A NUTRITIONAL SURVEY OF COMMERCIALLY AVAILABLE GRASS-FINISHED BEEF

By

Sara M. Bronkema

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirement
for the degree of

Animal Science – Master of Science

2018

ABSTRACT

A NUTRITIONAL SURVEY OF COMMERCIALLY AVAILABLE GRASS-FINISHED BEEF

By

Sara M. Bronkema

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 aim, a survey of grass-finishing production systems throughout the U.S. was conducted, and the beef finished on participating farms was analyzed for its nutritional composition, including fatty acid (FA), mineral and fat-soluble vitamin content. Annual production capacity of farms ranged from 25 to 5,000 cattle, with a mean age of cattle at harvest of 26.8 ± 2.3 months. An array of finishing diets included grazing exclusively perennial pasture, the incorporation of annual forage crops, as well feeding a variety of harvested forages and supplementation of non-starch feed byproducts. The ratio of omega-6 (*n*-6) to omega-3 (*n*-3) FA in beef samples averaged by producer ranged from 1.8 to 28.3, with an overall sample set median of 4.1. Only *n*-3 FA varied between harvest season, with a greater amount found in beef harvested in the spring. Mineral content was highly variable by season and producer, due to the inherent variation of soil and forage mineral content. Mean α-tocopherol content was 610.6 μg/100 g beef, and mean β-carotene content was 32.2 μg/100 g. The amount of these antioxidants also varied between producers, but tended to be greater in beef finished solely on fresh forages. This survey indicates that commercially available GFB can vary in its nutritional composition due to the diverse practices used to grass-finish cattle.

ACKNOWLEDGEMENTS

There are a number of people who made this journey possible. First, I would like to thank my advisor Dr. Jason Rowntree. I first met Dr. Rowntree in a dusty, hot show ring the summer prior to beginning my freshman year at MSU. Throughout my undergraduate degree, he was a willing mentor, and helped me pursue my interests in sustainable agriculture and always encouraged me to stand up for what I believed, and to never forget from Whom I draw my strength. When I expressed my interest in research, he offered the ultimate opportunity to mesh my interests in sustainable agriculture and meat science in this project. To the rest of my committee, Drs. Buskirk, Fenton, and Schweihofer, thank you for providing your insights and expertise along the way. Your support and the opportunities provided to me at MSU made this a worthwhile experience.

Other members of the MSU Animal Science department, including Dr. Swanson and Jen Dominguez, as well as Sarah Wells deserve my appreciation. One of the primary factors that drove me to pursue this degree was the chance to teach other students along the way, and you are the ones who taught me, inspired me, supported me, and most of all gave me the opportunity to do so. I would also like to thank Rachel Martin for being an "awesome, amazing, helpful, chock-full-of-advice roommate." Your insight, mentorship, and friendship were invaluable.

To my family, thank you for your steadfast support, for the prayers and laughter and especially the haven from the craziness that is grad school.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

