CONJUGATED LINOLEIC ACIDS ALTER BODY COMPOSITION DIFFERENTLY ACCORDING TO PHYSIOLOGICAL AGE IN MOULARD DUCKS

A Thesis
presented to
the Faculty of California Polytechnic State University,
San Luis Obispo

In Partial Fulfillment
of the Requirements for the Degree
Master of Science in Agriculture

by

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June 2013

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ABSTRACT

Conjugated linoleic acids alter body composition differently according to physiological age in Moulard ducks

Jeffrey Allen Fesler

Surveys have shown poultry meat to be the most popular animal food product world-wide due to its nutritional, sensory and economical characteristics (Mulder 1997). Within the last 10 years, consumption and related production has increased globally (percentage increase) for chicken meat (53%), turkey meat (13%), duck meat (67%), and goose meat (53%) (Scanes, 2007). A problem exists in that poultry producers are unable to prevent excess fat deposition and this leads to decreased profitability. The generation of fat directs energy away from the production of muscle which delays the time required to produce a salable product. In addition, poultry do not respond favorably to growth promoting technologies because they are harvested prior to sexual maturity.

Conjugated linoleic acid (CLA) could be used as an animal supplement to improve the health content of meat products because research has shown dietary CLA to reduce milk fat content in cows and lower body fat in rodents, swine, poultry and humans (Dugan and Aalhus, 1997; Park et al., 1997; Blankson et al., 2000; Bauman et al., 2000; Badinga et al., 2003). CLA is also reported to have anticarcinogenic effects. Of immense significance are studies performed with rodents where rats received a CLA mixture (30% 9,11-CLA and 30% 10,12-CLA) 2 weeks prior to chemically induced carcinogenesis, and mammary tumors were decreased in incidence by more than 50% in

number when compared to the control (Ip et al., 1996). However, proposed mechanisms by which CLA exerts its lipid modifying effects *in vivo* and *in vitro* are diverse and conflicting. The lipid modulating capability of CLA varies in effectiveness across species, breed, age at starting diet, sex, and environmental conditions (Bauman et al., 2000). In addition, *in vitro* testing conditions are not consistent between research groups and variables such as, media type, age of cells, and trial length are highly variable (Belury, 2002). Results are further complicated by CLA often being fed as a mixture containing several isomers, which makes it difficult to propose a mechanism(s) for the action of a single isomer without including the possibility of a synergistic effect (Mersmann, 2002).

In the current trial, we investigated how CLA affects body fat accretion in the duck. We designed our experiment to include two distinct age groups of ducks to determine if the metabolic modifying action of CLA varied with age of dietary induction. The effect of CLA on growth and development was measured using several methodologies. First, tissues dissected from ducks fed CLA were compared to tissues from animals receiving the non-CLA (control) diet and differences in size and weight were analyzed. Next, the concentration of nonesterified fatty acids and glucose metabolites was measured in blood serum to investigate if CLA affected the energy balance in birds. Last, probes for key genes involved in lipid metabolism were cloned from the chicken, and real-time PCR was implemented to determine if CLA affected the mRNA abundance of those genes at the time of harvest.

Keywords: conjugated linoleic acid, body composition, adipose, duck, fatty acid synthase, acetyl Co-A carboxylase

ACKNOWLEDGMENTS

I would like to thank all those who helped me with my Master's thesis:

To Dr. Daniel Peterson for his guidance and the opportunity to complete this research.

To Dr. Brooke Humphrey for his expertise in poultry management.

To Dr. Scott Steinmaus for his insight and perspective.

To my family for their continued support of all my endeavors.

To Guillermo Gonzales (Sonoma Foie Gras), Dr. Bart Cousins (BASF), Dr. Matt Burd, Dr. Allan Pettey, Dr. Mark Edwards, Tasha Johnson, Shavahn Loux, Carrie Kimmel, Ryan Jackson, Thomas Mellin, Amanda Saribalis, Courtney Berger, Steve Soderstrom, Jake Olson, Aleece Diaz, Ashli Middleton, Kelly Shaw, Karen Shaw, Becky Edman, Rachel Junsten, Ashley Knoll, Laura Shuey, Monaza Asghar, Marguerite Basso, Brent Fujimoto, Kat Hillman, Kelly Martorana, the Peterson lab group and all the other individuals that put their time into this research.

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Chapter 1 – Review of the Literature

Introduction to CLA

CLA was first discovered in 1935 where researchers found that some fatty acids in milk contained double bonds that had a conjugated arrangement (Booth et al., 1935). This discovery, although novel, was not reexamined until 1978 where Pariza et al. discovered a substance found in cooked beef that possessed antimutanogenic properties. Upon identification of this substance, it was determined to be a fatty acid with conjugated bonds and was later identified as conjugated linoleic acid (Pariza et al., 2000). CLAs are a group of positional and geometric isomers of octadecadienoic (linoleic) acid having double bonds that are conjugated or contiguous (Park et al., 1997). The two double bonds in CLA can be in C positions 8 and 10, 9 and 11, 10 and 12 or 11 and 13, thus giving rise to the designation of a conjugated diene (Roche et al., 2001). All configurations cis-trans, trans-cis, and trans-trans are possible in each positional system.

Formation and Occurrence of CLA

CLA is formed naturally through the biohydrogenation of unsaturated fatty acids by bacteria in the rumen of animals (Figure 1; Harfoot and Hazlewood, 1997). The *cis*-9, *trans*-11 (9,11-CLA) and *trans*-10, *cis*-12 (10,12-CLA) isomers are formed as intermediates in the biohydrogenation process with the *cis*-9 to trans-11 modification being the predominant isomer found in dairy products and ruminant meat (Table 1;

Kritchevsky, 2000). In addition, individual isomers of CLA can be commercially synthesized by alkaline isomerization of linoleic acid,chemically through dehydration of castor oil, and by oxidation of linoleic acid free radicals in the presence of sulfur-rich proteins (Padley, 1997; Wallace, 2007; Dormandy and Wickens, 1987).

Health Benefits of CLA

In recent years, the advancement of chemical purification techniques has allowed researchers the ability to use pure CLA isomers in animal and cell culture experiments. This has led to the general consensus that all of the known physiologic effects of CLA are induced by 2 isomers: 9,11-CLA and 10,12-CLA (Figure 2; Pariza, 2004). The 10,12-CLA isomer has been shown to reduce adipose content in several animal models whereas the 9,11-CLA isomer is depicted as having anti-carcinogenic effects (Bauman et al., 2000). However, due to availability and cost of pure CLA isomers, CLA preparations (unless stated otherwise) for many experiments typically consist of a mixture of CLA isomers (usually 30% 9,11-CLA, 30% 10,12-CLA, 22% oleic acid methyl ester, 6% palmitic acid methyl ester, 4% steric acid methyl ester, 2% linoleic acid methy ester, 6% compilation of other cis-cis and trans-trans isomers).

Anticancer Effects of CLA

Animals with chemically induced cancer were among the first experimental models used to test the anticarcinogenic effects of CLA. Early experiments demonstrated that brushing CLA on the skin of mice 7 days, 3 days or 5 minutes prior to the topical application of dimethylbenzathracene (administered for mammary tumor induction) caused a significant reduction in the number of papillomas when compared to animals in the control group (Ha et al., 1990). This finding led researchers to determine if similar effects could be induced when CLA was fed as part of the diet. Of these experiments, Ip and colleagues demonstrated that chemically-induced mammary tumors in rats could be inhibited by 1% dietary CLA when fed for 34 days prior to a 10 mg intraperitoneal dose of methylnitrosourea to induce mammary tumors (Ip et al., 1996). In a similar study, a 1% CLA mixture fed to rats from 3 weeks to 21 weeks of age exerted a protective effect to decrease the incidence of mammary tumors by 51% and reduce the density of the ductal-lobular tree of tumorigenic mammary epithelial cells by 20% after receiving a dose of dimethylbenz[a]anthrac at day 55 of the experiment (Thompson et al., 1997). Oddly, antitumorigenic action of CLA is not effective in all animal models. For example, in severe combined immunodeficient mice inoculated with human breast adenocarcinoma cells, 1% dietary CLA decreased tumor growth by 73% (Visconneau et al., 1997), yet in $Apc^{Min/+}$ mice (a model for intestinal cancer) 1% CLA had no effect on tumor number or

size (Petrik et al., 2000). Likewise, CLA had no effect on tumor growth in Balb/c mice infused with metastatic mammary tumor cells at 8 weeks of age fed 0.9% CLA (Wong et al., 1997).

