can impact color and water holding capacity.
Chemical States and Properties of Meat Color

Color in fresh meat is primarily because of the chemical properties of myoglobin. Myoglobin is a water soluble, sarcoplasmic muscle protein that is physiologically responsible for the storage of oxygen in the cells of the living animal, and is the color pigment of skeletal muscle. Myoglobin constitutes 95% of muscle pigment in well-bled animal carcasses (Francis and Clydesdale, 1975).

The structural characteristics of myoglobin consist of an iron-porphyrin ring with a heme structure in the center and an attached globin protein (Kagen et al., 1973). The heme group function is to bind oxygen within the muscle. Myoglobin in living tissue is in equilibrium in reduced myoglobin, which is purple, and oxymyoglobin, which is bright red. Both of these forms contain iron in the ferrous state (Fe²⁺) (Hunt, 1991).

In meat, myoglobin is present in one of three chemical states, which are dynamic in nature (Figure 2). When the meat surface is initially cut, myoglobin is in the reduced state, deoxymyoglobin. When oxygen contacts the exposed meat surface, it binds the sixth coordination site on myoglobin causing the meat to "bloom" and form oxymyoglobin. In this reaction, iron is maintained in the ferrous state. If oxygen is removed from meat, such as in vacuum packaging, the
oxymyoglobin converts back to deoxymyoglobin. A third form of myoglobin, which involves oxidation of iron from the ferrous (Fe^{2+}) to the ferric (Fe^{3+}) state is termed metmyoglobin and assumes a brownish-red color. Metmyoglobin is commonly found in meat products, which have been in the retail shelf for extended periods of time and in contact with atmospheric conditions, mainly oxygen. This brown color usually occurs when at least 60% of myoglobin is converted to metmyoglobin (Hunt, 1980). It is possible to reduce metmyoglobin to oxymyoglobin through the activity of metmyoglobin reductase (Renerre, 1999). Likewise, if metmyoglobin reductase is coupled with oxygen removal, it can be converted to the deoxymyoglobin state. During meat storage, the reducing activity of these reactions can be greatly depleted. This is likely due to low pH conditions that interrupts enzyme activity. There may also be losses of substrate and cofactors required to drive the reactions (Renerre, 1999). Often, this conversion from oxymyoglobin to metmyoglobin is considered undesirable by meat consumers. The concentration of myoglobin in domestic livestock species is quite variable with beef having the highest concentration, 4-10 mg/g wet tissue, followed by lamb and pork at 4-8 mg/g and .5-3 mg/g of wet tissue, respectively (Romans et al., 1995). These
concentrations correspond to the darker red color observed in beef compared with lamb and the pinkish-gray color of pork compared with lamb and beef. Color is a critical marketing tool for pork products and influences purchases by consumers. Consumers tend to select pork of "normal" color (grayish to reddish pink) compared with pale, soft and exudative (PSE) and dark, firm and dry (DFD) products (Frye, 1999). PSE pork has many causes, but a major factor is a combination of carcass heat and low pH which enhances autoxidation of myoglobin and can lead to color fading and even water loss (Renerre, 1999).

Measurement of meat color has evolved from visual, subjective methods to computer-driven objective methods (Hunt, 1991). Subjective visual classification of fresh meat has been used extensively. With respect to pork, a five point descriptive color scale is commonly used to evaluate color of the loin muscle (NPPC, 1991). More recently, this scale has been expanded in an effort to discern color differences with a greater degree of accuracy (NPPC, 1999b). Furthermore, visual appraisal of fresh whole muscle, fresh ground, and cooked meat by trained sensory panels has proven effective for classifying meat into comprehensive color classes (AMSA, 1978). One problem with human evaluation of color is the day-to-day emotional or mental state of the evaluator and variation
between evaluators. Furthermore, different environmental conditions can change the color properties of a meat sample. These disadvantages led to the development of objective instrumentation, which could consistently measure meat color with minimal degrees of variation (Kropf et al., 1984).

Probably the most common methods of quantifying color today are with instrumental reflectance methodology. Hunter lab and CIE values attempt to quantify color based on hue, chroma and intensity and assign L*, a* and b* values on a numerical scale (Hunt, 1991). L* values quantify the lightness or darkness of a product and range from 0 (black) to 100 (white). Redness and greenness of meat is quantified with a* values and occur as red if the value is > 0 and green if the value is < 0. Yellowness and blueness are quantified with b* values and occur as yellow if values are > 0 and blue if the value is < 0 (Hunter lab, 1996). In addition to these L*, a* and b* values, reflectance methods also allow the researcher to estimate what percentage of each of the three states of myoglobin are present on the surface of a meat product. This methodology gives K (absorption coefficient) / S (scatter coefficient) values which are quantification of reflectance values at known isobestic points for myoglobin across the visual spectrum (Hunt, 1980). Spectral wavelengths of
interest are 474, 525, 572, and 610 nm, which represent isobestic points for oxy- and metmyoglobin, oxy-, met- and deoxymyoglobin, deoxy- and oxymyoglobin, and deoxy- and metmyoglobin, respectively. Knowing the reflectance at these isobestic points allows one to quantify one the three states because the other two states will give the same reflectance value at a given spectral wavelength (Hunt, 1991). The measurement of meat color has been used as a sorting tool to determine which products may go to export markets or to further processed products (Frye, 1999).

Water Holding Capacity

A comprehensive discussion of water-holding capacity is certainly beyond the scope of this paper. However, we should realize the relationship between water holding capacity, pH and color formation in meat. Hamm, (1960) stated that water holding capacity of muscle effects the quality of meat during almost all processing operations after slaughter-transport, storage, aging, grinding, salting, curing, heating, freezing, thawing, drying.

Water holding capacity is defined as the ability of meat to retain the natural water within the muscle fiber matrix (Hamm, 1960). This aspect of meat quality is important for
two reasons. Water is the largest component, 75%, of meat and makes up a majority of the weight that is marketed at the grocery store (Offer and Trinick, 1983). Additionally, water, in combination with intramuscular fat, is highly correlated to meat juiciness. Therefore, water is critical to consumer satisfaction of pork. A loss of water in postmortem, fresh pork is one factor, which causes PSE pork (Offer and Trinick, 1983).

The main structural component of meat is the myofibril. The myofibril and its associated water accounts for approximately 70% of meat volume of which 30% is protein (Offer and Trinick, 1983). The balance of this volume is accounted for mainly by water (Hamm, 1960). The majority of water in meat is located between the actin and myosin filaments. The interfilament space can change in size according to pH, sarcomere length, ionic strength, osmotic pressure and whether the muscle is relaxed or contracted (Offer and Trinick, 1983). This interfilament space can swell or shrink according to pH (Bendall and Swatland, 1989). A low pH causes the interfilament space to shrink, thus leaving less space for water to be held. Conversely, the addition of salts, especially NaCl, will cause an interfilament swelling and allow for maximal water uptake in most muscle foods (Hamm,
1960). Offer and Trinick (1983) confirmed what Hamm (1960) had hypothesized when they concluded that the myofibril can swell to over twice its normal size in the presence of salt. This swelling was directly related to the increased water uptake in the meat fiber matrix, thus showing that the myofibril is the site of water holding and water uptake in meat systems. Knowing where water is held in the muscle can give us direct insight to the causes of water loss and subsequently a decline in meat quality. We know that water loss in PSE pork is related to undesirable eating characteristics in pork, namely decreased tenderness and juiciness (Bendall and Swatland, 1989). This water loss is likely caused by denaturation of the myofibrillar proteins due to low pH and high temperature conditions in postmortem glycolysis of muscle (Renerre, 1999). These postmortem muscle conditions are especially evident in pigs with the Halothane gene (Leach et al., 1996) or the Napole effect (McKeith et al., 1998) but can occur in pork from "normal" genotype pigs because of rough pre-slaughter handling or inadequate postmortem chilling (Grandin, 1997; D'Souza et al., 1998). In relationship to color, one might hypothesize that an increase in water loss would also change the reflectance properties of
a meat surface, thus greatly influencing both subjective and objective measurement of meat color.

**Lipid Oxidation and Fresh Meat Flavor**

Flavor is critical to consumer acceptance of fresh pork products and can be strongly influenced by a wide array of factors (Jeremiah et al., 1990). Rapid oxidative processes can cause problems in consumer acceptance of cooked meat products and has been associated with warmed-over flavor (WOF) (Pearson et al., 1983). Warmed over flavor is a general term describing the off-flavors produced in cooked and reheated meat products. Lipid peroxidation can be defined as a free radical chain reaction that proceeds stepwise through initiation, propagation, branching and termination (Kanner, 1994). Free radical formation is a self-propagating system and after one free radical is formed, it is difficult to stop the reactions of oxidation (McMillin, 1996). Hydrogen peroxide (H$_2$O$_2$), hydroxyl radical (HO•), ferryl (Fe$^{IV}$), and free metal ions can function as prooxidants in meat systems (Kanner, 1994). Lipid oxidation in meat is a major cause of muscle food deterioration (Melton, 1983). The reactions of lipid oxidation eventually end with the termination step, but tissue damage has already occurred and meat quality has
already been negatively affected (Kanner, 1994). Both phospholipids and triacylglycerols are components of lipids, which are responsible for formation of off-flavors in meat (Pearson et al., 1983). Meat products that are high in unsaturated fatty acids tend to be more prone to lipid oxidation compared with products with high levels of saturated fatty acids (Melton, 1983). This is because double bonds in unsaturated fatty acids tend to be less stable and will more readily give up H' ions to prooxidants (McMillin, 1996). Li and Watkins (1998) reported a change in fatty acid profile of bone tissue in rats from an unsaturated to a saturated fatty acid composition. CLA might also change the fatty acid profile in muscle tissue from supplemented animals. One could hypothesize that fresh meat products from CLA-supplemented animals could be less susceptible to oxidation because of a greater amount of saturated fats. In these saturated fats, there would be no double bonds available for free radical formation resulting in less oxidation. Furthermore, there may be fewer short chain fatty acids contributing to volatile compounds, which cause warmed-over flavor.

Lipid oxidation can be measured by several methods which include determination of peroxide value (PV) and the thiobarbituric acid (TBA) test (Melton, 1983). The PV method
utilizes a iodometric technique (AOCS, 1990) and reports values as meq of iodine/kg of fat. The TBA method is commonly used in determining lipid oxidation in muscle foods by measuring volatile compounds, especially malonaldehyde, produced from the oxidation of lipids and isolated by a distillation procedure (Tarladgis et al., 1960). Reports show that trained sensory panelists can detect 0.5 mg malonaldehyde/kg meat and untrained panelists can detect 1.0 mg malonaldehyde/kg meat making TBA levels a valuable measure of off-flavor in cooked meat products (Green and Cumuze, 1981). At these levels, off-flavors can develop.

A number of strategies have been proposed and reported to increase oxidative stability in muscle foods (Decker and Mei, 1996; Gray et al., 1996; Pearson et al., 1983). Gray et al. (1996) reported that supplementation of Vitamin E (α-tocopherol) in the diet at supranutritional levels will result in increased lipid stability in meat products from poultry, beef cattle, veal calves and pigs. Vitamin E functions as a naturally occurring free-radical scavenger in biological systems (Decker and Mei, 1996).

Decker and Mei (1996) also reported that ascorbate can maintain myoglobin in the reduced state by inactivating free radicals and regenerating α-tocopherol in meat systems.
Ascorbate at low levels can also act, however, as a prooxidant by reducing iron to its ferrous state. Thus, ascorbate is variable in its function as an antioxidant.

Furthermore, chelating agents might be added to meat systems to bind to free metals which might act as prooxidants and cause free radical production (Pearson et al., 1983). One such chelator is EDTA, which has the ability to bind iron into a ring structure and thus removing it from participation in the oxidation reaction (Pearson et al., 1983).

Given this information on lipid oxidation problems and its mechanism of action, it seems possible that any compound or molecule that could conserve natural antioxidants or participate as an antioxidant would prove useful in fresh meat systems.