KEY TO ABBREVIATIONS

AA ..... amino acid

AGA ..... American Grassfed Association

ALA ..... alpha-linolenic acid

ARA ..... arachidonic acid

BHT ..... butylated hydroxytoluene

BMR ..... brown midrib

C14:0 ..... myristic acid

C14:1 ..... myristoleic acid

C16:0 ..... palmitic acid

C16:1 ..... palmitoleic acid

C18:0 ..... stearic acid

C18:1 ..... oleic acid

C18:2*n*-6 ..... linoleic acid

C18:3*n*-3 ..... alpha-linolenic acid

C20:4*n*-6 ..... arachidonic acid

C20:5*n*-3 ..... eicosapentaenoic acid

C22:5*n*-3 ..... docosapentaenoic acid

C22:6*n*-3 ..... docosahexaenoic acid

Ca ..... calcium

CFB ..... conventionally-finished beef

CHD ..... coronary heart disease

CLA ..... conjugated linoleic acid

Cu ..... copper

CVD ..... cardiovascular disease

DHA ..... docosahexaenoic acid

DPA ..... docosapentaenoic acid

EFA ..... essential fatty acid

EPA ..... eicosapentaenoic acid

FA ..... fatty acid

FAME ..... fatty acid methyl ester

Fe ..... iron

GC-MS ..... gas chromatography-mass spectrometry

GFB ..... grass-finished beef

HCl ..... hydrochloric acid

K ..... potassium

LA ..... linoleic acid

LCPUFA ..... long-chain polyunsaturated fatty acid

LDL ..... low density lipoprotein

Mg ..... magnesium

Mn ..... manganese

MUFA ..... monounsaturated fatty acid

n-3 ..... omega-3

n-6 ..... omega-6

n-6:*n*-3 ..... omega-6 to omega-3 fatty acid ratio

Na ..... sodium

P ..... phosphorus

P:S ..... polyunsaturated fatty acid to saturated fatty acid ratio

PL ..... phospholipid

PUFA ..... polyunsaturated fatty acid

S ..... sulfur

Se ..... selenium

SFA ..... saturated fatty acid

SH ..... soybean hulls

TG ..... triacylglycerol

USDA ..... United States Department of Agriculture

Zn ..... zinc

CHAPTER 1

LITERATURE REVIEW

1.1. Introduction

The intent of this review is to present an overview of grass-finished beef (GFB), including production strategies and the growing market, and critically evaluate the nutritional aspects of this specialty product. Furthermore, this review surveys the factors influencing the nutrient composition of the final product.

The GFB sector of the beef industry is growing at a rapid pace, with retail sales doubling every year from 2012 to 2016, according to a privately funded report by the Stone Barns Center (Cheung et al., 2017). This growth is mainly due to interest from consumers in the perceived environmental footprint of food production and a clearer understanding of the effect of diet on human health (Cheung et al., 2017; Umberger et al., 2009). Mounting consumer interest in the source of their food has resulted in close scrutiny of production methods and health claims of products. Ultimately, the goal of this review is to create a better understanding of the variability of grass-finished beef production methods and nutritional composition, to aid the industry in understanding the opportunities and challenges faced in the market.

1.2. Beef nutrient composition

Beef is an excellent source of protein, minerals, vitamins, and fat. It has more protein per serving than pork or chicken (Table 1.1) and unlike most foods obtained from plant sources, it does not have a limiting amino acid (AA). Thus, beef is a complete and efficient source of protein, containing a balance of all eight AA (Williamson et al., 2005). Moreover, beef contains a number of important minerals and vitamins. Beef provides twice as much iron and zinc as pork and chicken, and is a source of trace minerals such as chromium, cobalt, copper, magnesium, nickel, phosphorus and selenium (USDA, 2016a; Williamson et al., 2005). Most of the iron in meat is haem iron, a bioavailable form of iron that is more easily absorbed in the body

(Williams, 2007; Williamson et al., 2005). Beef contains a number of B vitamins; in particular, it is a rich source of vitamin B12. In addition, beef provides small amounts of vitamins A, D, and E to the human diet (Williamson et al., 2005).

Table 1.1. Nutrient values for beef, pork, and chicken.

Nutrients (per 100 g)	Beef[1]	Pork[2]	Chicken[3]
Water (g)	70.3	73.6	74.9
Energy (kcal)	155	127	111
Protein (g)	22.3	22.0	20.3
Fat (g)	6.60	3.71	2.70
Iron (mg)	2.07	0.65	0.88
Zinc (mg)	3.58	1.86	1.19

[1] Values for beef, short loin, porterhouse steak, separable lean only, trimmed to 1/8" fat, all grades, raw
[2] Values for pork, fresh, loin, center loin (chops), bone-in, separable lean only, raw
[3] Values for chicken, roasting, meat only, raw
(USDA, 2016b)

