

SAĞLIK BİLİMLERİ DERGİSİ JOURNAL OF HEALTH SCIENCES Erciyes Üniversitesi Sağlık Bilimleri Enstitüsü Yayın Organıdır



Araştırma Yazısı

2018; 27: 149-155

*BONE MARROW FATTY ACID COMPOSITION OF GRASS-FED CATTLE : NUTRITIONAL APLICATION MERADA BESLENEN SIĞIRLARIN KESIM SONRASI KEMIK ILIĞI YAĞ ASIT KOMPOZISYONU: BESLENMEYE UYGULANMASI

Osman KUCUK^{1,*}, Loren CORDAIN², Kevin T. FULTON², Daniel C. RULE³

¹Department of Animal Nutrition and Nutritional Diseases, Erciyes University, School of Veterinary Medicine, Kayseri, TURKEY

²Department of Health and Exercise Science, Colorado State University, Fort Collins, Colo. USA

³Department of Animal Science, University of Wyoming, Laramie, Wyo. USA

ABSTRACT

The objective of the present work was to investigate how grass feeding in cattle influences the fatty acid (FA) composition in marrow tissue. For a split plot designed experiment, marrow was obtained from the femur, humerus, radius and tibia of four crossbred steers slaughtered at 28 mo of age and fed on mixture of cool season grasses and legumes. There were no bone type by location within bone interactions (P > 0.42), and the only within bone effect was for trans-vaccenic acid (TVA) where values for medial bone were lowest (P = 0.03) and highest for distal and proximal bone. Total monounsaturated FA and desaturation index were lower (P = 0.01) and total saturated FA were greater (P = 0.01) for proximal vs. distal bone marrow lipids. Marrow conjugated linoleic acid (CLA) was greater (P = 0.02) for distal bones (1.32 %) than for proximal bones (0.86 %). Overall, proportions of 18:3 n-3, CLA, and TVA were 0.60 %, 1.09 % and 2.50 %, respectively. We conclude that the FA profile of marrow in grass-fed cattle represents a healthy, nonatherogenic, animal-based fat source.

Keywords: Bovine bone marrow; *n*-3 Fatty acids; Conjugated linoleic acid; Grass-fed cattle; Fatty acid composition

ÖZ

Bu çalışmanın amacı, merada beslenen sığırların kemik iliği yağ asidi (YA) kompozisyonunu nasıl etkilediğini araştırmak idi. Buğdaygil ve baklagil otlarından oluşan bir merada otlayan ve 28 aylık yaşta kesime gönderilen kısırlaştırılmış besi sığırlarından elde edilen femur, humerus, radius ve tibia kemik ilikleri split plot deneme düzeneğinde toplanmıştır. Kemik türü ve örnek alınan kemik bölgesi arasında interaksiyon tespit edilmemiş olup (P > 0,42), sadece *trans*-vaksenik asit (TVA) için örnek alınan kemik bölgelerde distal ve proksimal kemikler için en yüksek ve medail kemikler için en düşük (P = 0.03) değerler bulunmuştur. Proksimal kemik bölgelerindeki kemik iliğinde distal kemik bölgelerindekine oranla desaturasyon indeksi ve toplam tekli doymamış YA oranı daha düşük (P = 0,01) ancak total doymuş YA oranı daha fazla (P = 0,01) tespit edilmiştir. Distal kemik bölgelerindeki ilikte bulunan konjuge linoleik asit (CLA) proksimal bölgelerdeki iliklere oranla daha fazla bulunmuştur (P = 0,02, %0,86'e karşı %1,32). Genel olarak 18:3, n-3, CLA, TVA oranları sırasıyla %0,60, % 1,09 ve %2,50 olarak bulunmuştur. Çalışma sonuçlarına göre merada beslenen sığırların kemik iliğinde bulunan yağ asitlerinin sağlıklı, aterojenik olmayan hayvansal kaynaklı yağlar olduğu sonucuna varılmıştır.

Anahtar kelimeler: sığır kemik iliği, n-3 yağ asitleri, konjuge linoleik asit, merada beslenen sığır, yağ asidi kompozisyonu

*Part of data was published in Western Section, American Society of Animal Science Conference in Laramie, Wyo, USA in 2008 (Proc. West. Sect. Amer. Soc. Anim. Sci 59:207-209).

