FINISHING DIETS WITH ELEVATED LEVELS OF a -LINOLENIC ACID INCREASE FEED EFFICIENCY AND ADIPOSE LIPOGENESIS BUT DO NOT ALTER BEEF CARCASS QUALITY

A Dissertation

by

SHAWN LOUIS ARCHIBEQUE

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

August 2003

Major Subject: Nutrition

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Approved as to style and content by:

Stephen B. Smith (Chair of Committee)	Gordon E. Carstens (Member)
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ABSTRACT

Finishing Diets With Elevated Levels of α-Linolenic Acid Increase Feed Efficiency and Adipose Lipogenesis but Do Not Alter Beef Carcass Quality. (August 2003)

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Forty-five Angus steers (358 kg BW) were utilized in a completely randomized block design with a 3 x 3 factorial arrangement of treatments to evaluate the hypothesis that differing dietary α-linolenic acid (from corn, flaxseed plus corn, or milo) and whole cottonseed (**WCS**) inclusion (0, 5, or 15% DM) would interact to alter fatty acid metabolism and deposition of conjugated linoleic acid (**CLA**) in subcutaneous (**s.c.**) and interfasicular (**i.f.**) adipose tissues, and thereby decrease carcass quality score. During the feeding period (135 d), steers receiving flaxseed or corn diets had a greater gain:feed ratio (0.119 and 0.108, respectively) than steers receiving the milo diet (0.093). Following transportation to a local abattoir and overnight starvation, there was less decrease in weight in flaxseed-fed steers (1.51%) than in steers fed the corn (2.89%) or milo diets (3.11%). Ribeye area of steers fed milo was less than that of steers fed the corn or flaxseed diets. Lipogenesis from acetate in s.c. adipose tissue was greater in steers fed flaxseed (5.42 nmol•h^{-1}•10^5 $cells^{-1}$) than in the corn (3.10 nmol•h^{-1}•10^5 $cells^{-1}$) or milo (1.92 nmol•h^{-1}•10^5 $cells^{-1}$) groups. Stearoyl-CoA desaturase (**SCD**) activity in s.c. adipose tissue was unchanged between the 0% WCS group (88.1 nmol•mg protein^{-}

^{1}•7 min^{-1}) and the 15% WCS group (20 nmol•mg protein^{-1}•7 min^{-1}). The i.f. saturated fatty acid percentages increased with increasing levels of WCS. The i.f. *cis*-9, *trans*-11 CLA percentage increased with increasing WCS in the steers fed the corn diet, whereas it remained unchanged or even decreased slightly in the steers fed the flaxseed or milo-based diets. Steers fed flaxseed had a greater s.c. adipose concentration of vaccenic acid (18:1*trans*-11) than the steers fed milo. Steers fed flaxseed also had greater s.c. and i.f. percentages of α-linolenic acid (18:3, n-3) than steers fed either of the other grain sources. Increased dietary α-linolenic acid from flaxseed may have increased s.c. adipocyte volume by stimulating lipogenesis. These data indicate that rations formulated to provide increased levels of α-linolenic acid (i.e., flaxseed) will increase feed efficiency and lipogenesis from acetate without altering either the quality or composition of the beef carcasses.

This dissertation is dedicated to my son, who completed my life in ways I never imagined.

In Broken Images
Robert Graves
Collected Poems, 1959, p.94

He is quick, thinking in clear images;
I am slow, thinking in broken images.

He becomes dull, trusting to his clear images;
I become sharp, mistrusting my broken images.

Trusting his images, he assumes their relevance;
Mistrusting my images, I question their relevance.

Assuming their relevance, he assumes the fact;
Questioning their relevance, I question the fact.

When the fact fails him, he questions his senses;
When the fact fails me, I approve my senses.

He continues quick and dull in his clear images;
I continue slow and sharp in my broken images.

He in a new confusion of his understanding;
I in a new understanding of my confusion.

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CHAPTER I

INTRODUCTION

Cattle fed in Australian feedlot as many as 300 d do not achieve the level of marbling observed in identical breed types fed in U.S. feedlots for only 120 d (Greg Chappell, Australian Angus stud producer; personal communication). We propose that Australian cattle are being fed a combination of whole cottonseed and some source of α-linolenic acid. Whole cottonseed contains the cyclopropene fatty acid, sterculic acid, which is a potent inhibitor of stearoyl CoA desaturase (**SCD**, Raju and Reiser, 1972). The *trans*-10, *cis*-12 isomer of CLA depresses SCD gene expression (Lee et al., 1998). Their combined effects could be sufficient to produce the reduced SCD activity we have documented in adipose tissue from Australian feedlot cattle (Yang et al., 1999). This would provide the biochemical basis for the occurrence of hard fat in Australian beef carcasses (Smith et al., 1998), and may also explain the lesser ability of Australian cattle to deposit marbling. We previously demonstrated that the adipose tissue concentration of SCD increases immediately preceding lipid filling of subcutaneous adipose tissue in feedlot Angus steers (Martin et al., 1999.). Ding and Mersmann (2000) demonstrated that oleic acid (the primary product of SCD) stimulated lipid filling in porcine preadipocytes in culture. Thus, depression of SCD activity may ultimately lead to reduced marbling. This, along with an increase in CLA concentration would provide a more healthful fatty acid composition.

This dissertation follows the style and format of the Journal of Animal Science.

The purpose of this investigation was to document the interaction between CLA and whole cottonseed on SCD gene expression in bovine tissues, and how this may affect the accumulation of marbling. The study will demonstrate how actively selecting for CLA isoform production may yield a leaner beef product that contains elevated concentrations of CLA. This would be useful for people who are concerned with lowering total dietary fat in combination with increasing their intake of CLA.

CHAPTER II

REVIEW OF LITERATURE

Stearoyl-CoA Desaturase

Stearoyl-CoA desaturase (**SCD**) is a microsomal enzyme that catalyzes the NADH and O_2 dependent desaturation of saturated fatty acids at carbon 9. This process predominantly yields 16:1n-7 and 18:1n-9 from 16:0 and 18:0 respectively. With the majority of dietary fatty acids being saturated by rumen microflora (Ekeren et al., 1992), SCD is the primary source of long chain unsaturated fatty acids. Adipose tissue and liver appear to be the major site of activity in the higher vertebrates (Ntambi, 1995). Three isoforms of the SCD gene have been identified in the mouse; SCD1 (Ntambi et al., 1988), SCD2 (Kaestner et al., 1989), and SCD3 (Zheng et al., 2001). There is a very different tissue distribution of these isoforms, even though there is a 85-88% homology in amino acid sequence. The SCD1 isoform is expressed at a greater rate in bovine adipose tissue than in liver (St. John et al., 1991), with the rates of activity in bovine adipose occurring at similar rates to those seen in rat liver. In mice, SCD3 is expressed in the skin (Zheng et al., 2001), Haderian (Miyazaki et al, 2001), and the preputial glands (Miyazaki et al., 2002). The SCD2 is expressed in B-lymphocytes, but not in mature T-lymphocytes (Tebbey and Buttke, 1992; Tebbey et al., 1994). To the author's knowledge, the relevance of the tissue distribution of these isoforms has not yet been established. Species, breed, and diet have altered both activity and gene expression of SCD in a variety of models. This review will focus on dietary factors that effect this activity and its role in adipose development.

Sterculic Acid

Cotton oil contains the cyclopropenoic fatty acids; sterculic acid [8-(2-octyl-1-cyclopropenyl) octanoic acid] and malvalic acid [7-(2-octyl-1-cyclo-propenyl) heptanoic acid]. The cyclopropenoic fatty acids comprise 0.5-1.0% of the total lipid (~65% malvalic acid and ~35% sterculic acid; Cao et al., 1993). Sterculic acid has demonstrated potent inhibition of SCD both in vivo and in vitro (Reiser and Raju, 1964; Raju and Reiser, 1972; Griinari et al., 2000). However, this inhibitory activity is removed when the sterculic acid is first heated (Cao et al., 1993). At this time, the actual mechanism of SCD inhibition by sterculic acid is unknown, however Enrique Gomez et al. (2002) postulate that sterculic acid directly inhibits SCD activity by a turnover-dependent reaction. However, in this study which utilized 3T3-L1 preadipocytes, the processes required for adipocyte differentiation, and SCD gene expression were not altered by sterculic acid addition.

Formation and Intake of Conjugated Linoleic Acid

The two major isomers are the *cis*-9, *trans*-11 and the *trans*-10, *cis*-12 isomers, with 80 to 90% of the total CLA of milk fat present as the *cis*-9, *trans*-11 isoform (Parodi, 1977; Sehat et al., 1998). Conjugated linoleic acid derives from a limited number of reactions. The primary source of *cis*-9, *trans*-11 octadecadienoic acid production in ruminants is via biohydrogenation of linoleic acid (18:2n-6) by ruminal microorganisms to 18:1*trans*-11 (Shorlund et al., 1955) and subsequent desaturation to 18:2*cis*-9, *trans*-11 by Δ^9 desaturase (Bauman et al., 1999). The primary source of *trans*-10, *cis*-12 octadecadienoic acid production in ruminants is proposed to be via

biohydrogenation of α-linolenic acid (18:3n-3) (Bauman et al., 1999). *Butyrivibrio fibrisolvens* produces CLA isomers as an intermediate in the biohydrogenation of linoleic acid and α-linolenic acid to stearic acid in the rumen (Chin et al., 1992; Gurr, 1987; Viviani, 1970)

Effects of Conjugated Linoleic Acid on Lipoprotein Cholesterol Metabolism

The possible effects of CLA on cholesterol metabolism have not received little attention relative to the other possible effects of CLA. Lee et al. (1994) first reported that feeding 0.5 g CLA/d to rabbits fed a semi-purified diet containing 14% fat and 0.1% cholesterol reduced LDL cholesterol and triglycerides, and the occurrence of atherosclerosis. This suggested that CLA, in addition to reducing adiposity, could reduce important risk factors in the development of coronary heart disease in humans. This was supported by Nicolosi et al. (1997), who fed up to 1.1% CLA to hypercholesterolemic hamsters. Nicolosi et al. (1997) observed reduced LDL plus very low density lipoprotein (VLDL) cholesterol in CLA-fed hamsters, without any effect on high density lipoprotein (HDL) cholesterol. Again, there was less early atherosclerosis in the CLA-fed hamsters.

Conjugated Linoleic Acid and Adipose Development

Over the past few years, there has been a massive interest in the effects of conjugated linoleic acid (**CLA**) upon many physiological processes. This explosion in scientific interest traces its origins back to the initial paper of Ha et al. (1987), which implicated the possible anticarcinogenic nature of CLA. A more recent interest in CLA has been focused on the effects that this compound may have on overall lipid

metabolism of mammals. Pariza et al. (1996) reported that mice, rats, and chicks fed diets containing 0.5% CLA plus 5.0% corn oil for 4 to 8 wk experienced body fat reductions of 57 to70, 23, and 22%, respectively. In a related experiment, mice were fed a 20% tallow diet containing 0.1% CLA vs a 20% tallow diet supplemented with 0.6% CLA for 4 wk. The 0.6% CLA-supplemented mice exhibited significantly reduced body fat accumulation (-46%) and increased lean body mass (+9%). Albright et al. (1996) indicated that CLA levels in rat fat pads and muscle each returned to normal physiologic levels within 2 to 4 wk after withdrawal of CLA supplementation, dependent on the CLA isomer used. Belury and Kempa-Steczko (1997) reported significantly lower body weights in rats as a result of 1.0 and 1.5% dietary CLA supplementation for 6 wk. Although food intake among treatment and control groups was similar, the feed-to-weight gain ratio (feed efficiency) increased 1.5 to 2.0-fold in CLA-supplemented diet groups.

Conjugated linoleic acid has also been shown to affect differentiation in murine preadipocytes and porcine stromal vascular cells (Ding et al., 2000), but the effects of CLA on bovine preadipocyte differentiation is still unknown. A multitude of in vitro studies have shown that treatment of murine preadipocytes with a mixture of CLA isomers (20 to 200 μM) decreased the proliferation (Brodie et al., 1999; Satory and Smith, 1999; Evans et al., 2000) and accumulation of lipid (Brodie et al., 1999; Choi et al., 2000; Evans et al., 2000; Park et al., 1997; 1999a,b). Mixed isomers of CLA have also reduced mRNA levels of adipocyte fatty acid binding protein and PPARγ2 in 3T3-L1 preadipocytes (Brodie et al., 1999; Choi et al., 2000). In contrast to this convention,

Satory and Smith (1999) reported an increase in the de novo lipogenesis of 3T3-L1 preadipocytes when treated with a mixture of CLA isomers.

More recently, isomer specific studies have determined that the *trans*-10, *cis*-12 isomer of CLA is the bioactive isomer affecting lipid metabolism. The *trans*-10, *cis*-12 isomer of CLA reduces stearoyl-CoA desaturase (**SCD**) activity (Choi et al., 2000) and gene expression (Choi et al., 2000, Lee et al., 1998), increased lipolytic activity (Park et al., 1999b), and decreased both lipoprotein lipase activity and triacylglycerol content of 3T3-L1 adipocytes (Park et al., 1999b). Therefore, some of the discrepancies in the literature concerning the effect of CLA on the differentiation of preadipocytes may be due to a variation in the isomeric content of CLAs that were used in varying studies. There is substantial information describing the role of CLA in the regulation of murine preadipocyte differentiation but, to our knowledge, there is no report describing a similar role in the differentiation of bovine preadipocytes.

The *trans*-10, *cis*-12 isomer of CLA depresses SCD-1 gene expression (Lee et al., 1998). This SCD isozyme recently has been shown to play a major role in genetically obese (ob/ob) mice (Cohen et al., 2002), and may play a role in a metabolic regulation of lipid filling that is extraneous to any transcription regulation of peroxisome proliferator activated receptors that CLA may exert. Although the exact metabolic mechanism for the role of depressed SCD activity is not yet known, a possible explanation espoused by Cohen et al. (2002) is that there will be a decrease in the cellular concentrations of malonyl CoA, which would lead to a decrease in de novo lipogenesis. This theory is based on the concept that the increase in saturated fatty acids

will allosterically inhibit acetyl-CoA carboxylase, which in turn will depress the intracellular concentration of malonyl CoA (Lunzer et al., 1977). This could also possibly increase the oxidation of fatty acids by removing the inhibitory effect of malonyl-CoA upon the carnitine palmitoyl-CoA transferase system (McGarry et al., 1977), which is the rate limiting step for the import and oxidation of fatty acids within the mitochondria of the cell. The increase expected in carnitine palmitoyl-CoA transferase activity in such a scenario is seen in mice fed a CLA supplemented diet (Chin et al., 1994; Park et al., 1997).

Therefore, the literature demonstrates a decrease in the differentiation of murine preadipocytes into mature adipocytes when cultured in CLA, but this has not yet been shown in bovine preadipocytes. Also, it is shown that CLA can inhibit the expression of SCD, which leads to an inhibition of lipogenesis. Our objectives were to provide additional, mechanistic information concerning the effects of CLA on adiposity in growing feedlot cattle. We proposed to increase CLA concentrations (especially the *trans*-10, *cis*-12 isomer) by providing a diet enriched in α-linolenic acid.

