INFLUENCE OF CONJUGATED LINOLEIC ACID SUPPLEMENTATION ON BODY COMPOSITION OF WEANED PIGS

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Christina E. Lindsey

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Christina E. Lindsey

APPROVED:

Marcy M. Beverly, PhD
Thesis Director

Stanley F. Kelley, PhD
Committee Co-Director

Mark J. Anderson, PhD
Committee Member

Kyle J. Stutts, PhD
Committee Member

John B. Pascarella, PhD
Dean, College of Science and
Engineering Technology

DEDICATION

This thesis is dedicated to my parents, Laverne and Luis Espindola Jr. It was their support to enter a graduate program to pursue my dream. A dream worth having is a dream worth pursuing. It was their hard work every day and sacrifice of their dreams for me to have mine. For them, I am forever grateful and blessed to have their support all these years.

This thesis is also dedicated to my husband, Adrian Lindsey. From the early mornings to afternoon feedings, a summer worth of memories was made with you, I cannot thank you enough for all the support you have given me.

ABSTRACT

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Cross bred gilts (n=32), that were approximately 8 wk old with a mean BW of 16 kg, were used in a randomized complete block design to evaluate the effects of dietary supplementation of conjugated linoleic acid (CLA) on body composition of weaned, growing pigs. The pigs were blocked by weight and randomly assigned to a treatment group; control treatment group (n=8), 0.33% CLA (n=8), 0.66% CLA (n=8), and 1.0% CLA (n=8). Feed was distributed via self-feeders, ad libitum, and CLA supplementation was administered as an oral drench daily at 0600. Body weights were recorded every 7 d, 10th rib back fat thickness was measured every 14 d via ultrasonography, longissimus muscle area (LMA) was measured via ultrasonography on d 0, 42, and 84. Data was analyzed using the mixed procedure of SAS. There was no main effect of CLA supplementation on BW (P = 0.73), 10th rib back fat thickness (P = 0. 36) or LMA (P = 0.69); however, there was a difference in the 10th rib back fat thickness on d 70 and 84 of the trial. Pigs supplemented with 0.66% ($P \leq 0.02$) and 1.0% ($P \leq 0.05$) CLA had reduced back fat thickness compared to the control group. These data indicate that CLA supplemented at varying rates for 84 d has no effect on BW or LM, but may reduce back fat thickness measured at the 10th rib. Further studies are needed to explore the effects of dietary supplementation of CLA on body composition of growing, weaned pigs.

KEY WORDS: Swine; CLA; BW; LMA; Fat thickness

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CHAPTER I

Introduction

In recent years, obesity has become a major social concern. The American Heart Association has deemed this condition a disease. With childhood obesity on the rise, a treatment is needed to combat this problem. Children that are obese are found to suffer from health issues as young adults and carry these issues with them into adulthood, essentially shortening their overall life expectancies. A possible avenue to combat obesity is through the supplementation of conjugated linoleic acid (CLA). Previous studies have shown that CLA has decreased fat levels through an increase in lipid metabolism. This research showed success on rodent, models; however the effects of dietary supplementation on humans are still in question.

The closest animal research model to a human is swine (*Sus domesticus*). Due to metabolic similarities in digestion and a monogastric digestive system, swine can serve as a model of the physiological changes that occur in body composition when supplemented with a diet containing CLA these findings can be applied to humans. Present studies involving the supplementation of CLA in swine have evaluated circulating fatty acid profiles and overall fat level. Their digestive systems and physiological processes work much the same way as humans, with the process and breakdown of lipids and proteins. Stangl et al. (1999) suggested that the selection of pigs as a model reflects humans physiologically much better than other animal models as well as in *in vitro* assays.

CHAPTER II

Literature Review

Obesity continues to be a widespread issue and leading health concern in the United States. Childhood obesity, defined as having a body mass index in the 95th percentile for age and gender, has increased in the last four decades (Kelsey et al., 2014). The consequences of childhood obesity can go farther than childhood, and in turn, lead to adult obesity. The diseases that are associated with obesity at any age are those that are related to the cardiovascular system, type II diabetes, retinal and renal complications, non-alcoholic fatty liver disease, asthma, sleep apnea, polycystic ovarian syndrome, cancer, and other obesity-related disorders (Kelsey et al., 2014). Obesity affects the genders differently. As puberty begins, the patterns of weight gain and body composition begin to differ. These differences can greatly affect how obesity related issues would be dealt with later in life. Researchers are looking for possible treatments to alleviate the problem. One possible solution is conjugated linoleic acid (CLA) which, in different animal studies, has been shown to decreased body weight and increased lean muscle tissue. CLA is being marketed as a nutritional supplement for humans with claims for fat reduction, hypolipidemic, bodybuilding, and anticatabolic effects (Mougios et al., 2001). Research in the supplementation of CLA began in the 1980's and proved to possess convincing effects on body composition that was seen in numerous animal models such as pigs (Dugan et al., 1997) and mice (Park et al., 1997). With the positive results in the animal research, human data remains to be inconsistent (Wang and Jones, 2004).

