THE EFFECT OF ANABOLIC IMPLANT AND DIETARY LIPID SOURCE ON INTRAMUSUCLAR LIPID DEPOSITION IN FINISHED BEEF CATTLE

by

KERRY RUTH SMITH

(Under the Direction of T. Dean Pringle)

ABSTRACT

Neither anabolic implant nor lipid supplementation altered adipose cellularity. The mRNA abundance of acetyl-CoA carboxylase, stearoyl-CoA desaturase, and lipoprotein lipase in beef intramuscular adipose tissue was not altered by anabolic implant or dietary lipid source. According to this research, these treatments did not have a direct effect on enzyme expression and lipogenesis.

KEY WORDS: Beef, Intramuscular lipid, Lipogenesis, Adipose cellularity, Anabolic implant, CLA, Corn oil

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DEDICATION

Glory to God…….Victory in Jesus

I dedicate my dissertation to
my Lord, God, and Savior.
Without the grace, love, strength, and peace of God
this dissertation would not have been possible.

[1]I will praise you with my whole heart;
before the god's I will sing praises to You.

[2]I will worship toward Your holy temple, and praise Your name
for your loving kindness and Your truth;
for You have magnified Your
word above all Your name.

[3]In the day when I cried out, You answered me,
and made me bold with strength in my soul.

[4]All the kings of the earth shall praise You, O Lord,
When they hear the words of Your mouth.

[5]Yes, they shall sing the ways of the Lord,
for great is the glory of the Lord.

[6]Though the Lord is on high, yet he regards the lowly;
But the proud He knows from afar.

[7]Though I walk in the midst of trouble, You will revive me;
You will stretch out Your hand against the wrath of my enemies,
And your right hand will save me.

[8]The Lord will perfect was concerns me;
Your mercy, O Lord, endures forever;
Do not forsake the works of Your hands.

Psalms 138 (NKJV)

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My Heavenly Father has blessed me richly with my parents. Mom and Dad I will never be able to put on paper enough words to describe how much you mean to me. Mom, you are a walking heart and you amaze me with all that you do. I have said this before and I will say it again, if I can be only half the woman you are I will go along way in life. Daddy, you are my chief counselor on this earth and just as David was a man after God's own heart, you and I share the same heart. I believe y'all have the heart God intended all parents to have. I thank y'all for you unending love, support, encouragement and friendship. If parenting is a spiritual gift, God generously bestowed the ability to the two of you. To my sister, Kelly, you are an amazing older sister who time and time again shows me the true spirit of generosity. I love you so much and thank you for the joyous blessing you have given the family in your son Kaleb.

I have always been surrounded by a tremendous amount of love and support thanks to my entire family. To my grandparents, Granddaddy, Grandmother, MawMaw and Papaw (whom we lost this past summer but is now in heaven), y'all are the rock and foundation on which all our family stands on. Your unconditional love, kindness and dedication is what defines the word family to me. To all of my aunts, uncles and cousins, God has blessed me exceedingly abundantly with you all.

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good person. I wish every student going through the education system would have a mentor like you.

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INTRODUCTION

Anabolic implants have been extensively integrated into the management practices of the finishing phase of U.S. beef production as a cost effective way to enhance animal performance (Samber et al, 1996; Duckett and Andrae, 2000) and lean:fat through an increase in longissimus and carcass muscle yield (Johnson et al., 1996a,b; Roeber et al., 2000). Despite the return on investment, research is varied on the impact of implantation on carcass quality and palatability. Some studies have indicated a reduction in marbling score due to combination trenbolone acetate and estradiol implants (Morgan, 1997; Roeber et al., 2000), while other studies have that shown implants have no effect on marbling score (Johnson et al., 1996a; Duckett et al., 1997). Duckett et al. (1999) reported implanting with a combination implant reduced intramuscular lipid amount and composition; however, when increases in longissimus muscle area were taken in account, differences were not significant. Duckett and Andrae (2000) reported a strong relationship ($r^2 = 0.68$) between reduction in marbling score and increase in longissimus muscle area. This would imply implantation has an indirect effect on intramuscular lipid content by distributing similar amounts of lipid over a greater lean muscle mass. However, little research exists on the direct effect of implanting on lipid deposition, information that would be valuable in determining implant programs that minimize negative effects on quality grade.

Beef lipid contains unique fatty acid intermediates, collectively termed conjugated linoleic acid (**CLA**) and *trans*-vaccenic acid, produced during ruminal biohydrogenation

of dietary unsaturated lipids to saturated products (Bauman et al., 1999). Conjugated linoleic acid refers to positional and geometric isomers of linoleic acid. Two prominent isomers have been shown to have health benefits as either anticarcinogenic (*cis*-9, *trans*-11 CLA; Ha et al., 1987) or antiobesity (*trans*-10, *cis*-12 CLA; Wiegand et al., 2001). Ruminant fat in milk and meat products are among the leading natural sources of CLA (Chin et al., 1992; Ritzenthaler et al., 2001). Dairy cattle research has shown vegetable oil or CLA salt supplementation to increase the *cis*-9, *trans*-11 CLA isomer in milk fat (Kelly et al., 1998). Altering intramuscular fatty acid composition, by enhancing unsaturated fatty acid content and CLA, would be beneficial to the beef industry and human health. Chouinard et al. (1999) and Baumgard et al. (2001) reported dietary CLA changed lipid metabolism and reduced milk fat synthesis. Research has shown CLA supplementation reduces lipid accumulation by adipocytes due to a single-isomer effect of *trans*-10, *cis*-12 CLA isomer (Park et al., 2000; Pariza et al., 2001). Changing beef intramuscular lipid composition is limited by ruminal biohydrogenation since only small to moderate changes in intramuscular lipid composition has been attributed to nutritional effects.

Longissimus muscle intramuscular lipid content is an essential aspect of beef palatability and fed cattle value-based marketing. However, limited information is available on the regulation of intramuscular lipid deposition because of the intrinsic difficulties in measuring this adipose depot (small sample size and difficulty in obtaining it). Intramuscular lipid is in low concentration (5 to 80 mg lipid per g of muscle tissue) in most muscles. Fat accretion is the balance between lipid synthesis (lipogenesis) and breakdown (fatty acid oxidation/lipolysis). Three key enzymes involved in lipid uptake

and biosynthesis for fat storage are acetyl CoA carboxylase, stearoyl CoA desaturase, and lipoprotein lipase. Molecular studies of intramuscular adipose tissue are needed to determine the origin of fatty acids and to determine the mechanisms by which fat synthesis occurs. Assessment of adipocyte cellularity and mRNA expression of key lipogenic and lipolytic enzymes of intramuscular is needed.

In this research, three trials were used to study the effects of nutrition and hormone treatments on lipogenesis. A nutrition trial examined supplementation of linoleate-rich corn oil and rumen-protected CLA in feedlot cattle, while hormone trials, using growth promotants, examined the use of a strong combination implant regimen in finished steers and heifers. The objective of each study was to determine the effect of treatment on changes in carcass quality, cellularity of subcutaneous and intramuscular depots, and mRNA expression of acetyl CoA carboxylase, stearoyl CoA desaturase, and lipoprotein lipase in intramuscular adipose tissue.

CHAPTER 1

LITERATURE REVIEW

Intramuscular lipid content of the longissimus muscle is an essential aspect of beef palatability and fed-cattle value-based marketing. The visible intramuscular fat, located in the perimysial connective tissues between muscle fiber bundles, is commonly called marbling. Currently, U. S. beef production utilizes a subjective measure of marbling score to segregate and price beef carcasses as marbling score has been shown across a wide range of levels to be related to beef palatability (Smith et al., 1984; Savell et al., 1987). Intramuscular lipid content of muscle is an incessantly varying trait in cattle revealing heritabilities of up to 0.65 when evaluated among cattle of the same age have been reported indicating the importance of genetics in intramuscular adipose tissue development (Marschall, 1999). Due to the valuable role of intramuscular fat in beef production, numerous trials have been conducted to determine the factors affecting intramuscular lipid deposition, as well as the ability of several marker-assisted quantitative trait loci (**QTL**) to enhance longissimus intramuscular fat.

Fat Accumulation in Beef Cattle: Lipogenesis

Adipose tissue is composed largely of fat, as its primary function is to store lipid. Fat accretion is the balance between fat synthesis (lipogenesis) and breakdown (fatty acid oxidation/lipolysis). In ruminant animals, lipogenesis occurs in the adipocyte and acetate is the predominant substrate utilized for fatty acid synthesis (Mersmann, 1979). Adipose tissue mass can increase by hyperplasia (cell proliferation) or hypertrophy (cell enlargement through lipid accumulation). The apparent increase in adipocyte number may be due to preadipocytes filling

with lipid or actual differentiation or proliferation of newly stimulated preadipocytes (Hood, 1982).

Hypertrophy is due to adipocytes accumulating triacylglycerols, which are formed by the esterification of glycerol 3-phosphate and fatty acids. The fatty acids utilized for accumulation of storage lipid in intramuscular adipose tissue originate from three sources: 1) plasma free fatty acids, 2) fatty acids from triacylglycerols transported in plasma lipoproteins, or 3) *de* novo fatty acid synthesis within the adipose tissue (Hood, 1982). Briefly, in *de novo* fatty acid synthesis, acetate carbons enter fatty acid biosynthesis via malonyl-CoA production through the acetyl-CoA carboxylase reaction and then into palmitate production through fatty acid synthase. Once palmitate is produced, a single two-carbon elongation synthesizes stearate and then oleate is produced by desaturation at the ninth carbon atom (methyl end).

Three key enzymes involved in lipid uptake and biosynthesis for fat storage are lipoprotein lipase (**LPL**), acetyl CoA carboxylase (**ACC**), stearoyl CoA desaturase (**SCD**). Providing free fatty acids to adipose tissue is LPL, which catalyzes the hydrolysis of triglycerides from circulating lipoprotein particles (Auwerx et al. 1992). The rate-limiting enzyme of fatty acid synthesis or lipogenesis is ACC. In this initial biotin-dependent step, acetyl CoA is converted to malonyl-CoA, the substrate for fatty acid synthesis (Abu-Elheiga et al., 2001). The fatty acid composition of muscle and adipose tissue is greatly influenced by the regulation of SCD. The SCD enzyme catalyzes the rate-limiting step in the biosynthesis of monounsaturated fatty acid (**MUFA**) by inserting a *cis*-double bond in the fatty acyl-CoA substrate Δ^9 position (Kim and Ntambi, 1999).

Management Practices to Alter Intramuscular Lipid Deposition and Composition

Two common production practices to alter the composition of foods of animal origin are through dietary nutrition and the use of growth promotants. The feeding regime for fattening influences intramuscular adipose development (Cianzio et al., 1985; Nishimura et al., 1999). Lipogenesis is stimulated by a high carbohydrate diet, whereas it is inhibited by polyunsaturated fatty acids (**PUFA**) and by fasting (Kersten, 2001). In beef production, guaranteeing satisfactory nutrition is first priority but increasing the efficiency of gain:feed and protein deposition has commonly required the use of anabolic implants. The effects of dietary lipid source and use of anabolic implants in beef cattle intramuscular lipid deposition and composition will be reviewed.

Dietary Lipid Source

The primary nutrients obtained through consumption of red meat are protein and water; however, the consumption of meat can be significant source of dietary fat due to intramuscular fat (marbling) as well as the inclusion of fat in ground and processed meat products. Fatty acid composition of adipose tissue has been the focus of numerous studies largely due to the impact of dietary fat on human blood lipids. Six fatty acids make up over 92% of the total fatty acid content of beef intramuscular lipid (Duckett et al., 1993). The six major fatty acids of beef intramuscular lipid are (in order of contribution): oleic (C18:1), palmitic (C16:0), stearic (C18:0), linoleic (C18:2), plamitoleic (C16:1) and myristic (C14:0). The percentages of these fatty acids are presented in **Figure 1.1**. Overall, intramuscular lipid contains 45% MUFA, 44% saturated fatty acids (SFA), 5% odd- and branched-chain fatty acids (OCFA) and 5% PUFA (Duckett et al., 1993). The SFAs are considered hypercholesterolemic or cholesterol elevating (Hegsted, 1965); however, stearic acid, which is saturated, has been shown to lower serum

cholesterol levels compared to other saturated fatty acids (Hegsted, 1965; Bonanome and Grundy, 1988).

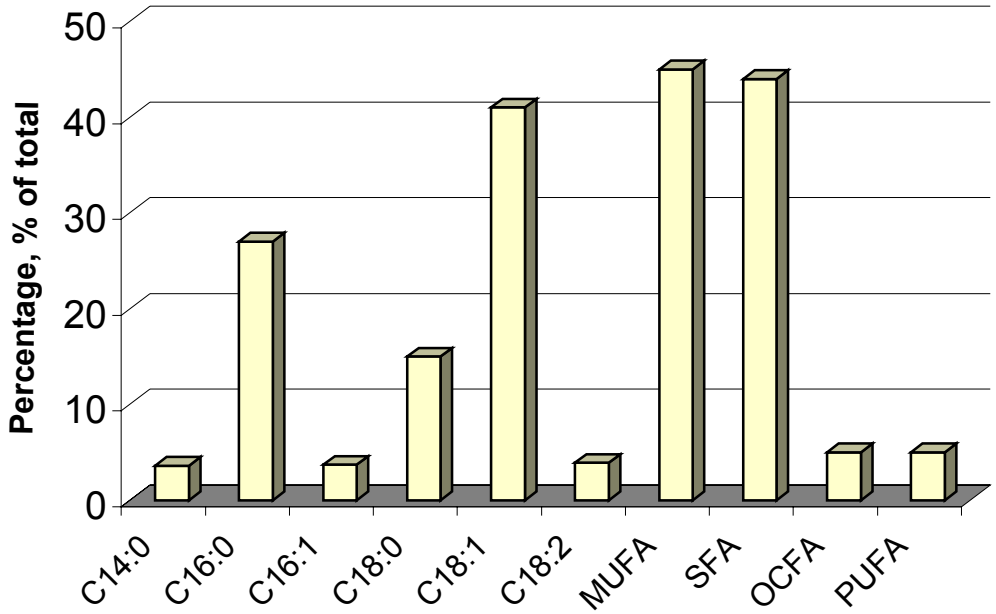

Figure 1.1. Fatty acid composition of beef intramuscular lipid. (Duckett et al., 1993).

Beef lipid contains unique fatty acid intermediates, collectively termed conjugated linoleic acid (CLA) and *trans*-vaccenic acid, produced during ruminal biohydrogenation of dietary unsaturated lipids to saturated products (Bauman et al., 1999). Conjugated linoleic acid refers to positional and geometric isomers of linoleic acid. Two predominant isomers have been shown to have anticarcinogenic (*cis*-9, *trans*-11 CLA; Ha et al., 1987) or antiobesity (*trans*-10, *cis*-12 CLA; Wiegand et al., 2001) properties. Ruminant fat in milk and meat products are among the leading natural sources of CLA (Chin et al., 1992; Ritzenthaler et al., 2001). In ruminant animals, CLA is produced as the first intermediate in the biohydrogenation of dietary linoleic

acid by an isomerase from rumen bacteria such as *Butyrivibrio fibrisolvens* (**Figure 1.2**). Complete biohydrogenation of linoleic acid results in stearic acid (C18:0). Bovine adipose tissue also has the SCD enzyme that can convert stearic acid to oleic acid (**Figure 1.3**; St. John et al., 1991).

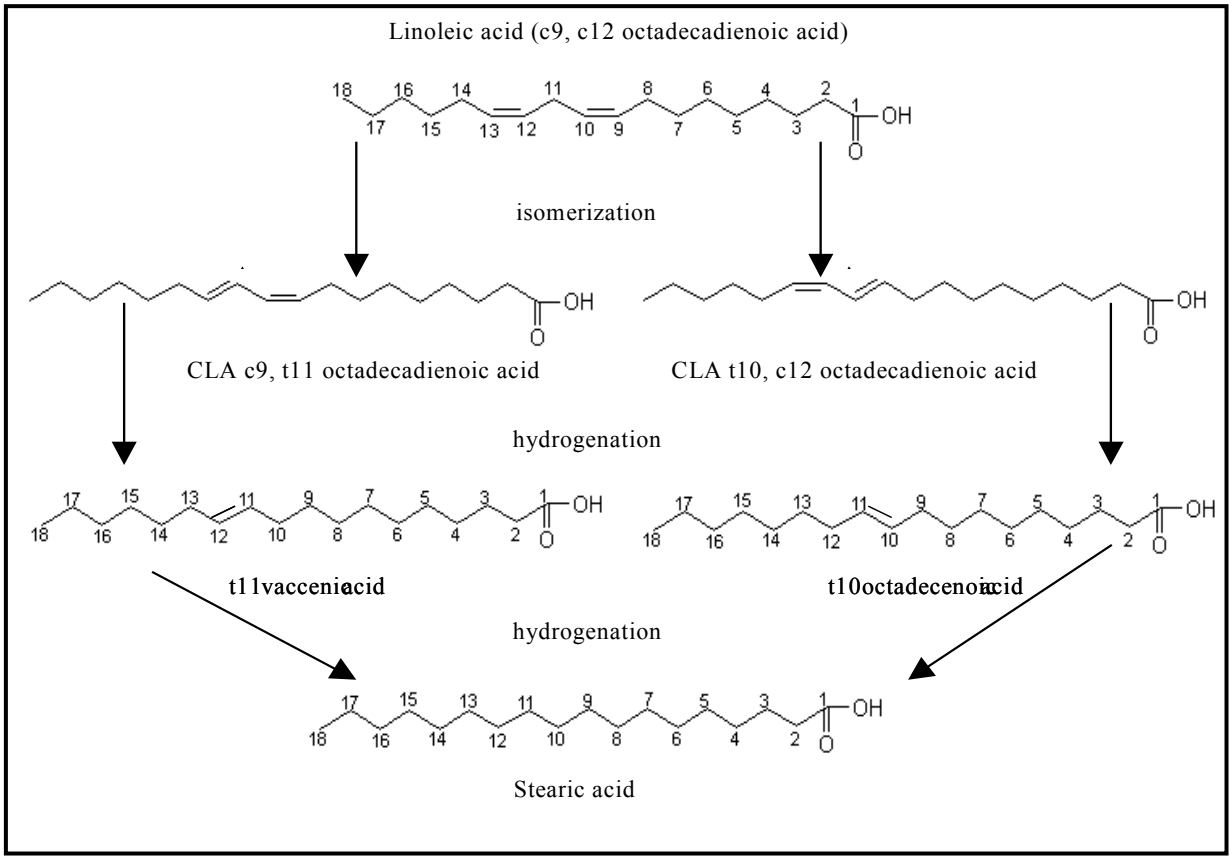

Figure 1.2. Ruminal biohydrogenation of dietary linoleic acid. (Adapted from Bessa et al., 2000.

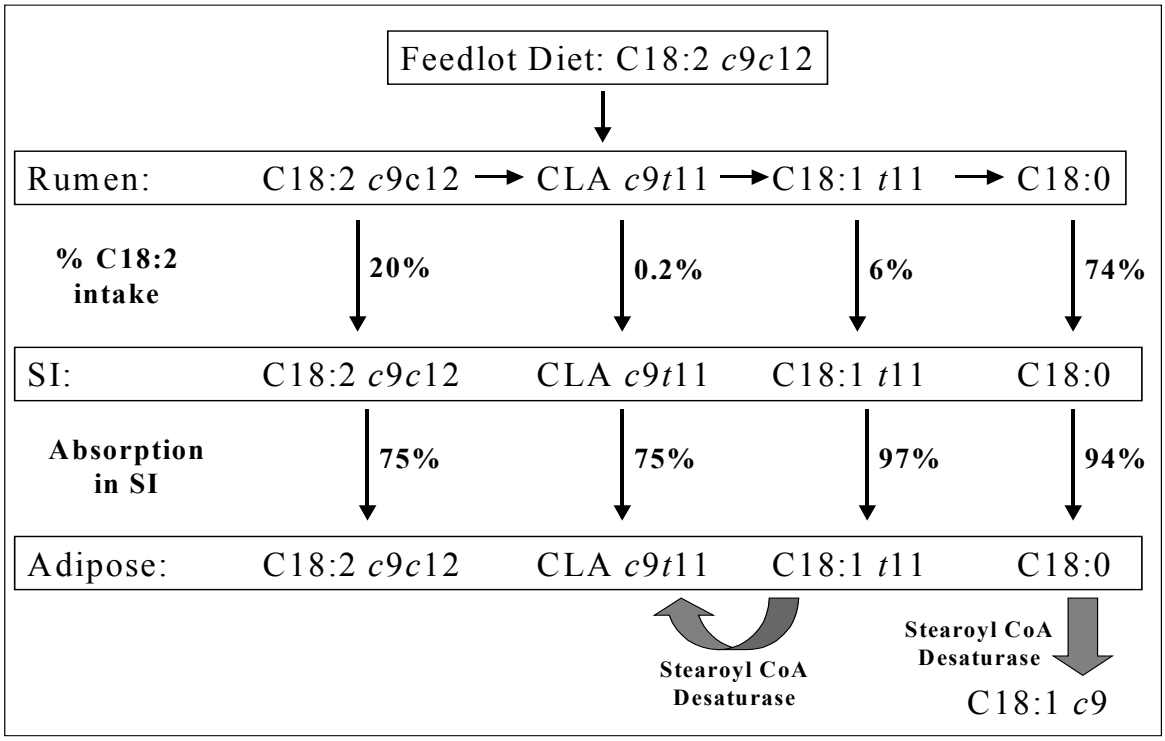

Figure 1.3. Linoleic acid from diet to adipose tissue deposition. (Adapted from Scollan et al., 2001 and Duckett et al., 2002)

Altering the fatty acid composition of intramuscular adipose tissue to increase MUFA and CLA would be advantageous due to potential human health benefits. However, ruminal biohydrogenation is a limitation in changing intramuscular composition. Only small to moderate changes in composition have been documented through modification of nutrition and/or management systems. Fatty acid content of the diet, digesta and intramuscular lipid is presented in **Figure 1.4**. The predominate fatty acid of cattle diets is linoleic (C18:2); however, when the digesta and intramuscular lipid are assayed, the predominate fatty acids are stearic (C18:0) and oleic (C18:1), respectively.