CHAPTER III

FINISHING DIETS WITH ELEVATED LEVELS OF α-LINOLENIC ACID INCREASE FEED EFFICIENCY BUT DO NOT ALTER BEEF CARCASS QUALITY

Overview

We hypothesized that there would be an interaction between dietary α-linolenic acid (18:3n-3) and whole cottonseed (WCS) on the metabolism and deposition of subcutaneous and interfasicular adipose tissue. Forty-five Angus steers (358 kg BW) were utilized in a completely randomized block design with a 3 x 3 factorial arrangement of treatments. The factors included the dietary inclusion rate of whole cottonseed (0, 5, or 15% DM) and the type of energy source (corn, flaxseed plus corn, or milo) fed for 135 d. Plasma 18:3n-3 increased throughout the feeding period in the steers fed flaxseed ($P < 0.01$). Plasma 18:1*trans*-11 was initially increased ($P < 0.01$) in the steers fed flaxseed or corn, yet there was no detectable plasma 18:2*cis*-9, *trans*-11 in any of the steers used in this experiment. During the feeding period, steers receiving flaxseed or corn diets had a greater ($P < 0.01$) ratio of weight gained to feed consumed (0.119 and 0.108, respectively) than steers receiving the milo diet (0.093). There was a tendency ($P < 0.06$) for the gain:feed ratio to decrease with increased percentage of WCS in steers fed the milo diet. There were no differences in the ADG or final live weight among treatment groups. Following transportation to a local abattoir and overnight deprivation of food, there was a reduced ($P < 0.01$) percentage decrease in weight in the steers fed the flaxseed diet (1.51%) than in the steers fed the corn (2.89%)

or milo diets (3.11%). Marbling score was not affected by WCS ($P = 0.14$) nor was there an interaction between grain source and WCS ($P = 0.16$). There was an interaction for lean maturity ($P < 0.02$), which decreased with increasing percentages of WCS in steers fed the corn or milo diets, but was unchanged in steers fed flaxseed. Ribeye area of steers fed milo was less ($P < 0.01$) than that of steers fed the corn or flaxseed diets. These data indicate that rations formulated to provide increased levels of α-linolenic acid (i.e., flaxseed) will increase feed efficiency without altering either the quality or composition of the beef carcasses. Additionally, the inclusion of WCS in milo diets may cause a decrease in efficiency and less salable lean.

Introduction

There recently have been a variety of attempts to increase the CLA concentration in the tissues of a variety of meat producing animals. These have included CLA-enhanced pork (Demaree et al., 2002), poultry (Du et al., 2001) and dairy products (Perfield et al., 2002). There has been little research has focused on the effects of increased CLA production in finished beef cattle (Duckett et al., 2002) and the effects of this upon performance. We were interested in the interaction of α-linolenic acid and whole cottonseed (**WCS**), which contains the cyclopropene fatty acid, sterculic acid, a potent inhibitor of stearoyl CoA desaturase (**SCD**; Raju and Reiser, 1972). Also, α-linolenic acid may serve as a source of conjugated linoleic acid (**CLA**) production (Baumann et al., 1999) and the *trans*-10, *cis*-12 isomer of CLA depresses SCD gene expression (Lee et al., 1998). The combined effects of sterculic acid and CLA could be sufficient to produce the reduced SCD activity that has been documented in adipose

tissue from Australian feedlot cattle (Yang et al., 1999). This would provide the biochemical basis for the occurrence of hard fat in Australian beef carcasses (Smith et al., 1998), and may also explain the lesser ability of Australian cattle to deposit marbling. It has been demonstrated previously that SCD gene expression in adipose tissue increases immediately preceding lipid filling of subcutaneous adipose tissue in feedlot Angus steers (Martin et al., 1999.). Ding and Mersmann (2001) demonstrated that oleic acid (the primary product of SCD) stimulated lipid filling in porcine preadipocytes in culture. Thus, depression of SCD activity may ultimately lead to reduced marbling.

The purpose of this investigation is to document the interaction between readily available feed commodities with differing amounts of α-linolenic acid and how this may affect carcass quality. Our hypothesis was that the diets high in α-linolenic acid and WCS would have the least amount of marbling, but would have improved yield grades.

Materials and Methods

Animals and Experimental Procedures

Forty-five Angus steers (358 kg BW) were used in this experiment. Care handling and sampling of the steers was approved by the Texas A&M University IAACUC (AUP#2001-75). Steers were randomly assigned to pens and treatments on d 1 after arriving at the feedlot. Dietary treatments consisted of a 3 x 3 factorial arrangement of grain source and whole cottonseed inclusion (0, 5, or 15% DM) in the diet. The three grain sources included a cracked corn diet, a cracked corn diet that

contained 10% DM as flaxseed, and a ground milo diet. All diets were formulated (Table 3.1) to be isonitrogenous, and meet or exceed all nutrient requirements for

Table 3.1 Dry matter and chemical composition of diets containing one of three grain sources (corn, corn with flaxseed, or milo) and one of three concentrations (0, 5, or 15 %DM) of whole cottonseed (**WCS**) in a total mixed ration.

	Grain/%WCS								
	Corn			Flaxseed			Milo		
Item	0	5	15	0	5	15	0	5	15
WCS	0	5	15	0	5	15	0	5	15
Cottonseed hulls	15	15	15	15	15	15	15	15	15
Rolled corn	68.6	65.2	58.3	62.0	58.6	51.7	0	0	0
Flaxseed	0	0	0	10	10	10	0	0	0
Ground milo	0	0	0	0	0	0	74.7	71.0	63.5
Molasses	4	4	4	4	4	4	4	4	4
Soybean meal	9.1	7.5	4.4	5.7	4.1	1	3.9	2.6	0
Trace mineral salt	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7
Vitamin premix	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Limestone	2.5	2.5	2.5	2.5	2.5	2.5	1.6	1.6	1.7
Nutritional composition[ab]									
Crude protein, %	12.3	12.3	12.3	12.3	12.3	12.3	12.3	12.3	12.3
Ether extract, %	3.19	3.89	5.31	6.86	7.57	9.00	2.63	3.37	4.85
NEm, Mcal/kg	1.89	1.90	1.90	1.91	1.91	1.91	1.74	1.76	1.78
NEg, Mcal/kg	1.09	1.07	1.04	1.06	1.04	1.00	1.19	1.19	1.17

[a] Percentage of dry matter.
[b] Calculated values based on NRC (1996)

growing steers (NRC, 1996). Steers were housed in groups of three in partially covered pens equipped with individual Calan gate feeders (American Calan, Northwood, NH). All diets were fed once daily in the morning in amounts adequate to allow ad libitum access to feed. Steers were gradually switched over a 7-d period to a high-concentrate

finishing diet (the corn based, 0% whole cottonseed diet). Following this adjustment (d 1 of treatment), steers were switched over a 7-d period to their respective treatment diets. Steers were weighed on d 0, 28, 56, 84, 107, and 133; and bled via jugular venipuncture on d 0, 28, 56, 84, and 107. Steers were blocked by 133-d weight with one steer from each treatment in each block. On the evening of d 135, the block of steers with the largest weight was transported to the Rosenthal Meat Science and Technology Center and housed overnight with access to water. The following day, these steers were slaughtered, with the same process repeated with the remaining four blocks of steers. The percentage difference between 133-d weight and final live weight immediately prior to slaughter was determined and is referred to as shrink in this paper.

Carcass Characteristics

Carcasses were weighed immediately following the slaughter process to determine hot carcass weight and then chilled at 4^{o}C for 48 h. USDA carcass measures and grades were measured by standard techniques (USDA, 1997) by trained Texas A&M University personnel. Quality and yield evaluations included determinations of skeletal maturity, lean maturity, marbling, quality grade, fat thickness, ribeye area, kidney pelvic and heart fat (**KPH**), preliminary yield grade, final yield grade and dressing percentage.

Plasma Fatty Acid Composition

Total lipid of 1 mL of plasma was extracted by method of Folch et al. (1957). The fatty acids were methylated as described by Morrison and Smith (1964), and the resulting fatty acid methyl esters (**FAME**) were analyzed using a Varian gas chromatograph (model CP-3800 fixed with a CP-8200 autosampler, Varian Inc., Walnut

Creek, CA). Fatty acid methyl esters were separated with a fused silica capillary column CP-Select [100 m • 0.25 mm (i.d.)] (Chrompack Inc., Middleburg, The Netherlands), with helium as the carrier gas. The injector temperature and flame ionization detector temperatures were 270 and 300°C, respectively with a 60:1 split:splitless ratio on the injector. Total run time was 48 min with the first 32 min at 180°C, and then increased at 20°C/min to 225°C. Individual FAME were identified using multiple standards from Nu-Chek Prep, Inc. (Elysian, MN). Individual FAME were quantified as a percentage of total FAME analyzed.

Statistical Analysis

The GLM procedure of SAS (SAS Inst. Inc., Cary, NC) was used for statistical analysis of data. The data for plasma fatty acid composition were analyzed as a split plot design with grain source, WCS inclusion rate, and the grain x WCS interaction as whole plot variables and day of treatment and the associated two-way interactions as the sub-plot. The three way day x grain x WCS was not significant and was not included in the model, without significantly altering the error sum of squares ($P > 0.05$). The model for all other variables included the following independent variables: block, grain source, WCS inclusion rate, and the grain source x WCS inclusion rate interaction. When treatment effects were significant ($P < 0.05$), means were separated using the pdiff statement of GLM; and a tendency for treatment to elicit a response was noted when $P < 0.10$.

Results

The diets for this study were formulated to be as similar in nutritional composition as was feasible (Table 1). As such, there was little difference in the protein concentrations of the diets, but a noticeable difference in the amount of dietary fat provided by each of the diets. For this reason, these feedstuffs inevitably altered the overall dietary energy concentration, which ranged from 1.74 to 1.91 Mcal NEm/kg and 1.00 to 1.19 Mcal NEg/kg. Yet, these feedstuffs were successful in altering the dietary fatty acid composition as desired (Table 3.2), with the flaxseed diet containing approximately 23% of the total fatty acids as 18:3n-3.

Table 3.2. Fatty acid composition of diets[a]

	Corn			Flax			Milo		
	0	5	15	0	5	15	0	5	15
	g/100g[b]								
12:0	0.02	0.02	0.03	0.02	0.02	0.01	0.04	0.04	0.03
14:0	0.15	0.33	0.57	0.09	0.32	0.37	0.18	0.46	0.72
16:0	14.10	16.89	20.65	9.28	14.39	14.93	15.88	19.34	23.15
18:0	2.64	2.64	2.62	3.40	3.04	3.09	2.16	2.35	2.48
18:1n-9	24.26	22.90	21.23	20.62	20.87	20.03	31.58	27.08	21.99
18:2n-6	56.87	55.76	53.87	34.52	43.97	41.26	47.66	49.08	50.08
18:3n-3	1.72	1.41	0.97	32.01	17.37	20.29	2.41	1.61	1.46
CLA *cis*9,*trans*11	nd	0.01	0.03	nd	nd	nd	0.05	0.01	nd

[a] Values represent arithmetic mean of duplicate as-fed samples.
[b] Values are g/100 g of identified fatty acids.
[c] Not detected

As intended in the formulation of the diets, there were significant differences in plasma fatty acid composition. Plasma 14:0 (Table 3.3) was higher ($P < 0.01$) in the corn- and flaxseed-fed steers (0.90 and 0.71, respectively) than in the milo-fed steers (0.66).

Table 3.3 Percentage of total fatty acids from plasma of Angus steers fed corn, flaxseed with corn, or milo based finishing rations with varying concentrations of whole cottonseed (**WCS**).

	Grain/%WCS												
	Corn			Flaxseed			Milo				*P*-value		
Item	0%	5%	15%	0%	5%	15%	0%	5%	15%	SE	Grain	WCS	GxWCS[y]
14:0	0.94	0.85	0.92	0.72	0.65	0.75	0.64	0.61	0.72	0.05	0.01	0.14	0.91
16:0	17.5	17.5	18.1	14.9	14.9	15.8	16.3	15.5	16.2	0.41	0.01	0.09	0.78
16:1	0.78	0.80	0.70	0.74	0.63	0.66	0.64	0.62	0.65	0.04	0.05	0.64	0.69
18:0	30.6	31.5	31.5	32.5	33.8	31.3	38.3	37.0	35.5	0.74	0.01	0.32	0.39
18:1*trans*11	1.52	1.07	0.37	1.23	1.26	1.59	0.49	0.50	0.77	0.22	0.02	0.78	0.13
18:1n-9	8.19	7.18	6.69	6.96	7.77	6.84	8.11	7.83	8.53	0.42	0.11	0.70	0.31
18:2	40.2	40.8	41.3	38.2	35.8	37.8	35.1	37.6	36.7	0.97	0.01	0.66	0.31
18:3	0.38	0.40	0.32	4.77	5.25	5.24	0.43	0.35	0.85	0.16	0.01	0.14	0.18

[y] Grain x percentage whole cottonseed interaction.

[z] Not detected.

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Even though there were detectable amounts of 18:1*trans*-11 in the plasma, there were no detectable CLA isomers in any of the plasma samples analyzed.

There was a decrease in plasma 16:0 by d 84 and d 107 (Figure 3.1), and both the flaxseed- and milo-fed steers had a greater decline than did the corn-fed steers (Day x Grain, $P < 0.01$). Unlike plasma 16:0, plasma 18:0 (Figure 3.2) was greatest in the milo-fed steers at all timepoints after treatment had begun. There were no apparent differences between the corn- and flaxseed-fed steers, except for a transient difference at d 84 (Day x Grain, $P < 0.01$). Plasma 18:1n-9 (Figure 3.3) appeared to vary until d 84 and d 107, at which time, the milo-fed steers had a greater percentage 18:1n-9 than either the corn- or flaxseed-fed steers. Plasma 18:1*trans*-11 (Figure 3.4) initially increased in both the corn and flaxseed steers by d 28 and d 56, whereas, in milo fed steers the 18:1*trans*-11 decreased to nearly undetectable levels. Only steers fed flaxseed maintained elevated levels of 18:1*trans*-11 (Day x Grain, $P < 0.01$). The percentage of plasma 18:2n-6 (Figure 3.5) was consistently greater in corn-fed steers than in the other treatment groups, until d 107, at which time it did not differ from the flaxseed-fed steers (Day x Grain, $P < 0.01$). Furthermore, there was a marked increase in plasma 18:2n-6 by d 84. As expected, the steers fed flaxseed exhibited a continual increase in plasma 18:3n-3 during the timecourse of the study (Figure 3.6), whereas plasma 18:3n-3 was nearly undetectable in the milo- and corn-fed steers (Day x Grain, $P < 0.01$).

