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Enhancing Conjugated Linoleic Acid Content in Beef by Altering Dietary Forage Level, Dietary Unsaturated Fat Supplementation, or Feeding Rumen Protected Conjugated Linoleic Acid.
(Under the Direction of SUSAN K. DUCKETT)

Conjugated linoleic acid (CLA) acts as an anticarcinogen and repartitioning agent. CLA is naturally occurring in ruminant fat and results from the incomplete ruminal biohydrogenation of linoleic acid. In experiment 1, six duodenally cannulated steers were used in a 3 X 2 factorial arrangement (12, 24, or 36% forage; 2 or 4% oil) to determine a the dietary forage and oil level that increased CLA and C18:1-*trans* flow to the small intestine. In experiment 2, thirty-six heifers were fed one of three diets: 1) control (12% forage), 2) control plus 4% corn oil, or 3) control plus 2% rumen protected CLA for the last 32 or 60 days of finishing. Dietary treatments did not alter animal performance, carcass characteristics, or meat quality. Total CLA percentage was of longissimus muscle was greater for rumen protected CLA fed animals.

INDEX WORDS: Conjugated Linoleic Acid, Beef, Biohydrogenation, Fatty Acid Composition

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by

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REVIEW OF LITERATURE

Ruminant products, such as beef and milk, are perceived to be a major source, compared to other meat sources, of saturated fat in human diets. The difference in lipid composition between ruminants and non-ruminants tissues is due to their dissimilar gastrointestinal systems. Unlike monogastric animals who's fat composition tends to mirror that of their diets, ruminant fat makeup is very different from that of their diets. When ruminants consume unsaturated fatty acids, they are usually hydrogenated to saturated fat in the rumen (Jakobsen 1999). Consequently, a feedlot steer can consume primarily linoleic (C18:2 c9, c12) acid, yet the digesta at the small intestine will be mostly stearic (C18:0) acid. Since eighteen carbon fats are greater than 75% absorbable, the lipid present in beef is nearly 40% saturated. Much of the monounsaturated fat content of beef is due to desaturation of stearic to oleic acid in the adipose tissue (Demeyer and Doreau, 1999).

The process by which polyunsaturated fats are ultimately hydrogenated into saturated fats is termed ruminal biohydrogenation. As products with higher levels of saturated fat like meat and milk, became associated with human health concerns such as heart disease, research efforts were initiated to increase the unsaturated fat content, but the biohydrogenation process limits these efforts. However, recent discoveries of a biohydrogenation intermediates having the potential for improving human health has lead researchers to investigate ruminal biohydrogenation further. Conjugated linoleic acid, the biohydrogenation intermediate of interest, has resulted in a large body of research into its origins and how altering ruminal biohydrogenation may enhance its content in meat and milk products.

Conjugated Linoleic Acid and Human Health

Conjugated octadecadienoic or conjugated linoleic acids (CLA) are a group of positional and geometric isomers of linoleic acid. They are characterized by double bonds that are separated by a single carbon-carbon bond rather than a methylene group. Bonds may possess *cis-cis*, *cis-trans,* or *trans-trans* geometries (Figure 1.1; Bessa et al., 2000). CLA has most recently received attention following discoveries of its potential impacts on human health.

Work in the 1980's designed to find potential carcinogens in fried hamburger actually discovered compounds that reduced the incidence of cancer in animal models. Following further investigation, CLA, present in the beef fat, was determined to be the anticarcinogenic agent. Topical application of crude CLA extract (Pariza and Hargraves, 1985) or synthetic CLA (Ha et al., 1987) reduced the proliferation of epidermal cancer in mice with induced epidermal papilomas. Further research showed that CLA exhibited anticarcinogenic effects on mammary, stomach and prostate cancer cells of human origin (Whigham et al., 2000).

Ha et al. (1990) analyzed CLA incorporated into animal tissues to determine the biologically active isomer(s). Although all isomers were present in the adipose tissue, only the *cis*-9, *trans*-11 (c9, t11) isomer was present as a membrane phospholipid. Thus, the c9, t11 isomer of CLA was determined to be the isomer of interest in cancer studies.

This conclusion corresponds with naturally occurring CLA composition; consisting primarily of c9, t11 (approximately 80-90% of milk fat CLA; Parodi, 1977 and 60% in beef fat; Shanta et al., 1994) and because CLA taken directly from beef fat or butter has been successfully used as an anticarcinogenic agent. Furthermore, synthetic CLA, also used in laboratory situations, has a high content of c9, t11.

Besides c9, t11's anticarcinogenic effects, CLA has been found to have additional health benefits. As an immune modulator, consumption of CLA may help preserve lean body mass during immune response and lessen the immune reaction to allergens (Cook et al., 1993). Research involving mice and hamsters has shown potential antiatherosclerosis effects from CLA (Whigham et al., 2000). Researchers have also explored CLA as an energy-repartitioning agent. When mice were fed 0.5% CLA (approximately equal concentration c9, t11 and *trans*-10, *cis*-12 isomers), supplemented mice exhibited a decrease in body fat while body water and protein increased (Park et al., 1999a). In a follow-up study, mice were fed CLA consisting of primarily c9, t11 or *trans*-10, *cis*-12 (t10, c12). The same changes in body composition were observed in the mice supplemented with additional t10, c12 concluding it is the CLA isomer responsible for energy repartitioning (Park et al., 1999b). In a review by Whigham et al. (2000), additional studies involving rats, pigs, and initial work in humans related to CLA reported changes in body composition.

Current CLA Consumption

Given the wide range of potential benefits from CLA, studies have been completed to determine how much CLA humans currently consume and how much would be required to realize health benefits. In the United States, reported intakes seem to be rather variable ranging from 20 to 290 mg/d, partially due to different means of data collection (Ritzenthaler et al., 2001). In an effort to compare measurement techniques Ritzenthaler et al. (2001) measured CLA consumption by adults (Figure 1.2). In all cases food duplicates reported mor CLA consumed compared to written records. Using food duplicates men and women consumed approximately 212 mg and 151 mg, respectively, of CLA per day. Intake of c9, t11 was 193 and 140 mg per day for men and women, respectively.

Using animal models and data, Ip et al. (1994) found a dose dependent response in inhibition of mammary tissue tumors when rats were fed between 0.05 and 0.5% dietary CLA. Considering the total quantity test subjects consumed and, on average, diets containing about 0.03 mg of c9, t11 per 100 g of dry diet, total consumption of c9, t11 may need to be about 620 and 441 mg/d for men and women, respectively (Ritzenthaler et al., 2001). These numbers are based on extrapolation, as little human CLA research is available. It is also noted that in laboratory animal research models extremely high doses of carcinogens are administered, meaning the effective level for humans in contact with fewer carcinogens may be considerably lower.

To determine if increased CLA consumption also led to increased blood and tissue accumulation in humans, researchers in Sweden used men who volunteered information about their milk consumption as well as adipose and blood serum samples (Jiang et al., 1999). These researchers found a significant correlation (r = 0.42) between the amount of c9, t11 present in adipose tissue and milk intake

By using food duplicate studies Ritzenthaler et al. (2001) noted the foods contributing ninety-seven percent of CLA was of animal origin, with 60% being from dairy products and 32% from beef. Similarly, c9, t11 came predominantly from dairy (68%) and secondarily from beef (25%) sources.

Formation of Conjugated Linoleic Acid in Ruminants

We have long known CLA is a unique fat naturally occurring in ruminant tissues and products. With the focus on human health and notions such as "the optimum approach to conquering cancer is prevention" (Parodi 1997) attention has turned to enhancing naturally occurring CLA levels.

CLA, specifically c9, t11 and t10, c12, arises from the incomplete ruminal biohydrogenation (BH) of linoleic and to a lesser extent linolenic acid. In 1966 Kepler et al. expanded on previous work regarding biohydrogenation of long chain unsaturated fats by rumen microorganisms and the conversion of linoleic acid to various eighteen carbon fatty acids by *Butyrivibrio fibrisolvens*. Their work, since repeated and further developed, resulted in the pathway outlining the conversion of linoleic acid to stearic acid (Figure 1.3).

Upon ingestion of fat, microbial lipases hydrolyze ester bonds, resulting in free fatty acids. Unsaturated fatty acids containing a *cis* bond in the 9 and 12 position are then subject to isomerization by rumen microbes having linoleate isomerase activity. The isomerization results in the c9, t11 or t10, c12 isomers of CLA. The *cis* bond is rapidly hydrogenated resulting in an accumulation of c18:1 *trans* (10 or 11). The final step of BH of linoleic acid is the hydrogenation of the *trans* bond resulting in stearic acid (Bauman et al., 1999).

Since Kepler et al.'s initial work, many other rumen bacteria capable of isomerization and hydration have been discovered. Consequently, there are numerous known and presumably unknown BH pathways for a variety of unsaturated fatty acids present in ruminant diets. The variety of microorganisms present, unsaturated fatty acids fed and potential BH intermediates and end products may help explain the vast array of CLA isomers. However, related to biologically active isomers, another fatty acid of interest is linolenic acid. Linolenic acid undergoes a series of events including isomerization and reductions, again potentially resulting in c9, t11, c18:1 *trans* or ultimately stearic acid (Bauman et al., 1999).

Fatty acids undergoing BH have the potential of escaping the rumen at any point. Consequently, one method of CLA accumulation in fat of ruminant origin is escape of CLA from the rumen and subsequent absorption at the small intestine. However, because the rumen is a highly reduced environment, the *cis* bond is readily hydrogenated. Fewer rumen microorganisms have the ability to hydrogenate the resulting monenes such as *trans*-vaccenic acid (TVA; C18:1 *trans*-11) making this the rate-limiting step in BH. Despite this limitation the majority of c18:1 *trans* is hydrogenated to stearic acid thus terminating the BH pathway (Bauman et al., 1999).

The CLA, resulting from incomplete ruminal BH, is able to pass through the rumen and be absorbed through the small intestine becoming available for incorporation into triglycerides. Once eighteen carbon lipids reach the small intestine they are readily absorbed (Demeyer and Doreau, 1999). However, discrepancies existed between the amount of c9, t11 present at the duodenum (escaped complete BH) and the quantity present in ruminant fat tissue or milk; simply stated not enough c9, t11 escapes to account for the concentration in ruminant products. Griinari et al. (2000) postulated the activity of stearoyl CoA desaturase or Δ^9 desaturase present in the adipose tissue was responsible for the additional c9, t11 found in ruminant fat. After infusion of TVA into the abomasum of lactating dairy cows the concentration of c9, t11 increased. Additionally, infusion of a potent Δ^9 desaturase inhibitor (sterculic acid) resulted in a significant decrease in c9, t11 production. Using quantitative measurements they concluded that approximately 64% of the CLA in milk fat was from the desaturation of TVA.

Backed by Griinari et al. (2000) and Kepler et al.'s (1966) research, natural CLA sources are traced to ruminal escape of BH intermediates. Besides natural animal sources, CLA can be made synthetically by reacting linoleic acid with a base in the presence of heat. Chin et al. (1992) reports about 95% of linoleic acid will be converted to CLA, with approximately 43% being the c9, t11 isomer and 44% being t10, c12. However, consistent with the rise in functional foods, or foods possessing more than just the six essential nutrients, the focus has been to enhance the CLA content in ruminant products; improving natural sources (Bauman et al., 1999).

Altering Conjugated Linoleic Acid Content in Ruminant Products

Prior to the interest in CLA for human health promotion, it was noted that the CLA content in milk fat differed based on season, location, animal diets, and even from animal to animal within the same herd (Bauman et al., 1999). For the most part the differences were a result of diet.

Dietary Forage Level

In beef cattle, the most obvious differences in CLA concentrations exist between grain and grass fed cattle. Using steers fed five different diets ranging from entirely grass to 80% concentrate, French et al. (2000) observed increasing forage content also led to a linear increase in CLA concentration in the longissimus. They summed up the relationship with the following equation: CLA concentration = -0.79(kg of concentrate intake) + .98 (r = 0.83) with CLA (g/100g of fatty acid methylesters) varying from 0.37 to 1.08. In dairy cattle, increasing the CLA content in milk fat by increasing dietary intake of forages, especially fresh pasture is well documented (Bauman et al., 1999, Kelly et al., 1998a, Lawless et al., 1998).

In addition to decreasing CLA concentration in milk fat, decreasing forage, without adding a buffer, led to increased flow of 18:1 *trans* at the duodenum (Kalscheur et al., 1997a). The BH of total unsaturated eighteen carbon fatty acids was 10% lower in low forage (25%) diets compared to high forage (60%) diets or low forage diets supplemented with a buffer. The cows also had significantly lower ruminal pH (5.83) on a low forage versus a high forage (60%) diet (pH = 6.12). These observations seem to point to ruminal pH playing a role in the completeness of BH.

Early work involving low forage diets, lipid metabolism, and ruminal microorganism populations indicate that the lower pH leads to decreased BH and increased lipid flow from the rumen. Latham et al. (1972) recorded about a 60% decrease in BH between low (10%) and high (45%) forage diets. The concentrations of lipid in the rumen contents were two to three times greater on low forage diets; explained by the dietary lipid content as concentrates tend to have more lipid compared to forages.

Research points to increasing forage level as a means to increase CLA content in ruminant fats. However, lower forage diets also decrease BH, increasing TVA flow to the duodenum. These opposing views illustrate the need for additional research and the complexity of the rumen environment. Another important observation is also within these papers: animal variation. Kelly et al. (1998a) and Lawless et al. (1998) both point out individual cows varied substantially. Lawless et al. (1998) reported, within the same treatment groups, milk CLA content varying from 6.8 to 25.7 mg/g on pasture, 10.6 to 33.5 mg/g on pasture plus rapeseed, and 8.8 to 30.5 mg/g on pasture and soybean diets.

Dietary Lipid

The comparisons between effects of low and high forage diets point to the second method of increasing CLA content in fat of ruminant origin; dietary lipid quantity and composition. In an *in vitro* study Beam et al. (2000) found that the rate of linoleic acid BH in ruminal contents decreased with each additional percentage of added linoleate. Increasing linoleic acid led to a shift in BH end products and also seemed to interfere with linoleate disappearance. Beam et al. (2000) concluded that the quantity of linoleic acid fed plays a key role in the amount and type of BH products available for intestinal absorption.

Butyrivibrio fibrisolvens cultures did not produce significant amounts of c9, t11 until the linoleic acid concentration was high enough to inhibit BH. With concentrations greater than 350 µm BH was inhibited, CLA content was significant, and cultures were no longer viable. Kim et al. (2000) also reported that *B. fibrisolvens* lyses when it reaches stationary phase; however, its membrane fractions were still able to isomerize linoleic acid to CLA. Also noted are characteristics of the microbe; they are celluolytic and are thus present in high concentrations in ruminants consuming large amounts of forages, a management scenario leading to increased CLA production.

Besides quantity of lipid present in the diet, CLA production and BH responses vary with type of fat. To compare oleic, linoleic, and linolenic acid’s ability to increase CLA production, dairy cows were fed diets containing 5.3% sunflower, peanut, or linseed oil. Sunflower oil (high in linoleic acid) increased CLA in milk fat nearly 500% compared to traditional diets. It was also significantly greater than either peanut (oleic) or linseed (linolenic) oil additions (Kelly et al., 1998b).