**Effects of pH on Postmortem Meat Quality**

Measuring pH decline and ultimate pH have been useful methods for predicting meat quality in fresh pork and both have visible effects on color formation and water holding capacity of meat. The rate of decline of pH measures how quickly lactic acid is formed through glycolysis, usually between 0 and 24 hr in the postmortem muscle. A rapid decline in pH has been implicated in the promotion of PSE pork,
because of water losses caused by acidic conditions that can
denature muscle proteins and impede their water holding
ability and color formation (Bendall and Swatland, 1989). The
relationship between PSE and pH was established initially in
the late 1950s when it was determined that PSE pork was the
product of a two to four times faster pH decline in postmortem
pork carcasses compared with normal (Bendall and Swatland,
1989). PSE has been shown to be a common occurrence in pigs
with the porcine stress syndrome (PSS). Porcine stress
syndrome is caused by a defect in a gene known as the
halothane gene (Leach et al., 1996). Pigs with this condition
are easily excitable and exhibit more rapid postmortem pH
decline (Fisher et al., 2000; Louis et al., 1993). In
general, halothane positive pigs are leaner and produce more
muscle, but their propensity toward meat quality problems has
precluded their use in most United States breeding systems.

Another genetic condition that is reportedly the cause of
PSE is because of low ultimate pH ($pH_u$) (Monin and Sellier,
1985) is the Napole effect. This condition, known as the
Napole or Hampshire effect, is PSE resulting from normal pH
decline, but an abnormally low ultimate pH (McKeith et al.,
1998). This low $pH_u$ has been linked to high glycolytic
potential (GP) in the muscle prior to death. This high GP is
associated with the Rendement Napole (RN) gene and was initially identified in lines of pigs with Hampshire ancestry (Lundstrom et al., 1996).

Pre-harvest handling can have profound effects on pH decline (Berg, 1998). Pigs exposed to less stress prior to death will not exhibit as great a body temperature increase, thus decreasing the negative conditions in muscle at death. On the farm handling of pigs can prove useful in maintaining pork quality, because pigs which are less excitable and adjusted to loud noises are less likely to develop PSE pork postmortem (Grandin, 1997). Also, pigs that are allowed to rest after transport to the packing plant and prior to stunning are also less likely to produce PSE pork (Grandin, 1997). Pigs that are negatively moved, electrically prodded, in the packing plant have more instances of PSE compared with pigs moved without electric prods (D'Souza et al., 1998). This increase in PSE is likely due to body temperature rises in the excited animal which, in association with pH decline postmortem, can cause denaturation of muscle proteins, subsequently causing water loss of meat (Offer and Trinick, 1983).

Both chilling method and time from stunning to chilling are important factors in the maintenance of desirable pork
quality attributes. As mentioned previously, the pork carcass exhibits a temperature rise directly after exsanguination. The act of bleeding the animal removes the "radiator" or cooling system of the carcass and the subsequent glycolysis creates heat within the muscles. This rise in temperature in conjunction with the decline in pH can cause denaturation of muscle proteins and a loss of water and pale color (D'Souza et. al, 1998). To avoid these detrimental quality aspects, it seems critical to decrease the time from stunning to chilling in order to minimize heat production in the muscle. D'Souza et. al (1998), subjected pork carcasses to a 45 min (normal) and 70 min (delayed) processing time from stunning to chilling. Carcasses with the delayed processing exhibited lower (P< .05) muscle glycogen levels and paler meat compared with 45 min (normal) processed pigs.

In addition to reduced processing time, the method of chilling has been investigated in terms of enhancing meat quality attributes. Frye et. al (1985), compared boneless pork loins rapid chilled in a brine (bc), carbon dioxide cabinet (cc) and carbon dioxide snow (cs) at 1, 3 and 5 hours of processing delay from hot boning to chilling. No differences were observed for pH post-chilling, but loins chilled at 1 h post-stunning maintained a higher muscle pH
compared with the 3 and 5 h delay times. However, this ultra-
quick chilling resulted in a product with higher shear force
values, caused by a cold shortening of the pork loin and a
subsequent toughening of the meat. This study suggests that
rapid chilling methods should be used on whole carcasses
showed data for pork quality of carcasses subjected to one of
the three chilling methods, which included a blast chill,
conventional chill and brine chilling. Blast chilling was for
3 h at -34° C, then 21 h at 2° C. Conventional chilling was 24
h at 2° C and brine chilling was 3 h under a -5° C brine shower
and 21 h at 2° C. A more rapid chilling, blast or brine,
resulted in increased muscle color in the ham, but had no
effect on the loin color. The blast method resulted in
increased firmness for all cuts; loin, leg and shoulder
compared with conventional and brine chilling.

This review of meat quality attributes demonstrates the
complex chemistry involved in the conversion of muscle to
meat. However, it also demonstrates that it might be possible
to change this chemistry to improve meat quality and
ultimately improve consumer acceptance of fresh meat products.
For pork meat production to be profitable at each step,
producers and processors must take advantage of increased
growth, performance and meat quality in concert. While it is likely that there is no "silver bullet" for producing the perfect pig for high quality, healthful pork, it seems that a nutritional supplement such as CLA has the potential to optimize various aspects of swine production.

References


Table 1. Concentration of CLA (%) in common foods.

<table>
<thead>
<tr>
<th>Foodstuff</th>
<th>Total CLA (mg/g fat)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground Beef</td>
<td>4.3</td>
</tr>
<tr>
<td>Lamb</td>
<td>5.6</td>
</tr>
<tr>
<td>Pork</td>
<td>0.6</td>
</tr>
<tr>
<td>Chicken</td>
<td>0.9</td>
</tr>
<tr>
<td>Egg Yolk</td>
<td>0.6</td>
</tr>
<tr>
<td>Turkey</td>
<td>2.5</td>
</tr>
<tr>
<td>Medium Cheddar Cheese</td>
<td>4.1</td>
</tr>
<tr>
<td>Mozzarella Cheese</td>
<td>4.9</td>
</tr>
<tr>
<td>Homogenized Milk</td>
<td>5.5</td>
</tr>
<tr>
<td>Butter</td>
<td>4.7</td>
</tr>
<tr>
<td>Safflower Oil</td>
<td>0.7</td>
</tr>
<tr>
<td>Sunflower Oil</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Adapted from Chin et al., 1992.
Linoleic Acid  
(cis-9, cis-12 octadecadienoic acid)  
\[ \downarrow \text{isomerization} \]  
CLA (conjugated linoleic acid)  
(cis-9, trans-11 octadecadienoic acid)  
\[ \downarrow \text{hydrogenation} \]  
Oleic Acid  
(trans-11 octadecenoic acid)  
\[ \downarrow \text{hydrogenation} \]  
Stearic acid  
(octadecanoic acid)

Figure 1. Pathway of biohydrogenation of linoleic acid to stearic acid by rumen microbes (From Kelly et al., 1998).
Figure 2. Flow chart for dynamic nature of states of myoglobin in fresh meat systems (Adapted from Hunt, 1991).
CHAPTER 2. CONJUGATED LINOLEIC ACID INCREASES MARBLING AND FIRMNESS SCORES AND CHANGES FATTY ACID PROFILE OF LOINS FROM GROWING-FINISHING SWINE

A paper to be submitted to the Journal of Animal Science

B. R. Wiegand, F. C. Parrish, Jr., J. C. Sparks and D. R. Zimmerman

Abstract

Conjugated linoleic acid (CLA), a collective term for geometric and positional isomers of linoleic acid, was supplemented to growing-finishing barrows (n = 92) at 0.75% of the diet. Carcass, meat quality, physical, chemical and sensory data were collected and analyzed. CLA was supplemented over different body weight gains and included a control 0 kg (T1) and CLA at 28, 57 and 86 kg (T2, T3 and T4, respectively). Subjective quality measures increased linearly for marbling (P < 0.03) and firmness (P < 0.07) with increasing time on CLA. No differences were observed for subjective color. Objective Hunter color scores were not different for L* or a* values, but were higher (P < 0.05) for b* values with CLA supplementation. No differences were observed for 24-h pH. Lipid oxidation values (TBA) were lower (P < 0.05) for CLA supplemented pigs when T1 (control) and T4
(longest fed) diets were compared. Fatty acid analysis showed a linear increase \((P < 0.0001)\) for saturated fatty acids and CLA isomers in loin tissue and a linear increase \((P < 0.01)\) for saturated fatty acids and CLA isomers in subcutaneous fat tissue. Sensory panel characteristics of loin chops were not different from controls. Improved meat quality characteristics of marbling and firmness with CLA supplementation might result in increase premiums in a value-based pricing system.

Keywords: conjugated linoleic acid, pork quality

**Introduction**

Much research has been done to measure pork composition and produce more muscle per carcass. Some fast growing lean animals, however, have a propensity toward pork quality problems (e.g. pale, soft, exudative pork). Current research is focusing on maintaining the genetic growth potential of pigs without compromising pork quality. One approach to improving pork meat quality is the supplementation of naturally occurring feed additives, like conjugated linoleic acid (CLA), in the growing-finishing diet. CLA consists of positional and geometric isomers of linoleic acid, an 18:2
fatty acid. The c-9, t-11 isomer was thought to be the biologically active form of CLA because it was the only isomer found in the phospholipid portion of tissue (Ha et al., 1990). More recently, Park et al., (1999), reported increased muscle and decreased fat in rats with supplementation of the t-10, c-12 isomers. CLA has been shown to improve growth rates of rats as well as serve as an anticarcinogenic compound when tumors were introduced into rats (Ip et al., 1991). Additionally, CLA has been hypothesized to have an antioxidant effect (Ha et al., 1990). The autooxidation of CLA forms furan fatty acids which can protect cells against peroxide attack (Yurawecz, 1996). However, other research has disputed the antioxidant capabilities of CLA (Banni et al., 1998). The possibility of an antioxidant effect with CLA prompted us to examine the effects CLA might have on fatty acid oxidation and color stability parameters of pork loins. The supplementation of CLA in growing-finishing barrows has been shown to improve growth and carcass characteristics (Cook et al., 1998; Thiel et al., 1998). Our hypothesis tested in this study was that CLA at a constant level in the growing-finishing diet would increase color, flavor, shelf life, and decrease lipid oxidation in pork chops from supplemented pigs. The objectives for this study were to measure growth, carcass and
meat quality characteristics of growing-finishing pigs fed CLA at 0.75% of the diet for different body weight gains.

Materials and Methods

Animals. This project was carried out in accordance with Iowa State University Animal Care and Use Committee Guidelines. Ninety-two Yorkshire x Landrace x Duroc x Hampshire barrows were randomly assigned by litter to four treatment groups with five replications per treatment. Pigs were housed in a total confinement, slatted floor facility in 20 adjacent pens. Treatment groups were based on weight gain on CLA diet and included a control diet at 0 kg (T1) and CLA supplementation at 28, 57 and 86 kg of body weight gain (T2, T3, and T4, respectively). CLA-60 isomers (Conlinco, Inc. Detroit Lakes, MN) were in an oil form and replaced soy oil in the treated diets. Treated diets contained 1.25% of the source or 0.75% of CLA isomers. Diet composition is presented in Table 1. All pigs remained on their respective diet until 115 kg of body weight at which time they were humanely slaughtered at Hormel, Inc. in Austin, MN. Due to different growth rates, pigs were slaughtered in two groups at 30 days apart. Carcasses were chilled 24 h postmortem before carcass measurements were taken. The left side of each carcass was
ribbed between the 10th and 11th rib and loineye area, 10th rib, 1st rib and last rib fat were measured.

*Procedures and Analysis.* At 24 h postmortem, whole bone-in loins were removed from the left side of each carcass. Loins (IMPS 410) were weighed, boxed and shipped under refrigerated conditions to the Iowa State University meat laboratory. At 48 h postmortem, whole loins were subjectively evaluated for color, marbling and firmness at the 10th and 11th rib face according to a five-point descriptive scale (NPPC, 1991). All T1 and T4 loins were cut into 2.54 cm chops. Chops were deboned and trimmed to 0.62 cm of subcutaneous fat. Chops were placed in Viskase® vacuum bags (n = 2 chops/package). The vacuum packages were assigned to 1, 14 or 28 days of retail storage. Packages were boxed and stored for their respective time at 2°C. Three 1.27 cm chops were also cut from each loin for measurement of pH, water holding capacity, lipid oxidation (TBA) and proximate analysis. These chops were stored in vacuum under the same conditions as were the 2.54 cm chops.