There also were several changes in plasma fatty acids over the time of the experiment due to WCS inclusion. The 5% WCS fed steers tended ($P < 0.09$) to have less plasma 16:0 (15.95 g/100 g fatty acids) than the steers fed either 0 or 15% WCS

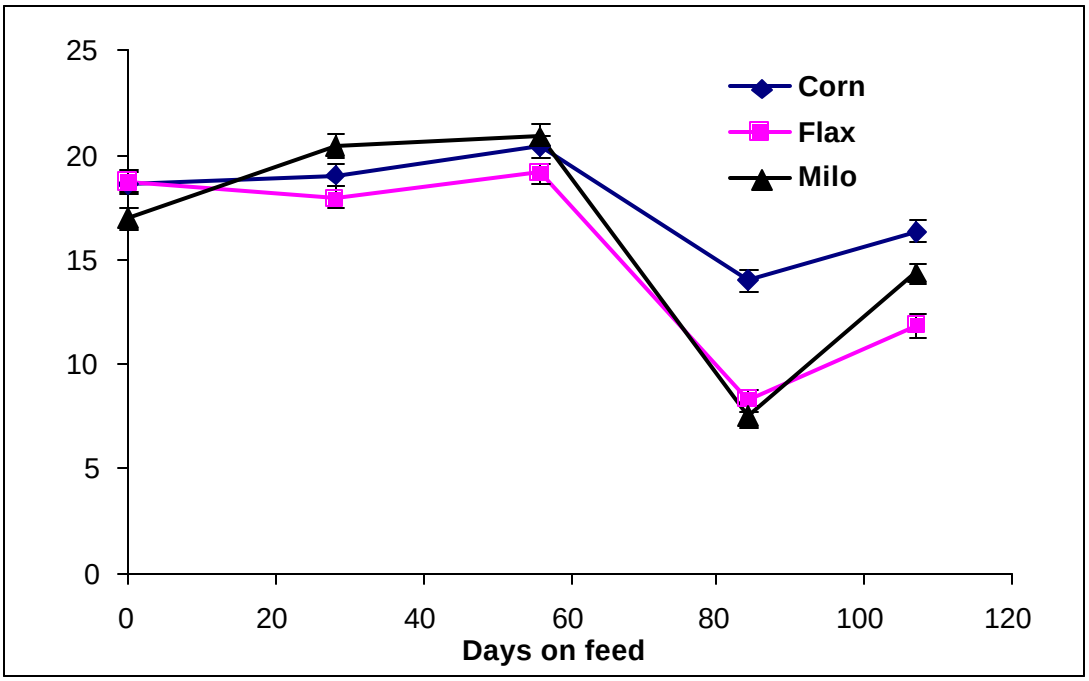

Figure 3.1 Plasma 16:0 from Angus steers fed corn, flaxseed (**Flax**), or milo based finishing diets over a 107-d period.

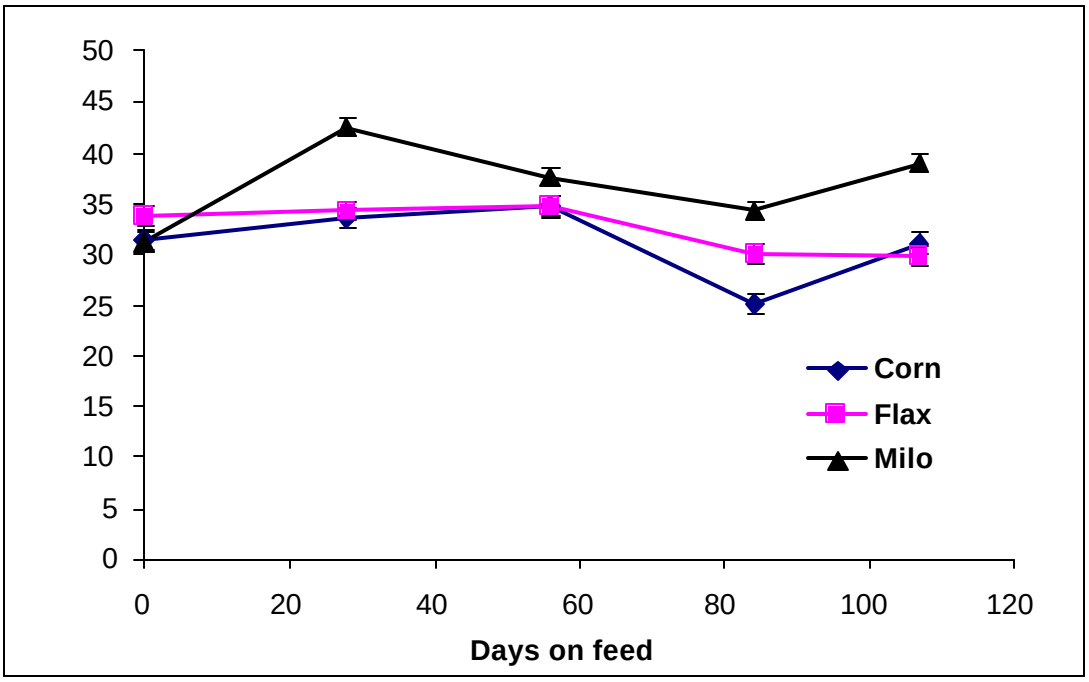

Figure 3.2 Plasma 18:0 from Angus steers fed corn, flaxseed (**Flax**), or milo based finishing diets over a 107-d period.

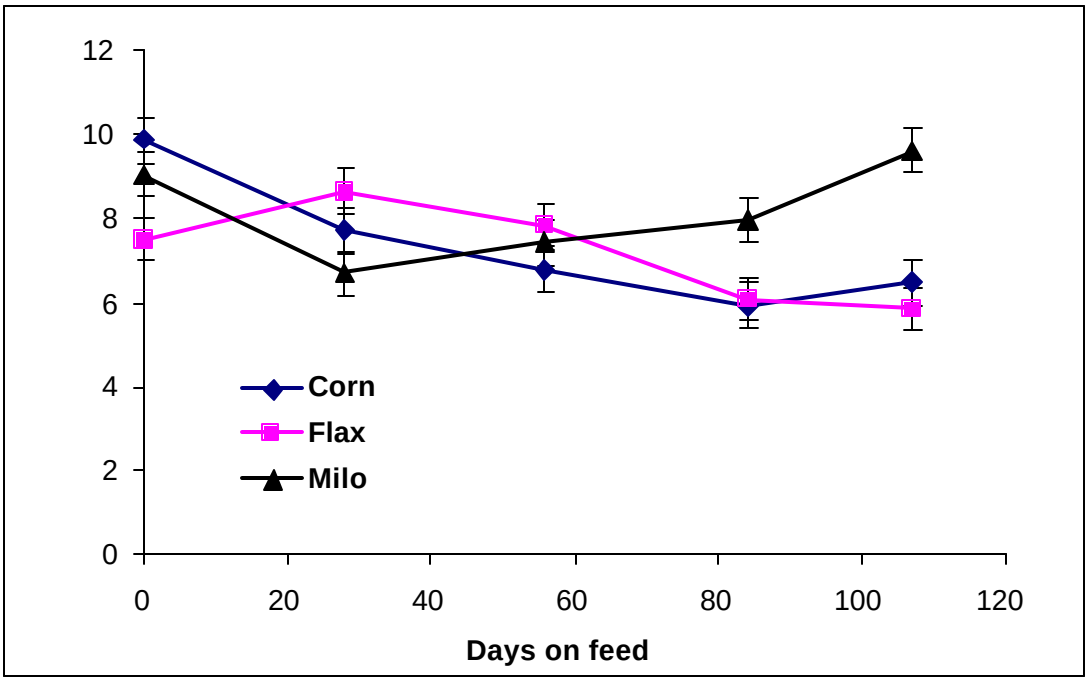

Figure 3.3 Plasma 18:1n-9 from Angus steers fed corn, flaxseed (**Flax**), or milo based finishing diets over a 107-d period.

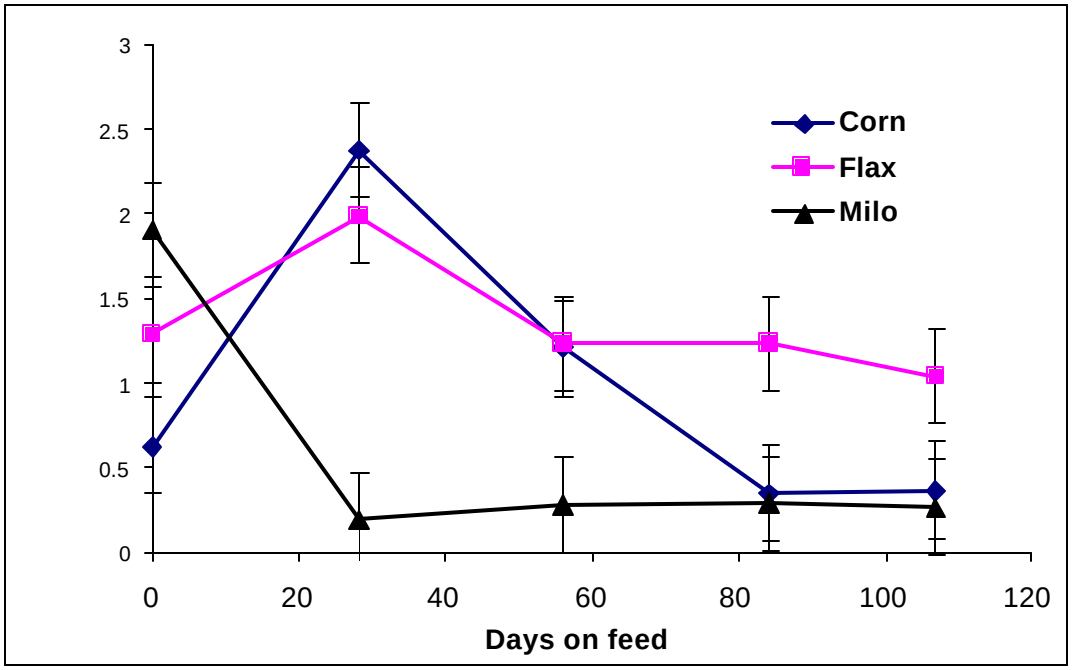

Figure 3.4 Plasma 18:1*trans*-11 from Angus steers fed corn, flaxseed (**Flax**), or milo based finishing diets over a 107-d period.

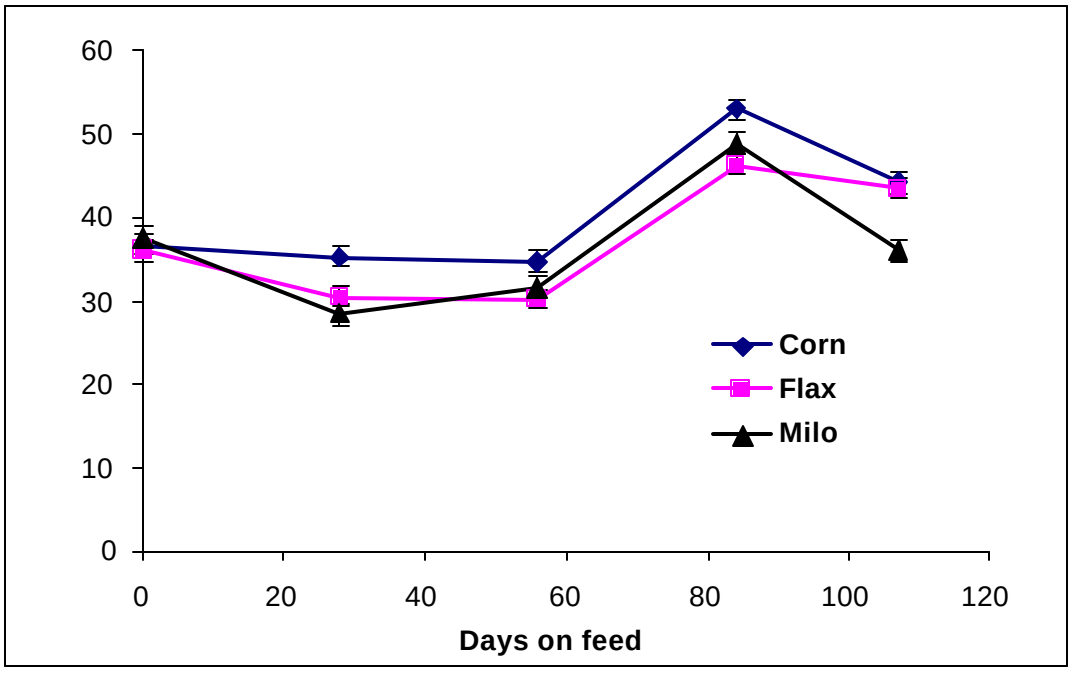

Figure 3.5 Plasma 18:2n-6 from Angus steers fed corn, flaxseed (**Flax**), or milo based finishing diets over a 107-d period.

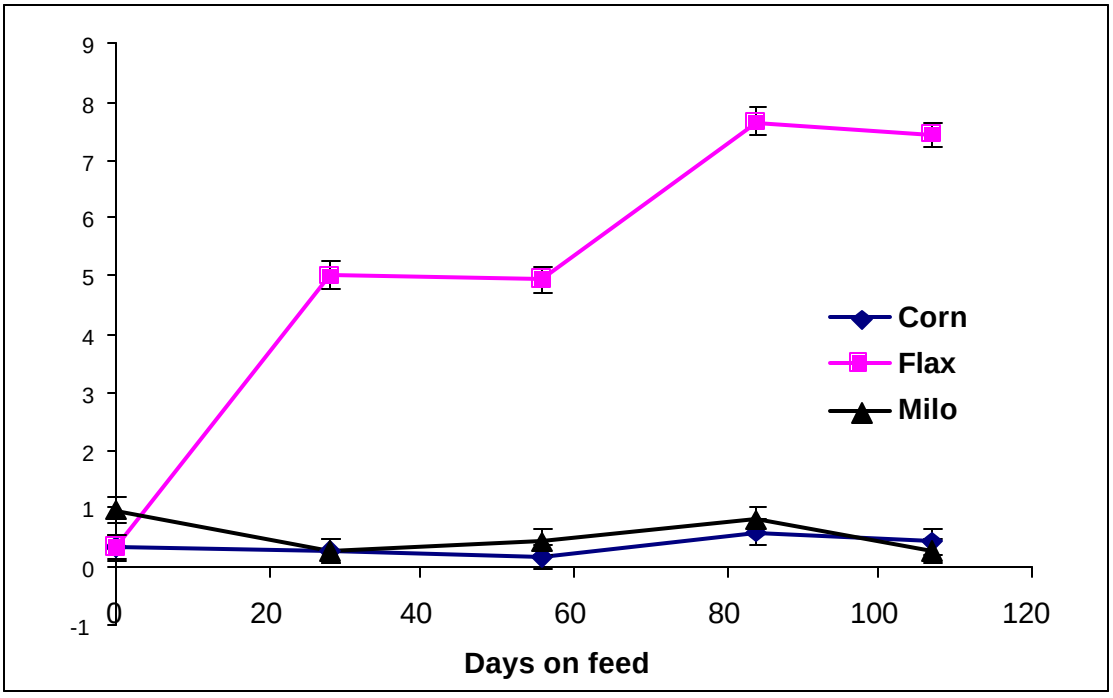

Figure 3.6 Plasma 18:3n-3 from Angus steers fed corn, flaxseed (**Flax**), or milo based finishing diets over a 107-d period.

(16.24 and 16.72 g/100g fatty acids, respectively). Steers receiving 15% WCS had lower plasma 16:1n-6 (Figure 3.7) than the other two treatments until d 84. At that time, plasma 16:1n-6 decreased at all WCS levels (Day x WCS, $P < 0.01$). Steers receiving 15% WCS had less plasma 18:0 (Figure 3.8) than the other two treatments at d 84 and 107 (Day x WCS, $P < 0.01$). The concentration of 18:1*trans*-11 decreased by d 28 in steers receiving 15% WCS, by d 56 in the steers receiving 5% WCS, and by d 84 in steers not receiving WCS (Day x WCS, $P < 0.01$).

Even though there were differences in dietary energy and ether extract, there were no differences ($P > 0.10$) in final live weight (Table 3.4, 472 kg) or ADG (1.14 kg/d). However, there tended ($P < 0.06$) to be a decrease in feed efficiency with increased WCS in the milo fed steers from (0.11 to 0.08 kg gain/kg feed), while there was no effect of WCS on gain:feed in the steers receiving either the corn or flaxseed. The steers fed flaxseed had less ($P < 0.01$) shrink (1.51%) compared to either the corn (2.89%) or milo (3.11%) fed steers. There was also a decrease ($P < 0.05$) in shrink from 3.26 to 1.86% when steers received 0 or 5% WCS.

