



## A Nutritional Survey of Commercially Available Grass-Finished Beef<sup>1</sup>

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**Abstract:** Consumer interest in the source of their food, its environmental footprint, and the impact of diet on health has supported the growth of the grass-finished beef (GFB) industry. Studies have concluded that GFB has distinct nutritional differences from conventionally-finished beef. As the GFB industry continues to expand, it is vital to continue to explore the nutritional complexities and variation in the product. To achieve this, a survey of grass-finishing production systems throughout the USA was conducted, and beef finished on the participating farms was analyzed for its nutritional composition, including fatty acid (FA), mineral and fat-soluble vitamin contents. Samples were analyzed from 12 producers and annual production capacity of farms ranged from 25 to 5,000 cattle, with a mean age of cattle at harvest of  $26.8 \pm 2.30$  mo. An array of finishing diets included grazing exclusively in perennial pasture, incorporating annual forage crops, and feeding a variety of harvested forages with supplementation of non-starch feed byproducts. Beef muscle tissue FA content was analyzed by gas chromatography-mass spectrometry (GC-MS). The mean ratio of omega-6 (*n*-6) to omega-3 (*n*-3) FA in samples varied significantly by producer, ranging from 1.80 to 28.3 ( $P < 0.0001$ ), with an overall sample set median of 4.10. A selection of minerals including iron, magnesium, and potassium were analyzed by ICP emission spectroscopy and mineral content significantly differed by producer for all minerals ( $P < 0.001$ ). Mean  $\alpha$ -tocopherol and  $\beta$ -carotene content was 610.6  $\mu\text{g}/100$  g beef and 32.2  $\mu\text{g}/100$  g, respectively. The amount of these antioxidants also varied between producers ( $P < 0.0001$ ), but tended to be greater in beef finished solely on fresh forages. This survey indicates that commercially available GFB can vary in nutritional composition due to the diverse practices used to grass-finish cattle.

**Keywords:** grass-fed beef, grass-finished beef, fatty acids, mineral, antioxidant, forage

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## Introduction

The grass-finished beef (GFB) industry continues to grow in response to consumer demand, in which retail sales have doubled annually from 2012 to 2016 (Cheung et al., 2017). There are a number of factors underlying the popularity of GFB, including perceptions surrounding its healthfulness, environmental impact, and animal welfare. Numerous studies have outlined the differences

between GFB and conventionally-finished beef (CFB), indicating that GFB has higher proportions of nutrients beneficial to human health (Chail et al., 2016; Duckett et al., 2013; Duckett et al., 2009; Ponnampalam et al., 2006). However, a majority of GFB nutritional profile analysis have been limited to controlled research trials and while the United States Department of Agriculture (USDA) reports a standard nutritional value for GFB, limited studies have been conducted to assess the nutritional qualities of commercially available GFB.

A grass-fed animal is generally defined as one that has only consumed forages from birth to harvest. Producers

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use a wide range of management strategies to raise and market cattle under a GFB label. Because of these inherent differences in production practices, there is great flexibility in labeling GFB products as “grass-fed”, “grass-finished”, or “pasture-raised” (Cheung et al., 2017) thus leading to consumer confusion regarding the distinction between the similar labels. There is a need for clear characterization of the nutritional qualities of the product sold to consumers associated with the grass-fed label. Additionally, there is a vital need for the promotion of accurate information about labeling claims from the GFB industry. Therefore, the intent of this study was to gain a greater understanding of the production methods and nutritional variability of GFB, a product consumers are willing to pay a premium for (Umberger et al., 2009; McCluskey et al., 2005). A nationwide survey of beef producers, all marketing cattle under a GFB label, was conducted to tie production practice to the fatty acid (FA), mineral, and fat-soluble vitamin content of commercially available GFB.

## Materials and Methods

This project received Exempt 2 status from the Michigan State University Institutional Review Board, IRB# x16-1273e, October 12th, 2016.

### Sample collection

A number of GFB producers nationwide were identified to participate in a confidential survey regarding their production methods and asked to submit samples of beef for nutritional analyses. Beef samples ( $n = 750$ ) were collected from ten states (GA, IA, MT, NE, NV, OH, OK, OR, TX, WI) and represented a broad area across the United States. Two sample collection periods were established: fall (September 2016 through February 2017;  $n = 390$ ), and spring (June through August 2017;  $n = 360$ ). The sampling periods served to account for geographical and climatic variability across regions where beef was sampled. Any producers that submitted less than 7 samples were excluded from the analyses, resulting in a sample size of 385 for fall and 355 for spring from twelve producers total. Samples were collected at fabrication, and carcass hanging times varied (24 to 96 h). A collection protocol was sent to all processors in the study. Two 56g samples per animal were requested cut from the anterior portion of the strip loin (IMPS/NAMP 180 Beef Loin, Strip Loin). Samples were individually bagged and frozen. Samples spent an average of 31 d in freezer before shipment. Samples were verified frozen on arrival and stored at  $-80^{\circ}\text{C}$  until sample analysis.

### Survey

To gain a greater understanding of the management and processing associated with the beef samples, 27- and 5-question confidential surveys were developed for participating producers and processors, respectively. The surveys were administered online using Qualtrics Version 2016 (Provo, UT), and emailed to participants with instructions for completion. Michigan State University’s Institutional Review Board approved the survey (IRB# x16-1273e). The survey assessed production methods and included questions regarding farm size, pasture, and forage supply, grazing strategies, number of cattle harvested annually, finishing diet, and other management strategies. Aging, capacity and other processing strategies were also surveyed. The survey response rate was 75%. All producers provided 7 or greater beef samples.