Lipid content was determined by hexane extraction on dried sample using a Soxhlet method (AOAC, 1990). Moisture was determined as the weight difference on samples after 18 h in an 80°C drying oven.
At the appropriate day of storage, chops were removed from vacuum and placed on styrofoam trays and wrapped with oxygen-permeable polyvinyl chloride film. Chops were placed in the retail self-service case (4°C) for 24 h after being repackaged. After the 24 h period, objective colors were measured by using the Hunter Lab Color system (Hunter Associates Laboratory, Inc. Reston, VA) with a 2.54 cm objective and a 10° light source. \( L^* \), \( a^* \) and \( b^* \) values were measured at the 1, 14 and 28 day storage times.

WHC was measured by using the Carver Press Method on 1, 14, and 28 d of storage time. This method uses a 0.3-g sample, which is pressed at 3000 psi for 3 min on 125 mm diameter filter paper (Kauffman et al., 1986). The areas of the pressed sample and expressed moisture were traced on the filter paper. The areas of these tracings were determined with a planometer (Model K & E 4236, Keuffel-Esser Co., Germany). A ratio of water to meat areas was calculated giving a measure of WHC.

A pH measurement was also made on each package of chops at all three days (1, 14 and 28) of storage with a Fisher Accumet 925 pH meter. The pH method used 10 g of homogenized muscle in 90 ml of distilled water. Duplicate pH samples were used for each chop.
Lipid oxidation was measured at 1, 14, and 28 d of storage by using thiobarbituric acid (TBA) and a distillation apparatus (Tarladgis et al., 1960). Malonaldehyde, a product of lipid oxidation, was measured with a Beckman DU 640 spectrophotometer (Fullerton, CA) at a wavelength of 532 nm.

Fatty acid profile of subcutaneous fat and loin muscle samples from the 10th and 11th rib junction was determined with gas chromatography. Lipids were extracted from the respective samples using the Folch extraction method (AOAC, 1990). This method used a 0.5 g sample which was homogenized with a Polytron homogenizer in 10 ml chloroform:methanol at 2:1 (v/v) (Folch 1) and 25 μl butylated hydroxy toluene (BHT) (Aldrich Chemical Co.). The homogenized sample was placed in 50 ml tubes with teflon-lined caps and incubated for 2 h at 4°C. Samples were then filtered through Whatman #1 paper into a 100 ml graduated cylinder. The sample tube and paper were rinsed with Folch 1. Twenty-five percent volume (of sample solution) of 0.8% NaCl was added to each cylinder and shaken 10 times to mix the solution. The solution was allowed to phase separate (2-4 h) and the lipid layer (top) was removed by pipette. Samples (10 ml) were pipette transferred to scintillation vials. Vials were placed on a 50°C hot plate
to evaporate the chloroform under a stream of nitrogen gas. The concentrated sample and vial were weighed.

Fatty acids were prepared for gas chromatography determination by using fatty acid methylation with sodium methoxide. One ml of hexane was added to each of the scintillation vials from the Folch extraction. Then 2 ml of sodium methoxide was added to each vial and the vials were vortexed at low speed. Vials were incubated in a heat block at 50° C for 10 min. Five ml of deionized water and 0.1 ml of glacial acetic acid was added to each vial. Lipids were extracted with two successive washings of 3 ml of hexane per vial. Two g of anhydrous sodium sulfate was added to each vial to remove any residual water. One ml of the fatty acid methyl esters (FAME) were transferred to gas chromatography vials and stored at 4° C until loading on the gas chromatograph (GC).

All FAME were analyzed with a Varian 3350 (Varian Chromatography Systems, Walnut Creek, CA). FAME were identified by comparison to their retention times with authentic standards (Nu-Chek-Prep, Elysian, MN). The GC was fitted with a 8200cx autosampler and a 60 m x 0.317 mm column (J & W Scientific, Folsom, CA). One µl of sample was injected
onto the column at an injector temperature of 250° C and a detector temperature of 220° C.

Sensory characteristics were evaluated for the T1 and T4 chops. Chops were cooked in a broiler set at 176° C to 71°C internally, and cut into 2.54 cm cubes for sensory evaluation. A panel consisting of 10 human subjects evaluated day 28 chops for tenderness, flavor, juiciness, off-flavor and pork flavor according to an 8-point descriptive scale (AMSA, 1995).

Statistical analysis. Statistical analysis was performed with the GLM procedure of SAS (1990). The statistical model included fixed effects of treatment, replication and day of storage time when appropriate. The model for sensory analysis included fixed effects of treatment, replication, day of panel and panelist. Contrasts were used to determine linear and quadratic relationships for means with regard to treatment group. Data are presented as least squares means with standard errors attached. Means were considered statistically different at P < 0.05. Furthermore, the correlation procedure of SAS (1990) was used to determine the correlation between subjective marbling and firmness scores.
Results and Discussion

Least squares means for subjective quality measures of color, marbling and firmness are shown in Table 2. Linear treatment differences were observed for marbling (P < 0.03), where marbling scores increased with longer feeding time on CLA. Proximate analysis verified these marbling scores, with hexane extractable lipid increasing with increasing time on CLA. Additionally, a linear trend (P < 0.07) was observed for firmness values, where firmness tended to increase with longer feeding time on CLA. Cook et al. (1998) reported no changes in marbling or firmness scores due to CLA supplementation to pigs. In general, fat from CLA-supplemented pigs was firmer than fat from control diet pigs as determined by visual observation. This could be due to an increase in saturated fat in the CLA supplemented-pigs (Eggert et al., 1999). This increase in saturated fat and total intramuscular fat could be an explanation for the increased subjective firmness scores. To test this hypothesis, a correlation was determined between marbling and firmness scores. The correlation was high (0.93), indicating a strong relationship between subjective marbling and firmness scores. No differences were observed for subjective color values between carcasses from control and CLA-diet animals (P = 0.95). Cook et al. (1998) reported
increases, however, in subjective color scores. Subjective values for color were objectively measured with the Hunter Color system (Table 3). Due to processing logistics, only loin chops from treatments 1 (control) and 4 (longest fed) were further measured for self-service shelf stability. When day of retail storage was included in the model, no statistical differences were observed between treatments at each day (1, 14 and 28). Day was removed from the model and the T1 and T4 groups were compared. Chops from CLA supplemented pigs had higher b* values ($P < 0.05$) compared with control chops. This higher b* value corresponds to a more yellow product which is a deviation from the desirable gray color of pork. The higher b* values may be the result of increased marbling in the loin face due to CLA supplementation. Furthermore, CLA chops tended to have higher $L^*$ and $a^*$ values compared with control chops. These values correspond to lighter and redder products, respectively. Thiel et al., 1998 reported increases in $a^*$ values with increasing levels, from 0.12% to 1.0% in the diet suggesting that dietary CLA may protect meat color.

Values for pH, water holding capacity and lipid oxidation (TBA) are presented in Table 4. Least squares means for pH were not different for treatment groups at 1, 14 and 28 days
of retail storage. Means ranged from 5.70 and 5.80 indicating acceptable pork quality was observed for control and CLA carcasses. These pH values were consistent those in the literature that indicated acceptable pork quality (Bendall and Swatland, 1989).

Water holding capacity, the ability of meat to bind water, is related to juiciness and cooking attributes of pork (Hamm, 1960). WHC ratio values, a smaller ratio indicating a higher WHC, do not differ between treatments, but tend to decrease over time of retail storage. The reason for improved WHC over time of storage is yet unresolved.

Results shown in Table 4 indicate that lipid oxidation, based on TBA values, was lower (P < 0.05) for 1 d chops from CLA supplemented pigs compared with chops from control pigs. TBA values were not different for 14 d and 28 d chops when T1 (control) and T4 (longest fed) diets were compared. These TBA values were well below 0.5 to 1.0 mg malonaldehyde/kg of tissue, a value that might indicate an unacceptable product (Tarladgis et al., 1960; Greene and Cumuze, 1981). Arachadonic acid is decreased by CLA supplementation (Cook et al., 1993) and arachadonic acid is one of the unsaturated fatty acids that contribute to malonaldehyde production and TBA values (Greene and Cumuze, 1981). The low TBA values in
loin samples from CLA-fed pigs at 1 d may be because of a decrease in arachadonic acid and an increase in saturated fatty acids in the loin tissue samples from CLA-fed pigs (Table 6). This is significant because arachadonic acid (20:4) is more prone to lipid oxidation compared with saturated fatty acids because the double bonds in its structure are subject to free radical attack. Thus, the TBA test may have only detected small levels of volatiles from lipid oxidation.

Fatty acid profile of loin muscle tissue at the 10th and 11th rib and subcutaneous fat samples are shown in Tables 6 and 7. The GC analysis of fatty acids showed a linear decrease (P < 0.0008) in polyunsaturated fatty acids (PUFA), a linear increase (P < 0.0001) in saturated fatty acids (SFA) and a linear increase (P < 0.01) in total CLA isomers. These data verify previous reports with regard to fatty acid profile changes in loin muscle because of CLA supplementation (Eggert et al., 1998). Fatty acid analysis of subcutaneous fat samples from the 10th and 11th rib junction showed a linear increase (P < 0.0001) in SFA and a linear increase (P < 0.0001) in total CLA isomers. One might hypothesize that the change from unsaturated to saturated fatty acids could be because of a decrease in delta-9 desaturase activity (Baumgard
et al., 1999; Li et al., 1998). The incorporation of CLA isomers into muscle and fat tissue may prove positive in terms of making pork a "functional food" with respect to CLA intake and potential decreases in cancer and heart disease.

Sensory panel results are summarized in Figure 1. No significant differences were observed between control and CLA loin chops, when based on an eight-point descriptive scale. These results indicate that supplementation of CLA to pigs does not significantly affect sensory characteristics of pork loin chops.

**Conclusions**

Loins from all pigs had acceptable pork quality, as indicated by the pH and TBA values, accompanied by Hunter a* color values that tended to increase with CLA supplementation. CLA chops had higher degrees of marbling and firmness scores. Furthermore, fatty acid profiles of loin tissue and subcutaneous fat samples were changed to a more saturated profile with CLA supplementation. Consequently, certain aspects of pork quality can be improved by using dietary CLA at 0.75% in the growing-finishing diet. However, the impact of changes in fatty acid profile with CLA supplementation is yet to be seen.
Implications

Improvements in loin marbling and firmness could lead to increased premiums or decreased price deductions for producers selling in a value-based system. Other research should be done to identify mechanistic control of CLA responses of pigs in the growing-finishing phase.

References


Table 1. Diet composition and calculated analysis at different body weights.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>28 to 57</th>
<th>57 to 86</th>
<th>86 to 115</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>68.76</td>
<td>83.47</td>
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<td>Soybean meal</td>
<td>27.38</td>
<td>12.99</td>
<td>11.76</td>
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<tr>
<td>Dicalcium phosphate</td>
<td>1.24</td>
<td>.82</td>
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<td>Calcium carbonate</td>
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<tr>
<td>Vitamin premix(^a)</td>
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<tr>
<td>Trace mineral premix(^b)</td>
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<td>Tylan 40</td>
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<td>Lysine·HCl</td>
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<td>.09</td>
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<td>Oil(^c)</td>
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Calculated analysis:

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<th>28 to 57</th>
<th>57 to 86</th>
<th>86 to 115</th>
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<tr>
<td>ME, kcal/kg</td>
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<td>3382</td>
<td>3395</td>
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<tr>
<td>Lysine, %</td>
<td>1.00</td>
<td>.73</td>
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<tr>
<td>Calcium, %</td>
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<td>.58</td>
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</tr>
<tr>
<td>Phosphorus, %</td>
<td>.60</td>
<td>.47</td>
<td>.42</td>
</tr>
</tbody>
</table>

\(^a\) At .2% of diet contributes per kilogram of diet: 4,400 IU vitamin A; 1,100 IU vitamin D; 6.6 mg riboflavin; 17.6 mg pantothenic acid; 33 mg niacin; 22 µg vitamin B12.