Hot carcass weight tended ($P < 0.10$; Table 3.5) to be less for milo-fed steers (286 kg) than the flaxseed-fed steers (307 kg). There were no differences in the skeletal maturity of the steers, although lean maturity decreased (grain x WCS, $P < 0.02$) from A^{84} to A^{60} in the milo fed steers as WCS increased from 0 to 5%, decreased from A^{76} to A^{54} in the corn fed steers receiving 0 or 15% WCS, and remained unchanged in the steers fed flaxseed. Also, two steers fed the combination of milo and 15% WCS were deemed to be dark cutters, and thus lean maturity was not assessed. However, these

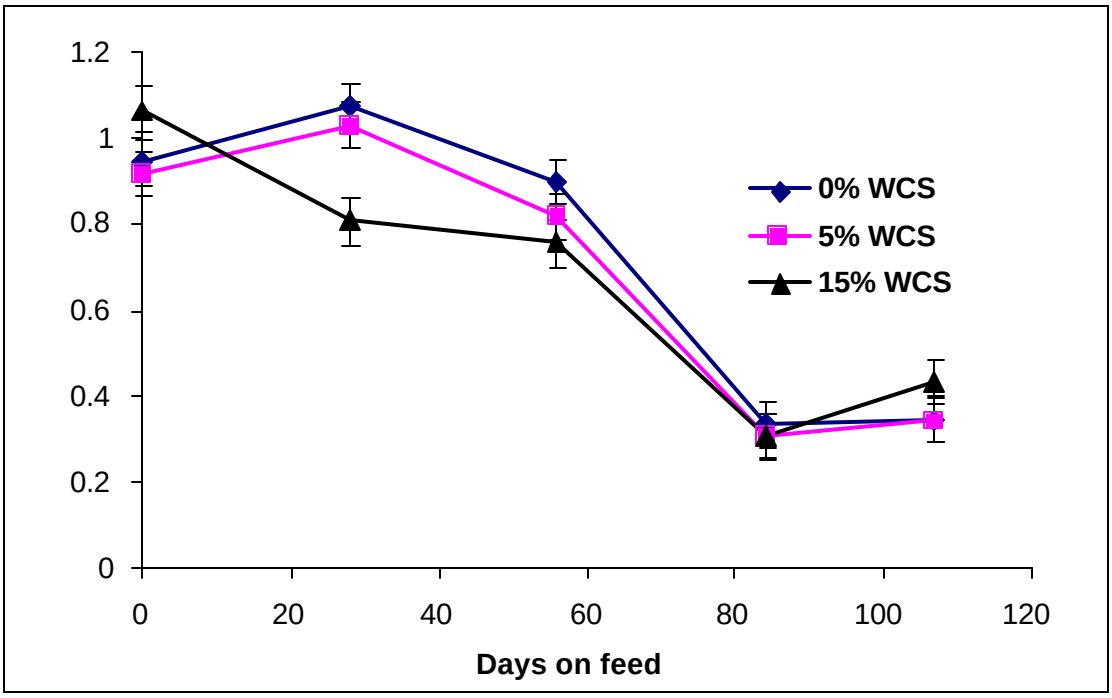

Figure 3.7 Plasma 16:1n-7 from Angus steers fed either 0, 5, or 15% dietary whole cottonseed (**WCS**) over a 107-d period.

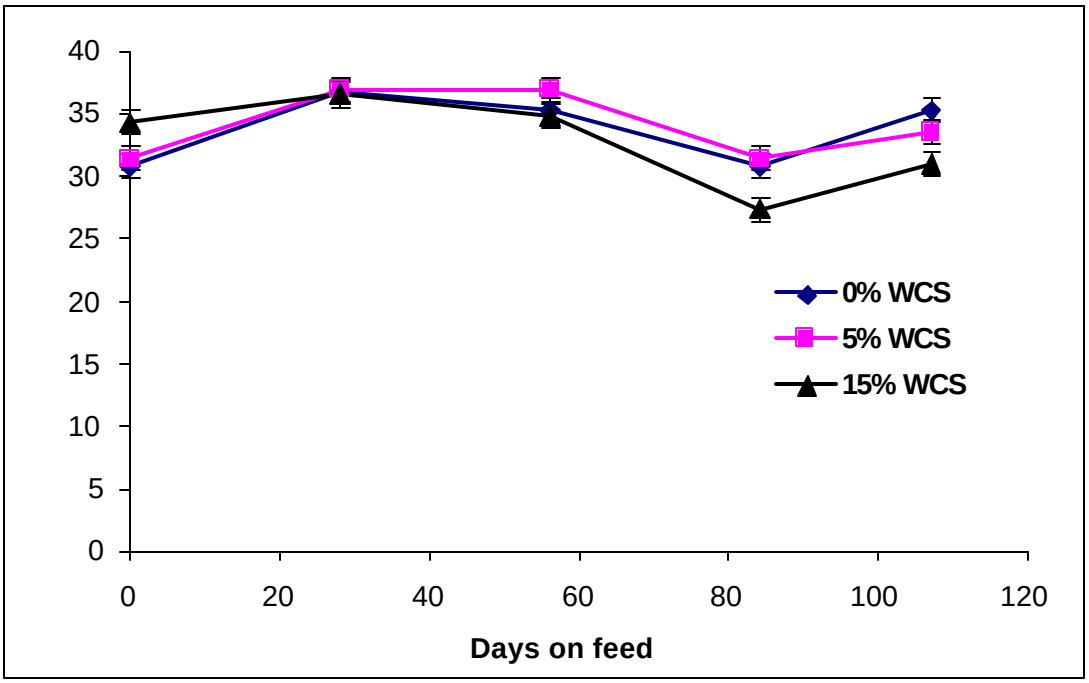

Figure 3.8 Plasma 18:0 from Angus steers fed either 0, 5, or 15% dietary whole cottonseed (**WCS**) over a 107 d period.

Table 3.4 Growth characteristics of Angus steers fed one of three grain sources (corn, corn with flaxseed, or milo) and one of three concentrations (0, 5, or 15 %DM) of whole cottonseed (**WCS**) in a total mixed ration.

	Grain/%WCS												
	Corn			Flaxseed			Milo				*P*-value		
Item	0%	5%	15%	0%	5%	15%	0%	5%	15%	SE	Grain	WCS	GxWCS[z]
Live wt., kg	450	469	490	488	499	480	460	452	463	18	0.39	0.88	0.63
ADG, kg	0.94	1.01	1.27	1.19	1.24	1.08	1.04	0.95	1.51	0.26	0.88	0.50	0.69
Feed efficiency, kg gain/kg fed	0.11	0.10	0.11	0.11	0.13	0.11	0.11	0.09	0.08	0.01	0.01	0.27	0.06
Shrink, %	3.32	1.33	4.02	2.36	1.34	0.83	4.09	2.91	2.32	0.68	0.01	0.05	0.11

[z] Grain x percentage whole cottonseed interaction.

Table 3.5 Carcass characteristics from Angus steers fed one of three grain sources (corn, corn with flaxseed, or milo) and one of three concentrations (0, 5, or 15 %DM) of whole cottonseed (**WCS**) in a total mixed ration.

	Grain/%WCS												
	Corn			Flaxseed			Milo				*P*-value		
Item	0%	5%	15%	0%	5%	15%	0%	5%	15%	SE	Grain	WCS	GxWCS[z]
Hot carcass wt., kg	283	292	310	308	314	300	285	283	290	12	0.10	0.72	0.58
Skeletal maturity[a]	168	158	160	160	154	168	154	156	150	5	0.13	0.56	0.35
Lean maturity[ad]	176[ef]	170[efg]	154[g]	156[g]	166[fg]	166[fg]	184[e]	160[fg]	165[efg]	8	0.37	0.13	0.02
Overall maturity[a]	172	164	160	160	158	166	168	158	154	5	0.36	0.15	0.26
Marbling[b]	470	502	394	532	364	396	430	408	426	44	0.63	0.14	0.16
Quality grade[c]	407	395	373	436	360	385	393	376	395	25	0.61	0.37	0.45
Fat thickness, cm	0.95	1.46	1.28	1.67	1.20	1.46	1.18	1.13	1.31	0.21	0.33	0.85	0.27
Ribeye area cm^2	72.19	72.98	80.47	76.13	78.90	77.84	70.48	67.46	69.82	0.43	0.01	0.33	0.41
KPH, %	2.2	2.5	2.1	2.4	2.9	2.7	2.1	2.4	2.7	0.3	0.20	0.25	0.66
Final Yield grade	2.78	3.38	2.89	3.48	3.21	3.22	3.12	3.21	3.38	0.27	0.41	0.80	0.50
Dressing %	62.9	62.3	63.3	63.2	63.0	62.6	61.9	62.6	62.4	0.6	0.47	0.96	0.68

[a] A = 100; B = 200; C = 300; D = 400; E = 500.

[b] Practically Devoid = 100; Traces = 200; Slight = 300; Small = 400; Modest = 500; Moderate = 600; Slightly Abundant = 700; Moderately Abundant = 800; Abundant = 900.

[c] Standard = 200; Select =300; Choice = 400; Prime = 500.

[d] Two steers in the Milo 15 treatment group were deemed dark cutters and lean maturity was not assessed.

[efg] Least square means without a common superscript differ ($P < 0.05$).

[z] Grain x percentage whole cottonseed interaction.

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differences were not enough to influence overall maturity, which was unaffected by treatment. Similarly, marbling was not affected by treatment, which combined with the lack of effect on maturity, led to an overall lack of effect on quality grade (Select[91]). There was no dietary treatment effect on fat thickness (1.29 cm). However, the ribeye area of steers fed milo (69.25 cm^2) was less ($P < 0.01$) than that of steers fed either the corn (75.21 cm^2) or flaxseed (77.62 cm^2) diets. The percentage of KPH (2.4%) and yield grade (3.19%) were not affected by treatment. Similarly, there was no effect due to treatment on dressing percentage either (62.7%). There were no liver condemnations or other carcass defects

Discussion

There has been substantial interest in increasing the amount of CLA in animal products for human consumption. Thus far, research has focused primarily on feeding CLA directly to monogastric animals (Park et al., 1999; Blankson et al., 2000) or on increasing the CLA content of ruminant products such as milk (Corl et al., 2001; Duckett et al., 2002). This interest is the largely result of research documenting the numerous beneficial effects of CLA in animal and human models. These effects include the anticarcinogenic effects of both the *cis*-9, *trans*-11 and *trans*-10, *cis*-12 isomers (Pariza and Hargraves, 1985; Ha et al., 1987; Ip et al., 2002), and the body composition repartitioning action of the *trans*-10, *cis*-12 isomer of CLA (Park et al., 1999; Ostrowska et al., 1999). There are several studies supporting the theory of endogenous formation of the *cis*-9, *trans*-11 isomer of CLA by SCD in dairy cattle (Corl et al., 2001; Griinari et al., 2000). Although these and others (Duckett et al., 2002) typically have shown an

increase in ruminal 18:1*trans*-11 concentration, followed by a subsequent rise in either plasma or milk 18:2*cis*-9,*trans*-11, we were unable to detect 18:2*cis*-9,*trans*-11 in plasma, although 18:1*trans*-11 was readily apparent in all treatments except for the milo-fed steers. This suggests that either our sensitivity is not high enough to detect 18:2*cis*-9,*trans*-11 or that the proposed alteration of 18:1*trans*-11 is not occurring in the first-pass metabolism of the intestine. The ground milo diet provided a greater surface area for more complete ruminal biohydrogenation of unsaturated fatty acids to 18:0 to occur, which may be the reason why there was no increase in the plasma 18:1*trans*-11 of these steers even though there was reasonably similar precursor (18:2n-6) concentrations in all diets.

The plasma 18:2n-6 content was similar in magnitude to the results of Chang et al. (1992). Much like that study, the plasma fatty acids of the steers in this study reflected the dietary fatty acid composition. While there was an understandable reduction to unsaturated fatty acids due to ruminal biohydrogenation, substantial differences in dietary fatty acid composition were reflected in similar alterations in plasma fatty acid composition. The most notable example of this was the elevated 18:3n-3 in the steers fed flaxseed. In addition, although there was elevated plasma 18:3n-3 by d 28, this fatty acid continued to increase until d 84. This suggests that studies of initial changes in metabolism or gene expression due to increased 18:3n-3 could see results as early as 28 d after feeding flaxseed-containing diets.

Supplementation of fat to finishing diets for cattle is generally limited to 5% to prevent decreased feed intake and animal performance (Esplin et al., 1963; Garrett et al.,

1976). However, Zinn (1989) demonstrated increased weight gain and feed efficiency when steers were fed up to 8% yellow grease or blended animal-vegetable fat. All the diets in this study were lower than 8%, except the flaxseed diet containing 15% WCS. The milo diet had the lowest lipid content and milo-fed cattle had the lowest feed efficiency. The tendency for feed efficiency to decrease with increasing WCS in the milo-fed steers may have resulted from a moderate ruminal acidosis brought on by the increased lipid inclusion in conjunction with the fine particle size of the ground milo. The response of corn-based diets to fat supplementation has been less consistent than diets based on other grains, and this may be the result of the higher basal energy from corn than is in other grain sources, such as milo (Krehbiel et al., 1995a,b; Andrae et al., 2000). However, the milo used in this study was more thoroughly processed than the other grains, which should increase its digestibility, and thus, overall energy derived from this diet. Therefore, it is likely that the differences between milo-fed cattle and the other treatment groups were not simply due to alterations in the energy content of the diet.

Shrink was decreased by both flaxseed and WCS inclusion in the diet. Both of these factors increased the total ether extract of the diets. As such, it is feasible to assume that this may have played in integral role in this reduction in loss. One possibility would be the substitution of lipid for other macronutrients, which would provide additional energy during the final overnight feed withdrawal.

Carcass composition of the steers from this study was within the range of what is currently being produced by the industry, according to the most recent National Beef

Quality Audit (**NBQA**, McKenna et al., 2002). However, according to the NBQA, the carcasses of the steers from this study would rank in the lower 18% of fed cattle. This was reflective of the relatively short feeding period used in the present study. This is supported by the consistently young maturity of all the animals in the study. Cianzio et al. (1985) reported that intramuscular adipose tissue develops later than subcutaneous, intermuscular, or internal fat. Therefore, it may be that there was insufficient time for treatments to affect carcass composition and quality due to the short feeding period.

Implications

These data indicate that rations formulated to provide increased levels of α-linolenic acid (i.e., flaxseed) will increase feed efficiency without altering either the quality or composition of the beef carcasses. Additionally, the inclusion of WCS in milo diets may cause a decrease in efficiency of gain and less salable lean. However as evidenced by the very young lean and skeletal maturity of these animals, possible differences in treatment may not have become apparent because the tissues did not have adequate time to adapt the changes induced by treatment. This implies that future studies of this nature should be much lengthier in time frame.