CLA is a member of the omega 6 fatty acid family, a polyunsaturated fatty acid that is a part of a group of geometric isomers of linoleic acid (Wang and Jones, 2004).

CLA is a term used to describe geometric and positional isomers of the 18-carbon fatty acid with two conjugated double bonds. The term conjugated is describing the structure in the middle of the acyl chain that makes the chain more rigid because of the inflexibility in the methylene carbon between the two double bonds (Subbaiah et al., 2011). The structure of CLA is different from its precursor of linoleic acid, a leading member of the omega 6 fatty acid family (Hansen, 2002), which can offer different biological effects on animal and human health. The two isomers most studied for their biological effects are *cis-9, trans-11* CLA, the predominant isomer produced in ruminant mammals also known as bovinic acid (Bassaganya-Riera et al., 2002), and *trans-10, cis-12* CLA (Cordero et al., 2011). Although there are two isomers, they act individually, giving different effects to the overall cell absorption and function (Martins et al., 2011). The trans-10, cis-12 CLA isomer affects changes in body composition while the cis-9, trans-11 isomer was not correlated with body composition changes but rather increased growth (Park et al., 1999; Whigham et al., 2000). With the understanding that the two isomers act independently, Wang and Jones (2004) reported that the ratio of isomers affected the overall results in mice, introducing the idea of the independent actions of the isomers. CLA has been supplemented to improve production and meat quality such as feed conversion ratio, growth rate, average daily gain and intramuscular fat in swine (Martin et al., 2008). This fatty acid is produced in the rumen of ruminants and occurs naturally in the lipid fraction of ruminant meat and milk (Slujis et al., 2010). CLA is derived from linoleic acid via biohydrogenation, which happens in the rumen of cattle. This is influenced by microbial digestion, specifically by *Butyruvibrio fibrisolvens* (Dugan et al., 1999). There are positive biological effects associated with CLA, that include anti-carcinogenic properties, anti-atheroenic effects, anti-inflammatory, and anti-diabetogenic effects, especially in animal models (Corl et al., 2008). Previous data from Mougios et al. (2001), provides evidence for a fat lowering effect of CLA on healthy humans and a tendency toward lowering serum lipids, although there were undesirable effects including a decrease in high density lipoprotein (HDL)- cholesterol that was statistically significant.

CLA has shown to be a biologically active molecule that offers many different aspects to both human and animal health. CLA exerts direct effects on adipocytes, the principal sites of fat storage, and skeletal muscle cells, the principal sites of fat combustion by increasing lipolysis and enhancing fatty acid oxidation (Park et al., 1999). CLA modifies the expression and the activity of stearoyl coenzyme A desaturase that converts the saturated fatty acids to their respective Δ9 monounsaturated fatty acids leading to higher saturated fatty acid and lower monounsaturated fatty acid concentration in pigs fed CLA-containing diets (Cordero et al., 2011). CLA inhibits the Δ9 desaturase, which is a vital role in lipogenesis and adipogenesis (Subbaiah et al., 2011). CLA is a newly recognized nutrient that functions to regulate energy retention and metabolism (Stangl et al., 1999). Dietary supplementation of CLA has shown to affect overall body composition and appears in some studies to be an anti-carcinogen and displays anti-obesity effects (Park et al., 1999).

Feeding 0.05 - 1.0% of mixed CLA increased feed efficiency and reduced back fat without affecting total body weight in pigs fed longer than 10 wk. Feeding 0.07 - 0.5% to growing pigs for eight weeks increased feed efficiency and lean body mass while reducing fat deposition (Evans et al., 2002; Dugan et al. 1997). In a study performed by Whigham et al. (2000), supplementing pigs with 1.0% of dietary CLA resulted in reduced feed intake, improved feed efficiency, deposition of less subcutaneous fat, and an increase in lean muscle compared to pigs fed a diet supplemented with sunflower oil. Dietary supplementation of CLA improved average daily gain and gain:feed in pigs fed from 26 to 114 kg. Belly firmness increased by the addition of CLA to the diet due to a shift toward a higher concentration of saturated fatty acids and lower concentrations of unsaturated fatty acids (Thiel-Cooper et al., 2001).