\(^b\) At .05% of the diet contributes in ppm: 75 Zn, 87.5 Fe, 30 Mn, 8.75 Cu, 0.1 I.

\(^c\) Soybean oil or CLA-60 oil in their respective treatment.
Table 2. Least squares means\(^a\) and standard errors for subjective scores\(^b\) of loin color, marbling, firmness and percentage lipid at differing weight gain on CLA.

<table>
<thead>
<tr>
<th>Trt</th>
<th>Color</th>
<th>Marbling</th>
<th>% Lipid</th>
<th>Firmness</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 0 kg</td>
<td>2.43</td>
<td>2.04(^c)</td>
<td>4.02(^c)</td>
<td>2.36</td>
</tr>
<tr>
<td>T2 28 kg</td>
<td>2.31</td>
<td>2.18(^d)</td>
<td>4.40(^d)</td>
<td>2.27</td>
</tr>
<tr>
<td>T3 57 kg</td>
<td>2.47</td>
<td>2.35(^a)</td>
<td>4.76(^a)</td>
<td>2.45</td>
</tr>
<tr>
<td>T4 86 kg</td>
<td>2.38</td>
<td>2.31(^e)</td>
<td>5.06(^e)</td>
<td>2.49</td>
</tr>
<tr>
<td>SEM</td>
<td>.10</td>
<td>.10</td>
<td>.09</td>
<td>.06</td>
</tr>
</tbody>
</table>

\(^a\) Values within a column with different superscripts are significant at \(P < .05\).

\(^b\) Based on National Pork Producer 5-point scale.

Table 3. Least squares means\(^a\) for Hunter \(L^*\), \(a^*\) and \(b^*\) values of overwrapped loin chops independent of storage day at 4\(^\circ\) C

<table>
<thead>
<tr>
<th>Trt(^b)</th>
<th>(L^*)</th>
<th>(a^*)</th>
<th>(b^*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>51.53</td>
<td>6.16</td>
<td>11.76(^c)</td>
</tr>
<tr>
<td>T4</td>
<td>52.18</td>
<td>6.53</td>
<td>12.15(^d)</td>
</tr>
<tr>
<td>SEM</td>
<td>.34</td>
<td>.17</td>
<td>.09</td>
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</tbody>
</table>

\(^a\) Values within columns with different superscripts significant at \(P < .05\)

\(^b\) T1 = 0 kg gain on CLA, T4 = 86 kg gain on CLA.
<table>
<thead>
<tr>
<th>Trt&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Day</th>
<th>pH</th>
<th>WHC</th>
<th>TBA&lt;sup&gt;c&lt;/sup&gt;</th>
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</thead>
<tbody>
<tr>
<td>T1</td>
<td>1</td>
<td>5.70</td>
<td>3.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.098&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>T4</td>
<td>1</td>
<td>5.70</td>
<td>3.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.081&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>T1</td>
<td>14</td>
<td>5.80</td>
<td>2.77&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.098&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>T4</td>
<td>14</td>
<td>5.79</td>
<td>2.92&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.158&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>T1</td>
<td>28</td>
<td>5.77</td>
<td>2.59&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.187&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>T4</td>
<td>28</td>
<td>5.76</td>
<td>2.39&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.132&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SEM</td>
<td></td>
<td>0.02</td>
<td>0.13</td>
<td>0.003</td>
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</table>

<sup>a,b</sup> Means with different letter within a column are significant at P < .05
<sup>c</sup> TBA values expressed as mg malonaldehyde/kg sample
<sup>d</sup> T1 = 0 kg weight gain on CLA, T4 = 86 kg weight gain on CLA.
Table 6. Fatty acid profile (%)\(^a\) of pork loin tissue from 10th and 11th rib junction.

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>0</th>
<th>28</th>
<th>56</th>
<th>87</th>
<th>Linear</th>
<th>Quadratic</th>
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<tbody>
<tr>
<td>14:0</td>
<td>1.53</td>
<td>1.88</td>
<td>2.03</td>
<td>2.03</td>
<td>.001</td>
<td>.07</td>
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<tr>
<td>16:0</td>
<td>30.39</td>
<td>33.18</td>
<td>35.49</td>
<td>36.70</td>
<td>.0001</td>
<td>.24</td>
</tr>
<tr>
<td>18:0</td>
<td>13.67</td>
<td>14.70</td>
<td>15.02</td>
<td>14.27</td>
<td>.11</td>
<td>.007</td>
</tr>
<tr>
<td>18:1</td>
<td>44.88</td>
<td>40.27</td>
<td>38.82</td>
<td>38.99</td>
<td>.0001</td>
<td>.004</td>
</tr>
<tr>
<td>18:2</td>
<td>8.22</td>
<td>8.74</td>
<td>7.78</td>
<td>6.52</td>
<td>.002</td>
<td>.03</td>
</tr>
<tr>
<td>18:3</td>
<td>0.27</td>
<td>0.23</td>
<td>0.16</td>
<td>0.12</td>
<td>.001</td>
<td>.89</td>
</tr>
<tr>
<td>20:4</td>
<td>1.03</td>
<td>0.41</td>
<td>0.34</td>
<td>0.66</td>
<td>.03</td>
<td>.001</td>
</tr>
<tr>
<td>CLA(^b)</td>
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<td>0.58</td>
<td>0.35</td>
<td>0.71</td>
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<tr>
<td>SFA(^c)</td>
<td>45.60</td>
<td>49.76</td>
<td>52.54</td>
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<td>.03</td>
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<tr>
<td>PUFA(^d)</td>
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<td>9.39</td>
<td>8.29</td>
<td>7.30</td>
<td>.0008</td>
<td>.29</td>
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</tbody>
</table>

\(^a\) Expressed as weight percentage of sample injected on gas chromatograph column
\(^b\) Conjugated linoleic acid isomers
\(^c\) Saturated fatty acids
\(^d\) Polyunsaturated fatty acids.
Table 7. Fatty acid profile (%)\(^a\) of pork subcutaneous fat tissue from 10th and 11th rib junction.

<table>
<thead>
<tr>
<th>Weight gain on CLA, kg</th>
<th>P-value</th>
<th>Fatty Acid</th>
<th>0</th>
<th>28</th>
<th>56</th>
<th>87</th>
<th>Linear</th>
<th>Quadratic</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td></td>
<td></td>
<td>1.67</td>
<td>2.29</td>
<td>2.77</td>
<td>3.00</td>
<td>.0001</td>
<td>.08</td>
</tr>
<tr>
<td>16:0</td>
<td></td>
<td></td>
<td>27.84</td>
<td>31.35</td>
<td>30.34</td>
<td>32.51</td>
<td>.01</td>
<td>.52</td>
</tr>
<tr>
<td>18:0</td>
<td></td>
<td></td>
<td>13.98</td>
<td>15.56</td>
<td>17.16</td>
<td>17.82</td>
<td>.003</td>
<td>.58</td>
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<td>39.17</td>
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<td>27.53</td>
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<td>18:2</td>
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<td></td>
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<td>15.12</td>
<td>14.63</td>
<td>14.74</td>
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</tr>
<tr>
<td>18:3</td>
<td></td>
<td></td>
<td>0.93</td>
<td>0.79</td>
<td>0.59</td>
<td>0.53</td>
<td>.0001</td>
<td>.56</td>
</tr>
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<td>20:4</td>
<td></td>
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<td>0.37</td>
<td>0.63</td>
<td>0.53</td>
<td>0.26</td>
<td>.64</td>
<td>.22</td>
</tr>
<tr>
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<td></td>
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<td>0.50</td>
<td>2.45</td>
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<td>49.19</td>
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<td>16.54</td>
<td>15.76</td>
<td>15.52</td>
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\(a\) Expressed as weight percentage of sample injected on gas chromatograph column

\(b\) Conjugated linoleic acid isomers

\(c\) Saturated fatty acids

\(d\) Polyunsaturated fatty acids.
Figure 1. Mean sensory attributes of CLA and control pork loins at 1 d shelf storage at 4°C.
CHAPTER 3. CONJUGATED LINOLEIC ACID IMPROVES FEED EFFICIENCY, AVERAGE DAILY GAIN AND DECREASES LAST RIB FAT IN SWINE WITH VARYING HALOTHANE STATUS

A paper to be submitted to the Journal of Animal Science

B. R. Wiegand, F. C. Parrish, Jr. and T. J. Baas

Abstract

Growing-finishing barrows (n = 64) of three stress genotypes (negative, carrier, or positive) were supplemented with a control diet or a diet containing 0.75% conjugated linoleic acid (CLA 60). Growth, carcass, meat quality, and sensory data were collected and analyzed. Average daily gain (ADG) was higher (P < 0.05) for negative genotype pigs fed CLA versus negative genotype pigs on the control diet. For the carrier and positive genotype pigs, ADG was lower (P < 0.05) for the CLA-fed pigs compared with the control diet pigs. Gain to feed ratio (G:F) was higher for negative genotype pigs fed CLA compared with negative, control-diet pigs. No differences were observed for G:F within the carrier and positive genotypes regardless of experimental diet. No differences were observed for loineye area between any of the genotype or diet groups. Tenth rib fat depth was lower (P < 0.05) for carrier, control-diet pigs compared with positive, control-diet pigs. No other differences were observed for
tenth rib fat depth. Last rib fat depth was significantly higher (P < 0.05) for negative genotype pigs fed CLA versus negative, control-diet pigs. Last rib fat depth was not different in the other genotype or diet groups. For subjective meat quality scores of the loineye, only marbling scores were different with no changes observed in color or firmness in any of the groups. Marbling scores were higher (P < 0.05) for negative and carrier, control-diet pigs compared with both positive-genotype feeding groups. Proximate analysis for fat and moisture content in loin chops evidenced no differences between any of the feeding groups. Similarly, there were no differences for Hunter L*, a* or b* values over the 3 d of self-service retail storage. These study results indicated that CLA supplementation has little or no effect on growth, carcass or meat quality parameters for carrier and positive stress genotype pigs. However, negative stress genotype pigs responded favorably to CLA supplementation for ADG and G:F parameters.

Keywords: swine, stress genotype, conjugated linoleic acid
Introduction

Recent research has shown that CLA, a geometric isomer of linoleic acid, may serve as a growth promoting nutritional supplement (Eggert et al., 1998; Thiel et al., 1998; and Wiegand et al., 2000). In addition, CLA has been shown to decrease backfat depth and improve certain aspects of meat quality. To better understand the changes in meat quality, namely color, marbling, and firmness of the loin, we designed the current study using pigs of known stress genotype. This population of pigs had the propensity toward meat quality problems, especially pale, soft, and exudative (PSE) meat. Such a population can serve as a good model for studying and understanding factors concerning meat quality and ultimately consumer appeal. Based on previous research (Thiel et al., 1998; Cook et al., 1998) we hypothesized that CLA supplementation at 0.75% of the diet would improve feed efficiency, decrease subcutaneous fat, and improve fresh pork color. The objective of this study was to investigate the effect of dietary CLA on growth, carcass and meat quality characteristics in stress-genotype pigs.
Materials and Methods

This project was carried out in accordance with guidelines of the Iowa State University Animal Care and Use Committee. Grower barrows (n = 64) were assigned by stress genotype (negative, carrier, or positive according to DNA test results) to a control diet or a diet supplemented with 0.75% conjugated linoleic acid (CLA 60, Conlinco Inc., Detroit Lakes, MN). CLA replaced soy oil in the control diet and the diets were isocaloric. Pigs were paired and penned in 32 adjacent pens in a gutter flush grower-finisher facility (Bilsland Swine Breeding Farm, Madrid, IA). Pigs went on the feeding trial at an average weight of 40 kg and were harvested at 115 kg at Hormel Foods Inc., Austin, MN. Body weights were recorded every two weeks of the feed trial to evaluate average daily gain and feed efficiency. Carcass data including dressing percentage, loineye area, tenth rib fat depth and last rib fat depth were recorded 24 h postmortem. Whole bone-in loins (IMPS 410) were removed from the right side of the carcass and shipped to the Iowa State University Meat Laboratory. At 36 h postmortem, subjective quality scores for color, marbling and firmness of the loin at the tenth rib interface (NPPC, 1991) were recorded.
Furthermore, L*, a* and b* color values were measured at this time using a Minolta colorimeter with a 10° light source. Loins were deboned (IMPS 412B) and vacuum packaged for 21 d at 4° C. At 21 d of cold storage, loins were sliced into 2.54 cm chops and placed in pairs on styrofoam trays with oxygen permeable polyvinyl chloride overwrap for 3 d at 4° C in a self-service retail case. Hunter L*, a* and b* values were measured with a Hunter Lab Scan (Hunter Associates, Reston, VA) at 1 d and 3 d of retail storage.