Makale Geliş Tarihi : 11.01.2018 Makale Kabul Tarihi: 06.06.2018 **Corresponding Author:** Prof. Dr. Osman KUCUK, Erciyes University School of Veterinary Medicine Department of Animal Nutrition and Nutritional Diseases, Kayseri, 38039 TURKEY

Tel.: +90 352 207 6666, ext:29691; Fax: +90 352 337 2740. E-mail: osmankucukwy@yahoo.com

Bone Marrow Fatty Acid Composition In Grass-Fed Cattle After Slaughter: Nutritional Implications

1. INTRODUCTION

High fat-diet plays an important role in the development of cancer, cardiovascular diseases and diabetes (1). The type of fatty acid (FA) has a more important role in determining coronary heart disease (CHD) risk than the total amount of fat in the diet (2). Diets high in some but not all (3) saturated FA (SFA) raise plasma low-density lipoprotein (LDL) cholesterol concentrations, which has frequently been shown to be associated with an increased risk for CHD (4). However, high SFA diets also reduce the total cholesterol/ high-density lipoprotein cholesterol ratio, particularly when they replace high glycemic load carbohydrates, thereby lowering the CHD risk (5). Frequently when SFA from animal foods replace high glycemic load carbohydrates, they lower small dense LDL and reduce triglycerides, both of which reduce the risk for CHD (5). Dietary intake of oleic acid (18:1 cis-9), conjugated linoleic acid (CLA, 18:2 cis-9, trans-11, common name rumenic acid), and *n*-3 polyunsaturated FA (PUFA), such as α -linolenic acid (18:3 all cis-9, 12, 15) are recommended in leu of certain SFA (5) to reduce CHD risk. Additionally, some (18:1 trans-9), but not all (18:1 trans-11) trans-FA increase CHD risk and increasingly a number of studies indicate that replacement of SFA by linoleic acid (18:2 n-6) may adversely affect CHD outcome (3,6,7). Interest in consumption of beef from grass-fed cattle is increasing because of its leanness (and hence higher protein content) and healthier FA profile than conventionally fed beef cattle. Among ruminant animals, several studies have examined the FA concentration of bone marrow in caribou (8,9), Dall sheep (10), desert big horn sheep (11), deer, antelope, and elk (12) and domestic range-fed cattle (13). Based upon reported FA profiles of ruminant bone marrow it represents a healthy choice for human consumption due to its high content of oleic acid (43 % to 78 % of total fatty acids) and cholesterol lowering SFA, stearic acid (18:0; 14 % to 21 %) (8-13). A need exists to make comprehensive measurements of the FA spectrum in bone marrow lipids to determine its potential as a source of healthful dietary animal fats, particularly because grass-fed beef retailers frequently include bone marrow as one of their byproduct foods. Despite marrow's high fat content, without knowledge of its FA composition, conclusions regarding its capacity to influence CHD risk are unclear. Our hypothesis suggests that marrow FA of grass-fed cattle will contain proportions of n-3 PUFA, CLA, and trans-vaccenic acid (TVA; 18:1 trans-11, the precursor of rumenic acid) that are comparable to beef lipids of grass-fed cattle, and will vary according to bone type and location. Our objective was to determine the FA composition of marrow lipids of humerus, femur, tibia, and radius, within the proximal, medial, and distal locations of each bone, of four grassfed steers and to contrast our FA data with published values for beef FA of grass-fed cattle.

2. METHODS AND MATERIALS

2.1 Tissue samples

Bones were sampled from Galloway steers harvested at 28 months of age (550 kg \pm 20). Steers had grazed a mixture of cool season grasses and legumes. The mixture contained smooth brome, meadow brome, orchard grass, festuolium, manska pubescent wheatgrass, Garri-

son creeping foxtail, and alfalfa. The steers were not supplemented during grazing. Cattle were harvested by a state inspected, custom processor in Broken Bow, Nebraska according to USDA guidelines for humane slaughter of cattle. Cattle were euthanized by stunning with captive bolt followed by exsanguination. Bones were cut with a band saw during carcass fabrication into thirds to represent the proximal, medial, and distal sections of the humerus, femur, tibia, and radius. Grazed forage composition was known, and records of live weight at harvest were available. All cattle handling and processing was accomplished by private enterprise and state inspected processor; thus, no university personnel were involved in any live animal aspects, and were provided the samples of bone only. Therefore, there was no requirement for animal care and use approval.

2.2 Fatty acid analysis

Approximately 50 mg of marrow from each bone from each side was weighed into a 16 x 125 mm borosilicate tube with Teflon lined caps that contained 1.0 mg of glyceryl-tritridecanoate as internal standard (Sigma-Aldrich, St. Louis MO). Samples were subjected to direct transesterifcation using 0.2 M methanolic-KOH as catalyst (Murrieta et al. 2003); water was used instead of saturated sodium chloride during extraction of FA methyl esters with 2.0 mL of HPLC-grade hexane (Sigma -Aldrich, St. Louis MO). Fatty acid methyl esters were separated with a GLC (Agilent Technologies, Santa Clara, CA) equipped with a 100 m capillary column (SP-2560, Supelco, Inc., Bellefonte, PA) and flame ionization detector as described by Murrieta et al. (14). Identification of individual FA methyl esters was accomplished using commercially available standards (Matreya, Inc., Pleasant Gap, PA), which were quantified using ChemStation Software (Agilent Technologies, Santa Clara, CA).