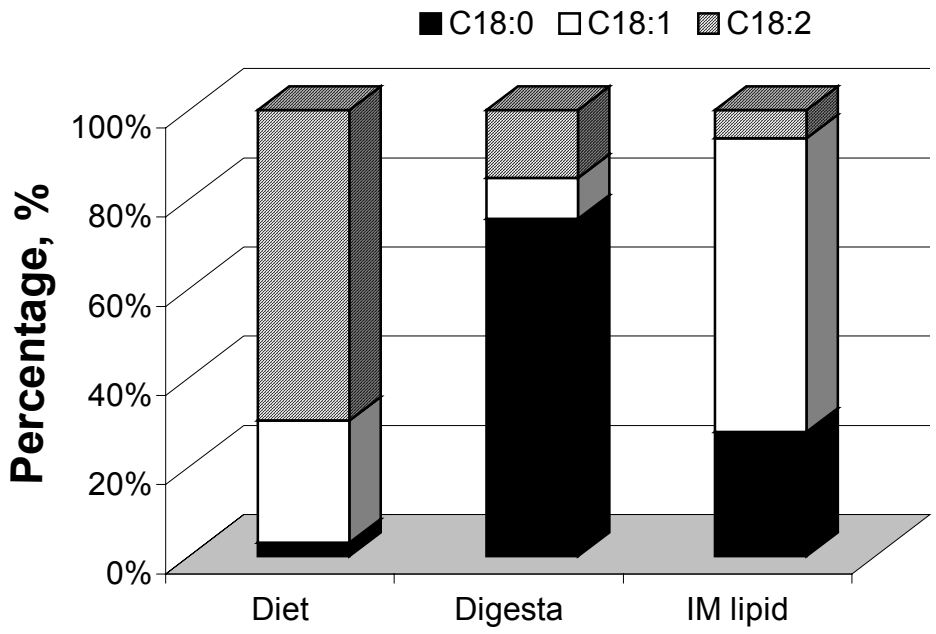

Figure 1.4. Fatty acid composition of diet, digesta and intramuscular lipid. Adapted from Kennington et al. (2000) and Andrae et al. (1998).

Supplemental Linoleate-Rich Corn Oil and Rumen-Protect CLA Salt: Specific feedlot diets can be developed to alter intramuscular lipid deposition and composition. Supplements utilized in typical feedlot systems that have been shown to change lipid composition are protected fats or high levels of unsaturated fatty acids. Both types of supplements would increase the level of unsaturated fatty acid escaping ruminal biohydrogenation. The addition of corn oil or high-oil corn to a typical corn feedlot diet increased ruminal biohydrogenation of 18-carbon unsaturated fatty acids (Dukett et al., 2002). Further, corn oil addition to a diet increased linoleic acid biohydrogenation and duodenal flow of specific isomers of octadecenoic and conjugated linoleic acids. Fatty acid composition of the diet has also been shown to alter CLA content of beef tallow (Pariza et al., 2001), lamb subcutaneous adipose tissue (Mir et al., 2000), and pork intramuscular lipids (Joo et al., 2002). The majority of the research on CLA supplementation of

ruminants has been done in dairy cattle. This work has shown that supplementation of CLA salts or vegetable oils increases the *cis*-9, *trans*-11 CLA isomer in milk fat (Kelly et al., 1998; Corl et al., 2001). Chouinard et al. (1999) and Baumgard et al. (2001) have shown dietary CLA supplementation alters lipid metabolism and results in depressed milk fat synthesis. Research has shown that CLA supplementation induces a reduction in lipid accumulation by adipocytes (Pariza et al., 2001) due to a single-isomer effect of the *trans*-10, *cis*-12 CLA isomer (Park et al., 2000). However this isomer is primarily found in synthetic CLA.

The Use of Anabolic Implants in Beef Cattle

History of Anabolic Implants: Hormones are substances secreted by glands or other tissues into body fluid to be carried to other organs or tissues, where they cause a specific effect. Hormones regulate growth and development through chemical reactions involved in physiological, biochemical and behavioral changes. Growth and development of the body is greatly impacted by hormones produced by the testes and ovaries. When compared to females, male animals usually grow faster and more efficiently, mature later and have a higher lean:fat. However, in meat animal production such as beef cattle, intact males are castrated to modify behavioral problems. Castrated males and females are less efficient producers of meat and have more fat and less muscle deposited during growth compared to intact males. Therefore, animal scientists have sought to enhance efficiency of growth and carcass composition of beef cattle by utilizing naturally occurring and synthetic estrogens and androgens. The principal hormones produced by the testes are androgens and hormones produced by the ovaries are estrogens and progesterone. Androgens stimulate muscle protein synthesis and decrease fat accretion. Estrogens have no effect on muscle protein synthesis but encourage fat deposition. Both androgens and estrogens promote bone salt deposition and cause epiphyseal plate closure when levels are high; however,

estrogens are more effective than androgens at advancing skeletal maturity. Synthetic estrogens (estradiol, zeranol or estradiol benzoate), singly or in combination with synthetic progestins and androgens, increase lean muscle mass by decreasing fat accretion and stimulating muscle growth. The mechanism of action for these repartitioning agents remains to be elucidated.

Today, anabolic implants are utilized almost exclusively in US beef production; however, the use of implants for enhancing meat production was first recorded with poultry production. Diethylstilbestrol (**DES**) was the first implant studied and Lorenz (1943) reported implanting cockerels increased breast and leg fat content by 300%, compared to non-implanted cockerels. Dinussion et al. (1948, 1950) was the first to publish studies on the effect of DES on growth rate of beef cattle. In heifers, DES improved weight gain and feed efficiency as well as increased appetite, body length and width, carcass maturity and sexual behavior.

Raun and Preston (1997) reviewed the history of hormonal modifier use and discussed the timeline of DES in cattle production. The FDA approved oral administration of DES in late 1954 and by December of 1955, an estimated six million cattle were being fed DES. By 1957, DES implants received FDA approval. Use of DES increased until 1972 when negative press and packer complaints concerning reduced carcass quality caused a reduction in the number of DES implanted cattle. Under the weight of bad publicity, the Food and Drug Administration (FDA) was forced to ban oral DES in 1972 and DES implants in 1973.

The banning of DES facilitated development of other anabolic implants. Currently 24 different anabolic implants are registered with the FDA for use in beef cattle production (**Table 1.1**). Implants can be classified according to the nature (estrogens, androgens, progestins, or combination) and dosage/potency of active ingredient(s) (**Table 1.2**). Anabolic implant products

Table 1.1. Registered hormonal growth promotants with FDA for use in beef cattle in the USA, by active ingredient, dosage, registered trade name, and proper beef group for use[a]

Ingredient	Dosage, mg	Trade name	Proper beef group
Single ingredient implants			
Estradiol[b]	25.7	Compudose 200[g]	Steers, Heifers
	43.8	Encore[h]	Steers, Heifers
Zeranol[b]	36	Ralgro[i]	Cattle
	72	Ralgro Magnum[i]	Steers
Trenbolone acetate[c]	140	Component T-S[fh]	Steers
	200	Finaplix-H[j]	Heifers
		Component T-H[fh]	
Combination ingredient implants			
Estradiol benzoate[be] / Progesterone[d]	10 / 100	Synovex-C[k]	Steers, Heifers
		Component E-C[fh]	
		Implus-C[h]	
	20 / 200	Synovex-S[k]	Steers
		Component E-S[fh]	
		Implus-S[h]	
Estradiol benzoate[be] / Testosterone propionate[c]	20 / 200	Synovex-H[k]	Heifers
		Component E-H[fh]	
		Implus-H[h]	
Estradiol[b] / Trenbolone acetate[c]	24 / 120	Revalor-S[j]	Steers
		Component TE-S[fh]	
	14 / 140	Revalor-H[j]	Heifers
	16 / 80	Revalor-IS[j]	Steers
	8 / 80	Revalor-IH[j]	Heifers
	8 / 40	Revalor-G[j]	Steers, Heifers
		Component TE-G[fh]	
Estradiol benzoate[be] / Trenbolone acetate[c]	20 / 200	Revalor-200[j]	Steers
	28 / 200	Synovex Plus[k]	Feedlot Steers, Heifers

[a]Adapted from Montgomery et al., 2003.
[b,c,d]Estrogen, Androgen, and Progestin group, respectively.
[e]Estradiol benzoate contains 71.4% estradiol as calculated on formula weight (Herschler et al., 1995).
[f]Available with Tylosin Tartate at 29 mg (Tylan; Elanco Animal Health, Indianapolis, IN).
[g]Elanco Animal Health.
[h]Vet life (Winterset, IA).
[i]Schering Plough (Madison, NJ).
[j]Intervet Inc. (Flemmington, NJ).
[k]Ft. Dodge Animal Health (Overland Park, KS).

Table 1.2. Classification of various implant types by category and relative potency

Implant	Category	Relative potency
Compudose, Ralgro, Encore, Implus-C, Synovex-C, Component E-C	Estrogen	Mild
Implus-S, Synovex-S, Component E-S, Ralgro Magnum	Estrogen	Strong
Component T-S, Finaplix-H, Component T-H	Androgen	—
Implus-H, Synovex-H, Component E-H, Revalor-S, Revalor-H, Revalor-G, Revalor-IH, Revalor-IS, Component TE-S, Component TE-G	Combination	Mild
Synovex Plus, Revalor-200	Combination	Strong

[a]Adapted from Montgomery et al., 2003; Duckett et al., 1997; Morgen, 1997.

have been extensively integrated into the management practices of all phases of beef cattle growth: from suckling calves, to grazing cattle and most commonly in feedlot cattle. In a 1999 survey of feedlots, the National Animal Health Monitoring System of USDA reported that 96% of the cattle processed were implanted at least once (NAHMS, 2000). Implants are administered subcutaneously on the posterior side of the ear mid-way between the ear tip and base. Typically, implants are in small pellet form with each individual implant consisting of multiple pellets (usually six) containing specific growth promotants designed for slow, prolonged release of the active ingredients.

Anabolic Implant Mode of Action: Anabolic steroids are approved for beef cattle production to improve efficiency through a repartitioning of energy towards lean muscle growth. Johnson et al. (1996b), Kerth et al. (2003), and Montgomery et al. (2003) and have recently reviewed the use of estrogen (**E_2**) and trenbolone acetate (**TBA**) anabolic implants as growth promotants in cattle. Mechanisms by which implants promote growth are not clear, but studies have suggested insulin-

like growth factor I (**IGF-I**) may be involved (Breier et al., 1988; Hayden et al., 1992; Hongerholt et al., 1992). Insulin-like growth factor I is a somatotropin-dependent anabolic peptide that stimulates proliferation and differentiation in muscle and other tissues (Florini et al., 1991). In addition to somatotropin (**ST**) regulation, IGF-I is also regulated by binding to one of six insulin-like growth factor binding proteins (IGFBP; Baxter, 1991). The E_2 implantation of cattle has been shown to increase ST (Breier et al., 1988; Hayden et al., 1992) and IGF-I (Breier et al., 1988) circulating concentrations. However, implantation of TBA or TBA + E_2 did not increase ST (Hunt et al., 1991; Hayden et al., 1992; Hongerholt et al., 1992) but did raise circulating IGF-I levels (Hunt et al., 1991; Hongerholt et al., 1992). Androgens, such as TBA, enhance cell membrane androgen receptors that increase cellular production of protein and in conjunction, adreno-corticotrophic hormone (**ACTH**) production is reduced. Decreased circulating cortisol levels have been proposed to aid protein accretion by decreasing protein catabolism (Jones et al., 1991; Isaacson et al., 1993). Thus, androgen implants appear to be both anabolic and anti-catabolic. Anabolic agents have also been reported to increase satellite cell number, water concentration and nitrogen content (Perry et al., 1991; Johnson et al., 1996a, 1998).

Blood steroid concentrations from separate TBA and E_2 implants are unlike concentrations produced by a combination implant (**TBA + E_2**; Heitzman and Harwood, 1977; Harrison et al., 1983). Combination implants increased serum levels of both IGF-I and IGFBP-3 as well as increased mitogenic activity of sera compared to non-implanted steers (Johnson et al., 1996b). Johnson et al. (1996b) reported increases in IGF-I levels almost immediately after implantation, during d0 to 40. Even at d115, combination implanted steers had higher concentrations of IGF-I than non-implanted steers. A combination implant improves growth

above the capacity of either hormone independently (Gerken et al., 1995) and the net gain is to that also of either E_2 or TBA alone. The increase in muscling of implanted cattle is most likely the net result of both protein synthesis and decreased protein degradation, with the strongest effect being on protein synthesis (Kerth et al., 2003). The importance of circulating IGF-I and IGFBP (Douglas et al., 1991; Koea et al., 1992) verses locally produced IGF-I and IGFBP (Daughaday and Rotwein, 1989) has been widely deliberated. Recently, Kerth et al. (2003) reported anabolic implant strategies could directly affect muscle protein synthesis and degradation through an autocrine rather than paracrine effect.

Industry Significance of Anabolic Implants: The use of anabolic implant in beef production is acknowledged as the greatest return on investment other than guaranteeing satisfactory nutrition (Montgomery et al., 2003). Economic advantages of implanted verses non-implanted cattle fluctuate but are usually $15 to $40 per animal (NAHMS, 2000). Anabolic implants are used to enhance economically important production traits such as average daily gain, feed efficiency and protein deposition (Samber et al, 1996; Duckett and Andrae, 2000) as well as lean:fat through an increase in longissimus and carcass muscle yield (Johnson et al., 1996a,b; Roeber et al., 2000). In a summary of implant strategies, Duckett and Andrae (2000) reported the largest increase (19 to 20%) in ADG was for feedlot cattle implanted once or twice with a combination implant. It has been reported that steers (Hermesmeyer et al., 2000) and heifers (Popp et al., 1997; Mader et al., 2000) implanted once with a mild or strong combination implant had heavier carcasses and larger longissimus muscle areas compared to non-implant steers and heifers. Dolezal (1997) showed combination implants, used initially and as a reimplant, produced the greatest increase in carcass weight and ribeye area in yearling steers. Mader et al. (1994) also found reimplanting yearling heifers with a combination implant resulted

in heavier carcass weights and increases longissimus muscle areas compared to nonimplanted controls. Other studies have shown little effect of anabolic implants on subcutaneous fat depth (Herschler et al., 1995; Foutz et al., 1997) and dressing percentage (Perry et al., 1991; Johnson et al. 1996a; Scheffler et al., 2003). Herschler et al. (1995), Foutz et al. (1997), and Scheffler et al. (2003) found no difference for percentage of kidney, pelvic and heart fat (**KPH**); conversely, others have reported implants decrease the percentage of KPH fat in implanted cattle (Johnson et al., 1996a; Duckett et al., 1999; Roeber et al, 2000). The decrease in internal fat found by some studies could be the result of an increase in carcass weight as KPH is expressed as a percentage of carcass weight. Yield grade is influenced by longissimus area in relation to carcass weight. Roeber et al. (2000) found with anabolic implants use, both of these measures increase, thus final yield grade would not be expected to change.

Anabolic implants are used to improve profitability through an enhancement of feed efficiency and carcass value. Carcass value is directly related to lean:fat, marbling deposition and meat tenderness. Marbling has and will continue to be for some time an essential aspect of fed cattle value-based marketing. Despite reductions in beef cattle production costs, concerns have been raised regarding the use of implants and their potential negative effects on USDA quality grade attributed to reduced marbling score and/or advanced skeletal and lean maturity (Belk, 1992; Duckett et al., 1997; Foutz et al., 1997). These effects have been especially worrisome in combination TBA + E_2 implants. In steers and heifers, the response of marbling scores to the use of a single anabolic implant has been variable. Johnson et al. (1996a) and Duckett et al. (1997) reported the use of a combination implant did not effect marbling is steers and heifers, respectively. However, Herschler et al. (1995) and Mader et al. (2000) documented a reduction in marbling score in steers and heifers implanted once with a mild or strong combination

implant. A review by Morgan (1997) of data from steers receiving a mild or strong combination re-implant, reported a decrease in marbling score by 26 points and 24% fewer carcasses grading low Choice compared to non-implanted controls. Dolezal (1997) and Roeber et al. (2000) also noted marbling score decreased with reimplantion. In contrast, other studies have shown re-implantion to have no effect on quality grade (Gerken et al., 1995; Johnson et al., 1996a). Duckett et al. (1999) reported implanting with a combination implant reduced intramuscular lipid amount; however, when increases in ribeye area were taken into account, the differences were not significant. A summary of implant usage by Duckett and Andrae (2000) showed a relationship ($r^2 = 0.68$) between reductions in marbling score and increases in ribeye area. This would imply implantation has an indirect effect on intramuscular lipid by diluting a similar amount of fat over a greater lean muscle mass.

Future Research

More research is needed to determine mechanisms controlling fat deposition in livestock cattle. Limited information is available on the regulation of intramuscular lipid deposition because of the inherent difficulties in measuring this adipose depot (small sample size and difficulty in obtaining it). Intramuscular lipid is in low concentration (5 to 80 mg lipid per g of muscle tissue) in most muscles. Serial slaughter research has revealed intramuscular lipid deposition can double in a relatively short period of time (Duckett et al., 1993). Molecular studies of intramuscular adipose tissue are needed to determine the origin of fatty acids and to determine the mechanisms by which fat synthesis occurs. Research as moved from applied to basic in order to determine the mechanisms of how an organism's code can be activated so that its information can be copied and its instruction executed. Only a few genes in a given cell are ever expressed. Genes (DNA) are first transcribed into messenger RNA (mRNA) and mRNA

then serves as the template for the cell to translate “the message” into protein. While indirect effects of anabolic implants on lipogenesis have been reported, direct effects of lipid source and anabolic implant on lipogenesis has not been examined. In order to evaluate this potential direct effect, assessment of intramuscular adipocyte cellularity and mRNA expression of lipogenic and lipolytic enzymes is needed.

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CHAPTER 2

MATERIALS AND METHODS

Animals, Diets, and Experimental Design

Implant Heifer Trial: Yearling Angus heifers (n = 10; 386 kg) sired by high marbling EPD bulls (marbling EPD ≥ +.3) were purchased and transported to the University of Georgia (UGA) Wilkins Beef Cattle Unit to evaluate the effect of implanting on intramuscular fat deposition. After an adjustment period, heifers were randomly allotted to serve as controls or implanted with Synovex-Plus (Fort Dodge Animal Health, Overland Park, KS; 28 mg estradiol benzoate and 200 mg of trenbolone acetate). Heifers designated for the implant group were first implanted on d 0 and re-implanted on d 55. Heifers were fed a high concentrate diet (84% concentrate, 12.5% crude protein, 1.2 NEg (Mcal/kg)) for 108 d.

Implant Steer Trial: Calf-fed Angus steers (n = 18; 279 kg) sired by high marbling EPD bulls (marbling EPD ≥ +.3) were purchased and transported to the University of Georgia (UGA) Wilkins Beef Cattle Unit to evaluate the effect of implanting on intramuscular fat deposition. After an adjustment period, steers were randomly allotted to serve as controls or implanted with Synovex-Plus (Fort Dodge Animal Health, Overland Park, KS; 28 mg estradiol benzoate and 200 mg of trenbolone acetate). Steers designated for the implant group were first implanted on d 0 and re-implanted on d 73. Steers were fed a high concentrate diet (84% concentrate, 12.5% crude protein, 1.2 NEg (Mcal/kg)) for 141 d.

Dietary Lipid Supplementation Heifer Trial: Angus × Hereford heifers (n=36; 365 kg) attained from the Northwest Georgia Experiment Station (Calhoun, GA) were used in a

completely randomized design to determine the effect of dietary lipid sources on lipid deposition of feedlot cattle. Heifers were fed a basal diet for an initial 56 d feeding period and then were allotted to one of three dietary treatments (DM basis) for the last 32 or 60 d before harvest: 1) basal diet containing 88% concentrate and 12% grass hay (CON); 2) basal diet plus 4% corn oil (OIL); or 3) basal diet plus 2% rumen-protected CLA salt (SALT), containing 31% CLA-60. Treatment effects were evaluated in a 3 × 2 factorial arrangement with three diets (CON, OIL, or SALT) and two feeding period lengths (32 or 60 d).

The rumen-protected CLA supplement was composed of a mixture of Ca-salts of palm oil and CLA, containing 22.1% palmitate, 4.8% stearate, 27.4% oleate, 7.1% linoleate, and 31% CLA isomers (27.2% cis-9, trans-11; 32.8% trans-10, cis-12; 10.6% trans-8, cis-10; 18.95% cis-11, trans-13; and 10.5% various trans, trans CLA isomers). Dietary treatments were prepared to be isonitrogenous; with an equal percentage of concentrate being removed as supplemental lipid was incorporated. Heifers at trial initiation were implanted with Synovex-H (20 mg of estradiol benzoate and 200 mg of testosterone; Ft. Dodge Animal Health, Ft. Dodge, IA) and were fed melengesterol acetate (Pharmacia, Kalamazoo, MI) at 0.45 mg·heifer^{-1}·day^{-1} and rumensin-80 (Elanco Animal Health, Greenfield, IN) at 250 mg of monensin activity·heifer^{-1}·day^{-1} throughout the trial. Animal weights were recorded at 28 d intervals throughout the feeding trial.

After 89 d on feed (32 d of dietary treatment), six heifers per dietary treatment (n=18) that had ≥1.27 cm s.c. fat thickness (as measured by real-time ultrasound) were slaughtered. The remaining heifers (six heifers/treatment; n=18) were fed their diet treatments for an additional 28 d to reach a similar s.c. fat thickness end point and then slaughtered.

Performance and Ultrasound

Animal weights were recorded throughout the feeding trials. For the implant heifer and steer trials, real-time ultrasound measurements of longissimus muscle area (ULMA), fat thickness (UFT) and intramuscular lipid (UIMF) percentage were recorded throughout the feeding trial. An American Association of Ultrasound Practitioners-certified technician collected images between the 12th and 13th rib interface using an Aloka® 500V (Corometrics Medical Systems, Wallingford, CT) machine with an Aloka® UST-5049-3.5 (17.2-cm linear) probe. Images were interpreted using Beef Information Manager software (version 3.0; Critical Vision, Inc., Atlanta, GA).

Harvest, Carcass Data and Sample Collection

Upon completion of the finishing period, the cattle were transported to the University of Georgia Meat Science and Technology Center (Athens, GA) where they were harvested under federal inspection using humane procedures. Fasted live animal weights were recorded prior to slaughter.

Immediately (15 min) after exsanguination, a portion of the longissimus muscle (6th to 9th rib) was removed and intramuscular (IM) adipose tissue was dissected, frozen in liquid nitrogen and stored at -70°C for subsequent mRNA analysis. Samples of IM and subcutaneous (SC) adipose tissue were also taken and assayed for tissue cellularity. The carcasses were chilled at 4°C for 48 h, and trained UGA personnel recorded USDA quality and yield grade factors —including, hot carcass weight; adjusted fat thickness; LMA; percentage kidney, pelvic, and heart fat; marbling score; and skeletal and lean maturity. Yield and quality grades were calculated according to USDA standards (USDA, 1997). One steak (2.54 cm thick) was removed from the longissimus muscle between the 11th and 12th rib of the right side of each carcass. Each

steak was trimmed of all external fat, pulverized in liquid nitrogen and stored at -20°C until lipid analysis could be completed.