The *in vivo* variability of CLA's anticarcinogenic efficacy prompted researchers to investigate the activity of CLA *in vitro*. Using a variety of carcinogenic cell lines, CLA has been shown to inhibit the growth of MCF-7 breast cancer cells, human colorectal and prostate cancer cells, gastric cancer cells, Caco-2 colon cancer cells, and NMU rat mammary adenocarcinoma cells in culture (Chujo et al., 2003, Palombo et al., 2002, Liu et al., 2002, Kim et al., 2002, Ip et al., 2000). 9,11-CLA is reported to impart a time and dose dependent response. In a study examining MCF-7 cells (human breast cancer cell line), CLA was shown to inhibit the growth and proliferation of MCF-7 cells when the concentration of CLA was increased over the course of 12 days (comparison based on culture mediums supplemented with equivalent concentrations of linoleic acid) (Schultz et al., 1992). It has also been suggested that the action of CLA is tissue specific because some experiments have shown CLA to have no effect on the growth of cancer cells. For example, the tumorogenic activity of M4Beau (human melanoma cell line), PC3 (prostatic adenocarcinoma), DLD-1 (colon adenocarcinoma) or A-549 (lung non-small cell carcinoma) was not inhibited when supplemented with CLA (De La Torre et

al., 2005). Varying media conditions may also affect CLA delivery into cells which could obscure the anticarcinogenic activity of CLA.

The identification of the underlying molecular and cellular mechanisms by which isomers of CLA impart anticancer effects has yet to be identified but warrants further investigation. Researchers have postulated that mechanisms may include the modulation of eicosanoid synthesis and signal transduction, up-regulation of genes dependent on the transcription factor peroxisome proliferator-activated receptor, inhibition of DNA adduct formation induced by exposure to carcinogens and induction of apoptosis (Ochoa et al., 2004; Durgam and Fernandes, 1997). Several investigators support the idea that CLA disrupts the arachidonic cascade and inhibits eicosanoid synthesis (Azain, 2003; Belury, 2002; Ip et al., 1996; Kritchevsky, 2000). Eicosanoids modulate tumorigenesis in many tissues including mammary gland, skin, prostate and colon (Fischer, 1995). The most important eicosanoid precursor is arachidonic acid, which is synthesized from linoleic acid (a promoter of mammary tumors) by elongation and desaturation (Akalln and Tokusoglu, 2003). CLA accumulation in liver, mammary, and lipid fractions of human sera has led researchers to postulate that CLA decreases eicosanoid synthesis by limiting the amount of fatty acid substrate available for the production of arachidonic acid (Belury, 2002). Additionally, CLA has been shown to enhance PPAR-γ activation which

leads to decreased production of inflammatory mediators responsible for tumor growth, migration and differentiation (Yu et al., 2002; Coussens and Werb, 2002). Using rodents as the primary *in vivo* model for investigating the initiation, promotion and progression stages of carcinogenesis, 9,11-CLA appears to have a greater affinity to inhibit carcinogenesis in distinct species and tissue types (Belury, 2002). In particular, dietary CLA has been shown to reduce the growth rates of mammary and prostate cancer cells when implanted in mice (Visconneau et al.,1997), yet have no effect on tumorigenesis in the proximal colon or cecum of rats (Yang et al., 2001). In addition, other variables such as percent dietary inclusion, isomer purity, duration of administration and treatment application are thought to influence the inhibitory properties of CLA (Kritchevsky, 2000). Perhaps with the availability of pure CLA isomers, future studies using different animal models and culture media *in vitro* will aid in elucidating potential mechanisms for the action of CLA to provide more conclusive evidence for the role of CLA as an anticarcinogenic therapeutic.

CLA and Atherosclerosis

Reports that CLA exhibited antioxidant properties *in vivo* (Ip et al., 1991) and *in vitro* (Ha et al., 1990) and knowledge that oxidized derivatives of cholesterol (Cook and MacDougal, 1968; Imai et al., 1976) and oxidized LDL (Steinberg et al., 1989) were atherogenic prompted several investigations into the effects of CLA on atherosclerosis

(Kritchevsky, 2000). Compelling evidence that CLA could be used to combat atherosclerosis was derived from animal experiments in which rabbits and hamsters were fed an atherogenic diet. In one experiment, rabbits supplemented with 0.5g CLA/day were fed an atherogenic diet (14% fat and 0.1% cholesterol) from 12 to 22 weeks of age and had significantly lower LDL and triacylglycerol levels which resulted in 30% less cholesterol deposition in the aorta in comparison to animals receiving the basal diet without CLA (Lee et al., 1998). Additionally, CLA decreased the severity of established atherosclerosis in the arch and thoracic aorta by 31% and 30%, respectively (Kritchevsky, 2000). In a similar study, hypercholesterolaemic hamsters fed a 1.1% CLA mixture had 26% less cholesterol and 26% less aortic streak formation (Nicolosi et al., 1997). Interestingly, the antiatherogenicity of CLA in rabbits and hamsters is not consistent with findings in mice. In C57BL/6 mice (a model for diet-induced obesity) fed an atherogenic diet, CLA promoted fatty streak formation and increased serum HDL cholesterol (Munday et al., 1999). This finding is peculiar in that high serum HDL cholesterol is commonly found in animals showing decreased atherosclerotic development and plaque formation (Paigen et al., 1987; Luria et al., 1991). Considering that CLA increased fatty streak formation by a mechanism independent of the serum lipoprotein profile in C57BL/6 mice, yet decreased atherosclerotic plaque formation in rabbits and hamsters,

indicates that CLA cannot be regarded as antiatherogenic until more data is collected and the mechanism(s) for its differential action across species is discovered.

CLA and Diabetes

The use of CLA for the treatment of diabetes continues to be a possible alternative to prescribed therapeutics, yet its action to attenuate hyperglycemia and hyperinsulinemia is confounded by physiological changes in the test subject. For example, Zucker Diabetic Fatty (ZDF) rats are commonly used to evaluate novel diabetic therapeutics and/or dietary treatments because they spontaneously develop diabetes at 7–12 weeks of age due to β-cell decompensation (Ohneda et al., 1995). However, experiments in ZDF rats where hyperglycemia and insulinemia appear to be mitigated by dietary CLA also report decreased feed intake and body weight which by itself could account for this response (Tsuboyama-Kasaoka et al., 2000; Houseknecht et al., 1998; Ryder et al., 2001). Despite these findings, CLA has been shown to have functional similarities to insulin sensitizing thiazolidinediones (troglitazone) that are commonly prescribed to diabetics. In an experiment using pre-diabetic ZDF rats, 1.5% CLA and troglitazone normalized impaired glucose tolerance and improved hyperinsulinemia (Houseknecht et al., 1998). In the same experiment, researchers looked at the expression of genes known to be activated by troglitazone and found that CLA induced the same genes. Of particular interest was the dose-dependent activation of peroxisome proliferator-activated receptor-γ (PPAR-γ).

PPAR-γ is a nuclear receptor that binds fatty acid ligands (or troglitazone) and executes transcriptional functions as heterodimers with retinoid X receptor (RXR; (Kersten et al., 2000)). PPAR-γ is considered the master regulator of adipocyte differentiation, because it induces anabolic processes such as TAG synthesis, glucose uptake, and fatty acid uptake resulting in differentiation into an adipocyte (Berger et al., 1999). Troglitazones are known to activate PPAR-γ at nanomolar concentrations (Forman et al., 1995; Lehmann et al., 1995). The functional similarities of CLAs to troglitazones, specifically the action of CLA to attenuate symptoms of insulin-resistance and type II diabetes in animals (Tontonoz et al., 1997) may implicate PPAR-γ as a primary target for the mechanistic action of CLA.