Previous research performed by Corino et al. (2003) on heavy pigs for the production of Parma Ham was performed on subjects that began at 97 kg and increased to 172 kg of body weight. The block design of three dietary treatments was performed on 36 Large White pigs. The supplementation rates were control, 0.25%, and 0.5% of CLA on an as-fed basis. The mixture of CLA used was a 65% mixture of equal parts of *cis-9, trans-11* and *trans-10, cis-12* isomers. The pigs were slaughtered and a chemical analysis was performed to understand the chemical and sensory aspects that CLA supplementation could cause on the products such as taste and texture after products were smoked or cured. Chemical and sensory aspects of products were not affected by supplementation.

Research performed by Eggert et al. (2001), used 30 genetically lean pigs that incorporated three dietary treatments of CLA supplemented, sunflower oil supplemented, and a sunflower oil diet consumed by pigs of the CLA group. The supplementation rate was 1.0% of both oils. The length of the research trial was 7 weeks in length. Results indicated that the supplementation did not affect back fat thickness or longissimus muscle area. This could be due to the length of the trial. With a research period of only 7 weeks, the body would not absorb and integrate the supplement in to the cells.

The prevalence of obesity has increased in all racial and ethnical groups, but in women, obesity is greater among members of racial and ethnic minority populations than in non-Hispanic, white women (Johnson et al., 2006). Early onset of puberty is considered an intermediary factor on the life-course path to a number of diseases in adulthood including hormone-related cancers (Cheng et al., 2012). Female children who are obese at a young age do not grow to their maximum height once puberty is reached and are more likely to experience early puberty (Johnson et al., 2006; Papadimitrou et al., 2006). The white adipose tissue produces several hormonal proteins, including leptin, with numerous effects on metabolism, immune response, reproduction, and feeding behavior in rodents and humans (Di Giancamillo et al., 2009). There are numerous pubertal hormones associated with weight gain and growth. In some aspects, the supplementation of CLA can be harmful to the body. Leptin is a hormone involved in maintaining blood glucose levels by inducing insulin-mediated glucose disposal; therefore, it is reasonable to consider that reductions of plasma leptin levels by CLA affect insulin sensitivity (Wang and Jones, 2004).

CHAPTER III

Purpose and Objective

Childhood obesity has doubled in the last thirty years. Previous research has been conducted with conjugated linoleic acid (CLA) as a dietary supplement and it has been reported to reduce obesity through an increase in lipid metabolism, in rodents models. With limited data on monogastric animals, specifically swine and humans, the main question is if supplementing CLA will increase the metabolism of lipids and change body composition in juvenile pigs which have similar digestive and metabolic systems to humans and can serve as a model of the response to CLA supplementation in humans. The purpose of this study was to quantify the effects of CLA as a dietary supplement on body composition of weaned, growing pigs as a model of growing, prepubertal humans.

CHAPTER IV

Materials and Methods

Animals

Approval from The Institutional Animal Care and Use Committee at Sam Houston State University on procedures involving animals was obtained (Protocol #: 14-10-17-1028-3-01). Thirty-two female, crossbred pigs, approximately 8 wk of age, with an initial BW of 16 kg were randomly assigned to one of four treatment groups based on body weight (BW). For this study, a randomized complete block design was performed with four treatment groups.

Dietary Treatments

CLA supplementation was based upon the pigs having a daily DMI of 5.0% of their body weight. CLA was supplemented daily at a rate of 0% (control), 0.33%, 0.66% and 1.0% of the diet through the use of an oral drench. The supplementation rate equals 0.015% BW for the treatment group receiving 0.33% CLA supplementation; 0.033% BW for the treatment group receiving 0.66% CLA supplementation; and 0.05% BW for the treatment group receiving 1.0% CLA supplementation. Each pen contained one subject from each treatment group; resulting in four pigs per pen. All treatment groups were subjected to a 7-d adaptation period prior to the start of the study. The CLA source (BASF Corp., Florham Park, New Jersey) being utilized contained 55% CLA, a mixture of *cis-9, trans-11; trans-10, cis-12* CLA. The subjects had *ad libitum* access to water and feed through a self-feeder that was cleaned and filled weekly. The feed in use was a commercial pelleted ration (Nutrena® Minneapolis, MN) that met all nutritional requirements during this growth phase. Feed nutrients included: crude protein at 16.0%, minimum Lysine concentration of 0.85%, crude fat at 3.0%, and crude fiber at 7.0%.

Measurements

Over a 12-wk period, BW was collected weekly and CLA supplementation was adjusted accordingly. Ultrasonic measurements of back fat depth at the 10th rib were performed every 14 d and longissimus muscle area (LMA) was measured on d 0, 42, and 84 of supplementation. The ultrasound device was an Aloka SSD-500V (Corometrics Medical Systems, Wallingford, CT) with a 3.5-MHz, 12.5-cm linear array transducer and standoff (Superflab; Mick Radio-Nuclear Instruments Inc., Mount Vernon, NY). Vegetable oil was used as a conductive medium. The same technician made all measurements of LMA and back fat thickness throughout the study. When illness occurred, animals were quarantined and treated, but maintained on their CLA treatment.

