Effects of supplemental rumen-protected conjugated linoleic acid or corn oil on fatty acid composition of adipose tissues in beef cattle¹

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ABSTRACT: Thirty-six Angus × Hereford heifers $(365 \pm 60 \text{ kg})$ were used to determine the effects of supplemental dietary lipid sources on fatty acid composition of i.m., perianal (p.a.), and s.c. lipid depots. Lipid was supplied to diets as either corn oil or a rumenprotected conjugated linoleic acid (CLA) salt for two specific treatment periods of either the final 32 or 60 d on feed. Following an initial 56-d feeding period, heifers were fed one of three dietary treatments (DM basis): 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 (RPCLA) containing 31% CLA. The trans-10, cis-12 CLA concentration was greatest (P < 0.05) for heifers fed RPCLA and OIL diets and least (P < 0.05) for CON, regardless of time on dietary treatment. Heifers fed supplemental RPCLA had greater (P < 0.05) total CLA content than either CON- or OIL-fed heifers. Adipose tissue concentration of *trans*-11 vaccenic acid (TVA) was less (P <0.05) for CON than OIL or RPCLA, which did not differ (P > 0.05). Percentages of C18:1 *trans*-10 were least (P = 0.05). < 0.05) in i.m. lipid compared with p.a. and s.c., which did not differ (P > 0.05). Following 60 d of lipid supplementation, heifers fed OIL and RPCLA had lower (P <

0.05) concentrations of oleic acid and total monounsaturated fatty acids (MUFA) compared with CON. The ratio of *cis*-9, *trans*-11 CLA:TVA was higher (P < 0.05) for heifers fed 60 vs. 32 d, but did not differ (P > 0.05)between adipose depots. Feeding OIL increased (P <0.05) adipose concentration of C18:2 fatty acid, whereas feeding RPCLA increased (P < 0.05) total CLA isomers by 22%. Intramuscular lipid contained the lowest (P <0.05) percentage of cis-9, trans-11 CLA, total CLA, C18:1 cis-9, C18:1 trans-10, and TVA. Total CLA and *cis*-9, *trans*-11 CLA isomers were increased (P < 0.05)in p.a. and s.c. adipose depots, whereas i.m. adipose tissue contained increased (P < 0.05) amounts of total PUFA. Results from this study indicate that short-term lipid supplementation to feedlot cattle can increase adipose tissue CLA concentrations, but only marginally (8.3 to 17.5%). Moreover, observed decreases in oleic acid and total MUFA concentrations of adipose tissues from heifers fed rumen-protected CLA or corn oil suggest that lipid supplementation may decrease Δ^9 desaturase activity in adipose tissues, which in turn would lower the conversion of TVA to cis-9, trans-11 CLA isomer.

Key Words: Beef, Conjugated Linoleic Acid, Fatty Acid Composition

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Introduction

Fatty acid (**FA**) composition of adipose tissue can be influenced by many factors, including nutritional status, depot, species, and time on feed (Duckett et al., 1993; Aharoni et al., 1995; Scollan et al., 2001). Manipulation of FA content through dietary lipid supplementation may provide producers with a means of

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supplying beef products containing enhanced levels of unsaturated FA. Edible beef products contain relatively low levels of PUFA and high levels of saturated fatty acids (**SFA**; Rule et al., 2002). From the standpoint of human nutrition, enhancing the unsaturated FA content of beef translates to a more healthful product. Dietary intake of SFA has been attributed to elevated serum cholesterol level and increased risk of cardiovascular disease in humans (Hegsted et al., 1965).

During ruminal biohydrogenation of dietary unsaturated lipids, unique FA intermediates, termed conjugated linoleic acid (**CLA**), as well as *trans* octadecenoic acids, are produced in addition to saturated end products (Bauman et al., 1999). Ruminant milk and meat products represent the largest natural source of CLA, and their concentration in bovine adipose depots is of interest to human health. In particular, the *cis*-9, *trans*-

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11 CLA isomer has been shown to possess anticarcinogenic (Ha et al., 1987) properties. Research in dairy cattle has shown that supplementing rumen-protected CLA or vegetable oils increases the *cis*-9, *trans*-11 isomer concentration in milk fat (Kelly et al., 1998; Enser et al., 1999; Corl et al., 2001). However, limited research is available on the effects of CLA supplementation on tissue composition in ruminants consuming high-concentrate diets. Therefore, the objective of this study was to determine the effects of supplemental corn oil or rumen-protected CLA salt for two specific time periods on subsequent tissue FA concentrations, with particular interest in CLA content of various adipose depots.

