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Conjugated linoleic acid content of beef from cattle fed diets containing high grain, CLA, or raised on forages☆

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Abstract

Twenty Angus crossbred steers were assigned to one of four treatments and followed from weaning to slaughter to study the effect of diet on the conjugated linoleic acid ($C_{18:2}$ *cis*-9, *trans*-11 and $C_{18:2}$ *trans*-10, *cis*-12 CLA isomers) content and quality of beef. During the adaptation period, treatments 1 (CTL), 2 (CLA), and 3 (GPS) received a diet consisting of 520 g corn silage, 213 g alfalfa hay, 250 g rolled barley, and 17 g mineral–vitamin premix/kg of dry matter (DM). Treatment 4 (PS) received alfalfa hay only. During the finishing period, CTL and CLA steers received a diet consisting of 123 g corn silage, 67 g alfalfa hay, 764 g rolled barley, and 46 g mineral–vitamin premix/kg of diet dry matter. In addition to the basal diet, CLA steers received 84 g/head/day of a synthetic mixture of partially rumen protected CLA isomers. The GPS and PS treatments were finished solely on pasture. Subcutaneous adipose tissue samples were collected from the M. longissimus dorsi at the end of the adaptation period, and both adipose and muscle tissues were collected from the longissimus and M. semitendinosus of each carcass at slaughter for fatty acid analysis. Beef tissues from PS and GPS steers had 466% and 218% more CLA ($C_{18:2}$ *cis*-9, *trans*-11 isomer) at slaughter compared with beef tissues from CTL steers, respectively. Supplementing synthetic CLA did not increase $C_{18:2}$ *cis*-9, *trans*-11 CLA content of beef, but increased *trans*-10, *cis*-12 CLA by 380% compare to beef from CTL animals. A trained taste panel detected no differences in tenderness or juiciness among treatments. However, beef from PS received higher off-flavor scores than other treatments. Raising cattle on forage and pasture with no grain supplementation enhances beef CLA content. Additionally, finishing cattle on pasture increased the vitamin E content of beef by 300% compared to beef from animals finished on a traditional high-grain diet.

Keywords: Beef; Conjugated linoleic acid; Pasture; Meat; Fat

1. Introduction

Conjugated linoleic acid (CLA) is a group of naturally occurring fatty acid isomers found in many foods. However, foods of ruminant origin have been

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found to contain the largest quantities of CLA (Chin et al., 1992). Conjugated linoleic acid may have potential human health benefits. The $C_{18:2}$ *cis*-9, *trans*-11 isomer of CLA has been shown to have anticarcinogenic properties in animal models (Ip et al., 1994; Visonneau et al., 1997; Corl et al., 2003). The $C_{18:2}$ *trans*-10, *cis*-12 isomer has been reported to increase the lean to fat ratio in growing animals (Park et al., 1999; Azain et al., 2000). Antidiabetic effects of CLA isomers have also been reported (Houseknecht et al., 1998; Ryder et al., 2001). For these reasons, increasing the CLA content of foods may increase their nutritive and therapeutic values.

Conjugated linoleic acid isomers are formed in the rumen as intermediates in the biohydrogenation of long-chain unsaturated fatty acids (Kepler and Tove, 1967). In addition, $C_{18:2}$ *cis*-9, *trans*-11 CLA is also synthesized endogenously through the desaturation of $C_{18:1}$ *trans*-11 by the Δ^9-desaturase enzyme (Griinari et al., 2000; Corl et al., 2001). It has been demonstrated that the CLA content of milk fat can be increased by grazing cows on pasture or by adding supplemental oils high in linoleic or linoleic acid to the diet of dairy cows (Kelly et al., 1998a; Dhiman et al., 1999a). It has assumed that CLA content could be increased in beef through similar dietary adjustments. Dhiman et al. (1999b) and Beaulieu et al. (2002) attempted to accomplish this through the addition of soybean oil to the diets of finishing steers for 105 days; however, results showed no significant difference in total CLA content among treatments. French et al. (2000) conducted a study for 85 days and showed that decreasing the amount of grain in the diet increased the concentration of CLA in the intramuscular fat of beef cattle. However, there is little information concerning the long-term influence (including both adaptation and finishing periods) of grains and forages on CLA content of beef.

The CLA content of beef may also be increased by feeding ruminally protected CLA supplements, making CLA available for absorption in the intestine and deposition in tissues.

The objective of the present research was to identify the long-term influence of grain and grazed grass in the diets of beef steers on the fatty acid profile of beef, including CLA content and also to determine what effect adaptation diet has on fatty acid profile. The secondary objective was to evaluate the quality of beef from cattle fed partially rumen-protected CLA supplement or raised on forages.

2. Materials and methods

2.1. Animals and treatments

Twenty weaned steer calves with an average weight of 235 kg (range 206–266 kg) were blocked according to body weight and randomly assigned by block to one of four treatments (CTL, CLA, GPS, and PS). There were two feeding periods: a adaptation and a finishing period. During the adaptation period, CTL, CLA, and GPS animals were all housed together and fed a total mixed ration (TMR) consisting of 520 g/kg corn silage, 213 g/kg alfalfa hay, 250 g/kg rolled barley, and 17 g/kg mineral–vitamin premix on a dry matter (DM) basis. The PS animals were housed as a group and were fed a diet containing alfalfa hay and free choice minerals and vitamins. Animals in all groups had ad libitum access to feed. After 195 days on the adaptation ration, steers were switched to their respective finishing diets. At the end of the adaptation period, steers in CTL, CLA, GPS, and PS treatments weighed 453±29, 450±28, 440±35, and 376±12 kg, respectively.

