EFFECTS OF SUPPLEMENTAL LIPIDS ON LEPTIN LEVELS AND FATTY ACID COMPOSITION OF BOVINE ADIPOSE DEPOTS

by

MARGARET HOPSON GILLIS

(Under the Direction of Susan K. Duckett)

ABSTRACT

Lipid was supplied to feedlot cattle diets as either corn oil or rumen-protected conjugated linoleic acid (CLA) for two specific treatment periods of 32 d or 60 d prior to harvest. Thirty-six heifers were fed one of three diets: 1) control, 2) 4% corn oil, or 3) 2% rumen protected CLA. The cis-9, trans-11 CLA isomer has been shown to be a potent anticarcinogen. Leptin is a 16kDa protein secreted by adipocytes that serves as a circulating signal of whole body energy homeostasis. Supplementing lipid to finishing cattle diets altered fatty acid composition of tissues and increased CLA deposition; however, changes were variable between adipose depots as well as length of supplementation. Serum leptin levels were not affected by dietary treatment. Animals supplemented with corn oil contained higher amounts of leptin in adipose tissues. Supplementing lipid to finishing diets did not alter feedlot performance or carcass characteristics.

INDEX WORDS: Conjugated linoleic acid, Beef cattle, Leptin, Fatty acid composition

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B.S.A., The University of Georgia, 1999

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

MASTER OF SCIENCE

ATHENS, GEORGIA

2002

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December 2002

ACKNOWLEDGEMENTS

I would like to thank my major professor, Susan Duckett, for allowing me the opportunity to work under her guidance for the past two years. It has been a privilege to be your student. Thank you especially for your wonderful counsel, friendship, and “subtle” encouragement.

To my Meats group peers, Carolina, Jaime, and Kerry, thank you for all your help with laboratory, computer, and stress management skills. It has been a pleasure to have the opportunity to work and become friends with such a great group of women.

Thank you also to Mike Mathis, Dean Pringle, and Matt Wooster for all your help with sample collection and to Gina Burden for her assistance with data analyses.

To my parents and brother, without your unconditional love and support this would not have been possible. Thank you for always believing that I could do whatever I set my mind to, even when I couldn’t see the light at the end of the tunnel! Special thanks to my brother Jim for always making it look easy; our experience as roommates is one that I’ll never forget. In hindsight, I can honestly say it was a true learning experience. Thanks for the jovial company, wonderful meals (as well as the accompanying sink full of dirty dishes), and lively entertainment.

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CHAPTER 1

REVIEW OF LITERATURE

Introduction

Fatty acid composition and lipogenic activity of adipose tissue can be influenced by many factors including, but not limited to: nutritional status, depot, species, individual variation, age, as well as endocrine status of the animal. Manipulation of the fatty acid content of beef through dietary lipid supplementation may provide producers with a means for supplying consumers with beef products containing enhanced levels of unsaturated fatty acids. Edible beef products contain relatively low levels of polyunsaturated fatty acids (PUFA) and high levels of saturated fatty acids (SFA). From the standpoint of human nutrition, enhancing the unsaturated fatty acid content of beef translates to a more healthful product; dietary intake of SFA by humans is considered to contribute to elevations in serum cholesterol level and increased risk of cardiovascular disease. In particular, current human dietary guidelines recommend that dietary n-3 PUFA intake be increased. By definition, the ruminant animal presents a unique situation related to the metabolism and subsequent deposition of dietary lipid as adipose tissue. Through a series of isomerization and desaturation reactions, the process of rumen microbial biohydrogenation (BH) converts unsaturated dietary linoleic acid to stearic acid and palmitoleic acid to palmitic acid. Figure 1 shows the pathway of ruminal BH of dietary linoleic (C18:2) acid to stearic (C18:0) acid. This results in the flow of fatty acids to the duodenum being primarily saturated (Demeyer and Doreau, 1999). Figure 2 depicts the fatty acid composition of diet, digesta, and intramuscular lipid. Adipose tissues of ruminant animals are not representative of feedlot diets. The fate of dietary linoleic acid following ruminal BH is presented in Figure 3.

During the processes of ruminal BH of dietary unsaturated lipids, unique fatty acid intermediates termed conjugated linoleic acid (CLA) possessing anticarcinogenic effects are produced in addition to the saturated end products (Bauman et al., 1999). Whether tissue CLA content can be increased to a level that would negate the effects of SFA in bovine adipose tissues remains to be determined. Thus, the intrinsic nature of the rumen environment complicates attempts to alter adipose tissue fatty acid composition through dietary lipid source. This review will focus on the effects of dietary lipid source as related to tissue fatty acid deposition.

Background

Adipose tissue is a remarkable endocrine organ that is inherently in a state of metabolic flux. In ruminant animals, its composition is dependent on: 1) the extent of ruminal biohydrogenation as well as intestinal flow rates of dietary lipids and 2) de novo synthesis of fatty acids from acetate precursors in adipose tissue (Enser et al., 1999). Adipose tissue is the primary site of lipogenesis, with enzyme activity playing a major role in the assimilation and accumulation of storage lipids. In ruminants, acetate is the principal precursor of de novo fatty acid synthesis. The rate-limiting step is catalyzed by acetyl CoA carboxylase (ACC), which produces malonyl CoA. In a series of steps, fatty acid synthase (FAS), together with reducing equivalents (NADPH) generated by the pentose phosphate pathway and isocitrate cycle, catalyze the formation of palmitate from malonyl CoA. A fatty acid elongase enzyme acts by carrying out the two carbon additions that result in stearic and arachidonic acid production. Additionally, lipoprotein lipase (LpL) is responsible for hydrolysis of blood triacylglycerides for either oxidation or adipose tissue synthesis (Vernon et al., 1999).

Stearoyl CoA desaturase (SCD), also called Δ^9 desaturase, activity in adipose tissue is responsible for insertion of double bonds at the n-9 position of fatty acids, but never beyond. Tissue activity of the Δ^9 desaturase enzyme results in oleic acid production from stearic acid and palmitoleic acid from palmitate. It also catalyzes the formation of the cis-9, trans-11 CLA isomer from trans vaccenic acid (TVA), which is another intermediate product produced from ruminal BH of dietary linoleic acid. Therefore, the activity of these enzymes, and in particular the Δ^9 desaturase enzyme, are of importance when considering manipulation of the fatty acid composition of bovine adipose tissue depots. The CLA isomers are simply variations of the positional and geometric isomers of octadecadienoic acid. CLA has been shown to possess potent anticarcinogenic effects in laboratory models. The cis-9, trans-11 isomer is the most predominantly active CLA and possesses the tumor reducing effects (Vernon, et al., 1999). Other isomers, namely trans-10, cis-12 CLA have been implicated as having repartitioning effects. Because ruminant meat and milk products represent the largest natural source of CLA, the concentration of these fatty acid isomers in adipose tissue is receiving much attention. Current literature suggests additional information is needed for understanding the role of adipose tissue enzyme activity in fatty acid deposition in adipose depots.

Lipid Digestibility

Numerous trials have targeted changes in fatty acid deposition through supplementation of finishing diets with unsaturated fats. In order to achieve such results, supplemental unsaturated fats, which are preferentially saturated by rumen microorganisms, must either be protected from ruminal biohydrogenation (as calcium soaps or formaldehyde encased lipids) or be present in amounts high enough to result in sufficient escape to the intestinal tract for absorption. Zinn et al. (2000) reported that independent of supplementation level, increased intestinal digestibility of fat corresponded to reduced ruminal biohydrogenation. Stearate, oleate, and linoleate flow to the duodenum associated with increased dietary lipid was inversely proportional to the extent of biohydrogenation. Similarly, Duckett et al. (2002) found BH to be highest for linolenic acid and lowest for oleic acid in steers supplemented with high oil corn. Intestinal flow of palmitate, stearate, oleate, linoleic, linolenic, and arachidonic acid was greater for steers fed high oil corn. Additionally, they reported increased production of both TVA and the cis-9, trans-11 isomer of CLA in the digesta, which were highly correlated ($r^2=0.74$).

Corn-based finishing diets

Increasing intestinal flow of dietary unsaturated fats increases the potential for intestinal absorption and tissue deposition. In the U.S., feedlot diets are primarily corn based, with linoleate being the predominant fatty acid. Williams et al. (1983) reported lowered percentages of 18:0 and increased proportions of 18:1 in soft tissue of grain-finished beef steers. Similarly, Sumida et al. (1972) found increased 16:1 and 18:1 levels and decreased 18:0 content in subcutaneous (SQ) depots of feedlot steers. However, increasing the level of dietary concentrate corresponded to linear increases of SFA (corresponding to increased 16:0) in intramuscular (IM) lipid of steers fed diets for 85 d (French et al., 2000). Andrae et al. (2001) also showed higher SFA content in IM lipid, which was primarily due to increased 16:0 percentages for typical high concentrate diets compared to feeding high-oil corn varieties.

Forage-based diets

In contrast to grain-based feeding programs, forage-based diets have consistently been shown to increase the PUFA content of adipose tissue (Williams et al., 1983; Marmer et al., 1984; Yang et al., 1999; and French et al., 2000). Compared to concentrate rations, the fatty acid profile of forage contains reduced levels of 14:0, 16:0, 18:0, and 18:1. The high concentration of PUFA associated with grass-fed beef can be attributed to the enhanced linolenic acid content of grass and grass silages. French et al. (2000) found the IM lipid content of beef longissimus muscle had reduced palmitate and SFA concentrations and enhanced proportions of total PUFA, n-3 fatty acids, and the cis-9, trans-11 isomer of CLA. Reduced palmitic acid in both IM and SQ lipid depots and lowered SFA: PUFA ratios were also reported by Yang et al. (1999) and French et al. (2000).

Rumen-protected lipids

The seed coats of some oilseeds may provide similar protection from ruminal BH as do calcium salts and formaldehyde-protected fats. Feeding 30 % dietary whole cottonseed increased linoleic acid and total PUFA in perinephric fat of beef steers and reduced the total unsaturated fatty acid content of SQ lipid (Huerta-Leidenz et al., 1991). Addition of dry rolled high-oil corn containing linoleic acid to finishing diets increased odd-chain fatty acids, linoleic acid, arachidonic acid, and total PUFA content of IM lipid depots (Andrae et al., 2001). Feeding protected cottonseed oil resulted in lowered palmitoleic and oleic acid in SQ adipose tissue. Stearic acid, linoleic acid, and total SFA were increased in SQ lipid (Yang et al., 1999). Full fat canola seeds rich in oleic acid were demonstrated by Rule et al (1994) to lower both palmitic acid and palmitoleic acid content and increase stearate, linoleic acid, and eicosapentaenoic acid in the pectoral muscle of finishing steers.

Long-chain polyunsaturated fatty acids

Fish meal (FM) and fish oil (FO) supplementation to finishing diets has gained recent attention due to its n-3 fatty acid content and the potential for incorporation of eicosapentaenoic acid (EPA) and docosahexaenocic acid (DHA) into ruminant tissues. Mandell et al. (1997) fed FM at either 5 or 10 % of the diet for different time periods to steer finishing diets. Although the inclusion of fish meal depressed dry matter intake, feed efficiency was not affected. Palmitic acid concentration was enhanced by both advancing time on test diet as well as increasing dietary intake of FM. Stearate, linoleate, arachidonic acid proportions were decreased by feeding FM. EPA and DHA incorporation into muscle lipids was positively correlated to advancing dietary FM level and length of supplementation. Enser et al. (1999) and Scollan et al. (2001) reported effects of FO or whole linseed (high linoleic acid content) supplementation as compared to Megalac, a highly saturated supplementation derived from palm oil. All unsaturated fat supplementation resulted in dramatic increases (2-3 fold) in IM CLA content as compared to Megalac. TVA and linolenic acid production were also higher in adipose tissue from animals fed the more unsaturated fats. They also report a high correlation (r^2=0.62) between trans-vaccenic acid and CLA production in adipose tissues that agrees with the observations of Duckett et al. (2002) for fatty acid concentration of digesta.