Materials and Methods

Experimental Design

Thirty-six Angus \times Hereford heifers (365 ± 60 kg; 13 mo of age) were obtained from the NW Georgia Experiment Station (Calhoun) and used in a completely randomized design. The University of Georgia Animal Care and Use Committee approved animal handling procedures for this trial. Effect of dietary lipid source on subsequent FA composition of i.m., perianal (p.a.), and s.c. adipose depots was evaluated in a 3×2 factorial arrangement with three dietary treatments supplied for two specific time periods. For the purposes of this study, heifers were fed treatment diets for the last 32 or 60 d before slaughter, which corresponded to a total of 89 or 118 d on feed, respectively. Heifers were randomly allotted to dietary treatments (12 heifers per treatment) at trial initiation. Following 89 d on feed (32 d of lipid supplementation), heifers (six heifers per treatment) that had 1.27 cm or greater s.c. fat thickness were slaughtered. The remaining heifers (six heifers per treatment) were fed the treatment diets to reach a similar fat thickness endpoint (≥ 1.27 cm), which required an additional 28 d on the dietary treatments, and were then slaughtered. This approach allowed us to attain the same compositional endpoint for each supplementation length. Effects of length of lipid supplementation on tissue FA composition were analyzed by directly targeting the period of i.m. lipid development. Research has shown that i.m. lipid deposition occurs in a nonlinear manner across time on feed. Duckett et al. (1993) observed a doubling in i.m. lipid between d 84 and 112 in Angus \times Hereford steers fed feedlot diets. Feeding periods beyond 112 d did not improve carcass quality traits, but instead resulted in s.c. lipid accumulation and economic losses associated with excess carcass trim.

Dietary Treatments

Following an initial feeding period of 56 d where heifers received the basal, high-concentrate diet, heifers were fed one of three dietary treatments (DM basis): 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 (**RPCLA**; Agribrands Purina Canada Inc., Ontario, Canada), containing 31% CLA. The OIL supplement was composed of 58% C18:2, 27% C18:1 *cis*-9, 11% C16:0, 2% C18:0, and 2% C18:3, whereas the RPCLA supplement contained 31% CLA, of which 27.2% was *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 and effects of supplementation length and dietary treatment on performance traits, carcass characteristics, and leptin concentrations of feedlot heifers utilized in this trial have been previously reported (Gillis et al., 2004). As supplemental lipid was included in treatment diets, an equal proportion of concentrate was removed. Synovex-H implants (20 mg of estradiol benzoate and 200 mg of testosterone; Fort Dodge Animal Health, Fort Dodge, IA) were administered to all animals at trial initiation.

Heifers were housed by treatment group in pens (six heifers per pen) outfitted with individual Calan gate feeders (American Calan, Inc., Northwood, NH). Heifers were allowed free access to diets, with fresh rations weighed and provided at 0800 (refusals were recorded daily). Heifer weights and feed samples were obtained before feeding at approximately 28-d intervals throughout the trial. Feed samples were lyophilized, ground through a Wiley mill equipped with a 1-mm screen, and stored at -20° C for subsequent proximate analysis and FA profiling.

Sample Collection

Heifers were transported to the University of Georgia's Meat Science and Technology Center (Athens) following overnight feed withdrawal. Live animal weights were recorded before slaughter. Heifers were slaughtered according to humane, industry-accepted procedures, and adipose tissue samples were removed from the s.c. (12th rib) and p.a. regions of the left side before chilling and were immediately frozen. Rib sections were obtained from right sides, and 2.54-cm-thick steaks were removed, trimmed, and stored at -20° C for determination of lipid concentration and FA composition.

Fatty Acid Composition

Adipose and muscle tissue samples were frozen in liquid nitrogen and pulverized using a Waring blender before lipid extraction. Total lipids were extracted in duplicate from samples using organic solvents according to the procedures of Folch et al. (1957), with the following modification: the solvent to sample ratio was 10:1. Lipid extracts were stored at -80°C for subsequent determination of FA composition.

Lipid extracts, containing approximately 5 mg of lipid, were transmethylated according to the method of Park (1994). Previous research in our laboratory has shown that this method does not alter the *cis-trans* bond arrangements of CLA during methylation (Duckett et al., 2002). Briefly, in situ extraction and transmethylation of sample extracts to fatty acid methyl esters (**FAME**) was achieved using sodium methoxide followed with boron trifluoride. Others (Kramer et al., 1997) have similarly shown that the use of a base followed by acidic catalysts results in complete conversion of FA to methyl esters without bond rearrangement.

Analysis of FAME was performed using an Agilent 6850 gas chromatograph equipped with an automatic sampler (Agilent, Wilmington, DE) according to conditions outlined by Duckett et al. (2002). Retention times were compared to those for known standards (Matreya, Pleasant Gap, PA; Nu-Chek Prep, Elysian, MN; Sigma Chemical Co., St. Louis, MO). Fatty acids were quantified based upon the inclusion of an internal standard (methyl tricosanoate) during methylation and expressed as a percentage of total FA.

Statistical Analysis

Data were subjected to ANOVA for a completely randomized design using the GLM procedure of SAS (SAS Inst., Inc., Cary, NC), with individual heifer serving as the experimental unit. The model included the effects of dietary treatment, adipose depot, length of supplementation, all two-way interaction terms, and the three-way interaction of treatment, depot, and length of supplementation. Least squares means were generated and separated using the PDIFF option of SAS for main or interactive effects. Significance was determined at $(P \le 0.05)$, whereas differences of (P > 0.05) to $(P \le 0.10)$ were considered as trends.