CLA and Body Composition

Data from numerous experiments have shown that crude mixtures of CLA isomers decrease body fat accretion when fed to growing mice, hamsters, pigs and chickens (House et al., 2005). *In vivo* and *in vitro* studies have demonstrated that 10,12-CLA is responsible for inhibiting body fat accretion by modifying the activities of lipogenic and oxidative gene targets transcriptionally controlled by PPAR-α, SREBP-1c, and PPAR-γ (Belury, 2002).

Several researchers have postulated that the beneficial effects on body composition imparted by 10,12-CLA are due to a PPAR-α mediated mechanism resulting

in increased transcript abundance of genes responsible for β-oxidation (Moya-Camarena et al., 1999). Carnitine palmitoyltransferase (CPT) and acyl-CoA oxidase (ACO) are key enzymes involved in the β-oxidation of fatty acids and experiments have reported increased ACO activity in liver homogenates of growing mice and geese (Degrace et al., 2006; Zhang et al., 2009), and increased CPT mRNA transcript abundance in the skeletal muscle of rodents (Brown et al., 2003; Declercq et al., 1987; Evans et al., 2002; Martin et al., 2000; Park et al., 1997).

Additionally, 10,12-CLA is thought to interfere with sterol regulatory element binding protein 1c (SREBP-1c) which is believed to potentiate adipogenesis by upregulating PPAR-γ. Sterol regulatory element binding proteins (SREBP) are membrane bound transcription factors that enter the nucleus and activate the transcription of genes involved in cholesterol and fatty acid synthesis (Yoshikawa et al., 2003). There are 3 forms of SREBP: SREBP-1a, 1c and SREBP-2. SREBP-1a and SREBP-2 are known to activate genes responsible for cholesterol synthesis while SREBP-1c is a transcription factor activating genes encoding enzymes involved in fatty acid synthesis and driving the formation of TAG and phospholipids. The transcriptional activity of SREBP-1c is driven by the binding of fatty acid ligands to a liver x receptor (LXR) binding site which then forms an obligate heterodimer with RXR in the promoter area for SREBP-1c (Desvergne et al., 2006). Researchers postulate that 10,12-CLA has an antagonistic effect on the transcription of SREBP-1c by competitively inhibiting the

binding of fatty acid ligands to the LXR binding domain (Roche et al., 2002). Additionally, the upregulation of PPAR-α and resulting β-oxidation of fatty acids is thought to decrease lipogenesis by limiting SFA substrate availability for LXR (Tsuboyama-Kasaoka et al., 2000). Regardless of transcriptional mechanism to inhibit body fat accretion, researchers have determined that dietary CLA decreases the mRNA expression of several key enzymes involved in lipogenesis (Wang and Jones, 2004; Takahashi et al., 2003). Lipoprotein lipase (LPL), acetyl-CoA carboxylase-α (ACC), and fatty acid synthase (FAS) catalyze several coordinated reactions involved in the synthesis of fat. LPL is responsible for cleaving fatty acids from circulating lipoproteins in the bloodstream so that they can enter the adipocyte to be oxidized or serve as building blocks for lipid synthesis (Mersmann, 2002), while ACC and FAS are key enzymes in the lipogenic pathway. CLA has been shown to inhibit LPL activity in 3T3-L1 adipocytes and suppress ACC expression in hamsters, dairy cows, mice, and piglets (Baumgard et al., 2000; Corl et al., 2008; Lin et al., 2004; Zabala et al., 2004). Acetyl CoA carboxylase (ACC) is an essential control point in the synthesis of fatty acids because it catalyzes the carboxylation of acetyl CoA to malonyl CoA which is an irreversible step towards the synthesis of fatty acids and the suppression of ACC would suggest decreased production of fatty acids. Ironically, experiments with hamsters and mice have shown increased FAS expression while experiments in rats have shown decreased FAS mRNA transcript abundance with both trials reporting a decrease in adipose mass suggesting that FAS

expression is differentially regulated in CLA fed animals (Azain et al., 2000; Kang et al., 2004; Zabala et al., 2004).

In vitro studies using rodent derived clonal preadipocytes (3T3-L1 cells) support that 10,12-CLA is able to inhibit fat storage by preventing differentiation into an adipocyte (Bretillon et al., 1999; Choi et al., 2000). Under normal growth conditions, preadipocytes will proliferate and differentiate into adipocytes, and researchers have shown CLA to arrest this process in 3T3-L1 cells from day 2 to 8 of treatment in a dose dependent manner (Brodie et al., 1999). Thus, lipid filling is decreased, due to fewer mature adipocytes available for triacylglycerol absorption.

CLA as a Fat Repartitioning Agent for Livestock

If the mechanism of action for CLA can be determined, the potential for using CLA as a fat repartitioning agent could be instrumental in increasing profitability for food animal producers. Commercial livestock operations use key traits such as growth, feed efficiency, and body size to select animals for their breeding stock. Utilizing these criteria, producers still have difficulty identifying animals that excel in repartitioning nutrients towards the generation of lean meat and not fatty tissues. Adipose tissue is not a valued end product, and within the last 30 years consumers have identified that consuming products high in saturated fats can lead to future health complications

(Moloney, 2002). Genetic selection and growth promoting technologies have helped to reduce carcass fat and improve feed efficiency for livestock operations in the U.S. (Cartwright, 1991). Growth promoters are bioactive supplements administered at or around sexual maturity that modify the metabolism of ingested nutrients to positively influence the conversion of feedstuffs into animal products (Buttery and Dawson, 1990). Recombinantly derived somatotropin, β-adrenergic agonists, and anabolic steroids are growth promoters that have shown the greatest commercial value to significantly improve lean content of meat in mammals (NRC, 1994). However, a problem exists in that growth promoters are not applicable in poultry due to the young harvesting age of meat production birds. Keeping in mind that the cellular mechanisms driving adiposity are often similar among animals (Cartwright, 1991), it is possible to theorize that CLA could be used as a metabolic modifier in the poultry industry to decrease undesirable lipid depots. Hence, future studies designed to identify the underlying mechanism(s) for CLA's modulating action would be warranted from an economic stand point and could increase the effectiveness of producing healthier meat products in the poultry industry.

Chapter 2 – Conjugated linoleic acids alter body composition differently according to physiological age in Moulard ducks*

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* Accepted June 6, 2013 for publication in Poultry Science

Abstract

Conjugated linoleic acids (CLA) have been shown to have remarkable yet inconsistent metabolic effects in mice, rats, hamsters, chickens, cattle, and humans. In particular, effects on lipogenesis vary with tissue, physiological state and specie. In this study we tested the hypothesis that CLA would differentially affect ducks of the same genetic background but of differing age. Growing (7 wk) and maintenance (11 wk) Moulard ducks were grouped by age and fed a standard diet supplemented with either 5% soybean oil (control) or 5% CLA isomer mixture. Animals were harvested after 3 weeks or 6 weeks for assessment of body composition including adipose, liver, viscera, and empty carcass weight. Serum nonesterified fatty acid (NEFA) and glucose concentrations were evaluated, and gene targets were cloned from the duck to use in quantifying mRNA abundance for genes involved in lipogenesis (fatty acid synthase, FAS; acetyl-CoA carboxylase, ACC) and lipid oxidation (carnitine palmitoyl transferase-1, CPT-1) in liver tissue from maintenance animals. After 3 weeks, the growing CLA group exhibited a 24% decrease in dissectible adipose tissue ($P < 0.05$) while maintenance animals showed no significant diet effect. After 6 weeks, the growing CLA group exhibited a 20% increase in liver mass compared to the control ($P < 0.05$), but no diet effect on adipose tissue. Maintenance animals receiving dietary CLA had a 42% decrease in adipose tissue mass after 6 weeks, increased serum NEFA, ACC and CPT-1 mRNA after 3 and 6 weeks ($P < 0.05$), and increased FAS mRNA after 3 weeks of

treatment ($P < 0.05$). These data indicate that CLA have potent effects on lipid metabolism in ducks, but that these effects differ dependent on physiological age.