Discussion

When interpreting the results of dietary lipid supplementation on fatty acid composition of adipose tissue depots, it is consequential that lipogenic enzyme activities be examined. While much remains to be discovered, inferences may be drawn in regard to potential changes in enzymatic regulation of fatty acid deposition as related to dietary lipid source. Chakrabarty et al. (1972) showed that ACC activity was proportional to marbling scores, which are related to adipocyte cellularity. Vernon et al. (1999) reported inhibition of ACC activity by both endocrine regulation as well as feedback inhibition from fatty acid production. Additionally, Barber et al. (2000) demonstrated that ovine ACC and LpL activities were highly correlated to adipocyte size, regardless of adipose tissue depot. They also documented significant correlation between oleic acid per adipocyte and both SCD mRNA per adipocyte and adipocyte size. St. John et al. (1991) reported highest SCD activity in the adipose tissue of ruminants, which was reflected in higher oleate and lower stearate concentrations. No detectable difference in SCD activity was found between breed types, despite varying levels of fat thickness. However, adipocyte cellularity was not different between breeds. According to work by Barber et al. (2000), the lack of breed variation in SCD activity may be attributed to the reported similarities in mean adipocyte size and volume. Similarly, Cameron et al. (1994) found that Wagyu cattle, despite increased adipose MUFA content, did not differ in SCD activity from Angus cattle. Further, they also found no significant differences in adipose cellularity or RNA concentration between breeds. May et al. (1994) found that SQ and IM preadipocytes of Wagyu cattle contained significantly higher rates of DNA synthesis than Angus cattle and that rates (measured by 3-methyl histidine incorporation into DNA) were higher in the SQ than IM preadipocytes. The Wagyu cattle had a greater number of adipocytes possessing smaller diameters than did Angus cattle. Furthermore, they demonstrated that FAS activity was greater in SQ fat of the Wagyu cattle, which was consistent with the observation that DNA synthesis in lipid-filling cells was greater in both IM and SQ adipose tissue of Wagyu. Rate of lipogenesis was not different between breeds, which suggests that Wagyu cattle may have a prolonged capacity to proliferate preadipocytes or improved genetic capability for hyperplasia.

Yang et al. (1999) reported positive linear correlation between SCD and palmitoleate ($r^2 = 0.52$) and total MUFA ($r^2 = 0.48$), which was substantiated by results of feeding rumen-protected cottonseed oil (CSO) containing high linoleic acid content. CSO feeding resulted in increased stearic acid and linoleic acid content of SQ adipose tissue. An inverse correlation was determined between SCD and linoleic acid ($r^2 = 0.50$) and SCD and total MUFA ($r^2 = 0.48$) Feeding the protected CSO also decreased SCD activity by greater than 50%. Rumen-protected CSO contain cycloprenoids, which may have also contributed to reduced SCD activity; additionally, some PUFA, including linoleic acid, are thought to inhibit SCD activity by depressing SCD gene expression. Forage-fed cattle, on the other hand, had a greater than 60% increase in SCD activity which was reflected in higher tissue incorporation of palmitoleic acid and oleic acids.

Knowledge of the biochemical pathways of lipogenesis and ruminal biohydrogenation are essential for interpretation of the effects of dietary lipid source. For instance, the increased TVA and CLA concentrations in the digesta of steers fed high oil (HO) corn varieties (Andrae et al., 2001) suggests that increasing linoleic acid content in the diet may have caused a decrease in ruminal BH by saturating the rumen environment with increased substrate. The resulting production of intermediates of biohydrogenation may have caused the improved flow of these fatty acids to the duodenum. In comparison, the increase in adipose tissue TVA and CLA reported by Enser et al. (1999) for steers fed

FO or whole linseed + FO suggests lipogenic enzyme regulation. The two diets were similar in dietary linoleic acid content and BH of linoleic acid was similar between treatments. These results suggest an improvement SCD activity or as concluded by the authors, an inhibition of tissue reductases that saturate these fatty acids. Regardless of the pathway, it appears that FO supplementation alters lipogenic pathways in adipose tissue.

Implications

The complexity that the ruminant animal represents in terms of lipid metabolism and deposition is unique. Numerous highly regulated pathways that are still not entirely understood mediate the effects of dietary fatty acid content on lipid BH and intestinal absorption, uptake by tissues from the bloodstream, and subsequent adipose tissue deposition. Current literature suggests that there are considerable discoveries yet to be made in the area of ruminant nutrition in relation to dietary fat supplementation. Research has clearly demonstrated that dietary lipid supplements can alter the fatty acid composition of beef products. However, increased PUFA content of beef associated with grass-based or fish oil supplemented diets has been shown to contribute to off-flavors in aroma volatiles and shorter shelf life. Additionally, the corresponding trans fatty acid increases correlated with CLA enrichment of tissues may limit consumer acceptability of these products. It is indicated that additional research, particularly in regard to greater tissue enrichment with CLA, is necessary.

Leptin

Leptin, a 16 kDa protein, is synthesized and secreted primarily by adipocytes, functions as a regulator of energy homeostasis, and serves as a circulating signal of adiposity through coordination with neuroendocrine function (Houseknecht et al., 1998;

Barb et al., 2002). Circulating leptin level has been shown to be well correlated to adiposity as well as adipocyte size for animals in a state of positive energy balance. However, research as to how dietary manipulations may alter circulating leptin concentrations or carcass characteristics is limited, particularly in ruminant animals consuming high-concentrate diets.

Although the mechanism of leptin action is still poorly understood, it appears to be associated with hypothalamic control of feed intake and may serve as the link between adipocytes and the central nervous system. Leptin has been connected with dose dependent decreases in feed intake, body mass, and fat depots (Barb, 1999). Furthermore, in contrast to other treatments, depletion of fat depots is maintained for several weeks following treatment with leptin (Azain et al., 1997).

Serum leptin concentrations are regulated by many different factors. Androgens, catecholamines, cAMP, long-term GH, fasting conditions, and cold exposure decrease serum leptin concentration. Estrogen, insulin, glucocorticoids, short-term GH, overeating, Interleukin-1, and impaired kidney function serve to increase levels of circulating leptin in vivo. The role of leptin in pituitary control may provide insight to its effects on feed intake and growth. Growth hormone-releasing hormone (GHRH) and somatotropin release-inhibiting factor (SRIF) regulation is controlled by the median eminence of the medulla oblongata and the combination of peripheral signals (including leptin) in GH secretion also occurs in the median eminence (Barb, 1999). In pigs, leptin treatment has been linked to elevations in GH concentrations, reduced feed intake, and improvements in metabolic rate (Barb et al., 1998).

Cohen et al. (2002) recently reported that leptin treatment in mice down-regulates gene expression of SCD-1. When *ob*/*ob* leptin deficient mice were crossed with mice possessing a mutation in SCD-1 gene, offspring were reported to have a 40% decrease in body fat mass and a 75% increase in energy expenditure, which the authors attribute to increased liver fatty acid oxidation. Untreated *ob*/*ob* mice had a 700% increase in SCD enzyme activity compared to wild type mice; however, leptin administration resulted in normalized SCD enzyme activity in these mice. Although the pathway by which leptin mediates these effects remains unknown, Cohen et al. (2002) suggest SCD-1 repression is achieved via reductions in cellular levels of malonyl-CoA, which in turn decreases fatty acid biosynthesis as well as fatty acid oxidation.

Conjugated linoleic acid supplementation has been linked to reductions in circulating leptin concentrations in humans (Medina et al., 2000) and laboratory models (Yamasaki et al., 2000; Rahman et al., 2001). However, Cha and Jones (1998) demonstrated that feeding fish oil or safflower oil to Sprague-Dawley rats resulted in leptin values 60% higher than those animals fed beef tallow.

Circulating leptin values are highly associated with adiposity and have been suggested as a factor for correlation to carcass characteristics. Kawakita et al. (2001) found backfat thickness (r = 0.28) as well as marbling score (r = 0.39) to be positively correlated with the rate of leptin increase in Wagyu steers. Similarly, positive correlations between backfat thickness, marbling score, KPH and leptin concentrations in feedlot steers and heifers have been suggested. Feeding corn oil to rats resulted in increased plasma leptin levels and up regulated mRNA leptin expression in comparison with feeding fat free diets (Iritani et al., 2000). In human adipose tissue, subcutaneous adipocytes expressed greater amounts of leptin mRNA than omental adipocytes (Montague et al., 1998; Gottscling-Zeller et al., 1999). In laboratory animals, epididymal and retroperitoneal adipose tissue express higher leptin mRNA levels than inguinal depots (Villafuerte et al., 2000; Zhang et al., 2001). In cattle, the opposite has been observed, with visceral adipocytes displaying greater leptin tissue expression than subcutaneous adipocytes (Chilliard et al., 2002; Ren et al., 2002).

The discovery of the obesity gene and its product, leptin has raised issues concerning potential applications for its use in animal agriculture (Zhang et al., 1994). Connections between leptin and regulation of energy balance, reproductive status, thermiogenesis, and hematopoiesis have been well established (Houseknecht et al., 1998). Metabolic modifiers are of particular interest in the area of meats and muscle biology due to their potential ability to direct nutrients from adipose tissue towards lean protein accretion. Although more research must be conducted, leptin has shown tremendous potential as a repartitioning agent in adipocytes. As with other metabolic modifiers, development of cost effective as well as efficient delivery systems must be developed before any practical benefits may be realized.