Fat is the most energy dense nutrient in the human diet. When consumed in moderation, it is a valuable source of essential fatty acids and fat-soluble vitamins. Fat is comprised of saturated, monounsaturated, and polyunsaturated fatty acids, along with triglycerides and cholesterol. Beef contains higher amounts of saturated fat and lower amounts of polyunsaturated fat than either pork or chicken, while all three protein sources have significantly less polyunsaturated fat than salmon, one of the most commonly consumed seafoods (Table 1.2). The difference in fat profiles between species is due to the digestive tract differences of the animals. As ruminants, cattle evolved eating a diet mainly consisting of forages and have a specialized digestive tract that allows them to process this high-fiber diet. The feed they consume is broken down by microbial fermentation in the rumen. Lipids are broken down into individual fatty acids through a process called lipolysis. Unsaturated fatty acids are subsequently converted to saturated fatty acids through biohydrogenation (Figure 1.1) - the addition of hydrogen ions to the fatty acid - by ruminal microbes

(Figure 1). Thus, cattle yield meat and dairy products with more saturated fat than their monogastric counterparts, despite consuming diets high in unsaturated fat. (Jenkins et al., 2008).

Table 1.2. Fatty acid content of beef, pork, chicken, and salmon.

Nutrients (per 100 g)	Beef[1]	Pork[2]	Chicken[3]	Salmon[4]
Saturated fatty acids (g)	2.557	1.098	0.670	3.050
Monounsaturated fatty acids (g)	2.840	1.346	0.830	3.770
Polyunsaturated fatty acids (g)	0.353	0.415	0.670	3.886

[1] Values for beef, short loin, porterhouse steak, separable lean only, trimmed to 1/8" fat, all grades, raw
[2] Values for pork, fresh, loin, center loin (chops), bone-in, separable lean only, raw
[3] Values for chicken, roasting, meat only, raw
[4] Values for fish, salmon, Atlantic, farmed, raw
(USDA, 2016b)