During the finishing period, steers in CTL and CLA treatments were housed in separate pens and were fed a basal TMR consisting of 123 g/kg corn silage, 67 g/kg alfalfa hay, 764 g/kg rolled barley, and 46 g/kg mineral–vitamin premix on a DM basis. In addition to the basal diet, CLA animals received 84 g/animal each day of a calcium salt of synthetic CLA isomers (Ca-CLA). The Ca-CLA was top-dressed on the TMR. The Ca-CLA was a mixture consisting of 350 g/kg $C_{18:2}$ *cis*-9, *trans*-11, 490 g/kg $C_{18:2}$ *trans*-10, *cis*-12, and 160 g/kg miscellaneous CLA isomers. Steers in GPS and PS treatments grazed on pasture and did not receive any supplemental grain during the finishing period. Animals in GPS and PS treatments received free choice minerals and vitamins.

After a finishing period of 130 days, animals were slaughtered at the Animal Slaughter Facility, Utah State University, Logan, UT, USA. Mean live body weights at the time of slaughter were 595±45, 587±45, 515±50, and 503±4 kg for CTL, CLA, GPS, and PS treatments, respectively. Animals were

slaughtered simultaneously as the amount of good-quality pasture was declining and it was desirable to maintain CLA levels that would be found in beef raised on normal pasture throughout the regular growing season. The experimental protocol was approved and conducted under established standards of the Utah State University Institutional Animal Care and Use Committee.

2.2. Diets, feed sampling, and analysis

The ruminal protection of the Ca-CLA was determined with an in vivo technique that was previously used to determine ruminal protection of formaldehyde-protected CLA (Gulati et al., 2000). Results from this study showed that 47% of Ca-CLA was protected from ruminal degradation at 24 h. A value of 50% ruminal protection was assumed in determining the amount of Ca-CLA supplement to feed to steers in the present study. It should be noted that protection level was determined using duodenally cannulated sheep. In a review by Demeyer and Doreau (1999), it is stated that when diets are high in unsaturated fatty acids and rumen pH is low, these factors will increase Ca-salt dissociation. Therefore, ruminal protection level for cattle fed high-grain diets may actually be lower than 50%.

Daily feed offered and feed refused was measured for steers in the CLA group to determine how much of the Ca-CLA was consumed by the animals. Samples of feed refusals were collected three times a week and analyzed for fatty acid profile including CLA. The CLA consumption calculated from the CLA contents of feed offered and refused was an average of 83.5 g/animal/day, suggesting that the majority of the Ca-CLA was consumed.

Animals in GPS and PS treatments grazed on pasture together during the finishing period. Pasture samples (4 samples/0.4 ha) were taken once a month from the pasture by clipping plots and analyzed for botanical and chemical composition. The average botanical composition of pasture was 1396 kg/ha live grass, 35 kg/ha live forbs, 37 kg/ha live weeds, and 787 kg/ha dead material on a DM basis. The average total available forage estimated was 2255 kg of forage DM/ha. Live grasses included Kentucky bluegrass (*Poa pratensis*), tall fescue (*Festuca arundinacea*), and orchardgrass (*Dactylis glomerata*). Live forbs consisted predominantly of white clover (*Trifolium repens*).

Representative samples of individual feed ingredients of the TMR in CTL, CLA, and GPS treatments and hay in the PS treatment were collected monthly. The partial DM contents of the individual ingredients were determined by oven-drying at 60 °C for 48 h. During analysis, the samples were dried at 105 °C for 8 h to determine absolute DM. Chemical analyses were expressed on the basis of this final DM. Dried feed samples were ground using a 1-mm screen and analyzed for crude protein (CP) using a Leco Nitrogen Analyzer (Model CHN-1000; Leco, St. Joseph, MI). The forage samples were analyzed for neutral detergent fiber (NDF) and acid detergent fiber (ADF) with the ANKOM^{200} fiber analyzer (ANKOM Technology, Fairport, NY, USA), using the basic procedure of Van Soest et al. (1991). Sodium sulfite was not used in the procedure for NDF determination, but pre-treatment with heat stable amylase (Type XI-A from *Bacillus subtilis*; Sigma-Aldrich, St. Louis, MO, USA) was included. Fatty acid analysis on feeds and Ca-CLA was performed using a procedure described by Sukhija and Palmquist (1988). Analysis was conducted in a gas chromatograph (Model 6890, Hewlett-Packard, Wilmington, DE, USA) fitted with a flame ionization detector. Gas chromatography conditions were the same as described by Dhiman et al. (1999a).

The chemical composition of the TMR was calculated from the nutrient analysis of individual dietary ingredients. The chemical composition of the adaptation TMR fed to animals in CTL, CLA, and GPS treatments was: DM=654 g/kg fresh feed and CP=95, NDF=511, and ADF=261 g/kg DM. The chemical composition of the alfalfa hay provided to PS animals was: DM=895 g/kg fresh feed and CP=176, NDF=492, and ADF=352 g/kg DM. The chemical composition of the finishing TMR fed to animals in CTL and CLA treatments was: DM=874 g/kg fresh feed and CP=115, NDF=415, and ADF=158 g/kg of DM. The average chemical composition of the pasture offered to animals during the finishing period in GPS and PS treatments was: DM=334 g/kg fresh feed and CP=117, NDF=654, and ADF=406 g/kg DM. Average fatty acid compositions of the adaptation TMR, finishing TMR, alfalfa hay, and pasture are given in Table 1.