Keywords: conjugated linoleic acid, body composition, adipose, duck, fatty acid synthase, acetyl Co-A carboxylase, carnitine palmitoyl transferase

Introduction

Conjugated linoleic acid (CLA) isomers, a group of positional and geometric isomers of linoleic acid, have been studied extensively in a variety of experimental models due to their ability to modulate cancer, atherosclerosis, obesity, immune function and diabetes (Brown and McIntosh, 2003). CLA is a natural food component found in the lipid fraction of dairy products and other ruminant fats and consists of more than 16 possible geometric isomers with cis-9, trans-11 CLA (9,11-CLA) and trans-10, cis-12 CLA (10,12-CLA) being the most abundant in commercial preparations (Bauman et al., 2000).

The mechanism of action for CLA isomers to decrease body fat accretion in chickens, pigs, and rodents (Park et al., 1999; Du and Ahn, 2002; Corl et al., 2008) or to inhibit milk fat production in ruminants (Peterson et al., 2004) is not fully understood. Studies *in vitro* and *in vivo* have led researchers to postulate that 10,12-CLA affects two key transcription factors in the peroxisome proliferator-activated receptor (PPAR) family (α, γ) as well as sterol regulatory element binding protein 1c (SREBP-1c) to promote or inhibit the expression of downstream target genes responsible for β-oxidation and lipogenesis (Belury, 2002). Support for this concept has been documented in animal

experiments where dietary CLA has been shown to increase mRNA abundance for oxidative enzymes responsive to PPAR-α when fed to growing mice (Moya-Camarena et al., 1999), rats (Rahman et al., 2001), and geese (Zhang et al., 2009). Similarly, other studies have documented decreased PPAR-γ mRNA abundance in mice (Kang and Pariza, 2001) and SREBP-1c mRNA abundance in hamsters (Zabala et al., 2007) when CLA was fed as part of the diet. Alternatively, *in vitro* studies have reported that the addition of 10,12-CLA to the media of murine 3T3-L1 cells (Brodie et al., 1999), as well as porcine (Brandebourg and Hu, 2005) and human preadipocytes (Brown et al., 2003) inhibited cell differentiation and decreased PPAR-γ mRNA expression. Interestingly, a CLA mixture was also shown to increase lipid filling in the 3T3-L1 model, underscoring the complexity of the biological activity of CLA (Satory and Smith, 1999). The ability for 10,12-CLA to attenuate lipogenesis in the mammary gland was characterized using the bovine MAC-T mammary epithelial cell line where 10,12-CLA decreased mRNA transcript abundance for key genes involved in lipid synthesis, an effect postulated to be secondary to decreased proteolytic activation of SREBP-1c (Peterson et al., 2004). Taken together, these findings present considerable evidence that adipogenesis can be controlled, at least in part, by CLA through SREBP-1c and PPAR mediated pathways.

The application of CLA to decrease body fat accretion is of particular interest to food animal producers, as excess carcass fat decreases feed efficiency and profitability (Harris and Newman, 1994). Over the last 50 years, food animal producers have

implemented growth-promoting technologies to decrease production costs by improving feed efficiency, and enhancing lean content to satisfy consumer demand (Avendaño-Reyes et al., 2006). Interestingly, growth-promoting technologies (anabolic steroids, β-adrenergic agonists) are of limited utility in poultry because meat birds are harvested prior to sexual maturity (Buttery and Dawson, 1990). Hence, poultry producers have relied on stringent genetic selection to propagate high growth birds which unfortunately has led to animals that are proportionally fatter than unimproved lines (Cartwright, 1991). The ability of CLA to decrease adipogenesis *in vivo* and *in vitro* may provide a promising method to decrease fat accretion and improve carcass quality in growing birds.

In the present study, we assessed for the first time the effects of CLA in the Moulard duck, a specie not subjected to the intensive selection that broilers and turkeys have seen, and one that is often maintained beyond physical maturity under normal production practices. The broad objective of the study was to determine whether CLA affected body composition in growing or maintenance Moulard ducks. To help us understand potential mechanisms underlying the reduction we observed in adipose tissue mass in maintenance ducks, we cloned and sequenced portions of the duck fatty acid synthase (FAS), acetyl Co-A carboxylase (ACC), and carnitine palmitoyl transferase-1 (CPT) genes to assess their mRNA abundance in liver tissue from the maintenance group of animals by quantitative PCR, and measured serum glucose and non-esterified fatty acid (NEFA) concentrations.

Materials and Methods

Animals and Diets

All animal use was approved by the California Polytechnic State University Animal Care and Use Committee. For this experiment, 7 week old (n=60) and 11 week old (n=60) male Moulard ducks were provided by Sonoma Foie Gras (Sonoma, CA). Animals were grouped by age and randomly assigned to 20 floor pens (pens numbered 1 to 20, 10 pens/age group, 6 birds/pen, 1.83 m^2/bird). Diets were isoenergetic and differed only by the inclusion of either 5% conjugated linoleic acid (CLA diet) or 5% soybean oil (soy oil diet) as the source of fat (Table 2). Soybean oil was chosen as the lipid source for animals in the control group based on a similar content of linoleic acid and its commercial availability, and the oils were assumed to be isoenergetic. Diets were assigned based on pen number, where odd numbered pens received the CLA diet and even numbered pens received the soy oil diet (5 pens/diet/age group). Research from modeling growth characteristics in Moulard ducks has shown that growth potential is maximized from 5 to 10 weeks of age with a plateau effect occurring at 11 weeks of age (Marie-Etancelin et al., 2008). Thus ducks starting dietary treatment at 7 weeks of age were termed "growing" while ducks starting diets at 11 weeks of age were termed "maintenance" to signify that the animals were no longer growing and instead maintaining a body weight.

The CLA isomer mixture was a fatty acid methyl ester preparation supplied by BASF (LUTA-CLA 60) as an amber colored oil containing approximately 28% of the cis-9, trans-11 isomer, 28% of the trans-10, cis-12 isomer with the remaining 44%

containing oleic and other fatty acids. Animals were housed on a concrete floor layered with 3 to 5 cm of wood shavings. Each pen contained two bell-type waters that provided open access to water at all times. Throughout the trial, animals were maintained in a 14 hour light/10 hour dark cycle.

Soy oil and CLA diets were formulated to meet or exceed all nutritional requirements of ducks (NRC, 1994; Table 2). A basal diet was formulated and mixed that included all ingredients other than the oils, and each experimental diet was prepared every 7d by mixing either CLA or soy oil in a stainless steel gear driven commercial mixer (Hobart, Troy, OH). Diets were placed in sealed plastic containers and stored at 4°C to prevent oxidation of oil additives. Both diets were fed ad libitum as a mash.

Experimental Design

At day 21 and 42 after commencing dietary treatment, three ducks per pen for each age group were harvested in the morning by electrical stunning and exsanguination in accordance with industry standards. Feed was available at all times. Body weight was recorded by weighing the ducks in a box cart digital scale immediately prior to harvest. At the time of harvest, blood was collected and centrifuged at 825 g for 10 min at 4°C. Serum was collected and aliquots were frozen at -20°C for serum glucose and NEFA analysis. Five gram samples of right liver lobe were immediately snap frozen in liquid nitrogen and stored at -80°C for subsequent mRNA analysis. The liver weight was recorded by weighing the dissected right and left liver lobes on a digital scale. All adipose tissue from crop to vent was dissected and weighed. The viscera weight included

weights of heart, emptied intestine, lungs, kidneys, reproductive tract and all other organs removed from the abdominal cavity. The weight of the emptied carcass including skin and feathers was designated carcass weight. Feed intake was calculated by measuring the amount of feed required to refill feeders to a predetermined mark at the top of the feeder (measured 2 times/day/pen). Spillage could not be avoided and was assumed to be independent of dietary treatment.

Serum Analysis

Serum glucose concentration was determined by measuring glucose oxidase activity (Sigma Diagnostics, St. Louis, MO) and serum NEFA concentration was analyzed by a colorimetric method (Wako Pure Chemical Industries, Richmond, VA).