2.3 Statistical analysis

Data were analyzed by using the MIXED procedure of SAS for a split-plot designed experiment (15). Significant differences (P < 0.05) among treatment means were determined. Post-hoc analyses were done with Tukey's range test (P < 0.05). The model included bone, location within bone, carcass side, and the bone by location interaction; carcass side and side by bone interaction were used as random effects and LS means were reported.

3. RESULTS

There were no bone type by location within bone interactions (P > 0.42), and the only within bone effect was for TVA where values for medial bone were lowest (P =0.03) and highest for distal and proximal bone, which were not different (2.37 vs. 2.51 and 2.63 ± 0.07 (SEM) mg/100 mg of total FA, respectively). Fatty acid profiles of marrow lipids within location (distal, medial, and proximal) and within femur, humerus, radius, and tibia were similar (P = 0.19 to 0.99) for all FA. Therefore, only main effects of bone type were reported, which are presented in Table 1. Fatty acids were presented as weight percentage (mg of FA per 100 mg of total FA). The concentration of total FA (mg of total FA/ mg of bone marrow) and was not affected (P = 0.47) by bone type. Weight percentages of *cis*-MUFA (14:1 *cis*-9, 16:1 *cis*-9,

150

17:1 *cis*-10, 18:1 *cis*-9, and 18:1 *cis*-11) were greater (P = 0.01 to 0.02) for marrow lipids of radius and tibia than for femur and humerus. Conversely, weight percentages of 16:0, 17:0, and 18:0 were lower (P = 0.01 to 0.05) in

marrow lipids of radius and tibia compared with femur and humerus. Of the C18-trans isomers, only TVA acid was affected by bone type with marrow lipids of femur and humerus having greater (P = 0.02) weight percent-

Table 1. Main effects of bone marrow fatty acid profile due bone type from grass-fed steers¹

Fatty acid ²	Femur	Humerus	Radius	Tibia	SEM ³	<i>P</i> -value
14:0	2.66	2.72	2.40	2.63	0.17	0.62
14:1 <i>cis-</i> 9	0.30 ^a	0.28ª	0.59 ^b	0.55 ^b	0.03	0.01
15:0	0.83	0.88	0.72	0.75	0.03	0.11
15:1 cis-5	0.27	0.29	0.23	0.24	0.02	0.29
16:0	26.40 ^b	26.73 ^b	23.92ª	24.49ª	0.45	0.05
16:1 <i>cis-</i> 9	2.55ª	2.28ª	4.32 ^b	4.08 ^b	0.26	0.02
17:0	1.23 ^b	1.24 ^b	0.91ª	0.99ª	0.05	0.04
17:1 cis-10	0.42ª	0.36ª	0.77 ^b	0.70 ^b	0.03	0.007
18:0	16.38 ^b	18.03 ^b	9.26ª	11.08ª	0.66	0.006
18:1 trans-9	0.05	0.06	0.05	0.04	0.004	0.32
18:1 trans-10	0.11	0.11	0.11	0.11	0.007	0.80
18:1 trans-11	2.83 ^b	2.76 ^b	2.16ª	2.26ª	0.08	0.02
18:1 cis-9	38.04 ^a	37.04ª	46.14 ^b	43.44 ^b	1.01	0.02
18:1 cis-11	2.62 ^b	2.39ª	3.20 ^b	3.18 ^b	0.13	0.05
18:2 <i>cis</i> -9,12 (n-6)	1.30	1.33	1.29	1.31	0.05	0.96
18:2 cis-9,trans-11 (CLA)	0.91ª	0.81ª	1.33 ^b	1.31 ^b	0.07	0.02
18:3 <i>cis</i> -9,12,15 (n-3)	0.64	0.59	0.57	0.61	0.02	0.41
Unknown ⁴	0.73	0.81	0.58	0.62	0.05	0.10
Unknown ⁵	1.35	1.22	1.37	1.47	0.10	0.48
Unknown ⁶	0.35	0.10	0.06	0.14	0.10	0.32
Total, mg/g	879.21	949.64	928.71	893.33	30.96	0.47
SFA ⁷	47.51 ^b	49.60 ^b	37.21ª	39.94ª	1.18	0.01
MUFA ⁸	47.09 ^a	45.39ª	57.59 ^b	54.61 ^b	1.21	0.01
cisMUFA ⁹	44.20 ^a	42.64 ^a	55.26 ^b	52.20 ^b	1.26	0.01
transMUFA ¹⁰	3.00 ^b	2.92 ^b	2.33ª	2.42ª	0.08	0.02
PUFA ¹¹	2.85ª	2.73ª	3.20 ^b	3.23 ^b	0.08	0.04
PUFA/SFA ¹²	0.060 ^a	0.055ª	0.087 ^b	0.088 ^b	0.004	0.02
n-6/n-3 PUFA ¹³ Desaturation index ¹⁴	2.09 0.48ª	2.33 0.46ª	2.32 0.59 ^b	2.21 0.56 ^b	0.09 0.03	0.39 0.001

¹Values presented as mg of fatty acid/ 100 mg total fatty acids.