Table 1
Fatty acid composition of TMR, alfalfa hay, and pasture

Item	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	Total fat, g/kg DM
	Fatty acid, g/100 g of total fatty acids reported							
Adaptation TMR	1.60	22.4	0.70	2.53	21.5	40.5	11.5	34.4
Finishing TMR	0.94	22.7	0.41	2.11	21.4	46.4	6.0	31.8
Alfalfa hay	1.84	25.8	0.40	4.18	24.9	15.5	27.8	22.4
Pasture	2.61	24.4	1.45	3.58	12.5	23.3	32.2	54.5

Total fatty acid content of the Ca-CLA supplement was 700 g/kg of DM. The Ca-CLA supplement consisted of $C_{14:0}$, $C_{16:0}$, $C_{16:1}$, and $C_{18:0}$ (10.7 g), $C_{18:1}$ (18.2 g), $C_{18:2}$ (8.0 g), $C_{18:2}$ *cis*-9, *trans*-11 (21.5 g), $C_{18:2}$ *trans*-10, *cis*-12 (30.2 g), miscellaneous CLA isomers (9.9 g), and long-chain fatty acids ($C_{20:0}$, $C_{20:1}$, $C_{22:0}$, and $C_{24:0}$; 1.5 g) per 100 g of total fatty acids.

2.3. Tissue sample collection and fatty acid analysis

Subcutaneous adipose tissue biopsies adjacent to the longissimus dorsi (LD) were collected at the end of the adaptation period. To collect tissue biopsies, the steers were anesthetized at incision sites and tissue samples were surgically removed from the LD on the right side of each animal. Samples were stored at −20 °C until further analysis could be conducted.

After slaughter, muscle and adipose tissue samples were dissected from the LD and semitendinosus (ST) on the left side of each carcass immediately after placement in the cold room. Tissue samples were stored in 20-ml glass scintillation vials at −20 °C until further analysis.

Lipid extraction and washing of the extract was conducted according to Folch et al. (1957) with some modifications. After homogenization, 5 ml of a 40 g/l KCl solution was added to the samples and the mixture was vortex-mixed. For the muscle tissue samples, another 5 ml of 40 g/l KCl solution was added to precipitate the protein out. Thirty milligrams of fat was derivatized to methyl esters by mixing with 5 ml of 40 ml/l HCl–MeOH (Chin et al., 1992). The methyl esters were extracted with 5 ml of hexane and 1 ml of distilled water. The hexane extract was washed twice with distilled water and dried over anhydrous sodium sulfate.

Gas chromatography conditions and analysis procedures were the same as described previously. Because $C_{17:0}$ is naturally present in beef, selected tissue samples were analyzed with and without heptadecanoic acid as an internal standard to ensure the accuracy and recovery. The CLA isomers reported are $C_{18:2}$ *cis*-9, *trans*-11 and $C_{18:2}$ *trans*-10, *cis*-12.

2.4. Sensory evaluation

For sensory evaluation, four 2.5-cm-thick top loin steaks were cut from the left side of each carcass at 24 h postmortem. Steaks were vacuum-packaged and aged in a cooler for 7 days. At the end of the 7-day aging period, steaks were frozen until the time of sensory evaluation.

Top loin steaks from some of the animals in the study along with a standard United States Department of Agriculture (USDA) average choice top loin steak and an eye of round steak were used in a training session to select potential taste panelists. Samples were cooked on an electric grill with a surface temperature of 163 °C to an internal temperature of 74 °C. Cooked steaks were cut into 1.5-cm square subsamples and served hot to the panelists. Potential panelists evaluated each sample for tenderness, juiciness, intensity of beef flavor, and intensity of off-flavor. Panelists evaluated tenderness and juiciness on an eight-point scale where 1=extremely tough and extremely dry and 8=extremely tender and extremely juicy. Intensity of beef flavor and intensity of off-flavor were rated on a seven point scale where 1=no beef flavor and no off-flavor and 7=extremely intense beef flavor and off-flavor.

The final taste panel evaluation was conducted with 10 panelists selected from the potential panelist pool in five different sessions (once a day for 5 consecutive days). During each session, each of the four treatment samples and a USDA choice steak were served. The USDA choice steak used each day came from the same animal and was served at each

taste panel session to account for day-to-day variation. Procedures for cooking and serving were the same as described for the training session. Panelists evaluated samples in private booths illuminated with red light, thereby decreasing their ability to distinguish visual differences in the samples. Characteristics and point scales used for evaluation were the same as those used during the training session.

2.5. Color stability and vitamin E analyses

A top loin steak and tenderloin steak were cut from the left side of each carcass 24 h postmortem. Steaks were vacuum-packaged and stored in the cooler until color stability analysis. Intensity of color in meat was measured by placing in a foam tray and covering with polyvinyl chloride film. The trays were placed under incandescent lights at 3 °C for 15 days. Readings were taken on days 1, 5, 7, 9, 12, and 15. Day 1 of the color readings corresponded to 2 days post-rigor. Hunter color L*, a*, and b* values were taken using a Hunter Lab miniscan portable color meter (Hunter Lab, Reston, VA, USA). The L* represents lightness in meat color on a scale from 0 to 100, where 0 corresponds to pure black and 100 corresponds to pure white. A positive a* value indicates redness with redness increasing as the number gets farther from 0. A negative a* value indicates greenness with greenness increasing as the value gets farther away from 0. A positive b* value indicates yellowness with yellowness increasing as the value gets farther from 0. A negative b* value represents blueness with blueness increasing as the value gets farther from 0. Three readings were averaged to determine the L*, a*, and b* values for each location and each time point. Color stability was also determined for fat, using measurements taken from the fat cover over the top loin muscle.