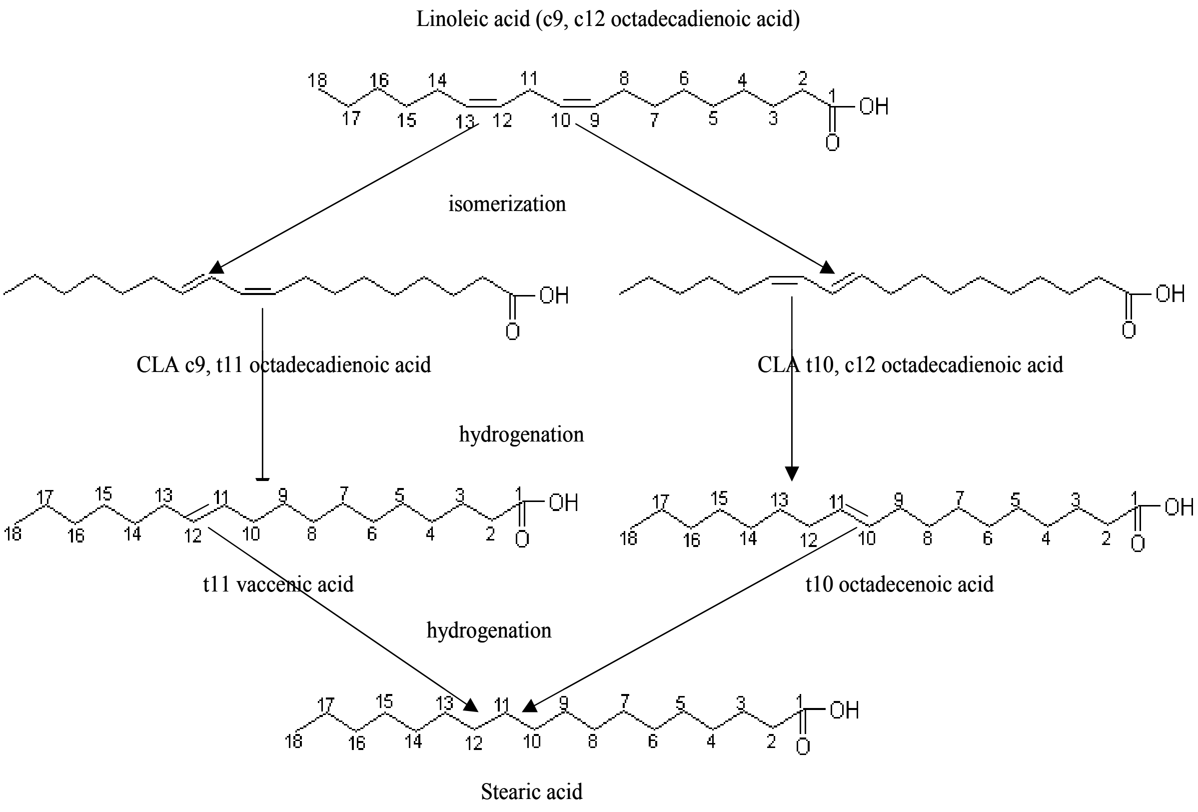

Figure 1.1 Pathway of ruminal biohydrogenation of dietary linoleic (C18:2) acid

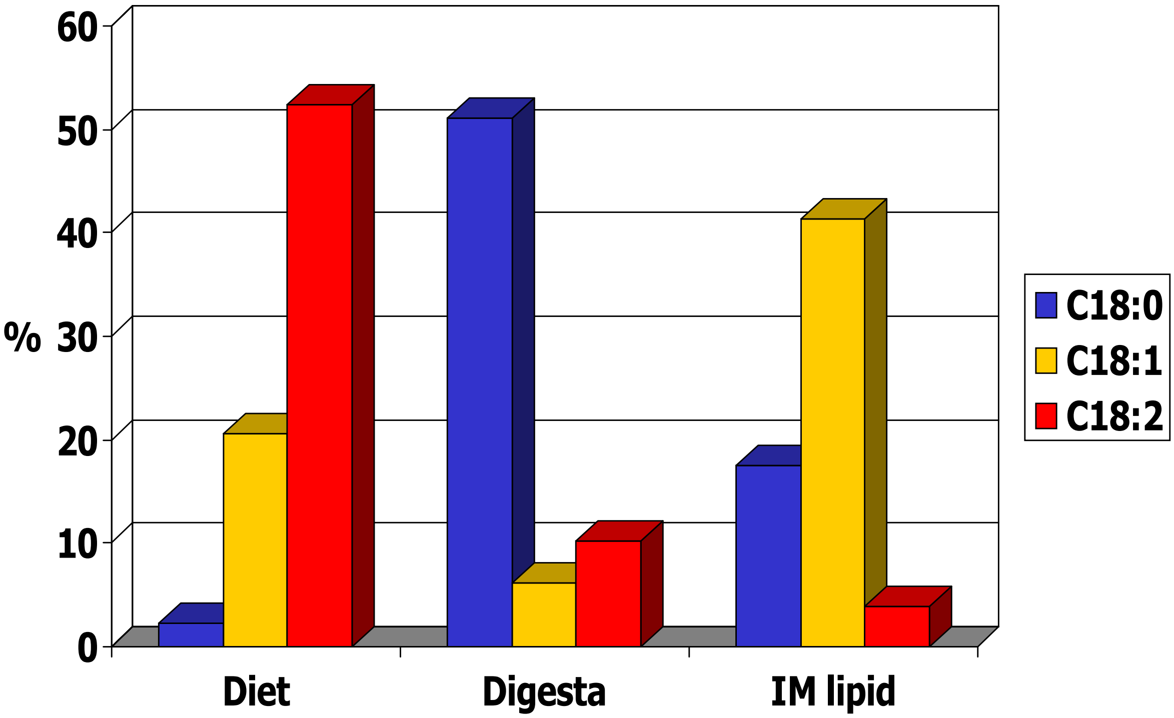

Figure 1.2 Fatty acid composition of diet, digesta, and IM lipid (Adapted from Andrae et al., 1998 and Kennington et al., 2000)

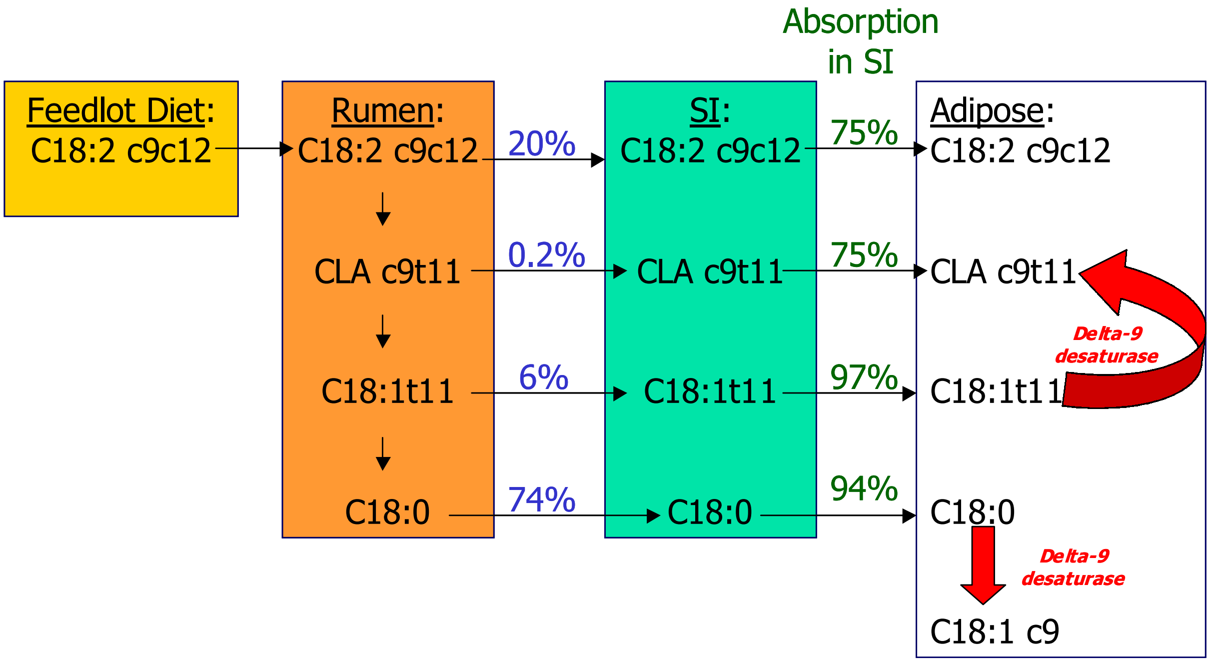

Figure 1.3 Fate of dietary linoleic acid following ruminal biohydrogenation (Adapted from Scollan et al., 2001 and Duckett et al., 2002)

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CHAPTER 2

EFFECTS OF SUPPLEMENTAL RUMEN-PROTECTED CONJUGATED LINOLEIC ACID OR LINOLEIC ACID ON BOVINE SERUM AND ADIPOSE TISSUE LEPTIN LEVELS[1]

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Abstract

Thirty-six Angus-cross heifers (366 kg) were used to determine effects of dietary lipid sources for specific lengths on serum and intramuscular (IM), perianal (PA), and subcutaneous (SQ) adipose tissue leptin concentrations. Following an initial feeding period of 56 d, heifers were fed one of three dietary treatments: 1) basal ration (CON), 2) basal ration plus 4% corn oil (OIL), or 3) basal ration plus 2% rumen-protected conjugated linoleic acid (CLA) with 31% CLA-60. Time on treatment was the last 32 or 60 d prior slaughter. Jugular blood samples were collected at 28 d intervals and serum subsequently harvested for leptin quantification via RIA. At harvest, IM, PA, and SQ adipose tissue samples were obtained. Data were analyzed with treatment, time on treatment diet, adipose depot, and all two- and three-way interactions in the model; differences were separated using least squares means procedure of SAS. Serum leptin levels increased ($P < 0.05$) across time-on-feed, but did not differ ($P > 0.05$) between heifers supplemented 32 d and 60 d. Leptin concentrations were higher ($P < 0.05$) during treatment periods than the period of dietary adaptation. Circulating leptin concentrations were not affected ($P > 0.05$) by dietary treatment at any time during the trial. However, leptin concentration in adipose tissue was greater ($P < 0.05$) for heifers fed OIL compared to either CON or CLA diets, which were similar ($P > 0.05$). Compared with adipose tissues from CLA salt supplemented animals, tissues from heifers fed corn oil contained 68% greater leptin concentration.

Introduction

During ruminal biohydrogenation of dietary unsaturated lipids, unique fatty acid intermediates termed conjugated linoleic acid (CLA) and *trans* vaccenic acid are produced in addition to saturated end products (Bauman et al., 1999, Enser et al., 1999; Scollan et al., 2001). Conjugated linoleic acid refers simply to the positional and geometric isomers of linoleic acid. In particular, the *cis*-9*, trans*-11 and *trans*-10, *cis*-12 CLA isomers have been shown to possess anti-carcinogenic and repartitioning properties, respectively. Ruminant milk and meat products represent the largest natural source of CLA isomers. Research in dairy cattle has shown that supplementing CLA salts or vegetable oils increases the *cis*-9*, trans*-11 CLA isomer in milk fat (Kelly et al, 1998; Enser et al., 1999; Corl et al., 2001). Additionally, research in lactating dairy cattle (Chouinard et al., 1999; Baumgard et al., 2001) as well as lactating sows (Bee, 2000) has shown that supplementing CLA to diets alters lipid metabolism and results in depressed milk fat synthesis.

Leptin, a 16 kDa protein synthesized and secreted by adipocytes, functions as a regulator of energy homeostasis and serves as a circulating signal of adiposity through coordination with neuroendocrine function (Houseknecht, et al., 1998; Barb et al., 2002). Circulating leptin concentrations are well correlated to adiposity as well as adipocyte size for animals in a state of positive energy balance. However, research as to how dietary manipulations may alter circulating leptin concentrations or carcass characteristics is limited, particularly in ruminant animals consuming high-concentrate diets. The objective of this study was to determine the effects of supplemental corn oil, composed predominantly of linoleic acid, or rumen-protected CLA on serum and adipose tissue leptin levels, animal performance, and carcass traits in feedlot beef cattle.

Materials and Methods

Animals and Diets. Thirty-six Angus x Hereford heifers (365 ± 60 kg) obtained from the NW Georgia Experiment Station, Calhoun were utilized in a completely randomized design to determine the effect of lipid source on leptin levels. Treatment effects on circulating leptin concentration were evaluated in a 3 x 2 factorial arrangement with three diets (Control, 4% corn oil, or 2% rumen-protected CLA salt) fed for the last 32 or 60 d prior to harvest, corresponding to time on feed of 88 or 116 d. Following an initial feeding period of 56 d, heifers were fed one of three dietary treatments: 1) basal ration containing 88% concentrate and 12% grass hay (CON), 2) basal ration plus 4% corn oil (OIL), or 3) basal ration plus 2% rumen-protected CLA salt (CLA), containing 31% CLA-60. Rumen-protected conjugated linoleic acid (CLA) salt was generously provided by Agribrands Purina Canada Inc. (Ontario, Canada). Animal handling procedures for this trial were approved by the University of Georgia Animal Care and Use Committee.

Ingredient and chemical composition of the three diets are shown in Table 1. Dietary treatments were formulated to be isonitrogenous; as supplemental lipid was included, an equal proportion of concentrate was removed. Synovex-H implants (20 mg estradiol benzoate and 200 mg testosterone; Ft. Dodge Animal Health, Ft. Dodge, IA) were administered to all animals at trial initiation.

Animals were housed by treatment groups in 6-animal pens outfitted with individual Calan gate feeders (American Calan, Inc., Northwood, NH). Heifers were allowed free access to diets, with fresh rations weighed and provided at 0800. Diet refusal was recorded weekly. Animal weights and feed samples were obtained at 28 d intervals throughout the trial. Feed samples were lyophilized, ground through a Wiley mill equipped with a 1-mm screen, and frozen at -20°C for subsequent proximate analysis and fatty acid profiling.