²Fatty acids denoted as number of carbons:number of double-bonds (if applicable).

 $^{3}n = 4.$

⁴Unknown fatty acids denoted as <16:0.

⁵Unknown fatty acids denoted as 16:0-18:0.

⁶Unknown fatty acids denoted as >18:0.

⁷SFA: Total Saturated Fatty acids (14:0 + 15:0 + 16:0 + 17:0 + 18:0).

⁸MUFA: Total Mono Unsaturated Fatty acids (14:1 + 15:1 + 16:1 + 17:1 + 18:1 *cis*-9 + 18:1 *cis*-11 + 18:1 *trans*-9 + 18:1 *trans*-10 + 18:1 *trans*-11).

⁹*cis*MUFA: Total *cis* Mono Unsaturated Fatty acids (14:1 + 15:1 + 16:1 + 17:1 + 18:1 *cis*-9 + 18:1 *cis*-11).

¹⁰transMUFA: Total trans Mono Unsaturated Fatty acids (18:1 trans-9 + 18:1 trans-10 + 18:1 trans-11).

¹¹PUFA: Total Polyunsaturated Fatty Acids (18:2 *cis*-9,12 + 18:2 *cis*-9,*trans*-11 + 18:3 *cis*-9,12,15).

 $^{12} {\rm PUFA/SFA} \left(18:2\ cis-9,12+18:2\ cis-9,trans-11+18:3\ cis-9,12,15\right) / \left(14:0+15:0+16:0+17:0+18:0\right).$

¹³n6/n3 PUFA (18:2 *cis*-9,12 / 18:3 *cis*-9,12,15).

¹⁴Desaturation index calculated as (16:1 + 18:1 *cis*-9 + CLA)/(16:0 + 16:1 + 18:0 + 18:1 *cis*-9 + 18:1 *trans*-11 + CLA).

ages than radius and tibia. Weight percentages of linoleic acid (18:2 *cis*-9, *cis*-12) were similar among the bones (P = 0.96). However, weight percentages of CLA (18:2 *cis*-9, *trans*-11) were greater (P = 0.02) in marrow lipids of radius and tibia compared with femur and humerus. For the comparisons described above, femur and humerus marrow FA compositions were similar (P > 0.05) as were marrow FA composition of radius and tibia.

Weight percentages of total SFA were lower (P = 0.01) in marrow of radius and tibia than femur and humerus; whereas, percentages of total MUFA and PUFA were greater ($P \le 0.04$) in marrow of radius and tibia. Total *trans*-MUFA weight percentages were greater (P = 0.02) in femur and humerus marrow lipids than in radius and tibia, which was the opposite trend observed for total cis-MUFA. The differences observed in weight percentages of SFA and PUFA resulted in a greater PUFA: SFA ratio (P = 0.02) in marrow FA of radius and tibia compared with femur and humerus. Ratios of n-6: n-3 PUFA were similar (P = 0.39) among bones as were weight percentages of 18:2 cis-9, cis-12 and 18:3 all cis-9, 12, 15 (P = 0.41 to 0.96). Desaturation index was greater (P = 0.41 to 0.96). 0.001) for radius and tibia compared with femur and humerus.

4. DISCUSSION

Weight percentages of SFA accounted for 37-49% of FA among bone types; these FA likely originated from a combination of de novo FA biosynthesis within the bone marrow along with deposition of dietary SFA. Forage lipids contain significant proportions of 16:0 (15 to 30%); whereas, 18:0 proportions typically range from less than 1.0% to about 5% (16-18). Fatty acid biosynthesis occurs in the marrow of long bones of rabbits (19) and could have occurred within the marrow of the cattle in the present study because the proportions of 16:0, 18:0, and 18:1 cis-9 were of similar magnitude found in bovine adipose tissue where de novo FA biosynthesis is responsible for much of the lipid in this tissue (20). Polyunsaturated FA of forages consist primarily of 18:2 n-6 (11 to 18%), and 18:3 n-3 (36 to 64%) (16,17,18,21); however, proportions of these FA, especially 18:3 n-3, will vary for different types of forages, as well as within a forage if contained in unharvested pasture or when stored as a hay. Pasture legumes, such as alfalfa, contain over 60% 18:3 n-3; however, lipids of harvested alfalfa contain about 36% of total FA as 18:3 n-3 (18; our unpublished data). Additionally, 18:0 of bone marrow lipids would occur through deposition of products of biohydrogenation of MUFA and PUFA produced in the rumen. Ruminal biohydrogenation of MUFA and PUFA is responsible for conversion of significant amounts of dietary unsaturated FA to SFA, as well as TVA, the precursor of rumenic acid (20). Kucuk et al. (22) reported substantial duodenal flow of SFA in sheep consuming forages supplemented with soybean oil, as well as greater than 90% intestinal absorption of 16:0 and 18:0.