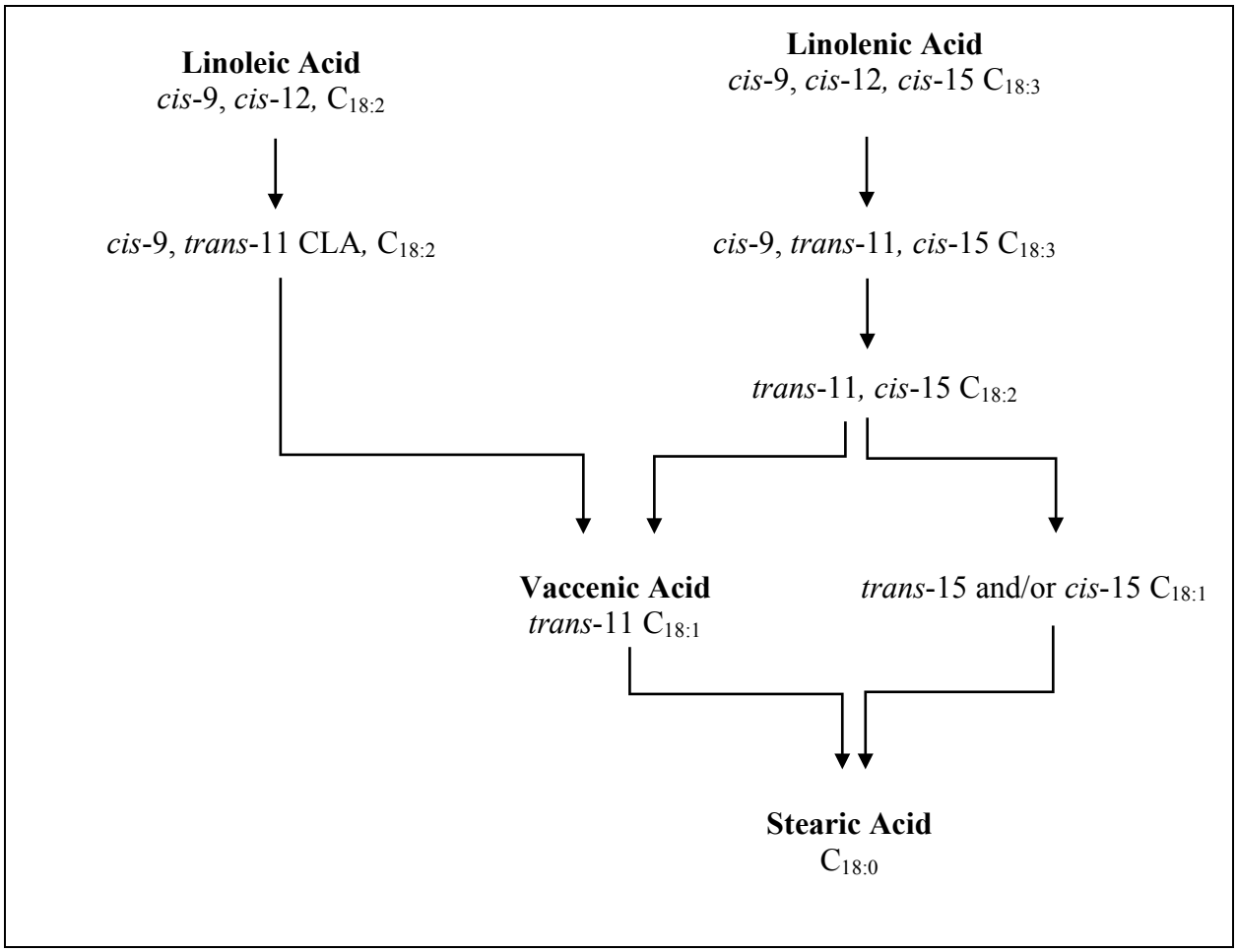

Figure 1.1. Biochemical pathways for the biohydrogenation of linoleic and linolenic acids in the rumen (adapted from Harfoot and Hazlewood, 1997).

In recent years, the nutrient profile of beef in relationship with human health has been challenged due to its levels of total and saturated fat, and its classification as a probable carcinogen by the World Health Organization (IARC, 2015). Thus, there is growing interest in improving the nutrient profile of beef so that it is more favorable for human health (Scollan et al., 2006). Beef nutrient composition is strongly correlated to how the animal was raised. The primary influence is the function of the animals' diet and thus innovation in altering the nutrient profile of beef has focused mainly on cattle nutrition. Many studies suggest that there are nutritive strengths to beef from cattle fed a diet consisting only of forages (Chail et al., 2016; Duckett et al., 2013; Duckett et al., 2009; Realini et al., 2004), such as lower fat content and decreased proportions of saturated fat.

1.3. Dietary fat and its role in human health

A debate that has dominated health discussions in the last 30 to 40 years is the negative aspects of saturated fat in the human diet and its link to cardiovascular disease (CVD). High consumption of saturated fat is associated with high total serum cholesterol and low-density lipoprotein cholesterol, which increases the risk of coronary heart disease (CHD) (Gropper, 2013). Dietary guidelines have long suggested lowering total fat intake as one way to reduce intake of saturated fat. However, while the *2015-2020 Dietary Guidelines for Americans* recommended that saturated fat not make up more than 10% of total energy intake, it rescinded the previously suggested upper limit of total fat intake, citing lack of evidence for reduced risk of cardiovascular disease (CVD) (USDHHS & USDA, 2015; Mozaffarian & Ludwig, 2015). Replacing dietary saturated fats with monounsaturated fats is another dietary recommendation associated with reduced risk of CHD (Hu et al., 1997). However, recent research indicates while this substitution has the potential for lowering total plasma cholesterol, it does not necessarily

provide cardioprotection (Degirolamo & Rudel, 2010; Gropper, 2013). Replacing saturated fat with polyunsaturated fat has also been recognized to reduced total plasma cholesterol and low density lipoprotein (LDL) cholesterol (Hodson, 2001; Jackson et al., 1978). A meta-analysis conducted by Mozaffarian et al. (2010) reported that increased polyunsaturated fats in the place of saturated fats in the diet reduced the incidence of CHD.