Cloning and Sequencing of Genes

Total RNA was isolated from frozen liver samples using a guanidinium thiocyanate-phenol-chloroform extraction (Chomczynski and Sacchi, 1987). Total RNA concentration was determined spectrophotometrically at 260nm. First-strand cDNA synthesis was completed using a Reverse Transcriptase kit (Promega, Madison, WI) according to manufacturer instructions with 1 μg total RNA using oligo (dT) as the primer. Primers for FAS, ACC, CPT, β-Actin, and 18S ribosomal RNA were designed according to published gene sequence information from the chicken and are reported in Table 3. The identity of the PCR products was confirmed by sequencing (Sequetech, Mountain View, CA), and percent homology to chicken, bovine, porcine and human sequence was compared using NCBI BLAST.

Quantitative Real-Time PCR

Real-time PCR was completed with the Applied Biosystems Fast SYBR Green Master Mix kit according to the manufacturer's instructions in a 25 μl reaction volume. Quantitative PCR primer pairs for FAS, ACC, CPT, β-Actin, and 18S ribosomal RNA were designed from cloned duck cDNA sequence for each gene of interest using Beacon Designer (Premier Biosoft International, Palo Alto, CA; Table 4). β-Actin was used as the endogenous control and non-template controls were included on each 96-well plate. Quantitative PCR was performed with a 7500 Fast Real-Time PCR System (Applied Biosystems, Carlsbad, CA) on liver samples from three animals for each dietary treatment. Data were analyzed using the $2^{-\Delta CT}$ procedure (Livak and Schmittgen, 2001) following verification that amplification efficiencies were similar. The cycle threshold values were calculated as the cycle number at which fluorescence of the sample exceeded threshold, which was determined by multiplying the SD of the baseline by 10.

Statistical Analysis

For each age group, data were analyzed using the general linear model in JMP (SAS Institute, Cary, NC) for the effects of pen location, diet, and treatment period duration as well as any interactions. In no case was there a significant effect that included pen location and it was subsequently dropped from the final statistical model, leading to a two-way ANOVA for the effects of diet, treatment period, and their interaction. When a main effect or interaction was significant in the two-way ANOVA ($P < 0.05$) individual unadjusted means comparisons were made using Fisher's LSD. All differences were considered significant at $P < 0.05$.

Results

Feed Intake

Feed intake was not affected by diet or treatment period for the growing or maintenance age groups of ducks ($P > 0.05$; Tables 5, 6).

Body Composition- Growing Animals

Dissectible adipose tissue mass was decreased after 3 weeks of treatment for growing animals receiving dietary CLA compared to control animals receiving the soy oil diet ($P < 0.05$; Table 5). Although an additional 3 weeks of treatment reduced the adipose deposition in animals receiving the CLA diet when compared to animals receiving the soy oil diet, this was not statistically significant. The control group of animals fed the soy oil diet also showed a reduction in dissectible adipose mass between the 3 and 6 week treatment periods, but this was not statistically significant ($P > 0.05$; Table 5). Liver mass was affected by diet and treatment period where dietary CLA led to a numerical increase in liver mass in growing ducks after 3 weeks when compared to the control, but this was not statistically significant ($P > 0.05$; Table 5). An additional 3 weeks of CLA treatment led to a decrease in liver mass that was numerically equivalent to the decrease seen in the control. Treatment period significantly increased final carcass weight and body weight in growing animals receiving either diet (Table 5).

Body Composition- Maintenance Animals

For the maintenance group of Moulard ducks, the effect of diet on dissectible adipose tissue was dependent on treatment period. Specifically, animals fed the soy oil diet gained adipose mass between the 3 and 6 week treatment periods (Table 6) while dietary CLA attenuated this adipose gain resulting in a significantly lower dissectible

adipose mass after 6 weeks of treatment as compared to animals receiving the soy oil diet ($P < 0.05$; Table 6). Liver weight, body weight, and carcass weight were not significantly different in either treatment group of adult Moulard ducks at any time point of the trial (Table 6). The CLA diet led to lower viscera mass compared to animals fed the soy diet ($P < 0.05$; Table 6).

Serum Glucose and NEFA

Serum glucose was not significantly different at 3 and 6 week harvest time points for the CLA and soy oil treatment groups for growing animals ($P > 0.05$; Table 5). Serum glucose was affected by treatment period in the maintenance group of animals. Specifically, after 6 weeks of treatment, serum glucose was significantly increased for animals in the maintenance group receiving either diet ($P < 0.05$; Table 6).

CLA treatment resulted in a numerical but non-significant elevation of serum NEFA in growing animals throughout the entire 6 week trial when compared to the control (Table 5). In the maintenance group of animals, dietary CLA significantly increased NEFA concentration at 3 and 6 week treatment periods ($P < 0.05$; Table 6).

mRNA Abundance

The cloned segments of the duck ACC, FAS, CPT, β-Actin and 18S rRNA ranged from 68% to 84% homology to bovine, porcine and human sequences, and between 94% and 100% homology to chicken sequence. Quantitative PCR was performed with liver tissue samples from the maintenance group of ducks as these animals exhibited the greatest magnitude effect on adipose mass after 6 weeks on the CLA diet. CLA led to an increase of FAS mRNA transcript abundance at 3 weeks of treatment (Figure 3; $P <$

0.05). After being on feed for an additional 3 weeks, FAS mRNA for CLA treated animals had decreased to a level near that of the control. ACC and CPT mRNA abundance were increased as a result of dietary CLA at the 3 week harvest time point when compared to animals of the same age in the control group (Figure 4, 5; $P < 0.05$). After 6 weeks of CLA treatment ACC and CPT mRNA levels remained elevated when compared to animals of the same age receiving the soy oil diet, but these differences were not significant.

Discussion

Effect of Diet on Body Composition in Growing Ducks

Growing animals receiving dietary soy oil or CLA had similar feed intake and increased body and carcass weights after being on the respective diets for 6 weeks. We expected this result as both treatment groups are in a physiological state of growth and therefore should increase in size until physically mature. This is in agreement with studies performed in mice, hamsters, piglets, chickens and humans where CLA modulated lipid metabolism without affecting body weight (Park et al., 1997; Thom et al., 2001; Du and Ahn, 2002; Zabala et al., 2004; Corl et al., 2008). Viscera mass was significantly decreased in growing animals in the 6 week treatment period group regardless of diet. Since both dietary groups were affected, it is assumed that this is a normal physiological change in Moulard ducks.

Although growing ducks in both dietary groups increased body mass over the 6 week trial, dissectible adipose tissue mass was significantly lower in the animals receiving the CLA diet in the first 3 weeks of treatment. At the end of the 6 week

treatment, the difference in adipose mass persisted but was not significant. A similar effect was reported in pigs where CLA reduced back fat thickness in growing gilts with no effect on live body weight (Ostrowska et al., 1999) and in mice where body fat was reduced by more than 50% in weanling mice receiving dietary CLA without affecting body weight (Park et al., 1997).

Several studies have shown dietary CLA to increase liver weight in growing mice (Degrace et al., 2004; Tsuboyama-Kasaoka et al., 2003) and broiler chickens (Badinga et al., 2003). In our study, CLA fed animals in the growing group had significantly larger liver mass than animals fed the soy diet after 6 weeks of treatment without a difference in feed intake. This finding is consistent with studies performed in laying hens where CLA fed animals had increased fat vacuolation and hepatic lipid infiltration resulting in increased liver weight (Cherian and Goeger, 2004). Additionally, experiments using mice attributed increased liver mass in CLA-fed animals to be a result of increased liver lipid accumulation (Delany and West, 2000) though such determination was not made in this experiment.

Effect of Diet on Body Composition in Maintenance Animals

CLA did not affect feed intake, body weight or carcass weight in physically mature ducks. These findings are similar to long term feeding trials conducted with 42 week old rats maintaining a body weight where animals fed 1.5% dietary CLA for 36 weeks did not display a significant difference in feed intake or body weight (Scimeca, 1998). Interestingly, in our trial there was a significant reduction in adipose mass in

response to the CLA diet in the maintenance animals after 6 weeks of treatment. There are limited data available involving the feeding of CLA to animals that are physically mature, and we are the first to report that CLA reduces adipose content in ducks in a maintenance state.