Statistical Analysis

The data were analyzed using the mixed procedure of SAS with repeated measures to determine differences in BW, 10th rib back fat thickness, and LMA among treatment groups. Data were also compared within day to determine differences between dietary treatments.

CHAPTER V

Results and Discussion

Body Weight

There was no difference in body weight among the treatment groups. Figure 1 shows the change in mean body weight by treatment for the duration of the trial.

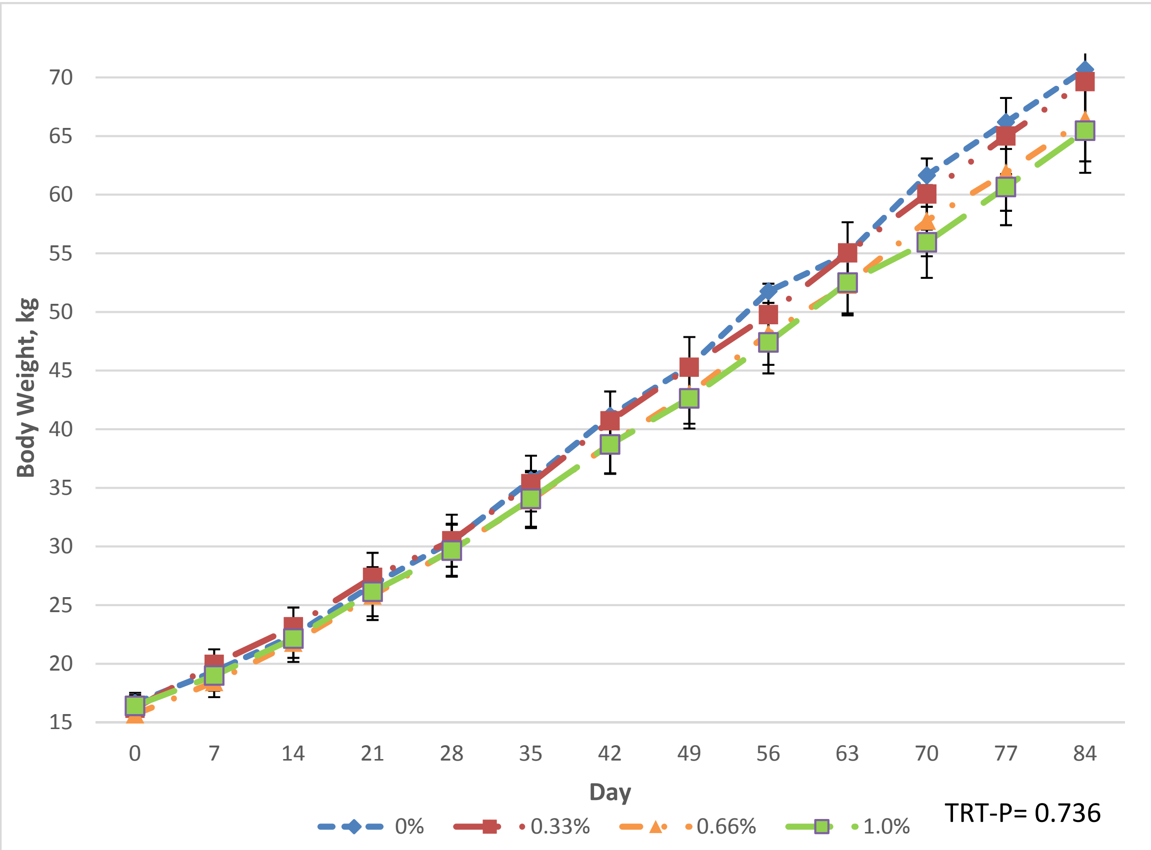

Figure 1: Weekly mean body weights (kg) of weaned, growing pigs consuming diets supplemented with 0% (control), 0.33%, 0.66%, or 1.0% CLA.

In comparison to other research in a similar area, Park et al. (1997) performed a study focusing on body composition of mice for 30 d. The mice were supplemented with a 0.5% CLA mixture with 5.0% corn oil, to reduce the rate of rejection. During the trial, the CLA-fed animals began to consume less feed around the same time, adding that the supplementation of CLA increased feed efficiency. Results indicate that the CLA supplemented group had a small reduction in body weight compared to the control group, but not at a significant level.

Morel et al. (2013) focused on average daily gain (ADG) of 48 pigs at a beginning weight of 19 kg during an 84-d trial. Two phases were completed during the course of the research. During phase 1, pigs were fed a diet with a 4.4% lipid content from d 1 to 56, and during phase 2, pigs were fed a diet with a 2.8% lipid content from d 57 to 84. The rate of supplementation for CLA was 2 g per kilogram of body weight. Results indicate that there was no difference in body weight.