Weight percentages of PUFA for the present work ranged from 2.56 to 3.38% among bone types and locations. These values were of lesser magnitude than metatarsal bone marrow PUFA reported for elk, deer, and antelope (12), as well as lower than PUFA of intramus-

cular lipids from grazing cattle (23). The PUFA : SFA ratio was 0.11 in intramuscular fat of beef and 0.15 in lamb (24); whereas, this ratio was 0.06 to 0.09 in bone marrow lipids of grass-fed cattle in the present study. Contrary to the results from the present work, Cordain et al. (12) reported PUFA: SFA ratios from 0.24 to 0.33 for metatarsal bone marrow lipids of elk, deer, and antelope. Grass-fed beef had PUFA: SFA ratios in intramuscular lipids from 0.23 (24) to 0.45 in intramuscular lipids of grazing cattle (25). Overall, PUFA : SFA ratios of bone marrow lipids in the present study were lower than values reported for bone marrow by others, as well as when compared with intramuscular lipids of grassfed beef. Additionally, these ratios were lower than desired ratios of 0.45 to 0.64 for humans (26). However, PUFA: SFA ratios that do not partition SFA into its nonatherogenic (18:0) and atherogenic FA (12:0, 14:0, 16:0) may be misleading. Our results show that when these variables are considered the PUFA to SFA (14:0, 16:0) ratio was 0.11 when values for all marrow locations were combined. Additionally, PUFA : SFA ratios that do not differentiate among 18-carbon FA and 20and 22-carbon FA have far less relevance for predicting CHD risk because 20:5 n-3 and 22:6 n-3 dramatically reduce CHD risk (27); whereas, high dietary intake of 18:2 n-6 may increase risk (28). Further the MUFA: PUFA: SFA ratios in regards to specific FA should be reported. In the present study the MUFA: PUFA: SFA (14:0, 16:0) ratio in marrow FA was 64 % : 4 % : 34 %, respectively. A similar calculation from intramuscular FA reported previously (16,23) revealed ratios of 49% : 14:% : 37 % (12:0, 14:0, 16:0) (16) and 53% : 13 % : 34% (14:0, 16:0) (23). Compared with these two studies (16,23), 18:2 *n*-6 was threefold less in the marrow lipids in the present study. By contrast, intramuscular FA of grass-fed beef had similar proportions of the atherogenic SFA, greater proportions of PUFA, and lesser proportions of MUFA than marrow lipids of grass-fed cattle. Moreover, the contrast revealed that the greater PUFA in grass-fed beef intramuscular lipids was compensated by greater MUFA in the marrow lipids, with the largest difference in total PUFA occurring for 18:2 n-6.

Increased intake of n-3 PUFA at the expense of n-6 PUFA is recommended for a healthy diet for humans, with the recommended dietary *n*-6: *n*-3 ratio below 4.0. The *n*-6: *n*-3 ratio of bone marrow lipids of grass-fed cattle from the present work averaged 2.24, which was similar to values reported for metatarsal bone marrow of elk, deer, and antelope (12). For bovine muscle, n-6: n-3 PUFA ratios in pasture finished cattle and of range-fed bison and beef cattle were lower, but within the same magnitude (23,25). These results indicate that n-6: n-3 ratios of bone marrow lipids were comparable to those of intramuscular lipids of grass-fed beef, and below levels recommended ranges for a healthy diet. However, although n-6: n-3 PUFA ratios suggest a relative comparator for healthfulness of the dietary FA, the quantity of either that is consumed is a more important measure of their healthfulness so that deficiencies of either are avoided (29).

Weight percentages of 18:2 n-6 and 18:3 n-3 in bone marrow lipids were about 50% that of the values reported for metatarsal bone marrow of elk, deer, and antelope (12). Miller et al. (13), however, reported con-

centrations of 18:2 *n*-6 and 18:3 *n*-3 that, after conversion to weight percentage, were similar to the values observed in the present study. Metatarsal bone marrow lipids from elk, deer, and antelope contained less 14:0, 16:0, and 18:0 (12) than observed in the present study, indicating species differences in bone marrow FA composition. Dietary factors likely played a significant role in the differences observed between wild ungulates and domestic livestock.