Real-time Ultrasound Data. Real-time ultrasound measures were collected across time on feed to assess SQ fat thickness, ribeye area, and IM fat percentage. Longitudinal scans for IM fat percentage and transverse scans for fat thickness and ribeye area prediction were obtained at the interface between the 12th and 13th rib by a certified technician using an Aloka 500-V ultrasonograph (Corometrics Medical Systems, Wallingford, CT) equipped with a 17-cm, 3.5-MHz linear probe. Beef Information Manager software, Version 3.0 (Critical Vision, Inc., Atlanta, GA) was used for image interpretation.

Serum Sampling. Blood samples were obtained by jugular venipuncture directly into collection tubes containing no anticoagulant on d 29, 56, 86, and 113 of the trial; fasted blood samples were obtained at slaughter. Samples were stored on ice prior to transport to the laboratory. Samples were stored overnight at 4ºC, centrifuged at 1600 x g, and serum harvested. Aliquots were stored at -80ºC for subsequent leptin quantification.

Leptin Assays. Following trial completion, serum samples were shipped on dry ice to the University of Missouri, Columbia for leptin analysis. Serum leptin concentrations were determined by radioimmunassay according to Delavaud et al. (2000). Assays were performed in duplicate aliquots of 200 µL with inter- and intra-assay coefficients of variation of less than or equal to 10%.

Carcass Data. Six heifers from each treatment were selected for harvest following 32 d on treatment diets. The selection criteria for animals harvested on d 88 of the trial included body weight (≥ 586 kg) and visual appraisal of backfat thickness (≥ 1.27 cm). The remaining 18 heifers were slaughtered on d 116 of the study following a 60-d treatment period. Animals were transported to the University of Georgia's Meat Science and Technology Center (Athens, GA) following overnight feed withdrawal. Live animal weights were recorded prior to harvest.

Subcutaneous adipose tissue samples were removed from the subcutaneous 12th rib and perianal region of the left side of carcasses prior to chilling and immediately frozen at -20ºC for subsequent leptin analyses. Hot carcass weights were recorded following trimming. Following a 48 hr chilling period, carcass measurements including: adjusted fat thickness, longissimus muscle area, marbling score, percentage of kidney, pelvic, and heart fat (KPH), skeletal maturity, and USDA quality and yield grades were determined by five trained evaluators. Rib sections (IMPS #107) were obtained from right sides and 2.54 cm thick steaks removed, trimmed, and frozen at -20°C for subsequent determination of total lipid and intramuscular leptin amounts.

Protein Assays and SDS-PAGE. Adipose tissue samples from SQ and PA depots and intramuscular lipid dissected from sections of longissimus muscle were homogenized in 1X lysis buffer with a ratio of 2:1 of tissue weight and centrifuged at 1600 x g for 15 min. at 4ºC to remove insolubles. Total protein concentration of sample lysates was determined using a Bio-Rad DC protein assay kit (Bio-Rad Laboratories, Herecules, CA). Adipose tissue extracts containing 30 μg total protein were diluted in SDS-sample buffer and subjected to SDS- PAGE under reducing conditions according to method of Laemmli

(1970) in 12% polyacrylamide gels. Prepared tissue proteins, prestained molecular weight protein marker (Bio-Rad) and leptin standard (Sigma Chemical Co., St. Louis, MO) were loaded onto gels and electrophoresis conducted for 35 min at 200 V. Protein bands were visualized using GelCode SilverSnap Stain kit (Pierce, Rockford, IL). Gel images were obtained and densitometry calculations performed using an Alpha Innotek Imager (San Leandro, CA); densitometry measures were standardized based upon the inclusion of the leptin standard.

Statistical Analysis. Data were analyzed using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC), with individual heifer serving as the experimental unit. Differences between treatment means were separated using least squares means procedure. Agreement between carcass and ultrasound variables was determined using the regression procedure of SAS. Pearson correlation analysis was used to analyze the relationship between serum leptin level and independent variables (SAS Inst. Inc., Cary, NC).

Results and Discussion

Performance data for feedlot heifers are presented in Table 2. Supplementing corn oil or rumen-protected CLA salt to finishing diets did not alter ($P > 0.05$) feedlot performance. Performance measurements were affected only by length of dietary supplementation, animals fed treatment diets 32 d prior to slaughter had higher ($P < 0.01$) ADG and daily DMI than heifers fed for 60 d. Feed efficiency values were similar ($P > 0.05$) between the two treatment lengths. Research in laboratory animals, swine, and dairy cattle demonstrated that CLA supplementation improved animal performance, reduced animal adiposity, and altered lipid metabolism. Although several positional and structural isomers of CLA exist, two in particular have been indicated as biologically active. The *cis*-9, *trans*-11 isomer has anticarcinogenic effects, while *trans*-10, *cis*-12 CLA isomer possesses repartitioning properties that reduced adiposity (Chouinard et al., 1999; Baumgard et al., 2001). Wiegand et al. (2001) showed CLA supplementation in swine increased gain:feed ratio compared to control animals, although no differences in ADG was observed between treatments. Feeding 2.5% rumen-protected CLA to feedlot steers for 130 d resulted in lower DMI and reduced ADG (Gassman et al., 2001).

Carcass characteristics were not different ($P > 0.05$) between diets or length of treatment (Table 3). Hot carcass weight, dressing percentage, backfat thickness, LM area, percentage KPH, YG, and QG were similar ($P > 0.05$) across time on treatment and among diets. Azain et al. (2000) observed reduced peritoneal and parametrial fat pad weights in Sprague-Dawley rats fed 0.25 or 0.5% CLA, which were attributed to decreased cell size rather than cell number. Similarly, subcutaneous as well as intramuscular lipid was reduced in barrows fed 0.12 to 1.0% CLA (Thiel-Cooper et al., 2001). Wiegand et al. (2001) also observed decreased backfat thickness in barrows fed 0.75% dietary CLA. Isomers of CLA containing a trans-10 bond have been implicated as those responsible for such repartitioning effects. Reductions in milk fat synthesis in lactating dairy cattle observed by Baumgard et al. (2001) were associated with increasing doses of abomasal trans-10, cis-12 CLA infusions.

Carcasses from animals supplemented with corn oil tended ($P = 0.07$) to have higher marbling scores compared to CLA salt fed, with control ration intermediate. Gassman et al. (2001) reported lower marbling scores in feedlot cattle when 2.5% rumen-protected CLA was fed. When high-oil corn was supplemented to finishing steers, marbling scores were increased compared to feeding a typical corn ration (Andrae et al., 2001).

Serum leptin concentrations across time on feed are shown in Figure 1. Circulating leptin concentrations did not differ ($P > 0.05$) among dietary treatment. Leptin values were greater ($P < 0.05$) for heifers supplemented for 60 d compared to 32 d. Serum leptin levels increased ($P < 0.05$) linearly across time on feed; however, values were similar ($P > 0.05$) during the first 56 d on feed when all animals received the basal diet and the last 32 or 60 d on dietary treatments.

Conjugated linoleic acid supplementation has been linked to reductions in circulating leptin concentrations in humans (Medina et al., 2000) and laboratory rodents (Yamasaki et al., 2000; Rahman et al., 2001). However, Cha and Jones (1998) demonstrated that feeding fish oil or safflower oil to Sprague-Dawley rats resulted in leptin values 60% higher than those animals fed beef tallow. Similarly, feeding corn oil to rats (Iritani et al., 2000) increased plasma leptin concentrations and up regulated leptin mRNA expression compared to feeding fat free diets.

Although no dietary effects on circulating leptin concentration were observed, a treatment effect was established at the tissue level. The effect of dietary treatment on adipose tissue leptin concentration is shown in Figure 2. Heifers fed supplemental corn oil had 68 % higher ($P < 0.01$) concentrations (μg leptin/μg protein in adipose tissue) of leptin in adipose depots compared to CLA and CON animals. An example of a 12% silver stained SDS-PAGE image is illustrated in Figure 3.

Adipose tissue leptin content did not differ ($P > 0.05$) between IM, PA, and SQ adipose depots (Figure 3). In human adipose tissue, subcutaneous adipocytes expressed greater amounts of leptin mRNA than omental adipocytes (Montague et al., 1998; Gottscling-Zeller et al., 1999). Additionally, Rosenbaum et al. (2001) showed that leptin expression was higher in subcutaneous compared with visceral adipose tissue in women. In laboratory animals, epididymal and retroperitoneal adipose tissue express higher leptin mRNA levels than inguinal depots (Villafuerte et al., 2000; Zhang et al., 2001). In cattle, the opposite has been observed, with visceral adipocytes displaying greater leptin tissue expression than subcutaneous adipocytes (Chilliard et al., 2001; Ren et al., 2002).

Circulating leptin concentrations are associated with adiposity and have been suggested as a factor for correlation to carcass characteristics. Ultrasound marbling scores across time on feed are shown in Figure 4. Serum leptin concentrations were not correlated ($P > 0.05$) with performance or carcass characteristics. However, there was a trend ($P = 0.08$) for a positive correlation ($r^2 = 0.30$) between the rate of leptin increase across time on feed and carcass marbling score. Additionally, circulating leptin concentrations at d 29 ($r^2 = 0.42$, $P < 0.05$), d 56 ($r^2 = 0.38$, $P = 0.05$), and d 86 ($r^2 = 0.48$, $P < 0.05$) were positively correlated with initial ultrasound intramuscular fat percentage measurements. Circulating leptin concentration at d 113 on feed for heifers fed experimental diets for 60 d, was associated ($r^2 = 0.55$, $P < 0.05$) with ultrasound marbling score. However, carcass marbling score was not highly correlated ($P = 0.08$) with the change in serum leptin concentrations over time. Similarly, the rate of increase in serum leptin concentration for animals fed dietary treatments for 60 d tended ($P = 0.06$) to be negatively correlated with total gain ($r^2 = -0.45$) as well as gain:feed ($r^2 = -0.44$). However, serum leptin concentrations for heifers supplemented 60 d were positively associated ($r^2 = 0.47$, $P = 0.05$) with dressing percentage. Additionally, percentage KPH fat was positively associated ($r^2 = 0.50$, $P < 0.05$) with the rate of increase in serum leptin concentrations across time for heifers supplemented 60 d prior to harvest.

Kawakita et al. (2001) found backfat thickness ($r = 0.28$) as well as marbling score ($r = 0.39$) to be positively correlated with the rate of increase in serum leptin concentrations in Wagyu steers. Figure 5 depicts the linear increase in backfat across time on feed. Ultrasound and as well as carcass backfat measurements were not correlated ($P > 0.05$) with circulating leptin concentration or rate of leptin increase over time on feed. Ultrasound longissimus muscle area measures across time on feed are shown in Figure 6. The standard error of prediction for ultrasound *vs.* carcass measurements was 0.26 cm for fat thickness, 6.42 cm^2 for ribeye area, and 0.57% for intramuscular lipid. These values are within range of the requirements for certification by the Beef Improvement Federation: 0.30 cm for fat thickness, 7.74 cm^2 for ribeye area, and 1.2% for intramuscular lipid. Subcutaneous fat thickness as well as longissimus muscle area increased in a linear manner across time on feed in a manner consistent with data reported by Duckett et al., 1993.

Implications

Serum leptin values were not consistently correlated with carcass and performance data. These results suggest that circulating leptin levels may not be indicative of carcass characteristics, including marbling score and subcutaneous fat thickness, in beef cattle fed finishing diets.

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Table 2.1 Composition of experimental dietary treatments expressed on a dry matter basis

	Dietary Treatment		
Item, %	CON	OIL	SALT
Ingredients			
Bermudagrass hay	12	12	12
Corn/Protein/Mineral Mix[a]	88	84	86
CLA salt[b]	-	-	2
Corn oil	-	4	-
Chemical Composition			
Dry matter	84.50	85.75	86.08
Crude protein	11.98	11.74	11.49
ADF	8.68	8.48	8.58
NDF	21.45	20.82	21.14
Ash	6.76	6.48	7.06
Total fatty acid	3.30	6.38	4.20

[a] Contained 81.4 % corn, 2.75 % soybean meal, 1.42 % limestone, 0.5% trace mineral 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. Supplied 0.4 mg MGA activity/hd/d and 250 mg Rumensin activity/hd/d.