Dairy cows consuming pasture also had increased CLA production when fed supplemental oil. Feeding both soybean and rapeseed concentrate resulted in significantly greater CLA in milk fat compared to the control (Lawless et al., 1998).

Feeding Rumen-Protected Lipids

In beef cattle, Enser et al. (1999) fed either Megalac® (increased saturated fats), fish oil or linseed oil. Both fish and linseed oil are high in omega-3 fatty acids. The unsaturated fats resulted in a 2-3 fold increase in CLA present in the intramuscular fat of feedlot steers compared to the saturated fat addition. All groups consumed similar amounts of linoleic acid, suggesting possibilities for BH of linolenic acid or interactions between long-chain more highly unsaturated fats present in fish oil and BH pathways.

Besides attempting to modify the rumen environment and contents to favor CLA production, rumen protected CLA salts have also been fed to dairy cows and feedlot animals. Gulati et al. (2000) determined that unprotected CLA was 80-90% hydrogenated when incubated with rumen contents. However, CLA encapsulated in a protein matrix was only about 30% hydrogenated. When feeding encapsulated CLA to sheep, abomasal CLA contents were approximately 3.5-4% greater. In milk goats, dietary inclusion of CLA led to a 30+% increase in both c9, t11 and t10, c12 in the milk.

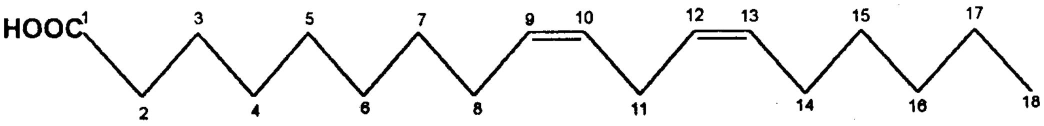
Calcium salts of CLA have also been included at 0, 1.0 or 2.5% of the feedlot diet resulting in a linear increase in i.m. lipid CLA as Ca-salt % increased. However, the CLA salt led to decreased daily gains, % choice carcasses, and feed intakes (Gassman et al., 2001). While Gulati et al. (2000) reported only 30% hydrogenation of protected CLA, in related work Gassman et al. (2001) reported 85% hydrogenation of CLA salts when fed to sheep. These differences in rumen bypass of CLA calcium salt are related to rumen pH as it alterers the dissociation of rumen protected CLA salts. Dissociation constants (pKd) for protected salts will vary based on the actual fat content; however, the difference between ruminal pH of a dairy cow consuming predominantly pasture is much different than a feedlot animal on a high concentrate diet resulting in varied hydrogenation of unsaturated lipids (Wu et al., 1991). Consequently, more work with protected CLA under rumen environmental conditions commonly seen in feedlot cattle is needed to clarify the role of pH on BH.

Besides increasing CLA, feeding protected salt resulted in increased concentrations of saturated fatty acids and decreased monounsaturated lipids (Gassman et al., 2001). The Δ^9 desaturase is inhibited by certain fatty acids, particularly those having 18 or more carbons and two or more double bonds: CLA, linoleic and linolenic acid (Ntambi 1999). In feeding studies, cattle fed rumen protected cottonseed oil had significantly more saturated fats (16:0 and 18:0) and less mono-unsaturated (16:1 and 18:1). The animal’s adipose tissue also had significantly less desaturase activity (Yang et al., 1999). If feeding CLA results in a beef or milk product that has an even higher saturated fat content, producers may lose one of the benefits of CLA: perceived health benefits for the consumer.

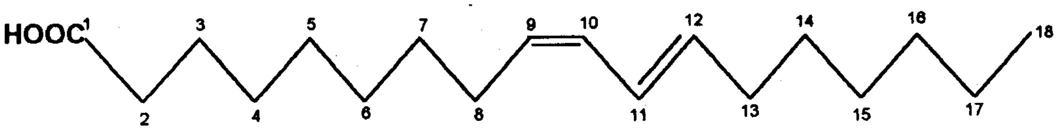
Addition of Ionophores

Research presently available is not in agreement as to the effects of ionophores on ruminal CLA production and BH. Zinn et al. (1988) observed no interactions between supplemental fat and monensin related to fat composition reaching the intestine. However, Fellner et al. (1997) reported that monensin inhibited linoleic acid BH rate resulting in an increase in c9, t11. Nonetheless, ionophores are common in typical feedlot diets and their effects on BH are important for modifying animal nutrition to enhance CLA production in a production scenario.

Ruminal biohydrogenation is proving to be a very complex process regulated by a number of factors ranging from forage to concentrate ratios, amount and type of lipid included in diets, ruminal pH, and even rumen microbial population to name only a few. Manipulating just one of these factors has led to increased CLA content in either milk or meat in many cases. However, changing the proven scenario slightly, for instance feeding dried forages rather than fresh pasture, results in a different outcome as far as product CLA content. These examples further illustrate the complexity of altering processes occurring in the rumen, which still holds many mysteries in itself. Certainly, with the potential of CLA giving ruminant products “functional food” status, providing management and/or nutritional techniques for producers to capture this market is valuable. At this point more research is needed in a variety of areas before high CLA meat, especially, and also milk will become the normal production outcome. The objectives of this research were to determine: 1)the effects of dietary forage and oil level on ruminal biohydrogenation and biohydrogenation intermediate production (CLA and C18:1 *trans*) and 2)the effects of feeding diets containing a dietary forage and oil level identified in objective 1 or rumen protected CLA on feedlot performance, tissue fatty acid composition and the quality attributes of resultant beef.



cis-9, *cis*-12 octadecadienoic acid (Linoleic acid)



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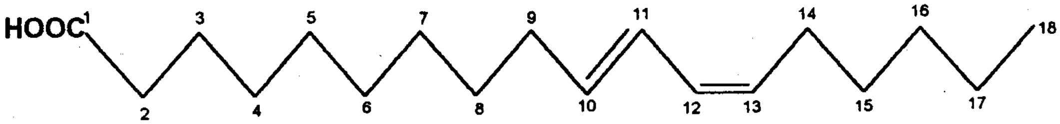
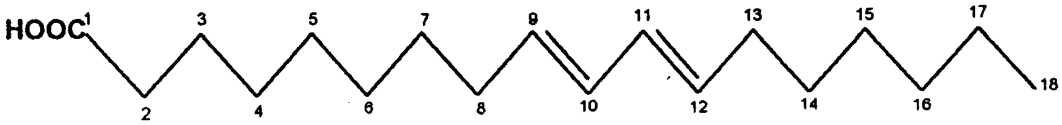


trans-10, *cis* -12 octadecadienoic acid



trans- 9, *trans*-11 octadecadienoic acid

Figure 1.1. Graphic representation of linoleic and conjugated linoleic acid isomers.[a]

[a] Adapted from Bessa et al., 2000.

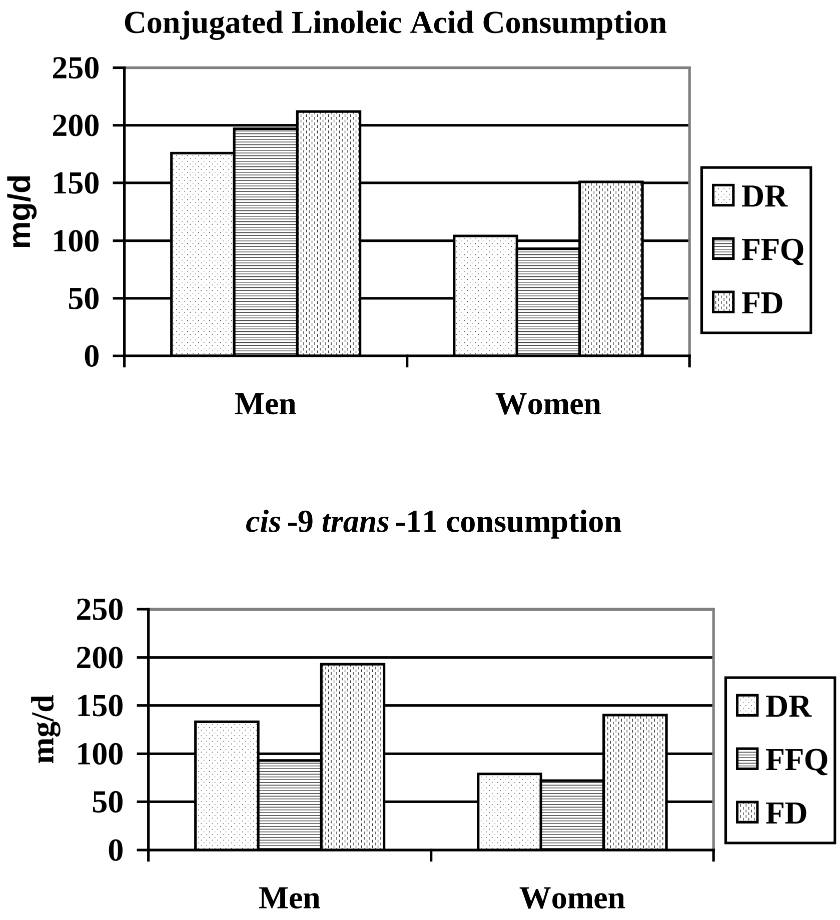


Figure 1.2. Total conjugated linoleic acid and *cis*-9, *trans*-11 isomer consumption by males and females and differences according to sampling technique.[a]

[a] Adapted from Ritzentahler et al., 2001
DR = dietary record; 3-day written
FFQ = food-frequency questionnaire
FD = food duplicates 3-day

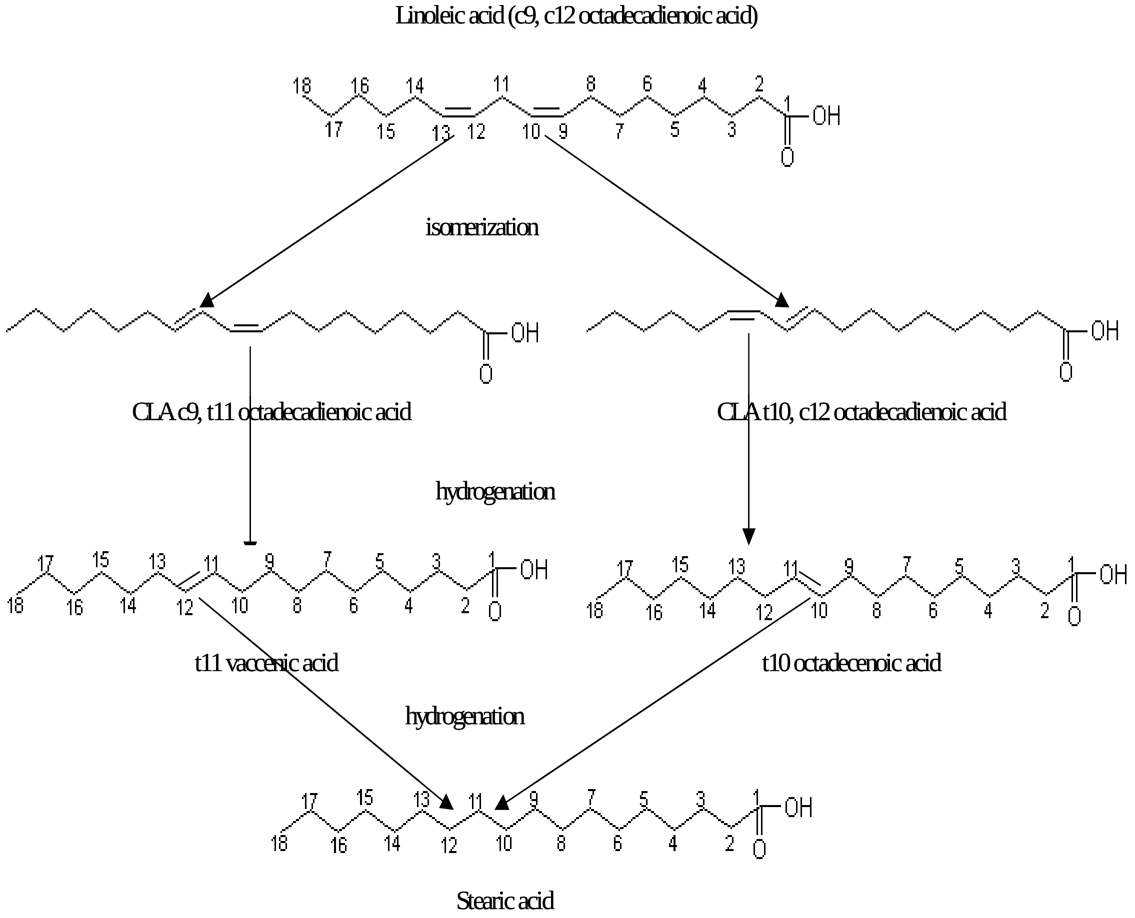


Figure 1.3 Ruminal biohydrogenation pathway for linoleic acid.

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CHAPTER 2

EFFECTS OF FORAGE AND OIL LEVEL ON RUMINAL BIOHYDROGENATION AND CONJUGATED LINOLEIC ACID PRODUCTION IN BEEF CATTLE FED FINISHING DIETS[1]

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Abstract

Six Hereford steers, cannulated in the proximal duodenum, were used to determine the effect of forage and oil level on conjugated linoleic acid (CLA) production and ruminal biohydrogenation. Steers were fed one of six treatment diets in a 3 X 2 factorial arrangement of treatments (grass hay level; 12%, 24%, or 36% and sunflower oil level 2% or 4%) in a 6 X 6 Latin Square design. All steers were fed treatment diets once daily. The remainder of the diet included steam rolled corn, monensin (250 $mg \cdot hd^{-1} \cdot d^{-1}$), and protein supplement (83% soybean meal, 12% limestone, and 5% trace mineral salt). Experimental periods included ten d of diet adaptation and four d of sampling. Data were analyzed with forage level, oil level and two-way interactions in the model. Duodenal concentration of the *cis*-9 *trans*-11 isomer of CLA did not differ ($P > 0.05$) among oil or forage levels. The *trans*-10 *cis*-12 CLA isomer content was greater ($P < 0.05$) in diets containing 12% forage compared to other forage levels. Diets containing 12% forage had greater ($P < 0.05$) c18:1 *trans* production than diets containing 36% forage with 24% forage being intermediate. Duodenal long-chain fatty acid flow (g/d) was greater ($P < 0.05$) in 4% vs 2% oil levels. Biohydrogenation of total 18 carbon fatty acids, oleic acid, and linolenic acid was lower ($P < 0.05$) in 12% forage compared to 36% forage with 24% forage being intermediate. Linoleic acid biohydrogenation was greatest ($P < 0.05$) for 36% forage and lowest for 12% forage. Oil level did not alter ($P > 0.05$) ruminal biohydrogenation or predominant CLA isomer production. Ruminal fatty acid digestibility was lower ($P < 0.05$) in 2% oil diets compared to 4%. Forage level did not alter ($P > 0.05$) ruminal dry matter, organic matter or fatty acid digestibility. Ruminal biohydrogenation increased with forage levels; however, the more extensive biohydrogenation did not alter *cis*-9 *trans*-11 CLA isomer formation. Low forage diets did increase ruminal production of c18:1 *trans* and the *trans*-10 *cis*-12 isomer of CLA. Increasing dietary oil levels did not alter ruminal biohydrogenation, but did increase the flow of total long-chain fatty acids to the small intestine.