Warner-Bratzler Shear Force

Implant Steer Trial: Boneless strip loins (IMPS #180) were obtained from one side of each carcass. All external fat was removed from the strip loin before slicing into 2.54 cm thick steaks. Steaks were vacuum-packaged and assigned to one of five aging times (1, 3, 7, 14 and 21 d). Following aging, steaks were frozen at -10°C until shear force evaluation.

Warner-Bratzler shear force (WBSF) was evaluated according to AMSA (1997). Steaks were thawed for 24 h at 4°C. Steaks were cooked on one side to an internal temperature of 40°C, turned, and cooked to a final internal temperature of 71°C on a Farberware Open Hearth Broiler (Farberware, Inc., Bronx, NY). Steaks were placed on plastic trays and allowed to equilibrate to room temperature (22°C). Six cores (1.27 cm in diameter) were removed from each steak parallel to the muscle fiber orientation. Each core was sheared once through the center, perpendicular to the muscle fiber orientation with a Warner-Bratzler shear machine (G-R Elec. Mfg., Manhattan, KS). The six shear force values from each steak were averaged for statistical analysis.

Muscle Lipid Analysis

Total lipids were extracted according to a modification of the Folch et al. (1957) method. Prior to this study, an experiment was conducted to determine a 10:1 ratio of sample to 2:1 chloroform:methanol was sufficient for lipid extraction (Smith, unpublished data). This extract, containing about 50 mg of lipid, was separated into triglyceride and phospholipid fractions according to the procedure of Larick et al. (1989). Fatty acid composition of longissimus muscle samples was determined by the direct transmethylation procedure of Park and Goins (1994). Methyl esters were separated by gas chromatography (Agilent 6850; Agilent Technologies,

Wilmington, DE) using a 100-m Supelco SP2560 (Supelco, Bellefonte, PA) capillary column (0.25 mm i.d. and 0.20 μm film thickness) as detailed by Duckett et al. (2002). Methyl tricosanoate (C23:0) was an internal standard included into each sample prior to methylation to quantify fatty acids.

Adipose Tissue Cellularity

Procedures outlined by Mersmann and MacNeil (1986) were used to determine adipocyte cellularity. Samples (50-mg) of SC and IM adipose tissue in duplicate were fixed by osmium tetroxide and separated by urea to determine adipocyte number, size distribution, diameter and volume. Cell fractions were measured electronically using a Coulter Counter (Coulter Electronics, Hialeah, FL) to determine adipocyte number and size (range of 20 to 240 μm). Due to the large number of particles <30 μm, only counts >30 μm were incorporated in cell number, diameter and volume calculations (Lee et al., 1994).

Ribonuclease protection assay (RPA) of ACC, SCD, and LPL mRNA from bovine IM adipose tissue

Total RNA was isolated from IM adipose tissue with the QIAzol lysis reagent (RNeasy® Lipid Tissue Midi Kit, QIAGEN Inc., Valencia, CA). The nonisotopic RPA outlined by Lee et al. (2002) was used for this experiment. Reverse-transcription PCR was used to synthesize antisense biotin-labeled riboprobes. In a 25 μl volume, 2 μg of total RNA was reverse transcirbed with 200 U of reverse transcriptase (Promega, Madison, WI) at 37°C for 1 hr. The cDNA synthesized was amplified using the primer pairs in which the antisense primers contained the bacteriophage T7 promoter sequence (27-bp) at the 5′ end (Kain et al., 1991). The sequences of the sense and antisense primers for ACC, SCD, LPL, and β-actin are in **Table 2.1**. The PCR reactions were completed in a 50 μL volume containing 5 μL of the RT product, 2.5 m*M* $MgCl_2$,

200 μ*M* dNTPs, 400 n*M* each of sense and antisense primers, and 2.5 U of Taq DNA polymerase (Sigma Chemical, St. Louis, MO). The thermal settings were as follows: 1 cycle at 94°C for 3 min, followed by 35 cycles at 94°C for 60 s, 58°C for 60 s, and 72°C for 60 s with a final extension at 72°C for 5 min. An *in vitro* transcription was accomplished using a MAXIscript™ labeling kit (Ambion, Inc., Austin, TX). The riboprobe reaction, in a 20 μL total volume, contained 5 μL of PCR products, 1× buffer, T7 RNA polymerase, 0.5 m*M* each of ATP, CTP, and GTP with 0.3 m*M* of UTP, and 0.2 m*M* biotin-labeled-16-UTP (Gibco BRL, Grans Island, NY). The reaction was executed for 1 hr at 37°C then incubated with DNase I for 15 min at 37°C. Transcripts were denatured (95°C for 3 min) and separated on 5% acrylamide/8 *M* urea denaturing polyacrylamide gels. After staining with ethidium bromide, the full-length riboprobe was excised and eluted from the gel in probe elution buffer (Ambion, Inc.) overnight at 37°C, precipitated, and quantified at 260/280 nm.

The RPA reaction was carried out using a RPA III™ kit (Ambion, Inc.). One multiprobe (ACC, SCD, LPL and β-actin standard) RPA per animal was completed since the expected product sizes were at least 10% different in bp length. Total RNA and 10-fold molar excess riboprobe was denatured (95°C for 3 min) and then allowed to hybridize at 56°C overnight in 10μL of hybridization buffer. The reactions were digested with RNase and then, through a simultaneous Ambion-patented procedure, RNase was inactivated and protected RNA was precipitated.

The protected RNA was separated by electrophoresis through an 5% acrylamide/8 *M* urea denaturing polyacrylamide gel and electrophoretically (XCell *SureLock*™ Mini-Cell with XCell II™ Blot Module Kit, Invitrogen, Carlsbad, CA) transferred onto a positively charged nylon

membrane (BrightStar-Plus™, Ambion, Inc.) with 0.5× TBE at 400 mA for 1 hr. Once the membrane was UV-crosslinked, nonisotopic detection was performed using BrightStar™ BioDetect™ kit (Ambion, Inc.) in which the membrane was incubated with streptavidin-alkaline phosphatase for 30 min and CDP-Star for 5 min at room temperature. The membrane was exposed using chemiluminescence of an Alpha Innotech Imager (San Leandro, CA) and the image was analyzed with Fluorchem™ 8000 (San Leandro, CA) software. The intensity of the target band was divided by the intensity of the β-actin standard band on each gel lane to calculate a ratio that was then compared between control and implanted heifers.

Statistical analysis

Implant Heifer Trial and Implant Steer Trial: Data were analyzed using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC), with individual animal serving as the experimental unit. Performance and ultrasound data were analyzed using the repeated measures analysis of the GLM procedure of SAS with differences in treatment (C vs. SP), time on feed (0, 28, 56, 73, 105, and 133 d) and their two-way interaction tested in the model. Change in ULMA, UFT and UIMF across time-on-feed was determined by regression analysis of SAS. Data for WBSF were analyzed using the repeated measures analysis of the GLM procedure of SAS with the model including treatment, aging time (1, 3, 7, 14 and 21 d), and their two-way interaction (degree of doneness as an independent covariate). For carcass traits, fatty acid concentration, total lipid percentage and mRNA enzyme data, the model analyzed the effect of treatment. Cellularity data were analyzed with treatment, tissue (IM vs. SC) and their two-way interaction in the model. Agreement between ultrasound and carcass variables was determined using regression procedures of SAS. Least squares means were separated using the PDIFF procedure of SAS and considered significant at $P \leq 0.05$ and a trend at $P > 0.05$ to $P = 0.10$.

Dietary Lipid Supplementation Heifer Trial: Data were analyzed using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC), with individual animal serving as the experimental unit. For carcass, fatty acid concentration, total lipid percentage and mRNA enzyme data, the model analyzed the effect of treatment (CON, OIL, or SALT), dietary lipid supplementation length (32 vs. 60 d), and their two-way interaction. Cellularity data were analyzed with treatment, dietary lipid supplementation length, tissue (IM vs. SC), and all possible interactions in the model. Least squares means were separated using the PDIFF procedure of SAS and considered significant at $P \leq 0.05$ and a trend at $P > 0.05$ to $P = 0.10$.

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Table 2.1. Sense (S) and antisense (AS) primers for synthesis of acetyl CoA carboxylase (ACC), stearoyl CoA desaturase (SCD), lipoprotein lipase (LPL), and β-actin riboprobes.

Gene		Primer sequences	PCR product size (bp)
ACC	S	5′-GATGGGCGGGATGGTCTCTTTTC-3′	436
	AS	5′-CCAAGCTTCTAATACGACTCACTATAGGTAGGGCAGGCTCCAGGTGACGATA-3′	
SCD	S	5′-TTCCCGACGTGGCTTTTTCTTCT-3′	337
	AS	5′-CCAAGCTTCTAATACGACTCACTATAGGCTCTCGGGGGTTGATGGTCTTGT-3′	
LPL	S	5′-TGTGAAATGCCATGACAAGTC-3′	277
	AS	5′-CCAAGCTTCTAATACGACTCACTATAGTGTGCTATTTGGCCACTATAC-3′	
β-actin	S	5′-GTTCAACACTCCTGCCATGTAT-3′	251
	AS	5′-CCAAGCTTCTAATACGACTCACTATAGGTAGCAGAGCTTCTCCTTGATG-3′	

CHAPTER 3

THE EFFECT OF ANABOLIC IMPLANTS ON INTRAMUSCULAR LIPID DEPOSITION IN FINISHED BEEF HEIFERS[1]

[1] Smith, K.R., S.K. Duckett, M. J. Azain and T.D. Pringle. To be submitted to *the Journal of Animal Science.*

ABSTRACT This study determined the effect of anabolic implants in finished beef heifers on changes in ultrasound measurements, carcass quality, cellularity of subcutaneous (SC) and intramuscular (IM) depots, and mRNA expression of acetyl CoA carboxylase (ACC), stearoyl CoA desaturase (SCD), and lipoprotein lipase (LPL) in IM adipose tissue. Ten Angus heifers (386 kg), sired by high marbling EPD bulls, were randomly allotted as controls (C) or implanted with Synovex-Plus (SP; 28 mg estradiol benzoate and 200 mg of trenbolone acetate) at d 0 and 55. Ultrasound measurements of LM area (ULMA), fat thickness (UFT) and intramuscular lipid (UIMF) were recorded at 28 d intervals during finishing. At 108 d, all heifers were harvested. A section was removed from the longissimus muscle (6^{th} to 9^{th} rib) immediately after harvest and IM adipose tissue was dissected from the muscle for subsequent mRNA analysis. Samples of SC and IM adipose tissues were collected for cell size determination. At 48 h postmortem, carcass data were collected and a steak removed (12^{th} rib) for lipid content and fatty acid composition analysis. Ultrasound data were analyzed using the GLM procedure of SAS with treatment, time and the two-way interaction in the model. Performance, carcass, fatty acid, total lipid and mRNA data were analyzed with treatment in the model. Cellularity data were analyzed with treatment, tissue and the two-way interaction in the model. Average daily gain was 36% greater ($P < 0.01$) for SP than C. For both C and SP, ULMA increased ($P < 0.05$) over time; however, SP increased at a faster rate than C (0.31 vs 0.23 cm^2/d). Additionally, UFT and UIMF increased ($P < 0.001$) over time, but did not differ ($P > 0.05$) between treatments. Slaughter and hot carcass weights tended to be greater ($P < 0.07$) for SP than C. SP had 23% larger ($P < 0.01$) REA and 10% greater ($P < 0.05$) overall maturity scores than C. Marbling score and quality grade were similar ($P > 0.05$) between treatments. Neither total lipid nor major fatty acid concentrations (oleic, palmitic, stearic and linoleic) differed ($P > 0.05$) between C and SP. Cell diameter distribution of

SC and IM was similar ($P > 0.05$) between treatments; however, cell diameter differed ($P < 0.05$) by depot. Total cell number, average cell diameter and average cell volume was similar across treatments and tissues ($P > 0.05$). Implantation did not alter ($P > 0.05$) the mRNA levels of ACC, SCD, and LPL in IM adipose tissue. Use of anabolic implants in heifers with genetic potential to marble did not alter ultimate IM lipid content, size, fatty acid composition, or expression of key lipogenic enzymes. This study suggests implanting does not have a direct effect on IM lipid deposition.

Key Words: Beef, Implant, Marbling, Lipogenesis, Adipose Cellularity

Introduction

Anabolic implants have been extensively integrated into the management practices of the finishing phase of U.S. beef production. Anabolic implants are used to enhance economically important production traits such as average daily gain, feed efficiency and protein deposition (Samber et al., 1996; Duckett and Andrae, 2000) as well as carcass lean:fat through an increase in longissimus and carcass muscle yield (Johnson et al., 1996a,b; Roeber et al., 2000). Despite the benefit of reducing beef cattle production costs, concerns have been raised regarding the use of implants and their potential negative effects on USDA quality grade. Reductions in quality grade have reportedly been through reduced marbling score and/or advanced skeletal and lean maturity (Belk, 1992; Duckett et al., 1997), especially in implants that combine trenbolone acetate (**TBA**) and estradiol ($\mathbf{E_2}$). In contrast, other studies have shown implants to have no effect on quality grade (Gerken et al., 1995; Johnson et al., 1996a). Duckett et al. (1999) reported implanting with a combination implant reduced intramuscular lipid amount and altered fatty acid composition; however, when expressed per unit of LM area the differences were not significant. A summary of implant usage by Duckett and Andrae (2000) showed a relationship ($r^2 = 0.68$) between reductions in marbling score and increases in LM area. This suggests implantation has an indirect effect on intramuscular lipid through an increase in lean muscle mass (i.e., a dilution of IM fat in a larger LM area). However, the direct effect of implanting on lipogenesis has not been examined suggesting a need for assessing the effects of anabolic implants on intramuscular adipocyte cellularity and mRNA expression of enzymes related to lipid accretion and deposition. This information will be valuable in designing implant regimes that minimize deleterious effects on quality grade. Therefore, the objective of this study was to determine the effect of anabolic implants in finished cattle on changes in ultrasound measurements, carcass quality, cellularity of

subcutaneous (**SC**) and intramuscular (**IM**) fat depots, and IM adipose tissue mRNA expression of acetyl CoA carboxylase (**ACC**), stearoyl CoA desaturase (**SCD**), and lipoprotein lipase (**LPL**).

Materials and Methods

Animals, Diets, Ultrasound, and Experimental Procedure

Yearling Angus heifers (n = 10; 386 kg) sired by high marbling EPD bulls (marbling EPD ≥ +.3) were purchased and transported to the University of Georgia (**UGA**) Wilkins Beef Cattle Unit to evaluate the effect of implanting on intramuscular fat deposition. After an adjustment period, heifers were randomly allotted to serve as controls (**C**) or implanted with Synovex-Plus (**SP**; Fort Dodge Animal Health, Overland Park, KS; 28 mg estradiol benzoate and 200 mg of trenbolone acetate). Heifers designated for the implant group were first implanted on d 0 and re-implanted on d 55. Heifers were fed a high concentrate diet (84% concentrate, 12.5% crude protein, 1.2 NEg (Mcal/kg)) for 108 d. At 28 d intervals across time-on-feed, live weight and real-time ultrasound measurements of LM area (**ULMA**), fat thickness (**UFT**) and intramuscular lipid (**UIMF**) percentage were recorded**.** An American Association of Ultrasound Practitioners-certified technician collected images using an Aloka® 500V (Corometrics Medical Systems, Wallingford, CT) machine with an Aloka® UST-5049-3.5 (17.2-cm linear) probe. Images were interpreted using Beef Information Manager software (version 3.0; Critical Vision, Inc., Atlanta, GA).

Harvest, Carcass Traits and Sample collection

Upon completion of the finishing period, the cattle were transported to the UGA Meat Science and Technology Center, where they were harvested under federal inspection using humane procedures. Immediately (<15 min) after exsanguination, a portion of the longissimus

muscle (6th to 9th rib) was removed and IM adipose tissue was dissected, frozen in liquid nitrogen and stored at -70°C for subsequent mRNA analysis. Samples of IM and SC adipose tissue were also collected for tissue cellularity measurement. Carcasses were chilled at 4°C for 48 h, and trained UGA personnel recorded USDA quality and yield grade factors —including, hot carcass weight; adjusted fat thickness; LM area; percentage kidney, pelvic, and heart fat (**KPH**); marbling score; and skeletal and lean maturity—according to USDA standards (USDA, 1997). One steak (2.54 cm), for lipid analysis, was removed from the longissimus muscle between the 11th and 12th rib of the right side of each carcass. Each steak was trimmed of all external fat, pulverized in liquid nitrogen and stored at -20°C for subsequent analysis.

Muscle Lipid Analysis

Total lipids were extracted according to a modification of the Folch et al. (1957) method. Prior to this study, it was determined that a 10:1 ratio of sample to chloroform:methanol (2:1) was sufficient for total lipid extraction (Smith, unpublished data). This extract, containing about 50 mg of lipid, was separated into triglyceride and phospholipid fractions according to the procedure of Larick et al. (1989). Fatty acid composition of longissimus muscle samples was determined by the direct transmethylation procedure of Park and Goins (1994). Methyl esters were separated by gas chromatography (Agilent 6850; Agilent Technologies, Wilmington, DE) using a 100-m Supelco SP2560 (Supelco, Bellefonte, PA) capillary column (0.25 mm i.d. and 0.20 μm film thickness) as detailed by Duckett et al. (2002). Methyl tricosanoate (C23:0) was included as an internal standard prior to methylation to quantify fatty acids.

Adipose Tissue Cellularity

Procedures outlined by Mersmann and MacNeil (1986) were used to determine adipocyte cellularity. Duplicate samples (50 mg) of SC and IM adipose tissue were fixed in osmium

tetroxide and dissociated in urea. A Coulter Counter (Coulter Electronics, Hialeah, FL) was used to determine adipocyte number of various sizes (range of 20 to 240 μm). Due to the large number of particles sized <30 μm, only counts >30 μm were incorporated in cell number, diameter and volume calculations (Lee et al., 1994).

*Ribonuclease protection assay **(RPA)** of ACC, SCD, and LPL mRNA from bovine IM adipose tissue*

Total RNA was isolated from IM adipose tissue with the QIAzol lysis reagent (RNeasy® Lipid Tissue Midi Kit, QIAGEN Inc., Valencia, CA). The nonisotopic RPA outlined by Lee et al. (2002) was used for this experiment. Reverse-transcription (**RT**) polymerase chain reaction (**PCR**) was used to synthesize antisense biotin-labeled riboprobes. In a 25 μl volume, 2 μg of total RNA was reverse transcribed with 200 U of reverse transcriptase (Promega, Madison, WI) at 37°C for 1 hr. The cDNA synthesized was amplified using the primer pairs in which the antisense primers contained the bacteriophage T7 promoter sequence (27 bp) at the 5′ end (Kain et al., 1991). The primer sequences for ACC, SCD, and LPL were reported in Lee et al. (2002) and Bonnet et al. (2001). The primer pairs were synthesized at the Molecular Genetics Instrumentation Facilities (UGA, Athens, GA). The sequences of the sense and antisense primers used for ACC, SCD, LPL, and β-actin are reported in **Table 3.1**. The PCR reactions were completed in a 50 μL volume containing 5 μL of the RT product, 2.5 m*M* $MgCl_2$, 200 μ*M* dNTPs (Sigma Chemical, St. Louis, MO), 400 n*M* each of sense and antisense primers, and 2.5 U of Taq DNA polymerase (Promega). The thermal settings were as follows: 1 cycle at 94°C for 3 min, followed by 35 cycles at 94°C for 60 s, 58°C for 60 s, and 72°C for 60 s with a final extension at 72°C for 5 min. An *in vitro* transcription was accomplished using a MAXIscript™ labeling kit (Ambion, Inc., Austin, TX). The riboprobe reaction, in a 20 μL total volume,

contained 5 μL of PCR products, 1× buffer, T7 RNA polymerase, 0.5 m*M* each of ATP, CTP, and GTP with 0.3 m*M* of UTP, and 0.2 m*M* biotin-labeled-16-UTP (Roche, Mannheim, Germany). The reaction was executed for 1 hr at 37°C then incubated with DNase I for 15 min at 37°C. Transcripts were denatured (95°C for 3 min) and separated on 6% acrylamide/7 *M* urea denaturing polyacrylamide gels. After staining with ethidium bromide, the full-length riboprobe was excised and eluted from the gel in probe elution buffer (Ambion, Inc.) overnight at 37°C, precipitated, and quantified at 260/280 nm.

The RPA reaction was carried out using a RPA III™ kit (Ambion, Inc.). One multiprobe (ACC, SCD, LPL and β-actin standard) RPA reaction per animal was completed, as the expected product sizes, based on the probe sequence, were at least 10% different in bp length. Total RNA and 10-fold molar excess riboprobe was denatured (95°C for 3 min) and then allowed to hybridize at 56°C overnight in 10 μL of hybridization buffer. The reactions were digested with RNase and then, through a simultaneous Ambion-patented procedure, RNase was inactivated and protected RNA was precipitated.

The protected RNA was separated by electrophoresis through a 6% acrylamide/7 *M* urea denaturing polyacrylamide gel (Invitrogen) and electrophoretically (XCell *SureLock*™ Mini-Cell with XCell II™ Blot Module Kit, Invitrogen) transferred onto a positively charged nylon membrane (BrightStar-Plus™, Ambion, Inc.) with 0.5× TBE at 400 mA for 1 hr. Once the membrane was UV-crosslinked, nonisotopic detection was performed using a BrightStar™ BioDetect™ kit (Ambion, Inc.) in which the membrane was incubated with streptavidin-alkaline phosphatase for 30 min and CDP-Star for 5 min at room temperature. The membrane was exposed using chemiluminescence of an Alpha Innotech Imager (San Leandro, CA) and the

image was analyzed with Fluorchem™ 8000 (Alpha Innotech, San Leandro, CA) software. The intensity of the target band was expressed as a percentage of the β-actin standard band intensity on each gel lane to calculate a ratio that was then statistically compared for treatment effects.

Statistical analysis

Data were analyzed using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC), with individual animal serving as the experimental unit. Performance and ultrasound data were analyzed using the repeated measures analysis of the GLM procedure of SAS with differences in treatment (C vs. SP), time on feed (0, 27, 55, 80, and 108) and their two-way interaction tested in the model. Change in ULMA, UFT and UIMF across time-on-feed was determined by regression analysis of SAS. For carcass traits, fatty acid concentration, total lipid percentage and mRNA enzyme data, the model analyzed the effect of treatment. Cellularity data were analyzed with treatment, tissue (IM vs. SC) and their two-way interaction in the model. Agreement between ultrasound and carcass variables was determined using regression procedures of SAS. Least squares means were separated using the PDIFF procedure of SAS and considered significant at $P \leq 0.05$ and a trend at $P > 0.05$ to $P = 0.10$.