### Fatty acid analysis

Analytical-grade reagents were purchased from Sigma-Aldrich (St. Louis, MO). Stearic acid- $d_{35}$  was used as internal standard (Sigma-Aldrich; St. Louis, MO). Oleic acid, and  $n$ -3 docosapentaenoic acid (DPA) standards were purchased from Cayman Chemical (Ann Arbor, MI, USA), and 9Z, 11E-Conjugated linoleic acid (CLA) standard was purchased from Matreya, LLC (State College, PA). All other FA standard curves were created using Supelco 37 Component FAME Mix (Sigma-Aldrich; St. Louis, MO). A modified version of the microwave assisted extraction (MAE) method used by Medina et al. (2015) was used to extract FAs from beef samples using the CEM Mars 6 microwave digestion system, equipped with a 24 vessel rotor and GlassChem vessel set (CEM Corporation; Matthews, NC; Medina et al., 2015). Strip loin samples were transferred to  $-20^{\circ}\text{C}$  for 24 to 48 h before processing. A representative core of the loin sample was taken, avoiding pockets of intramuscular fat. The core was trimmed to 400 mg, minced, and added to a microwave vessel. Eight mL of 4:1 (v/v) solution of ethyl acetate:methanol with 0.1% butylated hydroxytoluene was added to the vessels, and FAs were extracted using the following microwave parameters:  $55^{\circ}\text{C}$  for 15 min with initial ramp of 2 min at 400W maximum power. Vessel contents were filtered using Whatman lipid free filters (Weber Scientific; Hamilton, NJ) into a test tube containing 3.5 mL HPLC water. Samples were centrifuged at 2,500 g for 6 min and the top organic layer was transferred to a new tube. Samples were dried using a Digital Series SpeedVac System (ThermoFisher; Waltham, MA) to obtain extracted FAs.

Fatty acids were methylated as previously described (Ichihara and Fukubayashi, 2010). Briefly, samples were resuspended in toluene, transferred to a test tube containing internal standard, and 1 mL of 1.09M methanolic HCl was added. Samples were heated at 100°C for 1.5 h, cooled to RT, and neutralized with 5% (w/v) sodium bicarbonate. Two mL hexane was added and the upper organic phase was removed and dried to obtain fatty acid methyl esters (FAMES). FAMES were suspended in isooctane and transferred to GC vials. Samples were stored at -20°C until GC-MS analysis.

The PerkinElmer (Waltham, MA) 680/600S GC-MS equipped with an Agilent Technologies (Santa Clara, CA) DB-23, 30-m column was used for FAME quantification. The GC temperature parameters were as follows: initial temperature at 100°C for 0.5 min; ramp 7.0°C/min to 245°C; hold 2 min. Seven-point curves were created for all standards. Data analysis was conducted using MassLynx V4.1 SCN 714 (Waters Corporation; Milford, MA). Concentrations were normalized based on starting sample weight.

### Mineral analysis

For mineral analysis, beef samples were sectioned with 2 g dried overnight in a 75°C oven to determine dry sample weight, and 1 g digested overnight in an oven at 95°C with 2 mL of nitric acid. Digested samples were diluted with water to approximately 100 times the dried tissue mass. Mineral analysis was conducted as previously described (Wahlen et al., 2005) using an Agilent 7900 Inductively Coupled Plasma- Mass Spectrometer (Agilent Technologies Inc.). Concentrations of elements were calibrated using a 5-point linear curve comparing the analyte and internal standard response ratio, with standards obtained from Inorganic Ventures (Christiansburg, VA) and bovine muscle standards used as a control (National Institute of Standards and Technology, Gaithersburg, MD).

### Alpha-tocopherol and beta-carotene analysis

The antioxidants beta-carotene and alpha-tocopherol were analyzed as previously described (Rettenmaier and Schuep, 1992). Briefly, 0.5g tissue samples were mechanically homogenized in 2 mL of water, then frozen to lyse cells. After thawing, ethanol was added to an aliquot of the solution to precipitate proteins. Hexane was added to extract the vitamins. A measured portion of the hexane was evaporated under reduced pressure at 35°C. The remaining matter was suspended in chromatographic mobile phase and transferred to auto sam-

pler vials. A 5-point calibration curve was created using beta-carotene and alpha-tocopherol standards (Sigma-Aldrich) diluted to achieve absorbances of 0.18 to 0.22 at 450 nm and 0.09 to 0.11 at 292 nm, respectively. Samples were analyzed using a Waters 2 Acquity system and Waters Empower Pro Chromatography Manager software (Waters Corporation). Elution was isocratic using a mobile phase of acetonitrile:methylene chloride:methanol (70:20:10, v/v/v) and a Symmetry C18, 3.5 m, 2.1 x 50 mm analytical column (Sigma-Aldrich). The flow rate was 0.5 mL/min and UV absorption at 450 nm for beta-carotene and 292 nm for alpha-tocopherol.

