

Proximate, mineral and fatty acid composition of *semimembranosus* and cardiac muscles from grass- and grain-fed and zeranol-implanted cattle*

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Chemical composition (proximate, minerals, and fatty acids) of semimembranosus (SM) and cardiac muscle from cattle fed grass (G) and grain on grass without (GG) or with (GGP) zeranol implant were determined. The protein content of SM muscle from GG and GGP cattle was higher ($p \le 0.05$) while the moisture content was lower ($p \le 0.05$) than that from G cattle. The lipid content of cardiac muscle from GG cattle was higher ($p \le 0.05$) compared to that of G cattle. Grain supplementation (GG and GGP) increased the potassium level in SM muscle while a decrease was observed in cardiac muscle. Grain supplementation also increased linoleic acid but decreased polyunsaturated (PUFA) fatty acids in both muscles. There was 3 times more PUFA, particularly linolenic and arachidonic acids, in cardiac muscle than in SM muscle irrespective of the feed type. The concentrations of PUFA in SM and cardiac muscles were higher for G cattle than for GG and GGP cattle, which could make muscles from G cattle more prone to oxidation. © 1998 Elsevier Science Ltd. All rights reserved.

INTRODUCTION

Use of forage is an attractive proposition for cattle production in places where there is a natural abundance of grass throughout the year. Complete or partial grassfeeding of cattle is an important management tool during grain shortages and/or market fluctuations and can be used as an alternate finishing system for cattle producers in the Southeast USA. Zeranol, an anabolic agent derived from fermentation by the fungus Gibberella zeae, was used in beef cattle production to promote growth and to increase feed efficiency (Sharp and Dyer, 1971; Greathouse et al., 1983; Fumagalli et al., 1989). Extensive research was conducted to determine the palatability of forage-fed beef against grain-fed beef with or without implants (Melton et al., 1982; Larick and Turner, 1990; Xiong et al., 1996). Through these studies, it was established that beef from cattle raised exclusively on pasture has a less desirable flavor than beef from cattle receiving grain supplements or finished

on grain. A variety of flavor notes were used to describe the undesirable flavor, e.g. grassy, intense milky-oily, sour, and fishy (Brown *et al.*, 1979; Melton *et al.*, 1982; Larick *et al.*, 1987). The causes for undesirable flavor of forage-fed beef are still not completely understood, although it was attributed to variations in muscle fatty acid composition (Brown *et al.*, 1979; Westerling and Hedrick, 1979; Melton *et al.*, 1982), level and type of phospholipid (Larick and Turner, 1990), and differences in volatile content (Larick *et al.*, 1987; Maruri and Larick, 1992).

It thus seems clear that variations in muscle composition with changes in dietary regimen affect the stability, palatability and acceptability of beef. Some studies have shown that fatty acid composition of bovine fat is influenced by type of feed (Westerling and Hedrick, 1979; Melton *et al.*, 1982). Research investigating the effect of various feeding regimens on the composition (chemical, mineral, fatty acid) of beef heart muscle is limited. Such information is important as there was an increased interest in utilization of animal by-products, such as beef hearts, in further processed muscle foods. One of the limitations in utilization of beef hearts stems from its high susceptibility to oxidative deterioration caused by high concentrations of prooxidants (e.g. heme proteins and iron) and unsaturated lipid (e.g.

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arachidonic acid) (Srinivasan *et al.*, 1996). The objective of this research was to determine the effects of grassversus grain-feeding (with or without zeranol implantation) on proximate, mineral and fatty acid composition of beef cardiac and SM muscle.