CHAPTER IV

STEAROYL CO-A DESATURASE ACTIVITY OF BEEF STEERS FINISHED WITH CORN, FLAXSEED, OR MILO BASED DIETS DOES NOT CORRELATE WITH INDICES OF STEAROYL CO-A DESATURASE ACTIVITY BASED ON FATTY ACID RATIOS

Overview

Forty-five Angus steers (358 kg BW) were used in a completely randomized block design with a 3 x 3 factorial arrangement of treatments to evaluate the hypothesis that dietary α-linolenic acid (from corn, flaxseed plus corn, or milo) and whole cottonseed (**WCS**) inclusion (0, 5, or 15% DM) would interact to alter fatty acid metabolism and deposition of conjugated linoleic acid (**CLA**) in subcutaneous (**s.c.**) and interfasicular (**i.f.**) adipose tissues. Lipogenesis from acetate in s.c. adipose tissue was greater ($P < 0.01$) in steers fed flaxseed (5.42 nmol•h^{-1}•10^5 cells^{-1}) than in the corn (3.10 nmol•h^{-1}•10^5 cells^{-1}) or milo (1.92 nmol•h^{-1}•10^5 cells^{-1}) groups. Steers fed flaxseed or corn had a larger i.f. mean adipocyte volume ($P < 0.01$) than those fed milo and tended to have larger s.c. adipocytes ($P < 0.06$). There was an interaction ($P < 0.05$) wherein i.f. oleic acid (18:1n-9) was largely unchanged with increasing levels of WCS in the steers fed the corn or milo diets, but 18:1n-9 was decreased with increasing WCS in the steers fed the flaxseed diet. The i.f. CLA *cis*-9, *trans*-11 CLA percentage increased with increasing WCS in the steers fed the corn diet, whereas it remained unchanged or even decreased slightly in the steers fed the flaxseed or milo-based diets (interaction, $P < 0.02$). Steers fed flaxseed had greater ($P < 0.01$) s.c. adipose concentration of vaccenic

acid (18:1*trans*-11) than the steers fed milo and tended ($P < 0.07$) to have greater amount of vaccenic acid than steers fed corn alone. Steers fed flaxseed also had greater ($P < 0.01$) s.c. and i.f. percentages of the α-linolenic acid (18:3n-3) than steers fed either of the other grain sources. Stearoyl-CoA desaturase (**SCD**) activity in s.c. adipose tissue was unchanged between the 0% WCS group (88.1 nmol•mg protein^{-1}•7 min^{-1}) and the 15% WCS group (20 nmol•mg protein^{-1}•7 min^{-1}). Also, there was no correlation between SCD activity and s.c. SCD activity indices based on s.c. fatty acid composition (all, $P > 0.66$). The increases in saturated fatty acids in s.c. adipose tissue appeared to be a result of the decreased SCD activity in s.c. adipose tissue with increased inclusion of WCS, but this did not occur with increased dietary α-linolenic acid.

Introduction

The Dietary Reference Intakes (**DRI**, 2002) state that high levels of dietary saturated and total fat predispose people to an increased risk for heart disease. The DRI (2002) recommend that a diet low in saturated fat (< 20% of fat energy) can be accomplished by reducing the amount of animal fat intake. As such, there would be a definite benefit to producing beef with less total fat to minimize the reduction in beef intake in the above recommended diet.

Whole cottonseed (**WCS**) contains sterculic acid, which is a potent inhibitor of stearoyl CoA desaturase (**SCD**; Raju and Reiser, 1972). It has also been demonstrated that the *trans*-10, *cis*-12 isomer of conjugated linoleic acid (**CLA**) depresses SCD gene expression (Lee et al., 1998). Therefore, we postulated that the combined effects of dietary WCS and a CLA precursor, α-linolenic acid, could be sufficient to produce the

reduction in SCD activity that has been documented in adipose tissue of cattle with "hard fat" (Yang et al., 1999). Martin et al. (1999) previously demonstrated that the activity of SCD increases immediately preceding lipid filling of s.c. adipose tissue in feedlot Angus steers. Ding and Mersmann (2001) demonstrated that oleic acid (the primary product of SCD) stimulated lipid filling in porcine preadipocytes in culture. Thus, depression of SCD activity may ultimately lead to reduced adiposity in the finished carcass.

The purpose of this investigation was to document the interaction between dietary α-linolenic acid (a natural precursor for CLA production in ruminant systems) and WCS on SCD activity in bovine tissues, and how this may affect lipid synthesis. Our hypothesis was that providing increased dietary α-linolenic acid in combination with WCS would yield a leaner beef product that contains elevated concentrations of CLA. This would provide a product for beef consumers with less trimmable fat in combination with an increased concentration of CLA.

Materials and Methods

Animals and Experimental Procedures

Forty-five Angus steers (358 kg BW) were used in this experiment; care, handling and sampling of the steers was approved by the Texas A&M University IAACUC (AUP#2001-75). Steers were assigned randomly to pens and treatments on d 1 after arriving at the feedlot. Dietary treatments consisted of a 3 x 3 factorial arrangement of grain source and whole cottonseed inclusion (0, 5, or 15% DM) in the diet. The three grain sources included a cracked corn diet, a cracked corn diet that

contained 10% DM as flaxseed, and a ground milo diet. All diets were formulated to be isonitrogenous, and meet or exceed all nutrient requirements for growing steers (NRC, 1996). Steers were housed in groups of three in partially covered pens equipped with individual Calan gate feeders (American Calan, Northwood, NH). All diets were fed once daily in the morning in amounts adequate to allow ad libitum access to feed. Dietary composite samples were collected every 28 d for estimates of as-fed fatty acid composition. Steers were gradually switched over a 7-d period to a high-concentrate finishing diet (the corn based, 0% whole cottonseed diet). Following this adjustment (d 1 of treatment), steers were switched over a 7-d period to their respective treatment diets. Steers were blocked by final live weight with one steer from each treatment in each block. Each block of steers were slaughtered on sequential days.

Immediately following exsanguination, a section of hide covering the dorsal area on the left side, between the 5^{th} and 8^{th} ribs was removed. The longissimus muscle and the associated s.c. adipose from this region was excised and immediately placed in 1x Krebs-Henseleit bicarbonate buffer (pH = 7.4) with 5 mM glucose at 37°C. This sample was transported to the laboratory within 20 min, and s.c. and interfascicular (**i.f.**) adipose tissues were immediately dissected for analysis. Adipose samples used for lipogenesis from acetate were used immediately. All other adipose samples were processed and stored at –80°C until further analysis.

After evisceration, approximately 0.5 m of the duodenum immediately caudal from the pyloric sphincter was removed and emptied of contents. This sample was transported immediately to the laboratory on ice, rinsed gently with 1 x Krebs-Henseleit

bicarbonate buffer (pH = 7.4), and then lightly scraped to detach the epithelial cells, without incorporating the smooth muscle cells. These samples were weighed, processed, and stored at –80°C until further analysis.

Chemicals

All radiological chemicals were purchased from American Radiolabled Chemicals, Inc. (St. Louis, MO). All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO). All fatty acid standards were purchased from Nu-Chek Prep, Inc. (Elysian, MN). All solvents were AR grade or greater.

Lipogenesis

Lipogenesis from acetate was conducted as described by Page et al. (1997). Briefly, 1 g of tissue was incubated in 5 mM sodium acetate, 5 mM glucose, 10 mM Hepes buffer and 1 μCi [1-^{14}C] Acetate for 2 h at 37°C. The reaction was stopped by adding 3 mL of 5% trichloroacetic acid. Lipids were then extracted and radioactivity counted using a Beckman liquid scintillation spectrometer (Beckman, Palo Alto, CA). Rate of acetate incorporation was calculated and expressed on a cellular basis using the results from the cellularity analyses.

Fatty Acid Composition

Total lipid was extracted by the Folch et al. (1957) method. The fatty acids were then methylated as described by Morrison and Smith (1964), and the resulting fatty acid methyl esters (**FAME**) were analyzed using a Varian gas chromatograph (model CP-3800 fixed with a CP-8200 autosampler, Varian Inc., Walnut Creek, CA). Fatty acid methyl esters were separated with a fused silica capillary column CP-Sil88 [100 m • 0.25

mm (i.d.)] (Chrompack Inc., Middleburg, The Netherlands), with helium as the carrier gas. The injector temperature and flame ionization detector temperatures were 270 and 300°C, respectively. Total run time was 48 min with the first 32 min at 180°C, and then increased at 20°C/min to 225°C. Individual FAME were quantified as a percentage of total FAME analyzed.

Several indices of SCD activity were calculated using a variation of the estimator based on FAME ratios described by Corl et al. (2001). A total index was calculated as (14:1n-5 + 16:1n-7 + 18:1n-9 + 18:2*cis*-9,*trans*-11)/(14:0 + 16:0 + 18:0 + 18:1*trans*-11). In addition, indices based on 14 carbon (14:1n-5/14:0), 16 carbon (16:1n-7/16:0), 18 carbon (18:1n-9/18:0), and CLA (18:2*cis*9,*trans*11/18:1*trans*11) product:precursor ratios were also calculated.

Microsome Extraction and Stearoyl-CoA Desaturase Assay

Microsomal fractions of s.c. adipose, i.f. adipose, and intestinal mucosal tissues were prepared by homogenizing each tissue sample (1 to 1.5 g) with a Virtis homogenizer (The Virtis Company, Inc., Gardiner, N.Y.) in three volumes (wt/vol) of buffer. The buffer (pH = 7.4) used for microsomal extraction contained 0.25 M sucrose, 0.01 M potassium phosphate, 1 mM EDTA, and 1mM dithioerythritol. The homogenate was then centrifuged for 15 min at 5,000 x g. The supernate was decanted into a separate tube and the pellet and fat cake were discarded. This supernate was centrifuged for 30 min at 17,300 x g, and decanted into a separate tube, which was then centrifuged for 1 h at 104,000 x g. The supernate was discarded, the microsomal fraction was

collected and resuspended in 100 mM Tris-HCl buffer (pH = 7.4), snap frozen with liquid nitrogen, and stored at –80°C until further analysis.

The SCD activity of these fractions was determined as described by St. John et al. (1991) with modifications. The microsomal fractions were thawed in a 37°C water bath and 0.5 mL was incubated in a total volume of 1.5 mL of a solution containing 100 mM Tris-HCl (pH 7.4), 2 mM NADPH, 0.025 µCi [1-^{14}C]palmitoyl-CoA, and 0.25 µM palmitoyl-CoA. The incubations were continued for 7 min in a 37°C water bath and terminated by the addition of 3 mL of 10% KOH in MeOH. The samples were immediately placed in a 70°C water bath for 30 min and then acidified with 9 mL of 3 N HCl. Fatty acids were then extracted by three washes with 6 mL of n-pentane. The pentane phases were evaporated under nitrogen and methylated with 14% boron trifluoride in MeOH for 30 min at 70°C. Methyl esters were removed by the addition of 3 mL of distilled deionized water and then extracted by three washes with hexane. The hexane phases were evaporated under nitrogen and resuspended in 0.1 mL of hexane and separated by thin layer chromatography on a 5% $AgNO_3$ impregnated silica gel plate in a petroleum ether:diethylether solvent system (97:3, vol/vol). After separation, the plate was sprayed with 0.2% dichlorofluoroscein in ethanol to visualize the spots. The spots were scraped and counted using a Beckman liquid scintillation spectrometer.

Blanks (containing no microsomes) typically contain measurable dpm in the 16:1 spot, although no SCD product can be formed in the absence of microsomes. Therefore, the separation of FAME by the thin layer chromatography was confirmed by developing four preparations of an equal mixture of non-labeled 16:0:16:1 n-9 mixture with the

same developing system. The methyl esters from the spots were then extracted with hexane and analyzed by gas chromatography. This confirmed that there was 37.5% of the 16:0 remaining in the 16:1 spot. Because the spots were discrete with little tailing, we presume that radioactivity in the 16:1 spot of the blanks represents contamination of the radiolabeled palmitoyl-CoA. Therefore, all reaction 16:1 spots were corrected proportionately.

Cellularity

Adipocyte size, volume, and number were determined by the method of Etherton et al. (1977) with the modifications of Prior (1983). Adipose samples (0.1 g) frozen in liquid nitrogen were sliced into 1-mm thick sections on a chilled glass surface and placed in 20-mL scintillation vials coated with Sigmacoat (Sigma Chemical Co., St. Louis, MO). The tissues were rinsed three times with 37°C 0.154 M NaCl at 30-min intervals to remove free lipid. After the last rinse, 0.6 mL of 50 mM collidine-HCl buffer (pH 7.4) was forcibly added to each sample, followed by 1 mL of 3% osmium tetroxide in the collidine-HCl buffer. The samples were then incubated at 37°C for 96 h and then the osmium solution was removed and the tissue rinsed with 0.154 M NaCl until the solution appeared clear. The NaCl solution was then removed and samples were incubated in 10 mL of 8 M urea at 37°C for 96 h.

Statistical Analysis

The GLM procedure of SAS (SAS Inst. Inc., Cary, NC) was used for statistical analysis of data. The model included the following independent variables: block, grain source, whole cottonseed inclusion rate, and the grain source x whole cottonseed

inclusion rate interaction. A second model included SCD activity as a covariate along with the previous model to assess the relationship between SCD enzymatic activity and the indices of SCD activity based on FAME analysis. When treatment effects were significant ($P < 0.05$), a difference was determined and a tendency for treatment to elicit a response was noted when $P < 0.10$. Least square means of significant responses were separated using the pdiff statement of the GLM procedure.

Results

Providing α-linolenic acid (18:3n-3) in the form of flaxseed (Table 4.1) was used as a practical means of altering the levels of a natural substrate for ruminal production of the CLA *trans*-10, *cis*-12. There was over a fourfold increase ($P < 0.01$; Table 4.2) in i.f.

Table 4.1. Fatty acid composition of diets[a]

	Corn			Flax			Milo		
	0	5	15	0	5	15	0	5	15
	g/100g[b]								
12:0	0.02	0.02	0.03	0.02	0.02	0.01	0.04	0.04	0.03
14:0	0.15	0.33	0.57	0.09	0.32	0.37	0.18	0.46	0.72
16:0	14.10	16.89	20.65	9.28	14.39	14.93	15.88	19.34	23.15
18:0	2.64	2.64	2.62	3.40	3.04	3.09	2.16	2.35	2.48
18:1n-9	24.26	22.90	21.23	20.62	20.87	20.03	31.58	27.08	21.99
18:2n-6	56.87	55.76	53.87	34.52	43.97	41.26	47.66	49.08	50.08
18:3n-3	1.72	1.41	0.97	32.01	17.37	20.29	2.41	1.61	1.46
CLA *cis*9,*trans*11	nd	0.01	0.03	nd	nd	nd	0.05	0.01	nd

[a] Values represent arithmetic mean of duplicate as-fed samples.
[b] Values are g/100 g of identified fatty acids.

adipose tissue 18:3n-3 percentage in the steers fed flaxseed (0.88%) than in the corn (0.14%) or milo (0.17%) group, which confirms that our enriched source of 18:3n-3

increased the concentration of this fatty acid in i.f. adipose tissue. However, CLA *trans*-10, *cis*-12 was not detectible in any of the tissues sampled. The only detectible isoform of CLA in adipose tissue of these steers was the CLA *cis*-9, *trans*-11 isomer. In the i.f. adipose tissue of the steers fed corn, there was an increase in CLA *cis*-9, *trans*-11 with increased levels of WCS, whereas it remained unchanged in the flaxseed group and even decreased in the milo group (interaction, $P < 0.02$). However, a similar pattern for 18:1*trans*-11 percentages was not apparent; steers fed flaxseed had a greater ($P < 0.02$) percentage 18:1*trans*-11 (4.57%) than those fed milo (2.62%). The 18:1n-9 percentage of i.f. adipose decreased (interaction, $P < 0.05$) with increased inclusion of WCS in steers fed flax, whereas it remained static in steers fed milo and exhibited a quadratic response in steers fed corn. These alterations in i.f. adipose tissue fatty acids resulted in an increase ($P < 0.01$) from 52.1% to 55.2% total saturated fatty acids when dietary WCS increased from 0 to 15%. This was accompanied by a commensurate decrease in monounsaturated fatty acids in i.f. adipose tissue. Total polyunsaturated fatty acids had only a tendency ($P < 0.09$) to decrease with increased whole cottonseed inclusion. However, the polyunsaturated fatty acid percentage of i.f. adipose tissue was increased ($P < 0.01$) from 3.26% in the steers fed milo to 4.13% in the steers fed flaxseed.