Muscle tissue samples were taken at slaughter from the neck, stored at −20 °C, and analyzed for vitamin E (Liu et al., 1996b).

2.6. Statistical analyses

Statistical analysis was performed using the MIXED procedures of the SAS system version 8.2 (SAS, 1999–2000). Data on fatty acid profiles of tissue samples from the adaptation and finishing periods were analyzed separately. The experimental design used for fatty acid composition during the adaptation period was a randomized complete block and the statistical model used included block and treatment. For the finishing period, tissue and site were added to the model because two different tissues (adipose and muscle) and two different sites (LD and ST) were sampled. The model included block, treatment, tissue, sampling site, treatment×tissue interaction, treatment×site interaction, site×tissue interaction, and residual error. Data were not analyzed for a treatment×tissue×site interaction because a preliminary analysis of variance revealed no significance for this interaction. Where no significant interactions were found, only treatment means are reported.

The GLM procedure of the SAS System was used to analyze the results of the sensory evaluation where tenderness, juiciness, intensity of off-flavor, and intensity of beef flavor were the response variables. The statistical model included treatment, session, person, treatment×session interaction, treatment×person interaction, and residual error. Session and person were blocking effects.

Color stability analysis was completed using the MIXED procedure of the SAS system (SAS, 1999–2000). The response variables were the L*, a*, and b* values. The experimental design was a randomized complete block design with repeated measures. The statistical analysis for the color intensity of lean muscle cuts (untrimmed top loin steak and tenderloin steak) was performed using a model that included block, treatment, cut, day, treatment×cut interaction, treatment×day interaction, cut×day interaction, treatment×cut×day interaction, and residual error. The statistical analysis for fat color over the top loin was performed using a model that included block, treatment, day, treatment×day interaction, and residual error.

Significant differences were determined at a $P<0.05$ unless otherwise noted. The least squares means were separated using Tukey's t-test. Contrasts were created for obvious comparisons. During the adaptation period, CTL, CLA, and GPS treatments were compared to the PS treatment. Interactions are not reported and discussed unless they were of statistical significance.

3. Results and discussion

3.1. Fatty acid profile of subcutaneous adipose tissue at the end of the adaptation period

The levels of $C_{8:0}$ to $C_{16:0}$ fatty acids in subcutaneous adipose 8:0, $C_{18:1}$ *trans*, $C_{18:3}$ *cis* 9,12,15, $C_{18:2}$ *cis*-9, *trans*-11, and $C_{18:2}$ *trans*-10, *cis*-12 were higher in adipose from animals fed all forage compared with adipose tissue from animals fed forage plus grain diets. The $C_{20:3}$ *cis*-8,11,14 fatty acid was lower in adipose from animals fed the all-forage diet compared with adipose tissue from animals fed the forage plus grain diet. The concentrations of *cis* isomers of $C_{18:1}$ were higher in the subcutaneous adipose tissue of grain plus forage fed groups (CTL, CLA, and GPS) as compared to that of PS animals fed forage alone. The response of *trans* isomers of $C_{18:1}$ was opposite (Table 2).

Table 2
Fatty acid composition of subcutaneous adipose tissue from longissimus dorsi at end of adaptation period (g/100 g of total fatty acids)

Fatty acid[a]	Treatment[b]				S.E.M.[c]	P=F[d]
	CTL	CLA	GPS	PS		
$C_{8:0}$	0.14	0.12	0.10	0.16	0.02	0.43
$C_{10:0}$	0.03	0.03	0.03	0.04	0.06	0.65
$C_{12:0}$	0.07	0.08	0.08	0.10	0.02	0.70
$C_{14:0}$	4.51	3.89	4.19	3.98	0.25	0.34
$C_{15:0}$	2.23	1.87	1.92	1.18	0.27	0.09
$C_{16:0}$	35.0	33.3	34.3	35.2	0.69	0.26
$C_{16:1}$	7.99^{e}	7.13^{e}	7.52^{e}	4.81^{f}	0.37	0.01
$C_{17:0}$	0.91^{f}	0.97^{f}	0.90^{f}	1.82^{e}	0.08	0.01
$C_{17:1}$	1.04	1.02	1.04	1.15	0.04	0.19
$C_{18:0}$	8.62^{f}	9.45^{f}	8.37^{f}	12.99^{e}	0.76	0.01
$C_{18:1}$ *trans*	1.22^{f}	1.21^{f}	1.28^{f}	2.68^{e}	0.17	0.01
$C_{18:1}$ *cis*	36.5^{e,f}	39.0^{e}	38.3^{e}	33.6^{f}	1.17	0.02
$C_{18:2}$ *c*9,12	1.23	1.33	1.34	1.10	0.07	0.08
$C_{18:3}$ *c*6,9,12	0.00	0.014	0.005	0.020	0.008	0.32
$C_{18:3}$ *c*9,12,15	0.19	0.20	0.20	0.71	0.03	0.01
$C_{18:2}$ *c*9, *t*11, CLA	0.25^{f}	0.24^{f}	0.27^{f}	0.46^{e}	0.02	0.01
$C_{18:2}$ *t*10, *c*12, CLA	0.011^{f}	0.007^{f}	0.014^{f}	0.045^{e}	0.006	0.01
$C_{20:2}$ *c*11,14	0.013	0.011	0.00	0.002	0.006	0.37
$C_{20:3}$ *c*8,11,14	0.06^{e}	0.05^{e}	0.06^{e}	0.02^{f}	0.01	0.01
$C_{20:3}$ *c*11,14,17	0.028	0.113	0.036	0.003	0.04	0.31

[e,f] Means within rows with different superscripts differ at the P value indicated.