MATERIALS AND METHODS

Animals and sample preparations

Cross-bred Angus steers (n=21) from Angus sires and cross-bred dams were allotted to three feeding regimens with seven steers assigned to each regimen (treatment): (1) grass only, consisting primarily of Johnstone fescue (G); (2) grass supplemented with grain (GG); or (3) grass supplemented with grain and implanted with zeranol [trade name Ralgro for 6-(6,10-dihydroxyundecyl)- β -resorcyclic acid- μ -lactone, International Minerals and Chemical Corporation, Terre Haute, IN] (GGP). In Treatment 1, cattle were started on pasture in early May and finished in mid-October. The pasture arrangement for Treatment 2 was essentially the same as Treatment 1, except that cracked corn was provided in a self feeder while cattle were on pasture. The average daily consumption of cracked corn was 8.5 kg per animal for the duration of the study. Cattle receiving grain were started on a salt limited (10%) daily intake of 3.7 kg grain per animal. When salt was completely removed, cattle consumed 11.1 kg grain per animal per day for the last 60 days. Cattle in Treatment 3 received the same diet (grain on grass) as cattle in Treatment 2 except that they were given a single implant at the beginning of the experiment (May) containing zeranol (36 mg per animal) deposited subcutaneously on the back side of the ear.

After being on experiment for approximately 150 days, cattle from each dietary group were slaughtered at the University of Kentucky abattoir. Carcasses were electrically stimulated immediately after the dressing process (before splitting) to minimize muscle cold shortening and thaw rigor. Chemical analyses (described later) were performed using three randomly selected cattle for each dietary group from which left semimembranosus (SM) muscle was removed within 45 min post mortem (hot-boned). Both the SM muscle and hearts were cooled at 2°C for 24 h. The subcutaneous fat from SM muscle was completely removed. the А $10 \times 6 \times 2.5$ cm (L×WH) portion from the central part of the SM muscle was used for analysis. The caps and valves and the fat surrounding the heart muscle were thoroughly removed. The lean portions of both the SM and cardiac muscles were vacuum packaged in impervious polyethylene bags (Cryovac, Duncan, SC), and stored frozen $(-29^{\circ}C)$ until analyzed (within 3 months). After thawing overnight $(5^{\circ}C)$, the muscle samples were ground twice through a 4.7 mm hole plate to obtain a homogeneous mince.

Proximate composition

Proximate analyses of minced muscle were done at least in duplicate and reported as percent fat, moisture, protein and ash. Moisture content was determined using the air drying method of AOAC (1990) in which muscle samples were dried in a 100°C oven for 48 h. Protein content was determined with a Heraeus Macro Nitrogen Analyzer (UIC Inc., Joliet, IL). Dried muscle samples, after moisture determination, were used for total nitrogen determination. A conversion factor of 6.25 was employed to estimate the protein content from total nitrogen. Total lipid was extracted from muscle samples using a single-phase extraction procedure containing hexane-isopropanol (3:2 v/v) and weighed after evaporation of solvent as described by Kolarovic and Fournier (1986). Total ash content of dried muscle samples was determined by blast burning in a 550°C furnace as described in AOAC (1990).

Mineral analysis

The minced muscle samples used for proximate analysis were analyzed for mineral content (sodium, potassium, calcium, iron, and copper). Dried muscle samples (after moisture determination) were digested in a 100 ml micro-Kjeldahl flask with a mixture of 70% HNO₃ and 60% HClO₄ (4:1 v/v). Digestion was carried out in an exclusive perchlorate hood with a closed safety shield. After an overnight slow digestion at room temperature, the mixture was heated to 240°C for 15-30 min until dense white fumes were formed. The flask was cooled and diluted in a volumetric flask with 0.1 N HCl. Solutions of mineral standards were also prepared in 0.1 N HCl. A Model Unicam 929 atomic absorption spectrophotometer (Thermo Jarrell Ash Corporation, Franklin, MA) equipped with a 50 mm Universal Burner Head was used. Mineral content was reported as mg100g⁻¹ muscle by measuring absorbance using air-C₂H₂ flame, except for calcium where N₂O flame was used, as described in the manual supplied by the manufacturer (Thermo Jarrell Ash Corporation, Franklin, MA).