Table 4.2. Percentage of total fatty acids in interfasicular adipose tissue of Angus steers fed corn, flaxseed with corn, or milo based finishing rations with varying concentrations of whole cottonseed (**WCS**)

	Gain/%WCS												
	Corn			Flax			Milo				*P*-value		
Item	0%	5%	15%	0%	5%	15%	0%	5%	15%	SE	Grain	WCS	GxWCS[x]
12:0	0.06	0.06	0.07	0.06	0.07	0.08	0.06	0.06	0.07	0.01	0.48	0.27	0.91
14:0	3.22	2.86	3.36	3.12	3.51	3.99	3.32	3.27	3.34	0.29	0.26	0.25	0.52
14:1n-5	0.51	0.47	0.48	0.50	0.51	0.56	0.63	0.50	0.46	0.05	0.48	0.37	0.28
16:0	27.8	28.2	29.6	27.2	27.7	29.8	29.4	28.4	29.2	0.97	0.65	0.14	0.70
16:1n-7	2.09	2.22	2.13	2.13	2.52	2.07	2.49	2.23	2.30	0.19	0.48	0.61	0.51
18:0	21.7	20.4	22.6	20.6	20.1	21.9	19.7	21.5	21.7	1.07	0.68	0.21	0.65
18:1*trans*11	5.38	3.00	2.32	4.60	4.20	4.92	2.20	2.79	2.86	0.79	0.02	0.45	0.14
18:1n-9	34.9[ab]	39.3 [d]	36.0 [bc]	37.1 [bcd]	38.0 [cd]	32.5 [a]	39.0 [cd]	37.8 [bcd]	37.0 [bcd]	1.12	0.09	0.01	0.05
18:2n-6	3.81	3.14	2.90	3.33	2.33	2.94	2.60	2.89	2.65	0.30	0.07	0.14	0.18
18:3n-3	0.17	0.12	0.14	0.94	0.79	0.89	0.20	0.16	0.14	0.06	0.01	0.29	0.83
18:2*cis*9,*trans*11	0.29 [a]	0.35 [ab]	0.39 [bc]	0.43 [c]	0.32 [ab]	0.41 [bc]	0.45 [c]	0.35 [abc]	0.33 [ab]	0.04	0.27	0.24	0.02
SFA	52.8	51.5	55.6	51.0	51.4	55.7	52.5	53.2	54.3	1.17	0.76	0.01	0.50
MUFA	42.9	44.9	40.9	44.3	45.2	40.0	44.3	43.4	42.6	1.15	0.88	0.01	0.34
PUFA	4.27	3.60	3.44	4.70	3.44	4.25	3.25	3.40	3.13	0.34	0.01	0.09	0.24
Index[y]	0.71	0.82	0.70	0.78	0.80	0.63	0.81	0.77	0.74	0.04	0.51	0.01	0.12
14:1n-5/14:0	0.16	0.16	0.15	0.17	0.15	0.14	0.19	0.15	0.14	0.01	0.71	0.01	0.29
16:1n-7/16:0	0.08	0.08	0.07	0.08	0.09	0.07	0.08	0.08	0.08	0.01	0.54	0.20	0.50
18:1n-9/18:0	1.62	1.93	1.61	1.82	1.93	1.49	2.00	1.78	1.74	0.12	0.45	0.03	0.17
CLAind[z]	0.07[d]	0.13[bc]	0.18[ab]	0.11[cd]	0.08[cd]	0.11[cd]	0.23[a]	0.14[bc]	0.13[bcd]	0.03	0.02	0.63	0.01

[abcd] Least square means within a row with a different superscript are different ($P<0.05$).

[x] Grain x percentage whole cottonseed interaction.

[y] Desaturation index = (14:1 + 16:1 +18:1*cis*-9, 18:2*cis*-9,*trans*-11)/(14:0 + 16:0 + 18:0 + 18:1*trans*-11).

[z] CLA desaturation index = (18:2*cis*-9,*trans*-11)/(18:1*trans*-11).

Table 4.3. Percentage of total fatty acids in subcutaneous adipose tissue of Angus steers fed corn, flaxseed with corn, or milo based finishing rations with varying concentrations of whole cottonseed (**WCS**)

	Gain/%WCS												
	Corn			Flaxseed			Milo				*P*-value		
Item	0%	5%	15%	0%	5%	15%	0%	5%	15%	SE	Grain	WCS	GxWCS[x]
12:0	0.08	0.08	0.09	0.08	0.09	0.10	0.07	0.07	0.09	0.01	0.42	0.11	0.98
14:0	3.41	3.26	3.67	3.46	3.85	4.19	3.55	3.67	3.46	0.27	0.24	0.28	0.71
14:1n-5	0.51	0.53	0.47	0.49	0.71	0.51	0.59	0.40	0.54	0.10	0.64	0.89	0.36
16:0	28.0	28.4	29.8	27.5	27.5	29.3	29.8	29.3	29.3	1.02	0.27	0.35	0.74
16:1n-7	1.57	1.98	1.68	1.77	2.00	1.53	2.09	1.90	2.13	0.22	0.21	0.57	0.49
18:0	26.0	23.3	26.5	24.1	23.8	26.0	23.3	24.6	23.9	1.59	0.61	0.47	0.69
18:1*trans*11	5.44	3.47	2.77	5.09	4.75	5.73	2.58	3.05	3.41	0.85	0.01	0.67	0.26
18:1n-9	30.6	34.9	31.7	32.7	33.7	28.4	34.8	33.7	33.73	1.50	0.14	0.09	0.19
18:2n-6	4.01	3.52	2.92	3.50	2.49	2.94	2.68	2.89	2.75	0.33	0.04	0.13	0.22
18:3n-3	0.15	0.15	0.12	0.97	0.80	0.88	0.19	0.16	0.15	0.07	0.01	0.43	0.74
18:2*cis*9,*trans*11	0.27	0.39	0.35	0.41	0.39	0.36	0.38	0.33	0.35	0.04	0.26	0.89	0.20
SFA	57.4	55.0	60.0	55.1	55.2	59.6	56.7	57.6	56.9	1.69	0.83	0.09	0.41
MUFA	38.1	40.9	36.6	40.9	41.1	36.2	40.0	39.0	39.8	1.67	0.74	0.13	0.41
PUFA	4.43	4.06	3.39	4.88	3.69	4.18	3.25	3.38	3.25	0.36	0.01	0.13	0.28
Index[y]	0.57	0.68	0.57	0.64	0.66	0.51	0.67	0.63	0.65	0.04	0.46	0.13	0.27
14:1n-5/14:0	0.15	0.16	0.13	0.14	0.18	0.12	0.16	0.10	0.14	0.02	0.74	0.55	0.22
16:1n-7/16:0	0.06	0.07	0.06	0.06	0.07	0.05	0.07	0.07	0.07	0.01	0.34	0.37	0.41
18:1n-9/18:0	1.20	1.54	1.21	1.37	1.45	1.10	1.54	1.38	1.51	0.15	0.28	0.33	0.32
CLAind[z]	0.07	0.14	0.13	0.09	0.09	0.07	0.16	0.12	0.10	0.02	0.08	0.67	0.11

[x] Grain x percentage whole cottonseed interaction.

[y] Desaturation index = [(14:1 + 16:1 +18:1*cis* + 18:2*cis*-9, *trans*-11)/(14:0 + 16:0 + 18:0 + 18:1*trans*-11)].

[z] CLA desaturation index = [(18:2*cis*-9,*trans*-11)/(18:1*trans*-11)].

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The fatty acid composition of s.c. adipose tissue (Table 4.3) was not as responsive to interaction effects as i.f. adipose, although most of the main effects were similar between the two adipose depots. There was an increase in 18:3n-3 ($P < 0.01$) from 0.14% and 0.16% in s.c. adipose of the corn- and milo-fed steers, respectively, to 0.88% in the steers fed flaxseed. This was accompanied an increase ($P < 0.01$) in 18:1*trans*-11, from 3.01% in the steers fed milo to 5.19% in the steers receiving flaxseed, although there were no discernible effects of treatment on the s.c. percentage of CLA. There was a tendency ($P < 0.09$) for a decrease in 18:1n-9 concentration in s.c. adipose tissue to 31.3% in steers receiving 15% WCS compared to 34.1% in the steers receiving 5% WCS. There was also a tendency ($P < 0.09$) for the total saturated fatty acid percentage in s.c. adipose tissue to increase when steers were fed 15% WCS. Total polyunsaturated fatty acids were the lowest ($P < 0.01$) in steers receiving milo (3.29%), and were the same in s.c. adipose tissues of steers fed corn (3.96%) or flaxseed (4.25%).

Lipogenesis from acetate in s.c. adipose tissue (Table 4.4) was greater ($P < 0.01$) in steers fed flaxseed (5.42 nmol•10^5 cells•h^{-1}) than in steers fed either the corn (3.10 nmol•10^5 cells•h^{-1}) or milo (1.92 nmol•10^5 cells•h^{-1}) diets, whereas lipogenesis was unaffected by treatment in i.f. adipose tissue. The s.c. adipocytes of steers fed flaxseed

Table 4.4. Lipogenesis, cellularity, and stearoyl co-A desaturase (**SCD**) activity from various tissues of Angus steers fed corn, flaxseed with corn (**Flax**), or milo based finishing rations with varying concentrations of whole cottonseed (**WCS**)

	Grain/%WCS												
	Corn			Flaxseed			Milo				*P*-value		
Item	0%	5%	15%	0%	5%	15%	0%	5%	15%	SE	Grain	WCS	GxWCS[x]
Interfasicular													
Lipogenesis [y]	0.98	1.05	0.58	0.53	1.67	0.87	0.60	0.64	0.95	0.49	0.76	0.56	0.64
10^5 cells•g tissue^{-1}	29.9	23.2	25.4	32.8	24.0	28.5	22.2	21.6	28.8	4.1	0.46	0.23	0.63
Mean diameter,µm	56	63	61	58	59	61	49	53	45	4.6	0.01	0.57	0.81
Mean volume, pL	224	324	281	265	272	302	202	242	17.1	1.02	0.07	0.39	0.65
Peak diameter, µm	28	30	30	30	30	30	30	24	26	26	0.54	0.50	0.37
Peak volume, pL	106	122	110	118	112	130	112	120	116	7.6	0.42	0.88	0.16
SCD[z]	8.07	10.05	24.94	34.35	26.48	10.74	6.28	21.69	16.02	8.89	0.46	0.23	0.63
Subcutaneous													
Lipogenesis	3.46	2.72	3.12	4.92	5.66	5.69	2.37	1.38	2.02	1.14	0.01	0.91	0.95
10^5 cells•g tissue^{-1}	9.18	6.47	6.59	7.43	7.84	5.51	8.98	10.50	7.21	0.98	0.05	0.03	0.25
Mean diameter,µm	84	80	92	83	92	93	76	80	80	6	0.11	0.35	0.81
Mean volume, pL	0.67	0.72	0.87	0.67	0.86	0.92	0.55	0.69	0.65	0.09	0.06	0.06	0.84
Peak diameter, µm	60^{ab}	20^{c}	20^{c}	24^{bc}	70^{a}	48^{abc}	34^{abc}	20^{c}	22^{bc}	14	0.16	0.70	0.05
Peak volume, pL	1.42	2.01	1.65	1.52	2.22	2.02	1.43	1.65	1.69	0.22	0.27	0.02	0.85
SCD	88.87	90.73	34.76	116.72	22.02	71.49	58.69	90.31	110.64	15.06	0.84	0.78	0.30
Duodenum													
SCD	4.04	4.85	4.56	5.69	5.10	5.08	4.71	4.99	3.39	0.75	0.76	0.56	0.64

[x] Grain x percentage whole cottonseed interaction.

[y] Lipogenesis from acetate, nmol•10^5 cells•h.

[z] nmol•mg protein^{-1} • 7 min^{-1}.

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had fewer ($P < 0.01$) cells per gram of tissue and tended ($P < 0.06$) to have a greater mean cell volume than the adipocytes from the steers fed the other grain sources. The proportional diameter distribution of s.c. adipocytes demonstrated a modest shift towards larger cell diameters (Figure 4.1) for the steers fed flaxseed, with the effect being more apparent for cell volume distribution (Figure 4.2). Similarly, i.f. adipose mean diameter was greatest ($P < 0.01$) in the steers fed both flaxseed or corn (both 60 μm), and the smallest in the steers fed milo (49 μm). The i.f. adipocyte mean volume tended ($P < 0.07$) to be smallest in steers fed milo (205 pL) and largest in corn (276 pL) and flaxseed (279 pL) groups. These small differences make it difficult to discern any recognizable pattern within the proportional distribution of i.f. adipocyte diameter (Figure 4.3) and volume (Figure 4.4).

With increased WCS, s.c. peak diameter decreased in the steers fed corn, increased in steers fed flaxseed, and remained static in the steers fed milo (interaction, $P < 0.05$). Similarly, there was a shift towards a larger proportion of cells with greater diameters (Figure 4.5) and volume (Figure 4.6) for s.c. adipocytes of cattle fed WCS. Whole cottonseed did not affect peak diameter or volume, or the proportional distribution of i.f. adipose tissue (Figures 4.7 and 4.8). However, i.f. peak volume was greatest ($P < 0.01$) in the steers fed flaxseed (296 pL), and peak diameter was largest in the steers fed corn (29 μm) or flaxseed (30 μm), than in i.f. adipose tissue of steers fed milo (25 μm and 172 pL, respectively).

Stearoyl-CoA desaturase activity (Table 4.4) was not affected by treatment in intestinal duodenal cells or i.f. adipose tissue. There was greater ($P < 0.01$) s.c. adipose

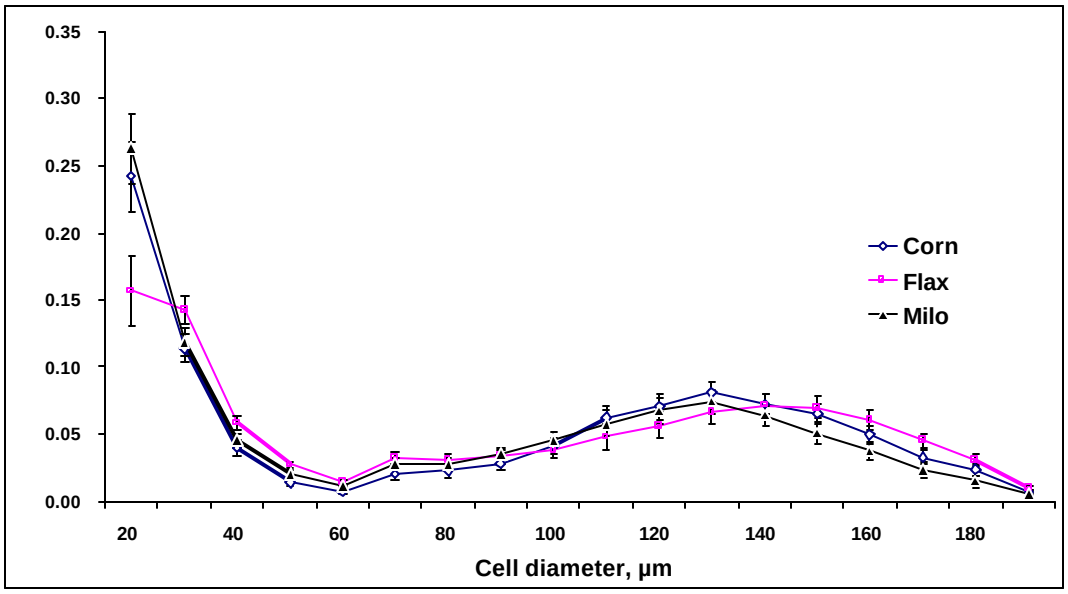

Figure 4.1. Proportional distribution of subcutaneous adipose tissue cells of differing diameters from Angus steers fed corn, flaxseed (**Flax**), or milo based finishing diets.