Results and Discussion

Implant regime

Anabolic implants are used in beef cattle to improve profitability through an enhancement of feed efficiency and carcass value. Carcass value is directly related to lean:fat and marbling deposition. Marbling has and will continue to be for some time an essential aspect of fed cattle value-based marketing. For this trial, an aggressive implant strategy was used to determine the direct effect of implants on intramuscular lipid deposition. Montgomery et al. (2003) summarized that a mild or strong combination (**TBA + E_2**) implant, as a single implant or

re-implant, enhanced feedlot performance and overall lean production by the greatest margin. An aggressive implant, Synovex-Plus, was used as it is considered a strong combination implant with the maximum concentration of trenbolone acetate and estradiol benzoate approved by FDA (Duckett et al. 1997; Montgomery et al. 2003). The most common implant scenario is a single implant at the beginning of the feeding period; however, for this study implant heifers not only received an implant at the beginning but were also re-implanted midway through the finishing phase.

Performance and ultrasound data

Weight and ultrasound measurements across time on feed are presented in **Table 3.2**. Initial weight and ultrasound measurements did not differ ($P > 0.05$) across treatment. During the first 55 d both heifer groups were similar in weight gain ($P > 0.20$). However, in the feeding period following reimplantation, implanted heifers tended to be heavier ($P = 0.07$) and ultimately had 11% greater ($P < 0.05$) final live weights than control heifers **(Table 3.3)**. Over the entire finishing period, ADG was 36% greater ($P < 0.01$) for SP than C. The increase in ADG due to implanting with a combination implant was similar to responses reported throughout the literature. In a summary of implant strategies, Duckett and Andrae (2000) reported the largest increase (19 to 20%) in ADG was for feedlot cattle implanted once or twice with a combination implant.

As expected, ULMA increased ($P < 0.05$) over time for both treatments; however, SP increased at a faster rate than C (0.31 vs 0.22 cm^2/d). Ultrsound fat thickness and UIMF increased ($P < 0.001$) over time for both treatments, with no rate of change difference ($P > 0.05$) noted between treatments. Implantation altered ($P < 0.06$) the magnitude of change in UIMF over time during finishing (**Figure 3.1**). Implanted heifers deposited 82% of their total UIMF prior to

reimplantation between d 27 to 55 ($P = 0.06$) and control heifers deposited 48% of their total UIMF between d 55 to 80 ($P < 0.05$). Ultrasound technology has been shown to be highly accurate in estimating live animal composition when done by a qualified technician. Ultrasound can be valuable tool to monitor and describe changes in carcass traits such as LMA, FT and IMF across time-on-feed to optimize management and marketing decisions (Nash et al., 2000; Greiner et al., 2003). For this study, the standard error of prediction for ultrasound verses carcass measures was 0.25 cm, 5.00 cm^2 and 0.56% for FT, LMA and IMF, respectively. These values would meet the requirements for Beef Improvement Federation certification (BIF, 1997) or American Association of Ultrasound Practitioners (R. Williams, personal communication).

Carcass attributes

Hot carcass weight tended to be greater ($P < 0.07$) for SP than C **(Table 3.3)**. Implanted heifers had 23% larger ($P < 0.01$) longissimus muscle area than control heifers. It has been reported that steers (Hermesmeyer et al., 2000) and heifers (Popp et al., 1997; Mader et al., 2000) implanted once with a mild or strong combination implant had heavier carcasses and larger longissimus muscle areas compared to non-implant steers and heifers. A review by Dolezal (1997) reported that combination implants used in yearling steers produced the greatest increase in carcass weight and ribeye area. Mader et al. (1994) also found reimplanting yearling heifers with a combination implant resulted in heavier carcass weights and increased longissimus muscle areas compared to controls. Dressing percentage, adjusted fat thickness and percentage of KPH fat did not differ ($P > 0.05$). Other studies have also shown the use of anabolic implants has little effect on subcutaneous fat depth (Herschler et al., 1995; Foutz et al., 1997) and dressing percentage (Perry et al., 1991; Johnson et al. 1996a; Scheffler et al., 2003). Herschler et al. (1995), Foutz et al. (1997), and Scheffler et al. (2003) found no difference for percentage of KPH

fat; however, others have reported implants decrease the percentage of KPH fat in cattle (Johnson et al., 1996a; Duckett et al., 1999; Roeber et al, 2000). The manner in which KPH was measured could contribute to the differences. This study and the aforementioned studies, with the exception of Scheffler et al. (2003), subjectively measured KPH percentage by trained personnel. The decrease in KPH may also be related to the increase in carcass weight, since KPH is expressed as a percentage of carcass weight. USDA yield grade was 17% lower ($P = 0.15$) for the implanted verses non-implanted heifers. In addition, 60% of the non-implanted heifers were USDA yield grade 4 while only 20% of the implanted heifers were USDA yield grade 4. Since adjusted fat thickness and other yield grade factors were similar across treatment, the decrease in yield grade appeared due to the increase in longissimus muscle area. Yield grade is influenced by longissimus muscle area in relation to carcass weight. Roeber et al. (2000) reported that both of these measures increase with anabolic implant use, thus final yield grade would not be expected to differ.

As for USDA quality grade attributes, SP had 10% greater ($P < 0.05$) overall maturity scores than C. Other studies have shown anabolic implants to advance skeletal maturity (Belk, 1992; Foutz et al., 1997). In this present study, marbling score was similar ($P > 0.05$) between implanted and non-implanted heifers. In steers and heifers, the response of marbling score to the use of a single anabolic implant has reportedly been variable. Johnson et al. (1996a) and Duckett et al. (1997) reported the use of a single combination implant did not effect marbling is steers and heifers, respectively. However, Herschler et al. (1995) and Mader (2000) documented a reduction in marbling score in steers and heifers implanted once with a mild or strong combination implant. A review by Morgan (1997) showed that steers receiving a mild or strong combination re-implant had a 26 point decrease in marbling score with 24% fewer carcasses

grading low Choice. Dolezal (1997) and Roeber et al. (2000) also noted marbling score decreases with reimplantion.

Muscle lipid characteristics

Total lipid content and triglyceride fraction percentage did not differ ($P > 0.05$) between C and SP **(Table 3.4)**. In Brangus steers implanted once, Gerken et al. (1995) observed no variation in longissimus crude fat percentages. However, Foulz et al. (1997) reported reduced crude fat percentages for combination implants. Total fatty acid percentage, composition of the major fatty acids (oleic, palmitic, stearic and linoleic) and saturated (SFA) fatty acid, monounsaturated (MUFA) fatty acid, and polyunsaturated (PUFA) fatty acid percentages were similar ($P > 0.05$) for implanted and non-implanted controls. This is in contrast to Duckett et al. (1999) who reported implanting increased stearic and linoleic acids and reduced oleic acid thus increasing SFA and reducing MUFA.

Adipose cellularity

Adipose tissue mass can increase by hyperplasia (cell proliferation) or hypertrophy (cell enlargement through lipid accumulation). The increase in adipocyte number may be due to preadipocytes filling with lipid or actual differentiation or proliferation of newly stimulated preadipocytes (Hood, 1982). In this study cell diameter distribution was similar ($P > 0.05$) between implanted and non-implanted heifers; however, cell diameter distribution differed ($P < 0.05$) by adipose depot **(Figure 3.2)**. Numerous studies have observed a hierarchy between depots in adipocyte size in which average SC diameter is greater than IM (Hood and Allen, 1973; Allen, 1976; Schiavetta et al., 1990; Mendizabal et al., 1999). Subcutaneous adipose tissue had a greater ($P < 0.05$) percentage of cells in the smallest diameter range (30 – 40 μm) and a second major population of cells in the diameter ranges >160 μm ($P < 0.05$). The a peak diameter

(the diameter with the greatest proportion of cells within that sub-population of cells) for SC fat was 170 μm. Conversely, IM adipose tissue had a greater ($P > 0.05$) percentage of cells in the middle diameter ranges (90 – 140 μm), with a peak diameter of 130 μm. These peak diameters were only 10 μm smaller than those observed by May et al. (1994) for Angus and Wagyu steers. Gilbert et al. (2003) reported peak diameters at approximately 100 μm and 150 μm for IM and SC adipose tissue, respectively. These smaller peak diameters for both tissues could be due to less fat deposition both subcutaneously and especially intramuscularly. At the cell diameter range of 160 –170 μm, there was a treatment by tissue interaction ($P < 0.05$). At this diameter range, for both C and SP, SC had a greater percentage of cells; however, SP had a much greater percentage of SC than IM cells at this range.

When adipocyte size distributions were determined for the present study, biphasic distributions were observed for both SC and IM **(Figure 3.2)** adipose tissue. As fat is deposited in cattle, a population of cells will accumulate lipid, increasing in diameter and volume. Once this population of cells attains a certain size due to hypertrophy, another population of smaller adipocytes becomes apparent demonstrating a biphasic diameter distribution (Allen, 1976). The occurrences of smaller adipocytes suggest reinitiation of hyperplasia or differentiation of preadipocytes (Allen, 1976). The exact cell size when this recruitment occurs is unknown for both SC and IM adipose tissue. Due to a discrepancy between SC and IM tissue as to when biphasic cell distribution is displayed, Allen (1976) and Schoonmaker et al. (2004) have reported that hyperplasia in SC tissue may be triggered at a larger adipocyte size compared to IM tissue.

In this study, no differences ($P > 0.05$) in average cell diameter or average cell volume **(Table 3.5)** were observed for implanted vs. non-implanted heifers nor SC vs. IM tissues. However, IM adipose tissue of implanted heifers had a 60% increase in the number of cells per

gram (P = 0.10). No data has been previously published to show the effect of implantation on adipose cellularity. Although not statistically different, implanted heifers did have numerically higher mean values for cell number and average cell volume. Marbling is conventionally thought of as a late-maturing fat depot that may not be fully developed at harvest (Hood and Allen, 1973; Cianzio et al., 1985; May et al., 1994). May et al. (1994) reported that IM adipose tissue contained more adipocytes per gram of tissue with a smaller mean cell diameter and volume relative to SC adipose tissue. Average cell diameter for SC and IM tissue did not differ statistically; however, SC tissue would be expected to have a larger average cell diameter and subsequent average cell volume. For this study, IM tissue had the numerically larger mean cell diameter but 36% of SC adipocytes were in the smallest diameter range (30 – 40 μm), which greatly impacted the mean diameter for SC.

Enzyme expression

Fat accretion is the balance between fat synthesis (lipogenesis) and breakdown (fatty acid oxidation/lipolysis). Three key enzymes involved in lipid uptake and biosynthesis for fat storage are LPL, ACC and SCD. Providing free fatty acids to adipose tissue is LPL, which catalyzes the hydrolysis of triglycerides from circulating lipoprotein particles (Auwerx et al. 1992). The rate-limiting enzyme of fatty acid synthesis or lipogenesis is ACC. In this initial biotin-dependent step, acetyl CoA is converted to malonyl-CoA, the substrate for fatty acid synthesis (Abu-Elheiga et al., 2001). The fatty acid composition of muscle and adipose tissue is greatly influenced by the regulation of SCD. The SCD enzyme catalyzes the rate-limiting step in the biosynthesis of MUFA by inserting a *cis*-double bond in the fatty acyl-CoA substrate Δ^9 position (Kim and Ntambi, 1999). The concentrations of mRNA for ACC, SCD and LPL from IM adipose tissue of implanted and non-implanted heifers were analyzed by ribonuclease protection

assay **(Figure 3.3)**. The mRNA abundance of the lipogenic enzymes (ACC, SCD, and LPL) in IM adipose tissue did not differ ($P > 0.05$) between implanted and control heifers **(Figure 3.4)**. Although not statistically different, implanted heifers had numerically higher mRNA levels for all three enzymes, as was seen with numerically higher mean values for cells per gram and average cell volume. To date, no data has been reported on the effects of implantation on mRNA expression in IM adipose tissue.

Implications

Anabolic implant enhanced animal performance and carcass protein accretion through an increase in longissimus muscle area. Implant did not alter intramuscular lipid deposition as measured by marbling score, total lipid content, fatty acid content, adipocyte cellularity or enzyme expression. Collectively, this study suggests that the use of anabolic implants does not have a direct effect on IM lipid deposition. It is also noteworthy that using a strong combination implant did not negatively affect quality grade in the cattle used in this study possibly because they had a greater than average potential to marble.

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Table 3.1. Sense (S) and antisense (AS) primers for synthesis of acetyl CoA carboxylase (ACC), stearoyl CoA desaturase (SCD), lipoprotein lipase (LPL), and β-actin riboprobes.

Gene		Primer sequences	PCR product size (bp)
ACC	S	5′-GATGGGCGGGATGGTCTCTTTTC-3′	436
	AS	5′-CCAAGCTTCTAATACGACTCACTATAGGTAGGGCAGGCTCCAGGTGACGATA-3′	
SCD	S	5′-TTCCCGACGTGGCTTTTTCTTCT-3′	337
	AS	5′-CCAAGCTTCTAATACGACTCACTATAGGCTCTCGGGGGTTGATGGTCTTGT-3′	
LPL	S	5′-TGTGAAATGCCATGACAAGTC-3′	277
	AS	5′-CCAAGCTTCTAATACGACTCACTATAGTGTGCTATTTGGCCACTATAC-3′	
β-actin	S	5′-GTTCAACACTCCTGCCATGTAT-3′	251
	AS	5′-CCAAGCTTCTAATACGACTCACTATAGGTAGCAGAGCTTCTCCTTGATG-3′	

Table 3.2. Weight and ultrasound measurements of longissimus muscle area (ULMA) and fat thickness (UFT) of control and implanted Angus heifers across time-on-feed

Measurement	d 0	d 27	d 55	d 80	d 108
			Weight, kg		
Control	405.51 ± 13.43	444.07 ± 14.50	495.96 ± 17.67	526.62 ± 17.11[a]	547.21 ± 18.59[c]
Implanted	416.85 ± 13.43	469.92 ± 14.50	525.44 ± 17.67	575.52 ± 17.11[b]	609.63 ± 18.59[d]
			ULMA, cm^2		
Control	53.74 ± 1.88[a]	61.55 ± 1.88[e]	68.32 ± 1.88[e]	76.26 ± 1.88[e]	79.55 ± 1.88[e]
Implanted	58.26 ± 1.88[b]	69.87 ± 1.88[f]	79.81 ± 1.88[f]	85.68 ± 1.88[f]	92.52 ± 1.88[f]
			UFT, cm		
Control	0.73 ± 0.18	0.87 ± 0.18	1.24 ± 0.18	1.37 ± 0.18	1.57 ± 0.18
Implanted	0.85 ± 0.18	1.08 ± 0.18	1.28 ± 0.18	1.38 ± 0.18	1.63 ± 0.18

[a,b]Within a column, means without a common superscript differ ($P < 0.10$).

[c,d]Within a column, means without a common superscript differ ($P < 0.05$).

[e,f]Within a column, means without a common superscript differ ($P < 0.01$).

Table 3.3. Performance and carcass traits of control and implanted Angus heifers

Trait	Control	Implanted	SEM	*P* > *F*
n	5	5		
ADG, kg/d	1.31[y]	1.78[z]	0.09	<0.01
Shrunk weight, kg	524.40[y]	583.30[z]	17.84	0.05
Hot carcass weight, kg	339.00	375.60	12.39	0.07
Dressing percentage	64.62	64.37	0.62	0.78
Adjusted fat thickness, cm	1.80	1.60	0.17	0.36
Longissimus muscle area, cm^2	70.84[y]	87.10[z]	2.40	<0.01
Kidney, pelvic and heart fat, %	1.90	2.10	0.29	0.64
USDA yield grade	3.98	3.30	0.31	0.16
Overall maturity[a]	162.00[z]	178.20[y]	4.92	0.05
USDA marbling score[b]	466.00	512.00	45.55	0.50

[a]Overall maturity scale: 100 = A^{00} and 200 = B^{00}.

[b]Marbling score: 400 = $Small^{00}$ and 500 = $Modest^{00}$.

[y,z]Within a row, means without a common superscript differ ($P < 0.05$).

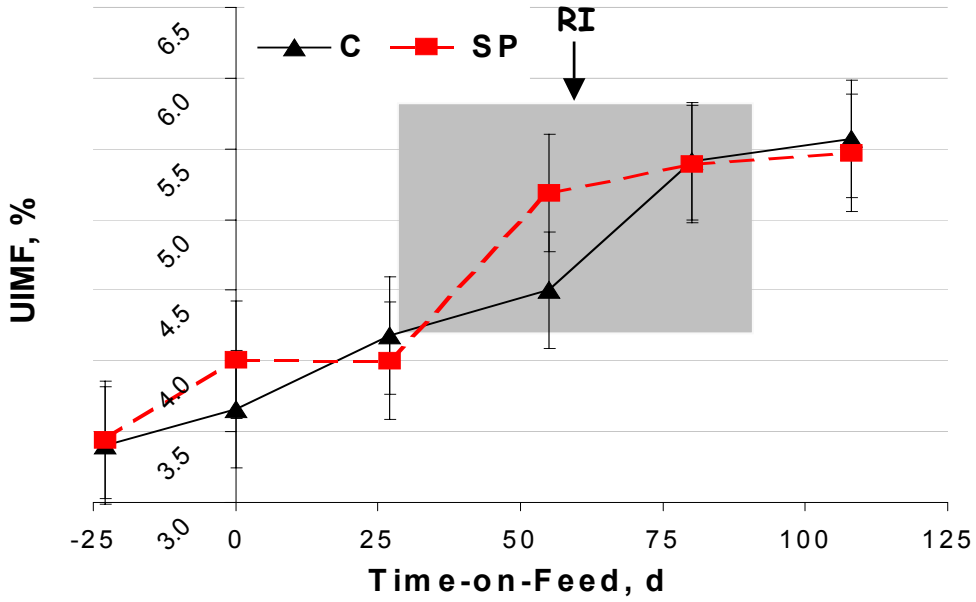

Figure 3.1. Ultrasound intramuscular fat (UIMF) percentage across time-on-feed for control (C) and implanted (SP) Angus heifers.

Table 3.4. Lipid characteristics of the longissimus muscle of control and implanted Angus heifers

Trait	Control	Implanted	SEM	*P* > *F*
n	5	5		
Total lipid content, %	6.39	6.32	0.43	0.91
Total fatty acid, %	5.82	5.73	0.55	0.90
Triglyceride fraction, %	91.63	91.37	0.01	0.87

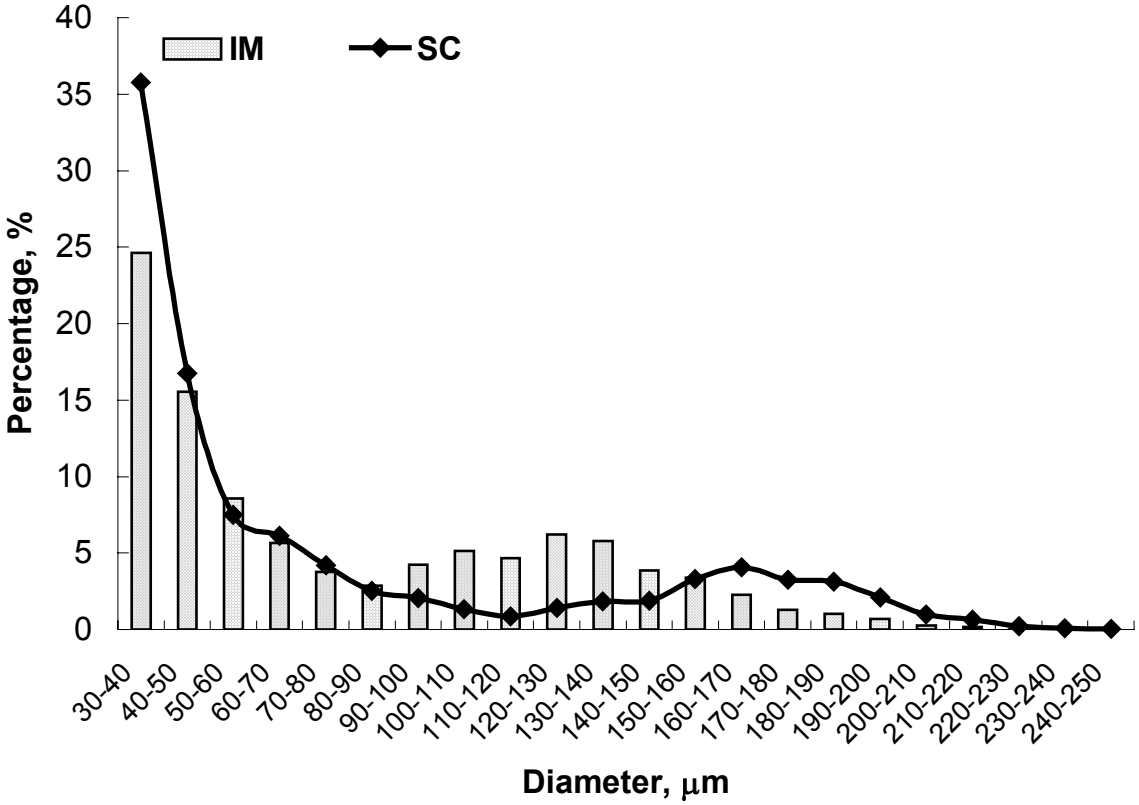

Figure 3.2. Cell diameter distribution for adipocytes from subcutaneous (SC) and intramuscular (IM) adipose tissues from Angus heifers.

Table 3.5. Adipose cellularity of intramuscular (IM) and subcutaneous (SC) tissue from control and implanted Angus heifers

	Control		Implanted			
	IM	SC	IM	SC	SEM	*P* > *F*
Number, 10^5 cells/g	6.53[y]	—	10.42[z]	—	1.49	0.10
Diameter, μm	81.99	74.75	80.50	78.59	4.51	0.56
Volume, 10^5 μm/cell	3.02	2.22	3.04	2.60	0.50	0.73

[y,z]Within a row, means without a common superscript differ ($P = 0.10$).