Fatty acid analysis

Fatty acid methyl esters were prepared by direct transesterfication of lipid (extracted for proximate analysis) using tetramethyl guanidine and methanol (1:4) as described in AOAC (1990). The methyl esters of fatty acids were analyzed using a Perkin-Elmer Auto System Gas Chromatograph (GC) (Perkin-Elmer, Norwalk, CT) using a DB-225 fused silica capillary column ($30 \text{ m} \times 0.25 \text{ mm}$ ID, phase thickness $0.25 \mu\text{m}$). The GC analysis was performed by temperature-programming from 190 to 220°C at a rate of 3°C min⁻¹, holding at 190°C for 8 min, and then at 220°C for 20 min. Samples (1 μ l) were injected into the GC column by split injection (split ratio of 100:1). The flow rate of carrier gas helium was 0.77 kg cm⁻² and the temperature for both injection port and flame ionization detector was 250°C. The TURBOCHROM software (PE-NELSON, Cupertino, CA) was used for data analysis. Individual fatty acids were identified by comparison with retention times of reference standards (Nu-Chek Prep, Elysian, MN) and reported as percentage of total lipid.

Statistical analysis

The experiment employed a completely randomized design in which each steer in the same dietary group was treated as a replicate. Statistical analyses were performed using the Statistix 3.5 software package for microcomputers (Analytical Software Inc., St. Paul, MN). The statistical model contained the main effects of feed and muscle type and the interactions of feed X muscle type. Computations were made using General Linear Models procedure. Main effect and individual means were separated by the test of least significant difference (significance level p < 0.05) as described by Snedecor and Cochran (1989).

RESULTS AND DISCUSSION

The effects of feeding regimen on carcass characteristics are presented in Table 1. All cattle were of similar age (16–18 months) but the average live weight and hot carcass weight of cattle finished on grain were higher (p < 0.05) than those of grass-fed cattle. The higher weight of grain-fed cattle was not unexpected and generally attributed to the high energy of the grain in the ration (Moody, 1976). Marbling score, which was based on intramuscular fat content of *longissimus dorsi* muscle, was higher (p < 0.05) for grain-fed cattle compared to that of grass-fed cattle. Several researchers have shown higher marbling scores or lipid content of *long-*

Table 1. Carcass characteristics of steers fed grass (G), grain on grass (GG), or grain on grass with zeranol implant $(GGP)^{a}$

Characteristic ^b	G	GG	GGP
Live weight (kg)	429 ^b	555 ^a	538 ^a
Hot carcass weight (kg)	242 ^b	305 ^a	313 ^a
Marbling score	3.9 ^b	5.4 ^a	4.9 ^a
Final USDA quality grade	Se ⁻	C ⁻	Se ⁺
Final USDA yield grade	2.2 ^b	2.9 ^a	2.9 ^a
Weight of heart ^c (kg)	1.3 ^b	1.7 ^a	1.6 ^a

^{*a*}The average age of steers used in the study for each dietary regimen was 16–18 months (A-maturity).

^{*b*}Carcass characteristics represent average values $(\pm SD)$ of 6 steers from each diet regimen.

^{*c*}Average weight of heart from three steers only.

Se: Select, C: Choice.

Means in the same row with the same superscripts are not significantly different (p > 0.05).

issimus dorsi muscle for feedlot cattle than that of range cattle (Westerling and Hedrick, 1979; Miller *et al.*, 1981).

Proximate composition of the *semimembranosus* (SM) muscle varied with changes in dietary regimen. The protein content of SM muscle from grain-finished cattle (GG and GGP) was higher ($p \le 0.05$) than that of grassfed cattle (G) (Table 2). Correspondingly, the moisture content was lower for the GG and GGP than for the G cattle. On a moisture-free basis, the protein content of the SM muscle from GG (89.7%) and GGP (89.1%) cattle was still higher than that of the G (87.7%) cattle. The ash content was similar for both the G and GG cattle. Lipid content of GG and GGP muscle showed higher values than that of G muscle; however, the difference was non-significant (p > 0.05). This could be a result of either the quantity of the intramuscular fat in the SM muscle being too low to be accurately determined or to the small number (3) of samples used in the study, or to both. Proximate composition of cardiac muscles showed minor variations between G, GG, and GGP cattle except that the lipid content of cardiac muscle from GG was significantly ($p \le 0.05$) greater than that of G. The higher lipid content may have been caused by corn supplementation, although the administration of the anabolic agent (zeranol) tended to diminish (p > 0.05) that effect.