Ruminant products represent the primary source of CLA for humans (30) with *cis*-9 *trans*-11 CLA (rumenic acid) the dominant CLA isomer in beef (31). Rumenic acid provides a range of potential health promoting biological properties including antiatherogenic and anticarcinogenic activities (32-34). Ritzenthaler et al. (31) proposed CLA intakes of 620 mg/day for men and 441 mg/ day for women as necessary for cancer prevention. For humans, one serving of whole milk (227 mL) and one serving of cheese (30 g) daily provides 90 mg of CLA (35). Similarly, the amounts of CLA in beef ranges only from 1.2 to 12.5 mg/g fat (36). The intake of CLA in the human diet can be increased by greater consumption of foods of ruminant origin, as well as through increasing the CLA content of the ruminant products. Pasture-fed cows produced milk with 500% greater CLA content compared with cows fed a diet containing 50% harvested forage (hay and silages) plus 50% grain (37). Beef obtained from steers raised on pasture was similarly greater in proportions of rumenic acid compared with steers fed high-concentrate diets (23,38,39).

Bone marrow CLA values of the grass-fed cattle were of similar magnitude to results reported for metatarsal bone marrow from wild ungulates (12), but were greater than values reported for longissimus muscle lipids of feedlot or range-fed beef (23). In addition, weight percentages of CLA, 16:1 cis-9, and 18:1 cis-9 were greater in bone marrow lipids obtained from the extremities (distal) than from bones within the core (proximal) of the cattle; whereas, TVA, 16:0, and 18:0 were greatest in the core lipids. This result suggests that delta-9 desaturase activity could have been lower in marrow of proximal bones. Enzyme activity of delta-9 desaturase in bone marrow has not been reported. However, for the present study the desaturation index was 22% greater (P = 0.001) for marrow FA of radius and tibia than for femur and humerus (Table 1).

Trans-vaccenic acid weight percentages in marrow lipids of the present work were similar to values reported for metatarsal bone marrow lipids of antelope and deer (12). However, Cordain et al. (12) reported much higher values for marrow 18:1 *trans*-11 from elk, which were twice that reported for longissimus muscle of range-fed bison or beef cattle (23).

Compared with the other FA present in the bone marrow lipids 18:1 *cis*-9 was proportionally the greatest. Compared with the grass-fed cattle of the present study, 18:1 *n*-9 weight percentages of greater magnitude were observed in metatarsal bone marrow of antelope, deer, and elk (12), as well as metatarsal bone marrow lipids of Caribou, which had values greater than 70% oleic acid (8). The greater magnitude of values for 18:1 *n*-9 in Caribou bone marrow lipids could be attributed to these animals living in far greater temperature extremes than many of the other species reported. Values for 18:1 *n*-9 in the present study were comparable to those reported by Miller et al. (13) and less than values reported by Mello et al. (40). Bone marrow levels of 18:1 n-9 were comparable to muscle-associated 18:1 n-9 (23,25).

For ungulates radius and tibia would be considered extremities while femur and humerus would be considered the core. Marrow of bones located further out toward the extremities had a different FA profile than marrow of bones located within the core of the animal such that within the core a greater proportion of SFA and lesser proportions of predominately MUFA occurred. Similar observations have been reported for adipose tissue depots in cattle wherein perirenal adipose tissue lipids contain greater 18:0 and less 18:1 cis-9 than subcutaneous adipose tissue (41). In addition, for bone marrow FA reported for the present work, radius and tibia FA profiles, as well as femur and humerus FA profiles were similar. Additionally data from wild North American ungulates show that the relative degree of saturation decreases distally in both the front and rear legs (8,10,11), perhaps as a result of increasing proximal to distal body temperatures (42).

Bone cross-sections are currently sold by retailers of grass-fed beef. The marrow is used as an ingredient in a number of recipes, which call for a fat source to enhance texture and flavor. Results of the present study showed that there was no difference in *n*-3 PUFA between the long, heavy core bones and the lighter bones of the extremities. However, weight percentages of MUFA and CLA were greater in marrow lipids of extremity bones than in the heavier core bones. Moreover, SFA were higher and MUFA were lower in the heavier core bones, which could impact the nutritional quality of this fat source.