Park et al. (1999) conducted a 30-d trial with mice. Mice were supplemented with CLA mixture containing different concentrations of various isomers of CLA. Two experiments were conducted during the 30-d study. In Experiment 1, mice were supplemented with 0.5% CLA, 0.3% CLA-1, or 0.25% CLA-2. In Experiment 2, mice were supplemented with 0.5% CLA-SF, 0.5% CLA-DC, or 0.9% CLA-DC. Increases in body weight were similar among the treated and control mice. The authors noted that the supplemented groups did not consume as much feed as the control group. This could indicate that feed efficiency was improved similar to results reported in a previous study by Park et al. (1997).

Eggert et al. (2001) performed a 7-wk study using 30 genetically lean pigs with a beginning weight of 75 kg. Supplementation rates were 1.0% CLA, 1.0% Sunflower oil, or a sunflower oil supplement at a restricted amount equal to that consumed by pigs in the CLA group. The supplementation did not affect the feed intake or feed efficiency. The authors proposed that no differences were observed due to the short duration of the trial. With the trial lasting only 7 wk, the body did not have adequate time to absorb and utilize the supplement.

Corino et al. (2003) used heavier pigs with a beginning weight of 97 kg with a supplementation rate of 0%, 0.25%, and 0.5% of CLA, using a 65% CLA with a mixture of isomers. Results indicate that the supplementation did not affect overall carcass weight or dressing percentage.

Back Fat Thickness

Figure 2 shows the mean 10th rib back fat thickness, measured bi-weekly by treatment group. There was no main effect of treatment on back fat thickness.

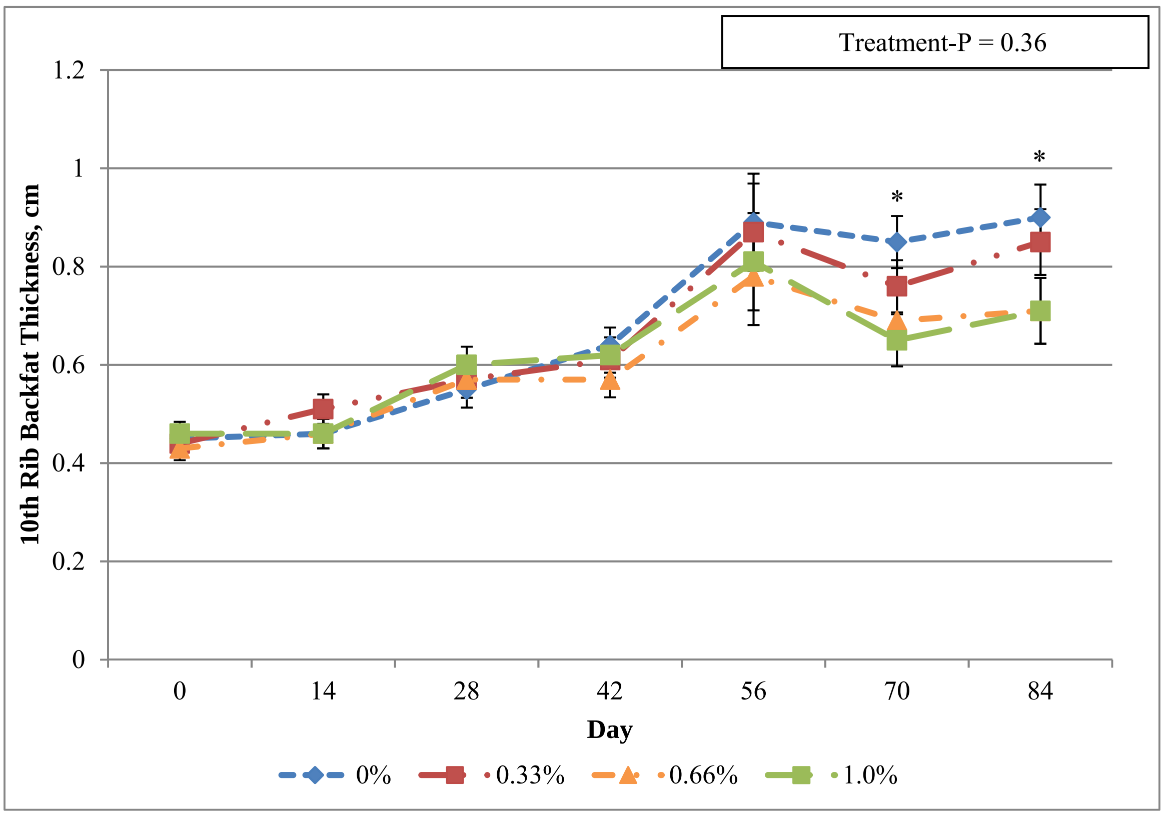

Figure 2: Bi-weekly mean 10th rib back fat thickness (cm) of weaned, growing pigs consuming diets supplemented with 0% (control), 0.33%, 0.66%, or 1.0% CLA.