Results

Fatty acid concentration by adipose tissue depot is shown in Table 1. All two and three-way interactions with adipose depot and dietary treatment or time on treatment were nonsignificant (P > 0.05). Intramuscular adipose depots contained the lowest (P < 0.05) percentage of total lipid, whereas p.a. contained the highest (P < 0.05) total lipid concentrations. Margaric (C17:0) acid and total odd-chain FA concentrations were lowest (P < 0.05) in i.m. lipid and highest (P < 0.05)0.05) in p.a. depots. Palmitic (C16:0) acid concentration was lower (P < 0.05) in p.a. depots than in i.m. or s.c. depots, which did not differ (P > 0.05). Stearic (C18:0) acid levels were greater (P < 0.05) in i.m. lipid than in p.a. or s.c. depots, which were similar (P > 0.05). Total SFA were highest (P < 0.05) in i.m. lipid and lowest (P< 0.05) in p.a. depots. Concentrations of myristic (C14:0) and pentadecyclic (C15:0) acids did not differ (P > 0.05)between adipose depots.

Myristoleic (C14:1, *cis*-9) acid concentration was greater (P < 0.05) in p.a. lipid than i.m. or s.c. depots. Intramuscular adipose tissue was composed of lower (P < 0.05) concentrations of myristoleic, palmitoleic, oleic,

and cis-12 octadecenoic (C18:1, cis-12) acid than p.a. or s.c. depots. Intramuscular lipid contained the lowest (P< 0.05) concentrations of trans-10 octadecenoic acid and *trans*-11 vaccenic acid (**TVA**) compared with p.a. and s.c. depots; however, p.a. adipose tissue contained the highest (P < 0.05) concentration of TVA. The content of total monounsaturated fatty acid (MUFA) was lower (P < 0.05) in i.m. tissue compared with either p.a. or s.c., which were similar (P > 0.05). Linoleic and arachidonic acid concentrations were higher (P < 0.05) in i.m. lipid compared with p.a. or s.c., which did not differ (P > 0.05). Linolenic (C18:3) acid content was higher (P <0.05) in i.m. and p.a. adipose depots, and lowest (P <(0.05) in s.c. adipose tissue. Total PUFA were higher (P < 0.05) in the i.m. compared with the p.a. and s.c. lipids, which were similar (P > 0.05). Unidentified FA were greater (P < 0.05) in samples from i.m. and p.a. than s.c.

Conjugated linoleic acid concentration by adipose tissue depot is presented in Table 2. Concentration of cis-11, *trans*-13 isomers tended (P < 0.10) to be higher in s.c. than i.m. adipose tissues, with p.a. being intermediate. Perianal adipose depots contained the highest (P <(0.05), and i.m. lipid the lowest (P < 0.05), proportions of trans-8, cis-10 CLA isomers. Intramuscular lipid contained lower (P < 0.05) concentrations of cis-9, trans-11; trans-10, cis-12; cis, cis; and trans, trans CLA isomers than p.a. and s.c. Due to the fact that the *cis*-9, trans-11 CLA isomer predominates as a percentage of total CLA (approximately 58 to 71%), a similar trend was observed in total CLA amounts, with i.m. lipid containing the lowest (P < 0.05), and p.a. the highest (P < 0.05)< 0.05), levels. In general, i.m. adipose depots contained lower (P < 0.05) levels of CLA isomers than either p.a. or s.c. depots.

Effect of dietary treatment on FA composition of adipose tissue depots is presented in Table 3. The two-way interactions between dietary treatment and length of supplementation were significant (P < 0.05) for some FA, and are presented in Table 4. Total lipid concentrations did not (P > 0.05) differ by dietary treatment. Heifers fed supplemental lipid for 60 d before slaughter had greater (P < 0.05) total lipid than those supplemented 32 d. Supplementing heifers with corn oil or rumen-protected CLA salt resulted in higher (P < 0.05) concentration of trans-11 octadecenoic acid in adipose tissues compared to CON heifers. Tissues from CONfed heifers contained greater (P < 0.05) amounts of *cis*-11 octadecenoic acid (C18:1, cis-11) than RPCLA, with OIL being intermediate. Linoleic acid content was higher (P < 0.05) in adipose tissues from heifers fed OIL compared with CON or RPCLA, which did not differ (P > 0.05). Polyunsaturated FA were found at the highest (P < 0.05) levels in tissues from heifers supplemented with OIL and lowest (P < 0.05) amounts in tissues of CON heifers. Fewer (P < 0.05) unidentified FA were observed for OIL than CON or RPCLA. Other FA (C14:1, C17:0, C18:0, C18:1 cis-12, C18:3, and C20:4) were not altered (P > 0.05) by dietary treatment. Concentrations of C17:0 and C18:1 cis-11 FA were lower