[b] CLA Salt provided by Agribrands Purina Canada Inc.

Table 2.2 Effect of time on treatment diet on performance of feedlot heifers

	Dietary Treatment				Time-on-Treatment		
Item	CON	OIL	SALT	SEM	32 d	60 d	SEM
ADG, kg	1.44	1.37	1.47	0.10	1.52^a	1.33^b	0.04
DDMI, kg	9.89	10.34	10.02	0.22	10.48^a	9.69^b	0.18
Gain:feed	0.146	0.133	0.144	0.006	0.143	0.139	0.005

^{ab}Means in the same row that lack a common superscript vary ($P < 0.01$).

Table 2.3 Effect of dietary treatment on carcass characteristics

	Dietary Treatment				Time-on-Treatment		
Item	CON	OIL	SALT	SEM	32 d	60 d	SEM
HCW, kg	294	301	299	4.9	300	296	4.0
Dressing percentage	61.3	61.0	61.4	0.37	61.5	61.0	0.30
Adj. fat thickness, cm	1.6	1.5	1.5	0.09	1.7	1.5	0.07
Longissimus area, cm^2	68.0	70.9	72.8	2.16	70.8	70.3	1.76
KPH percentage	2.2	2.0	2.3	0.15	2.1	2.2	0.12
Yield grade	3.7	3.4	3.4	0.14	3.6	3.4	0.12
Marbling score[a]	5.4^{cd}	5.8^{c}	5.3^{d}	1.7	5.7	5.3	1.4
Quality grade[a]	4.9	5.3	4.8	0.21	5.2	4.8	0.17
% Choice	73	92	83		94	61	
% CAB[b]	9	33	8		24	11	

[a] Marbling score/quality grade numerical code: 4.0 to 4.99 = Slight: Select; 5.0 to 5.99 = Small: Choice$^-$; 6.0 to 6.99 = Modest: Choice°. Skeletal maturity = A.

[b] CAB = Certified Angus Beef; Modest^0 marbling score or greater.

[cd] Means within a row with uncommon superscripts vary (*P* = 0.07).

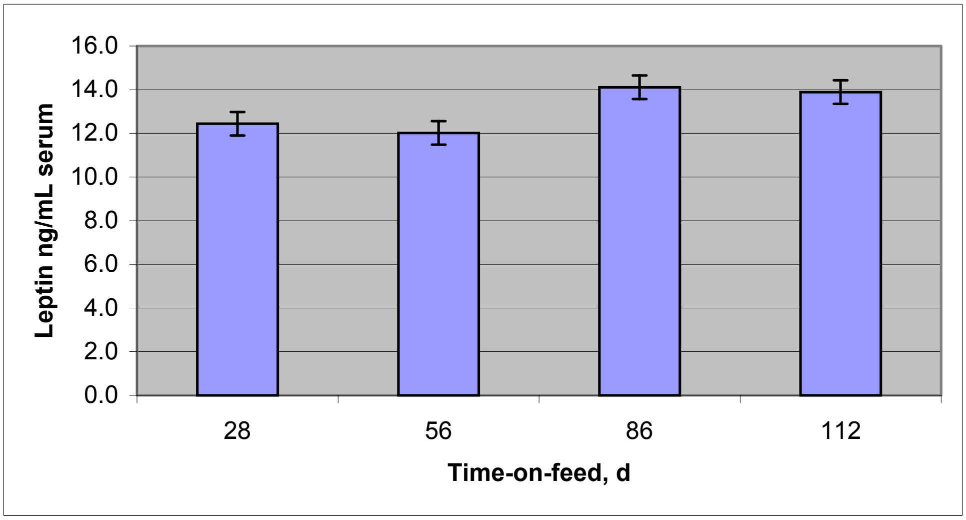

Figure 2.1 Effect of time on feed on serum leptin levels

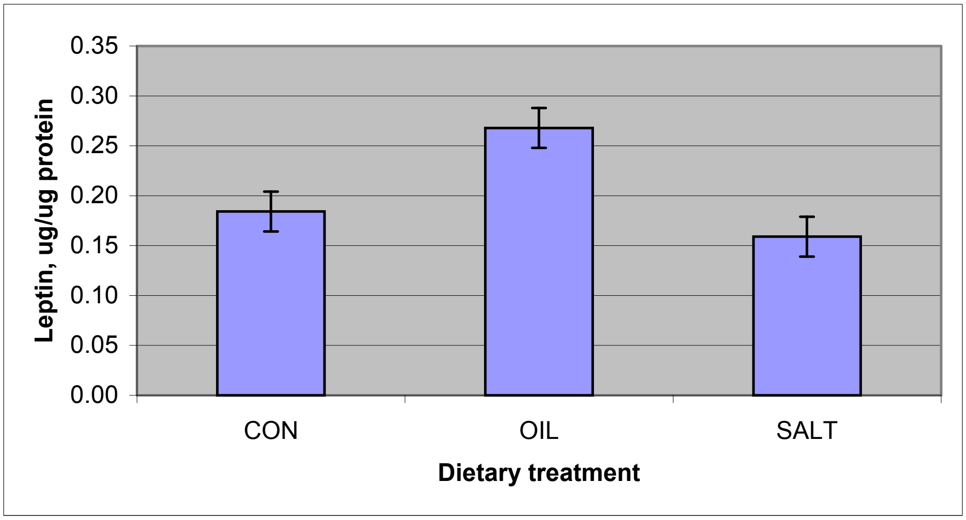

Figure 2.2 Effect of dietary treatment on adipose tissue leptin concentration

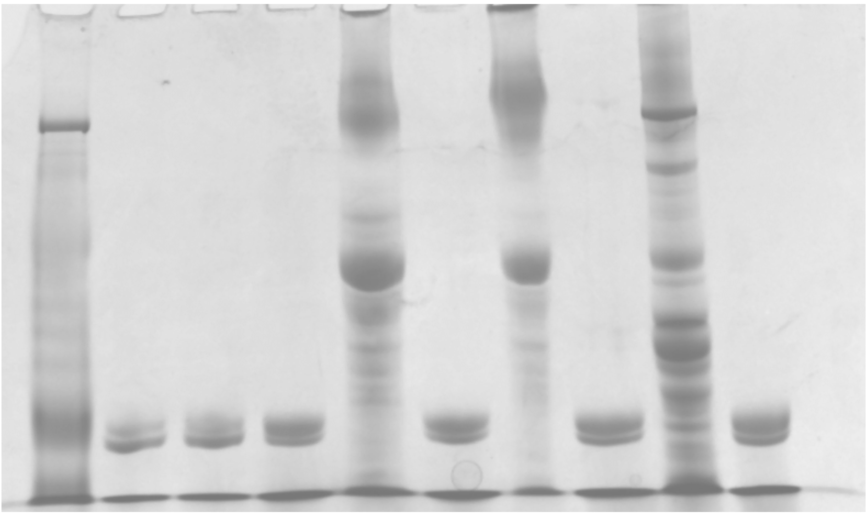

Figure 2.3 Silver stained 12% SDS-PAGE image. Lane 1: Wide-range molecular weight marker. Lanes 2, 3, 4, 6, 8, and 10: Leptin standards. Lane 5: Intramuscular sample. Lane 7: Perianal sample. Lane 9: Subcutaneous sample.

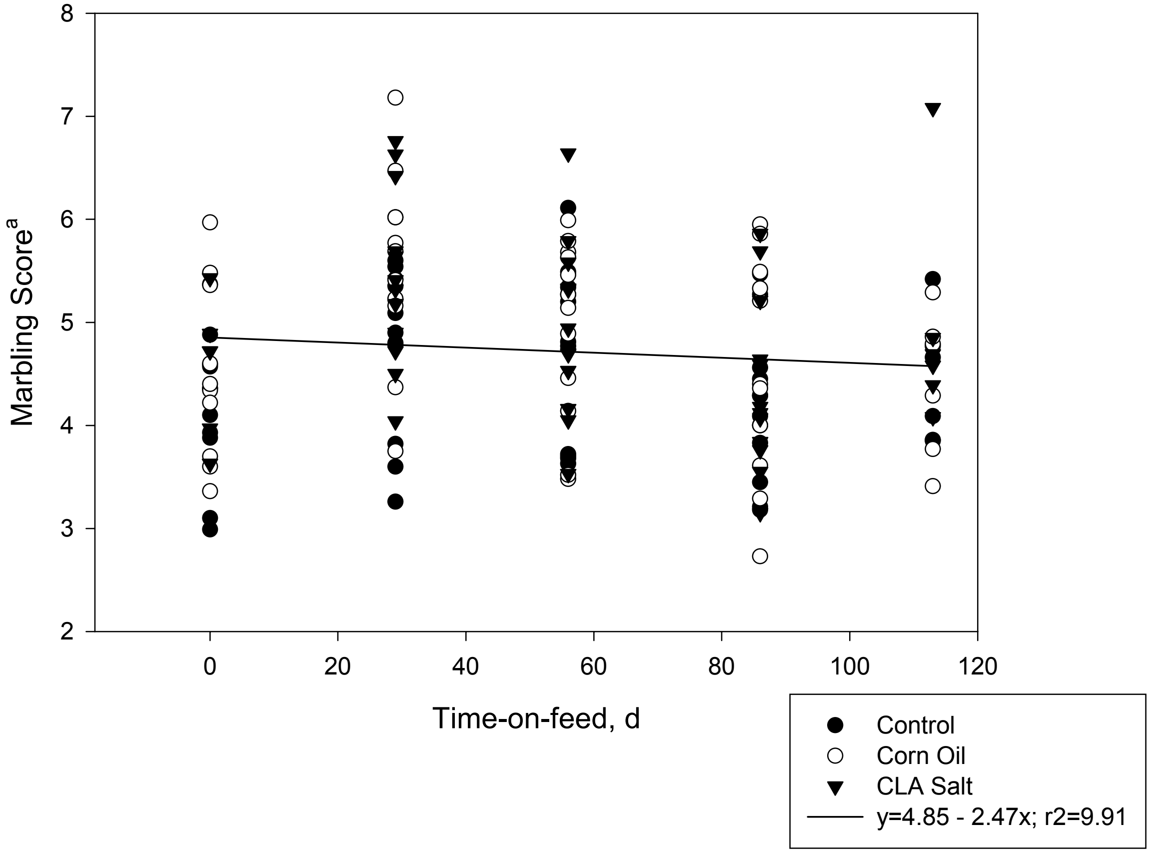

Figure 2.4 Change in marbling score across time on feed as measured by real-time ultrasound.