### Statistical analysis

Statistical analysis was conducted using Prism v7.0d for Mac OS X (GraphPad Software., La Jolla, CA). Producer comparisons were performed using Kruskal-Wallis test, correcting for multiple comparisons. Pearson correlation was computed using R v3.3.3. Macromineral and fatty acid values expressed are in mg/100g beef tissue, while microminerals and antioxidants are expressed in µg/100g tissue.

## Results and Discussion

### Survey-Production Methods

The size of the farms that participated in the survey varied significantly. The number of cattle marketed annually by respondents ranged from 25 to 5000 cattle (mean = 942, median = 600; Table 1). This is considerably greater than previously described survey respondents marketing an average of 40 and 25 head of cattle respectively (Gillespie et al., 2016; Steinberg and Comerford, 2009). In both of these studies, the focus was on producers who direct-marketed beef; the former surveyed producers nationwide, while the latter had a regional boundary. Our goals were to identify a broad range of producers varying in production capacity and identified branded programs, cooperatives, and even small producers, hence the criterion of > 7 samples necessary for study participation. The mean cattle age at harvest was 26.8 ± 2.30 mo which is older than previously reported mean cattle slaughter ages of 20.7 ± 4.70 and 20.8 ± 6.80 mo, respectively (Steinberg and Comerford, 2009; Lozier et al., 2005). In this survey, respondents indicated their primary breed of cattle as Angus or Angus cross, with one producer listing "British."

Finishing diets, defined as the diet fed for the last 60 d of finishing, are listed in Table 1. A wide variety

**Table 1.** Producer-reported data on farm capacity, age of cattle, and finishing diets

Producer	# of cattle marketed annually	Age at harvest, mo	Sample size, n =	Fall finishing diet	Spring finishing diet
1	300	NA <sup>1</sup>	25	Perennial pasture	--
2	600	23-24 (f) <sup>2</sup> 26 (sp)	75	BMR <sup>3</sup> forage sorghum, oat/pea/triticale silage, apple cider vinegar, cane molasses, soybean hulls	Oat/pea silage, alfalfa, BMR silage, cane molasses, soybean hulls
3	650	29.6	25	Perennial cool season grasses, annual cool season grasses and forbs, cover crop mix	NA
4	800	23-24	107	Summer annuals and warm season perennial pasture, plus cool season baleage OR cool season annuals and warm season annual baleage	--
5	NA	NA	81	NA	NA
6	1000	28	30	Forage sorghum silage, dry grass hay, soybean hulls	Forage sorghum silage, dry grass hay, soybean hulls
7	NA	NA	49	NA	--
8	5000	24-28	249	Seasonal forages	Winter annuals (barley, wheat) and sorghum sudan silage OR native pasture and BMR sudan
9	25	30	12	Cool season pasture (fescue-based) mixed clover, orchardgrass	Cool season pasture (fescue-based) mixed clover, orchardgrass
10	80	28	38	--	Grass-based forages
11	30	27	7	--	Perennial pasture with alfalfa, orchard grass, red and white clover, Johnson grass, and various forbs
12	NA	NA	22	--	NA

<sup>1</sup>NA indicates producer did not disclose information.

<sup>2</sup>Producer 2 indicated a difference in age between cattle harvested in the fall (f) vs spring (sp).

<sup>3</sup>BMR = brown midrib.

of finishing strategies were indicated, with some producers relying solely on perennial pastures, and others finishing cattle on annual crops or by feeding a diverse array of harvested forages. There is great variation with what is defined as 'grass-fed'. In 2016, the USDA (AMS, 2016) ceased their grass-fed label to ask individual entities to submit labeling standards. As a result, there is a broader description by label of defining grass-fed. For example, some of our respondents indicated supplementing non-starch feed byproducts.

Some management strategies were uniform across producers. For instance, all producers indicated they utilized a mineral program in their finishing strategies. Antibiotic usage was "only as needed" for 78% of respondents, and "never" for the remainder. Ionophores and growth promoters were excluded from use by all producers. This is similar to previously conducted surveys of grass-fed beef production, where no survey respondents used antibiotics as a feed additive, while 52% administered antibiotics to sick animals, and 99 and 95% did not use growth implants or feed additives such as ionophores, respectively, similar to Lozier et al., 2005; and Gillespie et al., 2016. None of the respondents indicated they implemented the technique of dry-aging the beef, in contrast to other surveys where the practice of

dry aging beef for 2 wk was nearly universal among GFB producers (Steinberg and Comerford, 2009; Lozier et al., 2005). This may be attributed to the relatively large scale of production, and aging carcasses for 2 wk may not be practical

### Fatty acid analysis

Total FA content in the beef samples was highly variable and ranged from 84.4 to 3,610 mg/100g beef (Table 2). Variation was expected, due to the framework of the survey with both large and small producers over a broad geographical representation and an array of finishing protocols. Mean total FA content was 723.4 mg/100g beef, comparatively lower than the 1,142 mg/100g and 2,982 mg/100g reported by De la Fuente et al. (2009) and Chail et al. (2016), respectively. We speculate that our survey came from cattle consuming less overall energy as compared to controlled settings with careful monitoring of dietary energy, leading to higher net energy consumption, and it is known that fat accumulation is correlated with energy intake (Pethick et al., 2014). Another reason the beef fat content was lower could be due to our sampling strategy. For our analysis, subsamples from the