Total fatty acids, %	i.m.	p.a.	s.c.	SEM
Total lipid	6.65^{f}	81.84^{d}	78.88^{e}	0.83
C14:0	3.20	3.41	3.29	0.08
C14:1 cis-9	0.05^{f}	$0.17^{ m d}$	$0.14^{ m e}$	0.01
C15:0	0.55	0.62	0.58	0.02
C16:0	27.11^{d}	25.72^{e}	26.77^{d}	0.21
C16:1 cis-9	$2.92^{\rm e}$	$3.26^{\rm d}$	3.23^{d}	0.09
C17:0	$0.54^{ m f}$	0.73^{d}	$0.69^{ m e}$	0.01
C18:0	14.92^{d}	$13.74^{ m e}$	13.78^{e}	0.25
C18:1 trans-10	1.58^{e}	1.99^{d}	2.12^{d}	0.12
C18:1 trans-11	0.75^{f}	1.13^{d}	$0.95^{ m e}$	0.03
C18:1 cis-9	$38.74^{ m e}$	40.41^{d}	40.10^{d}	0.32
C18:1 cis-12	$0.19^{\rm e}$	$0.37^{ m d}$	0.37^{d}	0.03
C18:2 cis-9, cis-12	2.19^{d}	$1.15^{ m e}$	$1.15^{ m e}$	0.05
C18:3 cis-9, cis-12, cis-15	0.20^{d}	0.20^{d}	$0.16^{ m e}$	0.01
C20:4	$0.47^{ m d}$	$0.04^{\rm e}$	$0.05^{ m e}$	0.02
Unidentified	4.89^{d}	4.72^{d}	$4.32^{ m e}$	0.10
SFA ^c	45.19^{d}	42.88^{f}	$43.85^{ m e}$	0.34
OCFA ^c	1.10^{f}	1.35^{d}	$1.28^{\rm e}$	0.02
MUFA ^c	45.88^{e}	49.03^{d}	48.61^{d}	0.34
PUFA	3.60^{d}	2.34^{e}	2.18^{e}	0.10

Table 1. Fatty acid composition by adipose depot^a

^aAll two- and three-way interactions were nonsignificant (P > 0.05).

^bi.m. = intramuscular; s.c. = subcutaneous; and p.a. = perianal.

 ^{c}SFA = saturated fatty acid; OCFA = odd-chain fatty acid; and MUFA = monounsaturated fatty acid. $^{d,e,f}W$ ithin a row, least squares means without a common superscript letter differ (P < 0.05).

(P < 0.05) in adipose tissues of heifers fed dietary treatments for 32 d compared with 60 d. Linolenic and TVA concentrations were higher (P < 0.05) in adipose depots from heifers supplemented with lipid for 32 than 60 d. Additional FA (C14:1 *cis*-9, C18:0, C18:1 *cis*-12, C18:2 *cis*-9, *cis*-12, C20:4, total PUFA and unidentified) were not (P > 0.05) affected by length of supplementation.

The effect of dietary treatment by length of lipid supplementation on adipose tissue FA concentration is shown in Table 4. Total lipid did not (P > 0.05) differ among treatments by length of lipid supplementation. The two-way interaction for dietary treatment and supplementation length was significant (P < 0.05) for myristic (C14:0), pentadecyclic (C15:0), palmitic (C16:0), palmitoleic (C16:1), trans-10 octadecenoic (C18:1 trans10), oleic (C18:1 *cis*-9), total SFA, and total MUFA. Concentrations of myristic acid were similar (P > 0.05) by time on treatment for RPCLA; however, heifers receiving CON diet had reduced (P < 0.05) levels of myristic acid, whereas OIL had higher (P < 0.05) levels as length of supplemented increased. Pentadecyclic and palmitic acid concentrations were lower (P < 0.05) at 60 than at 32 d for CON treatment. Length of supplementation did not (P > 0.05) alter pentadecyclic and palmitic acid concentrations of adipose tissues from heifers supplied OIL or RPCLA. Additionally, concentrations of total SFA did not (P > 0.05) differ by supplementation duration for OIL or RPCLA supplements; however, tissues from CON heifers contained lower (P< 0.05) total SFA at 60 vs. 32 d.

Table 2. Conjugated linoleic acid concentration by adipose depot^a

CLA, percentage				
of total fatty acids ^c	i.m.	p.a.	s.c.	SEM
trans-8, cis-10	0.108^{f}	0.269^{d}	$0.191^{\rm e}$	0.023
cis-9, trans-11	0.522^{e}	0.684^{d}	$0.677^{ m d}$	0.031
cis-11, trans-13	$0.015^{ m h}$	$0.024^{ m gh}$	$0.039^{ m g}$	0.008
trans-10, cis-12	$0.008^{\rm e}$	0.013^{d}	0.014^{d}	0.001
cis, cis isomers	$0.024^{\rm e}$	0.048^{d}	0.044^{d}	0.003
trans, trans isomers	0.060^{e}	0.132^{d}	0.126^{d}	0.003
Total CLA isomers	0.736^{f}	1.170^{d}	$1.091^{\rm e}$	0.022

^aAll two- and three-way interactions were nonsignificant (P > 0.05).