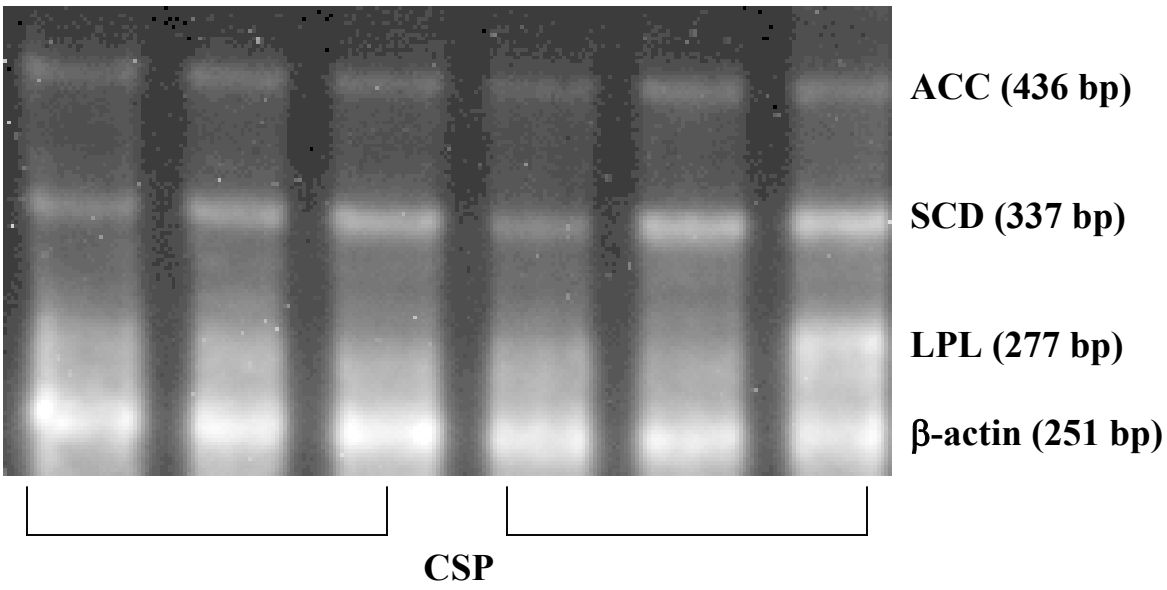

Figure 3.3. Ribonuclease protection assay (RPA) of acetyl Co-A carboxylase (ACC), stearoyl Co-A desaturase (SCD), lipoprotein lipase (LPL), and β-actin expression in intramuscular adipose tissue of control (C) and implanted (SP) Angus heifers.

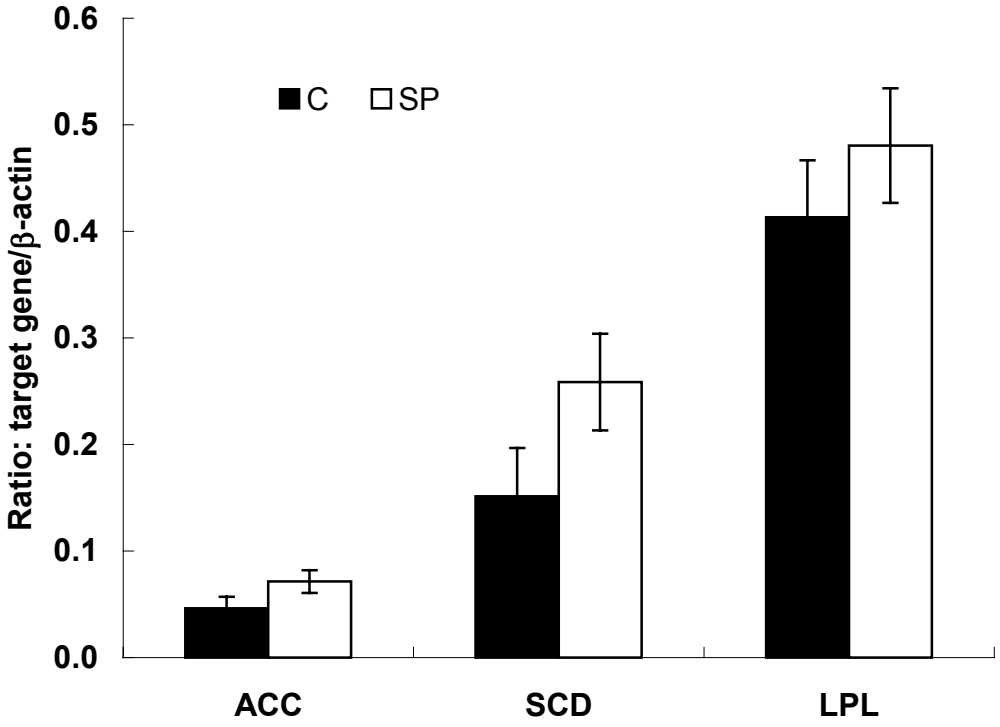

Figure 3.4. Densitomeric quantification of acetyl Co-A carboxylase (ACC), stearoyl Co-A desaturase (SCD), lipoprotein lipase (LPL) mRNA from intramuscular adipose tissue of control (C) and implanted (SP) Angus heifers. Data are expressed as means ± SE of five animals in each treatment.

CHAPTER 4

THE EFFECT OF ANABOLIC IMPLANTS ON INTRAMUSCULAR LIPID DEPOSITION IN FINISHED BEEF STEERS[1]

[1] Smith, K.R., S.K. Duckett, M. J. Azain and T.D. Pringle. To be submitted to *The Journal of Animal Science.*

ABSTRACT This study determined the effect of anabolic implants in finished cattle on carcass quality, cellularity of subcutaneous (SC) and intramuscular (IM) depots, and mRNA expression of acetyl CoA carboxylase (ACC), stearoyl CoA desaturase (SCD), and lipoprotein lipase (LPL) in IM adipose tissue. Angus steers (n=18; 279 kg), sired by high marbling EPD bulls, were allotted as controls (C) or implanted with Synovex-Plus (SP) at d 0 and 73. Weights were recorded at 28 d intervals during finishing. At 141 d, steers were harvested. A LM section was removed (6^{th} to 9^{th} rib) immediately after harvest and IM adipocytes dissected from the muscle for subsequent mRNA analysis. Samples of SC and IM adipose tissues were collected for determination of cell size. At 48 h postmortem, carcass data were collected and a steak removed (12^{th} rib) for lipid content and fatty acid composition analysis. Striploin steaks were removed and aged for Warner-Bratzler shear force (WBSF) analysis. Data were analyzed using the GLM procedures of SAS. Performance and WBSF data were analyzed using repeated measures with treatment, time on feed or aging and their two-way interaction in the model. Carcass, fatty acid, total lipid and mRNA data were analyzed with treatment in the model. Cellularity data were analyzed with treatment, tissue and the two-way interaction in the model. Final live weight was 4% greater ($P < 0.02$), ADG was 17% greater ($P < 0.01$) and hot carcass weight was 8% heavier ($P < 0.03$) for SP than C. The LM area tended to be larger ($P = 0.10$) for SP than C. Skeletal maturity was 10% greater ($P < 0.05$) for SP than C. Marbling score was similar ($P > 0.05$) between treatments. Steaks from C had lower ($P < 0.0001$) WBSF than SP and aging decreased WBSF ($P < 0.0001$); however, steaks from C and SP were <3.0 kg WBSF after only 3-d aging. Neither total lipid nor fatty acid concentration of oleic, palmitic, and linoleic differed ($P > 0.05$) across treatments; however, oleic concentration was higher ($P < 0.05$) for SP than C. Cell diameter distribution differed ($P < 0.05$) by depot. In addition, IM had more ($P < 0.0001$) cells

per gram but SC had larger ($P < 0.0001$) mean diameter and volume. Implantation did not alter ($P > 0.05$) the steady-state mRNA levels of key lipogenic enzymes (ACC, SCD, and LPL) in IM adipocytes. In this study, use of anabolic implants did not alter ultimate IM lipid content, cell size, fatty acid composition, or expression of key lipogenic enzymes. This study suggests implanting does not have a direct effect on IM lipid deposition.

Key Words: Beef, Implant, Marbling, Lipogenesis, Adipose Cellularity

Introduction

Anabolic implants have been extensively integrated into the management practices of the finishing phase of U.S. beef production as a cost effective way to enhance animal performance and protein deposition (Duckett and Andrae, 2000; Samber et al, 1996) as well as to increase carcass muscle yield (Johnson et al., 1996a,b; Roeber et al., 2000). Despite consistant increases in the return on investment, research on the impact of implantation on carcass quality and palatability varies. Intramuscular lipid content of the longissimus muscle is an essential aspect of beef palatability and fed cattle value-based marketing. Some studies have indicated a reduction in marbling score due to combination trenbolone acetate (**TBA**) and estradiol ($\mathbf{E_2}$) implant (Morgan, 1997; Roeber et al., 2000), while other studies have shown implants to have no effect on marbling score (Johnson et al., 1996a; Duckett et al., 1997). Duckett and Andrae (2000) reported a strong relationship ($r^2 = 0.68$) between the reduction in marbling score and increase in LM area. This would imply implantation has an indirect effect on intramuscular lipid by diluting a similar amount of intramuscular fat into a larger LM area. However, research does not exist on the direct effect of implanting on lipid deposition. Assessment of anabolic implant effects on intramuscular adipocyte cellularity and mRNA expression for lipogenic enzymes is needed. Recently, a trial was completed to assess the direct effect of a combination implant on these specific aspects of lipid deposition using heifers that had been backgrounded prior to being put on a feedlot diet (Smith et al., 2004). A second trial is needed to further study the effect of a combination implant on lipid deposition in younger, lighter weight steers fed for a longer period of time. The objective of this study was to examine the effect of anabolic implant in finished cattle on changes in ultrasound measurements, carcass yield and quality, cellularity of subcutaneous (**SC**) and intramuscular (**IM**) fat depots, and IM adipose tissue mRNA expression

of acetyl CoA carboxylase (**ACC**), stearoyl CoA desaturase (**SCD**), and lipoprotein lipase (**LPL**).

Materials and Methods

Animals, Diets, Ultrasound, and Experimental Procedure

Angus steers (n = 18; 279 kg) sired by high marbling EPD bulls (marbling EPD ≥ +.3) were purchased and transported to the University of Georgia (**UGA**) Wilkins Beef Cattle Unit to evaluate the effect of implanting on intramuscular fat deposition. After an adjustment period, steers were randomly allotted to serve as controls (**C**) or implanted with Synovex-Plus (**SP**; Fort Dodge Animal Health, Overland Park, KS; 28 mg estradiol benzoate and 200 mg of trenbolone acetate). Steers designated for the implant group were first implanted on d 0 and re-implanted on d 73. Integrity of the implants was monitored through out the trial by palpating the ears of the implanted steers. Steers were fed a high concentrate diet (84% concentrate, 12.5% crude protein, 1.2 NEg (Mcal/kg)) for 141 d. Across time-on-feed, live weight (0, 28, 56, 73, 105 and 133 d) and real-time ultrasound measurements (0, 28, 73, 105 and 133 d) of longissimus muscle area (**ULMA**), fat thickness (**UFT**) and intramuscular lipid (**UIMF**) percentage were recorded**.** An American Association of Ultrasound Practitioners-certified technician collected images between the 12^{th} and 13^{th} rib interface using an Aloka® 500V (Corometrics Medical Systems, Wallingford, CT) machine with an Aloka® UST-5049-3.5 (17.2-cm linear) probe. Beef Information Manager software (version 3.0;Critical Vision, Inc., Atlanta, GA) was used for image interpretation.

Harvest, Carcass Traits and Sample Collection

Cattle were transported to the UGA Meat Science and Technology Center (Athens, GA), fasted weights were recorded, and cattle were harvested under federal inspection using humane

procedures. Immediately (<15 min) after exsanguination, a portion of the longissimus muscle (6th to 9th rib) was removed and IM adipose tissue was dissected, frozen in liquid nitrogen and stored at -70°C for subsequent mRNA analysis. Samples of IM and SC adipose tissue were also collected for tissue cellularity measurements. Carcasses were chilled at 4°C for 48 h, and trained UGA personnel recorded USDA quality and yield grade factors —including, hot carcass weight; adjusted fat thickness; LM area; percentage kidney, pelvic, and heart fat (**KPH**); marbling score; and skeletal and lean maturity. Yield and quality grades were calculated according to USDA standards (USDA, 1997). One steak (2.54 cm) was removed from the longissimus muscle between the 11th and 12th rib of the right side of each carcass. Each steak was trimmed of all external fat, pulverized in liquid nitrogen and stored at -20°C for subsequent lipid analysis. Boneless strip loins (IMPS #180) were fabricated from one side of each carcass. All external fat was removed from the strip loin before slicing into steaks (2.54 cm). Steaks were vacuum-packaged and assigned to one of five aging times (1, 3, 7, 14 and 21 d). Following aging, steaks were frozen at -10°C until shear force evaluation.

Warner-Bratzler Shear

Warner-Bratzler shear force (**WBSF**) was evaluated according to AMSA (1997) guidelines. Steaks were thawed for 24 h at 4°C, cooked on one side to an internal temperature of 40°C, turned, and cooked to a final internal temperature of 71°C on a Farberware Open Hearth Broiler (Farberware, Inc., Bronx, NY). Steaks were then placed on plastic trays and allowed to equilibrate to room temperature (22°C). Six cores (1.27 cm in diameter) were removed from each steak parallel to the muscle fiber orientation. Each core was sheared once through the center, perpendicular to the muscle fiber orientation with a Warner-Bratzler shear machine (G-R Elec.

Mfg., Manhattan, KS). The six shear force values from each steak were averaged for statistical analysis.

Muscle Lipid Analysis

Total lipids were extracted according to a modification of the Folch et al. (1957) method. Prior to this study, it was determined that a 10:1 ratio of sample to chloroform:methanol (2:1) was sufficient for total lipid extraction (Smith, unpublished data). Fatty acid composition of longissimus muscle samples was determined by the direct transmethylation procedure of Park and Goins (1994). Methyl esters were separated by gas chromatography (Agilent 6850; Agilent Technologies, Wilmington, DE) using a 100-m Supelco SP2560 (Supelco, Bellefonte, PA) capillary column (0.25 mm i.d. and 0.20 μm film thickness) as detailed by Duckett et al. (2002). Methyl tricosanoate (C23:0) was included as an internal standard prior to methylation to quantify fatty acids.

Adipose Tissue Cellularity

Procedures outlined by Mersmann and MacNeil (1986) were used to determine adipocyte cellularity. Duplicate samples (50 mg) of SC and IM adipose tissue were fixed in osmium tetroxide and dissociated in urea. A Coulter Counter (Coulter Electronics, Hialeah, FL) was used to determine adipocyte number of various sizes (range of 20 to 240 μm). Due to the large number of particles sized <30 μm, only counts >30 μm were incorporated in cell number, diameter and volume calculations (Lee et al., 1994).

*Ribonuclease protection assay **(RPA)** of ACC, SCD, and LPL mRNA from bovine IM adipose tissue*

Total RNA was isolated from IM adipose tissue with the QIAzol lysis reagent (RNeasy® Lipid Tissue Midi Kit, QIAGEN Inc., Valencia, CA). The nonisotopic RPA outlined by Lee et

al. (2002) was used for this experiment. Reverse-transcription (**RT**) polymerase chain reaction (**PCR**) was used to synthesize antisense biotin-labeled riboprobes. In a 25 μl volume, 2 μg of total RNA was reverse transcribed with 200 U of reverse transcriptase (Promega, Madison, WI) at 37°C for 1 hr. The cDNA synthesized was amplified using the primer pairs in which the antisense primers contained the bacteriophage T7 promoter sequence (27 bp) at the 5′ end (Kain et al., 1991). The primer sequences for ACC, SCD, and LPL were reported in Lee et al. (2002) and Bonnet et al. (2001). The primer pairs were synthesized at the Molecular Genetics Instrumentation Facilities (UGA, Athens, GA). The sequences of the sense and antisense primers used for ACC, SCD, LPL, and β-actin are reported in **Table 4.1**. The PCR reactions were completed in a 50 μL volume containing 5 μL of the RT product, 2.5 m*M* $MgCl_2$, 200 μ*M* dNTPs (Sigma Chemical, St. Louis, MO), 400 n*M* each of sense and antisense primers, and 2.5 U of Taq DNA polymerase (Promega). The thermal settings were as follows: 1 cycle at 94°C for 3 min, followed by 35 cycles at 94°C for 60 s, 58°C for 60 s, and 72°C for 60 s with a final extension at 72°C for 5 min. An *in vitro* transcription was accomplished using a MAXIscript™ labeling kit (Ambion, Inc., Austin, TX). The riboprobe reaction, in a 20 μL total volume, contained 5 μL of PCR products, 1× buffer, T7 RNA polymerase, 0.5 m*M* each of ATP, CTP, and GTP with 0.3 m*M* of UTP, and 0.2 m*M* biotin-labeled-16-UTP (Roche, Mannheim, Germany). The reaction was executed for 1 hr at 37°C then incubated with DNase I for 15 min at 37°C. Transcripts were denatured (95°C for 3 min) and separated on 6% acrylamide/7 *M* urea denaturing polyacrylamide gels. After staining with ethidium bromide, the full-length riboprobe was excised and eluted from the gel in probe elution buffer (Ambion, Inc.) overnight at 37°C, precipitated, and quantified at 260/280 nm.

The RPA reaction was carried out using a RPA III™ kit (Ambion, Inc.). One multiprobe (ACC, SCD, LPL and β-actin standard) RPA reaction per animal was completed, as the expected product sizes, based on the probe sequence, were at least 10% different in bp length. Total RNA and 10-fold molar excess riboprobe was denatured (95°C for 3 min) and then allowed to hybridize at 56°C overnight in 10 μL of hybridization buffer. The reactions were digested with RNase and then, through a simultaneous Ambion-patented procedure, RNase was inactivated and protected RNA was precipitated.

The protected RNA was separated by electrophoresis through a 6% acrylamide/7 *M* urea denaturing polyacrylamide gel (Invitrogen) and electrophoretically (XCell *SureLock*™ Mini-Cell with XCell II™ Blot Module Kit, Invitrogen) transferred onto a positively charged nylon membrane (BrightStar-Plus™, Ambion, Inc.) with 0.5× TBE at 400 mA for 1 hr. Once the membrane was UV-crosslinked, nonisotopic detection was performed using a BrightStar™ BioDetect™ kit (Ambion, Inc.) in which the membrane was incubated with streptavidin-alkaline phosphatase for 30 min and CDP-Star for 5 min at room temperature. The membrane was exposed using chemiluminescence of an Alpha Innotech Imager (San Leandro, CA) and the image was analyzed with Fluorchem™ 8000 (Alpha Innotech, San Leandro, CA) software. The intensity of the target band was expressed as a percentage of the β-actin standard band intensity on each gel lane to calculate a ratio that was then statistically compared for treatment effects.

Statistical analysis

Data were analyzed using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC), with individual animal serving as the experimental unit. Performance and ultrasound data were analyzed using the repeated measures analysis of the GLM procedure of SAS with differences in

treatment (C vs. SP), time on feed (0, 28, 56, 73, 105, and 133 d) and their two-way interaction tested in the model. Change in ULMA, UFT and UIMF across time-on-feed was determined by regression analysis of SAS. Data for WBSF were analyzed using the repeated measures analysis of the GLM procedure of SAS with the model including treatment, aging time (1, 3, 7, 14 and 21 d), and their two-way interaction (degree of doneness as independent covariate). For carcass, fatty acid concentration, total lipid percentage and enzyme mRNA data, the model analyzed the effect of treatment. Cellularity data were analyzed with treatment, tissue (IM vs. SC) and their two-way interaction in the model. Agreement between ultrasound and carcass variables was determined using regression procedures of SAS. Least squares means were separated using the PDIFF procedure of SAS and considered significant at $P \leq 0.05$ and a trend at $P > 0.05$ to $P = 0.10$.

Results and Discussion

Implant regime

Anabolic implants are used to increase revenue of beef cattle production through an enhancement of both feed efficiency and carcass value. Carcass value is directly related to lean:fat and marbling deposition. Marbling has and will continue to be for some time an essential aspect of fed cattle value-based marketing. An aggressive implant strategy was utilized in this trial to investigate the direct effect of implants on intramuscular lipid deposition. Montgomery et al. (2003) summarized that a mild or strong combination (**TBA + E_2**) implant, as a single implant or re-implant, enhanced feedlot performance and overall lean production by the greatest margin. Synovex-Plus was used as it is considered a strong combination implant with the maximum concentration of trenbolone acetate and estradiol benzoate approved by FDA (Duckett et al. 1997; Montgomery et al. 2003). The most common implant scenario is a single implant at the

beginning of the feeding period; however, for this study implanted steers not only received an implant at the beginning but were also re-implanted midway through the finishing phase with the same implant.

Performance and ultrasound data

Weight and ultrasound measurements across time on feed are presented in **Table 4.2**. Initial weight and ultrasound measurements did not differ ($P > 0.05$) across treatment. After the first 73 d both steer groups were similar in weight gain ($P > 0.20$). However, in the feeding period immediately following reimplantation, implanted steers were heavier ($P < 0.05$) and ultimately had 4% greater ($P < 0.02$) shrunk final live weights than control steers **(Table 4.3)**. Johnson et al. (1996a) reported that combination implants improved feedlot performance and stimulated carcass protein accretion in feedlot steers and that by far the most rapid carcass protein gains were observed during the first 40 d after implanting with a combination implant. Implanted steers tended to have 11% higher ($P = 0.10$) ADG during d0 to 73 than control steers and following reimplantation (d73 to 133), ADG was 27% greater ($P < 0.02$) for SP steers compared to controls. Cumulative ADG was 17% greater ($P < 0.01$) for SP than C. The increase in ADG due to implanting with a combination implant was similar to responses reported throughout the literature. In a summary of implant strategies, Duckett and Andrae (2000) reported the largest increase (19 to 20%) in ADG was for feedlot cattle implanted once or twice with a combination implant.

As expected, ultrasound measurements of ULMA, UFT and UIMF increased ($P < 0.0001$) across time on feed for implanted and non-implanted steers; however, the rate of change did not differ ($P > 0.05$) between treatments. During the finishing period, UFT increased linearly for control steers (FT cm = 0.3583 + 0.0069 × time on feed, d; $r^2 = 0.7278$) and for implanted

steers (FT cm = 0.2987 + 0.0073 × time on feed, d; r^2 = 0.8231). Accretion of ULMA occurred linearly over time for control (LMA cm^2 = 47.7921 + 0.2148 × time on feed, d; r^2 = 0.8485) and implanted (LMA cm^2 = 45.8986 + 0.2445 × time on feed, d; r^2 = 0.7936) steers. Percentage of UIMF also increased over time on feed for control (IMF % = 3.2429 + 0.0163 × time on feed, d; r^2 = 0.4885) and implanted (IMF % = 3.6828 + 0.0154 × time on feed, d; r^2 = 0.4098) steers. Ultrasound technology has been shown to be highly accurate in estimating live animal composition when done by a qualified technician. Ultrasound can be valuable tool to monitor and describe changes in carcass traits such as LMA, FT and IMF across time-on-feed to optimize management and marketing decisions (Nash et al., 2000; Greiner et al., 2003). For this study, the standard error of prediction for ultrasound verses carcass measures was 0.13 cm, 4.98 cm^2 and 1.03% for FT, REA and IMF, respectively. These values would meet the requirements for Beef Improvement Federation certification (BIF, 1997) or American Association of Ultrasound Practitioners (R. Williams, personal communication).