**Table 2.** Fatty acid content of grass-finished beef (mg/100g beef)<sup>1</sup>

Fatty acid	Carbon #	Mean ± SEM	Min	Max
Total FA <sup>2</sup>		723 ± 17.7	84.4	3610
SFA <sup>3</sup>		321 ± 8.23	29.4	1790
Myristic	14:0	15.1 ± 0.51	1.13	97.2
Palmitic	16:0	203 ± 5.45	13.4	1100
Margaric	17:0	7.91 ± 0.24	0.90	55.3
Stearic	18:0	94.3 ± 2.16	14.7	554
MUFA <sup>4</sup>		320 ± 8.81	15.2	1710
Myristoleic	14:1n-7	4.21 ± 0.14	0.32	30.0
Palmitoleic	16:1n-7	23.8 ± 0.71	0.86	139
Oleic	18:1n-9	292 ± 8.03	13.6	1620
PUFA <sup>5</sup>		80.8 ± 1.10	25.2	224
Linoleic	18:2n-6	46.7 ± 0.94	11.9	168
ALA	18:3n-3	5.93 ± 0.15	0.28	29.6
Arachidonic	20:4n-6	16.8 ± 0.25	4.40	50.2
EPA	20:5n-3	3.56 ± 0.09	0.21	13.8
DPA	22:5n-3	4.01 ± 0.07	0.41	10.4
DHA	22:6n-3	0.33 ± 0.01	0.05	1.00
n-6 PUFA <sup>6</sup>		67.2 ± 1.21	17.1	220
n-3 PUFA <sup>7</sup>		13.6 ± 0.29	0.95	48.4
n-6:n-3 <sup>8</sup>		9.92 ± 0.47	1.16	96.1
CLA	18:2 cis-9 trans-11	1.53 ± 0.06	0.05	23.1

<sup>1</sup>FA = fatty acid, SFA = saturated FA, MUFA = monounsaturated FA, PUFA = polyunsaturated FA, ALA = alpha-linolenic acid, EPA = eicosapentaenoic acid, DPA = docosapentaenoic acid, DHA = docosahexaenoic acid, n-7 = omega-7, n-9 = omega-9, n-6 = omega-6, n-3 = omega-3, n-6:n-3 = n-6 to n-3 ratio, CLA = conjugated linoleic acid.

<sup>2</sup>Total FA = SFA + MUFA + PUFA.

<sup>3</sup>SFA = C14:0 + C16:0 + C17:0 + C18:0 + C23:0 + C24:0.

<sup>4</sup>MUFA = C14:1 + C16:1 + C18:1.

<sup>5</sup>PUFA = C18:2 + C18:3 + C20:3 + C20:4 + C20:5 + C22:5 + C22:6.

<sup>6</sup>n-6 PUFA = C18:2 + C20:3 + C20:4.

<sup>7</sup>n-3 PUFA = C18:3 + C20:5 + C22:5 + C22:6.

<sup>8</sup>n-6:n-3 = n-6 PUFA/n-3 PUFA.

loin samples were collected purposely avoiding large fat deposits in the lean tissue. Therefore, this sampling strategy may have resulted in the comparatively leaner samples than other reports.

Stearic acid, a saturated fatty acid (SFA) generally recognized to have a net neutral effect on serum cholesterol (Grande et al., 1970), accounted for an average of 13% of the total FA. Stearic acid is typically found in greater proportions in GFB than in CFB, constituting 13.1 to 17.7% of total FA (Garcia et al., 2008; Realini et al., 2004; Duckett et al., 2013). Concentrations of monounsaturated fatty acids (MUFAs) and SFAs were both 44% of total; previous studies have reported that GFB has significantly less MUFAs than SFAs (Duckett et al., 2013; Descalzo et al., 2005; Realini et al., 2004). Correlation coefficients of select variables are presented in Table 3. Saturated FA and MUFA were both more highly correlated with total fatty acid ( $r = 0.995$  and  $0.994$ , respectively) than polyunsaturated FAs (PUFA,  $r = 0.621$ ) indicating a lower PUFA:SFA ratio with increased total fatty acids, in agreement with previous reports (Warren et al., 2008; Duckett et al., 1993).

### Omega-6 and omega-3 FA content of GFB

The most prevalent PUFA in beef, linoleic acid (LA), an omega-6 (n-6) FA, was 6.5% of total FA. This is greater than reported for either GFB or CFB (2.6 and 2.7%, respectively; Duckett et al., 2013) though LA concentrations have been reported as high as 5.4% of total FA for GFB, and 4.7% for CFB as well (Descalzo et al., 2005). Alpha-linolenic acid (ALA) is the most prevalent omega-3 (n-3) FA in beef, and was 0.81% of total FA. This FA is typically found in great-

**Table 3.** Pearson correlation ( $r$ ) between fatty acid classes and antioxidants ( $P < 0.05$ )<sup>1</sup>

Fatty Acid	SFA	MUFA	PUFA	n-6	n-3	n-6:n-3	β-c	α-t
Total FA	0.995	0.994	0.621	0.604	-0.144	0.319	0.115	-0.179
SFA		0.983	0.587	0.572	-0.140	0.307	0.114	-0.168
MUFA			0.570	0.554	-0.132	0.285	-0.125	-0.178
PUFA				0.970	-0.222	0.550	-0.043	-0.204
n-6					-0.451	0.672	-0.042	-0.243
n-3						-0.680	-0.005	0.300
n-6:n-3							0.000	-0.165
β-c								0.651