Key Words: Conjugated Linoleic Acid, Biohydrogenation

Introduction

Conjugated linoleic acid (CLA) is the name given to a group of positional and geometric isomers of linoleic acid possessing two double bonds separated by a single carbon-carbon bond. CLA has received attention following its discovery as a potent anticarcinogen. The *cis*-9, *trans*-11 (c9, t11) isomer of CLA is the biologically active agent (Ha et al., 1990). In ruminant animals, c9, t11 is naturally produced as a result of the incomplete ruminal biohydrogenation (BH) of linoleic acid. Additionally, *trans* vaccenic acid (TVA; C18:1 *trans*-11), another intermediate in linoleic acid BH, has been shown to be desaturated to c9, t11 by the Δ^9 desaturase present in bovine adipose and mammary tissue (Griinari et al., 2000).

In an effort to increase the amount of CLA consumed by humans, research has been conducted to increase the quantity of CLA in both beef and milk. Feeding low fiber diets tend to decrease the extent of ruminal BH (Latham et al., 1972; Kalscheur et al., 1997a), resulting in an increased duodenal flow of 18:1 *trans* (Kalscheur et al., 1997a). However, in several dairy studies increasing dietary forage level led to increased CLA concentrations in the milk (Bauman et al., 1999). The addition of unsaturated vegetable oils has also been shown to increase CLA content of milk fat (Bauman et al., 1999). Adding unsaturated fat to feedlot diets led to increased quantities of CLA in beef (Enser et al., 1999). However, little research detailing the effects of varying forage and oil level, concurrently, on ruminal BH, TVA, and CLA production in feedlot diets. The objective of this study was to determine the effects of altering forage and oil levels on BH and flow of BH intermediates (CLA and TVA) to the small intestine in beef steers.

Materials and Methods

Animals. Six Hereford steers cannulated in the proximal duodenum were fed one of six diets in 3 x 2 factorial arrangement of treatments (grass hay level; 12%, 24%, or 36% and sunflower oil level; 2% or 4%) in a 6 x 6 Latin Square Design. Steers had 4 wk of recovery from local anesthesia and insertion of T-type cannula (Univ. of Wyoming, Laramie) into the proximal duodenum by a clinical veterinarian prior to beginning the study. Animals were housed indoors on concrete slatted floors and had ad libitum access to plain salt blocks. One steer was removed from the study after completing the third period due to complications with cannula placement, unrelated to dietary treatments. The University of Georgia Animal Use and Care committee approved the experimental animal protocol used in this study.

Diets. In addition to grass hay and sunflower oil, diets included a soybean meal based protein supplement and steam rolled corn. All diets were formulated for 12.5% CP and included monensin (250 $mg \cdot hd^{-1} \cdot d^{-1}$). Diet and chemical composition of the diets are presented in Table 2.1. Dietary treatments were fed once daily at 0800 at a level to limit refusals. Chromic oxide (10 $g \cdot hd^{-1} \cdot d^{-1}$) was added to the diets the last 10 d of each experimental period as an external marker for determining duodenal output.

Sample collection and analysis. Experimental periods were 14 d in length, with 10 d for diet adaptation and 4 d for sample collection. After diet adaptation, duodenal samples (200 mL) were taken 12 times over 4 d. Three evenly spaced samples were taken daily. Sampling times were shifted ahead each day resulting in 12 equally spaced samplings over a 24-h day. Duodenal samples were composited on an equal volume basis for each steer within each period and frozen (-20°C) for later processing. Diet samples were taken each morning during sampling as well as weighbacks collected and immediately frozen (-20°C). Feed samples were composited on an equal weight basis. Duodenal samples were thawed and separated into liquid and particle fractions. Fractions were lyophilized and recomposited on a DM basis. A portion of each feed sample was oven dried to determine DM (55°C in a forced air oven until dry). Remaining feed samples were lyophilized and ground through a Wiley mill (Arthur H. Thomas, Philadelphia, PA) fitted with a 1-mm screen. Duodenal and feed samples were frozen (-20°C) prior to subsequent ash (AOAC, 1990), fatty acid composition, and fiber analysis according to Goering and Van Soest (1970) using an Ankom 220 (Fairport, NY).

Duodenal and feed samples containing approximately 25 mg of lipid were converted to fatty acid methyl esters using the *in situ* preparation of FAME (Park and Goins, 1994). Methyl esters were separated by gas chromatography (Agilent 6850; Wilmington, DE) using a 100-m Supelco SP2560 (Supelco, Bellefonte, PA) capillary column (0.25 mm i.d. and 0.20 μm film thickness) under the following conditions: column oven temperature programmed from 150ºC to 165ºC at 1ºC/min, from 165ºC to 167ºC at 0.2ºC/min, from 167ºC to 225ºC at 1.5ºC/min and held at 225ºC for 5 min with a 1:100 split. The injector and detector were maintained at 250ºC. Hydrogen was the carrier gas at a flow rate of 1 mL/min. Individual fatty acids were identified by comparing retention times of known compounds (Sigma, St. Louis, MO; Supelco, Bellefonte, PA; Matreya, Pleasant Gap, PA). Concentrations were calculated based on an internal standard (methyl tricosanoate; C23:0) response and are presented as a percentage of total fatty acid (percentage weight basis) or by amounts (g).

Chromium concentration in duodenal samples and weighbacks were determined using a colorimetric assay (Fenton and Fenton, 1979). Concentrations of chromium and fatty acids in feed, refusals, and digesta were used to calculated digestibility and output of fatty acids. Percent biohydrogenation of total eighteen carbon fatty acids, oleic acid, linoleic acid, and linolenic acid were calculated according to Wu et al. (1991).

Statistical Analysis. Data were analyzed as a completely randomized experiment using the GLM procedure of SAS (SAS Inst. Inc., Cary NC). Animal served as the experimental unit for all variables measured. All data were analyzed with steer, period, forage level, oil level, and the forage x oil interaction in the model. Treatment means were separated using the least squares means procedure.

Results and Discussion

Ruminal digestibilites of DM, OM, long chain fatty acids, NDF, and ADF are presented in Table 2.2. Digestibility of DM, OM, long chain fatty acids, NDF and ADF was similar ($P > 0.05$) for all forage levels with no ($P > 0.10$) forage by oil level interaction. Kalscheur et al. (1997a) reported reductions in ruminal DM and OM digestibility for low fiber (25%) diets compared to high fiber (60%) or low fiber plus a buffer. Zinn et al. (2000) found high-fat supplementation with yellow grease and formaldehyde protected lipids reduced ruminal digestion of OM and NDF. Kalscheur et al. (1997b) reported no differences in ruminal digestibility of OM or DM when dairy cows were fed a control diet or 3% added high oleic sunflower oil, traditional sunflower oil, or vegetable oil. Fiber digestibility (NDF and ADF) did not differ ($P > 0.05$) between dietary treatments. However, numerical reductions in fiber digestibility were observed as forage level decreased. Previous work reported decreased NDF digestibility with low fiber (25%) diets (Kalscheur et al., 1997a). However, our NDF digestibility was much more variable in comparison (SEM = 5.27% v 3.30%). Ruminal fatty acid digestibility was greater ($P < 0.05$) for 4% compared to 2% oil. In a review of ruminal lipid metabolism, Jenkins (1993) reported a regression (r = 0.87) showing a loss of 13 g/100 g of lipid intake. Our calculated long chain fatty acid digestibility falls within those reported by researchers using various lipid sources fed from 0 to 20% of the diet (Jenkins 1993).

Intake (g/d) of specific long chain fatty acids is presented in Table 2.3. There were no interactions ($P > 0.10$) between forage and oil level for long chain fatty acid intake. Intake of myristic (C14:0), palmitic (C16:0), stearic (C18:0), oleic (C18:1 c9), linoleic (C18:2 c9, c12), arachidic (C20:0) and behenic (C22:0) acids as well as odd chain fatty acids (C13:0, C15:0 and C17:0) was similar ($P > 0.05$) among forage levels. Linolenic (C18:3 c9, c12, c15) acid intake was greater ($P < 0.05$) for 36% forage diet compared to 12%, with 24% forage being intermediate. Total intake of long chain fatty acids did not differ ($P > 0.05$) between forage levels. Intake of myristic and linolenic acids or odd chain fatty acid did not differ ($P > 0.05$) between oil levels. Increasing supplemental oil level increased ($P < 0.05$) intake of palmitic, stearic, oleic, linoleic, arachidic, and behenic acids. Intake of total long chain fatty acids was 40% greater ($P < 0.05$) for diets that contained 4% compared to 2% supplemental oil.

Ruminal BH of dietary unsaturated eighteen carbon fatty acids as influenced by dietary forage and oil level is shown in Table 2.4. Interactions between dietary forage and oil level were non-significant (*P* > 0.10). Dietary oil level did not alter (*P* > 0.05) ruminal biohydrogenation of total or individual unsaturated eighteen carbon fatty acids. In contrast, Wu et al. (1991) reported more extensive BH as the addition of a vegetable oil-animal fat blend (35% saturated fat) increased. Increasing forage level increased (*P* < 0.05) BH of total eighteen carbon fatty acids, oleic, linoleic, and linolenic acids. Diets containing 12% forage had lower (*P* < 0.05) BH for total eighteen carbon fatty acids, oleic, and linolenic acid when compared to diets with 36% forage, with 24% being intermediate. Linoleic acid BH was the lowest (*P* < 0.05) for 12% forage (83.73%) and the greatest (*P* < 0.05) for 36% forage (91.36%). Latham et al. (1972) reported that as ruminal pH declines the lipolytic activity of microorganisms and the extent of BH of linoleic and linolenic acid decline *in vitro*. Others (Kucuk et al., 2001; Kalschuer et al., 1997a) have shown that increased forage level increases BH of dietary unsaturated fatty acids. Similar BH levels for linolenic and total eighteen carbon fatty acids were reported for lactating dairy cows fed 60% or 25% forage with or without a buffer (Kalschuer et al., 1997a). However, these authors reported lower BH levels for oleic (47%) and linoleic (77%) acids.

Duodenal flows of individual fatty acids by forage and oil level are presented in Table 2.5. Interactions between dietary oil and forage level were observed (*P* < 0.10) for myristic, pentadecyclic (C15:0), palmitic, and total long chain fatty acids. Flow of myristic acid decreased (*P* < 0.05) as forage level increased in the 4% oil diets, but were similar (*P* > 0.05) among forage levels for 2% oil. Flow of pentadecyclic acid increased (*P* < 0.05) with greater forage in 2% oil diets but was similar (*P* > 0.05) among forage levels for 4% oil. Palmitic acid flow was greater (*P* < 0.05) for low forage with 4% oil and similar ($P > 0.05$) between forage levels for 2% oil. Flow of odd chain fatty acids were greater ($P < 0.05$) for higher forage levels (24 or 36%) with 2% oil and similar ($P > 0.05$) between forage levels for 4% oil.

Duodenal flows of long chain fatty acids by forage level or oil level is shown in Table 2.6. Forage level did not alter ($P > 0.05$) duodenal flow of palmitoleic (C16:1 c9), stearic, arachidic, eicosenoic (C20:1 c9), behenic, arachidonic (C20:4 c9, c12, c15, c18) total long chain, unidentified, or saturated fatty acids. Numerical increases in flow of stearic acid were observed with increased forage level. In agreement, Kalscheur et al. (1997a) reported a 10% increase in stearic acid flow to the duodenum with high fiber diets compared to low fiber diets. These differences reflect greater BH of unsaturated eighteen carbon fatty acids with increased dietary forage level. Flow of margaric acid was lower ($P < 0.05$) for the 12% forage compared to 24 and 36% forage. Daily flow of C18:1 *trans* was 80.9% greater ($P < 0.05$) for 12% forage than 36% forage with 24% forage being intermediate. Kalscheur et al. (1997a) found that the addition of a buffer resulted in similar C18:1 *trans* flow between high and low fiber diets, indicating the potential importance of a lowered ruminal pH on the accumulation of BH intermediates. Oleic and linoleic acid flow were greater ($P < 0.05$) for 12 and 24% forage levels than 36% forage. Lower BH of oleic and linoleic acids resulted in greater flow of these fatty acids to small intestine for the low forage diet. Conversely, flow of linolenic acid was greater ($P < 0.05$) in 36% diets compared to 12%, with 24% forage being intermediate. Despite increased BH with higher fiber diets, 36% forage diets provided nearly 29% more dietary linolenic acid resulting in greater flows to the small intestine. As a result of these BH patterns, 12% forage diets resulted in 69% greater ($P < 0.05$) flow of unsaturated fatty acids to the small intestine than 36% forage diets, with the 24% forage diets being intermediate.

Dietary oil level did not alter ($P > 0.05$) palmitoleic, margaric, stearic, C18:1 *trans*, linoleic, linolenic, arachidic, arachidonic, unidentified, or unsaturated fatty acid flow to the duodenum. Increasing dietary oil level tended ($P < 0.10$) to increase flow of oleic and eicosenoic (C20:1 c9) acids. Four percent oil resulted in greater ($P < 0.05$) duodenal flow of behenic and saturated fatty acids due to greater dietary supply. In agreement with Wu et al. (1991) increasing oil supplementation increased fatty acid flow to the small intestine. Total flow of long chain fatty acids was nearly 25% greater ($P < 0.05$) in 4% compared to 2% oil supplemented diets.

Ruminal production of CLA isomers by forage or oil level are shown in Table 2.7. Interactions between forage and oil level were non-significant ($P > 0.10$). Dietary oil level did not alter ($P > 0.05$) CLA flow. Forage level did not alter ($P > 0.05$) duodenal flow of *cis*-9, *trans*-11 (c9, t11), *cis*-9, *cis*-11, or total CLA. Despite differences in BH of linoleic acid, increasing forage level did not result in greater flow of c9, t11 CLA to the small intestine. Production of the *trans*-10, *cis*-12 (t10, c12) isomer of CLA was greater ($P < 0.05$) for 12% forage than 24 or 36%. Flow of *trans*-9, *trans*-11 (t9, t11) was greater ($P < 0.05$) for 12% than 36% forage with 24% being intermediate. Conversely, production of other CLA isomers was lower ($P < 0.05$) for 12% forage than 24 or 36%. There were numerical increases in total CLA production (+36.8%) for 12% forage compared to 36% due to increases in t10, c12 and t9, t11 isomers with low fiber diets. In lactating dairy cows, low fiber high concentrate diets have been found to increase the concentration of the t10, c12 isomer of CLA (Bauman et al., 1999).

Noting the relative magnitude of BH intermediates available for absorption at the small intestine may indicate the relative importance of each as sources for CLA present in body fat. While the c9, t11 isomer of CLA was present at 120 to 136 mg/d, c18:1 *trans* accounted for 25 to 45 g/d.

Implications

Increasing dietary forage level resulted in greater ruminal biohydrogenation of unsaturated eighteen carbon fatty acids. However, the more extensive biohydrogenation did not result in increased production of the *cis*-9, *trans*-11 isomer of conjugated linoleic acid. Conversely, lower forage diets did increase flow of the *trans*-10, *cis*-12 isomer of conjugated linoleic acid and C18:1 *trans* to the small intestine. Increasing supplemental oil level did not alter ruminal biohydrogenation, but did result in a greater flow of long chain fatty acids to the duodenum. This data suggests that increasing the flow, absorption, and desaturation of C18:1 *trans* may have a larger influence on tissue conjugated linoleic acid content compared to the direct deposition of conjugated linoleic acid isomers.