Park et al. (1997) performed research on mice for a length of 30 d. The supplementation rate was 0.5% CLA delivered with 5.0% corn oil. Results indicated a decrease in body fat of mice with CLA compared to the control group.

Figure 3 contains the mean 10th rib back fat thickness by treatment group on d 70 of the trial. The treatment groups supplemented with CLA at 0.66% and 1.0% had a decreased ($P \leq 0.05$) fat thickness on d 70 compared to control.

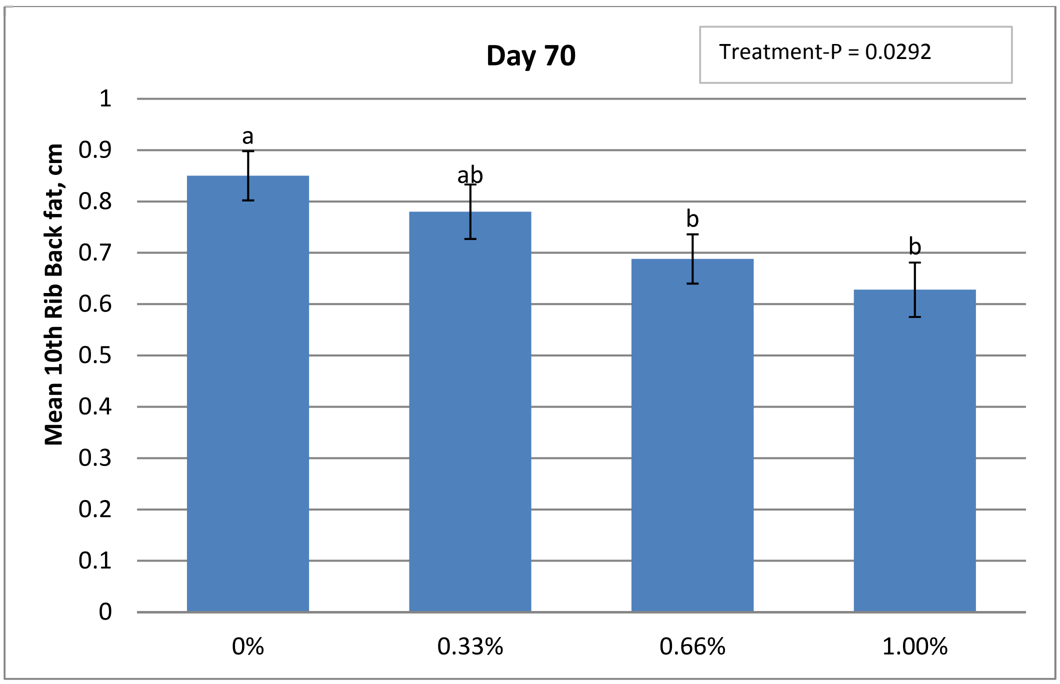

Figure 3: Comparison of 10th rib back fat thickness on d 70 of weaned, growing pigs consuming diets supplemented with 0% (control), 0.33%, 0.66% or 1.0% CLA.

Figure 4 contains the mean 10th rib back fat thickness by treatment group on d 84 of the trial. Similar to d 70, the treatment groups supplemented with CLA at 0.66% and 1.0% had a decreased ($P \leq 0.05$) fat thickness on d 84 compared to the control group.