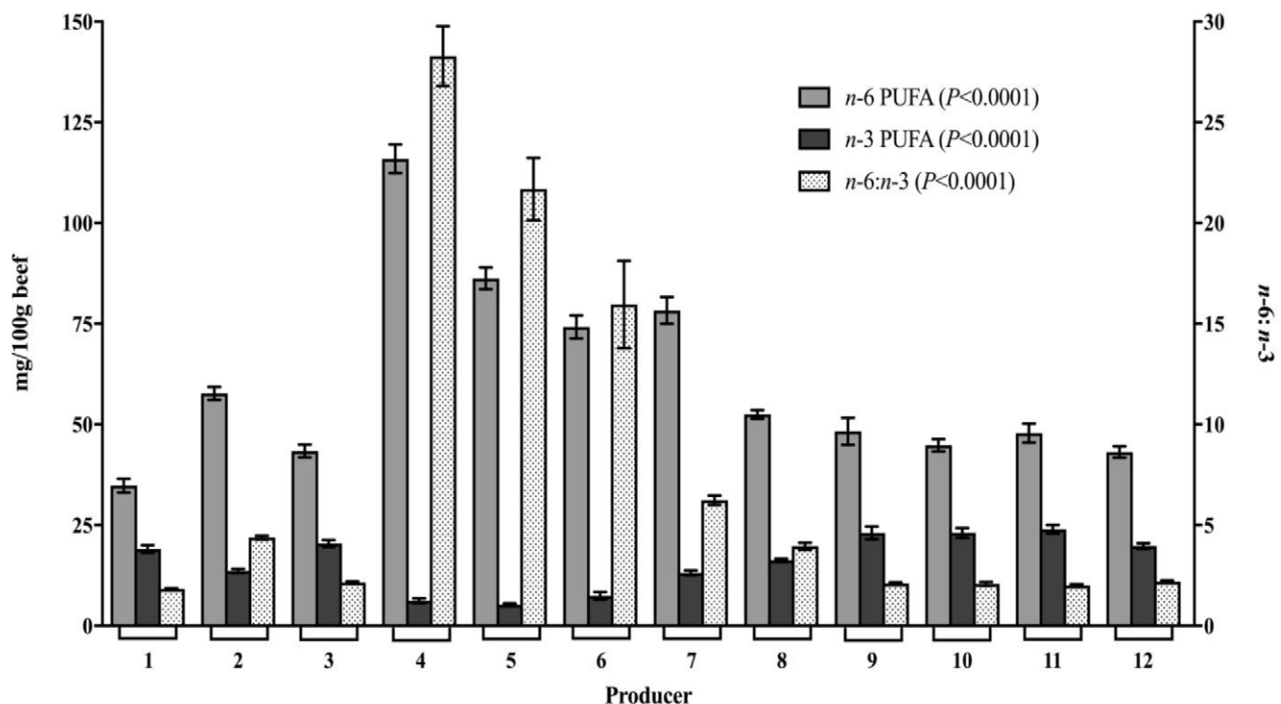
<sup>1</sup>FA = fatty acid, SFA = saturated FA MUFA = monounsaturated FA, PUFA = polyunsaturated FA, n-6 = omega-6 FA, n-3 = omega-3 FA, β-c = beta-carotene, α-t = alpha-tocopherol.

er concentrations in GFB than CFB, due to the higher *n*-3 content of GFB diets (Daley et al., 2010; Duckett et al., 2013). Both linoleic and ALA are essential fatty acids for humans and necessary for the synthesis of long chain PUFA in the body. However, the efficiency of this conversion is low, thus, it is important to obtain long chain FAs in the diet as well. Eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid, all *n*-3 FAs, were present in this sample set at concentrations of 0.49, 0.55, and 0.04% of total FA respectively compared to 0.55, 0.85, and 0.09% for these FAs in another report (Duckett et al., 2013).

The overall ratio of *n*-6 to *n*-3 FA (*n*-6:*n*-3 ratio; mean = 9.90, median = 4.10) was greater than expected for GFB. The mean *n*-6:*n*-3 ratios for individual producers varied widely ranging from 1.80 to 28.3. Research has consistently shown that cattle finished solely on grass have a lower *n*-6:*n*-3 ratio than CFB, with GFB typically having a ratio below 2, and CFB showing a ratio greater than 4 (Chail et al., 2016; Duckett et al., 2013; Warren et al., 2008; Leheska et al., 2008; Realini et al., 2004). The *n*-6:*n*-3 ratio is a strong indicator of feedstuffs used in finishing systems (Duckett et al., 2009) and ratio differences in GFB and CFB are generally attributed to greater amounts of linoleic acid in concentrates than forages (French et al., 2000; Warren et al., 2008). Some producers in this study

indicated supplementation of soybean hulls (SH) during the finishing phase, and others reported supplementation of SH outside of the finishing phase. The addition of a supplemental feedstuff with a higher linoleic content could result in an *n*-6:*n*-3 ratio similar to conventionally finished beef. In one study, cattle grazing on orchard grass and fescue supplemented with pelleted SH had a greater *n*-6:*n*-3 ratio than beef from cattle on a forage only diet (3.19 and 3.36 vs. 1.93; Baublits et al., 2006). However, cattle fed varying levels of SH before a 150 d finishing phase on forages have no reported differences in *n*-6:*n*-3 ratio (Duckett et al., 2009). Feeding SH during the finishing phase may improve performance, as forage finished cattle supplemented with SH had a greater average daily gain and finished with a higher yield grade (2.83 vs. 1.37) and quality grade (USDA Choice vs. USDA Standard) than those finished solely on grass (Pugh, 2003). It is possible that cattle supplemented with SH have a greater apparent biohydrogenation than those supplemented with corn, resulting in a higher content of stearic acid and CLA in beef (Kiesling, 2013). Many GFB programs, such as the American Grassfed Association, prohibit the supplementation of soy products to cattle under their label (American Grassfed Association, 2017).