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Table 2.1. Ingredient and chemical composition of feedlot type diets with varying forage and oil levels.[a]

	Treatments					
Forage level	12%	12%	24%	24%	36%	36%
Oil level	2%	4%	2%	4%	2%	4%
Grass hay	12.00	12.00	24.00	24.00	36.00	36.00
Protein supplement[b]	11.50	12.00	9.20	9.80	7.00	7.50
Sunflower oil	2.00	4.00	2.00	4.00	2.00	4.00
Corn, steam rolled	74.50	72.00	64.80	62.20	55.00	52.50
Monensin supple.,[c] kg · hd^{-1} · d^{-1}	0.45	0.45	0.45	0.45	0.45	0.45
	Chemical Composition[d]					
DM, %	86.72	86.71	86.59	86.90	86.79	87.00
CP, %	12.42	12.43	12.43	12.48	12.47	12.48
Total lipid, %	4.27	5.76	4.08	5.54	3.87	5.35
NDF, %	19.23	18.95	26.44	26.19	33.67	33.41
ADF, %	8.51	8.40	12.24	12.18	16.02	15.96
Ash, %	13.88	13.51	13.44	13.02	12.98	13.20

[a] Percentage of diet on a DM basis, fed as TMR once daily at 0800 hr.

[b] Composition of protein supplement: 83% soybean meal, 12% limestone, 5 % trace-mineralized salt (97% NaCl, 3,500 mg Zn/kg, 2,000 mg Fe/Kg, 1,800 Mn/kg. 350 mg Cu/kg, 100 mg I/kg, 90 mg Se/kg, and 60 mg Co/kg).

[c] Monensin supplement = 99.7% ground corn with 0.31% Rumensin-80 providing 250 mg · hd^{-1} · d^{-1} of Rumensin activity.

[d] All values except %DM are on a DM basis

Table 2.2. Effect of dietary forage and oil level on ruminal digestibility.

	% Forage				% Oil		
	12%	24%	36%	SEM	2%	4%	SEM
Duodenal output, g/d	3803.6	4380.4	4179.3	343.1	4112.3	4129.9	284.3
Intake, g/d	6081.80	6290.60	5794.80	408.90	6052.10	6059.40	328.9
DM, %	37.06	29.83	29.21	3.7	31.63	32.43	3.07
OM, %	40.15	35.75	35.13	3.45	36.8	37.22	2.86
Long chain fatty acid, %	13.53	0.56	9.92	5.32	1.23^{b}	14.78^{a}	4.28
NDF, %	5.95	18.57	24.99	6.57	17.86	15.15	5.44
ADF, %	7.56	16.51	20.13	7.08	13.76	15.71	5.87

^{ab}Means in the same row with uncommon superscripts differ ($P < 0.05$) due to oil level.

Table 2.3. Intake (g/d) of long chain fatty acids of steers fed varying forage and oil levels.

	% Forage				% Oil		
Item	12%	24%	36%	SEM	2%	4%	SEM
C14:0	0.29	0.31	0.30	0.02	0.28	0.32	0.19
C16:0	28.95	29.21	25.88	1.9	25.67^{f}	30.37^{e}	1.58
C18:0	9.59	9.70	8.62	0.59	7.36^{f}	11.25^{e}	0.49
C18:1 c9	117.06	116.13	100.47	7.15	86.00^{f}	136.44^{e}	5.92
C18:2 c9, c12	154.70	149.49	126.02	9.70	123.34^{f}	163.48^{e}	8.04
C18:3 c9, c12, c15	5.83^{d}	7.17^{cd}	7.50^{c}	0.47	6.63	7.03	0.39
C20:0	1.23	1.32	1.24	0.08	1.12^{f}	1.41^{e}	0.07
C22:0	1.57	1.69	1.55	0.10	1.19^{f}	2.01^{e}	0.08
OCFA[a]	0.16	0.20	0.20	0.02	0.18	0.19	0.12
Total Long Chain Fatty Acids	322.22	319.66	277.09	20.58	255.08^{f}	357.57^{e}	17.05

[a] OCFA: Total odd chain fatty acids (C13:0, C15:0, and C17:0).

[cd] Means in the same row with uncommon superscripts differ ($P < 0.05$) due to forage level.

[ef] Means in the same row with uncommon superscripts differ ($P < 0.05$) due to oil level.

Table 2.4. Effect of dietary forage and oil level on ruminal biohydrogenation of unsaturated eighteen carbon fatty acids.

	% Forage				% Oil		
	12%	24%	36%	SEM	2%	4%	SEM
Total C18's	73.08^{a}	79.03^{ab}	84.71^{b}	2.26	79.07	78.80	1.87
C18:1 c9	46.75^{b}	55.04^{ab}	65.96^{a}	5.59	56.76	55.08	4.63
C18:2 c9, c12	83.73^{c}	88.05^{b}	91.36^{a}	1.26	87.37	88.05	1.04
C18:3 c9, c12, c15	85.66^{b}	89.19^{ab}	90.71^{a}	1.24	88.27	88.77	1.03

[ab] Means in the same row with uncommon superscripts differ ($P < 0.05$) due to forage level.

Table 2.5. Duodenal flow (g/d) of long-chain fatty acids by dietary forage and oil level.

	2% Oil			4% Oil			
Item	12% Forage	24% Forage	36% Forage	12% Forage	24% Forage	36% Forage	SEM
C14:0	2.06^{c}	3.01^{bc}	2.10^{c}	4.91^{a}	3.42^{b}	2.23^{bc}	0.44
C15:0	0.98^{b}	1.77^{ab}	1.92^{a}	1.33^{b}	1.39^{b}	1.31^{b}	0.15
C16:0	31.43^{b}	44.78^{b}	30.60^{b}	68.2^{a}	51.21^{ab}	38.31^{b}	7.30
OCFA[d]	1.66^{c}	3.01^{a}	3.16^{a}	2.44^{ab}	2.58^{ab}	2.28^{bc}	0.27

[abc] Means within the same row with uncommon superscripts differ ($P < 0.05$).

[d] OCFA; total odd chain fatty acids (C13:0, C15:0 & C17:0).

Table 2.6. Duodenal flow (g/d) of long chain fatty acids of steers fed varying forage and oil levels.[a]

	% Forage				% Oil		
Item	12%	24%	36%	SEM	2%	4%	SEM
C16:1 c9	0.55	0.50	0.42	0.07	0.50	0.47	0.06
C17:0	0.70^{d}	1.02^{c}	0.9^{c}	0.09	0.90	0.90	0.07
C18:0	105.75	141.99	133.81	19.60	115.19	139.18	16.24
C18:1 t	45.73^{c}	36.13^{cd}	25.28^{d}	6.90	31.29	40.14	5.72
C18:1 c9	19.00^{c}	16.84^{c}	12.15^{d}	1.29	14.59^{h}	17.41^{g}	1.07
C18:2 c9, c12	14.44^{c}	11.42^{c}	7.55^{d}	1.28	10.50	11.55	1.06
C18:3 c9, c12, c15	1.12^{d}	1.43^{cd}	1.53^{c}	0.11	1.33	1.39	0.09
C20:0	1.33	1.61	1.66	0.16	1.40	1.66	0.13
C20:1 c9	0.45	0.32	0.20	0.05	0.27^{h}	0.38^{g}	0.04
C22:0	1.38	1.51	1.41	0.13	1.14^{f}	1.73^{e}	0.11
C20:4 c9, c12, c15, c18	0.30	0.38	0.42	0.06	0.37	0.36	0.05
Unidentified	14.42	17.81	16.11	1.53	15.27	16.60	1.26
Total Long-chain Fatty Acids	260.75	285.27	241.25	21.36	233.85^{f}	290.99^{e}	17.71
Saturated Fatty Acids	164.20	199.67	176.33	17.96	158.70^{f}	201.43^{e}	14.88
Unsaturated Fatty Acids	82.04^{c}	67.67^{cd}	48.40^{d}	8.63	59.64	72.34	6.94

[a] Fatty acids separated but being less than 0.30 g/d include: C8:0, C10:0, C14:1 c9, and C22:1 c9.

[cd] Means in the same row with uncommon superscripts differ ($P < 0.05$) due to forage level.

[ef] Means in the same row with uncommon superscripts differ ($P < 0.05$) due to oil level.

[gh] Means in the same row with uncommon superscripts differ ($P < 0.10$) due to oil level.

Table 2.7. Duodenal flows (mg/d) of conjugated linoleic acid isomers in steers fed varying forage and oil levels

	% Forage				% Oil		
Item	12%	24%	36%	SEM	2%	4%	SEM
cis -9, *trans* -11	127.50	136.50	120.57	19.27	125.92	130.47	15.97
trans -10, *cis* -12	169.86^{a}	76.14^{b}	20.27^{b}	19.95	75.48	102.04	16.54
cis -9, *cis* -11	0.00	2.38	16.89	7.13	7.17	5.67	5.91
trans -9, *trans* -11	178.28^{a}	129.51^{ab}	105.89^{b}	17.29	144.98	130.81	14.33
other*	45.86^{b}	103.11^{a}	118.07^{a}	17.37	81.18	96.84	14.39
Total	521.51	447.64	381.69	50.43	434.73	465.83	41.79

^{ab}Means in the same row with uncommon superscripts differ ($P < 0.05$) due to forage level.

**Trans, trans* and *cis, cis* isomers not able to be matched to standards.

CHAPTER 3

EFFECTS OF SUPPLEMENTAL LINOLEIC ACID OR RUMEN PROTECTED CONJUGATED LINOLEIC ACID ON PERFORMANCE AND TISSUE FATTY ACID COMPOSITION OF FEEDLOT HEIFERS[1]

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[1] Sackmann, J.R., S.K. Duckett, M.H. Gillis, C.E. Realini, and K.R. Smith. To be submitted to the *Journal of Animal Science.*

Abstract

Thirty-six Angus-Hereford cross heifers (365 kg) were fed linoleic acid or rumen protected conjugated linoleic acid (CLA) salt for the last 32 or 60 days of finishing to determine the effects on tissue CLA content, shelf life, sensory attributes, and animal performance. Dietary treatments were 4% corn oil (OIL), 2% rumen protected CLA salt (SALT) containing 31% CLA-60, or neither (CONT) arranged in a 3 X 2 factorial. Animals were individually fed fed a high concentrate diet including monensin (250 $mg{\cdot}hd^{-1}{\cdot}d^{-1}$) and MGA (Melengestrol acetate; 0.4 $mg{\cdot}hd^{-1}{\cdot}d^{-1}$). At 48 h postmortem carcass data, longissimus, and ground beef samples were obtained. Steaks (2.54 cm thick) and ground beef patties were vacuum packaged and frozen for subsequent fatty acid and sensory analysis. Samples were also overwrapped and held at 4°C for 19 d (steaks) and 8 d (ground beef) to measure changes in lipid oxidation (TBARS). Data were analyzed with treatment, time on treatment, and the two-way interaction in the model. In the longissimus, on a percentage basis, the *cis*-9, *trans*-11 isomer of CLA tended to be greater ($P < 0.10$) in SALT than OIL with CONT being intermediate. The *trans*-10, *cis*-12 isomer of CLA was greater ($P < 0.05$) in OIL fed for 60 d compared to SALT or OIL fed for 32 d or CONT regardless of time on treatment. *Trans*-10, *cis*-12 concentration in SALT fed for 60 d was also higher ($P < 0.05$) than OIL or SALT fed for 32 d and CONT for 60 d. SALT had greater ($P < 0.05$) concentrations of total CLA compared to OIL or CONT. Concentrations of total CLA or CLA isomers were not altered ($P > 0.05$) by dietary treatment in ground beef samples. The C18:1 *trans* concentration was greater ($P < 0.05$) in SALT vs CONT with OIL being intermediate for longissimus and ground beef. Linoleic acid content was greater ($P < 0.05$) in OIL compared to CONT with SALT being intermediate for longissimus. In ground beef samples the concentatration of linoleic and polyunsaturated fatty acids was greater ($P < 0.05$) in OIL compared to either SALT or CONT. Lipid oxidation was not altered ($P > 0.05$) by dietary treatment for longissimus samples. However, following 8 d of storage ground beef from OIL and SALT treatments had higher ($P < 0.05$) TBARS than CONT. Sensory off flavor scores were greater ($P < 0.05$) in OIL compared to CONT (0.906 vs 0.586; 9-point scale with 1 = extremely mild) with SALT being intermediate. Ground beef off flavor sensory scores did not differ ($P > 0.05$) among dietary treatments. Feed intake did not differ ($P > 0.05$) among treatmetns. SALT fed for 32 d had greater ($P < 0.05$) gain:feed and ADG compared to OIL or CONT. AT 60 d animal performance was similar ($P > 0.05$) between treatments. Supplementing linoleic acid or rumen protected CLA salt increased longissimus CLA content by 20% with minimal changes in shelf-life and sensory attributes, while CLA content of ground beef was unchanged.

Key Words: Conjugated linoleic acid, Beef

Introduction

The discovery of conjugated linoleic acid (CLA) as an anticarcinogenic, antiatherosclerosis, and energy repartitioning agent (Bauman et al., 1999) has led to attempts to increase the CLA content in foods we commonly consume. Conjugated linoleic acid is a group of geometric and positional isomers of linoleic acid possessing two double bonds separated by a single carbon-carbon double bond. Fats from ruminant animals, like those found in meat and milk, are natural sources of CLA. Dietary unsaturated fat undergoes biohydrogenation (BH) in the rumen. The incomplete ruminal BH of linoleic acid results in the *cis*-9, *trans*-11 (c9, t11) isomer of CLA and *trans* vaccenic (TVA; C18:1 *trans*-11) acid production. The c9, t11 isomer of CLA has been determined to be the biologically active isomer of CLA responsible for the anticarcinogenic properties (Ha et al., 1990). *Trans*-vaccenic acid has also been shown to be converted to c9, t11 by the Δ^9 desaturase enzyme present in both bovine adipose and mammary tissue (Griinari et al., 2000).

Several researchers (Kelly et al., 1998; Lawless et al., 1998; Gulati et al., 2000) have shown that CLA concentration can be enhanced in milk fat by the addition of unsaturated lipids or rumen protected CLA to the diet. Little research is available concerning adding lipid or rumen-protected CLA to feedlot diets.

Intramuscular lipid deposition has been shown to be non-linear. In Angus-Hereford cross steers i.m. fat percentage doubled between d 84 and 112 (Duckett et al., 1993). Similarly, in Angus heifers ultrasound data revealed a 50% increase in marbling deposition between d 56 and 84 (Smith et al., 2001). The objective of this study was to compare the effects of feeding supplemental unsaturated fat or rumen protected CLA salt for the final 32 or 60 d prior to harvest on animal performance, meat quality, and fatty acid composition of beef from feedlot heifers.