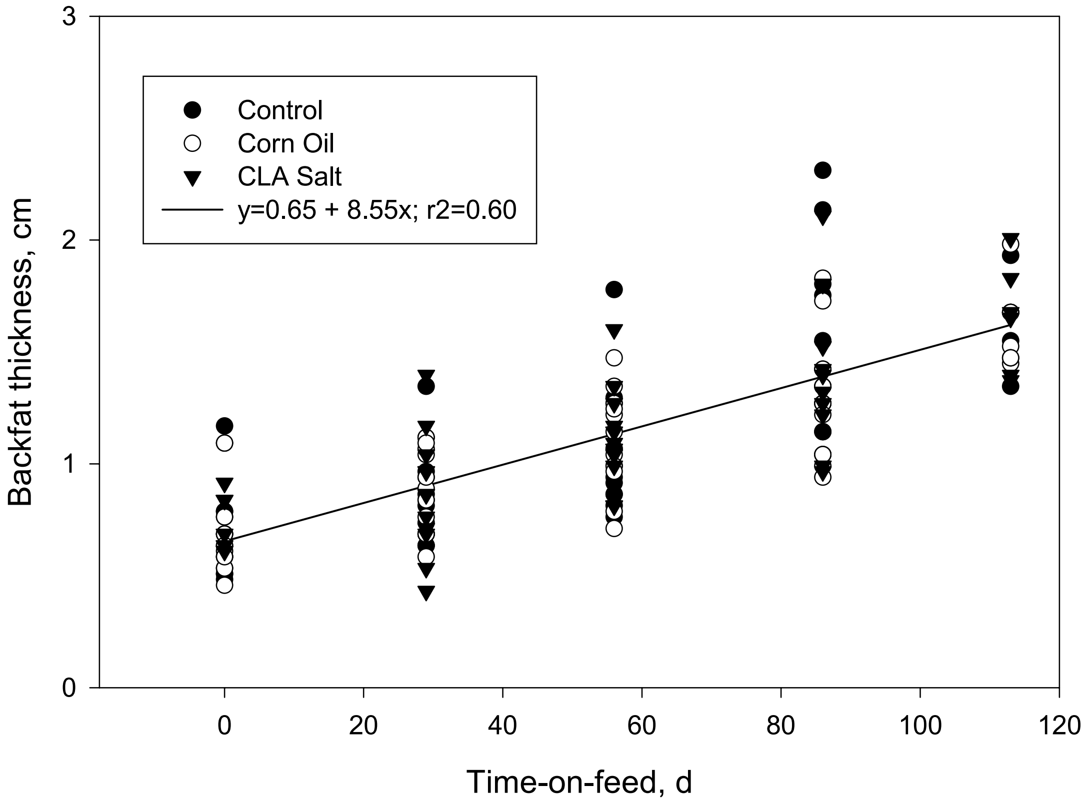

Figure 2.5 Change in subcutaneous fat thickness across time on feed as measured by real-time ultrasound.

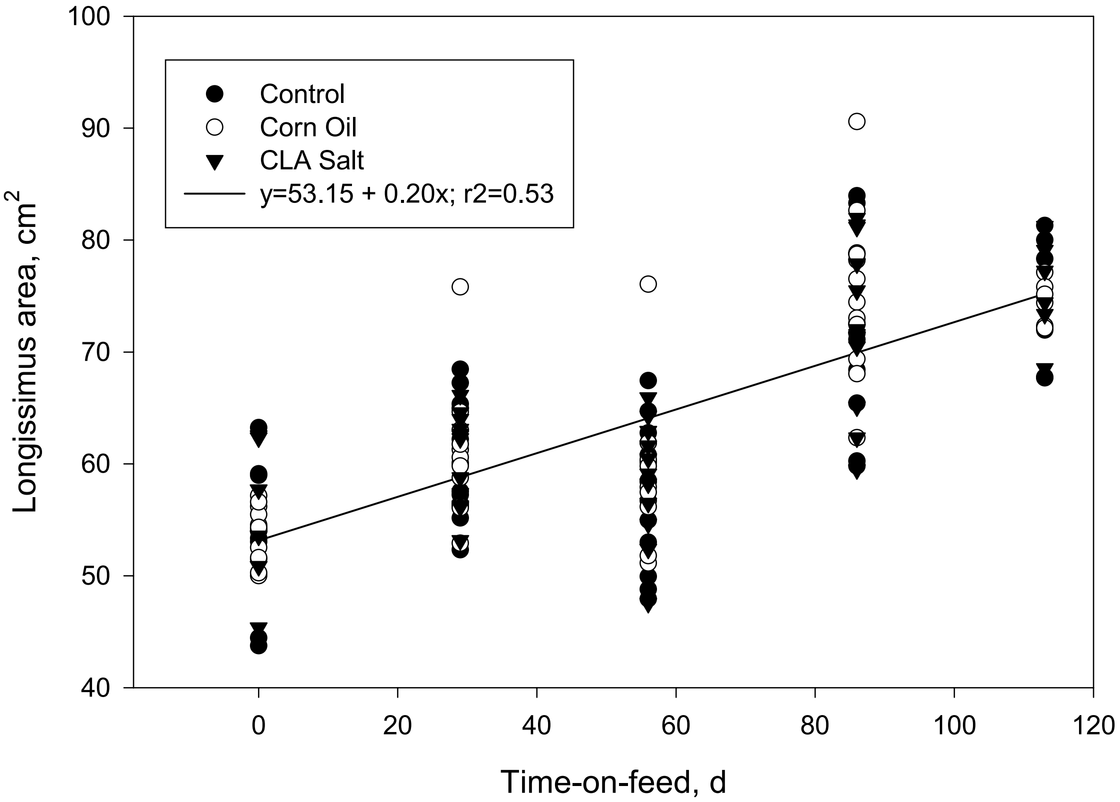

Figure 2.6 Change in longissimus muscle area across time on feed as measured by real-time ultrasound.

EFFECTS OF RUMEN-PROTECTED CONJUGATED LINOLEIC ACID OR LINOLEIC ACID SUPPLEMENTATION ON FATTY ACID COMPOSITION OF BOVINE ADIPOSE DEPOTS[1]

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

Abstract

Thirty-six Angus-cross heifers (366 kg) were used to determine effects of supplemental dietary lipid sources on fatty acid composition of intramuscular (IM), perianal (PA), and subcutaneous (SQ) lipid depots. Lipid was supplied to diets as either corn oil or rumen-protected conjugated linoleic acid (CLA) salt for two specific treatment periods of either 32 or 60 d. Following an initial feeding period of 56 d, heifers were fed one of three dietary treatments: 1) basal ration containing 88% concentrate and 12% grass hay (CON), 2) basal ration plus 4% corn oil (OIL), or 3) basal ration plus 2% rumen-protected CLA salt (CLA), containing 31% CLA-60. The cis-9, trans-11 CLA isomer and total CLA content was lowest ($P < 0.05$) in IM adipose tissue compared to PA and SQ, which did not differ ($P > 0.05$). The trans-10, cis-12 CLA concentration was highest ($P < 0.05$) for animals fed CLA and OIL diets for 60 d and lowest ($P < 0.05$) for CON, regardless of time on dietary treatment. CLA supplemented heifers had greater ($P < 0.05$) total CLA content than either CON or OIL fed animals. Adipose tissue concentration of C18:1 trans-11 was lower ($P < 0.05$) for CON than OIL or CLA, which were similar ($P > 0.05$). Percentages of C18:1 trans-10 were lowest ($P < 0.05$) in IM lipid compared to PA and SQ, which did not differ ($P > 0.05$). The ratio of cis-9, trans-11 to C18:1 trans-11 was higher ($P < 0.05$) for animals fed 60 d compared to 32 d, but did not differ (P > 0.05) between adipose depots. Supplemental corn oil increased ($P < 0.05$) adipose concentration of C18:2 fatty acid. Feeding rumen-protected CLA increased total CLA isomers by 22%. IM lipid contained the lowest ($P < 0.05$) percentage of cis-9, trans-11 CLA, total CLA, C18:1 cis-9, C18:1 trans-10, and C18:1 trans-11 fatty acids.

Introduction

Fatty acid (**FA**) composition of adipose tissue can be influenced by many factors including: nutritional status, depot, species, and time on feed (Duckett et al, 1993; Aharoni et al., 1995; Scollan et al., 2001). Manipulation of the FA content of beef through dietary lipid supplementation may provide producers with a means of supplying consumers with beef products containing enhanced levels of unsaturated fatty acids. Edible beef products contain relatively low levels of PUFA and high levels of saturated fatty acids (**SFA**). From the standpoint of human nutrition, enhancing the unsaturated FA content of beef translates to a more healthful product; dietary intake of SFA by humans is considered to contribute to elevations in serum cholesterol level and increased risk of cardiovascular disease (American Heart Association, 1986).

During ruminal biohydrogenation (**BH**) of dietary unsaturated lipids, unique FA intermediates termed conjugated linoleic acid (**CLA**) and *trans* vaccenic acid are produced in addition to saturated end products (Bauman et al., 1999). Ruminant milk and meat products represent the largest natural source of CLA and the concentration of these fatty acids in bovine adipose depots is of particular interest to human health. The *cis*-9*,* *trans*-11 and *trans*-10, *cis*-12 CLA isomers have been shown to possess anti-carcinogenic and repartitioning properties, respectively. Research in dairy cattle has shown that supplementing CLA salts or vegetable oils increases the *cis*-9*, trans*-11 isomer concentration in milk fat (Kelly et al., 1998; Enser et al., 1999; Corl et al., 2001). However, limited research is available on the effects of CLA supplementation on tissue composition in ruminants consuming high concentrate diets. The objective of this study was to determine the effects of supplemental corn oil or rumen-protected CLA salt on subsequent tissue FA concentrations, with particular interest in CLA content of various adipose depots.

Materials and Methods

Experimental Design. Thirty-six Angus x Hereford heifers (365 ± 60 kg) were obtained from the NW Georgia Experiment Station, Calhoun and utilized in a completely randomized design. The University of Georgia Animal Care and Use Committee approved animal handling procedures for this trial. Effect of dietary lipid source on subsequent fatty acid composition of intramuscular (IM), perianal (PA), and subcutaneous (SQ) lipid depots was evaluated in a 3 x 2 factorial arrangement with three diets (Control, 4% corn oil, or 2% rumen-protected CLA salt) supplied for two specific time periods. For the purpose of this study, heifers were fed treatment diets for the last 32 or 60 d prior to harvest, which corresponded to 88 or 116 d on feed, respectively. Research has shown that IM lipid deposition occurs in a nonlinear manner across time on feed. Duckett et al. (1993) observed a doubling in IM lipid between d 84 and d 112 in Angus x Hereford steers fed feedlot diets. Feeding periods beyond 112 d did not improve carcass quality traits, but instead resulted in SQ lipid accumulation and economic losses associated with excess carcass trim. Time on treatment effects on tissue fatty acid composition were analyzed by directly targeting the period of IM lipid development.

Diets. Following an initial feeding period of 56 d, heifers were fed one of three dietary treatments: 1) basal ration containing 88% concentrate and 12% grass hay (**CON**), 2) basal ration plus 4% corn oil (**OIL**), or 3) basal ration plus 2% rumen-protected CLA salt (**CLA**), containing 31% CLA-60. Rumen-protected conjugated linoleic acid (CLA) salt was generously provided by Agribrands Purina Canada Inc. (Ontario, Canada).

Ingredient, chemical, and fatty acid composition of the three diets are shown in Table 1. Treatment diets were formulated to be isonitrogenous; as supplemental lipid was included, an equal proportion of concentrate was removed. Synovex-H implants (20 mg estradiol benzoate and 200 mg testosterone; Ft. Dodge Animal Health, Ft. Dodge, IA) were administered to all animals at trial initiation.

Heifers were housed by treatment groups in 6-animal pens outfitted with individual Calan gate feeders (American Calan, Inc., Northwood, NH). Heifers were allowed free access to diets, with fresh rations weighed and provided at 0800; refusals were recorded weekly. Animal weights and feed samples were obtained at 28 d intervals throughout the trial. Feed samples were lyophilized, ground through a Wiley mill equipped with a 1-mm screen, and frozen at -20°C for subsequent proximate analysis and fatty acid profiling.

Sample Collection. Six heifers from each treatment were selected for harvest following 32 d on treatment diets. The selection criteria for animals harvested on d 88 of the trial included body weight ($\geq$ 586 kg) and visual appraisal of backfat thickness ($\geq$ 1.27 cm). The remaining 18 heifers were slaughtered on d 116 of the study following treatment length of 60 d. Animals were transported to the University of Georgia's Meat Science and Technology Center (Athens, GA) following overnight feed withdrawal. Live animal weights were recorded prior to harvest.

Adipose tissue samples were removed from the subcutaneous (at the 12th rib), and perianal region of the left side of carcasses prior to chilling and immediately frozen at -

20ºC for later analyses. Hot carcass weights were recorded following trimming. Following a 48 hr chilling period, carcass measurements including: adjusted fat thickness, longissimus muscle area, marbling score, percentage of kidney, pelvic, and heart fat (KPH), skeletal maturity, and USDA quality and yield grades were determined by five trained evaluators. Rib sections (IMPS #107) were obtained from right sides and 2.54 cm thick steaks removed, trimmed, and frozen at -20°C for determination of lipid concentration and fatty acid composition.