^bi.m. = intramuscular; s.c. = subcutaneous; and p.a. = perianal.

^cCLA = conjugated linoleic acid.

 d,e,f Within a row, least squares means without a common superscript letter differ (P < 0.05).

 $^{\rm g,h}$ Within a row, least squares means with uncommon superscripts differ (P < 0.10).

Table 3. Effect of dietary treatment on fatty acid composition of adipose tissues^a

	Dietary treatments ^b				Length of supplementation, d		
Total fatty acids, %	CON	OIL	RPCLA	SEM	32	60	SEM
Total lipid	55.29	56.27	55.82	0.83	53.96^{d}	57.63°	0.68
C14:1 cis-9	0.12	0.12	0.13	0.01	0.12	0.13	0.01
C17:0	0.67	0.65	0.65	0.01	$0.64^{ m d}$	0.68°	0.02
C18:0	13.91	14.08	14.45	0.25	14.35	13.94	0.25
C18:1 trans-11	0.84^{d}	0.98°	1.02^{c}	0.03	1.01°	0.88^{d}	0.03
C18:1 cis-11	1.73^{c}	$1.68^{\rm cd}$	1.62^{d}	0.03	$1.64^{ m d}$	1.71°	0.03
C18:1 cis-12	0.32	0.32	0.30	0.03	0.33	0.28	0.03
C18:2 cis-9, cis-12	1.34^{d}	1.76°	1.39^{d}	0.05	1.49	1.51	0.05
C18:3 cis-9. cis-12, cis-15	0.18	0.19	0.19	0.01	0.20°	$0.17^{ m d}$	0.01
C20:4	0.20	0.18	0.19	0.01	0.18	0.19	0.02
Unidentified	4.78°	4.41^{d}	4.73°	0.11	4.68	4.60	0.11
PUFA	$2.62^{\rm e}$	3.14°	2.85^{d}	0.08	2.89	2.85	0.06

^aAll two- and three-way interactions were non-significant (P > 0.05).

^bCON = control diet; OIL = control diet plus 4% corn oil; and RPCLA = control diet plus 2% rumenprotected conjugated linoleic acid salt.

 c,d,e Within a row, least squares means without a common superscript letter differ (P < 0.05).

Palmitoleic (C16:1, cis-9) acid adipose tissue content was not altered (P > 0.05) by supplementation length for CON or RPCLA; however, feeding OIL 60 d before slaughter increased (P < 0.05) palmitoleic acid tissue concentrations compared with 32 d of treatment. Adipose tissue levels of oleic acid (C18:1, cis-9) were similar (P > 0.05) among dietary treatments following 32 d of supplementation. Feeding OIL or RPCLA for 60 d before slaughter did not (P > 0.05) change tissue levels of oleic acid, whereas heifers fed CON diet for 60 d had increased (P < 0.05) levels of oleic acid. Similarly, feeding the basal diet a total of 60 d before slaughter increased (P < 0.05) total MUFA content of adipose depots. Length of lipid supplementation did not alter (P > 0.05)adipose MUFA concentrations in adipose tissues of heifers fed OIL or RPCLA.

Length of lipid supplementation did not (P > 0.05) alter *trans*-10 octadecenoic acid concentrations in adi-

pose tissues of cattle fed OIL or CON (Table 4). However, supplying 2% RPCLA to finishing diets for 60 d resulted in greater (P < 0.05) proportions of *trans*-10 octadecenoic acid compared with all other treatments. Regardless of time on treatment, RPCLA-fed heifers had higher (P < 0.05) tissue levels of *trans*-10 octadecenoic acid.

Table 5 shows the effects of dietary treatment on CLA content of adipose tissues. Adipose tissue from heifers fed OIL or RPCLA had greater (P < 0.05) concentrations of *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA compared with CON heifers. Total *cis*, *cis* and *trans*, *trans* CLA isomers were higher (P < 0.05) in concentration in adipose tissues of heifers fed RPCLA compared with CON or OIL, which were similar (P > 0.05). Other CLA isomers (*trans*-8, *cis*-10 and *cis*-11, *trans*-13) did not (P > 0.05) differ among dietary treatments. Total CLA isomers in adipose tissue were lowest (P < 0.05) for CON