Polyunsaturated fats are classified as omega-3 (*n*-3) or omega-6 (*n*-6) fats. Omega-3 polyunsaturated fatty acids are widely recognized to have health benefits, playing roles in infant development, and maintenance of mental health in adulthood (Simopoulos, 1991). Furthermore, *n*-3 polyunsaturated fatty acids have cardioprotective and anti-inflammatory properties, and the evidence for protection against heart disease has resulted in public health recommendations for increased consumption of fish, due to the abundance of n-3 fatty acids in seafood (Kremmyda et al. 2011; Kris-Etherton et al., 2003). Humans evolved on a diet relatively low in *n*-6 fats compared to current Western diets, and the balance of *n*-6 to *n*-3 fats is important for homeostasis (Simopoulos, 2002). While there is no daily reference value for the *n*-6:*n*-3 ratio, it is suggested that a healthy diet should have a ratio between 1:1 to 4:1 to reduce the risk of chronic diseases such as CVD, cancer, and inflammatory diseases. (Kremmyda et al., 2011; Daley et al., 2010; Simopoulos, 2002; Simopoulos, 1991).

Ruminant-derived food products contribute the largest portion of saturated fat to the human diet (Kris-Etherton & Innis, 2007). As public health policies recommend lowering consumption of saturated fat, interest has arisen in research to alter the fatty acid profile of beef. Much focus is on potential strategies to increase beneficial polyunsaturated fats and reduce saturated fats in beef to meet recommended nutritional requirements without significantly modifying current diets (Scollan et al., 2006; Shingfield et al., 2013).

1.4. Fatty acids: individual effects and dietary sources

Dietary fat, or triacylglycerol, is composed of three fatty acids (FA) attached to a glycerol backbone. Fatty acids can be categorized according to the number of carbon-carbon double bonds into saturated or unsaturated FA. Saturated fatty acids (SFA) have no double bonds. Monounsaturated fatty acids (MUFA) have one double bond, while polyunsaturated fatty acids (PUFA) have two or more double bonds.

The most common SFA found in beef are myristic (C14:0), palmitic (C16:0), and stearic acid (C18:0). Excess consumption of palmitic and myristic acid is known to increase serum cholesterol levels, which increases the risk of CVD and stroke, while stearic acid has been shown to have a net neutral impact on cholesterol levels (Gropper, 2013; Grande et al., 1970). The primary MUFA in beef is oleic acid (C18:1), but it also contains smaller amounts of myristoleic (C14:1) and palmitoleic acid (C16:1). Polyunsaturated fatty acids are categorized by the position of their first double bond; *n*-3 FAs have a double bond located three carbons from the methyl end, while the position of the double bond in *n*-6 FAs is on the sixth carbon. Linoleic acid (LA, C18:2) and alpha-linolenic acid (ALA, C18:3) are considered essential fatty acids (EFA) in the diet, as humans cannot synthesize them. Linoleic acid is an *n*-6 FA and is essential for the production of eicosanoids, or cell regulators, in the human body. It is an abundant FA in many foods, such as corn oil and also the most abundant PUFA in beef (Daley et al., 2010). Alpha-linolenic acid is an *n*-3 FA, found most abundantly in fish and seed oils. Long-chain polyunsaturated fatty acids (LCPUFA) include arachadonic acid (ARA, C20:4*n*-6), eicosopentaenoic acid (EPA, C20:5*n*-3), docosapentaenoic acid (DPA, C22:5*n*-3), and docosahexaenoic acid (DHA, C22:6*n*-3). Humans can synthesize these from dietary fatty acids, but due to low efficiency of this conversion, it is important to obtain these FA in the diet as well.