Fatty Acid Composition. Adipose and muscle tissue samples were frozen in liquid nitrogen and pulverized prior to lipid extraction. Total lipids were extracted from samples using organic solvents according to Folch et al. (1957) with a modified chloroform:methanol ratio of 10:1 of sample weight. Lipid extracts were stored at -80°C for subsequent determination of fatty acid composition.

Lipid extracts, containing approximately 25 mg of lipid, were transmethylated according to method of Park and Goins (1994). Previous research in our laboratory has shown that this method does not alter the cis - trans bond arrangements of CLA during methylation (Duckett et al., 2002). Briefly, *in situ* extraction and transmethylation of sample extracts to fatty acid methyl esters (FAME) was achieved using sodium methoxide followed with boron trifluoride. Others (Kramer et al., 1997) have similarly shown the use of base followed by acidic catalysts results in complete conversion of fatty acids to methyl esters without bond rearrangement.

Analysis of FAME was performed using an Agilent 6850 gas chromatograph equipped with an automatic sampler (Agilent, Wilmington, DE). A 100-m Supelco SP2560 capillary column (Supelco, Bellefonte, PA; 0.25 mm i.d. and 0.20 µm film thickness) was used to separate fatty acid peaks. Temperature of the column oven was ramped from 150 to 165°C at 1°C/min, from 165 to 167°C at 0.2°C/min, and from 167 to 225°C at 1.5°C/min and held at 225°C for 5 min. Temperature of the injector and detector was maintained at 250°C; sample injection volume was 1.0 µL with a split ratio 100:1. Hydrogen was the gas carrier with a flow rate of 1 mL/min. Analysis of FAME in the CLA Salt supplement resulted in incomplete resolution of the trans-8, cis-10 and cis-9, trans-11 CLA isomers. Consequently, cis-9, trans-11 CLA content of tissues of animals fed CLA salt may include trans-8, cis-10 CLA. Retention times were compared to those for known standards (Matreya, Pleasant Gap, PA; Nu-Chek Prep, Elysian, MN; Sigma Chemical Co., St. Louis, MO). Fatty acids were quantified based upon the inclusion of an internal standard, methyl tricosanoate, during methylation and expressed as a weight of total fatty acid.

Statistical Analysis. Data were subjected to analysis of variance for a completely randomized design using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC), with individual heifer serving as the experimental unit. The model included the effects of dietary treatment, depot, time on treatment, all two-way interaction terms, and the three-way interaction of treatment, depot, and time on treatment. Differences between treatment means were separated using least squares means procedure.

Results and Discussion

Performance data for feedlot heifers are presented in Table 2. Supplementing corn oil or rumen-protected CLA salt to finishing diets did not alter ($P > 0.05$) feedlot performance. Performance measurements were affected only by length of dietary supplementation, with those animals fed treatment diets 32 d prior to slaughter having higher ($P < 0.01$) overall ADG as well as daily DMI than heifers fed a total of 60 d. Feed efficiency values were similar ($P > 0.05$) between the two treatment lengths. Gassman et al. (2001) found reductions in ADG and FI when 2.5% rumen-protected CLA salt was fed to finishing steers. Similarly, these decreases in performance data were not accompanied by changes in feed efficiency values. Andrae et al. (2000) compared feeding a high oil corn hybrid to typical whole shelled corn and reported no differences in performance data of steers.

Carcass characteristics did not differ ($P > 0.05$) between dietary treatments or length of lipid supplementation (Table 3). Hot carcass weight, dressing percentage, backfat thickness, LM area, percentage KPH, YG, and QG were similar ($P > 0.05$) across time on treatment and between diets. Carcasses from animals supplemented with corn oil tended ($P = 0.07$) to have higher marbling scores compared to CLA salt fed, with control ration intermediate. Andrae et al. (2001) observed a similar trend for increased marbling score as well as quality grade in steers fed high oil corn. Gassman et al. (2001), however, reported decreased marbling score and lower percentages of animals grading choice for feedlot steers fed 2.5% rumen-protected CLA salt.

The effect of dietary treatment by length of lipid supplementation on adipose tissue fatty acid concentration is shown in Table 4. Following 32 d on dietary treatment, CON fed heifers had higher ($P < 0.05$) adipose tissue concentrations of myristic (C14:0) acid than OIL supplemented heifers. Heifers fed CON for 60 d had lowest ($P < 0.05$) levels of C14:0, regardless of time on treatment diet. At 60 d, OIL and CLA salt supplemented animals had tissue levels of myristic acid similar ($P > 0.05$) to CON and CLA animals at 32 d. Pentadecyclic (C15:0) acid levels were highest ($P < 0.05$) for heifers fed rumen-protected CLA salt 60 d and for OIL treatment regardless of supplementation length. Adipose tissues of heifers fed CON diet for 60 d contained lowest ($P < 0.05$) C15:0 concentrations. At 32 d on treatment, CLA salt supplemented heifers had lowest ($P < 0.05$) C15:0 tissue content. Pentadecyclic acid level for control treatment was similar ($P > 0.05$) at 32 d to OIL and CLA diets, regardless of time on treatment. Concentrations of palmitic (C16:0) acid were higher ($P < 0.05$) at 32 d for CON fed animals than OIL supplemented animals, with CLA intermediate. Following 60 d on dietary treatment, adipose tissue samples from OIL and CLA treated heifers contained higher ($P < 0.05$) amounts of C16:0 than CON heifers. Adipose tissues from heifers receiving CON treatment had lower ($P < 0.05$) C16:0 levels at 60 d compared with 32 d of treatment, while feeding corn oil caused palmitic acid content to increase ($P < 0.05$) with length of supplementation. Length of dietary treatment did not alter ($P > 0.05$) C16:0 concentration of adipose tissues from heifers supplied rumen-protected CLA salt. Total SFA content was higher ($P < 0.05$) in all tissues at 32 d and in tissues from CLA salt fed animals at 60 d on dietary treatment. Saturated fatty acid concentration was lowest ($P < 0.05$) in tissues of CON fed animals following 60 d of dietary treatment. Feeding corn oil or rumen-protected CLA salt a total of 60 d prior to slaughter did not alter ($P > 0.05$) total SFA content of adipose tissues. Duckett et al. (1993) reported linear increases in concentrations of C14:0, a linear decrease in C18:0, and cubic decreases in content of C15:0 and C16:0 fatty acid across time on feed in intramuscular lipid of beef cattle. Increasing levels of dietary concentrate resulted in increases in palmitic acid and total SFA content in IM lipid in feedlot steers (French et al., 2000).

Palmitoleic (C16:1, *cis*-9) acid adipose tissue content was greater ($P < 0.05$) for CON fed animals regardless of time on treatment diet and for OIL supplemented animals at 60 d of treatment (Table 4). Feeding 4% corn oil 60 d prior to harvest increased ($P < 0.05$) palmitoleic acid tissue concentrations compared to OIL fed at 32 d of treatment. Oleic acid (C18:1, *cis*-9) adipose tissue levels were similar ($P > 0.05$) between dietary treatments following 32 d of supplementation. Feeding OIL and CLA diets for 60 d prior to slaughter did not change ($P > 0.05$) tissue levels of oleic acid. Animals fed CON diet for 60 d had the highest ($P < 0.05$) concentrations of oleic acid in adipose tissues. Feeding the basal diet a total of 60 d prior to harvest increased ($P < 0.05$) total MUFA content for all adipose tissue depots. Length of lipid supplementation did not alter ($P > 0.05$) adipose MUFA concentrations in tissues of animals fed corn oil or rumen-protected CLA salt. Duckett et al. (1993) reported a linear increase in MUFA content of bovine IM lipid, which resulted primarily from an increase in concentration of oleic acid across time on feed in steers fed feedlot diets. Stearoyl CoA desaturase (SCD) is an enzyme responsible for double bond insertion at the n-9 position of fatty acids and the formation of *cis*-9, *trans*-11 CLA isomers from C18:1 *trans*-11 fatty acids produced during ruminal biohydrogenation of linoleic acid (Bauman et al., 1999). Some PUFA, including linoleic acid, are thought to inhibit SCD activity by down-regulating SCD gene expression.

Time on treatment diet did not alter ($P > 0.05$) C18:1 *trans*-10 concentrations in adipose tissues of cattle fed 4% corn oil or basal diets (Table 4). However, supplying 2% rumen-protected CLA salt to finishing diets for 60 d resulted in greater ($P < 0.05$) proportions of C18:1 *trans*-10 compared to all other treatments. Regardless of time on treatment, CLA salt fed heifers had higher ($P < 0.05$) levels of C18:1 *trans*-10 fatty acids.

Fatty acid concentration by adipose tissue depot is shown in Table 5. Margaric (C17:0) acid content was lower ($P < 0.05$) in IM lipid and higher ($P < 0.05$) in PA depots, with SQ intermediate. Total OCFA were greatest ($P < 0.05$) in PA depots and lowest ($P < 0.05$) in IM lipid, with SQ intermediate.

Palmitic acid concentration (Table 5) was lower ($P < 0.05$) in PA depots than in IM or SQ depots, which did not differ ($P > 0.05$). Stearic (C18:0) acid levels were greater ($P < 0.05$) in IM lipid than in PA or SQ depots, which were similar ($P > 0.05$). Total SFA were highest ($P < 0.05$) in IM lipid and lowest ($P < 0.05$) in PA depots, with SQ intermediate. Andrae et al. (2001) also found that IM lipid contained higher proportions of SFA, which was primarily due to increased C16:0 percentages in typical high concentrate diets compared to feeding high oil corn varieties.

Myristoleic (C14:1, *cis*-9) concentration was greater ($P < 0.05$) in PA lipid than IM depots, with SQ intermediate (Table 5). Intramuscular adipose tissue was composed of lower ($P < 0.05$) concentrations of myristoleic, palmitoleic, oleic, and C18:1, *cis*-12 fatty acids than PA or SQ depots. Subcutaneous and PA depots contained higher ($P < 0.05$) amounts of total MUFA compared to IM lipid. Intramuscular lipid contained lowest ($P < 0.05$) concentrations of C18:1 *trans*-10 and *trans*-11 compared to PA and SQ depots; PA adipose tissue contained the highest ($P < 0.05$) concentration of C18:1 *trans*-11 fatty acids. The content of total MUFA was lower ($P < 0.05$) in IM tissue compared to either PA or SQ, which were similar ($P > 0.05$). Linoleic acid was higher ($P < 0.05$) in intramuscular lipid compared with PA or SQ, which did not differ ($P > 0.05$). Similarly, total PUFA were higher ($P < 0.05$) in IM lipid compared to PA and SQ, which were similar ($P > 0.05$).

Conjugated linoleic acid concentration for adipose tissue depots is presented in Table 6. The *cis*-9, *trans*-11/*trans*-8, *trans*-10 CLA isomer content was lowest ($P < 0.05$) in IM lipid depots compared to PA and SQ, which were similar ($P > 0.05$) in concentration. Due to the fact that the *cis*-9, *trans*-11 CLA isomer predominates as a percentage of total CLA, a similar trend was observed in total CLA amounts, with IM lipid containing lower ($P < 0.05$) levels than PA and SQ, which did not differ ($P > 0.05$). The cis-11, cis-13 CLA isomer concentration was greater ($P < 0.05$) in PA and SQ than IM lipid. The concentration of cis-10, cis-12 CLA isomers was highest ($P < 0.05$) in PA depots and lowest ($P < 0.05$) in IM lipid, with SQ intermediate. In general, IM adipose depots contained lower ($P < 0.05$) levels of CLA isomers than either PA or SQ lipid. Madron et al. (2002) reported similar findings when comparing the fatty acid composition of longissimus IM lipid to SQ adipose tissue, with SQ depots containing higher levels of the *cis*-9, *trans*-11 CLA isomer compared to IM lipid.