Lipid content was determined by hexane extraction on dried samples using a Soxhlet method (AOAC, 1990). Moisture was determined as the weight difference in samples after 18 h in an 80° C drying oven.

Statistical Analysis

The GLM procedure of SAS was used to analyze data in a 3 x 2 factorial design with three genotypes and two diets (SAS, 1990). Data are presented as least squares means with standard errors. Repeated measures were used to analyze color data over time of retail storage.
Results and Discussion

Data analysis for growth parameters revealed a higher average daily gain (ADG) for negative genotype pigs fed CLA compared with negative, control-diet pigs (Table 2). In contrast, carrier and positive genotype pigs fed the CLA diet exhibited lower ADG compared with the control diet pigs. No differences were observed for ADG between the three stress genotypes. Leach et al. (1996) reported similar results for ADG where normal pigs exhibited higher ADG than positive genotype pigs. Gain to feed ratio (G:F) was higher (P < 0.05) for negative genotype pigs fed CLA compared with negative genotype pigs fed the control diet. These results are similar to our previous study (Sparks et al., 1998). No differences were observed for diet within the carrier and positive genotype or between the three stress genotypes.

It should be noted that only 51 pigs were actually harvested for this project. Of the thirteen pigs lost, eight were stress positive and the other five were stress carriers. These loses decreased the sample size of these two genotypes for carcass, meat quality and sensory characteristics. Carcass data, including loineye area, tenth rib fat depth, and last rib fat depth are shown in Table 3. Loineye area data showed no differences for genotype or diet classes. These
data are in agreement with other reports where carrier and positive genotype pigs exhibited larger loin eye area compared to stress negative pigs (Fisher et al., 2000; Leach et al., 1996). Tenth rib fat depth showed no differences for diet within any of the three genotypes. However, carrier genotype pigs fed the control diet had greater tenth rib fat depth compared with control diet, positive genotype pigs (2.58 cm vs 1.68 cm). Last rib fat depth values were quite variable and resulted in inconsistent differences with respect to diet and stress genotype. Negative pigs fed CLA had more (P < 0.05) last rib fat than negative genotype pigs fed the control diet (3.81 cm vs 2.71 cm). Additionally, carrier pigs fed the control diet had more last rib fat than positive genotype pigs, fed the control and CLA diet (3.48 cm vs 2.64 cm and 2.64 cm, respectively). Typically, tenth rib fat depth and last rib fat depth have been shown to change with stress-gene status where negative genotype pigs have the greatest fat depth and positive genotypes have the lowest depth, with carrier genotypes being intermediate (Fisher et al., 2000; Leach et al., 1996).

Subjective quality scores for the loin face at the tenth and eleventh rib (Table 4) showed few differences in respect to color, marbling and firmness. In fact no differences were
observed in subjective color or subjective firmness for the main effects of diet or genotype. Mixed results were observed for subjective marbling scores where negative genotype pigs fed CLA and carrier genotype pigs fed the control diet exhibited higher degrees of marbling compared to both diet groups within the positive genotype classification. Hexane extractable fat percentage (Table 5), used to verify subjective marbling values, revealed only numerical differences which tended to support differences in the subjective marbling scores.

Hunter color data analysis resulted in some unexpected differences with respect to genotype classes. Table 6 shows Hunter L*, a* and b* values of loin chops at 1 d and 3 d of self-service case storage after the whole loin was stored for 21 d at 4°C under vacuum packaging. Genotype was a non-significant main effect in the statistical model so Hunter values were pooled by diet effect. L* values were not different for either diet at 1 d or 3 d of storage. Fisher et al., 2000 reported that L* values increase linearly with stress negative, stress carrier and stress positive genotypes, respectively, indicating a lighter loineye color. At 3 d, a* values tended (P < 0.07) to be higher for chops from CLA-diet pigs compared with chops from control-diet pigs. Additionally,
b* values were higher (P < 0.02) for chops from control-diet pigs. These Hunter color results are consistent with previous data we have collected with respect to CLA supplementation (Wiegand et al., 1998).

**Implications**

The data from this study indicate that feeding CLA to pigs, which are negative for the stress gene, may have benefits in terms of average daily gain and feed conversion. However, due to death losses of a number of stress-positive pigs, it seems premature to draw conclusions for the efficacy of feeding CLA to improve carcass and meat quality characteristics of these genotype pigs. Certainly, the results for carcass and meat quality data are mixed and difficult to explain. Another study should be conducted to verify results from this project.

**References**


Table 1. Diet composition and calculated analysis at different body weights.

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<th>86 to 115</th>
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<td>Soybean meal</td>
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<td>.82</td>
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Calculated analysis:

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<tr>
<td>Phosphorus, %</td>
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</tbody>
</table>

$^a$ At .2% of diet contributes per kilogram of diet: 4,400 IU vitamin A; 1,100 IU vitamin D$_3$; 6.6 mg riboflavin; 17.6 mg pantothenic acid; 33 mg niacin; 22 µg vitamin B$_{12}$.

$^b$ At .05% of the diet contributes in ppm: 75 Zn, 87.5 Fe, 30 Mn, 8.75 Cu, 0.1 I.

$^c$ Soybean oil or CLA oil in their respective treatment.
Table 2. Average daily gain and gain:feed for three stress genotypes (n = 51)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Diet</th>
<th>ADG(^a)</th>
<th>G:F(^bc)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>Control</td>
<td>.84(^d)</td>
<td>.25(^d)</td>
</tr>
<tr>
<td>Negative</td>
<td>CLA</td>
<td>.92(^{ef})</td>
<td>.32(^{ef})</td>
</tr>
<tr>
<td>Carrier</td>
<td>Control</td>
<td>.93(^e)</td>
<td>.26(^{de})</td>
</tr>
<tr>
<td>Carrier</td>
<td>CLA</td>
<td>.85(^{df})</td>
<td>.27(^{de})</td>
</tr>
<tr>
<td>Positive</td>
<td>Control</td>
<td>.88(^e)</td>
<td>.24(^d)</td>
</tr>
<tr>
<td>Positive</td>
<td>CLA</td>
<td>.85(^{df})</td>
<td>.25(^d)</td>
</tr>
</tbody>
</table>

| SEM       | .02        | .01        |

\(^a\) Average daily gain (kg/d)

\(^b\) Gain to feed ratio (kg/kg)

\(^c\) Means within a column with different letters significant at (P > .05)

---

Table 3. Least squares means\(^a\) for loineye area, tenth rib and last rib fat depth of three stress genotypes (n = 51).

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Diet</th>
<th>Loineye Area(^a)</th>
<th>Tenth Rib(^c)</th>
<th>Last Rib(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>Control</td>
<td>45.34</td>
<td>2.45(^{de})</td>
<td>2.71(^{df})</td>
</tr>
<tr>
<td>Negative</td>
<td>CLA</td>
<td>44.24</td>
<td>2.52(^{de})</td>
<td>3.01(^e)</td>
</tr>
<tr>
<td>Carrier</td>
<td>Control</td>
<td>46.44</td>
<td>2.58(^d)</td>
<td>3.48(^{ef})</td>
</tr>
<tr>
<td>Carrier</td>
<td>CLA</td>
<td>48.56</td>
<td>2.32(^{de})</td>
<td>3.10(^{de})</td>
</tr>
<tr>
<td>Positive</td>
<td>Control</td>
<td>48.24</td>
<td>1.87(^e)</td>
<td>2.64(^d)</td>
</tr>
<tr>
<td>Positive</td>
<td>CLA</td>
<td>49.72</td>
<td>1.94(^{de})</td>
<td>2.64(^d)</td>
</tr>
</tbody>
</table>

| SEM       | 2.45       | .19                 | .26             |

\(^a\) Means within a column with different letter significant at P < .05

\(^b\) Expressed in cm\(^2\)

\(^c\) Expressed in cm
Table 4. Least squares means\textsuperscript{a} for subjective quality scores\textsuperscript{b} of loins from three stress genotypes (n = 51).

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Diet</th>
<th>Color</th>
<th>Marbling</th>
<th>Firmness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>Control</td>
<td>2.55</td>
<td>2.22\textsuperscript{ce}</td>
<td>2.22</td>
</tr>
<tr>
<td>Negative</td>
<td>CLA</td>
<td>2.62</td>
<td>2.87\textsuperscript{c}</td>
<td>2.88</td>
</tr>
<tr>
<td>Carrier</td>
<td>Control</td>
<td>2.30</td>
<td>2.50\textsuperscript{c}</td>
<td>2.30</td>
</tr>
<tr>
<td>Carrier</td>
<td>CLA</td>
<td>2.50</td>
<td>2.40\textsuperscript{ce}</td>
<td>2.30</td>
</tr>
<tr>
<td>Positive</td>
<td>Control</td>
<td>2.25</td>
<td>1.62\textsuperscript{de}</td>
<td>2.13</td>
</tr>
<tr>
<td>Positive</td>
<td>CLA</td>
<td>2.14</td>
<td>1.71\textsuperscript{de}</td>
<td>2.14</td>
</tr>
<tr>
<td>SEM</td>
<td></td>
<td>.28</td>
<td>.29</td>
<td>.26</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Means within a column with different letter significant at P < .05.

\textsuperscript{b} Based on National Pork Producers 5-point scale.

Table 5. Least squares means for fat and moisture percentage of loin chops from three stress genotypes (n = 51).

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Diet</th>
<th>Lipid %</th>
<th>Moisture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>Control</td>
<td>3.29</td>
<td>72.96</td>
</tr>
<tr>
<td>Negative</td>
<td>CLA</td>
<td>4.36</td>
<td>71.87</td>
</tr>
<tr>
<td>Carrier</td>
<td>Control</td>
<td>3.35</td>
<td>72.69</td>
</tr>
<tr>
<td>Carrier</td>
<td>CLA</td>
<td>4.28</td>
<td>73.01</td>
</tr>
<tr>
<td>Positive</td>
<td>Control</td>
<td>2.82</td>
<td>73.95</td>
</tr>
<tr>
<td>Positive</td>
<td>CLA</td>
<td>3.30</td>
<td>73.42</td>
</tr>
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<td>SEM</td>
<td></td>
<td>.61</td>
<td>1.02</td>
</tr>
</tbody>
</table>
Table 6. Least squares means$^a$ for Hunter color values of loin chops from CLA vs. control diet pigs (n = 51).

<table>
<thead>
<tr>
<th>Diet</th>
<th>Day$^b$</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1</td>
<td>44.96</td>
<td>6.33</td>
<td>10.71</td>
</tr>
<tr>
<td>CLA</td>
<td>1</td>
<td>46.24</td>
<td>6.64</td>
<td>11.26</td>
</tr>
<tr>
<td>SEM</td>
<td>.89</td>
<td>.34</td>
<td>.31</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3</td>
<td>47.32</td>
<td>5.00</td>
<td>10.07$^c$</td>
</tr>
<tr>
<td>CLA</td>
<td>3</td>
<td>47.32</td>
<td>5.73</td>
<td>10.92$^d$</td>
</tr>
<tr>
<td>SEM</td>
<td>1.04</td>
<td>.28</td>
<td>.26</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Means within a column with different letter significant at $P < .05$.