	$Dietary \ treatment \times length \ of \ supplementation^b$						
	32 d			60 d			
Total fatty acids, %	CON	OIL	RPCLA	CON	OIL	RPCLA	SEM
Total lipid	54.56	54.11	53.20	56.02	58.43	58.43	1.18
C14:0	$3.40^{\rm cd}$	3.21^{d}	$3.36^{\rm cd}$	2.83^{e}	3.54°	$3.46^{\rm cd}$	0.11
C15:0	$0.60^{\rm cd}$	0.65°	$0.56^{ m d}$	$0.48^{\rm e}$	$0.61^{\rm cd}$	$0.61^{\rm cd}$	0.03
C16:0	27.24°	26.36^{de}	26.46^{cd}	25.69^{e}	26.48^{cd}	26.97^{cd}	0.29
C16:1, cis-9	$3.22^{\rm cd}$	$3.02^{\rm d}$	$3.13^{\rm cd}$	$3.18^{\rm cd}$	3.40°	$2.89^{\rm d}$	0.12
C18:1 trans-10	$1.40^{ m ef}$	$1.78^{\rm e}$	2.22^{d}	1.25^{f}	$1.78^{\rm e}$	2.95°	0.17
C18:1 cis-9	39.51^{d}	$39.61^{\rm d}$	$38.97^{ m de}$	42.29°	39.88^{d}	$38.21^{\rm e}$	0.45
SFA ^a	44.75°	44.13^{cd}	44.21°	42.20^{e}	43.63^{d}	44.92°	0.49
MUFA ^a	$47.11^{\rm d}$	47.55^{d}	47.46^{d}	49.80 ^c	48.09^{d}	47.02^{d}	0.48

Table 4. Effect of supplementation length and dietary treatment on fatty acid composition of adipose tissues

^aSFA = saturated fatty acid; and MUFA = monounsaturated fatty acid.

^bCON = control diet; OIL = control diet plus 4% corn oil; and RPCLA = control diet plus 2% rumenprotected conjugated linoleic acid salt.

 c,d,e,f Within a row, least squares means without a common superscript letter differ (P < 0.05).

*							
Total fatty acids, %							
	CON	OIL	RPCLA	SEM			
trans-8, cis-10	0.200	0.181	0.186	0.022			
cis-9, trans-11	0.599^{d}	0.664^{c}	0.672°	0.028			
cis-11, trans-13	0.031	0.021	0.030	0.008			
trans-10, cis-12	$0.005^{\rm d}$	0.015°	0.015°	0.001			
Total cis, cis	0.036^{d}	0.034^{d}	0.044^{c}	0.003			
Total trans, trans	0.082^{d}	0.090^{d}	0.152°	0.003			
Total CLA ^b	0.919^{e}	$0.997^{ m d}$	1.080°	0.022			

 Table 5. Effect of dietary treatment on conjugated linoleic acid concentration in adipose tissues

 a CON = control diet; OIL = control diet plus 4% corn oil; and RPCLA = control diet plus 2% rumen-protected conjugated linoleic acid salt.

^bCLA = conjugated linoleic acid.

 c,d,e Within a row, least squares means without a common superscript letter differ (P < 0.05).

treatments and highest (P < 0.05) for RPCLA treatments. Supplementing corn oil or rumen-protected CLA to finishing diets increased total adipose tissue CLA levels by 8.5 and 17.5%, respectively. Concentrations of CLA isomers did not (P > 0.05) differ by length of supplementation (data not shown).

Discussion

In ruminant animals, FA composition of adipose tissue depots is dependent on 1) supply of dietary FA to depots as influenced by extent of ruminal biohydrogenation, as well as intestinal absorption rates, 2) de novo synthesis of FA from precursors supplied to adipose tissues, and 3) rate of desaturation by the adipose tissue enzyme, Δ^9 desaturase (Enser et al., 1999). Unsaturated FA, which are preferentially saturated by ruminal microorganisms, must either be protected from ruminal biohydrogenation (as calcium soaps or formaldehyde-encased lipids) or be present in amounts high enough to result in sufficient escape to the intestinal tract for absorption. Typically, 68 to 84% of dietary 18-carbon, unsaturated FA are biohydrogenated in the rumen of animals fed high-concentrate diets (Zinn et al., 2000; Duckett et al., 2002; Sackmann et al., 2003); however, treating unsaturated fats with formaldehyde protects them from ruminal degradation and reduces biohydrogenation levels to 54% (Zinn et al., 2000). Supplementing corn oil or substituting high-oil corn for typical corn varieties in high-concentrate rations increases the flow of linoleic acid to the small intestine (Duckett et al., 2002). Gulati et al. (2000) have shown that encapsulation of CLA isomers with a protein matrix reduced biohydrogentation from 70 to 30%, and increased milk CLA levels by 10-fold. Increasing intestinal flow of dietary unsaturated fats increases the potential for intestinal absorption and tissue deposition. Thus, this experiment was conducted to evaluate the short-term supplementation of rumen-protected CLA or corn oil in feedlot rations to enhance CLA content in beef. Short-term supplementation was evaluated based on previous research (Duckett et al., 1993), which

showed a twofold increase in i.m. lipid deposition during 84 and 112 d on feed in finishing steers serially slaughtered. Targeting the supplementation period of dietary lipid to coincide with period of heightened marbling deposition would potentially allow for altering FA composition of beef at a lower cost of supplementation.