There were several distinctions between proximate compositions of the SM and cardiac muscles. The moisture content of all cardiac muscle samples (G, GG, GGP) (78.8–79.2%) was higher ($p \le 0.05$) than that of the SM muscle (74.3-77.1%) (Table 2). This may be attributed to fluid associated with the higher concentration of heme compounds (myoglobin and cytochromes) in cardiac muscle (Pearson and Young, 1989). In contrast, the protein content of cardiac muscles was lower than that of the SM muscle. On a dry weight basis, the protein content of cardiac muscle samples (84.8-86.8%) was lower than that of respective SM muscle samples (87.1-89.1%). The ash content of cardiac muscle was also slightly lower ($p \le 0.05$) than that of the SM muscle. The lipid content was nearly two-fold more in cardiac muscle than in SM muscle irrespective of the feeding regimen. Since only the lean portions of the muscle were compared, the high concentration of mitochondria in cardiac muscle may have been the main reason for the observed differences in lipid content between the two muscles. The proximate composition of both the cardiac and SM muscle samples was within the range of values reported in the literature (Pearson and Young, 1989; USDA, 1990).

Mineral analysis revealed that potassium levels in the SM muscle from G cattle was lower than that of GG or GGP cattle (Table 3). In contrast, the potassium level in cardiac muscle from G was higher than that from GG and GGP cattle. The reasons for this opposite dietary effect on potassium content between cardiac and SM muscles were unclear. For minerals other than potassium, in both the SM and cardiac muscles, variations

Composition (%)	SM muscle			Cardiac muscle			
	G	GG	GGP	G	GG	GGP	
Moisture Protein Fat Total ash	$\begin{array}{c} 77.12\pm 0.91^{b}\\ 20.07\pm 0.16^{c}\\ 1.28\pm 0.10^{c}\\ 1.09\pm 0.07^{b} \end{array}$	$\begin{array}{c} 75.23 \pm 1.00^{c} \\ 22.23 \pm 0.51^{b} \\ 1.97 \pm 0.38^{c} \\ 1.11 \pm 0.04^{b} \end{array}$	$74.34 \pm 0.20^{\circ}$ 22.86 ± 0.06^{a} $1.40 \pm 0.21^{\circ}$ 1.16 ± 0.02^{a}	$\begin{array}{c} 79.24 \pm 0.62^{a} \\ 18.02 \pm 0.07^{d,e} \\ 3.62 \pm 0.37^{b} \\ 1.04 \pm 0.02^{c} \end{array}$	$\begin{array}{c} 78.99 \pm 0.75^{\rm a} \\ 17.82 \pm 0.13^{e} \\ 4.66 \pm 0.74^{\rm a} \\ 1.04 \pm 0.01^{\rm c} \end{array}$	78.78 ± 0.09^{a} 18.40 ± 0.03^{d} $3.91 \pm 0.10^{a,b}$ 1.05 ± 0.01^{c}	

 Table 2. Proximate composition of SM and cardiac muscles from steers fed grass (G), grain on grass (GG), and grain on grass with zeranol implant (GGP)

^{a,b,c,d}Means (\pm SD) within a row with the same superscript are not significantly (p > 0.05) different.

caused by dietary treatments were minimal. Differences in mineral content except copper between the SM and cardiac muscle were pronounced. Sodium levels were nearly 3 times higher in cardiac muscle than in SM muscle for all dietary regimens. In general, sodium level in beef cardiac muscle was reported to be higher (30%) than in top round muscle (USDA, 1990). The iron content in cardiac muscle was nearly twice as much as in SM muscle. The higher concentration of iron in cardiac muscle was probably a consequence of higher concentration of heme proteins. The concentration of potassium was the highest among minerals that were quantified in SM muscle. This agreed with published information (Pearson and Young, 1989). However, this was not the case in cardiac muscle where both potassium and sodium were present in large quantities.