Unlike animals in the growing group, CLA treatment did not increase liver mass in animals maintaining a body weight. This finding is interesting considering that ducks in the growing group harvested after 6 weeks of CLA treatment had increased liver mass and were 1 week younger than ducks in the maintenance group harvested after 3 weeks. Hence, we expected ducks in the maintenance group to follow a similar trend and to have increased liver weight. Although the animals from both groups were not the exact same age at the time of harvest, the effect of feeding duration can be considered. Perhaps the action of CLA to increase liver mass plateaus as animals enter physical maturity as observed in the maintenance group. This possibility warrants further investigation.

Effect of CLA on Serum Glucose and Nonesterified Fatty Acids

Circulating glucose and NEFA are precursors for the synthesis and oxidation of fat, respectively, and these metabolites can serve as indicators of energy balance in animals. Since glucose is the principal carbon source for lipogenesis in avian species, an animal in a positive energy balance will utilize glucose to replenish glycogen reserves and then convert excess glucose into fat. In contrast, an animal experiencing the initial stages of fasting will cleave fatty acids from lipid storage depots so they can be released into the blood as NEFA and oxidized into a usable form of energy in muscle and liver

tissues. Several researchers have postulated that CLA decreases adiposity by inhibiting glucose uptake and promoting lipolysis in adipocytes (Ostrowska et al., 1999; Mersmann, 2002) while concurrently increasing insulin sensitivity in muscle and liver tissues to accelerate glucose absorption and oxidation (Houseknecht et al., 1998). Here, we evaluated glucose and NEFA concentrations in CLA-fed animals to determine if this mechanism was plausible. In this study, glucose concentration was not significantly different for either treatment group at any time when measured in the growing group of animals. In the maintenance group of ducks, glucose concentrations were elevated after 6 weeks of treatment for animals receiving both diets when compared to values measured at 3 weeks. Since both dietary groups were affected, it is assumed that this is an age related physiological change. Nonetheless, all values were within normal ranges specified for avian species (210-550 mg/dL; Candeletta et al., 1993).

CLA led to a numerical but non-significant increase in NEFA concentration in growing animals harvested after 6 weeks of treatment. In contrast, CLA significantly increased NEFA concentrations at both time points for animals in the maintenance group. Increased NEFA concentrations indicate increased rates of lipolysis (Ostrowska et al., 1999; Azain et al., 2000) and may, in part, explain the lower adipose tissue mass in the maintenance group of animals after 6 weeks of treatment. The fact that adipose mass increased in the control group between 3 and 6 weeks while remaining the same for the CLA group between the 3 and 6 weeks may indicate that CLA-induced lipolysis prevented the accumulation of lipid in adipose tissue that occurred in the control group.

Effect of CLA on CPT, FAS and ACC mRNA

Elevated serum NEFA concentration and reduced adipose mass in the maintenance group of ducks led us to investigate mRNA abundance for the CPT gene to determine whether the increased NEFA might also be destined for oxidation. Rahman and coworkers suggested that in rodent models, CLA elevated fatty acid oxidation rates via an increase in activity of the CPT enzyme (Rahman et al., 2001). CPT is considered the rate-limiting enzyme for fatty acid oxidation and plays a central role in the partitioning of fatty acids between mitochondrial oxidation or the accumulation as long-chain acyl-CoA and complex lipids in the cytosol. The present study showed that CLA significantly increased the transcript abundance of CPT in liver tissue after 3 weeks of treatment in maintenance animals. In the second 3 week period, the difference persisted but was not significant. These findings parallel those reported in a similar specie where male goslings fed 2.5% CLA from hatch to 8 weeks of age demonstrated increased liver CPT abundance when compared to animals in the control group receiving a soy based diet (Zhang et al., 2009). Elevated CPT transcript abundance combined with increased serum NEFA could indicate a metabolic shift towards lipid oxidation rather than synthesis. If the animals were in an energy deficit, we would expect to see a decrease in the abundance of lipogenic enzymes ACC and FAS, because de novo fatty acid synthesis can be inhibited by decreased substrate availability (Bergen and Mersmann, 2005). It was very unexpected that CLA increased the transcript abundance of lipogenic genes FAS and ACC in liver tissue from the maintenance animals at the 3 week treatment

period when compared to the control, because CLA treated animals had less dissected adipose tissue indicating decreased lipogenesis. Although this difference was only significant at 3 weeks, other researchers have reported similar findings in growing mice fed a 1% CLA mixture that exhibited increased rates of fatty acid synthesis in the presence of decreased fat deposition (Delany and West, 2000). The simultaneous increases in abundance of mRNA for genes involved in both lipid oxidation and lipogenesis indicates the complex nature of the effects of CLA isomers on metabolism and warrants further investigation.

Based on our findings, it appears that CLA holds potential as a potent metabolic modifier in poultry species, yet further research to expand these findings is needed to determine the mechanism of action for CLA so improvements in feed efficiency and carcass quality can be optimized.

Acknowledgments

The authors gratefully acknowledge Guillermo Gonzales, Sonoma Foie Gras, for funding and supplying Moulard ducks for these experiments. In addition we thank all the members affiliated with the Peterson Laboratory Group at California Polytechnic State University-San Luis Obispo, including the following faculty members Brooke Humphrey, Scott Steinmaus, Matthew Burd, Allen Pettey, and Mark Edwards for assistance in bird care and experimental sampling.

Table 1: Concentration of CLA in representative foods[1]

Food	CLA (g/kg fat)*	9,11-CLA isomer (%)*
Meat		
Beef, fresh ground	4.3	85
Chicken	0.9	84
Pork	0.6	82
Turkey	2.5	76
Seafood		
Salmon	0.3	nd
Shrimp	0.6	nd
Trout	0.5	nd
Cheese		
Cheddar	3.6	92
Parmesan	3.0	90
Romano	2.9	92
Dairy Products		
Butter	4.7	88
Milk, homogenized	5.5	92
Yogurt	4.8	84
Vegetable oils		
Maize	0.2	39
Olive	0.2	47
Safflower	0.7	44

[1]Table adapted from Kritchevsky (2000)
nd, not detectable.
* after Chin *et al.* (1992)

Table 2: Composition of experimental diets[1]

	Diet	
Ingredient (%)	Soy Oil	CLA
Corn grain, ground	63	63
Soybean meal	22	22
Soy Oil	5	-
CLA[2]	-	5
Sand	6	6
Dicalcium phosphate	2	2
Ground Limestone	0.7	0.7
Salt, white loose	0.6	0.6
L-Threonine	0.3	0.3
DL-Methionine	0.2	0.2
Vit/Min Mix[3]	0.2	0.2

[1] Calculated ME = 3116 kcal/kg, CP = 15.5% for both diets
[2] The conjugated linoleic acid (CLA) mix contained 28% of the 9c, 11t isomer, and 28% of the 10t, 12c isomer (BASF, Florham Park, NJ).
[3] Vitamin/Mineral mix formulated to meet or exceed nutrient requirements.