We conclude that bone marrow is a rich source of FA in grass-fed beef, some of which are deemed healthful and of current biomedical importance, such as 18:3 *n*-3 and CLA. Conjugated linoleic acid and 18:3 *n*-3 were observed at proportions expected in meat lipids of grass-fed and feedlot finished beef; however, greater desaturation of SFA in the marrow lipids of bones of the extremities and thus, greater CLA synthesis from desaturation of TVA was likely in bones of the extremities and less in marrow lipids of the core bones. Results of the present work supported the hypothesis that *n*-3 PUFA and CLA are within the magnitude of meat lipids of grass-fed beef; however, FA profiles did not vary for location within bone, but were affected by location of bone within the core or extremities.

Acknowledgment

Authors thank Dr. Charles Murrieta and Mary Murrieta for expert technical assistance.

REFERENCES

- Rabhi N, Hannou SA, Froguel P, Annicotte JS. Cofactors as metabolic sensors driving cell adaptation in physiology and disease. Front in Endoc 2017; 8:304-311.
- Laaksonen DE, Nyyssönen K, Niskanen L, et al. Prediction of cardiovascular mortality in middleaged men by dietary and serum linoleic and polyunsaturated fatty acids. JAMA Intern Med 2005; 165:93-199.

- Hunter JE, Zhang J, Kris-Etherton PM. Cardiovascular disease risk of dietary stearic acid compared with trans, other saturated, and unsaturated fatty acids: a systematic review. Amer J Clin Nutr 2010; 91:46-63.
- 4. Wilson PW, D'Agostino RB, Levy D, et al. Prediction of coronary heart disease using risk factor categories. Circulation 1998; 97:1837-1847.
- 5. Baum SJ, Kris-Etherton PM, Willett WC, et al. Fatty acids in cardiovascular health and disease: a comprehensive update. J Clin Lipid 2012; 6:216-234.
- Field CJ, Blewett HH, Proctor S, Vine D. Human health benefits of vaccenic acid. Appl Physiol Nutr Metab 2009; 34:979-991.
- Ramsden CE, Zamora D, Leelarthaepin B, et al. Use of dietary linoleic acid for secondary prevention of coronary heart disease and death: evaluation of recovered data from the Sydney Diet Heart Study and updated meta-analysis. Brit Med J 2013; 4:346:e8707.
- Meng MS, West GC, Irving L. Fatty acid composition of caribou bone marrow. Comp Bioch Physiol B: Mol Biol 1969; 30:187-191.
- Soppela P, Nieminen M. The effect of wintertime undernutrition on the fatty acid composition of leg bone marrow fats in reindeer (Rangifer tarandus tarandus L.). Comp Biochem Physiol B: Mol Biol 2001; 128:63-72.
- West GC, Shaw DL. Fatty acid composition of dall sheep bone marrow. Comp Biochem Physiol B: Mol Biol 1975; 50B:599-601.
- 11. Turner JC. Adaptive strategies of selective fatty acid deposition in the bone marrow of desert bighorn sheep. Comp Biochem Physiol B: Mol Biol 1979; 62A:599-604.
- 12. Cordain L, Watkins BA, Florant GL, et al. Fatty acid analysis of wild ruminant tissues: evolutionary implications for reducing diet-related chronic disease. Eur J Clin Nutr 2002; 56:181-191.
- 13. Miller GJ, Frey MR, Kunsman JE, Field RA. Bovine bone marrow lipids. J Food Sci 1982; 47:657 -660.
- 14. Murrieta CM, Hess BW, Rule DC. Comparison of acidic and alkaline catalysts for preparation of fatty acid methyl esters from ovine muscle with emphasis on conjugated linoleic acid. Meat Sci 2003; 65:523-529.
- Kaps M, Lamberson W. Split-plot design. In: Kaps M, Lamberson W. (Eds.), Biostatistics for Animal Science. MA: CABI Publishing, Cambridge 2004; pp 342-354.
- 16. Nuernberg K, Dannenberger D, Nuernberg G, et al. Effect of a grass-based and a concentrate feeding system on meat quality characteristics and fatty acid composition of longissimus muscle in different cattle breeds. Livest Prod Sci 2005; 94:137-147.
- 17. Noci F, French P, Monahan FJ, Moloney AP. The fatty acid composition of muscle, fat, and subcutaneous adipose tissue of grazing heifers supplemented with plant oil-enriched concentrates. J Anim Sci 2007; 85:1062-1073.
- Weston TR, Derner JD, Murrieta CM, et al.Comparison of catalysts for direct transesterifi-

cation of fatty acis in freeze-dried forage samples. Crop Sci 2008; 48:1636-1641.