Materials and Methods

Animals. Thirty-six Angus-Hereford cross heifers (initial BW = 365 ± 60 kg) were used in a 3 x 2 factorial arrangement of treatments to determine the effects of dietary supplementation of oil or rumen protected CLA salt fed during the last 32 or 60 d of finishing on tissue CLA content, product shelf life, sensory attributes and animal performance. The heifers were acquired from the Northwest Branch Station (Calhoun, GA), stratified by weight into groups, and randomly assigned to one of three dietary treatments. The three dietary treatments were: 1) control, normal feedlot ration (**CONT**; 88% concentrate, 12% forage), 2) control ration with 4% added corn oil (**OIL**; 84% concentrate, 12% forage, 4% corn oil), and 3) control ration supplemented with 2% rumen protected conjugated linoleic acid (**SALT**; 86% concentrate, 12% forage, 2% CLA). Treatment diets were fed for the last 32 (**-32**) or 60 (**-60**) d of the feeding period. For the first 56 d on feed, all heifers received the CONT diet. At d 56, heifers were switched to the three dietary treatments (12/trt). At 88 d on feed, six heifers per treatment were removed for harvest based on visual estimates of fat thickness ($\geq$ 1.27 cm) or live weight ($\geq$ 586 kg). The remaining six heifers per treatment were fed for an additional 28 d and harvested at 116 d on feed.

Each heifer was fed individually throughout the trial using an electronic gate feeding system (American Calan, Inc., Northwood, NH). Heifers were trained to consume diets from their individual feeding stations as they were adapted to a high concentrate diet over a 21-d period prior to beginning the study. Each heifer also received Monensin (250 $mg \cdot hd^{-1} \cdot d^{-1}$) and MGA (Melengestrol acetate; 0.4 $mg \cdot hd^{-1} \cdot d^{-1}$) daily. Diet and chemical composition of the diets are presented in Table 3.1. Diets were fed once daily, with amount offered recorded daily and refusals recorded weekly. Performance traits of interest included: DMI, ADG, and feed efficiency. Rumen protected CLA was obtained from Agribrands Purina Canada Inc. (Ontario, Canada) and contained 31% CLA isomers by weight. Isomer composition for the rumen protected CLA was: 27.2% *cis*-9 *trans*-11, 32.8% *trans*-10 *cis*-12, 10.6% *trans*-8 *cis*-10, 19.0% *cis*-11 *trans*-13, and 10.5% various *trans*, *trans* isomers. Heifers were weighed and feed samples were taken at 30 d intervals across the feeding trial. A portion of each feed sample was oven dried (55°C until dry) to determine DM. The remainder was lyophilized, ground using a Wiley mill (Arthur A. Thomas, Philadelphia PA) equipped with a l-mm screen, and frozen (-20°C) for subsequent ash (AOAC, 1990) and fatty acid composition analysis.

All heifers were implanted with Synovex-H (20 mg estradiol benzoate and 200 mg testosterone; Ft. Dodge Animal Health, Ft. Dodge, IA) at the start of the trial. One heifer, assigned to CONT, was removed from the study due to an injury not related to treatment. The University of Georgia Animal Care and Use Committee approved the experimental animal protocol used in this study.

Carcass Characteristics and Meat Quality. Heifers were transported to the University of Georgia Meat Science Technology Center weighed and slaughtered after a 24 h feed withdrawal period. Hot carcass weights were recorded following trimming of any contamination, bruising, or blemishes. At 48 h postmortem carcass data including adjusted fat thickness, longissimus area, marbling score, percentage pelvic, kidney and heart (**KPH**) fat, skeletal maturity, USDA quality grade, and USDA yield grade were collected by trained evaluators. Carcass value was calculated based on current value based marketing grids (AMS, 2001). Rib sections (IMPS # 107) and plates (IMPS # 120) were removed from the right side. Ribs were cut into steaks (3-2.54 cm and 4-1.25 cm-thick) and trimmed of external fat. The 2.54 cm-thick steaks were vacuum packaged and aged for 14 d at 4ºC and then frozen (-20ºC) for subsequent sensory panel and proximate (including intramuscular fatty acid composition) analysis. The 1.25 cm-thick steaks were wrapped with oxygen permeable overwrap and placed in a 4ºC lighted cooler for 0, 5, 12 and 19 d to measure lipid oxidation using the TBARS procedure (Ahn et al., 1998 and Jo and Ahn, 1998). Plates were trimmed, ground (0.635 cm) and 4 patties (~120 g) were vacuum packaged and frozen at -20ºC for susbsequent proximate analysis, intramuscular lipid composition and sensory analysis. Eight patties (~120 g) were placed on Styrofoam trays and wrapped with oxygen permeable overwrap for shelf life analysis. Overwrapped samples were placed in a 4ºC lighted cooler and TBARS were measured at a 0, 2, 4 and 8 d.

Prior to proximate analysis, steaks and ground patties were pulverized in liquid nitrogen. Lipids were extracted using chloroform-methanol (Folch et al., 1957), modified by using a 10:1 ratio of chloroform-methanol to sample. Extract containing approximately 25 mg of lipid was converted to fatty acid methyl esters using the *in situ* preparation of FAME (Park and Goins, 1994). Feed samples containing about 25 mg of lipid were also prepared according to Park and Goins (1994). Methyl esters were separated by gas chromatography (Agilent 6850, Wilmington, DE) using a 100-m Supelco SP 2560 column (Supelco, Bellefonte, PA) capillary column (0.25 mm i.d. and 0.20 μm film thickness) under the following conditions: column oven temperature programmed from 150ºC to 165ºC at 1ºC/min, from 165ºC to 167ºC at 0.2ºC/min, from 167ºC to 225ºC at 1.5ºC/min and held at 225ºC for 5 min with a 1:100 split. The injector and detector were maintained at 250ºC. Hydrogen was the carrier gas at a flow rate of 1 mL/min. Individual fatty acids were identified by comparing to retention times of known compounds (Sigma, St. Louis, MO; Supelco, Bellefonte, PA; Matreya, Pleasant Gap, PA). Concentrations were calculated based on an internal standard (methyl tricosanoate; C23:0) and are presented as a percentage of total fatty acid (percentage weight basis) or by amount (g). The *trans*-8, *cis*-10 isomer of CLA present in the CLA salt (3.3%) could not be separated from the *cis*-9, *trans*-11 isomer using the above procedure, thus all values reported for *cis*-9, *trans*-11 from SALT fed heifers may also contain the *trans*-8, *cis*-10 isomer. Nitrogen content was determined using a Leco FP2000 nitrogen analyzer (Leco Inc, St. Joseph, MI), in which samples were oven dried for 30 min at 90ºC prior to analysis. Ash percentage was determined by difference after samples were heated overnight in a 500ºC oven.

Trained sensory evaluation was conducted according to the American Meat Science Association (AMSA, 1995). An eight-person taste panel was trained according to AMSA (1995) guidelines. Longissimus steaks were cooked to an internal temperature of 71ºC, and then cut into 1- x 1- x 2.54-cm cubes using a plastic grid (14 cm long x 12 cm wide x 4 cm deep, with slots spaced every 1.25 cm apart). Ground beef samples were cooked to an internal temperature of 81ºC and cut into six wedges per patty. Samples were served immediately to each panel member. Samples were evaluated for initial and overall tenderness, juiciness, beef flavor and connective tissue (ground samples only) using an eight-point scale (1 =extremely tough, extremely dry, extremely bland or abundant; 8=extremely tender, extremely juicy, extremely intense or none). Off-flavor was appraised on a nine-point scale (0=none; 8=extremely intense).

Statistical Analysis. Data were analyzed as a completely randomized experiment using the GLM procedure of SAS (SAS Inst. Inc., Cary NC). Animal served as the experimental unit for all variables measured. All data were analyzed with dietary treatment, time on treatment, and the two-way interaction in the model. Treatment means were separated using the least squares means procedure.

Results and Discussion

Heifer performance data by dietary treatment and time is presented in Table 3.2. Intake did not differ ($P > 0.05$) by dietary treatment, time on treatment, or the two way interaction. Gassman et al. (2001) reported reductions in feed intake with inclusion of 2.50% rumen protected CLA to feedlot diets. Addition of CLA SALT increased ($P < 0.05$) ADG for the first 32 d on treatment compared to OIL or CONT. Daily gains were similar ($P < 0.05$) among treatments fed for 60 d. In contrast, Gassman et al. (2001) found CLA supplementation decreased ADG. Feed efficiency (gain:feed) was improved ($P < 0.05$) for SALT fed for 32 d compared to OIL or CONT. However, gain:feed was similar ($P > 0.05$) among treatments fed for 60 d. In previous research, no differences were observed in feed efficiency between control and SALT supplemented feedlot cattle (Gassman et al., 2001).

Carcass traits by dietary treatment or time on treatment are presented in Table 3.3. Hot carcass weight, live weight, longissimus area, adjusted fat thickness, and quality grade did not differ ($P > 0.05$) among diets or time treatments. Marbling scores tended ($P < 0.10$) to be greater for OIL compared to SALT with CONT being intermediate. Andrae et al. (2001) found marbling scores increased in steers fed high oil corn compared to traditional corn. Gassman et al. (2001) found 2.50% CLA-salt fed steers had lower marbling scores compared to control groups and there was a numeric decrease in backfat measurements as percentage CLA fed increased. However, in that study continental-cross steers were fed CLA for an average of 133 d. Carcasses from heifers fed for 32 d tended ($P < 0.10$) to have greater marbling scores than those fed for 60 d. Previous work has shown marbling deposition to be non-linear. In Angus-Hereford steers i.m. fat percentage doubled between days 84 and 112 on feed, with no change for additional days on feed (Duckett et al., 1993). In Angus heifers the deposition curve was similar, however, 50% of marbling deposition took place between d 56 and 84 d on feed (Smith et al., 2001). The heifers used in this study appear to have reached a plateau in their marbling deposition as marbling scores did not increase with additional time on feed. Carcass values were determined using a value based marketing grid with no differences ($P > 0.05$) between dietary treatments; however, OIL treatments were valued, on average, at approximately $30 and $18 than SALT or CONT, respectively.

The interaction between diet and time on treatments was significant ($P < 0.10$) for dressing percentage, KPH, and yield grade (Table 3.4). Dressing percentage was highest ($P < 0.05$) for CONT-32 and lowest ($P < 0.05$) for CONT-60 with all other treatments being intermediate. Percentage of KPH was lower ($P < 0.05$) for OIL-32 than OIL-60, SALT-60, and CONT-32 with all others being intermediate. Numeric yield grades were lower ($P < 0.05$) for SALT-32 and CONT-32 than SALT-60 with all others being intermediate.

Fatty acid composition by diet and time on treatment is shown in Table 3.5. Myristic (C14:0), myristoleic (C14:1 c9), palmitic (C16:0), palmitoleic (C16:1 c9), stearic (C18:0), behenic (C22:0), unidentified, monounsaturated, and polyunsaturated fatty acids did not differ ($P < 0.05$) between diets or time on treatments. The percentage of C18:1 *trans*, oleic, and linoleic was not altered ($P > 0.05$) by time on treatment. Concentration of C18:1 *trans* (accounts for *trans*-9, -10, and -11) was greater ($P < 0.05$) in SALT compared to CONT with OIL being intermediate. Ruminal by-pass studies have shown that protected CLA is largely hydrogenated (~80%) when supplemented in high concentrate feedlot diets (Sackmann et al., 2002; Gassman et al., 2001). The addition of unsaturated fat has also been shown to increase 18:1 *trans* content in beef (Enser et al., 1999). Oleic acid content was greater ($P < 0.05$) in CONT than SALT with OIL being intermediate. Gassman et al. (2001) also found a decreased content of oleic acid in 2.50% CLA-salt supplemented heifers compared to control animals. The differences in oleic acid content may be the result of altering Δ^9 desaturase enzyme activity. In a review of Δ^9 desaturase activity, Ntambi (1999) explains eighteen carbon fats containing at least 2 conjugated double bonds in the 9th and 12th position (linoleic acid, present in corn oil) to reduce enzyme activity. In dairy research, the presence of t10, c12 CLA isomer has also been shown to decrease Δ^9 desaturase activity (Baumgard et al., 2001). Linoleic acid percentage was greater ($P < 0.05$) for OIL than CONT with SALT being intermediate. Steers fed high oil corn also had an increased of linoleic acid concentration in i.m. lipid (Andrae et al., 2001).

The interaction between diet and time on treatment for pentadecyclic, margaric, total odd-chain (OCFA), certain CLA isomers, linolenic, eicosenoic (C20:1 c9), and total saturated (SFA) fatty acid was significant ($P < 0.10$; Table 3.6). Pentadecyclic, margaric, and OCFA decreased ($P < 0.05$) with time on treatment for CONT but were similar ($P > 0.05$) for OIL and SALT regardless of time on treatment. Linolenic acid concentration was lower ($P < 0.05$) at 60 d on treatment compared to 32 d for CONT, with no change ($P > 0.05$) for SALT or OIL. Concentration of eicosenoic acid increased and total SFA decreased ($P < 0.05$) with time on treatment for CONT but were similar ($P > 0.05$) for SALT and OIL over time. The *trans*-10, *cis*-12 (t10, c12) isomer of CLA increased ($P < 0.05$) with time on treatment for OIL but did not change ($P > 0.05$) for

CONT or SALT treated heifers. The *trans*-9, *trans*-11 (t9, t11) CLA isomer increased with time on treatment for SALT but was similar ($P > 0.05$) for OIL and CONT.

The fatty acid profile for ground beef by dietary treatment or time on treatment is presented in Table 3.7. Linolenic acid concentration decreased ($P < 0.05$) with time on treatment. Total saturated fatty acid concentration tended ($P < 0.10$) to be greater at 32 d on treatment than at 60 d. Other fatty acids (myristic, myristoleic, palmitic, palmitoleic, stearic, C18:1 *trans*, linoleic, arachidonic, unidentified, and polyunsaturated fatty acids) were unaffected ($P > 0.05$) by time on treatment. Similar to intramuscular fat, C18:1 *trans* was greater ($P < 0.05$) for SALT than CONT, with OIL being intermediate. Linoleic acid and total polyunsaturated (PUFA) fatty acid concentrations were greater ($P < 0.05$) for OIL than CONT or SALT. In ground beef, other fatty acids (myristic, myrisoleic, palmitic, palmitoleic, stearic, linolenic, arachidonic, unidentified, and SFA) were unaffected ($P > 0.05$) by dietary treatment.

The dietary treatment by time on treatment was significant ($P < 0.10$) for pentadecyclic, margaric, oleic, t9 t11 CLA isomer, and OCFA interactions (Table 3.8). Pentadecyclic acid and OCFA concentration decreased ($P < 0.05$) with time on treatment for CONT with no change ($P > 0.05$) for SALT or OIL. Margaric acid percentage decreased ($P < 0.05$) with time on treatment for CONT and OIL but was similar ($P > 0.05$) for SALT. Oleic acid and monounsaturated fatty acid (MUFA) percent increased ($P < 0.05$) with time on treatment for CONT but did not change ($P > 0.05$) for SALT or OIL. Duckett et al. (1993) found increasing time on feed increased concentrations of MUFA in i.m. lipid. However, since SALT and OIL treatments remained similar over time an inhibition of the Δ^9 desaturase enzyme may have occured. The t9, t11 isomer of

CLA decreased ($P < 0.05$) with time on treatment for CONT while OIL and SALT were similar ($P > 0.05$) across time.