Fatty acid profiles of the SM and cardiac muscles with changes in dietary treatments are given in Table 4. The concentration of monounsaturated fatty acids increased in both the SM and cardiac muscles for grainfinished cattle (GG and GGP) when compared to that in grass-fed cattle (G). In contrast, the concentration of PUFA (with ≥ 2 double bonds) in both muscles decreased in GG and GGP. The increase in monounsaturated fatty acids in muscles of GG and GGP was primarily caused by increases in oleic acid [18:1 (n-9)], which may be attributed to high concentration of the fatty acid in cracked corn. SM muscle from GG and GGP also contained more palmitic acid (16:0) and a less common fatty acid [16:1 (n-7)], but less stearic (18:0) and linoleic [18:2 (n-6)] acids compared to that of G. It is possible that increases in oleic acid in SM muscle of GG and GGP occurred at the expense of either stearic or linoleic acid or both. Some previous researchers reported that feeding high energy grain diets resulted in increased concentrations of 18:1 (n-9) at the expense of 16:0 and (or) 18:0 in the lipid (Westerling and Hedrick, 1979; Schroeder *et al.*, 1980).

The pattern of fatty acid changes with variations in feeding regimen was different in cardiac muscle compared to the SM muscle. Beef heart from GG contained more 16:0 and 18:1 (n-9) and less 18:2 (n-6) and 20:4 (n-6) (arachidonic acid) than did beef heart from G. The increases in 16:0 and 18:1 (n-9) in cardiac muscle could be attributed to fatty acids in the cracked cornsupplemented diet. Implantation of zeranol in grain-fed cattle resulted in small increases in 14:0 (myristic), 16:0, and the 16:1 (n-7) fatty acids in SM muscle compared to that of grain-fed cattle without the implant. The reason(s) for these changes is not clear but it may be a result of the anabolic effect of zeranol which is known to regulate the synthesis of muscle components. Conjugated linoleic acid, a known anticarcinogen, was low in SM (0.3-0.4%) and cardiac (0.2–0.3%) muscle. Its concentration was not (p > 0.05) affected by feed type or implant.

There were several differences in fatty acid profiles between the SM and cardiac muscles from cattle within the same dietary regimen. For example, in grass-fed cattle, the distribution of saturated and monounsaturated fatty acids was 41.5 and 39.1% in the SM muscle, compared to 31.1 and 19.1% in the cardiac muscle. Most notably, the concentration of PUFA was nearly 3 times more in cardiac muscle (48.6%) than in SM muscle (16.6%). This was mainly caused by high concentrations of arachidonic and linoleic acids in cardiac muscle. The concentration of oleic acid was about

 Table 3. Mineral composition of SM and cardiac muscles from steers fed grass (G), grain on grass (GG), and grain on grass with zeranol implant (GGP)

Mineral $(mg \ 100 \ g^{-1} \ muscle)$	SM muscle				P value		
	G	GG	GGP	G	GG	GGP	
Sodium	46 ± 5^{b}	50 ± 4^{b}	$55\pm5^{\mathrm{b}}$	146 ± 44^a	135 ± 24^{a}	126 ± 3^{a}	0.0015
Potassium	147 ± 32^{c}	$191\pm32^{\rm b}$	227 ± 23^a	$160\pm24^{b,c}$	120 ± 43^d	102 ± 21^{d}	0.0001
Calcium	$10 \pm 2^{a,b}$	11 ± 2^{a}	11 ± 1^{a}	$5 \pm 1^{c,d}$	$7 \pm 1^{b,c}$	3 ± 1^{d}	0.0011
Iron	3.7 ± 0.4^{b}	$2.7\pm0.6^{\circ}$	$2.5\pm0.1^{\circ}$	5.4 ± 0.1^{a}	4.9 ± 0.7^{a}	$5.1\pm0.3^{\mathrm{a}}$	< 0.0001
Copper	$0.17 \pm 0.10^{a,b}$	$0.10\pm0.04^{\rm b}$	$0.15 \pm 0.05^{a,b}$	$0.21 \pm 0.02^{a,b}$	0.27 ± 0.03^a	$0.22\pm0.06^{a,b}$	0.1709

a,b,c,d Means (\pm SD) within a row with the same superscript are not significantly (p > 0.05) different.