$^b$ Day of storage in self-service case after 21 d in 4°C cooler storage.
CHAPTER 4. CONJUGATED LINOLEIC ACID IMPROVES FEED EFFICIENCY, DECREASES SUBCUTANEOUS, AND IMPROVES CERTAIN ASPECTS OF MEAT QUALITY IN STRESS-GENOTYPE PIGS

A paper to be submitted to the Journal of Animal Science

B. R. Wiegand, F. C. Parrish, Jr., J. E. Swan and T. J. Baas

Abstract

Conjugated linoleic acid (CLA) was supplemented to crossbred growing-finishing pigs (n = 64) at 0.75% of the total diet. Pigs were randomly assigned to the CLA or control diets based on stress genotype (negative, carrier or positive). Growth, carcass and meat quality data were analyzed. Gain:feed was significantly higher for CLA diet animals (350 g/kg feed) versus control diet animals (330 g/kg feed) independent of genotype (P < 0.05). No differences were observed for average daily gain for the diet or genotype classes. Postmortem pH was lower by 3 h for CLA supplemented pigs with no differences in ultimate pH. No differences were observed for ultimate pH between the three genotypes. Percent carcass shrink at 24 h postmortem was not affected by CLA. CLA supplemented pigs exhibited decreased 10th rib fat depth (2.34 cm vs 2.84 cm) and last rib fat depth (2.46 cm vs 2.72 cm) compared with control pigs (P < 0.05). Loin muscle area (LMA) was not
affected by CLA supplementation, but LMA was significantly
different for genotype, with positive genotype carcasses
having the largest LMA (45.02 cm²) and negative carcasses
having the smallest LMA (36.44 cm²). Carrier carcasses were
intermediate for LMA (40.76 cm²). NPPC subjective scores for
color were not affected by CLA, but color was significantly
different with scores of 1.50, 2.40 and 3.1 for positive,
carrier and negative genotypes, respectively. Subjective
marbling scores were significantly increased in all genotypes
with CLA supplementation. Hexane-extractable lipid analysis
verified the increases in marbling scores. Subjective
firmness scores were significantly higher for CLA supplemented
pigs and were highly correlated (0.89) to marbling scores. L* values were higher for stress positive pigs at 24 h
postmortem. Also, L* values were higher for CLA-fed pigs over
7 d of shelf storage. Sensory characteristics were not
different with CLA supplementation for tenderness, juiciness,
and flavor intensity. This study shows LMA is increased with
stress carrier and stress positive genotypes, but lean color
is negatively affected with the presence of the stress gene.
Additionally, CLA supplementation improves feed efficiency,
decreases backfat and improves pork quality attributes of
marbling and firmness of the longissimus muscle.
Keywords: conjugated linoleic acid, swine, meat quality

Introduction

Currently our research has focused on improving growth, compositional and quality characteristics of pigs by feeding conjugated linoleic acid (CLA) in the growing-finishing diet. In an effort to find a model for pork quality problems, we chose to work with a herd of known stress-genotype pigs. The stress gene has been a source of pork meat quality problems for many years (Louis et al., 1993). This population of pigs including genotypes which are negative, carriers and positive for the stress gene have proven to be a good source of pale, soft and exudative (PSE) pork and have allowed us to pursue methods for understanding and possibly overcoming pork quality problems. It is true that the stress gene is being selected against in most swine operations in the United States. PSE, however, is a problem which occurs in stress-free swine herds and understanding methods for decreasing its incidence certainly holds merit in today's industry. Based on previous research (Wiegand et al., 1998a; Wiegand et al., 1998b; Sparks et al., 1998; Park et al., 1999), we hypothesized that CLA fed at 0.75% in the diet would improve feed efficiency, decrease
backfat depth and improve pork quality characteristics of stress-genotype pigs. Consequently, our objective in this study was to measure growth, carcass and meat quality characteristics in stress-genotype pigs supplemented with 0.75% CLA in the growing-finishing diet.

**Materials and Methods**

All procedures of this project were in accordance with the guidelines of the Iowa State University Animal Care and Use Committee. Crossbred growing-finishing pigs (n = 64) were penned in pairs according to stress genotype (negative, carrier or positive) and diet (control or 0.75% CLA-60 (Conlinco, Inc. Detroit Lakes, MN)). The diet composition is shown in Table 1. Pigs started the feeding trial at an average weight of 40 kg and were harvested at an average weight of 106 kg. Body weights were recorded every two weeks to monitor average daily gain and to adjust protein percentage in the diet. Due to varying growth rates, pigs were harvested in four groups over a 30 d period at the Iowa State University Meat Laboratory.

**Post harvest carcass and quality measurements.**

Temperature and pH decline in the longissimus dorsi (10th and 11th rib) was monitored every hour beginning at
exsanguination and continuing for 24 h postmortem. Temperature was measured with a 10 cm steel probe attached to an electro-therm TM99A digital thermometer (Middlefield, CT). A pH-Star probe (SFK Technology, Inc., Cedar Rapids, IA) was used to measure pH decline. Hot carcass weight and 24 h chilled carcass weight were recorded to calculate dressing percentage and 24 h cooler shrinkage percentage. The left side was ribbed between the 10th and 11th rib and loineye area, 10th rib fat depth and color, marbling and firmness values were recorded (NPPC, 1991). Additionally, 1st rib fat, last rib fat depth and ham muscling score were recorded.

Postfabrication meat quality procedures
At 24 h postmortem, carcasses were fabricated into primal cuts and the right side loin (IMPS 410) was removed. The loin was deboned and 2.54 cm chops (IMPS 1412B) were removed for proximate analysis, Hunter Lab color L*, a* and b* values (Hunter Associates, Reston, VA), sensory panel and myoglobin determination. The remaining boneless loin section was vacuum packaged and stored at 3° C for 21 d. At 21 d of cold storage, the loin section was sliced into 2.54 cm chops and placed on styrofoam trays with polyvinyl-chloride overwrap for 1, 2, 3 and, 7 d in a retail self-service display case at 4°
C. Hunter color measurements were taken at each day of retail storage.

Percentage lipid and moisture were determined using hexane extraction methods and weight differences after sample vacuum drying at 80° C, respectively (AOAC, 1990).

Total myoglobin content of loin chops was determined by pulverizing a 10 g meat sample in liquid nitrogen and adding 100 ml of cold 40 mM potassium phosphate buffer (pH 6.80). The sample was blended in a Waring blender for 2 min and incubated at 4° C for 1 h. Samples were then centrifuged at 15,000 X g for 30 min. The supernatant was filtered through a Whatman number 1, 125 mm paper and 3 ml were transferred to a disposable cuvette. Samples were scanned from 300-700 nm on a Beckman DU 640 spectrophotometer (Fullerton, CA). Total myoglobin was calculated from the absorbance at 418 nm which represents myoglobin in the oxymyoglobin state (Hunt, 1999).

Sensory panel evaluation was determined by a 10-membered panel. Panelists evaluated 1 cm³ samples of loin chops which were cooked in a General Electric broiler set at 176° C. Chops were turned once when they reached an internal temperature of 35° C and cooked to a final internal temperature of 71° C. Panelists evaluated samples for
tenderness, juiciness, and flavor intensity based on an 8-point descriptive scale (AMSA, 1995).

**Statistical Analysis**

Statistical analysis of the data included a completely randomized design with a 3 x 2 factorial arrangement of three genotypes and two diets. Analysis of variance was performed with the General Linear Model (GLM) procedure of SAS (1990). The following model was fitted for main effects (diet, genotype) and interactions

\[ Y_{ij} = \mu + G_i + D_j + GD_{ij} + e_{ij} \]

Where \( Y_{ij} \) is the dependent variable, \( \mu \) = the overall mean, \( G_i \) = the \( i^{th} \) genotype effect, \( D_j \) = the \( j^{th} \) diet effect, \( GD_{ij} \) = the interaction between genotype and diet and \( e_{ij} \) = residual error. Data are presented as least squares means with standard errors attached. Means were considered different at \( P < 0.05 \). Additionally, color, pH and temperature data were analyzed with repeated measures over day of retail storage and hours of postmortem chilling, respectively.

**Results and Discussion**

Gain to feed ratio (G:F) and average daily gain (ADG) data are shown in Table 2. G:F was higher (\( P < 0.05 \)) for CLA-diet animals (350 g/kg) versus control diet animals (330 g/kg)
independent of genotype. Chin et al. (1994) reported improvement in feed efficiency for rats fed CLA. They attributed these changes to the ability of CLA to regulate energy metabolism and nutrient partitioning. The authors speculated that if body fat is decreased by CLA supplementation, then less energy would be required to maintain animal growth, thus making them more efficient. Park et al. (1997) subsequently verified these findings in a similar mouse study. It seems possible that these same mechanisms are responsible for the feed efficiency improvement observed in pig models. No differences were observed between the three stress genotypes for G:F. Also, no differences were observed for ADG in the diet or genotype classes. Similar results for G:F and ADG have been shown with CLA supplementation (Sparks et al., 1998).

Figure 1 illustrates pH decline from 30 min to 24 h postmortem in the longissimus at the 10th and 11th rib junction for each of the three stress genotypes (n = 64). The rate of decline is much steeper for carrier and positive genotype carcasses compared with the negative genotype. At 30 min postmortem, pH is lower (P < 0.05) for the stress positive pigs compared with the carrier and negative genotypes. By two h postmortem, pH values for carrier and positive carcasses are
lower (P < 0.05) than negative genotype carcasses. There are no differences between the three genotypes for pH at 24 h postmortem (ultimate pH). When the pH data are analyzed by diet (Figure 2), we found that at 3 h postmortem, the carcasses from CLA-fed pigs exhibited lower (P < 0.01) pH values, but no differences were observed between diets for ultimate pH. Also, within the stress negative genotype (Figure 3), pH values are lower (P < 0.02) at 3 h postmortem for carcasses from CLA-fed pigs compared with control-diet carcasses. There are no differences for ultimate pH with the negative genotype. One possible explanation, which might be suggested by these data, for the rapid pH decline in the CLA-fed pigs may be linked to glycogen utilization. One might hypothesize that if pigs have greater feed conversion (energy utilization), then perhaps they are able to store more glycogen compared with the control-diet pigs. This extra glycogen would presumably be available for postmortem muscle glycolysis (Maribo et al., 1999). This increased energy reserve could drive the production of lactic acid at a greater rate since more substrate is available. However, this rationale may be flawed in the fact that ultimate pH was not different between the CLA- and control-diet pigs. If glycogen were present in greater amounts, it would seem that more
lactic acid would be formed, resulting in a lower ultimate pH (Bendall, 1973). Still the possibility exists that at a given pH value and muscle temperature, glycolysis would stop because of denaturation of key enzymes in the reaction pathway (Bendall, 1973). If this were true, it could be possible for there to be residual glycogen in the muscle that was not metabolized to lactic acid. The muscle temperature \times pH threshold at which glycolysis stops is not well defined in the literature. Other possibilities for explaining the faster rate of pH decline with CLA may be associated with enzymes within the glycolytic pathway. There are many enzymes that drive the production of lactic acid. Some of the rate limiting enzymes, including phosphofructokinase (PFK), glucose-6-phosphate and pyruvate kinase may be present in a greater amount or might be more active in muscle from CLA-fed pigs. This could be the topic for future research in CLA metabolism.

It is interesting to note that the correlation between 30 min pH and ultimate pH is 0.16 (Table 3). This is a rather weak relationship indicating that ultimate pH is a poor predictor of rate of pH decline. This becomes important in that the rate of pH decline may be more important in pork quality prediction than ultimate pH. One might hypothesize
that a fast rate of decline causing acidic conditions in combination with high muscle temperature early postmortem may cause denaturation of proteins (Bendall, 1973), namely myoglobin, which is largely responsible for meat color (Renerre, 1999). These observations suggest that a measure of pH at 30 or 45 min and at 24 h postmortem would be useful in predicting pork quality given that a fast rate of decline and/or a low ultimate pH can be responsible for PSE conditions (Bendall and Swatland, 1989).