Conjugated linoleic acid concentrations, expressed at mg/g of lipid, in longissimus and ground beef are present in Table 3.9. The concentration of c9, t11/*trans*-8, *cis*-10 (t8, c10) and *cis*-9, *cis*-11 (c9, c11) isomers of CLA were greater ($P < 0.05$) for SALT than OIL with CONT being intermediate for longissimus. Time on treatment tended to reduce ($P < 0.10$) the concentration of c9, t11/t8, c10 and increase ($P < 0.05$) the percentage of other CLA isomers. Heifers used in this study were received directly from grass pasture and according to ultrasound measurements had an average of 0.76 cm of backfat at the start of the trial. The heifers' condition prior to application of treatment diets might explain why c9, t11 concentration tended to decrease over time. Research has shown that grass-fed beef has a higher concentration of CLA (French et al., 2000) and levels previously reported for tissues from feedlot animals are lower (0.37; French et al. 2000) than those reported here. The concentration of other CLA isomers were also greater ($P < 0.05$) for SALT than CONT or OIL in longissimus and ground beef. The rumen protected CLA salt contained 12.5% of other CLA isomers that are not normally produced during ruminal biohydrogenation. In ground beef, time on treatment also tended ($P < 0.10$) to alter c9, c11 deposition as 60 d was greater than 32 d on treatment. Adding the concentration of all CLA isomers resulted in a 20% greater ($P < 0.05$) total CLA concentration for SALT than CONT or OIL for the longissimus. However, total CLA percentage was similar ($P > 0.05$) across time on treatment in both longissimus and ground beef.

Longissimus and ground beef composition by dietary treatment and time on treatment are presented in Table 3.10. Longissimus from OIL tended ($P < 0.10$) to have less moisture than SALT with CONT being intermediate. Lipid, protein and ash content of longissimus muscle were similar ($P > 0.05$) between dietary treatments. However, numeric increases in lipid percentage of longissimus were observed for OIL which is in agreement with marbling score increases. Time on treatment did not alter ($P < 0.05$) moisture or percentage lipid, protein, or ash in longissimus. Similarly ground beef composition was not altered ($P < 0.05$) by dietary treatment. However, moisture, lipid, and ash percentage differed ($P < 0.05$) between 32 and 60 d on treatment. Lipid percentage was greater ($P < 0.05$) for 60 d, conversely moisture content were greater ($P < 0.05$) for 32d. These differences may be explained by differences in the amount of trimming done by personnel prior to grinding plates, as carcasses from -60 d heifers were not trimmed as well.

Figure 3.1 shows lipid oxidation (TBARS) of longissimus samples by dietary treatment. Following 19 d of storage, dietary treatment did not alter ($P < 0.05$) malonaldehyde concentration in longissimus steaks. Previous research involving CLA salt fed steers showed no differences in shelf-life between treated and control samples (Gassman et al., 2001).

Lipid oxidation of steaks by time on treatment is presented in Figure 3.2. Steaks from 32 d on treatment had higher ($P < 0.05$) TBARS values on d 12 than 60 d. At 0, 5, and 19, TBARS values were similar ($P > 0.05$) between time on treatments.

Ground beef sample lipid oxidation by dietary treatment is shown in Figure 3.3. Following 8 d of storage, TBARS were greater ($P < 0.05$) in OIL fed heifers compared to

CONT, with SALT being intermediate. The increased level of lipid oxidation may be a result of the elevated concentration of PUFA observed for OIL treatments.

Time on treatment also affected TBARS measurements in ground beef (Figure 3.4). At d 8, ground beef samples from heifers fed for 60 d samples had a greater ($P <$ 0.05) malonaldehyde concentration than 32 d. At d 0, 2, and 4 of storage, TBARS values were similar ($P > 0.05$) between time on treatment. The trend of decreased SFA as time on treatment increased may have resulted in the increased level of oxidation.

Sensory characteristics of steak and ground beef by dietary treatment and time on treatment are shown in Table 3.11. Initial tenderness of steaks decreased ($P < 0.05$) with greater time on treatment for CONT samples, while OIL and SALT had numeric increases. Overall tenderness for steaks from OIL treated heifers increased ($P < 0.05$) with increasing time on treatment. Steaks from CONT and SALT treated heifers had similar ($P > 0.05$) overall tenderness across time on treatment. Beef flavor was greater (P < 0.05) for SALT-60 compared to CONT-60, with all other treatments being intermediate. In ground beef samples, overall tenderness increased ($P < 0.05$) as time on treatment increased for OIL and SALT fed heifers, while CONT was unaltered ($P > 0.05$) by time on treatment. Beef flavor scores for ground beef samples increased ($P < 0.05$) with greater time on treatment for CONT heifers. Ground beef samples from heifers fed OIL or SALT had similar ($P > 0.05$) flavor scores across time.

Sensory results for longissimus and ground beef samples by dietary treatment or time on treatment are presented in Table 3.12. Juiciness and off flavors were not altered ($P > 0.05$) by time on treatment in steak samples. Juiciness was greater ($P < 0.05$) for CONT fed heifers compared to OIL, with SALT being intermediate. Off flavors were higher (*P* < 0.05) for OIL compared to CONT, with SALT being intermediate. However, all values for off flavor were less than 1 (extremely mild) indicating little potential consumer acceptance problems. Initial tenderness, off flavor, and connective tissue scores were similar (*P* > 0.05) for ground beef samples among dietary treatments. However, juiciness tended to be greater (*P* < 0.10) in OIL fed heifers compared to SALT, with CONT being intermediate. Time on treatment did not alter (*P* > 0.05) sensory scores for juiciness, off flavor or connective tissue in ground beef. Initial tenderness was greater (*P* < 0.05) for heifers receiving treatment diets for 60 d compared to 32 d. In sensory panels involving feedlot steers fed 1.0 or 2.5% CLA salt no differences in sensory attributes for tenderness, flavor, or overall acceptability were detected (Gassman et al., 2001).

Combining CLA concentration and lipid content of longissimus samples CLA available in a serving (100 g cooked/114 g uncooked) of beef from this study was determined (Figure 5). Although lower in c9, t11 concentration, OIL fed heifers tended to have greater marbling scores, thus one serving of steak from the OIL treatment would result in a greater intake (30.53 mg) of c9, t11. Steaks from animals fed SALT and CONT would supply 27.82 and 27.33 mg, respectively, of c9, t11 per serving. The c9, t11 comprised over 73% of total CLA in steak samples.

Implications

Feedlot diets supplemented with rumen protected conjugated linoleic acid increased total tissue conjugated linoleic acid concentration and heifer performance during the initial phase of supplementation. However, it also tended to decrease marbling score. Supplementing feedlot diets with unsaturated oil did not alter tissue conjugated linoleic acid concentration, however it did increase marbling score. Both supplementations had minimal effects on product quality and sensory attributes. On a consumption basis, increased marbling scores in oil supplemented heifers resulted in a larger increase in conjugated linoleic acid content per serving than increasing the concentration of conjugated linoleic acid by supplementing rumen protected salts of conjugated linoleic acid.

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Table 3.1. Ingredient composition of dietary treatments for heifers fed supplemental oil (OIL) or rumen protected CLA salt (SALT) during the last 32 or 60-d of finishing.[a]

	CONT	OIL	SALT
Hay, Bermuda grass	12	12	12
Corn/soybean meal[b]	88	84	86
Corn oil	-	4	-
CLA salt[c]	-	-	2
		Chemical Composition[d]	
DM, %	84.50	86.08	85.75
CP, %	11.98	11.49	11.74
Total lipid, %	3.30	6.38	4.20
ADF, %	8.68	8.48	8.58
NDF, %	21.45	20.82	21.14
Ash, %	6.76	6.48	7.06
		Fatty Acid Compostion[d]	
C16:0, %	13.38	13.33	13.58
C18:0, %	0.92	0.97	1.00
C18:1 c9, %	22.11	22.22	22.17
C18:2 c9, c12, %	52.30	52.35	51.31
C18:3 c9, c12, c15,%	3.86	3.80	3.83
		CLA isomers[c]	
cis -9, *trans*- 11, %	-	-	0.47
trans -10, *cis* -12, %	-	-	0.18
cis -9, *cis* -11, %	-	-	0.13
trans -9, *trans* -11, %	-	-	0.07
Total CLA, %	-	-	1.06

[a] Percentage of diet on a DM basis.

[b] Composition: 81.4% corn, 2.75% Soybean Meal, 1.42% Limestone, 0.5% trace-mineralized salt (97% NaCl, 3,500 mg Zn/kg, 2,000 mg Fe/Kg, 1,800 mg Mn/kg, 350 mg Cu/kg, 100 mg I/kg, 90 mg Se/kg, and 60 mg Co/kg), and 0.9% urea.

[c] CLA salt was supplied by Agribrands, Canada.

[d] All values except %DM are on a DM basis

Table 3.2. Effect of feeding supplemental oil (OIL) or rumen protected CLA salt (SALT) for 32 or 60 d on heifer performance.

Time on Treatment	-32d				-60d			
Diet	CONT	OIL	SALT	SEM	CONT	OIL	SALT	SEM
Feed Intake, kg	11.94	11.47	10.86	0.94	11.78	9.89	10.13	0.86
ADG, kg	1.44^{bc}	1.52^{b}	2.00^{a}	0.11	1.28^{bc}	1.18^{c}	1.25^{bc}	0.11
Gain:Feed	0.14^{b}	0.14^{b}	0.21^{a}	0.01	0.13^{b}	0.12^{b}	0.13^{b}	0.01

^{abc}Means in the same row with uncommon superscripts differ ($P < 0.05$).

Table 3.3. Effect of feeding supplemental oil (OIL) or rumen protected CLA salt (SALT) for either 32 or 60 d on carcass characteristics.

	Dietary Treatment				Time on Treatment		
Item	CONT	OIL	SALT	SEM	-32 d	-60 d	SEM
Live wt, kg	480.74	492.89	487.29	7.72	487.79	486.16	6.21
Hot carcass wt, kg	294.47	300.85	299.38	5.07	299.85	296.61	4.08
Adjusted fat thickness, cm	1.63	1.53	1.59	0.09	1.67	1.50	0.07
Longissimus area, cm^2	26.75	27.91	28.67	0.88	27.87	27.68	0.71
Marbling score[a]	5.40^{cd}	5.81^{c}	5.26^{d}	0.18	5.66^{e}	5.32^{f}	0.14
Quality grade[a]	4.85	5.33	4.83	0.21	5.12	4.83	0.18
Value[b], $/carcass	734.92	764.58	745.96	22.1	764.72	732.25	18.64

[a] Marbling score and quality grade code: 4.0 to 4.99 = Slight: Select; 5.0 to 5.99 = Small: $Choice^-$; 6.0 to 6.99 = Modest: $Choice^0$; all carcasses were A maturity.

[b] VBM grid/cwt: base price = $103.03, premiums; CAB $3.05, discounts; Yield Grade 4 $14.34, Select $10.37.

[cd] Means in the same row with uncommon superscripts differ ($P < 0.10$) due to dietary treatment.

[ef] Means within the same row with uncommon superscripts differ ($P < 0.10$) due to time on treatment.

Table 3.4. Effect of interactions between feeding supplemental oil (OIL) or rumen protected CLA salt (SALT) by time on treatment on carcass characteristics.

Time on Treatment		-32d				-60d		
Diet	CONT	OIL	SALT	SEM	CONT	OIL	SALT	SEM
Dressing Percentage, %	62.27^{b}	60.62^{bc}	61.31^{bc}	0.57	60.25^{c}	61.19^{bc}	61.57^{bc}	0.52
KPH^{b}, %	2.50^{b}	1.58^{c}	2.17^{bc}	0.23	1.92^{bc}	2.33^{b}	2.42^{b}	0.21
Yield Grade	3.77^{b}	3.20^{bc}	3.71^{b}	0.22	3.52^{bc}	3.61^{bc}	3.12^{c}	0.20

[a] KPH: kidney, pelvic, and heart fat.

[bc] Means in same row with uncommon superscripts differ ($P < 0.05$).

Table 3. 5. Effect of feeding supplemental oil (OIL) or rumen protected CLA salt (SALT) for either 32 or 60 d on longissimus muscle long chain fatty acid composition.[a]

	Dietary Treatment				Time on Treatment		
Item	CONT	OIL	SALT	SEM	-32 d	-60 d	SEM
C14:0, %	2.98	3.11	3.21	0.14	3.11	3.09	0.11
C14:1 c9, %	0.63	0.66	0.70	0.04	0.65	0.68	0.04
C16:0, %	25.74	25.59	25.89	0.40	26.07	25.41	0.32
C16:1 c9, %	2.95	2.94	2.89	0.11	2.94	2.90	0.09
C18:0, %	14.54	14.37	14.36	0.31	14.40	14.45	0.26
C18:1 t, %	0.90^{d}	1.38^{cd}	1.93^{c}	0.22	1.53	1.28	0.18
C18:1 c9, %	40.39^{c}	39.39^{cd}	38.17^{d}	0.61	38.97	39.66	0.50
C18:2 c9, c12, %	2.11^{d}	2.54^{c}	2.25^{cd}	0.13	2.27	2.33	0.11
C22:0, %	0.19	0.17	0.17	0.01	0.17	0.18	0.01
C20:4 c9, c12, c15, c18, %	0.51	0.45	0.50	0.05	0.48	0.50	0.04
Unidentified, %	6.69	6.58	7.03	0.16	6.77	6.77	0.13
Monounsaturated Fatty Acids, %	45.01	44.53	43.84	0.55	44.23	44.69	0.45
Polyunsaturated Fatty Acids, %	3.28	3.61	3.48	0.17	3.43	3.48	0.14

[a] Fatty acids separated but being less than 0.01% include: C8:0, C10:0, C12:0, C13:0, C20:0, and C22:1 c9.

[cd] Means within same row with uncommon superscripts differ ($P < 0.05$) due to dietary treatment.

Table 3.6. Effect of feeding supplemental oil (OIL) or rumen protected CLA salt (SALT) by time on treatment on longissimus muscle long chain fatty acid composition.

Time on Treatment	-32d				-60d			
Diet	CONT	OIL	SALT	SEM	CONT	OIL	SALT	SEM
C15:0, %	0.52^{a}	0.49^{a}	0.45^{ab}	0.04	0.39^{b}	0.43^{ab}	0.51^{a}	0.03
C17:0, %	1.43^{a}	1.32^{a}	1.19^{ab}	0.09	0.09^{b}	1.15^{ab}	1.27^{ab}	0.10
C18:2 t10, c12, %	0.02^{bc}	0.02^{c}	0.02^{c}	0.00	0.02^{c}	0.04^{a}	0.03^{ab}	0.00
C18:2 t9, t11, %	0.02^{c}	0.03^{c}	0.06^{b}	0.01	0.03^{c}	0.03^{c}	0.08^{a}	0.00
C18:3 c9, c12, c15, %	0.15^{a}	0.08^{b}	0.07^{b}	0.01	0.08^{b}	0.08^{b}	0.08^{b}	0.01
C20:1 c9, %	0.10^{b}	0.15^{a}	0.14^{ab}	0.01	0.18^{a}	0.15^{a}	0.16^{a}	0.01
SFA[d], %	46.86^{a}	45.42^{ab}	45.24^{ab}	0.89	44.02^{b}	45.12^{ab}	46.06^{a}	0.89
OCFA[d], %	1.96^{a}	1.83^{ab}	1.65^{bc}	0.11	1.49^{c}	1.60^{b}	1.79^{ab}	0.10

[abc] Means within the same row with uncommon superscripts differ ($P < 0.05$).

[d] SFA: total saturated fatty acids, OCFA: total odd chain fatty acids (C15:0 & C17:0).