The amount of *n*-6 and *n*-3 FAs, and the *n*-6:*n*-3 ratio were highly variable across producers ( $P < 0.0001$ ;



**Figure 1.** Producer comparison of mean  $\pm$  SEM *n*-6, *n*-3, and *n*-6:*n*-3 ratio.<sup>1</sup> *n*-6 = omega-6 fatty acids, *n*-3 = omega-3 fatty acids. *P*-values from Kruskal–Wallis analysis are shown demonstrating significant differences by producer in total *n*-6, *n*-3, and *n*-6:*n*-3 ratio.

Fig. 1). Beef from producers 4, 5, and 6 had the least  $n-3$  FAs, and greatest  $n-6:n-3$  ratio. Producers 4 and 6 indicated a broad variety of finishing diets, including stored forages and supplements while producer 5 did not indicate finishing diet. The ratios for these producers were greater than values previously reported for GFB; Chail et al. (2016), Duckett et al. (2013), and Realini et al. (2004), have reported  $n-6:n-3$  ratios of 3.44, 1.33, and 1.44 for GFB, respectively. Producers 1, 3, 9, 10, and 11 had the greatest amounts of  $n-3$  and the lowest  $n-6:n-3$  ratios (range = 1.80 to 2.20), which is consistent with other reported results for GFB. Producers 2 and 8 had mean  $n-6:n-3$  ratios of 4.40 and 3.90 respectively, and indicated supplementation of various harvested feedstuffs (Table 1), including forage silage. The results of the current study are consistent with reports that beef from cattle finished on fresh forages had greater  $n-3$  content and a lower  $n-6:n-3$  ratio than beef from cattle fed harvested grass silage ad-libitum, with both receiving a concentrate supplement daily (French et al., 2000).

Beef from producers 4, 5, and 6 had significantly greater  $n-6:n-3$  ratios than the remaining producers ( $P < 0.0001$ ), and higher than previously reported values for beef from feedlot finished cattle (6.01 and 4.84, respectively; Duckett et al., 2013; Duckett et al., 2009). Chail et al. (2016) reported an  $n-6:n-3$  ratio for feedlot finished beef of 5.74, along with USDA Choice grade beef obtained from a retailer with a  $n-6:n-3$  ratio of 15.2. The survey boundaries disallow inference as to the greater than expected  $n-6:n-3$  ratios of beef provided by some of the producers. However, the results of the current study indicate that cattle finished on fresh forages yield beef with a lower  $n-6:n-3$  ratio than those supplemented with harvested forages. An area of further interest would be to evaluate the  $n-6:n-3$  ratio of the forage finishing diets. The FA content in stored forages is highly variable; the primary variable affecting total FA content and the proportion of ALA of grass silage is due to the maturity of the grass at harvest; the more mature the forage, the lower the total FA content and proportion of ALA (Khan et al., 2012). The length of time that forages are wilted in preparation for ensiling is also associated with a decrease of ALA through oxidative loss (Khan et al., 2012; Khan et al., 2011; Van Ranst et al., 2009). Silage additives and inoculants (and thus the type and extent of fermentation) have little to no effect on FA content of forages in well-sealed silages (Van Ranst et al., 2009; Dewhurst & King, 1998). Furthermore, preserving grass as dry hay results in lower forage LA, ALA, total FA, and fat-soluble vitamin concentrations compared to grass preserved through ensiling (Villeneuve et al., 2013; Shingfield et al., 2005).