[a] Number of carbon double bonds; *c*=*cis* double bond; *t*=*trans* double bond.

[b] Treatment diets fed during adaptation period were: animals in CTL, CLA, and GPS received 733 g forage and 267 g mix grain/kg of dietary dry matter and animals in PS received alfalfa hay and free choice mineral block. Treatment diets fed during finishing period were: animals in CTL and CLA received 190 g forage and 810 g grain mix/kg of dietary dry matter. In addition to basal diet, animals in CLA received 84 g per head/day of synthetic mixture of partially rumen protected CLA isomers. Animals in GPS and PS treatments grazed on a pasture during finishing period.

[c] Standard error of the mean.

[d] P value.

Table 3
Fatty acid composition of beef tissues (average of adipose and muscle tissues from longissimus and semitendinosus) of carcass at slaughter from cattle fed diets containing high grain, CLA, or raised on forages (g/100 g of total FA)

Fatty acid[a]	Treatment[a]				S.E.M.[b]	P=F[c]
	CTL	CLA	GPS	PS		
$C_{8:0}$	0.153	0.151	0.164	0.172	0.03	0.95
$C_{10:0}$	0.026	0.025	0.023	0.026	0.003	0.92
$C_{12:0}$	0.047	0.049	0.043	0.050	0.003	0.39
$C_{14:0}$	3.70^{d}	3.36^{d,e}	3.34^{d,e}	3.23^{e}	0.10	<0.01
$C_{15:0}$	2.64^{d}	2.00^{e}	2.15^{e}	2.18^{e}	0.09	<0.01
$C_{16:0}$	29.49^{d}	28.95^{d,e}	27.77^{e,f}	27.20^{f}	0.35	<0.01
$C_{16:1}$	7.28^{d}	5.85^{e,f}	6.17^{e}	5.22^{f}	0.25	<0.01
$C_{17:1}$	1.27^{d}	1.13^{e,f}	1.09^{f}	1.23^{d,e}	0.03	<0.01
$C_{18:2}$	1.35	1.45	1.51	1.32	0.05	0.06
$C_{18:2}$ *t*10, *c*12	0.015^{f}	0.072^{d}	0.025^{e,f}	0.042^{e}	0.006	<0.01
$C_{18:3}$ *c*6,9,12	0.015^{e}	0.023^{e}	0.023^{e}	0.038^{d}	0.003	<0.01
$C_{20:2}$	0.006^{d,e}	0.012^{d}	0.009^{d,e}	0.002^{e}	0.002	<0.01
$C_{20:3}$ *c*8,11,14	0.075	0.059	0.062	0.064	0.005	0.17
$C_{20:3}$ *c*11,14,17	0.095	0.138	0.106	0.095	0.02	0.47
$C_{20:4}$	0.006^{e}	0.002^{e}	0.004^{e}	0.021^{d}	0.003	<0.01

[d–f] Means with different superscripts within the same row differ at the P value mentioned in the last column.

[a] Refer to footnotes of Table 2 for description of treatments. Fatty acids with treatment×site interaction are presented in Table 4.

[b] Standard error of the mean.

[c] P value.

3.2. Fatty acid profile of beef at slaughter

The levels of $C_{8:0}$ to $C_{12:0}$ fatty acids were similar in beef tissues (adipose and muscle from LD and ST) from animals in all treatments (Table 3). Beef from CTL animals contained either numerically or statistically higher proportions of $C_{14:0}$, $C_{15:0}$, $C_{16:0}$, $C_{16:1}$, and $C_{17:1}$ fatty acids compared with the other treatments.

The treatment×site interaction was significant for $C_{17:0}$, $C_{18:0}$, and $C_{18:1}$ *cis* and *trans* fatty acids in beef tissues (Table 4). The LD adipose and muscle tissues from PS animals contained higher concentrations of $C_{17:0}$ and $C_{18:0}$ fatty acids compared with any of the other treatment by site combinations. French et al. (2000) observed no difference in the concentrations of $C_{18:0}$ in intramuscular tissue of animals fed diets differing in forage and grain levels. However, others have shown that animals finished on forages have higher concentrations of $C_{18:0}$ compared with animals fed high grain, low forage diets (Melton et al., 1982).

The levels of $C_{18:1}$ *trans* fatty acids were higher in LD and ST adipose and muscle from PS animals compared with any of the other treatments (Table 4). In addition, LD adipose and muscle in PS beef was higher in $C_{18:1}$ *trans* fatty acids than ST (6.2 vs. 4.2 g/100 g of total fatty acids, respectively). Interestingly, while LD from PS steers contained the highest concentration of $C_{18:1}$ *trans*, it also contained the lowest concentration of $C_{18:1}$ *cis* compared with the other treatments×site combinations (Table 4). Feeding Ca-CLA in CLA increased the proportion of $C_{18:1}$ *trans* fatty acids in beef tissues compared with the CTL. These results suggest that raising beef animals on forage and pasture increased (300%) the proportions of $C_{18:1}$ *trans* fatty acids and decreased $C_{18:1}$ *cis* fatty acids compared with animals fed high-grain diets (CTL and CLA treatments).