Carcass attributes

Hot carcass weight was 8% greater ($P < 0.03$) for SP than C and SP steers tended to have larger ($P = 0.10$) LM areas than C steers (**Table 4.3**). Reports have shown that steers (Hermesmeyer et al., 2000) and heifers (Popp et al., 1997; Mader et al., 2000) implanted once with a mild or strong combination implant had heavier carcasses and larger longissimus muscle areas compared to non-implant steers and heifers. A review by Dolezal (1997) showed in yearling steers combination implants, used initially and as a reimplant, produced the greatest increase in carcass weight and ribeye area. Although implantation increased longissimus muscle area by 7% for this study, longissimus muscle area was not enlarged as extensively as in past research (Smith et al., 2004). Dressing percentage was similar ($P = 0.90$) between treatments.

Other yield grade factors—adjusted fat thickness and percentage of KPH—as well as final USDA yield grade did not differ ($P > 0.30$) between implanted and control steers. Other studies have also shown the use of anabolic implants has little effect on subcutaneous fat depth (Herschler et al., 1995; Foutz et al., 1997). Yield grade is influenced by longissimus area in relation to carcass weight. Roeber et al. (2000) found that anabolic implants, increased both of these measures and thus final yield grade would not be expected to change.

As for USDA quality grade attributes in this study, the implanted group had 10% greater ($P < 0.01$) skeletal maturity scores than the control group and marbling score was similar ($P = 0.90$) between treatments. This is consistent with findings from feedlot heifers of similar genotype (Smith et al., 2004). Other studies have also shown anabolic implants to advance skeletal maturity (Belk, 1992; Foutz et al., 1997). In steers and heifers, the response of marbling scores to implantation has been variable with a single anabolic implant. Johnson et al. (1996a) and Duckett et al. (1997) reported the use of a combination implant did not effect marbling is steers and heifers, respectively. However, Herschler et al. (1995) and Mader (2000) documented a reduction in marbling score in steers and heifers implanted once with a mild or strong combination implant. A review by Morgan (1997) of data from feedlot steers receiving a mild or strong combination re-implant, showed a decrease in marbling score of 26 points and 24% fewer carcasses grading low Choice compared to controls. Dolezal (1997) and Roeber et al. (2000) also noted marbling score decreased with reimplantion. Using a strong combination implant did not negatively affect quality grade in the cattle used in this study possibly because they had a greater than average potential to marble. This result is consistent with findings from feedlot heifers of similar genotype (Smith et al., 2004).

Warner-Bratzler Shear Force

Steaks from control steers had lower ($P < 0.0001$) WBSF values than steaks from implanted steers (2.16 vs. 2.56 kg). Research is conclusive that anabolic implants enhance beef cattle production efficiency; however, studies on the effects of implants on tenderness have revealed varied results. Some studies have shown implants containing trenbolone acetate and estradiol (androgenic and combination) to have no effect on beef tenderness of strip loin steaks (Belk and Savell, 1992; Gerken et al., 1995; Barham et al., 2003; Kerth et al. 2003). While other research has reported implanting beef cattle increased WBSF and decreased tenderness (Samber et al., 1996; Morgan, 1997; Platter et al., 2003). Foutz et al. (1997) determined steers implanted twice with trenbolone acetate implants would more likely yield tough steaks as compared to steers implanted once with a trenbolone acetate implant or two estradiol implants. Morgan (1997) reported, regardless of aging time, top loin steaks from "aggressively" implanted cattle were tougher than steaks from non-implanted or "conservatively" implanted cattle and steaks from implanted cattle had on average 0.5 kg higher shear force values than non-implanted cattle. In this study, steaks from implanted steers had higher shear force values than steaks from non-implanted cattle, the values on average were 0.4 kg higher and but only 0.2 kg higher after 14 d of aging.

As aging increased, WBSF values decreased ($P < 0.0001$; **Table 4.4**). The initial WBSF values at d 1 were higher ($P < 0.001$) than all other aging values. The WBSF values for 3-d aging were similar ($P = 0.50$) to WBSF values for 7-d aging; however, aging steaks at least 14 d decreased WBSF values ($P < 0.005$). Aging past d 14 did not decrease ($P = 0.99$) WBSF values. Control steers started with a d 1 WBSF value of 2.67 kg and decreased to a d 21 value of 1.88 kg, while the initial d 1 WBSF value of the implanted steers was 3.26 kg and declined to 2.08 kg

at d 21. The decrease in WBSF values, or improvement in tenderness over time, is consistent with the natural proteolytic aging process, and suggests implanted as well as non-implanted cattle benefit from aging. Miller et al. (2001) reported a 19-d aging period should be sufficient for steaks from implanted and non-implanted cattle to reach tenderness levels acceptable for 93 to 98% of consumers. Miller et al. (2001) also specified consumer satisfaction would be 93% when WBSF values were between 3.0 and 4.3 kg. In the present study, steaks from both the implanted and non-implanted steers would have been acceptable for a 100% guaranteed tender product (<3.0 kg WBSF) after only 3-d aging period.

Muscle lipid characteristics

Total lipid content did not differ ($P > 0.05$; **Table 4.4**) between implanted and non-implanted steers. In Brangus steers implanted once, Gerken et al. (1995) observed no variation in longissimus crude fat percentages. However, Foutz et al. (1997) reported that combination implants reduced crude fat percentages. Six fatty acids make up over 92% of the total fatty acid content (Duckett et al., 1993). The six major fatty acids of beef intramuscular lipid are (in order of contribution): oleic (C18:1), palmitic (C16:0), stearic (C18:0), linoleic (C18:2), plamitoleic (C16:1) and myristic (C14:0). Neither total lipid nor fatty acid concentration of oleic, palmitic, and linoleic differed ($P > 0.05$) across treatments; however, oleic concentration was higher ($P < 0.05$) for SP than C. In a previous study with feedlot heifers of similar genotype (Smith et al., 2004), the major fatty acid concentrations were not altered by implantation. However, Duckett et al. (1999) reported implanting increased stearic and linoleic acids and reduced oleic acid thus increasing SFA and reducing MUFA.

Adipose Cellularity

Adipose tissue can enlarge by hyperplasia (cell proliferation) or hypertrophy (cell enlargement through lipid accumulation). The increase in adipocyte number may be due to preadipocytes filling with lipid or actual differentiation or proliferation of newly stimulated preadipocytes (Hood, 1982). In this study cell diameter distribution was similar ($P > 0.05$) between implanted and non-implanted steers; however, cell diameter distribution differed ($P < 0.05$) by adipose depot **(Figure 4.1)**. Numerous studies have observed a hierarchy between depots in adipocyte size in which SC is greater in size than IM (Hood and Allen, 1973; Allen, 1976; Schiavetta et al., 1990; Mendizabal et al., 1999). Subcutaneous adipose tissue had a greater ($P < 0.05$) percentage of cells in the large diameter ranges (>140 μm), with a peak diameter (the diameter with the greatest proportion of cells within that sub-population of cells) of 160 μm. Intramuscular adipose tissue had a greater ($P > 0.05$) percentage of cells in the middle diameter ranges (50 – 120 μm), with a peak diameter of 110 μm. Gilbert et al. (2003) reported similar peak diameters of approximately 100 and 150 μm for IM and SC adipose tissue, respectively. May et al. (1994) used Angus and Wagyu steers and reported peak diameters of 140 and 180 μm for IM and SC adipose tissue, respectively. The peak diameters reported by May et al. (1994) were approximately 30 μm larger than the peak diameters shown in this study and in Gilbert et al. (2003), which is consistent with the greater age and adiposity of the cattle in the May et al. (1994) study.

When adipocyte size distributions were determined for the present study, biphasic distributions were observed for both SC and IM **(Figure 4.1)** adipose tissue. As fat is deposited in cattle, a population of cells will accumulate lipid, increasing in diameter and volume. Once this population of cells attains a certain size due to hypertrophy, another population of smaller

adipocytes becomes apparent demonstrating a biphasic diameter distribution (Allen, 1976). The occurrences of smaller adipocytes suggest reinitiation of hyperplasia or differentiation of preadipocytes (Allen, 1976). The exact cell size of when this recruitment occurs is unknown for both SC and IM adipose tissue. Due to a discrepancy between SC and IM tissue as to when biphasic cell distribution is displayed, Allen (1976) and Schoonmaker et al. (2004) have reported that hyperplasia in SC tissue may be triggered at a larger adipocyte size compared to IM tissue.

In this study, no differences ($P > 0.05$) were observed in average cell diameter or average cell volume **(Table 4.5)** for implanted verses non-implanted steers; however, implanted steers tended ($P = 0.08$) to have more cells per gram compared to control steers. No data has been previously published to show the effect of implantation on adipose cellularity. When comparing adipose tissue depots, IM tissue had more ($P < 0.0001$) cells per gram but SC tissue had larger ($P < 0.0001$) average diameter and volume. Marbling is conventionally thought of as a late-maturing fat depot that is not fully developed at harvest (Hood and Allen, 1973; Cianzio et al., 1985; May et al., 1994). May et al. (1994) reported IM adipose tissue, contained more adipocytes per gram of tissue with a smaller mean cell diameter and volume relative to SC adipose tissue.

Enzyme expression

Fat accretion is the balance between fat synthesis (lipogenesis) and breakdown (fatty acid oxidation/lipolysis). Three key enzymes involved in lipid uptake and biosynthesis for fat storage are LPL, ACC and SCD. Providing free fatty acids to adipose tissue is LPL, which catalyzes the hydrolysis of triglycerides from circulating lipoprotein particles (Auwerx et al. 1992). The rate-limiting enzyme of fatty acid synthesis or lipogenesis is ACC. In this initial biotin-dependent step, acetyl CoA is converted to malonyl-CoA, the substrate for fatty acid synthesis (Abu-Elheiga et al., 2001). The fatty acid composition of muscle and adipose tissue is greatly

influenced by the regulation of SCD. The SCD enzyme catalyzes the rate-limiting step in the biosynthesis of MUFA by inserting a *cis*-double bond in the fatty acyl-CoA substrate Δ9 position (Kim and Ntambi, 1999). The concentrations of mRNA for ACC, SCD and LPL from IM adipose tissue of implanted and non-implanted heifers were analyzed by ribonuclease protection assay **(Figure 4.2)**. The mRNA abundance of ACC, SCD, and LPL enzymes in IM adipose tissue did not differ ($P > 0.05$) between implanted and control steers **(Figure 4.3)**. Although not statistically different, implanted steers had numerically higher mRNA levels for all three enzymes. To date, no data has been reported on the effects of implantation on mRNA expression of IM adipose tissue other than previous work from our laboratory. In a past study, mRNA levels of ACC, SCD, and LPL in IM adipose tissue were also not altered by implantation in genetically similar feedlot heifers (Smith et al., 2004).

Implications

Anabolic implant enhanced animal performance and carcass protein accretion evidenced by an increase in longissimus muscle area. However, implanting did not alter intramuscular lipid deposition as measured by marbling score, total lipid content, fatty acid content, adipocyte cellularity or enzyme expression. Thus, this study suggests that the use of anabolic implants does not have a direct effect on IM lipid deposition. Also, it is notable that using a strong combination implant did not negatively affect quality grade in the cattle used in this study perhaps as they had a greater than average potential to marble. Further research is needed to fully understand the mode of action of anabolic implants on protein accretion and lipid metabolism.

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Table 4.1. Sense (S) and antisense (AS) primers for synthesis of acetyl CoA carboxylase (ACC), stearoyl CoA desaturase (SCD), lipoprotein lipase (LPL), and β-actin,riboprobes.

Gene		Primer sequences	PCR product size (bp)
ACC	S	5′-GATGGGCGGGATGGTCTCTTTTC-3′	436
	AS	5′-CCAAGCTTCTAATACGACTCACTATAGGTAGGGCAGGCTCCAGGTGACGATA-3′	
SCD	S	5′-TTCCCGACGTGGCTTTTTCTTCT-3′	337
	AS	5′-CCAAGCTTCTAATACGACTCACTATAGGCTCTCGGGGGTTGATGGTCTTGT-3′	
LPL	S	5′-TGTGAAATGCCATGACAAGTC-3′	277
	AS	5′-CCAAGCTTCTAATACGACTCACTATAGTGTGCTATTTGGCCACTATAC-3′	
β-actin	S	5′-GTTCAACACTCCTGCCATGTAT-3′	251
	AS	5′-CCAAGCTTCTAATACGACTCACTATAGGTAGCAGAGCTTCTCCTTGATG-3′	

Table 4.2. Weight and ultrasound measurements of longissimus muscle area (ULMA), fat thickness (UFT), and intramuscular fat (UIMF) of control and implanted Angus steers across time-on-feed

Measurement	d 0	d 28	d 57	d 73	d 105	d 133
			Weight, kg			
Control	282.74 ± 3.92	341.00 ± 7.87	390.24 ± 8.14	417.05 ± 7.92	463.87 ± 8.87[a]	486.86 ± 10.11[c]
Implanted	275.73 ± 3.92	338.88 ± 7.87	395.79 ± 8.14	425.17 ± 7.92	490.84 ± 8.87[b]	513.57 ± 10.11[d]
			ULMA, cm^2			
Control	46.45 ± 1.87	54.65 ± 1.87	61.03 ± 1.87	—	70.97 ± 1.87	75.29 ± 1.87
Implanted	44.84 ± 1.87	52.77 ± 1.87	61.68 ± 1.87	—	71.48 ± 1.87	77.68 ± 1.87
			UFT, cm			
Control	0.35 ± 0.08	0.54 ± 0.08	0.78 ± 0.08	—	1.11 ± 0.08	1.24 ± 0.08
Implanted	0.30 ± 0.08	0.51 ± 0.08	0.71 ± 0.08	—	1.05 ± 0.08	1.27 ± 0.08
			UIMF, %			
Control	3.39 ± 0.30	3.45 ± 0.30	4.16 ± 0.30	—	5.19 ± 0.30	5.28 ± 0.30
Implanted	3.85 ± 0.30	4.11 ± 0.30	4.23 ± 0.30	—	5.42 ± 0.30	5.79 ± 0.30

[a,b]Within a column, means without a common superscript differ ($P < 0.05$).

[c,d]Within a column, means without a common superscript differ ($P = 0.08$).

Table 4.3. Performance and carcass traits of control and implanted Angus steers

Trait	Control	Implanted	SEM	*P* > *F*
n	9	9		
ADG, kg/d	1.53[y]	1.79[z]	0.06	0.01
Shrunk weight, kg	494.31[y]	515.96[z]	11.27	0.02
Hot carcass weight, kg	299.12[y]	322.45[z]	6.81	0.03
Dressing percentage	63.05	62.97	0.47	0.90
Adjusted fat thickness, cm	1.35	1.40	0.06	0.64
Longissimus muscle area, cm^2	68.60	73.15	1.85	0.10
Kidney, pelvic and heart fat, %	2.67	2.44	0.15	0.31
USDA yield grade	3.48	3.46	0.10	0.92
Skeletal maturity[a]	144.44[y]	158.89[z]	3.64	0.01
USDA marbling score[b]	428.89	423.33	37.63	0.92

[a]100 = A^{00} and 200 = B^{00}.

[b]400 = $Small^{00}$ and 500 = $Modest^{00}$.

[y,z]Within a row, means without a common superscript differ ($P < 0.05$).

Table 4.4. Lipid and shear force characteristics of the longissimus muscle of control and implanted Angus steers

Trait	Control	Implanted	SEM	*P* > *F*
n	9	9		
Total lipid content, %	5.47	5.37	0.65	0.92
Total fatty acid, %	4.13	4.44	0.59	0.72
WBSF d1, kg	2.67[y]	3.26[z]	0.14	<0.01
WBSF d3, kg	2.31	2.67	0.14	0.07
WBSF d7, kg	2.09[y]	2.69[z]	0.14	<0.01
WBSF d14, kg	1.87	2.09	0.14	0.29
WBSF d21, kg	1.88	2.08	0.14	0.34

[y,z]Within a row, means without a common superscript differ ($P < 0.05$).

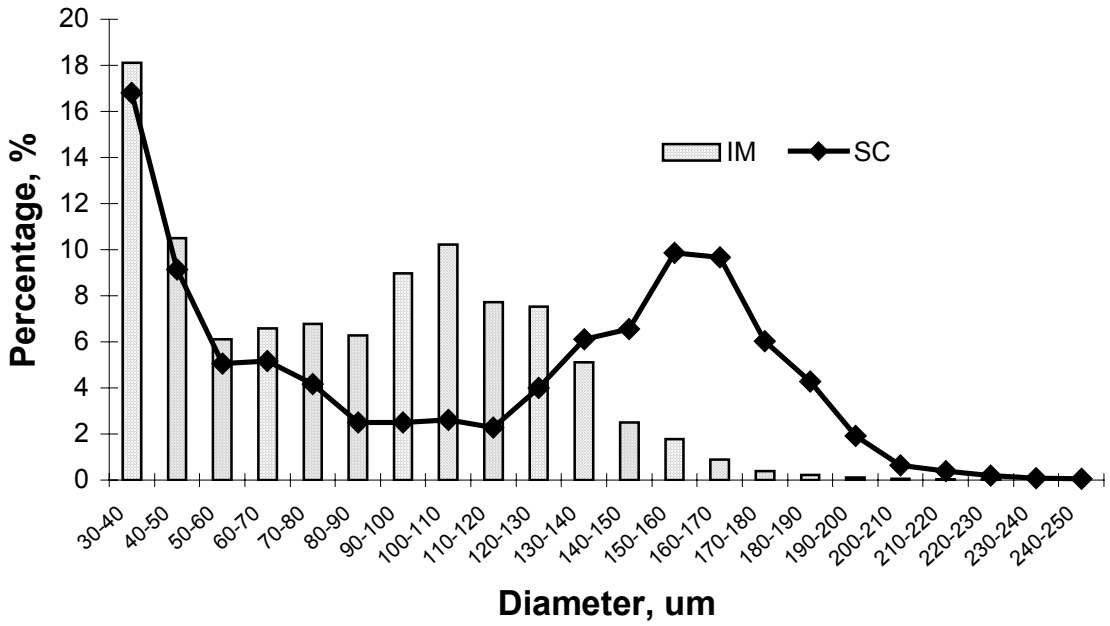

Figure 4.1. Cell diameter distribution for adipocytes from subcutaneous (SC) and intramuscular (IM) adipose tissues from Angus steers.

Table 4.5. Adipose cellularity of intramuscular (IM) and subcutaneous (SC) tissue from control and implanted Angus steers

	Control		Implanted			
	IM	SC	IM	SC	SEM	*P* > *F*
Number, 10^5 cells/g	5.70[a]	2.91[b]	6.67[a]	3.81[b]	0.52	0.94
Diameter, μm	84.11[a]	107.66[b]	85.18[a]	104.99[b]	2.16	0.39
Volume, 10^5 μm/cell	3.18[a]	6.66[b]	3.33[a]	6.14[b]	0.31	0.28

[a,b]Within a row, means without a common superscript differ ($P < 0.05$).

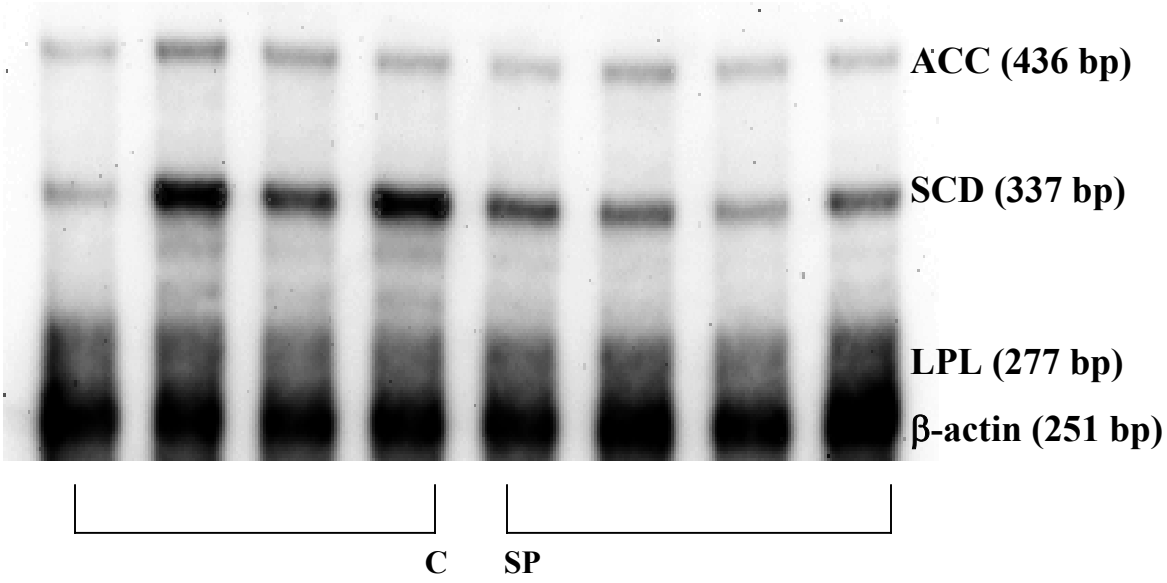

Figure 4.2. Ribonuclease protection assay (RPA) of acetyl Co-A carboxylase (ACC), stearoyl Co-A desaturase (SCD), lipoprotein lipase (LPL), and β-actin expression in intramuscular adipose tissue of control (C) and implanted (SP) Angus steers.

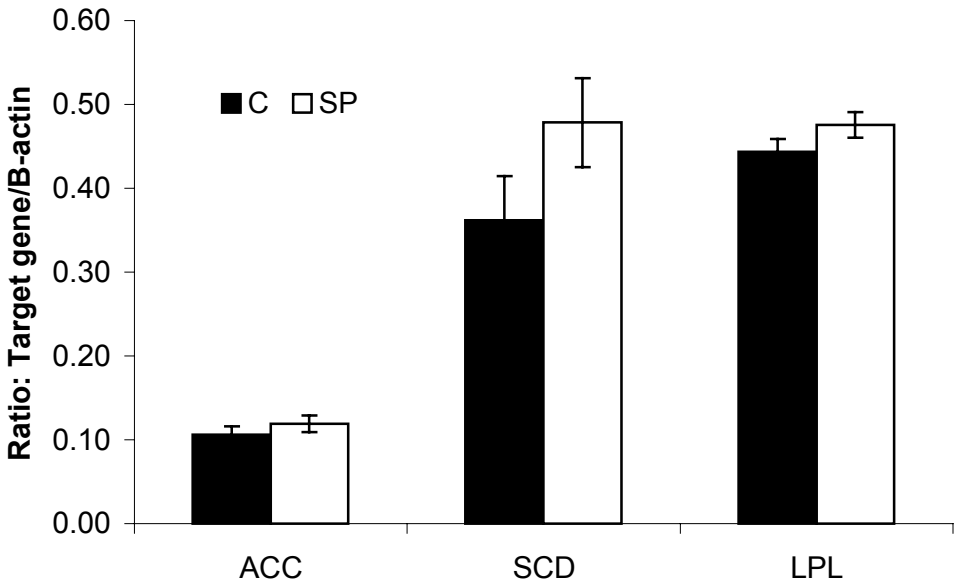

Figure 4.3. Densitomeric quantification of acetyl Co-A carboxylase (ACC), stearoyl Co-A desaturase (SCD), and lipoprotein lipase (LPL) mRNA from intramuscular adipose tissue of control (C) and implanted (SP) Angus steers. Data are expressed as means ± SE of five animals in each treatment.