Table 3.7. Effect of feeding supplemental oil (OIL) or rumen protected CLA salt (SALT) for either 32 or 60 d on ground beef long chain fatty acid composition.[a]

	Dietary Treatment				Time on Treatment		
Item	CONT	OIL	SALT	SEM	-32 d	-60 d	SEM
C14:0, %	3.14	3.25	3.41	0.12	3.27	3.26	0.10
C14:1 c9, %	0.78	0.81	0.84	0.06	0.78	0.85	0.05
C16:0, %	24.54	24.26	24.72	0.32	24.73	24.28	0.26
C16:1 c9, %	3.10	3.09	3.03	0.12	3.04	3.11	0.09
C18:0, %	14.73	14.72	14.71	0.36	14.79	14.58	0.30
C18:1 t, %	0.86^{d}	1.16^{cd}	1.41^{c}	0.12	1.09	1.21	0.10
C18:2 c9, c12, %	1.42^{d}	1.76^{c}	1.35^{d}	0.06	1.53	1.49	0.05
C18:3 c9, c12, c15, %	0.19	0.19	0.20	0.01	0.20^{e}	0.18^{f}	0.01
C20:4 c9, c12, c15, c18, %	0.13	0.13	0.11	0.01	0.13	0.11	0.01
Unidentified, %	6.48	6.49	6.54	0.13	6.54	6.47	0.11
Saturated Fatty Acids, %	44.57	44.27	45.01	0.47	45.07^{g}	44.17^{h}	0.36
Polyunsaturated Fatty Acids, %	2.51^{d}	2.91^{c}	2.53^{d}	0.08	2.67	2.62	0.06

[a] Fatty acids separated but being less than 0.01% include: C8:0, C10:0, C12:0, C20:0, C20:1 c9, C22:0 and C22:1 c9.

[cd] Means within same row with uncommon superscripts differ ($P < 0.05$) due to dietary treatment.

[ef] Means within same row with uncommon superscripts differ ($P < 0.05$) due to time on treatment.

[gh] Means within same row with uncommon superscripts differ ($P < 0.10$) due to dietary treatment.

Table 3.8. Effect of feeding supplemental oil (OIL) or rumen protected CLA salt (SALT) by time on treatment on ground beef long chain fatty acid composition.

Time on Treatment		-32d				-60d		
Diet	CONT	OIL	SALT	SEM	CONT	OIL	SALT	SEM
C15:0, %	0.58^{a}	0.56^{a}	0.52^{ab}	0.03	0.45^{b}	0.51^{ab}	0.56^{a}	0.03
C17:0, %	1.50^{a}	1.38^{a}	1.31^{ab}	0.08	1.18^{b}	1.16^{b}	1.34^{ab}	0.07
C18:1 c9, %	40.23^{b}	40.96^{b}	40.96^{b}	0.73	42.87^{a}	41.39^{ab}	40.11^{b}	0.59
C18:2 t9, t11, %	0.03^{a}	0.02^{bc}	0.02^{bc}	0.00	0.02^{c}	0.02^{c}	0.02^{ab}	0.00
MUFA[d], %	45.13^{b}	45.91^{b}	46.14^{b}	0.69	47.72^{a}	46.77^{ab}	45.72^{b}	0.56
OCFA[d], %	2.10^{a}	1.95^{a}	1.85^{abc}	0.10	1.64^{c}	1.70^{bc}	1.92^{ab}	0.09

[abc] Means within the same row with uncommon superscripts differ ($P < 0.05$).

[d] MUFA: total monounsaturated fatty acids, OCFA: total odd chain fatty acids (C15:0 & C17:0).

Table 3.9. Effect of feeding supplemental oil (OIL) or rumen protected CLA salt (SALT) for either 32 or 60 d on longissimus and ground beef conjugated linoleic acid composition (mg/g of lipid).

	Dietary Treatment				Time on Treatment		
Item	CONT	OIL	SALT	SEM	-32 d	-60 d	SEM
Longissimus							
C18:2 c9, t11/t8, c10	4.518^{cd}	4.291^{d}	4.878^{c}	0.188	4.747^{g}	4.378^{h}	0.153
C18:2 c9, c11	0.245^{ab}	0.222^{b}	0.297^{a}	0.020	0.246	0.263	0.018
C18:2 other CLA*	0.231^{d}	0.342^{cd}	0.414^{c}	0.050	0.260^{f}	0.399^{e}	0.047
C18:2 total CLA	5.444^{b}	5.434^{b}	6.542^{a}	0.209	5.830	5.783	0.184
Ground Beef							
C18:2 c9, t11/t8, c10	6.851	7.242	7.172	0.369	7.001	7.174	0.299
C18:2 t10, c12	0.245	0.280	0.340	0.043	0.293	0.283	0.035
C18:2 c9, c11	0.260	0.268	0.329	0.043	0.232^{f}	0.339^{e}	0.035
C18:2 other CLA*	0.211^{b}	0.358^{b}	0.586^{a}	0.054	0.409	0.361	0.043
C18:2 total CLA	7.774	8.324	8.638	0.361	8.156	8.335	0.294

*Other *trans*, *trans* and *cis, cis* isomers of CLA that were unable to be identified specifically.

^{ab}Means within same row with uncommon superscripts differ ($P < 0.05$) due to dietary treatment.

^{cd}Means within same row with uncommon superscripts differ ($P < 0.10$) due to dietary treatment.

^{ef}Means within same row with uncommon superscipts differ ($P < 0.05$) due to time on treatment.

^{gh}Means within same row with uncommon superscripts differ ($P < 0.10$) due to time on treatment.

Table 3.10. Effect of feeding supplemental oil (OIL) or rumen protected CLA salt (SALT) for 32 or 60 d on longissimus and ground beef composition.

Item	CONT	OIL	SALT	SEM	-32 d	-60 d	SEM
Longissimus							
Moisture, %	70.93^{cd}	69.46^{d}	71.05^{c}	0.58	70.41	70.55	0.47
Lipid, %	6.27	7.62	6.31	0.64	6.65	6.82	0.52
Protein, %	22.24	22.32	22.22	0.25	22.42	22.10	0.20
Ash, %	1.15	1.14	1.17	0.02	1.15	1.16	0.01
Ground Beef							
Moisture, %	55.62	53.95	53.11	1.10	55.62^{a}	52.84^{b}	0.89
Lipid, %	26.30	28.42	29.30	1.54	26.14^{b}	29.87^{a}	1.25
Protein, %	17.25	16.83	16.80	0.56	17.40	16.52	0.45
Ash, %	0.83	0.79	0.79	0.02	0.84	0.77	0.02

[ab]Means within same row with uncommon superscripts differ ($P < 0.05$) due to time on treatment.

[cd]Means within same row with uncommon superscripts differ ($P < 0.05$) due to dietary treatment.

Table 3.11. Effect of feeding supplemental oil (OIL) or rumen protected CLA salt (SALT) for 32 or 60 d by time on treatment for longissimus and ground beef sensory analysis.

Time on Treatment		-32 d				-60 d		
Diet	CONT	OIL	SALT	SEM	CONT	OIL	SALT	SEM
Longissimus								
Initial Tenderness[a]	5.91^{c}	5.04^{d}	5.42^{d}	0.17	5.38^{d}	5.60^{cd}	5.63^{cd}	0.14
Overall Tenderness[a]	5.59^{c}	4.88^{d}	5.25^{cd}	0.19	5.34^{cd}	5.50^{c}	5.44^{c}	0.16
Beef Flavor[a]	5.13^{cd}	5.08^{cd}	4.92^{cd}	0.15	4.73^{d}	4.81^{cd}	5.17^{c}	0.12
Ground Beef								
Overall Tenderness[a]	5.78^{d}	5.48^{e}	5.44^{e}	0.07	5.79^{d}	6.00^{c}	5.79^{d}	0.07
Beef Flavor[a]	4.45^{d}	4.81^{cd}	4.77^{cd}	0.13	4.98^{c}	4.77^{cd}	5.00^{c}	0.12

[a] Sensory ratings (8 = extremely juicy, extremely tender, extremely intense;
1 = extremely dry, extremely tough, or extremely bland)

[cde] Means within same row with uncommon superscripts differ ($P < 0.05$).

Table 3.12. Effect of feeding supplemental oil (OIL) or rumen protected CLA salt (SALT) for either 32 or 60 d on longissimus and ground beef sensory analysis.

	CONT	OIL	SALT	SEM	-32 d	-60 d	SEM
Longissimus							
Juiciness[a]	5.31^{c}	4.84^{d}	5.08^{cd}	0.10	5.01	5.15	0.15
Off Flavor[b]	0.59^{d}	0.91^{c}	0.74^{cd}	0.06	0.86	0.63	0.21
Ground Beef							
Juiciness[a]	5.36^{ef}	5.41^{e}	5.03^{f}	0.12	5.11	5.42	0.14
Initial Tenderness[a]	5.87	5.78	5.64	0.09	5.62^{h}	5.91^{g}	0.09
Off Flavor[b]	1.08	0.67	0.69	0.18	0.93	0.69	0.13
Connective Tissue[a]	6.73	6.84	6.81	0.12	6.75	6.84	0.08

[a] Sensory ratings (8 = extremely juicy, extremely tender, no connective tissue; 1 = extremely dry, extremely tough, or abundant connective tissue)

[b] Sensory ratings (8 = extremely intense; 0 = none)

[cd] Means within same row with uncommon superscripts differ ($P < 0.05$) due to dietary treatment.

[ef] Means within same row with uncommon superscripts differ ($P < 0.10$) due to dietary treatment.

[gh] Means within same row with uncommon superscripts differ ($P < 0.05$) due to time treatment.

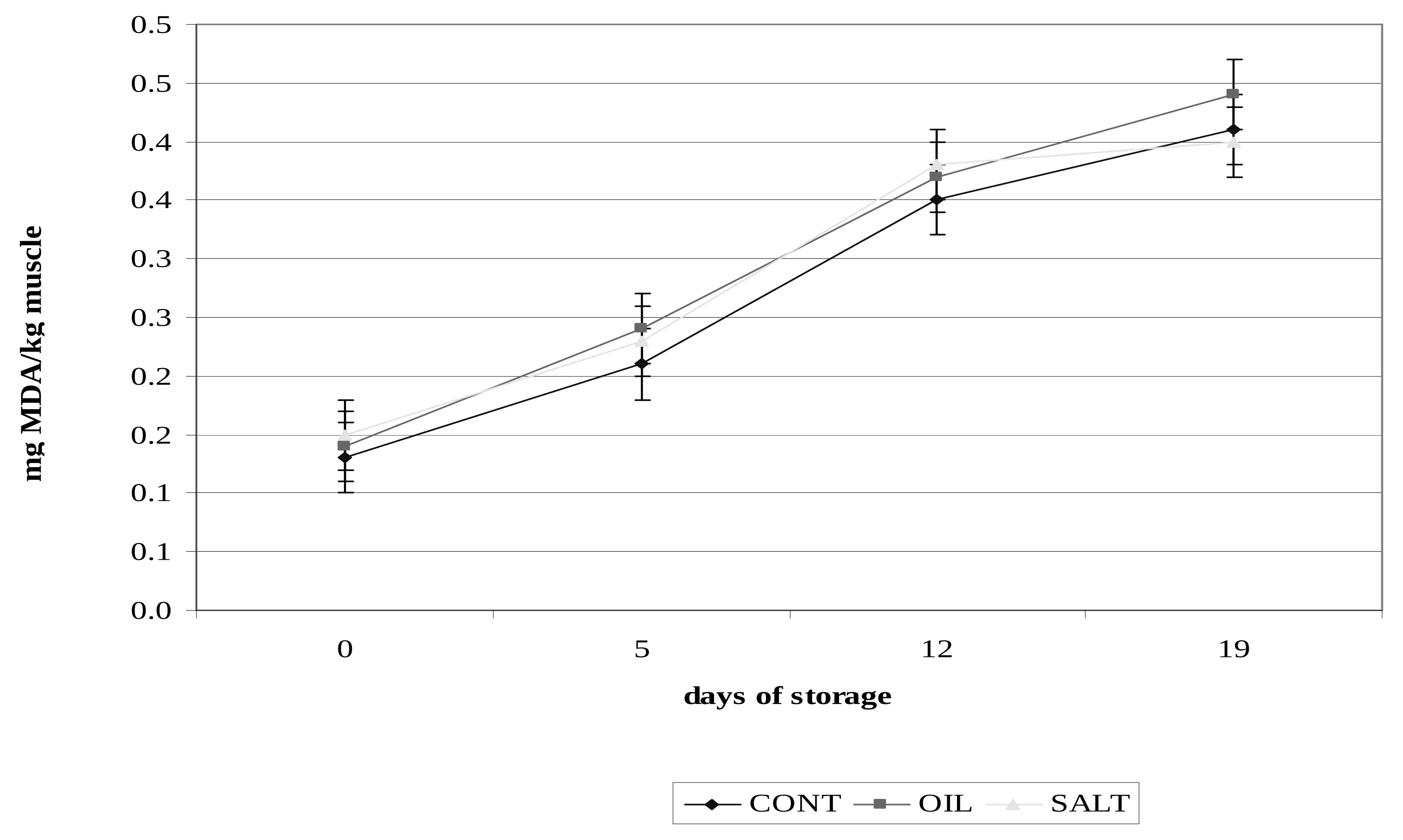

Figure 3.1. Effect of feeding supplemental oil (OIL) or rumen protected CLA salt (SALT) on TBARS measurements in the longissimus muscle.