The $C_{18:2}$ fatty acid was not different in beef tissues among treatments (Table 3), irrespective of tissue or site. The $C_{18:3}$ *cis*-6,9,12 (Table 3) fatty acid was higher in meat of PS animals compared with the other treatments, irrespective of tissue or site. Both adipose and muscle tissues in PS contained significantly higher proportions of $C_{18:3}$ *cis*-9,12,15 fatty acid than any of the other treatments. Treatment×tissue means were 0.20^{c}, 0.09^{d}, 0.14^{cd}, 0.14^{cd}, 0.40^{b}, 0.44^{b}, 0.87^{a}, and 0.84^{a} for adipose and muscle in CTL, CLA, GPS, and PS treatments, respectively. These results can be explained by the fact that green forages are rich in $C_{18:3}$ (Dhiman et al., 1999a; Ward et al., 2003), thereby resulting in higher levels of $C_{18:3}$ in adipose and muscle from PS animals.

Higher proportions of $C_{18:3}$ *cis*-9,12,15 fatty acid in grass-fed beef may be beneficial for human health because supplementation of omega-3 fatty acids has been shown to decrease mitogen-stimulated lymphocyte proliferation, enhance immune function (Thies et al., 2001), and help prevent heart disease (Albert et al., 1998). It is interesting to note that the mean for $C_{18:3}$ *cis*-9,12,15 fatty acid in GPS was only approximately 50% of that in PS (0.42 vs. 0.85). These results suggest that animals backgrounded and finished on forage and pasture alone have higher levels of $C_{18:3}$ fatty acids compared with beef backgrounded on grain and finished on pasture.

The treatment×site and treatment×tissue interactions were both significant for $C_{18:2}$ *cis*-9, *trans*-11 in beef tissues (Fig. 1). Both adipose and muscle tissues in PS had higher $C_{18:2}$ *cis*-9, *trans*-11 content than any of the other treatment×tissue combinations. In addition, tissues from both LD and ST sites in PS were higher in $C_{18:2}$ *cis*-9, *trans*-11 content than any of the other combinations. Generally, GPS and PS animals had 218% and 466% more $C_{18:2}$ *cis*-9, *trans*-11,

Table 4
Fatty acid composition of beef tissues (adipose and muscle) from *Longissimus dorsi* and *semitendinosus* of carcass at slaughter from cattle fed diets containing high grain, CLA or raised on forages, g/100 g of total FA

	Treatment[a]									
	CTL		CLA		GPS		PS			
Fatty acid[a]	LD[b]	ST[b]	LD	ST	LD	ST	LD	ST	S.E.M.[c]	*P*=F
$C_{17:0}$	1.11^{e}	1.05^{e}	1.18^{e}	1.13^{e}	1.13^{e}	1.04^{e}	1.67^{d}	1.20^{e}	0.05	<0.01
$C_{18:0}$	$9.60^{e,f}$	7.31^{g}	11.1^{e}	$7.99^{f,g}$	11.7^{e}	$8.61^{f,g}$	14.3^{d}	$8.44^{f,g}$	0.50	<0.01
$C_{18:1}$ *cis*	$41.9^{d,e}$	$43.5^{d,e}$	$41.3^{d,e}$	44.6^{d}	40.4^{e}	$42.6^{d,e}$	35.1^{f}	$42.4^{d,e}$	0.84	<0.01
$C_{18:1}$ *trans*	1.34^{i}	1.03^{i}	2.96^{g}	2.03^{h}	$3.59^{e,f}$	$3.08^{f,g}$	6.22^{d}	4.15^{e}	0.30	<0.01

[a] Refer to footnotes of Table 2 for description of treatments. Fatty acid with significant treatment × site interaction.
[b] LD=Longissimus dorsi; ST=semitendinosus.
[c] Standard error of the mean.
[d–i] Means with different superscripts in the same row differ at the *P* value mentioned in the last column.

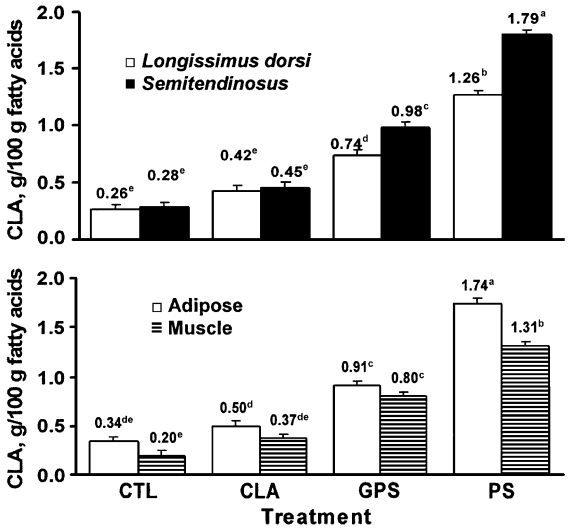

Fig. 1. Conjugated linoleic acid ($C_{18:2}$ *cis*-9, *trans*-11) content of beef from cattle fed diets containing high grain, CLA or raised on forages by site (*longissimus dorsi* and *semitendinosus*) and tissues (adipose and muscle) collected from *Longissimus dorsi* and *semitendinosus*.

respectively, compared with CTL animals. Feeding supplemental Ca-CLA to animals did not result in higher proportions of $C_{18:2}$ *cis*-9, *trans*-11 in adipose or muscle as compared to CTL.

One interesting observation is that while LD contained higher proportions of $C_{18:1}$ *trans* fatty acids, ST contained higher proportions of $C_{18:2}$ *cis*-9, *trans*-11 (Table 4; Fig. 1). These results suggest that there may be greater efficiency of conversion from $C_{18:1}$ *trans* into $C_{18:2}$ *cis*-9, *trans*-11 in the ST muscle compared with LD, indicating that there is possibly a difference in the activity of Δ^9-desaturase between these two sites.