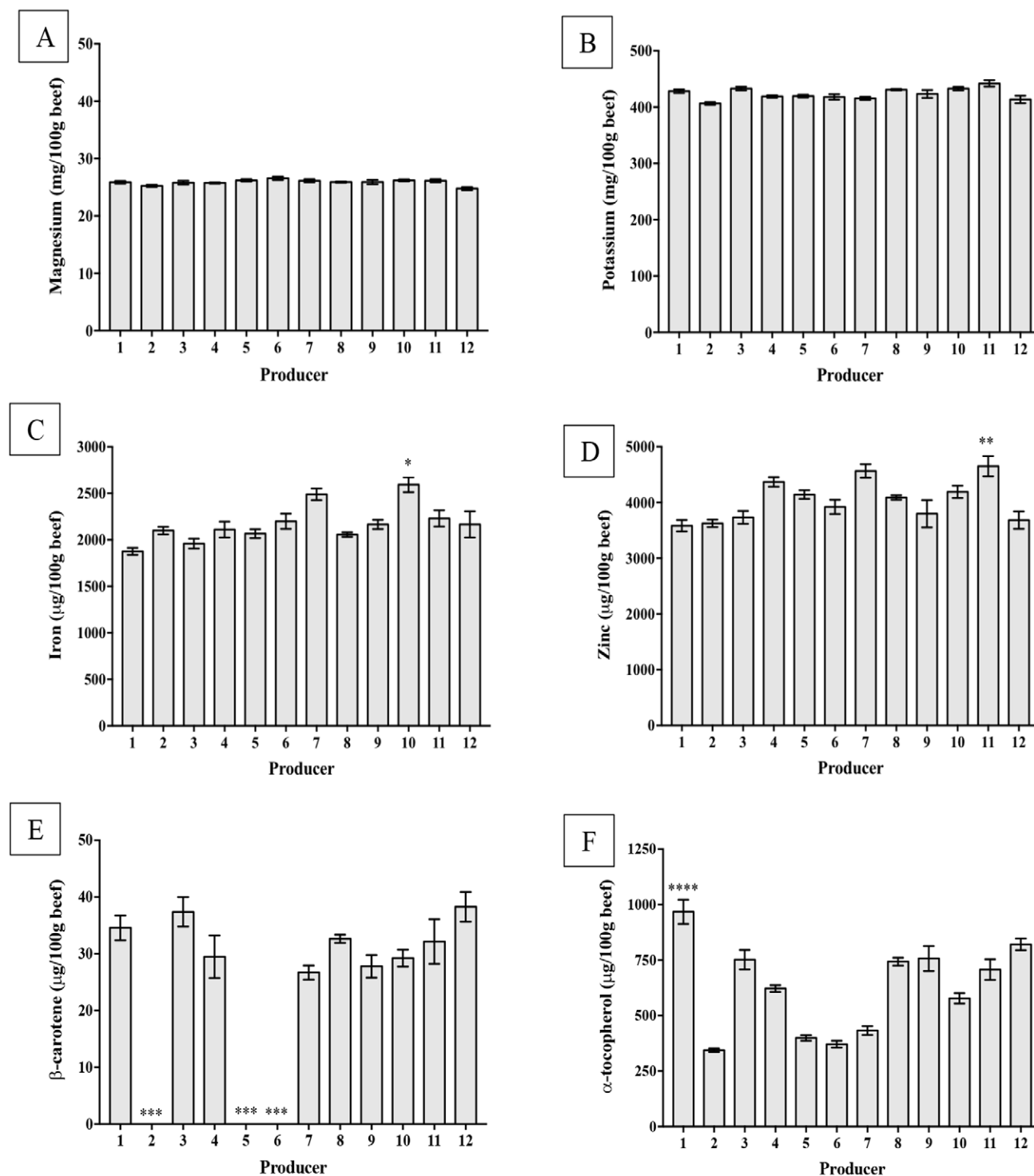
Interestingly, total FA was positively associated with the  $n-6:n-3$  ratio ( $r = 0.402$ ; Table 3), indicating that as grass-fed animals fatten, the  $n-6:n-3$  ratio increases rather than decreases as previously hypothesized, potentially limiting the benefit gained from any additional  $n-3$  FA (De Smet et al., 2004).

## Minerals

Beef mineral and antioxidant content is presented in Table 4. Mineral content was similar to previously reported levels for GFB though, in general, K, Mg, and Fe were greater, and Na, Cu, and Se were lower (Duckett et al., 2009; Leheska et al., 2008). Copper was below the limit of quantification for a number of samples, therefore only quantifiable data is presented ( $n = 435$ ). Producer comparisons of select minerals and antioxidants are presented in Fig. 2. Beef from all producers had similar levels of Mg (23 to 26 mg/100g beef), K (400 to 450 mg/100g beef), and Zn (3500 to 4500  $\mu$ g/100g beef). Producer 10 beef had significantly higher Fe ( $P < 0.0001$ ) than all other producers except 7 and 11. Due to the national scope of our study and since the nutrient composition of forages is dependent on numerous factors such as soil mineral content and moisture, climate, plant species and maturity, and leaf to stem ratio, the high level of variation we observed was expected (Kilcher, 1981; Preston, 2008). Schmidt et al. (2013) have reported that the mineral composition varied by forage species for P, K, Ca, Mg, S, Zn, Cu, Mn, Fe, and Na, while beef from cattle finished on these forages exhibited differences in Mg, Zn, and Na, with greater amounts found in beef finished on bermudagrass than on other forage species (Schmidt et al., 2013). This contrasts a previous report in which no

**Table 4.** Mineral and antioxidant content of grass-finished beef

Macrominerals, mg/100g	Mean $\pm$ SEM	Min	Max
Sodium	41.7 $\pm$ 0.29	25.0	93.0
Magnesium	25.9 $\pm$ 0.06	22.0	33.0
Phosphorus	210 $\pm$ 0.43	171	277
Sulfur	206 $\pm$ 0.50	173	274
Potassium	423 $\pm$ 0.84	330	526
Microminerals, $\mu$ g/100g			
Iron	2130 $\pm$ 19.6	1170	9830
Zinc	4080 $\pm$ 27.8	2200	6840
Copper	63.3 $\pm$ 0.65	49.0	250
Selenium	17.6 $\pm$ 0.26	6.0	68.0
Antioxidants, $\mu$ g/100g			
$\alpha$ -tocopherol	611 $\pm$ 9.90	161	1680
$\beta$ -carotene	32.2 $\pm$ 0.56	11.0	103