Table 4. Fatty acid composition (%) of total extractable lipids from lean portions of SM and cardiac muscles from steers fed grass (G), grain on grass (GG), and grain on grass with zeranol implant (GGP)

	SM muscle			Cardiac muscle			
Fatty acid	G	GG	GGP	G	GG	GGP	
14:0	1.2 ^{b,c}	2.3 ^b	2.9 ^a	1.7 ^{c,d}	0.7 ^d	0.7 ^d	
14:1(n-5)	0.3	0.7	1.0	Tr	Tr	Tr	
16:0	21.2 ^c	25.1 ^b	27.5 ^a	11.7^{e}	14.0 ^d	14.4 ^d	
16:1(n-7)	2.1°	4.9 ^b	5.3 ^a	1.2 ^d	1.5 ^d	1.2 ^d	
17:0	1.0	0.8	0.9	0.6	0.8	0.7	
17:1(n-8)	0.7	0.9	0.9	0.5	0.7	0.4	
18:0	19.1ª	11.9 ^b	12.8 ^b	17.7 ^a	19.2 ^a	18.8 ^a	
18:1(n-9)	35.7 ^b	42.4 ^a	39.6 ^a	17.3^{e}	24.0 ^c	21.1 ^d	
18:2(n-6)	10.0 ^c	5.9 ^d	4.9 ^d	30.3 ^a	24.0 ^b	24.7 ^b	
18:3(n-3)	0.6	0.5	0.3	1.2	0.9	0.6	
CLA	0.4	0.4	0.3	0.2	0.3	0.2	
20:3(n-6)	0.5	0.4	0.4	1.1	1.5	1.8	
20:4(n-6)	3.6°	1.7 ^c	1.3 ^c	13.8 ^a	9.3 ^b	12.4 ^a	
20:5(n-3)	0.3	0.3	0.1	0.8	0.8	0.5	
22:5(n-3)	0.4	0.4	0.3	0.7	0.9	0.9	
Saturated	41.5 ^a	39.3 ^{a,b}	43.2 ^a	31.1 ^{b,c}	33.9 ^b	33.9 ^b	
Monounsaturated	39.1 ^b	48.8 ^a	46.8 ^a	19.1 ^d	26.3°	23.0 ^{c,d}	
Polyunsaturated	16.5 ^c	10.5 ^d	8.5 ^d	48.6 ^a	38.4 ^b	41.5 ^b	

CLA: conjugated linoleic acid; Tr: traces ($\leq 0.1\%$).

a,b,c,d,eMeans within a row with the same superscript are not significantly (p > 0.05) different.

50% less in cardiac muscle than in SM muscle. A similar trend in the distribution of saturated and unsaturated fatty acids between the two muscles was observed in other dietary regimens (GG and GGP).

Overall, dietary treatments of beef cattle clearly exhibited an effect on the proximate composition of muscle, especially in protein and more so for semimembranosus than for cardiac muscle. Although changes in fat content were relatively minor, changes in fatty acid profiles in the SM and cardiac muscles resulting from alteration of the specific diet or growth implant may have an impact on the stability and shelf-life of further processed foods. This can be substantiated by the high concentration of PUFA (including both ω -3 and ω -6 fatty acids) in cardiac muscle from grass-fed cattle compared to that from grain-supplemented cattle. Therefore, the cardiac muscle from grass-fed cattle may be less oxidatively stable compared to cardiac muscle from grain-fed cattle with or without the zeranol implant. These factors must be considered in processing applications of animal by-products. The expected oxidative susceptibility of beef heart muscle, particularly that from grass-fed cattle, entails an inclusion of antioxidants whether the heart muscle is directly formulated into food or further processed before use. For example, if used for surimi (minced and washed tissue), preparation to enhance its economic value and functionality, it would be desirable to incorporate antioxidants into beef heart muscle during washing to minimize the development of oxidative rancidity and to extend the shelf-life of surimi.

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