Effect of dietary treatment on fatty acid composition of adipose tissue depots is presented in Table 7. Supplementing heifers with corn oil or rumen-protected CLA salt resulted in higher ($P < 0.05$) concentration of C18:1 *trans*-11 fatty acid compared with adipose tissues from CON animals. Tissues from CON fed animals contained greatest ($P < 0.05$) amounts of C18:1 *cis*-11, while SALT had the lowest ($P < 0.05$) levels; lipid from OIL treated heifers was similar ($P > 0.05$) to CON and CLA salt treatments. Linoleic acid (C18:2 *cis*-9, *cis*-12) content was higher ($P < 0.05$) in adipose tissues from animal fed corn oil compared to CON or rumen-protected CLA salt treatments, which were similar ($P > 0.05$). Andrae et al. (2001) reported similar increases in linoleic acid content of intramuscular lipid from steers fed high oil corn diets. Additionally, linoleic acid content was greater ($P < 0.05$) in IM lipid depots compared with PA or SQ adipose tissue depots, which were similar ($P > 0.05$). Levels of C18:2 *cis*-11, *cis*-13 were lower ($P < 0.05$) in lipid from heifers fed 4% corn oil compared with 2% rumen-protected CLA salt, while CON lipid was similar ($P > 0.05$) to OIL and CLA salt. Feeding rumen-protected CLA salt increased ($P < 0.05$) adipose tissue levels of C18:2 *cis*-8, *cis*-10. Total CLA isomer concentration was higher ($P < 0.05$) in adipose tissues of animal fed rumen-protected CLA salt compared with control and corn oil diets, which were similar ($P > 0.05$). Polyunsaturated fatty acids were found at the highest ($P < 0.05$) levels in tissues from heifers supplemented with 4% corn oil and lowest ($P < 0.05$) amounts in CON lipids, with CLA intermediate. Similarly, Andrae et al. (2001) reported elevated levels of polyunsaturated fatty acids in adipose tissue of cattle fed high-oil corn. Linoleic acid is an essential fatty acid, therefore, the increased concentrations in lipid depots of heifers supplemented with 4% corn oil demonstrates that short-term feeding was effective at altering fatty acid composition. However, oil supplementation did not increase *cis*-9, *trans*-11 CLA content. Beaulieu et al. (2002) also observed no change in lipid content of *cis*-9, *trans*-11 CLA isomers when 5% soybean oil was supplied.

Implications

Supplementing rumen-protected conjugated linoleic acid to finishing cattle diets alters fatty acid composition of adipose depots and increased CLA deposition. However, subsequent changes in lipid profiles were highly variable between adipose depots as well as supplementation lengths. Corn oil and rumen-protected CLA salt diets increased adipose concentration of C18:1 *trans*-11 fatty acid compared to control treatment, indicating the possibility of suppression of SCD activity with polyunsaturated fatty acid supplementation.

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Table 3.1 Composition of experimental dietary treatments expressed on a dry matter basis

	Dietary Treatment		
Item, %	CON	OIL	SALT
Ingredients			
Bermudagrass hay	12	12	12
Corn/Protein/Mineral Mix[a]	88	84	86
CLA salt[b]	-	-	2
Corn oil	-	4	-
Chemical Composition			
Dry matter	84.50	85.75	86.08
Crude protein	11.98	11.74	11.49
ADF	8.68	8.48	8.58
NDF	21.45	20.82	21.14
Ash	6.76	6.48	7.06
Total fatty acid	3.30	6.38	4.20

[a] Contained 81.4 % corn, 2.75 % soybean meal, 1.42 % limestone, 0.5% trace mineral 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. Supplied 0.4 mg MGA activity/hd/d and 250 mg Rumensin activity/hd/d.

[b] CLA Salt provided by Agribrands Purina Canada Inc.

Table 3.2 Effect of time on treatment diet on performance of feedlot heifers

	Dietary Treatment				Time-on-Treatment		
Item	CON	OIL	SALT	SEM	32 d	60 d	SEM
ADG, kg	1.44	1.37	1.47	0.10	1.52^{a}	1.33^{b}	0.04
DDMI, kg	9.89	10.34	10.02	0.22	10.48^{a}	9.69^{b}	0.18
Gain:feed	0.146	0.133	0.144	0.006	0.143	0.139	0.005

[ab] Means in the same row that lack a common superscript vary ($P < 0.01$).

Table 3.3 Effect of dietary treatment on carcass characteristics

	Dietary Treatment				Time-on-Treatment		
Item	CON	OIL	SALT	SEM	32 d	60 d	SEM
HCW, kg	294	301	299	4.9	300	296	4.0
Dressing percentage	61.3	61.0	61.4	0.37	61.5	61.0	0.30
Adj. fat thickness, cm	1.6	1.5	1.5	0.09	1.7	1.5	0.07
Longissimus area, cm^2	68.0	70.9	72.8	2.16	70.8	70.3	1.76
KPH percentage	2.2	2.0	2.3	0.15	2.1	2.2	0.12
Yield grade	3.7	3.4	3.4	0.14	3.6	3.4	0.12
Marbling score[a]	5.4^{cd}	5.8^{c}	5.3^{d}	1.7	5.7	5.3	1.4
Quality grade[a]	4.9	5.3	4.8	0.21	5.2	4.8	0.17
% Choice	73	92	83		94	61	
% CAB[b]	9	33	8		24	11	

[a] Marbling score/quality grade numerical code: 4.0 to 4.99 = Slight: Select; 5.0 to 5.99 = Small: $Choice^-$; 6.0 to 6.99 = Modest: Choice°. Skeletal maturity = A.

[b] CAB = Certified Angus Beef; $Modest^0$ marbling score or greater.

[cd] Means within a row with uncommon superscripts vary (*P* = 0.07).

Table 3.4 Effect of supplementation length and dietary treatment on fatty acid composition of adipose tissues

	32 d				60 d			
Fatty acid, %	CON	OIL	SALT	SEM	CON	OIL	SALT	SEM
C14:0	3.39^{a}	3.22^{b}	3.36^{ab}	0.11	2.82^{c}	3.54^{a}	3.46^{a}	0.11
C15:0	0.60^{ab}	0.65^{a}	0.56^{b}	0.03	0.48^{c}	0.61^{a}	0.61^{a}	0.03
C16:0	27.24^{a}	26.39^{b}	26.46^{ab}	0.29	25.66^{b}	26.48^{a}	26.97^{a}	0.29
C16:1, *cis*-9	3.21^{a}	3.02^{b}	3.13^{ab}	0.12	3.18^{a}	3.40^{a}	2.89^{b}	0.12
C18:1 *trans*-10	1.40^{cd}	1.78^{c}	2.22^{b}	0.17	1.27^{d}	1.78^{c}	2.94^{a}	0.17
C18:1 *cis*-9	39.51^{b}	39.59^{b}	38.97^{bc}	0.45	42.33^{a}	39.88^{b}	38.21^{c}	0.45
SFA[e]	44.79^{a}	44.13^{ab}	44.21^{a}	0.48	42.19^{c}	43.63^{b}	44.92^{a}	0.49
MUFA[e]	47.11^{b}	47.55^{b}	47.46^{b}	0.48	49.80^{a}	48.09^{b}	47.02^{b}	0.48

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

[e] SFA = saturated fatty acid; MUFA = monounsaturated fatty acid.

Table 3.5 Fatty acid composition by adipose depot

	Adipose depot			
Fatty acid, %	IM	PA	SQ	SEM
C14:1 *cis*-9	0.05^{c}	0.17^{a}	0.14^{b}	0.01
C16:0	27.11^{a}	25.72^{b}	26.77^{a}	0.21
C16:1 *cis*-9	2.92^{b}	3.26^{a}	3.23^{a}	0.09
C17:0	0.54^{c}	0.73^{a}	0.69^{b}	0.01
C18:0	14.92^{a}	13.74^{b}	13.78^{b}	0.25
C18:1 *trans*-10	1.58^{b}	1.99^{a}	2.12^{a}	0.12
C18:1 *trans*-11	0.75^{c}	1.13^{a}	0.95^{b}	0.03
C18:1 *cis*-9	38.74^{b}	40.41^{a}	40.10^{a}	0.32
C18:1 *cis*-12	0.19^{b}	0.37^{a}	0.37^{a}	0.03
C18:2 *cis*-9, *cis*-12	2.19^{a}	1.15^{b}	1.15^{b}	0.05
SFA[d]	45.22^{a}	42.87^{c}	43.85^{b}	0.34
MUFA[d]	45.89^{b}	49.04^{a}	48.59^{a}	0.34
OCFA[d]	1.09^{c}	1.35^{a}	1.28^{b}	0.02
PUFA[d]	2.83^{a}	2.09^{b}	2.07^{b}	0.06

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

[d] SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; OCFA = odd chain fatty acid; PUFA = polyunsaturated fatty acid.

Table 3.6 Conjugated linoleic acid concentration by adipose depot

	Adipose Depot			
Fatty acid, %	IM	PA	SQ	SEM
cis-9,*trans*-11/*trans*-8, *trans*-10	0.523^{b}	0.697^{a}	0.708^{a}	0.031
cis-11, *cis*-13	0.007^{b}	0.013^{a}	0.016^{a}	0.001
cis-10, *cis*-12	0.015^{c}	0.034^{a}	0.026^{b}	0.002
Total CLA^{d}	0.629^{b}	0.917^{a}	0.933^{a}	0.034

^{abc}Means within a row with uncommon superscripts differ ($P < 0.05$).

^{d}CLA = conjugated linoleic acid.

Table 3.7 Effect of dietary treatment on fatty acid composition of adipose tissues

	Dietary Treatment			
Fatty acid, %	CON	OIL	SALT	SEM
C18:1 *trans*-11	0.837^{b}	0.937^{a}	1.021^{a}	0.029
C18:1 *cis*-11	1.728^{a}	1.678^{ab}	1.621^{b}	0.029
C18:2 *cis*-9, *cis*-12	1.337^{b}	1.764^{a}	1.394^{b}	0.049
C18:2 *cis*-11, *cis*-13	0.011^{ab}	0.009^{b}	0.015^{a}	0.001
C18:2 *cis*-8, *cis*-10	0.000^{b}	0.000^{b}	0.003^{a}	0.001
Total CLA^{d}	0.745^{b}	0.816^{b}	0.917^{a}	0.033
PUFA^{e}	2.085^{c}	2.601^{a}	2.300^{b}	0.006

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

[d] CLA = conjugated linoleic acid.

[e] PUFA = polyunsaturated fatty acid.

CHAPTER 4

CONCLUSIONS

Circulating leptin concentrations were not affected ($P > 0.05$) by dietary treatment at any time during the trial. However, leptin concentration in adipose tissue was greater ($P < 0.05$) for heifers fed OIL compared to either CON or CLA diets, which were similar ($P > 0.05$). Compared with adipose tissues from CLA salt supplemented animals, tissues from heifers fed corn oil contained 68% greater leptin concentration.

The ratio of cis-9, trans-11 to C18:1 trans-11 was higher ($P < 0.05$) for animals fed 60 d compared to 32 d, but did not differ ($P > 0.05$) between adipose depots. Supplemental corn oil increased ($P < 0.05$) adipose concentration of C18:2 fatty acid. Feeding rumen-protected CLA increased total CLA isomers by 22%. IM lipid contained the lowest ($P < 0.05$) percentage of cis-9, trans-11 CLA, total CLA, C18:1 cis-9, C18:1 trans-10, and C18:1 trans-11 fatty acids.

Supplementing rumen-protected conjugated linoleic acid to finishing cattle diets alters fatty acid composition of adipose depots and increased CLA deposition. However, subsequent changes in lipid profiles were highly variable between adipose depots as well as supplementation lengths. Corn oil and rumen-protected CLA salt diets increased adipose concentration of C18:1 *trans*-11 fatty acid compared to control treatment, indicating the possibility of suppression of SCD activity with polyunsaturated fatty acid supplementation.