CHAPTER 5

THE EFFECT OF SUPPLEMENTAL CORN OIL OR RUMEN-PROTECTED CONJUGATED LINOLEIC ACID ON LIPID DEPOSITION OF FINISHED BEEF CATTLE[1]

[1] Smith, K.R., S.K. Duckett, M. J. Azain , J.R. Sackmann, M.H. Gillis, and T.D. Pringle. To be submitted to *The Journal of Animal Science.*

ABSTRACT This study determined the effect of dietary lipid source in finished cattle on carcass quality, cellularity of subcutaneous (SC) and intramuscular (IM) adipose depots, and mRNA expression of acetyl CoA carboxylase (ACC), stearoyl CoA desaturase (SCD), and lipoprotein lipase (LPL) in IM adipose tissue. Angus-crossed heifers (n=36; 365 kg) were fed a basal diet for an initial 56 d feeding period, then were allotted to 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 (SALT), containing 31% CLA-60. Six heifers per treatment (n=18) were harvested subsequent to a feeding period of either 32 d or 60 d. A LM section was removed (6^{th} to 9^{th} rib) immediately after harvest and IM adipose tissue dissected from the muscle for subsequent mRNA analysis. Samples of SC and IM tissues were collected for determination of cell size. At 48 h postmortem, carcass data were collected and a steak removed (12^{th} rib) for lipid content and fatty acid composition analysis by GLC. Carcass, fatty acid concentration, total lipid percentage and enzyme mRNA data, were analyzed with treatment, time on treatment, and their two-way interaction. Cellularity data were analyzed with treatment, time on treatment, tissue, and all possible interactions in the model. Total lipid content of the longissimus did not differ ($P > 0.05$) by treatment or time. Cell diameter distribution was similar ($P > 0.05$) among dietary treatments but differed ($P < 0.05$) by depot. No differences ($P > 0.05$) were observed in mean cell diameter or cells per gram for treatment or tissue. Mean volume was similar ($P > 0.05$) among dietary treatment; however, SC had a larger ($P < 0.05$) mean volume than IM. The mRNA expression of ACC, SCD and LPL was similar ($P > 0.50$) among diets. The mRNA expression of ACC and LPL did not differ ($P > 0.50$) by time on treatment; however, SCD expression was higher ($P = 0.06$) for 32 d vs 60 d.

Lipid supplementation to feedlot diets increased CLA content but did not alter total lipid, adipose cellularity or enzyme mRNA expression.

Key words: Beef, CLA, Lipogenesis, Adipose Cellularity

Introduction

Intramuscular lipid content of the longissimus muscle is an essential aspect of beef palatability and fed cattle value-based marketing. Beef lipid contains unique fatty acid intermediates, collectively termed conjugated linoleic acid (**CLA**) and *trans*-vaccenic acid, produced during ruminal biohydrogenation of dietary unsaturated lipids to saturated products (Bauman et al., 1999). Conjugated linoleic acid refers to positional and geometric isomers of linoleic acid. Two prominent isomers have been shown to have health benefits in the form of either anticarcinogenicity (*cis*-9, *trans*-11 CLA; Ha et al., 1987) or antiobesity (*trans*-10, *cis*-12 CLA; Wiegand et al., 2001). Ruminant fat in milk and meat products are among the leading natural sources of CLA (Chin et al., 1992; Ritzenthaler et al., 2001). Dairy cattle research has shown vegetable oil or CLA salt supplementation to increase the *cis*-9, *trans*-11 CLA isomer in milk fat (Kelly et al., 1998). Chouinard et al. (1999) and Baumgard et al. (2001) reported dietary CLA changed lipid metabolism and reduced milk fat synthesis. Altering intramuscular fatty acid composition, by enhancing unsaturated fatty acid content and CLA, could be beneficial to the beef industry and human health. Modification of beef intramuscular lipid composition is limited by ruminal biohydrogenation as research indicates only small to moderate changes in intramuscular lipid composition occur due to nutritional effects. However, the direct effect of lipid source on lipogenesis and lipolysis has not been examined, nor has the assessment of supplemental lipid on intramuscular adipocyte cellularity. Thus, the objective of this study was to determine the effect of supplemental corn oil, composed mostly of linoleic acid, or rumen-protected CLA on carcass quality, cellularity of subcutaneous (**SC**) and intramuscular (**IM**) fat depots, and IM adipose tissue mRNA expression of acetyl CoA carboxylase (**ACC**), stearoyl CoA desaturase (**SCD**), and lipoprotein lipase (**LPL**) in finished beef cattle.

Materials and Methods

Animals and Diets

Thirty-six Angus × Hereford heifers (365 kg) obtained from the Northwest Georgia Experiment Station (Calhoun, GA) were used in a completely randomized design to determine the effect of dietary lipid sources on lipid deposition of feedlot cattle. Heifers were fed a basal diet for an initial 56 d feeding period and then were allotted to one of three dietary treatments for the last 32 or 60 d before harvest: 1) basal diet containing 88% concentrate and 12% grass hay (**CON**); 2) basal diet plus 4% corn oil (**OIL**); or 3) basal diet plus 2% rumen-protected CLA salt (**SALT**), containing 31% CLA-60. Treatment effects were evaluated in a 3 × 2 factorial arrangement with three diets (CON, OIL, or SALT) and two feeding period lengths (32 or 60 d).

The rumen-protected CLA supplement, generously provided by Agribrands Purina Canada, Inc. (Ontario, Canada), was composed of a mixture of Ca-salts of palm oil and CLA, comprised of 22.1% palmitate, 4.8% stearate, 27.4% oleate, 7.1% linoleate, and 31% CLA isomers (27.2% *cis*-9, *trans*-11; 32.8% *trans*-10, *cis*-12; 10.6% *trans*-8, *cis*-10; 18.95% *cis*-11, *trans*-13; and 10.5% various *trans*, *trans* CLA isomers). Dietary treatments were prepared to be isonitrogenous; with an equal percentage of concentrate being removed as supplemental lipid was incorporated. **Table 5.1** shows the ingredient and chemical composition of the three dietary treatments. Heifers at trial initiation were implanted with Synovex-H (20 mg of estradiol benzoate and 200 mg of testosterone; Ft. Dodge Animal Health, Ft. Dodge, IA) and were fed melengesterol acetate (Pharmacia, Kalamazoo, MI) at 0.45 mg·heifer^{-1}·day^{-1} and rumensin-80 (Elanco Animal Health, Greenfield, IN) at 250 mg of monensin activity·heifer^{-1}·day^{-1} throughout the trial. Animal weights were recorded at 28 d intervals throughout the feeding trial.

Harvest, Carcass Data and Sample Collection

After 89 d on feed (32 d of dietary treatment), six heifers per dietary treatment (n=18) that had ≥1.27 cm SC fat thickness (as measured by real-time ultrasound) were slaughtered. The remaining heifers (six heifers/treatment; n=18) were fed their diet treatments for an additional 28 d to reach a similar SC fat thickness end point and then slaughtered. For harvesting, cattle were transported to the University of Georgia Meat Science (**UGA**) and Technology Center (Athens, GA). Fasted live animal weights were recorded prior to slaughter.

Immediately (<15 min) after exsanguination, a portion of the longissimus muscle (6^{th} to 9^{th} rib) was removed and IM adipose tissue was dissected, frozen in liquid nitrogen and stored at -70°C for subsequent mRNA analysis. Samples of IM and SC adipose tissue were also collected for tissue cellularity measurements. Carcasses were chilled at 4°C for 48 h, and trained UGA personnel recorded USDA quality and yield grade factors—including, hot carcass weight; adjusted fat thickness; LM area; percentage kidney, pelvic, and heart fat (**KPH**); marbling score; and skeletal and lean maturity. Yield and quality grades were determined according to USDA standards (USDA, 1997). One steak (2.54 cm), for lipid analysis, was removed from the longissimus muscle between the 11^{th} and 12^{th} rib of the right side of each carcass. Each steak was trimmed of all external fat, pulverized in liquid nitrogen and stored at -20°C for subsequent analysis.

Muscle Lipid Analysis

Total lipids were extracted according to a Folch et al. (1957) method. Prior to this study, it determined that a 10:1 ratio of sample to chloroform:methanol (2:1) was sufficient for total lipid extraction (Smith, unpublished data).

Adipose Tissue Cellularity

Procedures outlined by Mersmann and MacNeil (1986) were used to determine adipocyte cellularity. The adipose tissue for the first slaughter group, 32 d supplementation length, was frozen and stored -20°C prior to being assayed, while the adipose tissue of the second slaughter group, 60 d supplementation length, was assayed fresh. Duplicate samples (50 mg) of SC and IM adipose tissue were fixed in osmium tetroxide and dissociated and Coulter Counter (Coulter Electronics, Hialeah, FL) was used to determine adipocyte number of various sizes (range of 20 to 240 μm). Due to the large number of particles sized <30 μm, only counts >30 μm were incorporated in cell number, diameter and volume calculations (Lee et al., 1994).

*Ribonuclease protection assay (**RPA**) of ACC, SCD, and LPL mRNA from bovine IM adipose tissue*

Total RNA was isolated from IM adipose tissue with the QIAzol lysis reagent (RNeasy® Lipid Tissue Midi Kit, QIAGEN Inc., Valencia, CA). The nonisotopic RPA outlined by Lee et al. (2002) was used for this experiment. Reverse-transcription (**RT**) polymerase chain reaction (**PCR**) was used to synthesize antisense biotin-labeled riboprobes. In a 25 μl volume, 2 μg of total RNA was reverse transcribed with 200 U of reverse transcriptase (Promega, Madison, WI) at 37°C for 1 hr. The cDNA synthesized was amplified using the primer pairs in which the antisense primers contained the bacteriophage T7 promoter sequence (27 bp) at the 5′ end (Kain et al., 1991). The primer sequences for ACC, SCD, and LPL were reported in Lee et al. (2002) and Bonnet et al. (2001). The primer pairs were synthesized at the Molecular Genetics Instrumentation Facilities (UGA, GA). The sequences of the sense and antisense primers used for ACC, SCD, LPL, and β-actin are reported in **Table 5.2**. The PCR reactions were completed in a 50 μL volume containing 5 μL of the RT product, 2.5 m*M* $MgCl_2$, 200 μ*M* dNTPs (Sigma

Chemical, St. Louis, MO), 400 n*M* each of sense and antisense primers, and 2.5 U of Taq DNA polymerase (Promega). The thermal settings were as follows: 1 cycle at 94°C for 3 min, followed by 35 cycles at 94°C for 60 s, 58°C for 60 s, and 72°C for 60 s with a final extension at 72°C for 5 min. An *in vitro* transcription was accomplished using a MAXIscript™ labeling kit (Ambion, Inc., Austin, TX). The riboprobe reaction, in a 20 μL total volume, contained 5 μL of PCR products, 1× buffer, T7 RNA polymerase, 0.5 m*M* each of ATP, CTP, and GTP with 0.3 m*M* of UTP, and 0.2 m*M* biotin-labeled-16-UTP (Roche, Mannheim, Germany). The reaction was executed for 1 hr at 37°C then incubated with DNase I for 15 min at 37°C. Transcripts were denatured (95°C for 3 min) and separated on 6% acrylamide/7 *M* urea denaturing polyacrylamide gels. After staining with ethidium bromide, the full-length riboprobe was excised and eluted from the gel in probe elution buffer (Ambion, Inc.) overnight at 37°C, precipitated, and quantified at 260/280 nm.

The RPA reaction was carried out using a RPA III™ kit (Ambion, Inc.). One multiprobe (ACC, SCD, LPL and β-actin standard) RPA reaction per animal was completed, as the expected product sizes, based on the probe sequence, were at least 10% different in bp length. Total RNA and 10-fold molar excess riboprobe was denatured (95°C for 3 min) and then allowed to hybridize at 56°C overnight in 10 μL of hybridization buffer. The reactions were digested with RNase and then, through a simultaneous Ambion-patented procedure, RNase was inactivated and protected RNA was precipitated.

The protected RNA was separated by electrophoresis through a 6% acrylamide/7 *M* urea denaturing polyacrylamide gel (Invitrogen) and electrophoretically (XCell *SureLock*™ Mini-Cell with XCell II™ Blot Module Kit, Invitrogen) transferred onto a positively charged nylon

membrane (BrightStar-Plus™, Ambion, Inc.) with 0.5× TBE at 400 mA for 1 hr. Once the membrane was UV-crosslinked, nonisotopic detection was performed using a BrightStar™ BioDetect™ kit (Ambion, Inc.) in which the membrane was incubated with streptavidin-alkaline phosphatase for 30 min and CDP-Star for 5 min at room temperature. The membrane was exposed using chemiluminescence of an Alpha Innotech Imager (San Leandro, CA) and the image was analyzed with Fluorchem™ 8000 (Alpha Innotech, San Leandro, CA) software. The intensity of the target band was expressed as a percentage of the β-actin standard band intensity on each gel lane to calculate a ratio that was then statistically compared for treatment effects.

Statistical analysis

Data were analyzed using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC), with individual animal serving as the experimental unit. For carcass, fatty acid concentration, total lipid percentage and enzyme mRNA data, the model analyzed the effect of treatment (CON, OIL, or SALT), dietary lipid supplementation length (32 vs. 60 d), and their two-way interaction. Cellularity data of heifers fed for 32 d were analyzed separately from heifers fed 60 d due to the confounding effect of time on feed and frozen vs. fresh tissue. The model for cellularity data included treatment, tissue (IM vs. SC), and treatment by tissue interaction. Least squares means were separated using the PDIFF procedure of SAS and considered significant at $P \leq 0.05$ and a trend at $P > 0.05$ to $P = 0.10$.

Results and Discussion

Carcass Characteristics

Carcass data for this trial were previously reported by Gillis et al. (2004a) and are presented in **Table 5.3**. Briefly, final live weight, final USDA yield grade, yield grade factors—hot carcass weight, adjusted fat thickness, LM area and percentage of KPH—and

dressing percentage did not differ (P > 0.10) by diet treatment or by length of supplementation. Marbling score tended (P = 0.07) to differ by diet treatment; with corn oil supplemented heifers having higher marbling scores than CLA salt heifers and control heifers being intermediate.

Muscle Lipid Characteristics

Total lipid percentage of the longissimus muscle did not differ (P > 0.20) by diet treatment or by time on feed **(Table 5.3)**. Fatty acid data have been previously reported by Gillis et al., 2004b. Heifers supplemented with SALT had 22% greater (P < 0.05) total CLA content than control or OIL fed heifers. Corn oil supplementation increased (P < 0.05) linoleic acid concentration in adipose tissue, but did not alter (P > 0.05) level of CLA isomers. Fatty acid composition of the diet has also been shown to alter CLA amount in beef tallow (Pariza et al., 2001), lamb subcutaneous adipose tissue (Mir et al., 2000), and pork intramuscular lipids (Joo et al., 2002).

Adipose cellularity

Adipose tissue can enlarge by hyperplasia (cell proliferation) or hypertrophy (cell enlargement through lipid accumulation). The increase in adipocyte number may be due to preadipocytes filling with lipid or actual differentiation or proliferation of newly stimulated preadipocytes (Hood, 1982). Cellularity data of heifers fed for 32 d were analyzed separately from heifers fed 60 d due to the confounding effect of time on feed and frozen vs. fresh tissue. Mersmann and MacNeil (1986) reported, with porcine adipose tissue, that tissue stored frozen prior to being assayed had smaller mean diameters and a greater number of particles < 35 μm. In this study, cell diameter distribution of adipose tissue for both supplementation length groups was similar (P > 0.05) among CON, OIL and SALT heifers. Dietary treatment was significant only once, at diameter subgroup (170 – 180 μm) for the 32 d supplemented heifers, in which

CON had a higher percentage of particles than OIL, with SALT as an intermediate. In porcine adipose tissue, Smith et al. (2002) also reported no difference in adipose cellularity traits of CLA, corn oil, or beef tallow supplemented diets. In contrast, Azain and coworkers (Azain et al., 2000; Poulos et al., 2001; Sisk et al., 2001) reported CLA consistently reduced adipocyte volume in rodent models. To date no research has been published on the effects of feeding CLA or linoleate rich corn oil on cellularity of beef adipose tissue.

For this investigation, cell diameter distribution differed ($P < 0.05$) by adipose depot for both 32 d and 60 d supplemented heifers **(Figure 5.1** and **Figure 5.2)**. Numerous studies have observed a hierarchy between depots in adipocyte size in which SC is greater in size than IM (Hood and Allen, 1973; Allen, 1976; Schiavetta et al., 1990; Mendizabal et al., 1999). For the 32 d supplemented heifers, subcutaneous adipose tissue had a greater ($P < 0.05$) percentage of cells in the smaller diameter ranges (30 – 50 μm) and in the large diameter ranges (>150 μm; $P < 0.05$), with a peak diameter (the diameter with the greatest proportion of cells within that sub-population of cells) of 190 μm. Intramuscular adipose tissue had a greater ($P > 0.05$) percentage of cells in the middle diameter ranges (70 – 150 μm), with a peak diameter of 110 μm. For the 60 d supplemented heifers, subcutaneous adipose tissue had a greater ($P < 0.05$) percentage of cells in the large diameter ranges (>150 μm; $P < 0.05$) with a peak diameter of 190 μm and intramuscular adipose tissue had a greater ($P > 0.05$) percentage of cells in the middle diameter ranges (70 – 150 μm), with a peak diameter of 110 μm. Gilbert et al. (2003) reported peak diameters of 100 μm for IM tissue and 150 μm for SC tissue and May et al. (1994) observed for Angus and Wagyu steers peak diameters of 140 μm and 180 μm for IM and SC adipose tissue, respectively. Peak diameters can vary due to age and adiposity of the cattle, but the peak diameters for this study were within the ranges of other published studies.

Adipocyte diameter distributions showed biphasic distributions for both SC and IM **(Figure 5.1** and **Figure 5.2)** adipose tissue. As fat is deposited in cattle, a population of cells will accumulate lipid, increasing in diameter and volume. Once this population of cells attains a certain size due to hypertrophy, another population of smaller adipocytes becomes apparent demonstrating a biphasic diameter distribution (Allen, 1976). The occurrences of smaller adipocytes suggest reinitiation of hyperplasia or differentiation of preadipocytes (Allen, 1976). The exact point of when this recruitment occurs is unknown for both SC and IM adipose tissue. Reports have suggested reinitiation of hyperplasia in SC tissue may be triggered at a larger adipocyte size compared to IM tissue (Allen, 1976; Schoonmaker et al., 2004).

Table 5.4 presents adipose cellularity traits of IM and SC tissue from control, corn oil fed and CLA salt fed heifers supplemented for 32 d. Mean diameter and volume of CON, OIL, and SALT differed by tissue ($P < 0.05$) as IM adipose tissue had a larger diameter and volume than SC tissue. The interaction of treatment by tissue was also significant for mean diameter and volume. The SC tissue of CON heifers was similar to the IM tissue of CON and SALT heifers as well as was greater in diameter and volume than SC tissue of OIL and SALT heifers. Subcutaneous tissue would be expected to have a larger average cell diameter and subsequent average cell volume; however, for this study, IM tissue had the numerically larger mean cell diameter but 50% of SC adipocytes were in the smallest diameter range (30 – 40 μm), which greatly impacted the mean diameter for SC. Cells per gram differed by dietary treatment for SC tissue as OIL had a greater ($P < 0.01$) number of cells than CON with SALT as an intermediate. Corn oil supplemented heifers did have a higher total lipid content compared to the other dietary treatments which might correspond to a greater number of cells per gram. The elevated numbers of particles in the lowest diameter range, subsequent smaller diameter and volume, and increased

number of cells per gram for SC adipose tissue might be due to the tissue being frozen prior to being assayed as the results are comparative with the findings of Mersmann and MacNeil (1986).

Table 5.5 presents adipose cellularity traits of IM and SC tissue from control, corn oil fed and CLA salt fed heifers supplemented for 60 d. In this study, no differences ($P > 0.05$) in average cell diameter or volume were observed for dietary treatment. When comparing adipose tissue depots, SC tissue had larger ($P < 0.05$) mean diameter and subsequent volume. May et al. (1994) reported IM adipose tissue, contained more adipocytes per gram of tissue with a smaller mean cell diameter and volume relative to SC adipose tissue.

Enzyme expression

Fat accretion is the balance between fat synthesis (lipogenesis) and breakdown (fatty acid oxidation/lipolysis). Three key enzymes involved in lipid uptake and biosynthesis for fat storage are LPL, ACC and SCD. Providing free fatty acids to adipose tissue is LPL, which catalyzes the hydrolysis of triglycerides from circulating lipoprotein particles (Auwerx et al. 1992). The rate-limiting enzyme of fatty acid synthesis or lipogenesis is ACC. In this initial biotin-dependent step, acetyl CoA is converted to malonyl-CoA, the substrate for fatty acid synthesis (Abu-Elheiga et al., 2001). The fatty acid composition of muscle and adipose tissue is greatly influenced by the regulation of SCD. The SCD enzyme catalyzes the rate-limiting step in the biosynthesis of MUFA by inserting a *cis*-double bond in the fatty acyl-CoA substrate Δ^9 position (Kim and Ntambi, 1999). The concentrations of mRNA for ACC, SCD and LPL from IM adipose tissue of control, corn oil, and CLA salt fed heifers were analyzed by ribonuclease protection assay **(Figure 5.3)**.

To date, this investigation is the first to report the effects of dietary lipid source on mRNA expression of beef IM adipose tissue. Interactions between dietary treatment and

supplementation length for enzyme expression were not significant, thus enzyme expression was evaluated based on the main effects of treatment and time on feed. The enzyme density ratios of control, corn oil supplemented and CLA salt supplemented heifers are presented in **Figure 5.4**. The mRNA expression of SCD and LPL was similar (P = 0.50 and P = 0.95; respectively) among diets. Overall, ACC was also similar (P = 0.18) between diets; however, when comparing means, SALT fed heifers tended to have a higher ratio (P = 0.08) than OIL-fed heifers, with control heifers being intermediate. The mRNA expression of ACC and LPL did not differ (P = 0.50 and P = 0.72; respectively) by length of supplementation; however, SCD expression was higher (P = 0.06) for 32 d supplementation verses 60 d supplementation **(Figure 5.5)**. In this study, enzyme expression was not correlated (P > 0.05) with either carcass characteristics or total lipid content.