Initially, the significantly lower pH values resulting from CLA supplementation may be of concern with respect to their potential impact on pork quality parameters. The impact these pH data might have on loin color characteristics at 24 h postmortem are shown in Table 3. The correlation coefficient between 30 min pH and Hunter L* values is -0.50. This relationship is inverse indicating a lighter loin color with declining pH. Additionally, correlation coefficients between 24 h pH and Hunter a* and b* values are -0.56 and -0.59, respectively. These results are more difficult to interpret as one would likely not expect loin color to become more red (a*) while pH values decline. Fisher et al. (2000) have suggested that a* values increase because water is lost when pH values become low and meat pigment becomes more
concentrated in the resulting product. These results warrant that further investigation into meat color is needed to understand the impact of pH on Hunter color values.

Carcass data including percentage carcass shrink, backfat depth, and loineye area are presented in Tables 4, 5, and 6, respectively. No differences were observed for percentage carcass shrink at 24 h postmortem between any of the genotype or diet groups. Tenth rib fat depth is lower (P < 0.05) for CLA-fed pigs within each stress genotype. Last rib fat depth was also lower (P < 0.05) for CLA-fed pigs within all three stress genotypes. These data are similar to reports from other studies involving CLA supplementation (Thiel et al., 1998; Eggert et al., 1999; Sparks et al., 1998). The decrease in body fat may be attributed in part to a decrease in steroyl-CoA desaturase activity by the trans-10, cis-12 isomer of CLA (Lee et al., 1998). Park et al. (1999) also reported reduction in body fat while feeding the trans-10, cis-12 isomer. Pariza et al. (2000) have suggested that changes in fat metabolism may be linked to enhanced β-oxidation, but definitive evidence does not yet exist to support this hypothesis. Loin muscle area (LMA), at the 10th and 11th rib, was not effected by CLA supplementation. Two other studies, (Eggert et al., 1999; Cook et al., 1998) also reported no
differences in LMA with CLA supplementation. Studies in
rodent models have shown increases in whole body protein with
CLA supplementation (Park et al., 1999; Park et al., 1997).
If whole body protein increases in pigs fed CLA, these
increases do not seem to be expressed as increases in
longissimus muscle area. However, the possibility exists that
increased muscle accretion may occur in other muscle groups.
Furthermore, stress-positive carcasses exhibited larger (P <
0.05) LMA than stress-negative carcasses, with stress carrier
carcasses being intermediate. Leach et al. (1996) reported
similar increases for LMA in stress-genotype pigs.

Subjective meat quality of the loin face at the 10th and
11th rib, including color, marbling, and firmness are shown in
Table 8. No differences were observed in color for diet or
genotype effects. Marbling scores showed significant (P <
0.05) increases for CLA-fed pigs within each stress genotype.
Increases in marbling fat with CLA supplementation is probably
because of a change in fat metabolism or the area of fat
deposition, but no evidence currently exists to support this
idea. The same results were observed for subjective firmness
scores, which tended to increase for CLA-fed pigs within each
genotype. The relationship between marbling and firmness was
strong, resulting in a Pearson correlation coefficient of
0.92. This relationship suggests that by increasing marbling with CLA, one might also increase firmness of the loin face. This increase in firmness may be linked to an increase in saturated fat in pork products from CLA-fed pigs (Eggert et al., 1999).

Hexane-extractable lipid analysis was used to verify subjective marbling scores (Table 8). The main effect of diet produced an increase \( (P < 0.05) \) in lipid percentage with CLA-fed pigs producing 3.07\% lipid compared with 2.61\% lipid from the control-diet pigs. Lipid percentage increased within each genotype for CLA-pigs with negative genotype, CLA-fed pigs exhibiting the greatest increase and positive genotype, CLA-fed pigs exhibiting the smallest increase compared to control-diet pigs within their respective genotype groups. These increases in lipid percentage were similar to results from our previous study (Wiegand et al., 1998a). Proximate analysis also yielded differences in percentage moisture of loin chops, where chops from control diet pigs had higher \( (P < 0.04) \) percentage moisture compared with chops from pigs fed CLA, 73.0\% versus 72.4\%. These differences are evident within each genotype group, but no differences were observed for percentage moisture between the three stress genotypes. These
moisture results would likely be expected given the changes in lipid percentage in the CLA-fed pigs.

Figure 4 shows Hunter L* color development of center cut pork chops over 7 d of self-service case storage after 21 d of whole loin vacuum storage at 4° C. L* values increased sharply over storage time from 0 d to 1 d and then increased slowly out to 7 d. Chops from control diet pigs initially had lower (P < 0.05) L* compared with chops from CLA-fed pigs and these differences were observed at all five time points measured. Hunter a* values are shown in Figure 5 and show an increase in a* values in the first 24 h of self-service storage, with a gradual decrease in a* values over 7 d of storage. No differences in a* values were observed between chops because of diet. Table 9 shows Hunter L*, a*, and b* values at 24 h postmortem for the three stress genotypes. L* values were higher (P < 0.05) for stress-positive pigs compared with negative genotypes, while carrier pigs were intermediate in comparison. These results have been shown in previous studies with stress-genotype pigs (Murray et al., 1989; Fisher et al., 2000). Fisher et al. (2000) suggest that L* values are higher in stress-positive pigs because of an increased denaturation of sarcoplasmic proteins. This idea could be supported by our quantification of soluble myoglobin.
in the loin chop at 24 h postmortem (Table 10). Within the positive genotype, chops from the control diet pigs had higher (P < 0.05) soluble myoglobin values compared with chops from CLA-fed pigs, 0.90 mg/g versus 0.74 mg/g. This decrease in soluble myoglobin corresponds with the increase in L* values with CLA supplementation within the positive genotype.

Additionally, at 24 h postmortem, we observed increased a* values with positive-genotype loins exhibiting higher a* values compared with negative-genotype loins, while carrier loins were intermediate for a* values. Fisher et al. (2000) have suggested that the increased a* values in stress-positive pigs is the result of increased pigment concentration because of water loss in the chop. Our data do not support this explanation because we did not observe any significant increases in soluble myoglobin when comparing the three stress genotypes. However, within the positive genotype we did see a decrease in moisture percentage (Table 8) in loin samples from CLA-fed pigs, which may be linked to the lower pH early postmortem. These factors in combination may have resulted in increased water loss in the stress-positive, CLA-fed pigs which might have resulted in a loss of soluble myoglobin in this group of pigs.
Sensory data are shown in Table 11. The characteristics of tenderness, juiciness and flavor intensity were not affected by CLA supplementation. These results verify our previous study in which no differences were observed in sensory characteristics with CLA supplementation (Thiel-Cooper et al., 1999, Wiegand, et al., 1998).

**Implications**

The results from this study show that supplementation of CLA improves feed efficiency, decreases backfat and improves marbling and firmness scores of loin chops. These results seem to be dependent on stress gene susceptibility for certain measures of performance and meat quality. CLA supplementation also causes a lower pH early postmortem which may have resulted in the higher Hunter L* values for loin chops. Improvements in feed efficiency and decreased backfat in combination with improvements in certain meat quality characteristics, marbling and firmness, may make pork production more profitable if the price of CLA at 0.75% in the diet is nominal.
References


Hunt, M. E. 1999. Personal communication. Kansas State University, Manhattan, KS.


Table 1. Diet composition and calculated analysis at different body weights.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>40 to 57</th>
<th>57 to 86</th>
<th>86 to 106</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
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<td>83.47</td>
<td>85.01</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>27.38</td>
<td>.82</td>
<td>.57</td>
</tr>
<tr>
<td>Dicalcium</td>
<td>1.24</td>
<td>.82</td>
<td>.57</td>
</tr>
<tr>
<td>Calcium</td>
<td>.82</td>
<td>.77</td>
<td>.77</td>
</tr>
<tr>
<td>NaCl</td>
<td>.25</td>
<td>.25</td>
<td>.25</td>
</tr>
<tr>
<td>Vitamin premix</td>
<td>.20</td>
<td>.20</td>
<td>.20</td>
</tr>
<tr>
<td>Trace mineral</td>
<td>.05</td>
<td>.05</td>
<td>.05</td>
</tr>
<tr>
<td>Tylan 40</td>
<td>.05</td>
<td>.05</td>
<td>.05</td>
</tr>
<tr>
<td>Lysine·HCl</td>
<td>.00</td>
<td>.15</td>
<td>.09</td>
</tr>
<tr>
<td>Oil</td>
<td>1.25</td>
<td>1.25</td>
<td>1.25</td>
</tr>
<tr>
<td>Total</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
</tbody>
</table>

Calculated analysis:
- ME, kcal/kg: 3369, 3382, 3395
- Lysine, %: 1.00, .73, .65
- Calcium, %: .70, .58, .50
- Phosphorus, %: .60, .47, .42

^ At .2% of diet contributes per kilogram of diet: 4,400 IU vitamin A; 1,100 IU vitamin D₃; 6.6 mg riboflavin; 17.6 mg pantothenic acid; 33 mg niacin; 22 μg vitamin B₁₂.

^ At .05% of the diet contributes in ppm: 75 Zn, 87.5 Fe, 30 Mn, 8.75 Cu, 0.1 I.

^ Soybean oil or CLA oil in their respective treatment
Table 2. Least squares means for gain to feed (G:F) ratio and average daily gain (ADG) from three genotypes of pigs.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Diet</th>
<th>G:F</th>
<th>ADG (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Control</td>
<td>318&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.88</td>
</tr>
<tr>
<td>Normal</td>
<td>CLA</td>
<td>331&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.90</td>
</tr>
<tr>
<td>Carrier</td>
<td>Control</td>
<td>340&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.94</td>
</tr>
<tr>
<td>Carrier</td>
<td>CLA</td>
<td>350&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.95</td>
</tr>
<tr>
<td>Positive</td>
<td>Control</td>
<td>331&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.87</td>
</tr>
<tr>
<td>Positive</td>
<td>CLA</td>
<td>368&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.89</td>
</tr>
</tbody>
</table>

SEM<sup>c</sup> 4.1 0.06

<sup>a</sup>Means within a column with different letters are significant at P < 0.05.

<sup>b</sup>Expressed as g gain/kg feed

<sup>c</sup>SEM = standard error.

Table 3. Pearson correlation coefficients between pH and Hunter color values.

<table>
<thead>
<tr>
<th></th>
<th>30 min pH</th>
<th>24 hr pH</th>
<th>L&lt;sup&gt;*&lt;/sup&gt;</th>
<th>a&lt;sup&gt;*&lt;/sup&gt;</th>
<th>b&lt;sup&gt;*&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 min pH</td>
<td>1.0</td>
<td>0.16</td>
<td>-0.50</td>
<td>-0.56</td>
<td>-0.59</td>
</tr>
<tr>
<td>24 hr pH</td>
<td>0.16</td>
<td>1.0</td>
<td>-0.55</td>
<td>-0.37</td>
<td>-0.56</td>
</tr>
<tr>
<td>L&lt;sup&gt;*&lt;/sup&gt;</td>
<td>-0.50</td>
<td>-0.55</td>
<td>1.0</td>
<td>0.56</td>
<td>0.86</td>
</tr>
<tr>
<td>a&lt;sup&gt;*&lt;/sup&gt;</td>
<td>-0.56</td>
<td>-0.37</td>
<td>0.56</td>
<td>1.0</td>
<td>0.81</td>
</tr>
<tr>
<td>b&lt;sup&gt;*&lt;/sup&gt;</td>
<td>-0.59</td>
<td>-0.56</td>
<td>0.86</td>
<td>0.81</td>
<td>1.0</td>
</tr>
</tbody>
</table>
### Table 4. Least squares means for percentage cooler shrink of carcasses from three genotypes of pigs.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Diet</th>
<th>HCW (kg)(^a)</th>
<th>CCW (kg)(^b)</th>
<th>% Shrink</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Control</td>
<td>79</td>
<td>77</td>
<td>2.5</td>
</tr>
<tr>
<td>Normal</td>
<td>CLA</td>
<td>77</td>
<td>75</td>
<td>2.5</td>
</tr>
<tr>
<td>Carrier</td>
<td>Control</td>
<td>80</td>
<td>78</td>
<td>2.5</td>
</tr>
<tr>
<td>Carrier</td>
<td>CLA</td>
<td>80</td>
<td>78</td>
<td>2.5</td>
</tr>
<tr>
<td>Positive</td>
<td>Control</td>
<td>82</td>
<td>80</td>
<td>2.4</td>
</tr>
<tr>
<td>Positive</td>
<td>CLA</td>
<td>80</td>
<td>78</td>
<td>2.5</td>
</tr>
<tr>
<td>SEM</td>
<td></td>
<td></td>
<td></td>
<td>0.05</td>
</tr>
</tbody>
</table>

\(^a\) HCW = hot carcass weight.
\(^b\) CCW = chilled carcass weight.