Adipose tissue is the primary site of lipogenesis in ruminants, with enzyme activity playing a major role in the assimilation and accumulation of storage lipids. The adipose tissues utilized in this study (i.m., p.a., and s.c.) were selected based on their active accumulation during the late finishing period when our dietary treatments were administered. Intramuscular adipose tissues contained greater concentrations of SFA and PUFA than did p.a. or s.c. adipose tissues, whereas p.a. and s.c. adipose tissues contained greater concentrations of MUFA and odd-chain FA. The increase in total PUFA content of i.m. lipid is reflective of 90% greater levels of linoleic acid in i.m. adipose tissue compared with p.a. and s.c. depots. Others have reported similar differences in FA composition of various adipose depots in lamb (Bolte et al., 2002; Wachira et al., 2002) and beef (Sumida et al., 1972; Garcia et al., 2003). No interactions between adipose depot and dietary treatment or supplementation length were observed, which indicates that changes in depot FA composition due to diet or time are consistent across adipose depots. These results suggest that differences in enzymatic rates among adipose depots are likely responsible for the resultant changes in FA composition. Additionally, the results of the current study illustrate the importance of measuring changes in FA composition in those adipose tissues most likely to be consumed in the human diet. Quantifying FA composition of depots not typically consumed (p.a. or s.c.) may overestimate levels of FA present in a serving of beef.

Supplementing linoleic acid in the form of corn oil to finishing cattle during the last 32 or 60 d on feed increased linoleic acid concentrations in adipose tissues by 32%, which resulted in greater total concentration of total PUFA. Similarly, Bolte et al. (2002) reported 33% greater percentages of PUFA in adipose tissues (kidney, pelvic, and heart fat [KPH], s.c., and tailhead depots) of lambs fed linoleate-rich diets. Moreover, these authors reported FA composition data pooled across dietary treatments, and observed 26.7% greater concentration of total PUFA in tailhead fat compared with KPH and s.c. lipid. Bolte et al. (2002) also reported 55.2% greater levels of linoleic acid and 42.3% higher concentrations of PUFA in i.m. adipose tissue of lamb fed high-linoleate safflower seeds. Also, Garcia et al. (2003) reported greater concentrations of linoleic acid in i.m., s.c., and kidney fat depots of heifers fed 5% whole sunflower seeds compared with heifers fed no added fat. The greatest improvement was an increase in i.m. linoleic acid concentration of 60.6% when sunflower seed was the fat source, and increases of 40.9 and 47.8% for s.c. and KPH fat, respectively, were also observed. Andrae et al. (2001) reported similar increases in total PUFA (20.1%) and linoleic acid concentrations (18.7%) in i.m. lipid from steers fed high-oil corn diets. Duckett et al. (2002) reported an increased flow of linoleic acid and *trans*-octadecenoic acids to the duodenum of cattle fed high-concentrate diets containing high-oil corn or corn oil. The increased concentration of linoleic acid in lipid depots of heifers supplemented with 4% corn oil demonstrates that short-term feeding was effective in altering FA composition.

Supplementing lipid to feedlot diets, either as rumenprotected CLA or corn oil, for increased time periods (60 vs. 32 d) did not alter oleic acid, total SFA, or total MUFA concentrations in adipose tissues. In contrast, heifers fed CON in the present study had lower SFA, and higher oleic acid and MUFA content in adipose tissues as time-on-feed increased. Similarly, Gassman et al. (2000) reported a reduction of 17.8% in oleic acid concentration in i.m. adipose tissues of steers supplemented with CLA salt an average of 130 d compared with control animals. Duckett et al. (1993) reported a linear increase in MUFA content in bovine i.m. lipid, which resulted primarily from an increase in concentration of oleic acid across time-on-feed in steers fed high concentrate diets. The adipose tissue enzyme, Δ^9 desaturase, is responsible for insertion of a *cis* double bond at the ninth position from the carboxyl group of FA, converting stearate to oleate. Some PUFA, including linoleic acid, are thought to inhibit Δ^9 desaturase activity by downregulating gene expression (Yang et al., 1999; Choi et al., 2002; Smith et al., 2002). The decrease in MUFA concentrations observed in the current study for heifers fed supplemental lipid 60 d before slaughter suggests that Δ^{9} desaturase activity was depressed. Therefore, the activity of the Δ^9 desaturase enzyme is important when considering manipulation of the FA composition of bovine adipose tissue depots.

Ruminant milk and meat products are the largest natural source of CLA. By definition, the ruminant animal presents a unique situation related to the metabolism and subsequent deposition of dietary lipid as adipose tissue. Through a series of isomerization and desaturation reactions, the process of rumen microbial biohydrogenation converts unsaturated dietary linoleic acid to stearic acid. This process results in the flow of FA to the duodenum being primarily saturated (Demeyer and Doreau, 1999). During the processes of ruminal biohydrogenation of dietary unsaturated lipids, unique FA intermediates (CLA) possessing anticarcinogenic effects are produced in addition to the *trans*-octadecenoic acids and saturated end products (Bauman et al., 1999). Increasing the content of the *cis*-9, *trans*-11 CLA isomer, which is a potent anticarcinogenic agent (Ha et al., 1987), may be beneficial from the standpoint of human nutrition.