Table 3: Primer sequences for gene cloning and sequencing

Gene[1]	Primer Sequence (5'→ 3')	Product size (bp)	Designed according to chicken Genebank no.
FAS	Sense: TGCCTGCGTAGCCTTTGAAATGTG Antisense: GCTGAGTGCTTCACGGTTGATGTT	346	NM_205155
ACC	Sense: TTGCGTCGTCAATAGTGGCTCAGA Antisense: AGCAATCCCGACCAAAGAGAGAGA	405	NM_205505
CPT	Sense: ATCAACGAGTCAGACACCACAGCA Antisense:TCGCTGTTCAGAAGAGTTTCCATCCC	323	NM_001012898
18S	Sense: TCGCTCCCCTCCCGTTACTTG Antisense: CGCCTGCTGCCTTCCTTGG	318	AF173612
β-Actin	Sense: ACCCCTGTGATGAAACAAAACCC Antisense: GCGAGTAACTTCCTGTAACAATGC	265	NM_205518

[1]FAS, Fatty Acid Synthase; ACC, Acetyl Co-A Carboxylase; CPT, Carnitine Palmitoyl Transferase-1; 18S, 18S ribosomal RNA

Table 4: Primer sequences for quantitative PCR analysis

Gene[1]	Primer Sequence (5'→ 3')	Product size (bp)
FAS	Sense: ATGGGTCTCATTCCTTTGTGGCAG Antisense: AGCATTGACACGAGCCTCCAGAT	185
ACC	Sense: TGAAGAGGTTGGCTACCCTGTCAT Antisense: TTACGAAGATTGGAGAGCCTGGGA	143
CPT	Sense: ACTCTATCCACTTCCACGTCTCCA Antisense: TTCCATCCCGACAACAGAAGGT	178
18S	Sense: CTTTCGATGGTAGTGTCTGTGCCT Antisense: TCCTTGGATGTGGTAGCCGTTTCT	104
β-Actin	Sense: GGAATGGTGAAGGTATCAGCAGCAG Antisense: AAACCCATAAATGCGAGTAACCTTCC	175

[1]FAS, Fatty Acid Synthase; ACC, Acetyl Co-A Carboxylase; CPT, Carnitine Palmitoyl Transferase-1; 18S, 18S ribosomal RNA

Table 5: Growing Moulard performance during the course of the study[1]

	3 wk on diet		6 wk on diet			*P* value		
Variable	**Soy Oil**	**CLA**	**Soy Oil**	**CLA**	**SE**	**D*T**	**D**	**T**
Body Wt (kg)	3.70	3.54	4.36	4.23	0.06	0.97	0.10	<0.01
Carcass (kg)	2.99	2.83	3.63	3.50	0.49	0.87	0.04	<0.01
Adipose (g)	38.2	29.1	31.6	24.6	2.0	0.70	<0.01	0.054
Liver (g)	80.2	88.6	63.1	75.8	2.4	0.52	<0.01	<0.01
Viscera (g)	264.0	280.3	241.0	253.1	8.1	0.85	0.22	0.03
Serum glucose (mg/dL)	196.8	205.4	206.3	198.2	31.7	0.34	0.97	0.89
Serum NEFA (µM)	525.3	564.0	579.4	632.6	23.6	0.75	0.21	0.06
Feed intake (g/d/animal)[2]	51.3	51.9	43.4	49.7	2.1	0.35	0.26	0.10

[1]Values for each variable are means ± SE (n=15 birds/treatment). *P*-values are listed for the effects of diet (D), treatment period (T), or interactions (D*T). CLA = conjugated linoleic acid; NEFA = nonesterified fatty acids.

[2]Values are mean feed intake (g/bird per treatment group per d). Feed intake per pen was the amount of feed needed to refill each pen's feeder. These values were pooled weekly by pen and divided by the total number of birds in each pen and days to determine average gram intake/bird per treatment group per day (n=5).

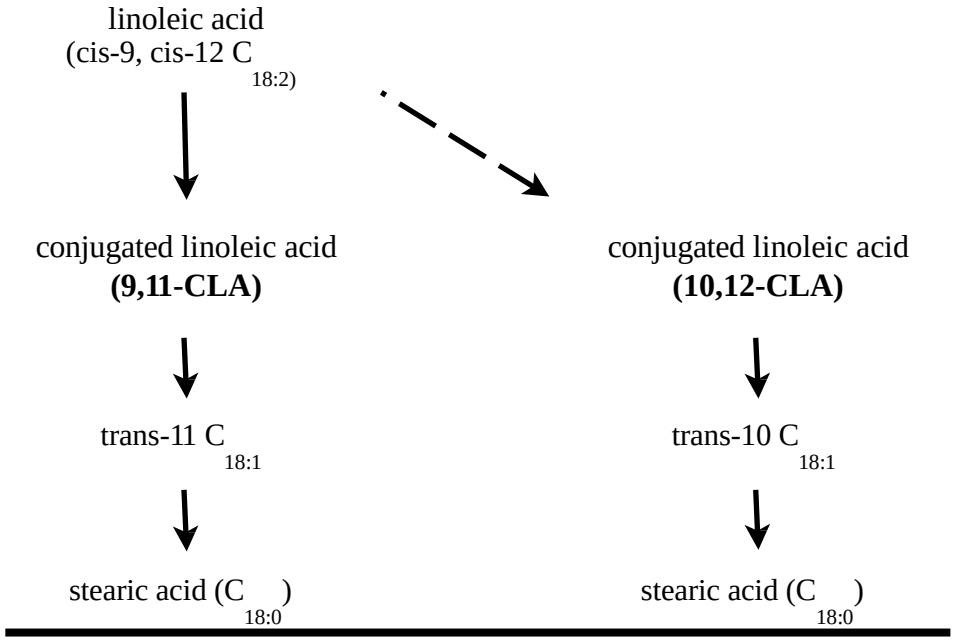
Table 6: Maintenance Moulard performance during the course of the study[1]

	3 wk on diet		6 wk on diet			*P* value		
Variable	**Soy Oil**	**CLA**	**Soy Oil**	**CLA**	**SE**	**D*T**	**D**	**T**
Body Wt (kg)	4.40	4.35	4.51	4.12	0.08	0.15	0.07	0.45
Carcass (kg)	3.74	3.68	3.82	3.53	0.06	0.26	0.09	0.60
Adipose (g)	14.0	18.2	31.6	18.1	2.5	0.02	0.22	0.02
Liver (g)	65.0	58.3	58.9	60.3	1.8	0.10	0.37	0.37
Viscera (g)	244.8	238.1	248.1	206.0	7.7	0.09	0.02	0.17
Serum glucose (mg/dL)	193.4	204.1	212.1	215.5	22.2	0.54	0.24	0.01
Serum NEFA (µM)	459.2	617.4	508.7	639.5	21.5	0.73	<0.01	0.21
Feed intake (g/d/animal)[2]	45.6	49.8	47.1	40.5	2.4	0.13	0.73	0.26

[1]Values for each variable are means ± SE (n=15 birds/treatment). *P*-values are listed for the effects of diet (D), treatment period (T), or interactions (D*T). CLA = conjugated linoleic acid; NEFA = nonesterified fatty acids.

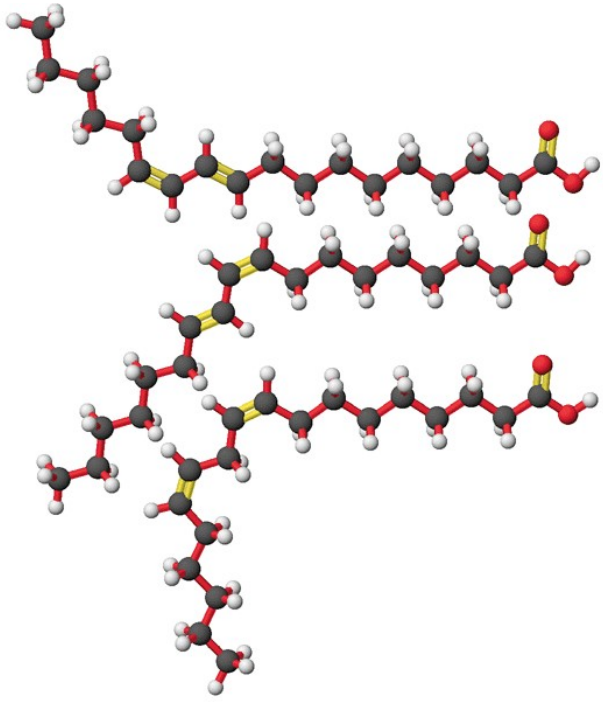
[2]Values are mean feed intake (g/bird per treatment group per d). Feed intake per pen was the amount of feed needed to refill each pen's feeder. These values were pooled weekly by pen and divided by the total number of birds in each pen and days to determine average gram intake/bird per treatment group per day (n=5).

Figure 1: Biohydrogenation of Linoleic Acid[1]



[1] Adapted from Bauman and Griinari 2003

Figure 2: Structure of Linoleic and Conjugated Linoleic Acids[1]



10,12-CLA

9,11-CLA

Linoleic acid (9,12)

[1]Figure adapted from the *Journal of Chemical Education* 1996; 73:A302-3.