The large increase in $C_{18:2}$ *cis*-9, *trans*-11 in beef tissues from animals raised on forage and pasture alone is similar in magnitude to the increase of $C_{18:2}$ *cis*-9, *trans*-11 in milk from dairy cows grazing on pasture (Kelly et al., 1998b; Dhiman et al., 1999a). The GPS animals were taken off the adaptation diet 5 months prior to slaughter and were on pasture with PS animals during the finishing period. This allowed ample time for rumen microorganisms to adjust to a pasture-based diet. Results from the current study suggest that feeding grain during adaptation may decrease expression of the mechanism responsible for the synthesis and incorporation of $C_{18:2}$ *cis*-9, *trans*-11 into tissues.

The levels of $C_{18:2}$ *trans*-10, *cis*-12 in beef tissues from animals fed supplemental Ca-CLA were 380%, 188%, and 71% higher than beef tissues from CTL, GPS, and PS animals, respectively (Table 3). The high level of $C_{18:2}$ *trans*-10, *cis*-12 in CLA animals is likely due to the high concentration (49.0 g/100 g of total CLA) of $C_{18:2}$ *trans*-10, *cis*-12 in Ca-CLA. French et al. (2000) reported that animals fed only grass had muscle tissue containing three times as much total CLA compared with that of animals fed high-grain diets. Raising beef animals on forage/pasture instead of high-grain diets increased total CLA by 466% in the current study.

In the present study, an acidic catalyst was used for fatty acid methyl ester preparation. Studies have shown that recovery of $C_{18:2}$ *cis*-9, *trans*-11 is lower when using an acid catalyst as compared to a base catalyst (Kramer et al., 1997; Murrieta et al., 2003). Though the values reported in this study are lower than would be determined using a basic catalyst for methylation, relative comparisons among treatments should still be valid. Treatment differences in CLA levels in muscle were not affected by use of acidic methylation catalysts (Murrieta et al., 2003).

Availability of $C_{18:2}$ *cis*-9, *trans*-11 from 100 g beef was calculated for CTL and PS treatments. Fat content values were estimated to be 5.6 g/100 g of fresh meat in CTL and 3.3 g/100 g of fresh meat in PS using a method described in Aberle et al. (2001). Wahrmund-Wyle et al. (2000) determined the cooking yield of top loin steak to be approximately 79% and this value was used in calculating the available CLA in meat. The average $C_{18:2}$ *cis*-9, *trans*-11 values used were 0.26 and 1.53 g/100 g of fat in CTL and PS treatments (Fig. 1). Based on our calculations, the $C_{18:2}$ *cis*-9, *trans*-11 available from CTL and PS beef was 12 and 40 mg/100 g of meat, respectively. These results suggest that despite the considerably lower total fat content of beef from forage fed animals (PS), the availability of $C_{18:2}$ *cis*-9, *trans*-11 is 3.3 times greater than beef from animals fed high-grain diets (CTL).

The 20-carbon fatty acids did not show a clear trend except that $C_{20:4}$ *cis*-5,8,11,14 was significantly higher in beef from animals raised on forage and pasture (PS) compared with beef from animals in the other treatments (Table 3).

3.3. Sensory characteristics of meat

Panelists rated the standard USDA choice steak as being more tender than steaks from the treatments in the present study (Table 5). However, there was no difference in tenderness or juiciness for steaks from animals in the experimental treatments. It has been shown that the degree of doneness has a greater effect on taste panel juiciness ratings than animal age or marbling score (Wulf et al., 1996).

Panelists rated the intensity of beef flavor higher in meat from animals fed a high-grain diet (CTL) than meat from animals raised on forage and pasture (PS; Table 5). The CLA, GPS, and PS treatments were rated similar for beef flavor. Animals raised on forage and pasture (PS) also had the highest off-flavor score, which was described as a "grassy" flavor by two of the more experienced panelists. Interestingly, steaks from animals in GPS, which were also finished on pasture, had no off-flavor detected by the panelists. This same response has been noted before where animals receiving only pasture had higher off-flavor scores compared with grain-fed animals or animals that were fed pasture and grain (Sapp and Williams, 1999).

Results from the current study suggest that experimental diet appeared to have little effect on the sensory characteristics of beef as perceived by taste panel members, with the exception of beef from PS animals having lower consumer-perceived beef flavor and a noticeable "grassy" flavor. An off-flavor can be a concern; however, this off-flavor is a perceived image based on personal preference. Feeding Ca-CLA to beef animals had no effect on the sensory characteristics of beef as perceived by taste panel members.

Table 5
Taste panel test results of meat from cattle fed diets containing high grain, CLA, or raised on forages

Test	Treatment[a]					S.E.M.[b]	P=F
	Standard	CTL	CLA	GPS	PS		
Tenderness[c]	6.06[d]	4.94[e]	4.70[e]	4.64[e]	4.40[e]	0.17	0.01
Juiciness[c]	4.60	5.06	4.74	4.60	4.28	0.19	0.08
Beef flavor[c]	4.26[de]	4.72[d]	4.26[de]	4.24[de]	3.74[e]	0.15	0.01
Off flavor[c]	1.32[e]	1.24[e]	1.34[e]	1.34[e]	1.82[d]	0.10	0.01

[d,e]Means with different superscripts differ significantly at the P value mentioned in the last column.

[a] Standard: United States Department of Agriculture average choice top loin steak. Refer to footnotes of Table 2 for a description of treatments. Each value represents the mean of 50 observations.

[b] Standard error of the mean.

[c] Higher value signifies more tenderness, juiciness, intense beef flavor, and intense off-flavor.