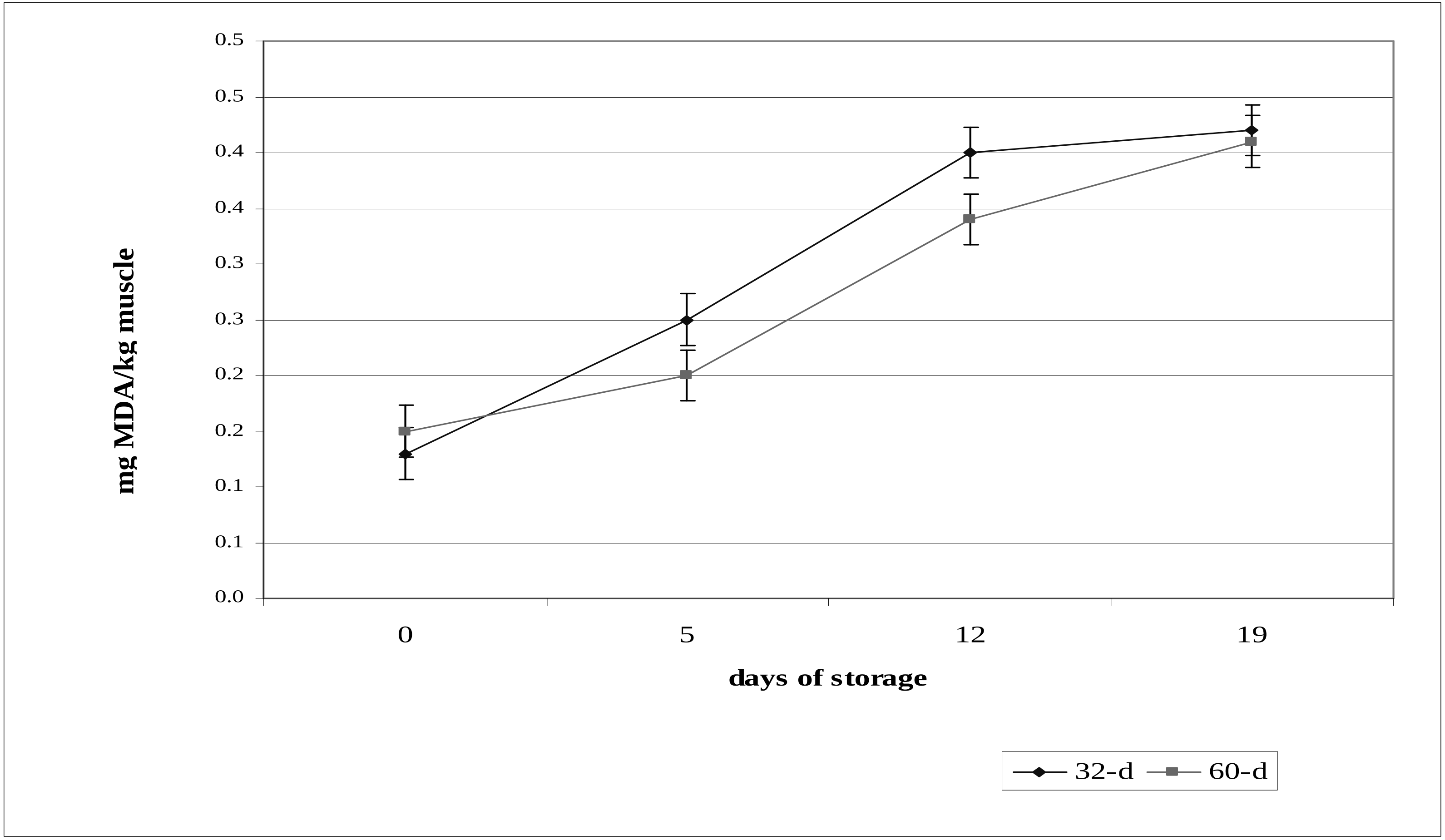

Figure 3.2. Effect of feeding supplemental oil or rumen protected CLA salt for either 32 or 60-d on TBARS measurements in the longissimus muscle.

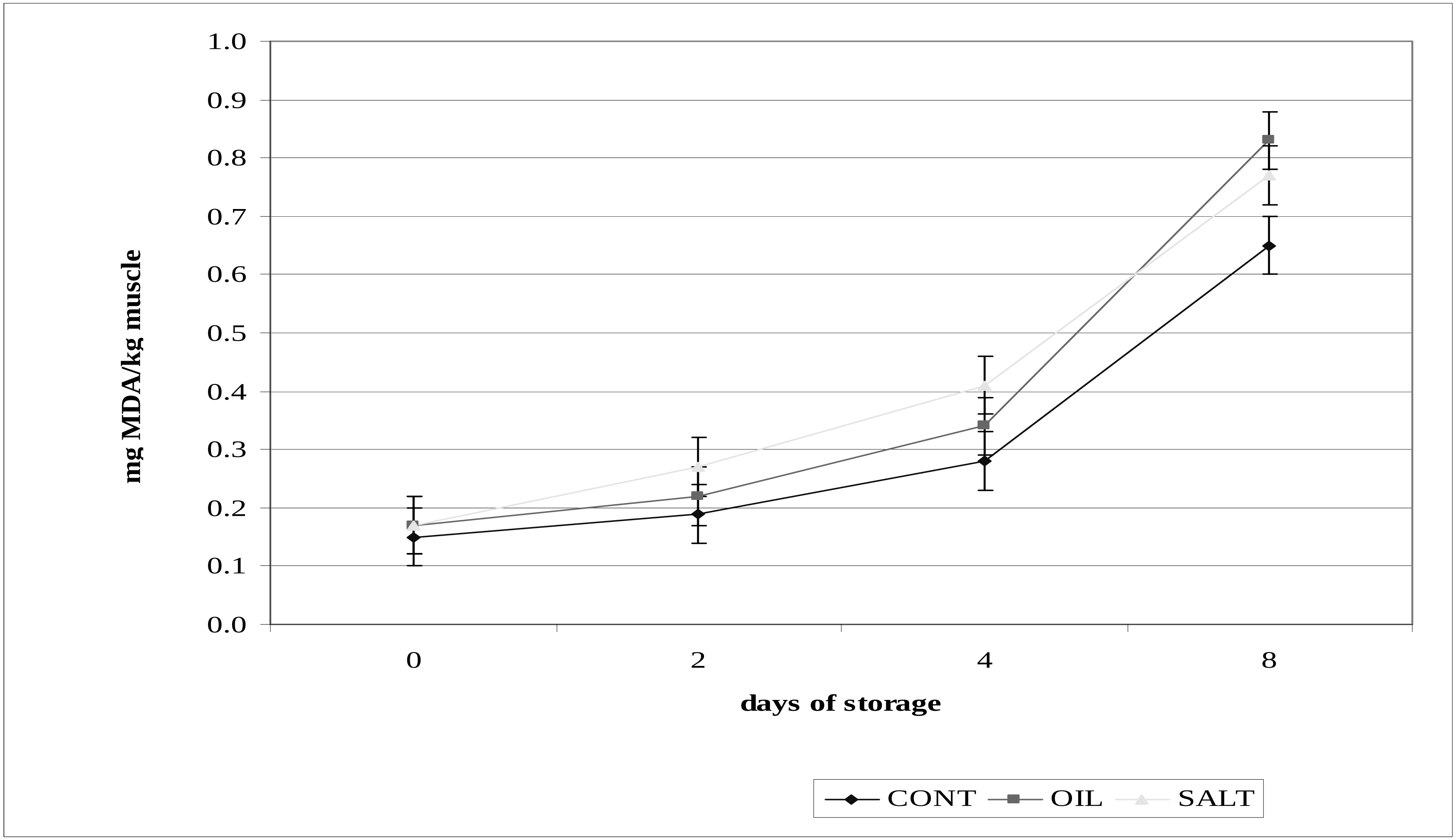

Figure 3.3. Effects of feeding supplemental oil (OIL) or rumen protected CLA salt (SALT) on TBARS measurements in ground beef.

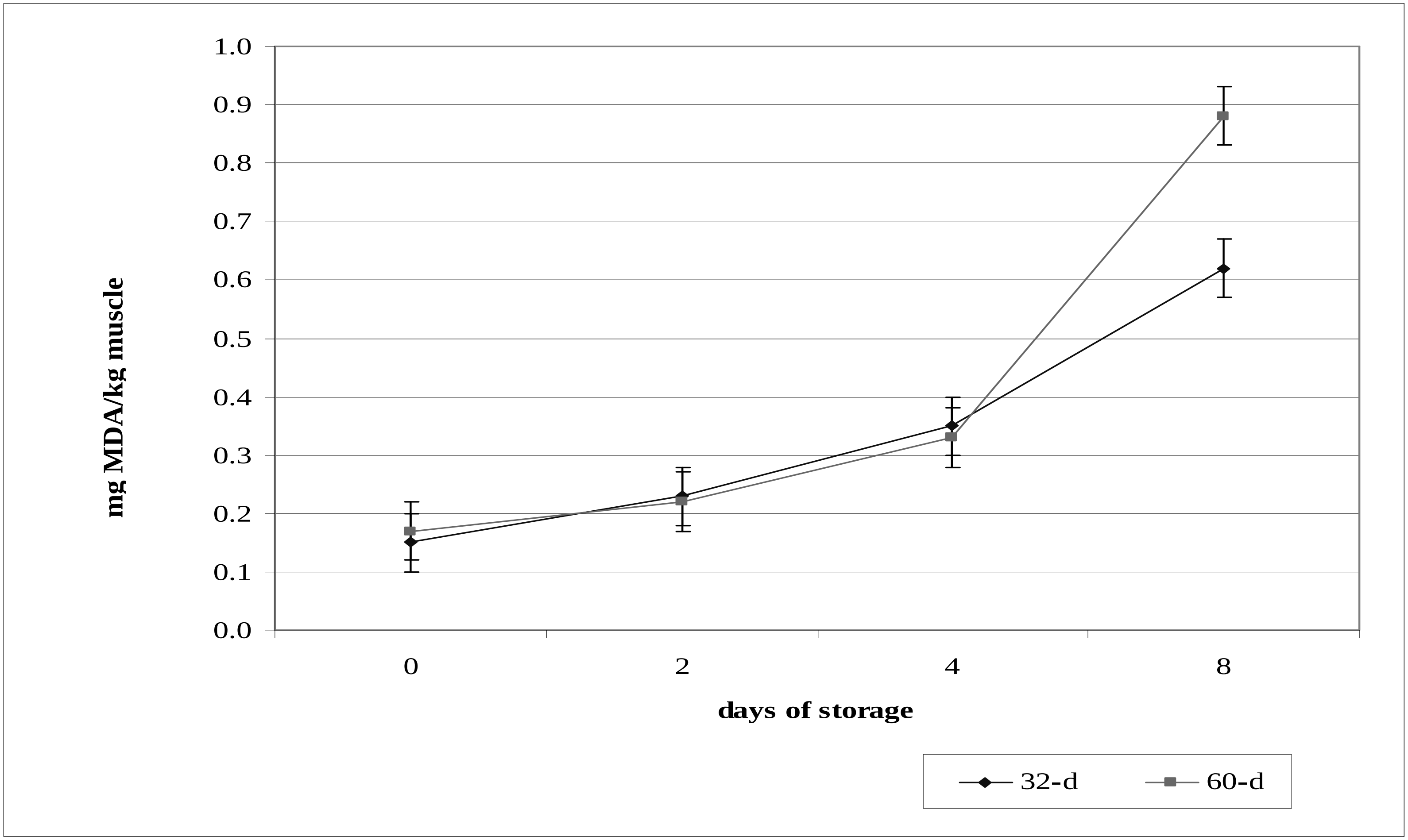

Figure 3.4. Effect of feeding supplemental oil or rumen protected CLA salt for either 32 or 60-d on TBARS measurements in ground beef.

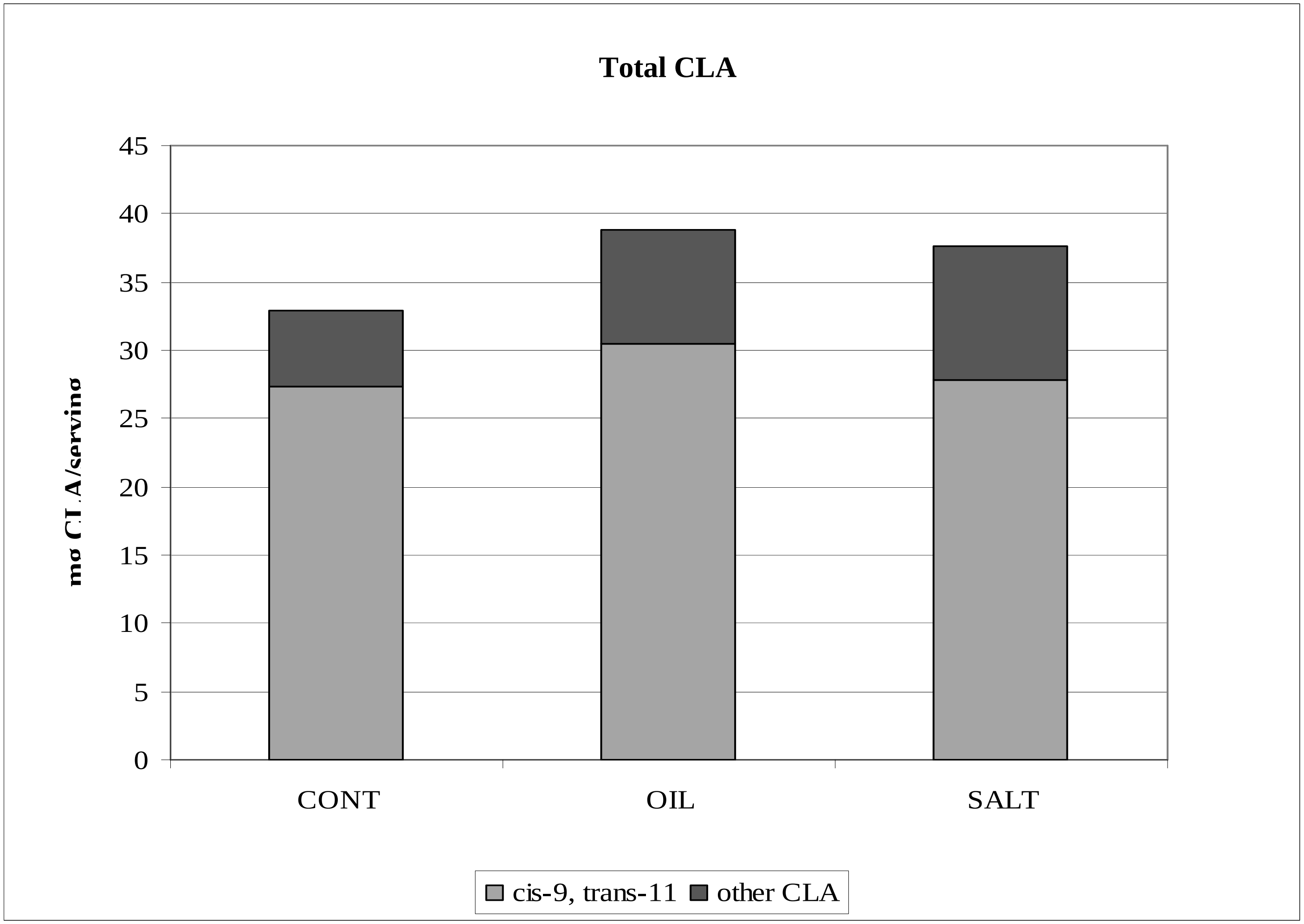

Figure 3.5. Effect of feeding supplemental oil (OIL) or rumen protected CLA salt (SALT) on CLA content per serving of steak.

CHAPTER 4

CONCLUSION

Decreasing dietary forage levels in feedlot type beef cattle diets resulted in decreased ruminal biohydrogenation. Forage level did not alter the production of the *cis*-9, *trans*-11 isomer of conjugated linoleic acid (CLA). However, lower forage diets resulted in an increased duodenal flow of C18:1 *trans* and the *trans*-10, *cis*-12 isomer of CLA. Supplemental oil level did not alter biohydrogenation or intermediate production. Feeding 4% compared to 2% supplemental oil resulted in an increased flow of total long-chain fatty acids to the small intestine. Based on these observations, a diet containing 12% forage and 4% supplemental oil was fed to feedlot heifers to evaluate animal performance, meat quality, and tissue fatty acid composition to heifers receiving no supplementation or 2% supplemental rumen protected CLA diet.

Heifers received treatment diets for the last 32 or 60 days of finishing. Dietary intakes did not differ among treatments. Heifers receiving CLA salt for the first 32 days had increased average daily gains and were more efficient compared to other heifers. Carcasses from oil fed heifers tended to have higher marbling scores and were more valuable. Lipid oxidation and taste panel results showed few differences of economic importance between treatments. The intramuscular lipid from CLA salt fed heifers had 20% more total CLA than either control or oil fed heifers. They also tended to have more *cis*-9, *trans*-11 CLA compared to oil fed heifers. However, on a serving basis, OIL fed heifers had a greater content of CLA per serving due to the increases in intramuscular lipid.