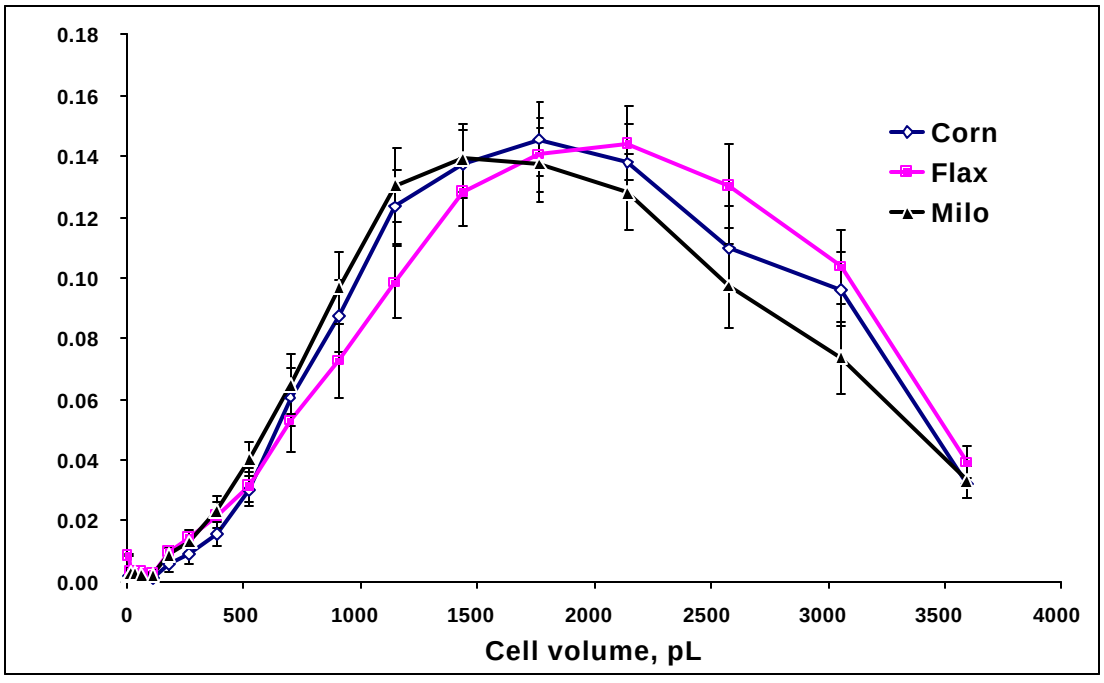

Figure 4.2. Proportional distribution of subcutaneous adipose tissue cells of differing volumes from Angus steers fed corn, flaxseed (**Flax**), or milo based finishing diets.

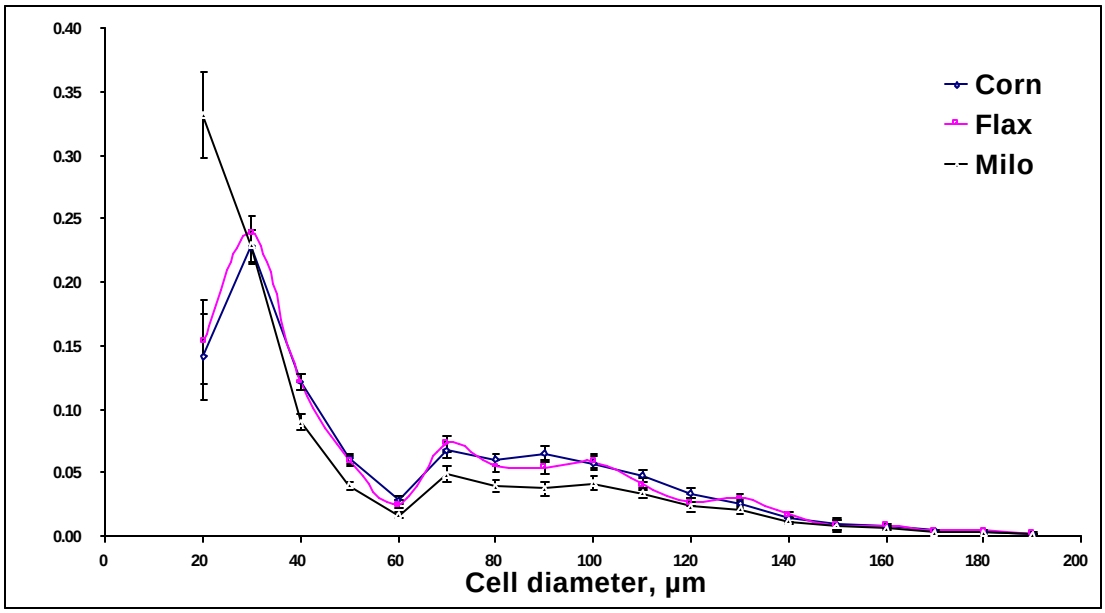

Figure 4.3. Proportional distribution of interfasicular adipose tissue cells of differing diameters from Angus steers fed corn, flaxseed (**Flax**), or milo based finishing diets.

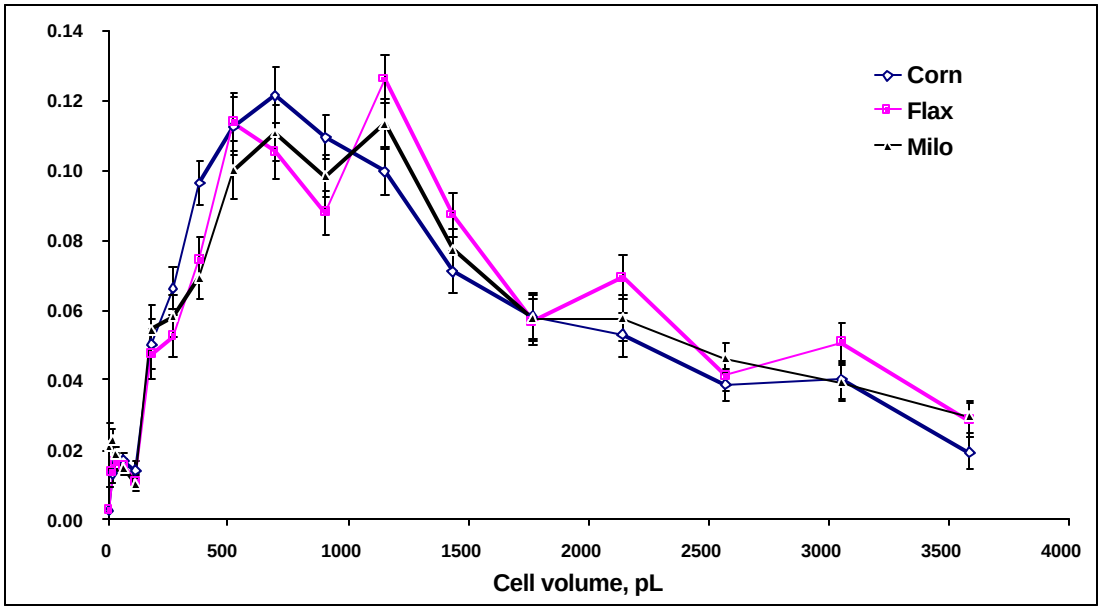

Figure 4.4. Proportional distribution of interfasicular adipose tissue cells of differing volumes from Angus steers fed corn, flaxseed (**Flax**), or milo based finishing diets.

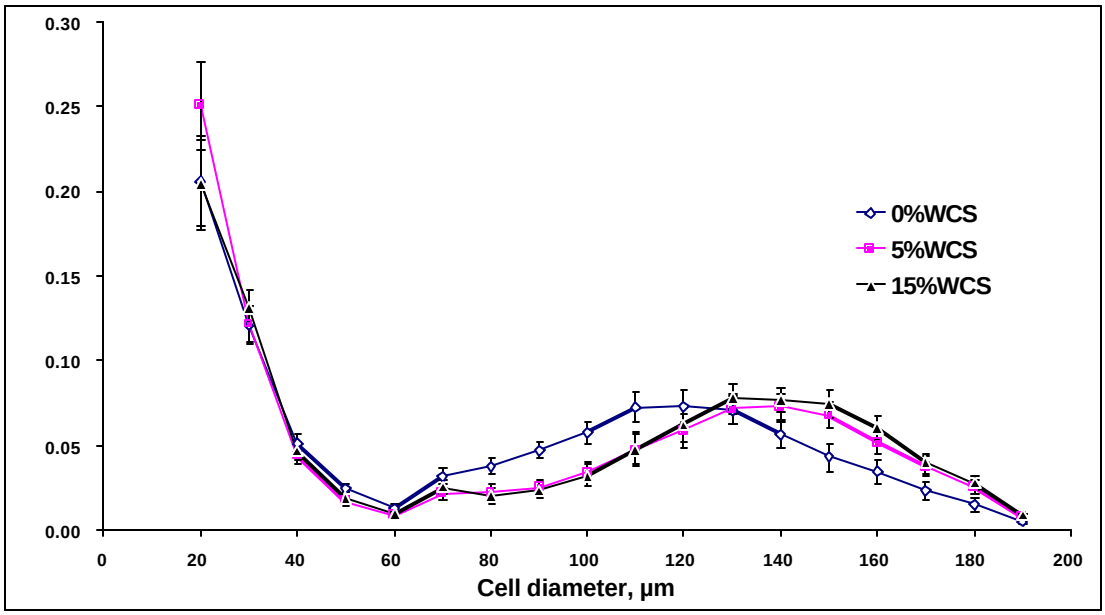

Figure 4.5. Proportional distribution of subcutaneous adipose tissue cells of differing diameters from Angus steers fed diets containing 0, 5, or 15% whole cottonseed (DM basis).

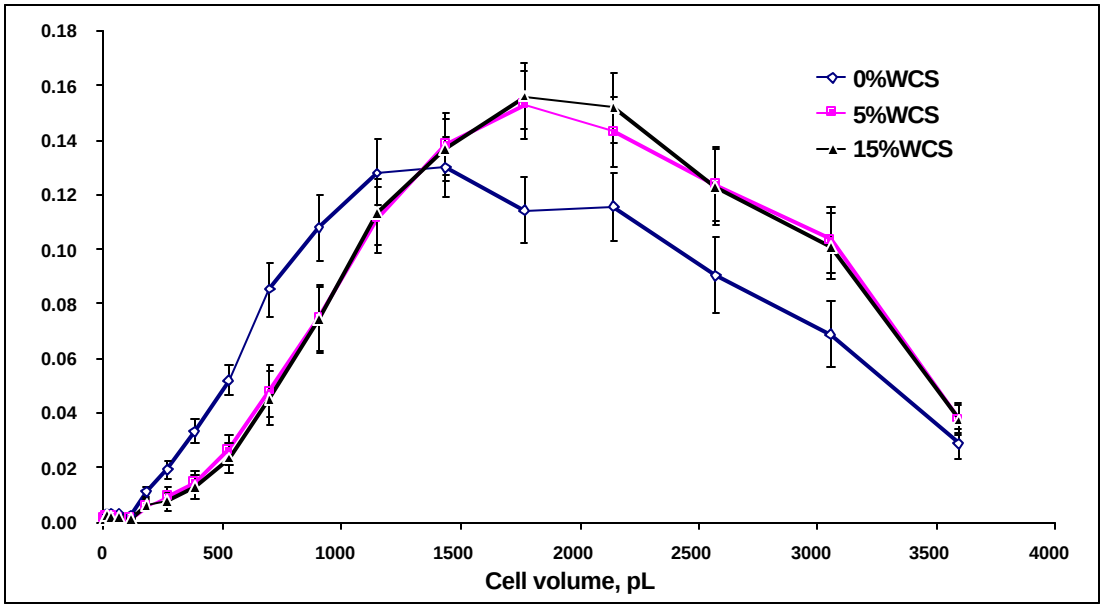

Figure 4.6. Proportional distribution of subcutaneous adipose tissue cells of differing volumes from Angus steers fed diets containing 0, 5, or 15% whole cottonseed (DM basis).

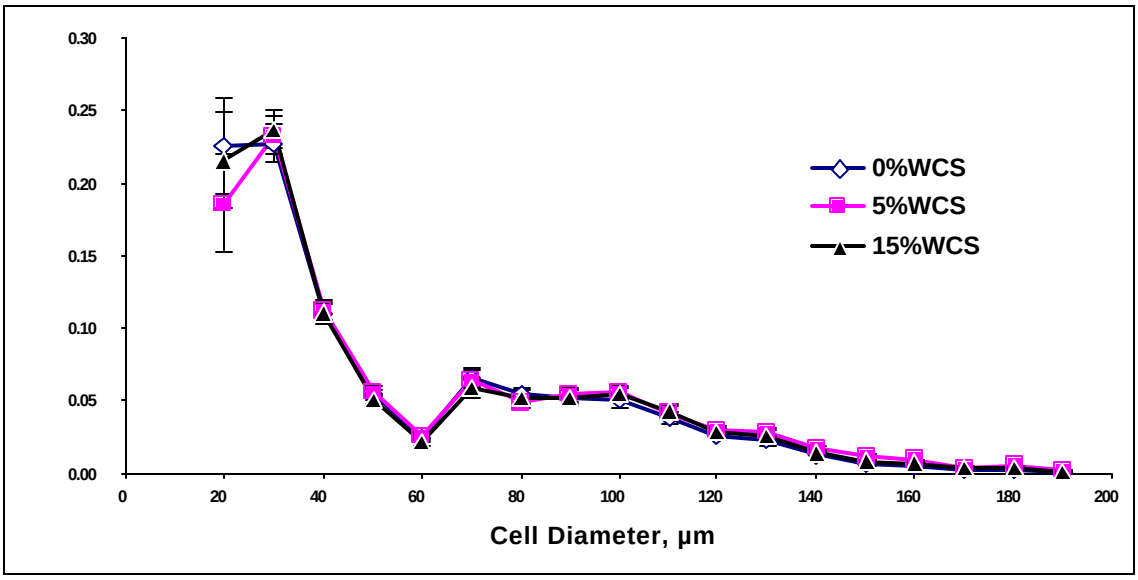

Figure 4.7. Proportional distribution of interfasicular adipose tissue cells of differing diameters from Angus s teers fed diets containing 0, 5, or 15% whole cottonseed (DM basis).