### Table 5. Least squares means and standard errors for 10th rib and last rib fat depth (cm) of carcasses from three genotypes of pigs.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Diet</th>
<th>Tenth rib(^a)</th>
<th>Last rib(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Control</td>
<td>2.97(^b)</td>
<td>2.66(^b)</td>
</tr>
<tr>
<td>Normal</td>
<td>CLA</td>
<td>2.54(^c)</td>
<td>2.51(^b)</td>
</tr>
<tr>
<td>Carrier</td>
<td>Control</td>
<td>2.71(^b)</td>
<td>2.79(^b)</td>
</tr>
<tr>
<td>Carrier</td>
<td>CLA</td>
<td>2.18(^c)</td>
<td>2.38(^c)</td>
</tr>
<tr>
<td>Positive</td>
<td>Control</td>
<td>2.87(^b)</td>
<td>2.66(^b)</td>
</tr>
<tr>
<td>Positive</td>
<td>CLA</td>
<td>2.23(^c)</td>
<td>2.51(^b)</td>
</tr>
<tr>
<td>SEM</td>
<td></td>
<td>0.13</td>
<td>0.15</td>
</tr>
</tbody>
</table>

\(^a\) Means within a column with different letters are significant at \(P < 0.05\).
Table 6. Least squares means and standard errors for loin muscle area (cm²) from carcasses of three genotypes of pigs.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Diet</th>
<th>Loin Muscle Area&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Control</td>
<td>35.57&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Normal</td>
<td>CLA</td>
<td>37.86&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Carrier</td>
<td>Control</td>
<td>39.80&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Carrier</td>
<td>CLA</td>
<td>41.67&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Positive</td>
<td>Control</td>
<td>44.11&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Positive</td>
<td>CLA</td>
<td>45.85&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>SEM</td>
<td></td>
<td>2.03</td>
</tr>
</tbody>
</table>

<sup>a</sup> Means within a column with different letters are significant at P < 0.05.

Table 7. Least squares means and standard errors for subjective quality scores of loins at the 10th and 11th rib interface of carcasses from three genotypes of pigs.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Diet</th>
<th>Color</th>
<th>Marbling&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Firmness&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Control</td>
<td>3.16</td>
<td>2.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.83&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Normal</td>
<td>CLA</td>
<td>3.00</td>
<td>2.50&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.00&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Carrier</td>
<td>Control</td>
<td>2.44</td>
<td>1.38&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.05&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Carrier</td>
<td>CLA</td>
<td>2.38</td>
<td>1.72&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.38&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Positive</td>
<td>Control</td>
<td>1.40</td>
<td>1.00&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.00&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Positive</td>
<td>CLA</td>
<td>1.65</td>
<td>1.37&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.50&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>SEM</td>
<td></td>
<td>0.35</td>
<td>0.13</td>
<td>0.24</td>
</tr>
</tbody>
</table>

<sup>a</sup> Means within a column with different letters are significant at P < 0.05.
Table 8. Least squares means for proximate analysis of loin samples from 10th and 11th rib interface (%) from three genotypes of pigs.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Diet</th>
<th>Moisture&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Lipid&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Control</td>
<td>73.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.86&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Normal</td>
<td>CLA</td>
<td>72.53&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.37&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Carrier</td>
<td>Control</td>
<td>73.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.55&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Carrier</td>
<td>CLA</td>
<td>72.50&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.04&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Positive</td>
<td>Control</td>
<td>72.77&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.41&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Positive</td>
<td>CLA</td>
<td>72.23&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.81&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>SEM</td>
<td></td>
<td>.12</td>
<td>.20</td>
</tr>
</tbody>
</table>

<sup>a</sup> Means within a column with different letters are significant at P < 0.05.

Table 9. Least squares means<sup>a</sup> and standard errors of Hunter L*, a*, and b* values for loin chops from three stress genotypes by experimental diet at 24 h postmortem.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Diet</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>Control</td>
<td>44.49&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.10&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>9.94&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Negative</td>
<td>CLA</td>
<td>47.86&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.67&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10.69&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Carrier</td>
<td>Control</td>
<td>49.50&lt;sup&gt;e&lt;/sup&gt;</td>
<td>5.48&lt;sup&gt;d&lt;/sup&gt;</td>
<td>11.41&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Carrier</td>
<td>CLA</td>
<td>50.43&lt;sup&gt;f&lt;/sup&gt;</td>
<td>5.80&lt;sup&gt;de&lt;/sup&gt;</td>
<td>11.59&lt;sup&gt;ef&lt;/sup&gt;</td>
</tr>
<tr>
<td>Positive</td>
<td>Control</td>
<td>52.22&lt;sup&gt;f&lt;/sup&gt;</td>
<td>5.85&lt;sup&gt;e&lt;/sup&gt;</td>
<td>12.01&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Positive</td>
<td>CLA</td>
<td>54.69&lt;sup&gt;f&lt;/sup&gt;</td>
<td>5.73&lt;sup&gt;de&lt;/sup&gt;</td>
<td>12.50&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>SEM</td>
<td></td>
<td>0.42</td>
<td>0.26</td>
<td>0.18</td>
</tr>
</tbody>
</table>

<sup>a</sup> Means within a column with different letters are significant at P < 0.05.

<sup>b</sup> SE = Standard error.
Table 10. Least squares means of soluble myoglobin (mg/g) for center loin chops.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Diet</th>
<th>Myoglobin^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>Control</td>
<td>0.87c</td>
</tr>
<tr>
<td>Negative</td>
<td>CLA</td>
<td>0.82c</td>
</tr>
<tr>
<td>Carrier</td>
<td>Control</td>
<td>0.78c</td>
</tr>
<tr>
<td>Carrier</td>
<td>CLA</td>
<td>0.79c</td>
</tr>
<tr>
<td>Positive</td>
<td>Control</td>
<td>0.90b</td>
</tr>
<tr>
<td>Positive</td>
<td>CLA</td>
<td>0.74c</td>
</tr>
<tr>
<td>SEM</td>
<td></td>
<td>0.03</td>
</tr>
</tbody>
</table>

^a Means within a column with different letters are significant at P < 0.05.

Table 11. Least squares means® and standard errors of loin chops sensory panel attributes of three stress genotypes by experimental diet.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Diet</th>
<th>T^c</th>
<th>J^c</th>
<th>FI^c</th>
<th>OA^c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>Control</td>
<td>6.35</td>
<td>5.76</td>
<td>5.41</td>
<td>5.36</td>
</tr>
<tr>
<td>Negative</td>
<td>CLA</td>
<td>5.53</td>
<td>5.03</td>
<td>5.00</td>
<td>5.45</td>
</tr>
<tr>
<td>Carrier</td>
<td>Control</td>
<td>4.97</td>
<td>4.56</td>
<td>4.92</td>
<td>4.88</td>
</tr>
<tr>
<td>Carrier</td>
<td>CLA</td>
<td>5.16</td>
<td>4.76</td>
<td>4.88</td>
<td>5.13</td>
</tr>
<tr>
<td>Positive</td>
<td>Control</td>
<td>5.22</td>
<td>4.22</td>
<td>5.01</td>
<td>4.22</td>
</tr>
<tr>
<td>Positive</td>
<td>CLA</td>
<td>5.09</td>
<td>3.81</td>
<td>4.67</td>
<td>4.68</td>
</tr>
<tr>
<td>SEM</td>
<td></td>
<td>0.40</td>
<td>0.38</td>
<td>0.20</td>
<td>0.18</td>
</tr>
</tbody>
</table>

^a Means within a column with different letters are significant at P < 0.05.

^b SEM = Standard error.

^c T = tenderness, J = juiciness, FI = flavor intensity, OA = overall acceptability.
Figure 1. Least squares means of pH decline of loin at 10th and 11th rib for three stress genotypes.
Figure 2. Least squares means of pH decline in loin at 10th and 11th rib by experimental diet.
Figure 3. Temperature decline in the loin at 10th and 11th rib for three stress genotypes.
Figure 4. Temperature decline in loin at 10th and 11th rib by experimental diet
Figure 5. Least squares means of pH decline in loin at 10th and 11th rib for normal pigs by experimental diet.
Figure 6. Hunter L* values of loin chops over time of self-service case storage by experimental diet.
Figure 7. Hunter a* values of loin chops over days of self-service storage by experimental diet.
GENERAL DISCUSSION

The data from all three experiments presented in this dissertation have shown improvements in various aspects of pork production when CLA is supplemented in the diet at 0.75%. However, at times the results seem to be dependent on the genetics of the pigs. In this discussion it is appropriate to discuss the impact of CLA on pork production in general. The improvements we see in gain:feed should have a positive impact on feed costs to the producer. Savings in total feed consumed by pigs will increase a producer's profit margin if the cost of CLA is nominal. Furthermore, since CLA supplementation results in backfat depth decreases, a producer that sells their pigs on a value-based system might see an improved premium. A number of packer grids use backfat depth (10th rib or last rib) as one factor in calculating the lean value of pig carcasses. The purchase of pigs with less subcutaneous fat will add value to carcasses for the packer because they will not have as much trim fat that would potentially go into waste or would be sold at a much lesser value compared to lean muscle meat cuts or trim. The results for meat quality improvement with CLA supplementation does not seem to be consistent across differing genetic lines of pigs. We did see
an increase in marbling and firmness of the loinear in all of
the stress-free pigs, but these improvements were not carried
through in the stress-carrier and stress-positive pigs.
Furthermore, other measures of meat quality including
postmortem pH decline, color development in the self-service
case and factors influencing water holding capacity were not
as well defined and need further research in order to make
sound management decisions with regard to CLA supplementation
in the diet of growing-finishing pigs.

Recommendations for Future Research

When a research project is initiated, the researchers
have a set of questions or hypotheses that they hope to
answer. Usually, in the course of their studies, a myriad of
other questions arise. The three experiments reported on in
this dissertation have presented us with numerous questions
which are beyond the scope of the current project, but which
direct impact on the feasibility of incorporating conjugated
linoleic acid into a swine production system. Future research
should try to identify the mechanism by which CLA decreases
subcutaneous fat in pigs while increasing intramuscular fat in
the loin muscle. Furthermore, a project designed to study the
influence of CLA supplementation on the reproductive
characteristics of pigs is certainly warranted. We would not want to incorporate a nutritional supplement that might have negative effects on the reproductive efficiency of a swine system. Also, one is always interested in the economic feasibility of a feed supplement. It is difficult to determine the per pig cost of CLA supplementation at the current time. This is mainly due to the fact that CLA is not produced in quantities largest enough to sustain its use as an animal feed ingredient in the United States. As information regarding the cost of production of CLA is elucidated, the swine industry should have a better estimate of its cost as a feed ingredient. This information will help determine if the benefits attained from CLA will outweigh its cost and in fact increase the profit margin for pork producers.
I would like to thank some very important persons for their support during my pursuit of my Doctorate in Meat Science. There have been good times and tough times, but hard work and good luck have seen me through.

I wish to thank my wife Teresa for her support while I have completed nine years worth of higher education. At times I'm sure it seemed like it might never end.

My fellow graduate students deserve many thanks as well. We all know that graduate education and thesis writing could never be completed without a little help from our friends.

To Dr. Joe Sebranek, if all else fails we always have hunting, storytelling and college basketball.

Many thanks to Drs. Steven and Elisabeth Lonergan. You always had time to listen, gave great constructive criticism and most of all, you are great friends.

Most of all, I wish to thank my major professor Dr. F. C. Parrish, Jr. You have certainly given me opportunities that few other institutions could offer. Our discussions on life were a little crazy at times, but I think we are both better of for having them. Thanks for the lifetime worth of knowledge and for this I can never repay or thank you enough.