Sources of EFA and LCPUFA include meat, eggs, seafood, and seed oils (Whelan & Rust, 2006). Both *n*-3 and *n*-6 PUFA are important in the regulation of inflammation, though *n*-6 FAs are typically pro-inflammatory, and *n*-3 FA anti-inflammatory (Calder, 2008). These FA compete for the same enzymes in the desaturase pathway (Figure 2). Thus, there is an innate competition between the two FA families, and an imbalance of one over the other can shift physiological states and promote inflammation, CVD, and autoimmune diseases (Simopoulos, 2002).

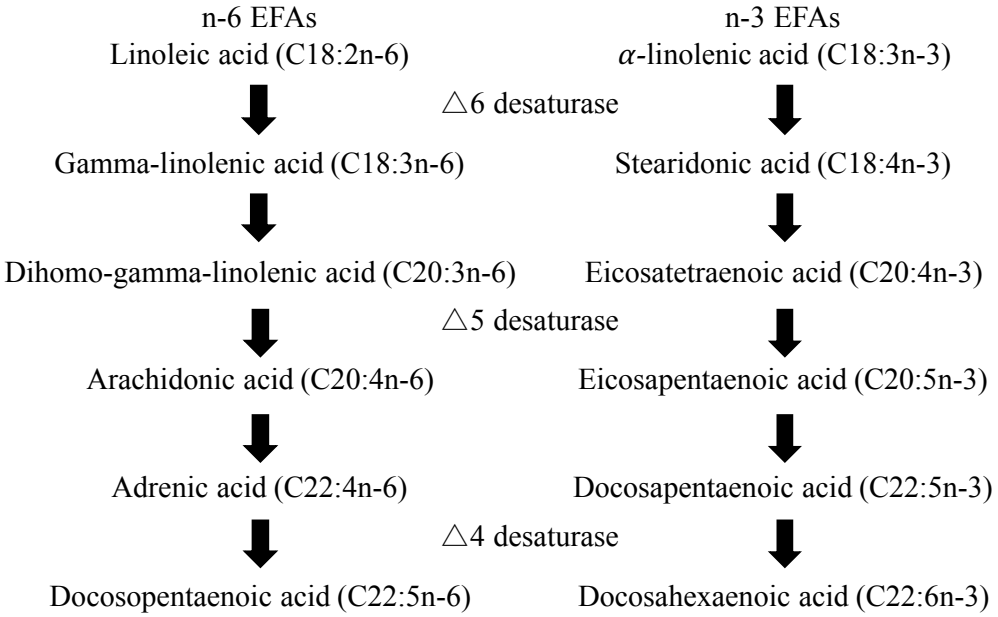

Figure 1.2. Desaturase and elongation pathway for linoleic and α-linolenic acid (adapted from Simopoulos, 1991).

1.5. Beef production strategies

Cattle are traditionally raised in several stages: calves nurse their mothers from birth until they are weaned at 6 to 10 mos. of age. After weaning, calves generally are placed in one of two management scenarios, both of which will be referred to as conventional finishing throughout this paper. The first is directly into a feedlot where they are fed a high-energy, balanced feed

ration that is made up of a variety of forages, grains, and supplements. The second is a background or stocker operation, where cattle typically graze forages until they are 12 to 16 mos. old before entering a feedlot. A smaller subset of beef producers finish cattle solely on forages without supplemental grain; this beef is referred to as grass-finished, or grass-fed beef (GFB).

There are no legal standards defining the term grass-fed. The United States Department of Agriculture (USDA) created a standard for GFB in 2007, but retracted it in 2016, stating that the label did not "facilitate the marketing of agricultural products in a manner that [was] useful to stakeholders or consumers," (USDA, 2016b). However, the USDA still oversees the labeling of GFB, requiring producers to create a standard or use a third-party certifier. Third-party certifiers have developed their own set of standards such as the American Grassfed Association's (AGA) definition and the Animal Welfare Approved label. While there is some variation between labels, the fundamentals are consistent; a grass-fed animal can consume only forages from birth to harvest (with the exception of mother's milk) and no grain. Forages can include perennial and annual grasses, legumes, brassicas, forbs, grain crops in the vegetative state, and crop residues without grain (AGA, 2017). Harvested forages, such as hay and ensiled forages are also acceptable sources of feed during periods when fresh forages are inaccessible. However, many standards require that ruminant animals graze fresh pasture to acquire the majority of their feed. Mineral and vitamin supplementation are acceptable and encouraged. While these standards are a good outline for producers to follow, they also provide opportunity to use a diverse range of management practices to raise their cattle.