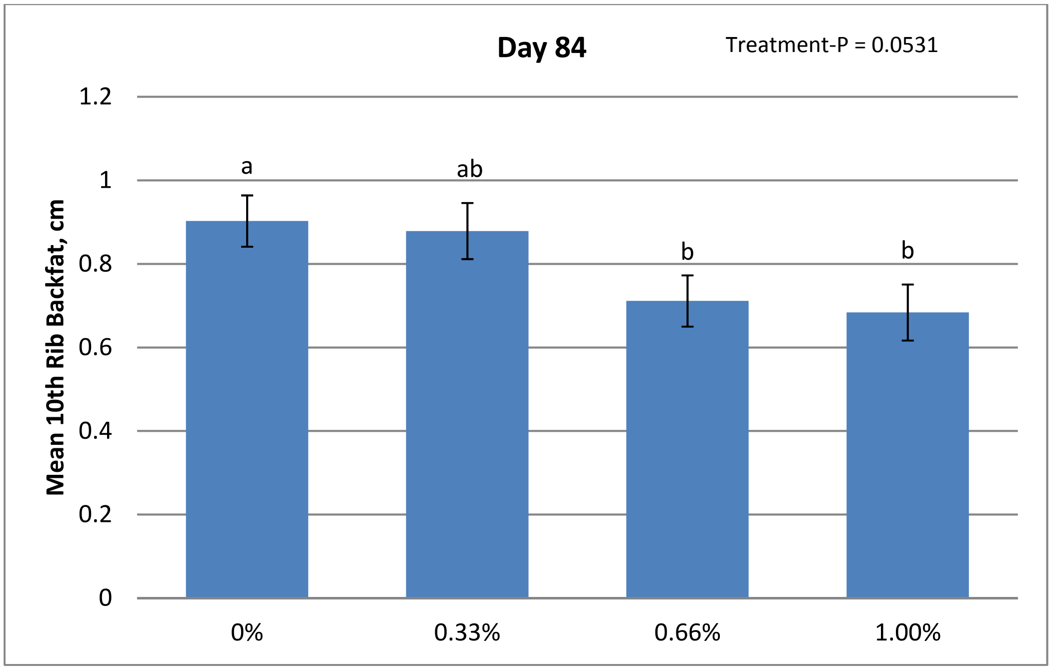

Figure 4: Comparison of 10th rib back fat thickness on d 84 of weaned, growing pigs consuming diets supplemented with 0% (control), 0.33%, 0.66%, or 1.0% CLA.

In comparison to previous research, Thiel-Cooper et al. (2001) used a supplementation rate of 0%, 0.12%, 0.25%, 0.50%, and 1.0% CLA in growing pigs. The source of CLA was a 60.5% CLA product with a mixture of isomers. Ultrasound measurements were taken at 52, 68, 91, and 114 kg. Results noted that the CLA-supplemented group had a lower 10th rib fat depth compared to the control group beginning at 91 kg and the difference was maintained through 114 kg at the 0.12% CLA supplementation level.

Park et al. (1999) conducted research on mice. Mice were maintained on a diet with a 6.0% fat content, using corn oil as a filler. The supplementation of crystallized CLA was used in this trial and it contained varying rates of CLA isomers. Two experiments were performed using various isomer distributions. In Experiment 1, mice were supplemented with 0.5% CLA, 0.3% CLA-1, or 0.25% CLA-2. In Experiment 2, mice were supplemented with 0.5% CLA-SF, 0.5% CLA-DC, or 0.9% CLA-DC. This research indicated a decrease in body fat in mice supplemented with CLA and it initiated the understanding that the CLA isomers effect the body differently when absorbed and utilized. The *trans-10, cis-12* CLA isomer affects changes in body composition while the *cis-9, trans 11* CLA isomer affects growth rates.

Corino et al. (2003) conducted a trial with pigs that had a beginning weight of 97 kg and an ending weight of 172 kg. The supplementation rate was a control, 0.25%, and 0.5% CLA on an as-fed basis. The length of the trial was not specified. In contrast to previous research results, this study indicated that back fat thickness was not affected by the supplementation of CLA.

Eggert et al. (2001), completed a study that focused on belly firmness. The research performed used 30 genetically lean pigs for 7 wk. The supplementation rate was 1.0% CLA, 1.0% sunflower oil, or a sunflower oil supplement at a restricted amount equal to that of the CLA supplemented group. The supplementation of CLA did not affect back fat thickness in these pigs. It was concluded by the authors that the use of genetically fatter pigs would result in a decrease of back fat thickness at a significant level.

Longissimus Muscle Area

Longissimus muscle area was not affected by CLA supplementation. Figure 5 contains the mean LMA for each treatment group on d 0, 42, 84 of the trial.