**Figure 2.** <sup>1</sup>Producer comparison of the mean  $\pm$  SEM mineral and fat-soluble vitamin content of GFB; A) magnesium content was similar among all producers B) potassium content was similar among all producers C) iron significantly varied by producer ( $P < 0.001$ ); \*iron was highest in samples from producer 10 D) zinc significantly varied by producer ( $P < 0.001$ ); \*\*zinc was highest in producer 11 E)  $\beta$ -carotene; \*\*\* $\beta$ -carotene values were below the lower limit of quantification for all samples from producers 2, 5, and 6 F)  $\alpha$ -tocopherol significantly varied by producer ( $P < 0.001$ ); \*\*\*\* $\alpha$ -tocopherol was highest in producer 1. Values below the lower limit of quantification were excluded from all analyses.

difference in mineral content between GFB and CFB was found (Duckett et al., 2009). All producers in this study indicated that they supplemented minerals

in their finishing programs, therefore inherent variation of soil mineral status by location, and the wide variation in forages fed to the cattle may persist in de-



termining GFB mineral content. In fact, it has been suggested that supplementing minerals to ruminants in locations where there are deficiencies in soils may improve beef mineral content (Preston, 2008). Despite variation in mineral composition, GFB remains a good source of Fe and Zn (Williamson et al., 2005).

### **Alpha-tocopherol and $\beta$ -carotene**

Two fat soluble vitamins present in beef of interest for their antioxidant properties are vitamins A and E. These are measured in the current study, directly for vitamin E ( $\alpha$ -tocopherol) and indirectly for vitamin A as its precursor,  $\beta$ -carotene. The content of both of these antioxidants are reported to be significantly greater in GFB than in CFB (Duckett et al., 2013; Descalzo et al., 2007; Yang et al., 2002). The GFB  $\alpha$ -tocopherol and  $\beta$ -carotene content are indicated in Table 4. The mean beef  $\alpha$ -tocopherol content was 611  $\mu\text{g}/100\text{ g}$  of tissue. Only 358 samples had  $\beta$  carotene content above the limit of quantification (11  $\mu\text{g}/100\text{ g}$ ), and the mean reflects those samples ( $n = 358$ ; 32.2  $\mu\text{g}/100\text{ g}$ ). The  $\alpha$ -tocopherol content reported in this study is greater than previously reported for GFB (343  $\mu\text{g}/100\text{ g}$  and 375  $\mu\text{g}/100\text{ g}$ ) by Duckett et al. (2013) and De la Fuente et al. (2009). The mean  $\beta$ -carotene reported here is lower than that found for beef cattle grazing mixed pasture at 57.8  $\mu\text{g}/100\text{ g}$  (Duckett et al., 2013). Previous research has found that carotenoid content of harvested feeds varies greatly depending on maturity at harvest and length of storage, though the content in fresh forages is typically great enough to meet nutritional requirements for cattle (Preston, 2008).

The  $\alpha$ -tocopherol and  $\beta$ -carotene content was highly variable between producer beef samples ( $P < 0.0001$ ; Fig. 2E-F). Beef from producers 2, 5, 6, and 7 had lower amounts of  $\alpha$ -tocopherol than all other producers ( $P < 0.0001$ ), though still within ranges reported by others for GFB (Duckett et al., 2013; De la Fuente et al., 2009; Descalzo et al., 2007; Yang et al., 2002). Producers 5 and 7 did not report their feeding strategies, but producers 2 and 6 indicated feeding more harvested forages than the remainder of the producers. Beef from producers 2, 5, and 6 had a  $\beta$ -carotene content below the limit of quantification for nearly all samples.  $\alpha$ -tocopherol content is reportedly greater in fresh forages than in harvested hays (Ballet et al., 2000). Duckett et al. (2013) reported no difference in  $\alpha$ -tocopherol in beef from cattle finished on mixed pasture, alfalfa, and pearl millet, but found that  $\beta$ -carotene was lower in beef from cattle finished on alfalfa (mean = 0.519  $\mu\text{g}/100\text{g}$ ). In a similar study, no

difference in  $\alpha$ -tocopherol content in beef finished on five different forage species, but reported a wider range of  $\beta$ -carotene levels (38 to 160  $\mu\text{g}/100\text{g}$ ; Schmidt et al., 2013). These results indicate that finishing with fresh forages can achieve greater content of the antioxidant  $\alpha$ -tocopherol, which is widely promoted as a health benefit of GFB, compared to finishing on harvested forages.

### **Conclusions**

This survey of commercially available GFB indicates that producers use a wide variety of feeding strategies to finish cattle, including fresh and harvested forages, and both annual forage crops and perennial pastures. The diversity of production strategies mirrors the variability in the nutritional profile of the beef. Beef sampled for this study contained greater proportions of MUFA and PUFA to total FA than has been previously reported for GFB. The  $n$ -6: $n$ -3 ratio was numerically lower in beef from producers who indicated finishing cattle solely on fresh forages compared to beef from producers who finished cattle on harvested feeds. Similarly, the content of  $\alpha$ -tocopherol and  $\beta$ -carotene was greater in beef from producers who reported finishing on fresh forages. Mineral content was highly variable, reflecting the diversity in forages fed and locations where forages were grown. This survey serves to gain a greater understanding of the trends and variation among GFB produced in the USA and marketed to consumers.

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