- Altman KI, Richmond JE, Salomon K. A note on the synthesis of fatty acids in bone marrow homogenates as affected by X-radiation. Bioch Biophysica Acta 1951; 7:460-465.
- Vernon RG. Lipid metabolism in the adipose tissue of ruminant animals. In: Christie WW. (Ed), Lipid metabolism in ruminant animals. Pergamon Press, New York 1981; pp 279-362.
- 21. Mel'uchova B, Blasko J, Kubinec R, et al. Seasonal variations in fatty acid composition of pasture forage plants and CLA content in ewe milk fat. Small Rum Res 2008; 78:56-65.
- Kucuk O, Hess BW, Ludden PA, Rule DC. Effect of forage:concentrate ratio on ruminal digestion and duodenal flow of fatty acids in ewes. J Anim Sci 2001; 79:2233-2240.
- 23. Rule DC, Broughton KS, Shellito SM, Maiorano G. Comparison of muscle fatty acid profiles and cholesterol concentrations of bison, beef cattle, elk, and chicken. J Anim Sci 2002; 80:1202-1211.
- 24. Scollan N, Hocquette J, Nuernberg K, et al. Innovations in beef production systems that enhance the nutritional and health value of beef lipids and their relationship with meat quality. Meat Sci 2006; 74:17–33.
- Muchenje V, Hugo A, Dzama K, et al. Cholesterol levels and fatty acid profiles of beef from three cattle breeds raised on natural pasture. J Food Comp Anal 2009; 22:354–358.
- Wood JD, Richardson RI, Nute GR, et al. Effects of fatty acids on meat quality: review. Meat Sci 2003; 66:21-32.
- Wang C, Harris WS, Chung M, et al. n-3 fatty acids from fish or fish-oil supplements, but not alpha linolenic acid, benefit cardiovascular disease outcomes in primary- and secondary-prevention studies: a systematic review. Amer J Clin Nutr 2006; 84:5-17.
- 28. Ramsden CE, Faurot KR, Zamora D, et al. Targeted alteration of dietary n-3 and n-6 fatty acids for the treatment of chronic headaches: A randomized trial. Pain 2013; 154:1010-1016.
- 29. Wijendran V, Hayes KC. Dietary n-6 and n-3 fatty acid balance and cardiovascular health. Ann Rev Nutr 2004; 24:597-615.
- Chin SF, Liu W, Storkson JM, et al. Dietary sources of conjugated dienoic isomers of linoleic acid, a newly recognized class of anticarcinogens. J Food Comp Anal 1992; 5:185-197.
- 31. Griinari JM, Bauman DE. Biosynthesis of conjugated linoleic acid and its incorporation into meat and milk in ruminants. In: Yurawcez MP, Mossoba MM, Kramer JKG, Pariza MW, Nelson GJ. (Eds), AOCS Press, Illinois USA, 1999; pp 180-200.
- 32. Ritzenthaler KL, McGuire MK, Falen R, et al. Estimation of conjugated linoleic acid intake by written dietary assessment methodologies underestimates actual intake evaluated by food duplicate methodology. J Nutr 2001; 131:1548-1554.
- De La Torre A, Gruffat D, Durand D, et al. Factors influencing proportion and composition of CLA in beef. Meat Sci 2006; 73:258-268.

- Hargrave-Barnes KM, Azain MJ, Milner JL. Conjugated linoleic acid induced fat loss dependence on delta 6-desaturase or cycolooxygenase. Obesity 2008; 16:2245-2252.
- 35. Luna P, Fontecha J, Ju´arez M, de la Fuente MA. Conjugated linoleic acid in ewe milk fat. J Dairy Res 2005; 72:415-424.
- 36. Raes K, De Smet S, Demeyer D. Effect of dietary fatty acids on incorporation of long chain polyunsaturated fatty acids, and conjugated linoleic acid in lamb, beef and pork meat: a review. Anim Feed Sci Techn 2004; 113:199-221.
- Dhiman TR, Anand GR, Satter LD, Pariza MW. Conjugated linoleic acid content of milk from cows fed different diets. J Dairy Sci 1999; 82:2146-2156.
- French P, Stanton C, Lawless F, et al. Fatty acid composition, including conjugated linoleic acid, of intramuscular fat from steers offered grazed grass, grass silage, or concentrate-based diets. J Anim Sci 2000; 78:2849-2855.
- Poulson CS, Dhiman TR, Cornforth D, Olson KC, Walters J. Influence of diet on conjugated linoleic acid content of beef. J Anim Sci 2001; 79:159-164.
- 40. Mello FC, Field RA, Forenze S, Kunsman JE. Lipid characterization of bovine bone marrow. J Food Sci 1976; 41:226-230.
- 41. Rule DC, Busboom JR, Kercher CJ. Effect of dietary canola on fatty acid composition of bovine adipose tissue, muscle, kidney, and liver. J Anim Sci 1994; 72:2735-2744.
- 42. Petrakis NL. Some physiological and developmental considerations of the temperaturegradient hypothesis of bone marrow distribution. Amer J Physic Anthr 1966; 25:119-130.