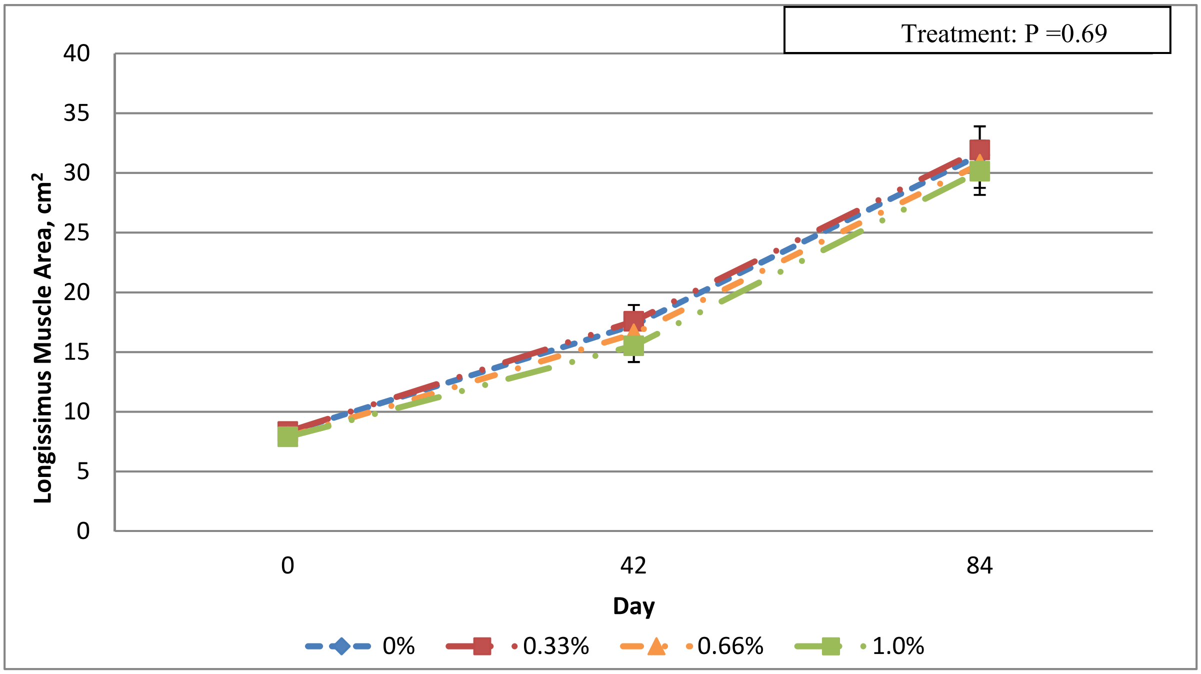

Figure 5: Day 0, 42, and 84 longissimus muscle area (LMA, cm^2) of weaned, growing pigs consuming diets supplemented with 0% (control), 0.33%, 0.66%, or 1.0% CLA.

No difference was detected in LMA among dietary treatments. From these results, we can interpret that muscle growth was similar throughout each of the treatment groups and the supplementation did not affect the growth and development of muscle of the pigs. Similar results were indicated in research performed by Martinez-Aispuro et al. (2014). The focus of that study was how a low protein diet with CLA supplementation would affect the overall growth and performance of pigs. The trial used 36 pigs with an initial weight of 17 kg and CLA supplementation at different phases of the life cycle. A 2x3 factorial design was used with two rates of CLA and three levels of crude protein. Six replicates per treatment were used for 21, 28, and 35 d during the nursery, growth and finishing phases. Results indicated that supplementing 0.5% or 2.0% CLA in the diet had no effect on LMA. The authors expected CLA supplementation to decrease back fat thickness and the resulting energy would be used to increase LMA. These results are supported by the current study as CLA supplementation had no effect on LMA.

In the research performed by Thiel-Cooper et al. (2001), pigs supplemented with CLA showed an increase in LMA at the 91 and 114 kg weight when compared to the control group when measured via ultrasound. Although this difference was noted on ultra sound measurements, the difference was not detected in carcass measurements; there was a decrease in LMA noted for the supplemented group.

Eggert et al. (2001), completed a study using 30 genetically lean pigs for 7 wks. The supplementation rate was 1.0% of a CLA, mixture containing 58% CLA. The results indicated that the supplementation did not affect LMA. This could be a result from the use of genetically lean pigs as well as the length of the trial.

CHAPTER VI

Conclusions and Implications

The results of this study indicate that the supplementation of CLA does not affect BW or LMA in young, growing pigs. However, supplementation of CLA at a rate of 0.66% of the diet or greater can reduce 10^{th} rib back fat thickness. With a difference detected at d 70 and 84, it can be concluded that if the length of the trial was extended the differentiation between the treatment groups would increase. With the subjects having ad libitum access to feed and water, this increased the opportunity to grow beyond normal amounts. This would increase the chance to develop more back fat due to the increase in available energy consumed, the use of approximately 8 wk old pigs would decrease the chance for abnormal growth. In their current growth phase, fat development would be minimal while overall growth is increasing. Further research is needed to determine if the length of time while supplementing the CLA needs to be extended since a level of difference was detected after d 70 of supplementation.

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VITA

Christina Espindola Lindsey

Education

Sam Houston State University

Master of Science in Agriculture May 2017

Thesis Title: Influence of Conjugated Linoleic Acid on Body Composition of Weaned Pigs.

Sam Houston State University December 2014

Bachelor of Science in Animal Science

Minor in Science

Experience

Sam Houston State University Huntsville, Texas

Research Assistant

Cared for and maintained research animals.

Assisted with data collection and analysis.

Processed blood samples for further lab analysis.

Performed lab analysis on plasma samples to measure fatty acid profile.

Professional Activities and Honors

Delta Tau Alpha, Agricultural Honor Society: Fall 2013- Fall 2014

Sigma Alpha, Professional Agriculture Sorority: Fall 2013- Fall 2014