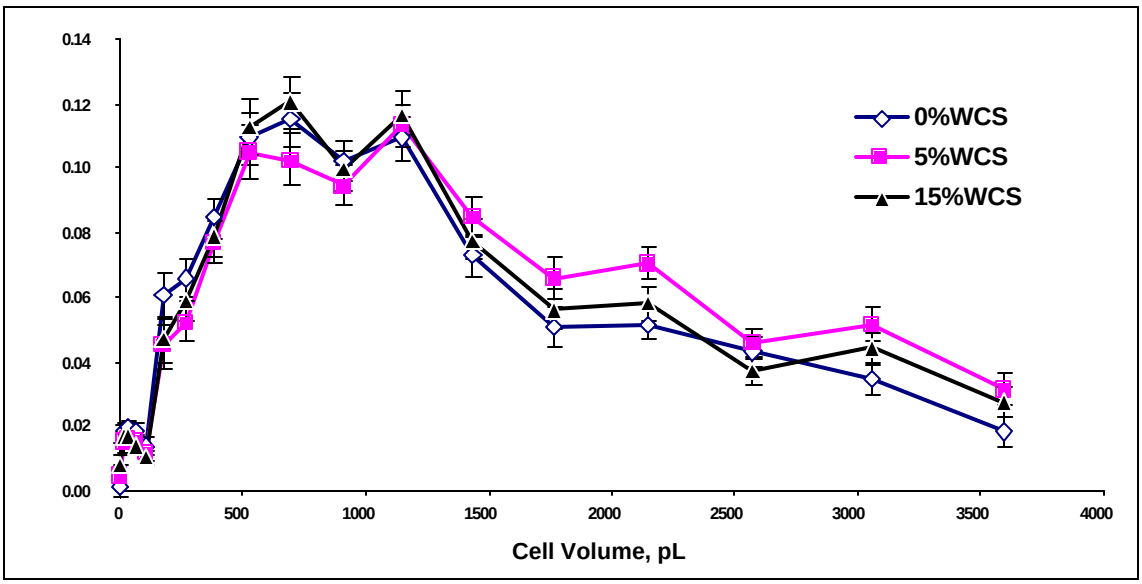

Figure 4.8. Proportional distribution of interfasicular adipose tissue cells of differing volumes from Angus steers fed diets containing 0, 5, or 15% whole cottonseed (DM basis).

SCD activity to 74 nmol•mg protein^{-1}•7 min^{-1} in steers fed flaxseed compared to 53 nmol•mg protein^{-1}•7 min^{-1} in milo-fed steers. There were larger differences in the various indices of SCD activity based on fatty acid composition. The i.f. ratio of total SCD products to substrates was less in the 15% WCS group (0.69) than in the 0 and 5% groups (0.76 and 0.79, respectively). The i.f. 14:1:14:0 ratio and the 18:1:18:0 ratio both were decreased ($P < 0.01$ and $P < 0.03$, respectively) by WCS, although the 16:1:16:0 ratio was not affected by WCS treatment. Whole cottonseed decreased the i.f. 18:2*cis*-9, *trans*-11:18:1*trans*-11 ratio from 0.23 to 0.13 in steers fed milo, whereas the ratio did not change in cattle fed flaxseed (0.10) and increased from 0.07 to 0.18 in steers fed the corn diet (interaction, $P < 0.02$). However, s.c. SCD indices were largely unaltered, with only a tendency ($P < 0.08$) for a decrease in the ratio of 18:2*cis*-9, *trans*-11 to 18:1*trans*-11 in steers fed flaxseed (0.08), compared to those fed milo (0.13).

There were very few significant relationships among linear comparisons between the measured SCD enzymatic activity and the various indices based on fatty acid composition (Table 4.5). The only significant ($P < 0.01$) relationship was an increase of 0.07 in the 18:1:18:0 ratio in i.f. adipose tissue with each subsequent 1 nmol•mg protein^{-1}•7 min^{-1} increase in i.f. adipose SCD enzymatic activity. There was a tendency ($P < 0.09$) for a positive relationship (0.04) between the ratio of total SCD products to substrates and the SCD enzyme activity of i.f. adipose tissue. However, a negative relationship (-1.517) tended ($P < 0.07$) to exist between the i.f. ratio of total SCD products to substrates and the SCD enzyme activity of intestinal mucosal cells.

Table 4.5. Linear relationship between stearoyl-CoA desaturase (**SCD**) activity and fatty acid indices of SCD activity from various tissues of Angus steers.

Tissue	Dependent	Independent[a]	Slope	Intercept[b]	*P*-value[c]
Subcutaneous	Index[y]	Subcutaneous	0.0068 (0.0208)	39.7	0.74
	14:1/14:0	Subcutaneous	0.0091 (0.0205)	12.6	0.66
	16:1/16:0	Subcutaneous	-0.0033 (0.0079)	6.87	0.68
	18:0/18:1	Subcutaneous	-0.0082 (0.0290)	59.1	0.78
	CLAindex[z]	Subcutaneous	0.0021 (0.0204)	8.47	0.92
	Index	Intestinal mucosa	-0.8172 (1.128)	70.0	0.47
	14:1/14:0	Intestinal mucosa	-0.3638 (0.5627)	16.7	0.52
	16:1/16:0	Intestinal mucosa	-0.1491 (0.1807)	7.84	0.42
	18:0/18:1	Intestinal mucosa	-3.283 (3.442)	165	0.35
	CLAindex	Intestinal mucosa	-0.7332 (0.5393)	11.4	0.18
Interfasicular	Index	Interfasicular	0.0391 (0.0224)	41.3	0.09
	14:1/14:0	Interfasicular	0.0009 (0.0151)	11.3	0.96
	16:1/16:0	Interfasicular	0.0173 (0.0106)	6.85	0.11
	18:0/18:1	Interfasicular	0.0748 (0.0279)	60.6	0.01
	CLAindex	Interfasicular	0.0425 (0.0401)	8.38	0.30
	Index	Intestinal mucosa	-1.517 (0.8159)	77.9	0.07
	14:1/14:0	Intestinal mucosa	0.1205 (0.2390)	12.3	0.62
	16:1/16:0	Intestinal mucosa	-0.1841 (0.1496)	8.39	0.23
	18:0/18:1	Intestinal mucosa	-4.043 (2.777)	181	0.16
	CLAindex	Intestinal mucosa	-0.3643 (0.6570)	11.3	0.58

[a] SCD activity of microsomes from indicated tissues (nmol • mg protein^{-1} • 7min^{-1}).

[b] A biased estimate of the intercept generated by the solution option in the general linear model procedure of SAS.

[c] Probability for type I error for the covariate (independent variable) in the ANCOVA.

[y] Desaturation index = [(14:1 + 16:1 +18:1*cis*)/(14:0 + 16:0 + 18:0)].

[z] CLA desaturation index = [(18:2*cis*-9,*trans*-11)/(18:1*trans*-11)].

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Discussion

Although there are studies supporting the theory of endogenous formation of the *cis*-9, *trans*-11 isomer of CLA by SCD in dairy cattle (Corl et al., 2001; Griinari et al., 2000), these studies are largely based on calculations of SCD activity. For example, using the ratio of desaturase products and substrates within a given tissue has been used extensively to estimate SCD activity (Corl et al., 2001; Griinari et al., 2000; Duckett et al., 2002). This approach would be correct if the tissue sampled was at equilibrium. However, living tissues are at steady state, with turnover and input from dietary sources and endogenous synthesis. As such, estimations of enzymatic activity based upon calculations of products and reactants must be confirmed by measurement of the actual enzyme activity. This has been demonstrated in previous instances. For example, it was largely believed that mammals possessed a Δ^4-desaturase because it was the only logical manner in which to form docosahexanoic acid (24:6n-3) from its precursor, docosapentanoic acid (24:5n-3). However, Sprecher and coworkers demonstrated the lack of the Δ^4-desaturase (Voss et al., 1991) and the presence of a variant pathway involving partial β-oxidation (Moore et al., 1995).

Of particular note is the fact that while the s.c. adipose of tissue of steers fed milo in this study had the highest 18:2*cis*9-*trans*-11:18:1*trans*-11 ratio (which is of particular interest to those trying to increase CLA production) the actual SCD activity was lowest for the tissue from these cattle. This is partially due to the lower 18:1*trans*-11 in the milo fed cattle, which is likely the result of complete ruminal hydrogenation, facilitated by the increased surface area of the ground milo compared to the other feedstuffs.

Therefore, had assumptions about enzymatic activity been based solely on the 18:2*cis*9-*trans*-11:18:1*trans*-11 ratio, we would have concluded incorrectly that milo increased SCD activity.

There has been substantial interest in increasing the amount of CLA in animal products for human consumption. Thus far, research has focused primarily on feeding CLA directly to monogastric animals (Park et al., 1999; Blankson et al., 2000) or on increasing the CLA content of ruminant products such as milk (Corl et al., 2001; Duckett et al., 2002). This interest is the largely result of research documenting the numerous beneficial effects of CLA in animal and human models. These effects include the anticarcinogenic effects of both the *cis*-9, *trans*-11 and *trans*-10, *cis*-12 isomers (Pariza and Hargraves, 1985; Ha et al., 1987; Ip et al., 2002), and the body composition repartitioning action of the *trans*-10, *cis*-12 isomer of CLA (Park et al., 1999; Ostrowska et al., 1999).

Hasler (2002) described the support for beef, lamb, dairy and turkey as a functional food as being weak based on current concentrations of CLA in these meats. As such, if there is a possibility of increasing the CLA content in ruminant products, such as beef, these products may more affectively serve as functional foods. In our study, we demonstrated an ability to increase the CLA content of bovine subcutaneous adipose tissue. However, the adipose of steers fed milo without WCS had the highest numerical concentrations of CLA, these concentrations decreased as WCS increased in the diet. This was similar to what was expected. The study was designed to increase intake of sterculic acid from WCS (Coleman, 1973), thereby decreasing SCD activity

and thus decreasing endogenous CLA production by desaturation of 18:1*trans*-11. A similar approach was direct addition of sterculic oil to the diet (Corl et al., 2001). However, the lack of decrease in CLA concentrations with increased inclusion of WCS in the subcutaneous adipose tissue of steers receiving either the flaxseed or corn diets was not as evident because these provided increased substrate for ruminal formation of CLA and had overall greater SCD activity in this tissue, which could possibly combine to alleviate the effects of WCS on fatty acid desaturation. The increase in s.c. SCD activity in flaxseed-fed steers may be the result of the increased 18:3n-3 binding to peroxisome proliferator-activated receptor γ, which increases SCD gene transcription in mouse liver (Miller and Ntambi, 1996).

Adipose tissue increases in mass by either increasing the number of adipocytes (hyperplasia) or by increasing the size (hypertrophy) of individual cells as they begin to fill with lipid (Hood and Allen, 1973). This situation should then, lead to the generation of two populations of cells within the adipose depot. These two populations would include a smaller sized group of mitotic cells as well as a group of larger, differentiated cells, which have started to accumulate lipid. Both of these cell populations were evident in this study, as seen in the proportional distribution of adipocytes with differing diameters. This biphasic cell distribution is consistent with results seen by several laboratories (Hood and Allen, 1973; Lee et al., 1983; May et al., 1994), but is in contrast with the results of Schiavetta et al., (1990) who described a single sized population of bovine adipocytes. However, Schiavetta et al. (1990) relied upon a single large aperture, which may be less likely to detect the very small cells that are detected using other

available techniques. The number of cells within the s.c. adipose was very similar to that noted by May et al. (1994), whereas there were decidedly more adipocytes per gram of i.f. adipose tissue in the current study, than reported by May et al. (1994). This is most likely because the steers used by May et al. (1994) were fed for 552 d, whereas the current steers were fed for only 130 d. Because the i.f. adipose depot is thought to develop at a later point in physiological maturity, these cells would not have had as long to accumulate lipid.

The lesser SCD enzymatic activity in the i.f. adipose relative to s.c. adipose tissue also may have been a result of the stage of development of this tissue. Stearoyl-CoA desaturase recently has been shown to play a major role in lipid filling of genetically obese (ob/ob) mice (Cohen et al., 2002), and may play a role in a metabolic regulation of lipid filling that is extraneous to transcriptional regulation. Although the exact metabolic mechanism for the role of depressed SCD activity is not yet known, a possible explanation is that there will be a decrease in the cellular concentrations of malonyl CoA, which would lead to a decrease in de novo lipogenesis Cohen et al. (2002). This theory is based on the concept that the increase in saturated fatty acids will allosterically inhibit acetyl-CoA carboxylase, which in turn will depress the intracellular concentration of malonyl CoA (Lunzer et al., 1977). This could also possibly increase the oxidation of fatty acids by removing the inhibitory effect of malonyl-CoA upon the carnitine palmitoyl-CoA transferase system (McGarry et al., 1977), which is the rate-limiting step for the import and oxidation of fatty acids within the mitochondria of the cell. Therefore, the metabolic systems with the greatest rates of SCD enzymatic activity

would be generating the most lipid. Our study supports this concept by demonstrating that the larger s.c. adipocytes, which have twice the SCD activity of i.f. adipocytes, have the greatest rate of lipogenic activity. This was further supported by the increased s.c. SCD from the flaxseed fed steers, which had largest adipocytes and greatest rates of lipogenesis.

Implications

These data demonstrate that increasing dietary α-linolenic acid by feeding flaxseed will increase lipid synthesis and adipose concentrations of α-linolenic acid, but will not alter the CLA content. Increased lipogenesis and adipocyte volume associated with an increase in SCD activity, which adds further support for the theory that increased SCD activity of adipose depots is associated with increased lipogenesis. However, indices of SCD activity based on fatty acid concentrations did not correlate with actual SCD activity, which implies that these indices are not valid measures of SCD activity in bovine s.c. or i.f. adipose tissues. These data imply that greater care needs to be taken when basing estimates of SCD activity solely upon ratios of the desaturase products and substrates. Furthermore, inclusion of WCS in milo-based diets may cause an increase in adipose tissue saturated fat that is not seen in finished steers fed diets with higher concentrations of α-linolenic acid. This implies that feed practices designed to generate a more health conscious beef product that is lower in total saturated fat should exclude the combination of milo and WCS.

CHAPTER V

CONCLUSION

These data indicate that rations formulated to provide increased levels of α-linolenic acid (i.e., flaxseed) will increase feed efficiency without altering either the quality or composition of the beef carcasses. Additionally, the inclusion of WCS in milo diets may cause a decrease in efficiency and less salable lean. However as evidenced by the very young lean and skeletal maturity of these animals, possible differences in treatment may not have become apparent because the tissues did not have adequate time to adapt the changes induced by treatment. This implies that future studies of this nature should be much lengthier in time frame.

These data demonstrate that increasing dietary α-linolenic acid by feeding flaxseed will increase lipid synthesis and adipose concentrations of α-linolenic acid, but will not alter the CLA content. Increased lipogenesis and adipocyte volume associated with an increase in SCD activity, which adds further support for the theory that increased SCD activity of adipose depots is associated with increased lipogenesis. However, indices of SCD activity based on fatty acid concentrations did not correlate with actual SCD activity, which implies that these indices are not valid measures of SCD activity in bovine s.c. or i.f. adipose tissues. These data imply that greater care needs to be taken when basing estimates of SCD activity solely upon ratios of the desaturase products and substrates. Furthermore, inclusion of WCS in milo-based diets may cause an increase in adipose tissue saturated fat that is not seen in finished steers fed diets with higher concentrations of α-linolenic acid. This implies that feed practices designed to generate

a more health conscious beef product that is lower in total saturated fat should exclude the combination of milo and WCS.

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