Research has implied CLA supplementation induces a reduction in lipid accumulation by adipocytes (Pariza et al., 2001) due to a single-isomer effect of *trans*-10, *cis*-12 CLA isomer (Park et al., 2000). The reduction in lipid accumulation in adipocytes has been correlated with the inhibition of the enzymes examined in this study. Research has indicated fatty acid composition of the diet can alter SCD enzyme activity of porcine SC adipose tissue (Klingenberg et al., 1995). Smith et al. (2002) reported dietary CLA depressed SCD enzyme activity in porcine adipose tissue but did not measure SCD mRNA and could not directly discern between direct effects of CLA supplementation on SCD enzyme activity or gene expression. Expression of the SCD gene is essential in the differentiation of 3T3-L1 preadipocytes (Ntambi et al., 1988). Others have shown SCD gene expression increased dramatically throughout adipocyte hypertrophy phases in SC tissue of post-weanling piglets (Smith et al., 1999) and calves (Martin et al., 1999). In mice fed CLA, Lee et al. (1998) observed a decrease in hepatic SCD mRNA levels. Similarly, Choi et al. (2000) and Park et al. (2000) reported decreased SCD enzyme

activity and gene expression with the supplementation of CLA. In 2001, Choi et al. reported that treating cultured human hepG2 cells with CLA did not alter SCD mRNA levels but did decrease SCD enzyme activity and concentrations of MUFA. The *trans*-10, *cis*-12 CLA isomer is considered a strong, direct inhibitor of SCD activity, compared to other isomers, whereas the *cis*-9, *trans*-11 CLA isomer has had no effect on SCD activity (Park et al., 2000).

Research has also shown in CLA-fed mice, heparin-releasable LPL and intracellular LPL enzyme activities had been significantly reduced (Xu et al., 2003). In 1997, Park et al. found a mixture of CLA isomers inhibited heparin-releasable LPL activity in cultured mouse 3T3-L1 adipocytes and later (Park et al., 1999) the inhibition effect was found to be due to one CLA isomer, *trans*-10, *cis*-12 CLA. Xu et al. (2003) also suggested that CLA may inhibit LPL gene expression in normal adipocytes *in vivo* and that CLA did not increase lipolysis *in vivo*, thus CLA might have an early effect on fat metabolism through a reduction in fat deposition and not an enhancement in fat release. Baumgard et al. (2002) demonstrated that treatment with *trans*-10, *cis*-12 CLA significantly decreased production of milk fat through a reduction in mRNA abundance of key enzymes by 39 to 54%, including ACC, LPL and SCD. Piperova et al. (2000) detected in mammary tissue, ACC enzyme activity and mRNA abundance of ACC was reduced when cows were fed a milk fat depressing diet. In adipose tissue of growing mice, Tsuboyama-Kasaoka et al. (2000) reported dietary CLA supplements decreased mRNA abundance for ACC.

To date no research has been published on the mRNA abundance of key lipogenic enzymes in beef IM adipose tissue to compare the findings of this study. According to milk fat synthesis studies, the supplementation of CLA salt should have lowered the mRNA abundance of ACC, SCD and LPL. However, this was not the case and although there was no difference due to dietary treatment, SALT heifers had higher mRNA levels than the other two treatments.

Modification of beef IM lipid composition is limited by ruminal biohydrogenation as research indicates only small to moderate changes in IM lipid composition due to nutritional effects. While the difference in *cis*-9, *trans*-11 CLA content was significant, the magnitude or importance of this change is not likely to be sufficient to alter gene expression.

Implications

Supplemental dietary lipid source as rumen-protected CLA or linoleate-rich corn oil added to feedlot diets did not alter muscle accretion or intramuscular lipid deposition as measured by marbling score, total lipid content, adipocyte cellularity or enzyme expression. Short-term addition of rumen-protected CLA or corn oil to finishing beef cattle diets altered fatty acid composition of the IM adipose tissue of the longissimus muscle. However, the degree of this change was not enough to modify gene levels. Thus, this study suggests that dietary lipid source does not have a direct effect on IM lipid deposition. Further research is needed to fully understand the mode of action of dietary nutrition lipid metabolism.

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Table 5.1. Composition of experimental dietary treatments (DM basis)

	Diet		
Item, %	CON[a]	OIL[a]	CLA[a]
Ingredient			
Bermudagrass hay	12	12	12
Corn, dry rolled	83	79	81
Protein/mineral mix[b]	5	5	5
CLA salt[c]	—	—	2
Corn oil[d]	—	4	—
Chemical Composition			
Dry matter	84.50	86.08	85.75
Crude protein	11.98	11.49	11.74
ADF	8.68	8.48	8.58
NDF	21.45	20.82	21.14
Total fatty acid	3.30	6.38	4.20

[a]Dietary treatment abbreviations: CON = control, OIL = 4% corn oil, and CLA = 2% rumen-protected CLA salt.

[b]Mix contained: 49.4% soybean meal, 25.5% limestone, 9% 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 16.1% urea. Supplied 0.45 mg of melengesterol acetate·heifer^{-1}·d^{-1}.

[c]CLA salt (mixture of Ca salts of palm oil and CLA) contained: 22.1% palmitate, 4.8% stearate, 27.4% oleate, 7.1% linoleate, and 31% CLA isomers (27.2% *cis*-9, *trans*-11; 32.8% *trans*-10, *cis*-12; 10.6% *trans*-8, *cis*-10; 18.95% *cis*-11, *trans*-13; and 10.5% various *trans*, *trans* CLA isomers).

[d]Fatty acid composition: 58% C18:2, 27% C18:1 *cis*-9, 11% C16:0, 2% C18:0, and 2% C18:3.

1

Table 5.2. Sense (S) and antisense (AS) primers for synthesis of acetyl CoA carboxylase (ACC), stearoyl CoA desaturase (SCD), lipoprotein lipase (LPL), and β-actin riboprobes.

Gene		Primer sequences	PCR product size (bp)
ACC	S	5′-GATGGGCGGGATGGTCTCTTTTC-3′	436
	AS	5′-CCAAGCTTCTAATACGACTCACTATAGGTAGGGCAGGCTCCAGGTGACGATA-3′	
SCD	S	5′-TTCCCGACGTGGCTTTTTCTTCT-3′	337
	AS	5′-CCAAGCTTCTAATACGACTCACTATAGGCTCTCGGGGGTTGATGGTCTTGT-3′	
LPL	S	5′-TGTGAAATGCCATGACAAGTC-3′	277
	AS	5′-CCAAGCTTCTAATACGACTCACTATAGTGTGCTATTTGGCCACTATAC-3′	
β-actin	S	5′-GTTCAACACTCCTGCCATGTAT-3′	251
	AS	5′-CCAAGCTTCTAATACGACTCACTATAGGTAGCAGAGCTTCTCCTTGATG-3′	

Table 5.3. Effect of dietary treatment and length of lipid supplementation on carcass characteristics, performance, and lipid characteristics of the longissimus muscle of control (CON), corn oil (OIL) supplemented, and CLA salt (SALT) supplemented crossbred Angus heifers

	Dietary treatment					Supplementation length			
Item	CON	OIL	SALT	SEM	P > F	32 d	60 d	SEM	P > F
n	12	12	12			18	18		
Shrunk weight, kg	480.31	492.45	486.86	7.47	0.53	487.35	485.72	6.10	0.85
Hot carcass weight, kg	294.17	300.54	299.09	4.91	0.64	299.58	296.28	4.01	0.57
Dressing percentage	61.25	61.04	61.44	0.37	0.75	61.49	61.00	0.30	0.25
Adjusted fat thickness, cm	1.63	1.53	1.59	0.09	0.73	1.67	1.50	0.07	0.11
Longissimus muscle area, cm^2	67.95	70.89	72.81	2.16	0.30	70.79	70.31	1.76	0.85
Kidney, pelvic and heart fat, %	2.21	1.96	2.29	0.15	0.28	2.08	2.22	0.12	0.44
USDA yield grade	3.65	3.40	3.42	0.14	0.42	3.56	3.41	0.12	0.39
USDA marbling score[b]	440^{yz}	481^{y}	426^{z}	17.01	0.07	466	432	14.00	0.10
Total lipid content, %	6.31	7.65	6.31	0.60	0.20	6.73	6.78	0.49	0.94

[a]100 = A^{00} and 200 = B^{00}.
[b]400 = $Small^{00}$ and 500 = $Modest^{00}$.
[y,z]Within a row, means without a common superscript tend to differ ($P = 0.07$).

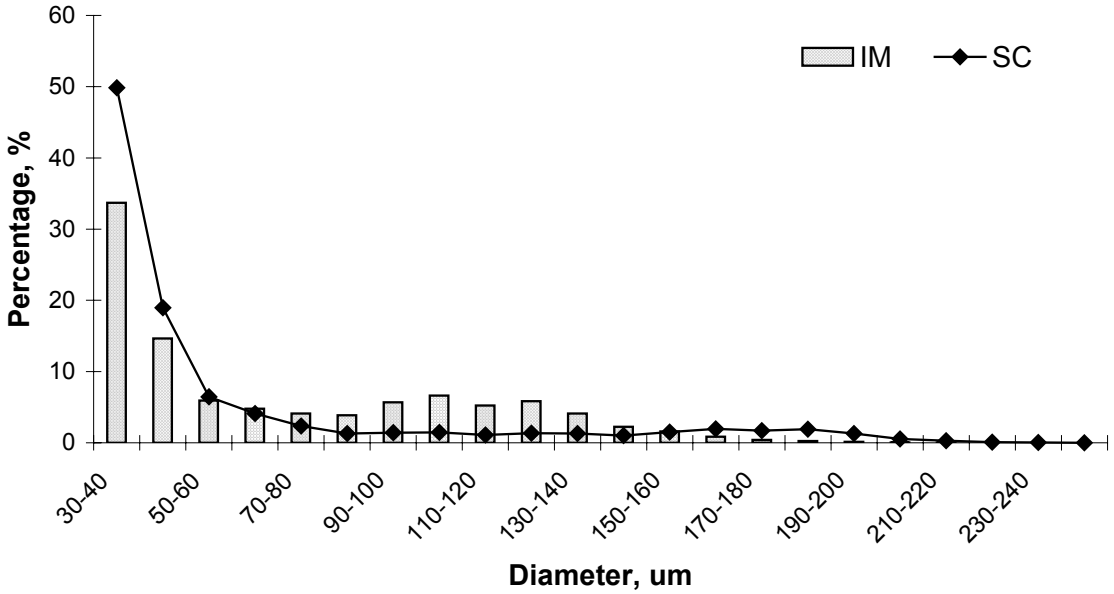

Figure 5.1. Cell diameter distribution for adipocytes from subcutaneous (SC) and intramuscular (IM) adipose tissues from crossbred Angus heifers supplemented for 32 d.

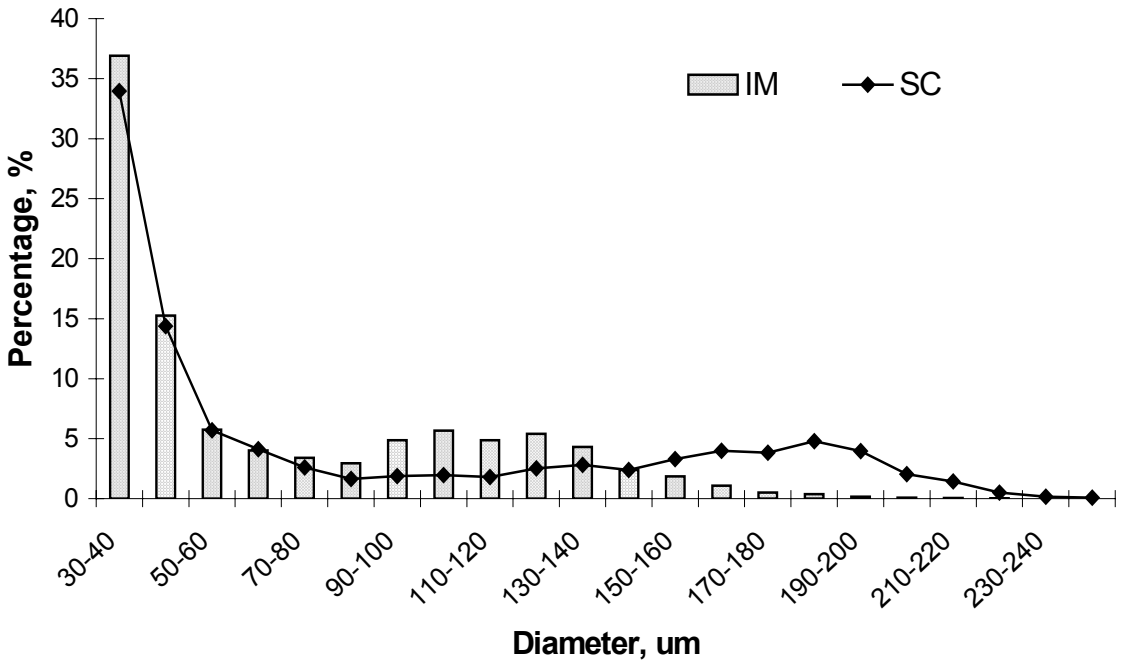

Figure 5.2. Cell diameter distribution for adipocytes from subcutaneous (SC) and intramuscular (IM) adipose tissues from crossbred Angus heifers supplemented for 60 d.

Table 5.4. Adipose cellularity of intramuscular (IM) and subcutaneous (SC) tissue from control (CON), corn oil supplemented (OIL) and CLA salt (SALT) supplemented Angus crossbred heifers supplemented for 32 d

	CON		OIL		SALT			
	IM	SC	IM	SC	IM	SC	SEM	*P* > *F*
Number, 10^5 cells/g	—	17.32[b]	—	25.30[a]	—	23.55[ab]	2.75	0.01
Diameter, μm	70.45[ab]	66.53[b]	75.26[a]	56.57[c]	70.73[ab]	59.11[c]	2.50	0.02
Volume, 10^5 μm/cell	1.85[ab]	1.58[b]	2.27[a]	0.96[c]	1.99[ab]	1.11[c]	0.20	0.05

[a,b, c]Within a row, means without a common superscript differ ($P < 0.05$).

Table 5.5. Adipose cellularity of intramuscular (IM) and subcutaneous (SC) tissue from control (CON), corn oil supplemented (OIL) and CLA salt (SALT) supplemented Angus crossbred heifers supplemented for 60 d

	CON		OIL		SALT			
	IM	SC	IM	SC	IM	SC	SEM	*P* > *F*
Number, 10^5 cells/g	7.93	8.37	6.86	6.76	7.43	6.49	0.94	0.77
Diameter, μm	68.46[a]	87.91[b]	74.02[a]	87.24[b]	69.04[a]	89.99[b]	4.24	0.63
Volume, 10^5 μm/cell	1.73[a]	3.67[b]	2.22[a]	3.66[b]	1.83[a]	4.14[b]	0.49	0.68

[a,b]Within a row, means without a common superscript differ ($P < 0.05$).

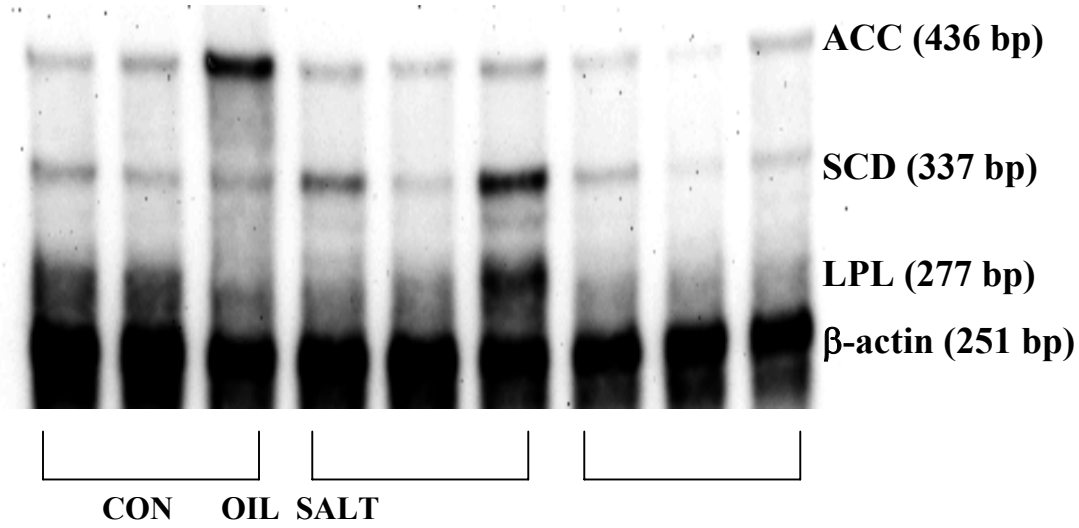

Figure 5.3. Ribonuclease protection assay (RPA) of acetyl Co-A carboxylase (ACC), stearoyl Co-A desaturase (SCD), lipoprotein lipase (LPL), and β-actin expression in intramuscular adipose tissue of control (CON), corn oil supplemented (OIL), and CLA salt supplemented (SALT) crossbred Angus heifers.

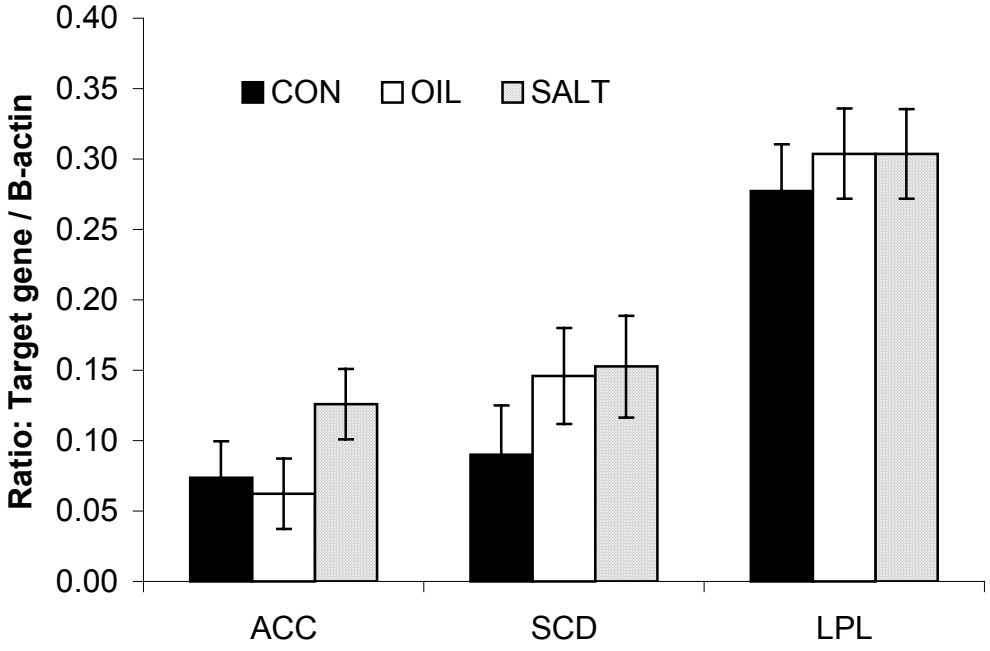

Figure 5.4. Densitomeric quantification of acetyl Co-A carboxylase (ACC), stearoyl Co-A desaturase (SCD), and lipoprotein lipase (LPL) mRNA from intramuscular adipose tissue of control (CON), corn oil supplemented (OIL) and CLA salt supplemented (SALT) crossbred Angus heifers. Data are expressed as means ± SE of twelve animals in each treatment.

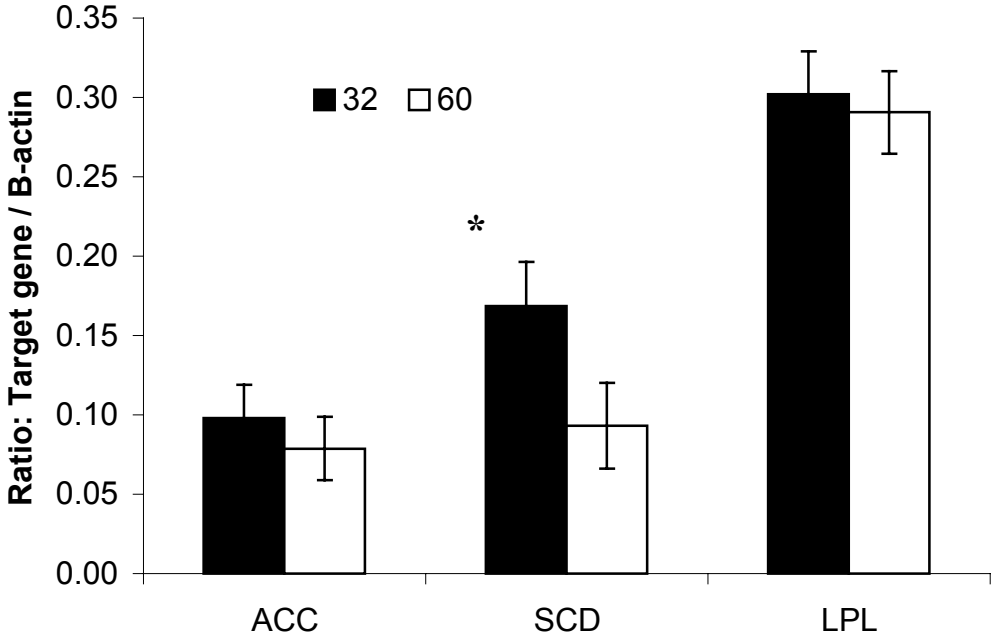

Figure 5.5. Densitomeric quantification of acetyl Co-A carboxylase (ACC), stearoyl Co-A desaturase (SCD), and lipoprotein lipase (LPL) mRNA from intramuscular adipose tissue of crossbred Angus heifers supplemented for 32 or 60 d. Data are expressed as means ± SE of twelve animals in each treatment. Mean with asterick is different from 60 d supplementation (P = 0.06).

CONCLUSION

Anabolic implant enhanced animal performance and carcass protein accretion through an increase in ADG and longissimus muscle area, respectively. However, implanting did not alter intramuscular lipid deposition as measured by marbling score, total lipid content, fatty acid content, adipocyte cellularity or enzyme expression. Collectively, this study suggests that the use of anabolic implants does not have a direct effect on IM lipid deposition. It is also noteworthy that using a strong combination implant did not negatively affect quality grade in the cattle used in this study possibly as they had a greater than average potential to marble. Supplemental dietary lipid source as rumen-protected CLA or linoleate-rich corn oil added to feedlot diets did not alter muscle accretion or intramuscular lipid deposition as measured by marbling score, total lipid content, adipocyte cellularity or enzyme expression. Short-term addition of rumen-protected CLA or corn oil to finishing beef cattle diets altered fatty acid composition of the IM adipose tissue of the longissimus muscle. However, the degree of this change was not enough to modify gene levels. Thus, this study suggests that dietary lipid source does not have a direct effect on IM lipid deposition. Further research is needed to fully understand the mode of action of anabolic implants and dietary nutrition on protein accretion and lipid metabolism.