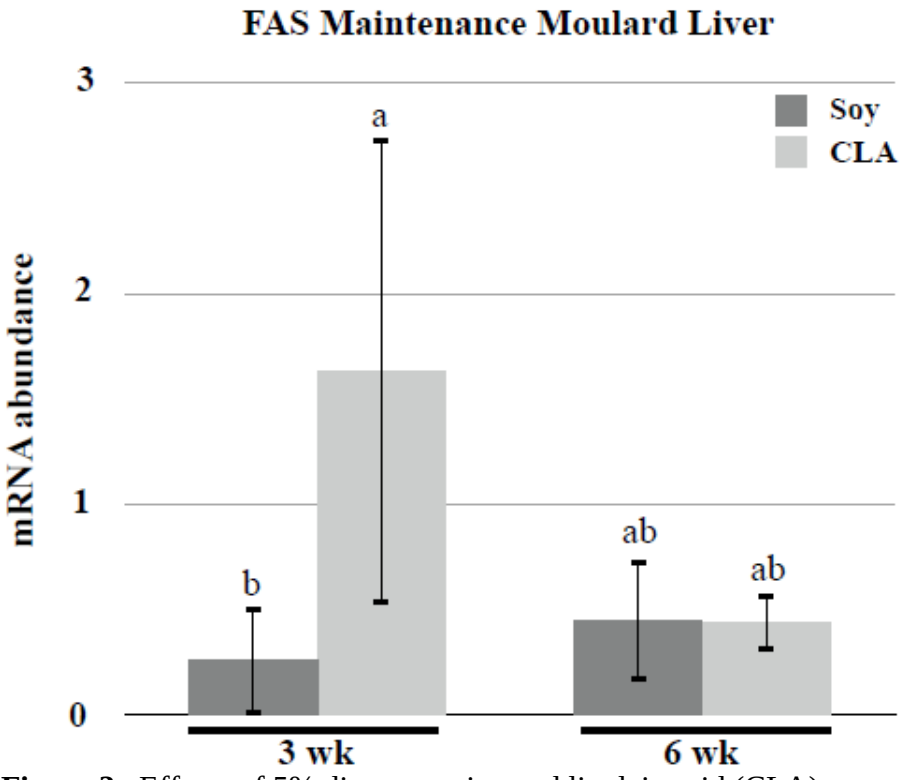


Figure 3. Effects of 5% dietary conjugated linoleic acid (CLA) or soy oil for 3 and 6 wk on fatty acid synthase (FAS) mRNA abundance in liver tissue from birds maintaining a BW. Data were normalized to the abundance of β-actin and expressed on an arbitrary unit scale. Error bars represent SEM; bars with differing letters (a,b) were significantly different ($P < 0.05$; n=3).

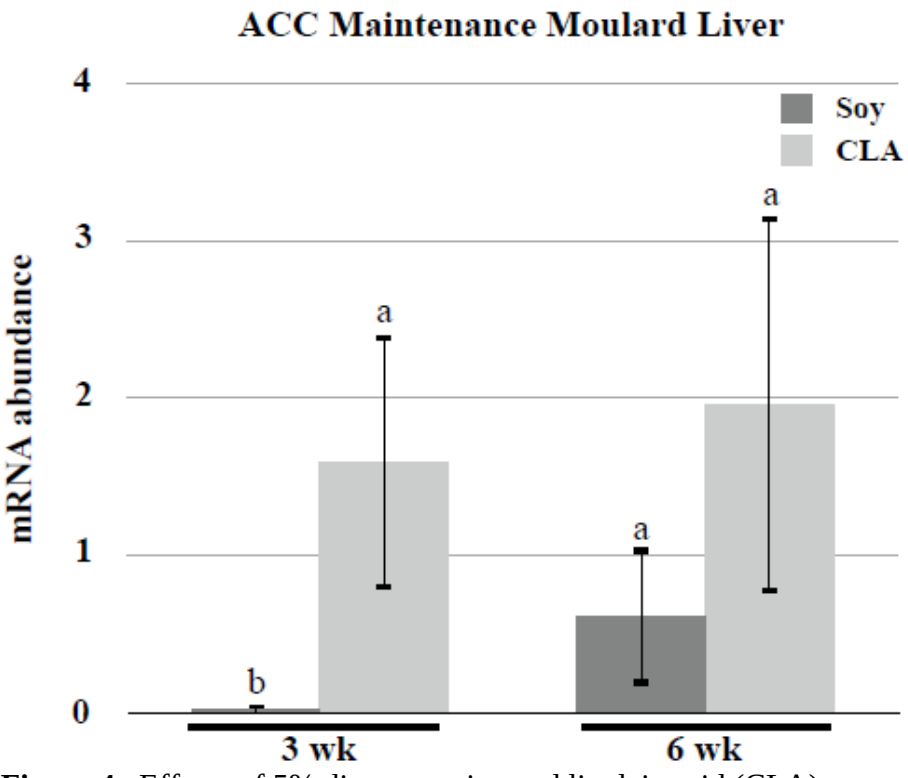


Figure 4. Effects of 5% dietary conjugated linoleic acid (CLA) or soy oil for 3 and 6 wk on acetyl-CoA carboxylase (ACC) mRNA abundance in liver tissue from birds maintaining a BW. Data were normalized to the abundance of β-actin and expressed on an arbitrary unit scale. Error bars represent SEM; bars with differing letters (a,b) were significantly different ($P < 0.05$; n=3).

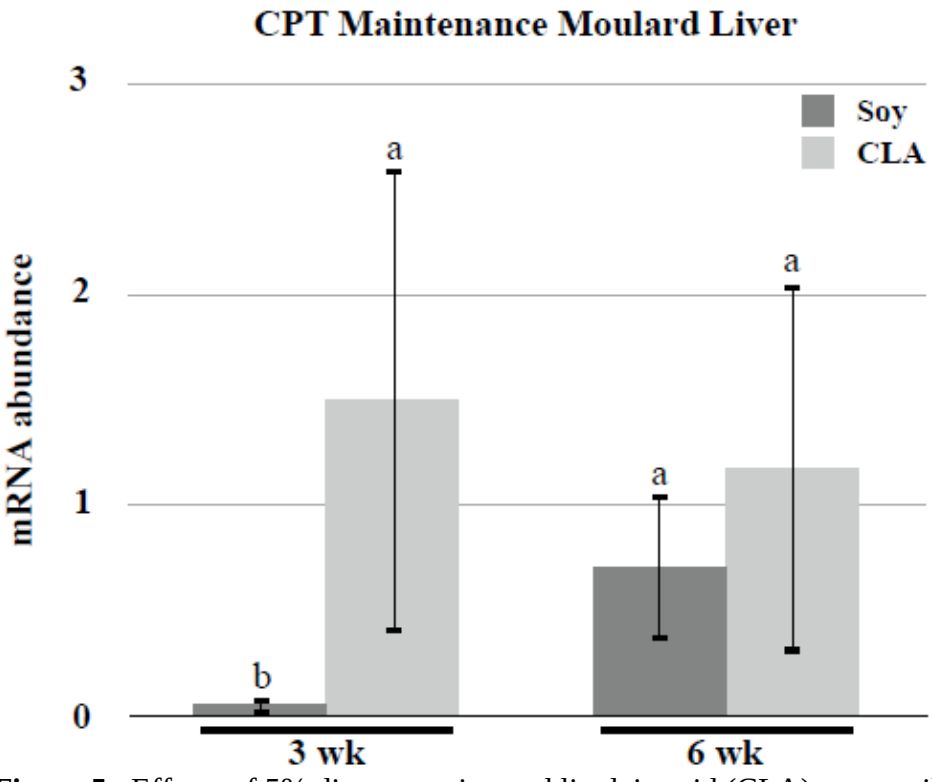


Figure 5. Effects of 5% dietary conjugated linoleic acid (CLA) or soy oil for 3 and 6 wk on carnitine palmitoyl transferase (CPT) mRNA abundance in liver tissue from birds maintaining a BW. Data were normalized to the abundance of β-actin and expressed on an arbitrary unit scale. Error bars represent SEM; bars with differing letters (a,b) were significantly different ($P < 0.05$; n=3).

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