Conjugated linoleic acid, as well as *trans*-10 octadecenoic acid and TVA, concentrations differed between adipose depots. Perianal and s.c. adipose depots contained greater concentrations of biohydrogenation intermediates, including all *trans*- and *cis*-octadecenoic acids and CLA isomers. Madron et al. (2002) reported similar findings when comparing the FA composition of i.m. to s.c. adipose tissue, with s.c. containing higher levels of the *cis*-9, *trans*-11 CLA than i.m. Bolte et al. (2002) also reported higher levels of CLA and *trans*octadecenoic acids in p.a. depots vs. s.c., which were both greater than kidney fat. These authors also found higher levels of CLA and *trans*-octadecenoic acids in the i.m. lipid of the semitendinosus vs. LM.

Supplementing rumen-protected CLA or corn oil during the final 32 or 60 d on feed increased the concentration of TVA by 12% for OIL supplemented heifers and by 21% for RPCLA compared to CON. The cis-9, trans-11 CLA isomer concentration was 11% greater for OIL and 12% for RPCLA compared with CON. Adipose tissue trans-10, cis-12 CLA concentration was twofold greater for OIL and RPCLA compared with CON. Various *cis*, *cis* and *trans*, *trans* isomers of CLA, as well as trans-10 octadecenoic acid and total CLA concentrations, in adipose tissues were greater in heifers fed rumen-protected CLA salts vs. the CON or OIL diets, indicating that RPCLA supplementation was effective in increasing adipose concentration of CLA isomers; however, changes were relatively small. Gassman et al. (2000) reported a 1.4-fold increase in LM CLA content when 2.5% rumen-protected CLA salt (containing 48% CLA isomers) was supplemented to finishing steers. When CLA salt was included at 1% of the diet, CLA isomer concentrations in the LM were 58% greater than controls (Gassman et al., 2000). Lambs fed high-oleate or -linoleate safflower seeds had greater concentrations of CLA isomers (cis-9, trans-11 and trans-10, cis-12) and TVA in adipose tissues (Bolte et al., 2002). These authors also found that CLA and TVA concentrations in adipose tissues were greater for high-linoleate vs. high-oleate seeds, indicating the substrate supply was important for increasing deposition of biohydrogenation intermediates. Garcia et al. (2003) reported increased cis-9, trans-11 CLA isomer concentrations in s.c. lipid of heifers fed diets containing whole sunflower seeds; however, they did not observe differences in kidney fat and were unable to detect CLA in i.m. lipid. Mir et al. (2002) reported large increases in adipose tissue cis-9, trans-11 CLA concentrations of Wagyu × Limousin steers consuming a barley-based diet supplemented with safflower oil. In contrast, Beaulieu et al. (2002) observed no change in lipid content of cis-9, trans-11 CLA isomers when 5% soybean oil was supplied to Angus × Wagyu feedlot heifers. Research in dairy cattle has shown that supplementing CLA salts or vegetable oils increases the cis-9, trans-11 CLA isomer concentrations in milk fat (Corl et al., 2001).

Trans-11 vaccenic acid can be desaturated to the cis-9, *trans*-11 isomer of CLA by the Δ^9 desaturase enzyme present in bovine mammary gland (Griinari et al., 2000). This enzyme is also present in bovine adipose tissues and is responsible for the desaturation of stearic acid to oleic acid, the predominant FA in beef tissues (St. John et al., 1991). Research in dairy cattle has shown that 64 (Griinari et al., 2000) to 78% (Corl et al., 2001) of the CLA in milk fat originated from desaturation of TVA. Estimates from our laboratories (Gillis et al., 2003a) suggest that over 86% of tissue CLA in beef originates from desaturation of TVA based on ratios of TVA:cis-9, trans-11 CLA in duodenal and adipose tissues. Results from this study indicate that shortterm lipid supplementation to feedlot heifers can increase adipose CLA concentrations but only to a minor extent (8.5 to 17.5%). The reductions observed in oleic acid and total MUFA concentrations of adipose tissues from heifers fed rumen-protected CLA or corn oil suggest that lipid supplementation may depress Δ^9 desaturase activity in adipose tissues, which would lower the conversion of TVA to the cis-9, trans-11 CLA isomer. Additional research is needed to determine the effects of lipid supplementation in ruminants fed high concentrate diets on adipose enzyme activity, specifically the Δ^9 desaturase enzyme.

Implications

Short-term supplementation of rumen-protected conjugated linoleic acid or corn oil to finishing cattle diets altered composition of various adipose depots. However, these supplementation strategies were only marginally effective in increasing adipose tissue concentrations of *trans*-11 vaccenic acid and conjugated linoleic acid, compounds important for human health.

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