3.4. Vitamin E content and color stability of meat

The mean vitamin E contents of beef were 1.33[b], 1.33[b], 5.38[a], and 5.33[a] mg/kg of fresh meat for CTL, CLA, GPS, and PS treatments, respectively. Meat from animals finished on pasture had a 300% increase (P<0.01) in vitamin E content when compared with animals finished on grain. Higher vitamin E concentrations have been shown to improve the color stability of beef (Liu et al., 1995).

The treatment×cut interaction was significant (P=0.01) for L* values (lightness) of lean muscle. The treatment×day interaction was significant (P< 0.01) for lean muscle within each response variable. Therefore, L*, a*, and b* values for lean muscle tissue over time are presented in Fig. 2. The L* values for lean muscle color leveled off over time, but there was no discernable trend among treatments.

Steaks from animals in the CTL treatment had more redness at day 1 compared with the other treatments. However, the a* values for lean muscle over time suggest that meat from animals finished on pasture (GPS and PS) retained their redness better than those finished on high-grain diets (CTL and CLA). Meat from CTL and CLA treatments both significantly dropped (P<0.01) in redness by the end of the 15-day test period (Fig. 3). Sapp and Williams (1999) and Lanari et al. (2002) reported a similar effect, where meat from pasture-raised animals retained its redness better than meat from animals that were fed high-grain diets. The improved redness retention is most likely associated with the naturally increased levels of vitamin E in meat from pasture-finished animals compared to meat from grain-finished animals. A study by Liu et al. (1996a) showed that feeding supplemental vitamin E improved red color retention. Meat from CLA animals had the least red color stability. These results suggest that feeding Ca-CLA, which is a rich source of unsaturated fatty acids, will decrease the red color in meat, mainly because of oxidation.

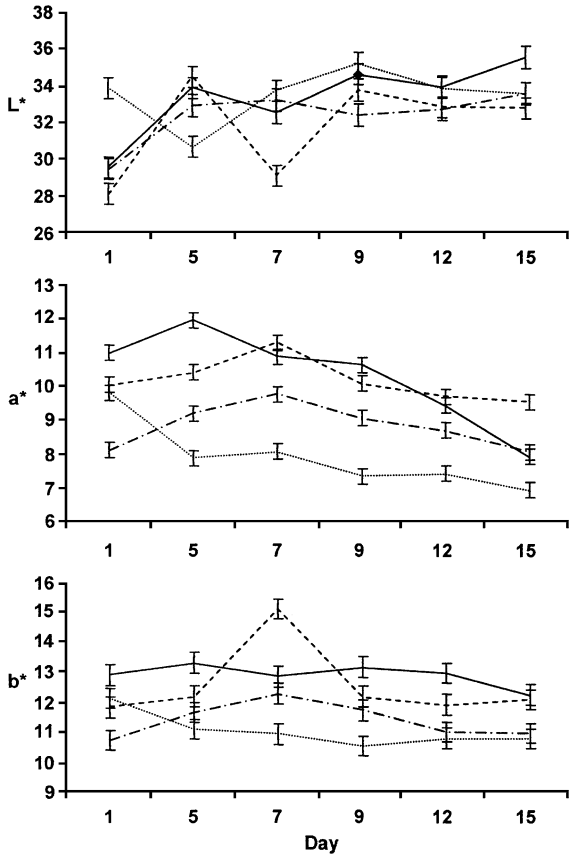

Fig. 2. Color stability of meat (L*, a*, and b* values of muscle) from cattle fed diets containing high grain, CLA, or raised on forages. (——) CTL; (·····) CLA; (– – – –) GPS; (– · –) PS. *P* values for all three interactions were <0.01.

When looking at yellowness (b*), the CTL treatment was very consistent across the entire period (Fig. 2) and did not differ between days. Meat from CLA animals decreased ($P<0.05$) in yellowness by day 5, then held steady until the end of the test period. A visual appraisal taken each day noted that meat from CLA treatment appeared to brown faster than any of the other treatments. Pasture-finished animals (GPS and PS) had meat that increased in yellowness towards the middle of the test period, then dropped back to levels that were not different from day 1.

Color stability was conducted on fat cover over the top loin (data not shown). The L* values for fat color became more consistent as time progressed and showed that fat from all treatments became lighter in color, except for CLA (data not shown). Fat from CLA steaks decreased in lightness by day 5 ($P<0.01$) but then increased afterwards to a level that was not different from day 1. Treatment mean a* values were 0.016^{a}, -0.549^{b}, -0.460^{b}, and -0.017^{a} ($P<0.01$) for CTL, CLA, GPS, and PS treatments, respectively. Treatment mean b* values were 5.64^{c}, 4.43^{d}, 6.81^{b}, and 8.92^{a} ($P<0.01$) for CTL, CLA, GPS, and PS treatments, respectively. Other researchers (Bennett et al., 1995) have also noted that fat from forage-fed beef is more yellow than fat from animals fed grain due to the presence of carotenes. All fat samples tended to increase in yellowness with time on display. One interesting observation was that CLA steaks were significantly less yellow than any of the other treatments, meriting further investigation.

4. Conclusions

The concentration of $C_{18:2}$ *cis*-9, *trans*-11 isomer of CLA in beef can be raised by as much as 466% by feeding forages and pasture only compared with beef from animals fed typical high-grain diets. Beef from animals raised on forage and pasture alone may have a perceived off-flavor. However, other beef quality characteristics were not affected by diet.

Meat from animals raised on forage and pasture retains its redness better than meat from grain-finished cattle. Additionally, finishing cattle on pasture increased the vitamin E content of beef by 300% compared to beef from animals finished on a traditional high-grain diet.

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