Cattle feed, general management practices, genetics, and harvest and processing methods are a few of the areas in which GFB production vary. In terms of forage and feed diversity, a survey of 149 producers of GFB in the U.S. found that producers relied on a range of different

forage types to feed their cattle. Producers ranked a cool season grass-clover mix as the most important in their production strategy, but perennial warm-season grasses, alfalfa, other legumes, and other forages were ranked second through fifth. “Other” responses included birdsfoot trefoil, lespedeza, vetch, peas, brassicas, sorghum-sudan grass, chicory, forbs, triticale, and more. These data indicate diversity as expected, with a range of growing conditions due to climate, but also represent the wide variation of practices within GFB production (Lozier et al., 2005). Within the GFB industry, health management practices are similar in terms of the absence of conventional beef production practices such as growth implants and feed additives. However, management practices such as the use of dewormers, fly and lice treatment, antibiotics for treatment of sick animals, and vaccinations varied (Lozier et al., 2005). Another survey of GFB producers in the Northeastern region of the United States reported that 48% of respondents did not use preventative vaccines on their farm (Steinberg & Comerford, 2009). Furthermore, the breed of beef animal raised varies between producers of GFB. Lozier et al. (2005) reported that producers were diverse in which breed of cattle they raised; while the most commonly raised were breeds like Angus and Hereford, 22 different breeds made up 44% of the responses. Steinberg and Comerford (2009) reported similar variability, with Black Angus the predominant breed being used by 29% of producers.

Production of GFB is typically more vertically integrated than conventionally raised beef; the majority of producers market the beef raised on their farm directly to consumers (Gillespie, 2016; Steinberg & Comerford, 2009). Thus, variation also occurs in harvest practices. Producers indicate that the most important factors influencing when an animal is harvested off pasture are body condition score and animal body weight, with the age of the animal less important. Consequently, the age of an animal at slaughter tends to vary (Lozier et al., 2005; Steinberg &

Comerford, 2009). Hanging of the whole beef carcass after harvest to age the product has fallen out of practice in large scale conventional beef production in order to streamline the process from feedlot to freezer. However, several surveys indicate that nearly all producers of GFB age their beef by this method for approximately two weeks before processing the meat (Steinberg & Comerford, 2009; Lozier et al. 2005).

While grass-finishing is a growing sector of the U.S. beef industry, the number of grass-finished cattle slaughtered each year is estimated to be approximately 232,000 of the 30.7 million total head of cattle slaughtered in 2016, or about 0.76% (Cheung et al., 2017; USDA, 2017). Conventionally finished beef yields a relatively consistent product, as cattle are raised in large groups and fed a carefully balanced diet. It is difficult for grass-fed beef producers to match the efficiency of grain-fed beef production. Supplying consistent, high levels of energy to the cattle through the production phase is challenging. Furthermore, because of the inherent inconsistency of cattle raised under the definition "grass-fed," the resulting beef produced varies widely. Thus, while there is an expanding body of research on the nutritional aspects of grass-fed beef, there is little understanding of how management practices as a whole ultimately affect the end product.

1.6. Grass-fed beef market

There is a growing consumer interest in GFB that is driven by perceived environmental and health benefits, as well as animal welfare concerns (Gwin et al., 2012; McCluskey et al., 2005; Umberger, et al., 2009). Proper grazing management can reduce soil erosion, improve soil organic carbon content, sequester atmospheric carbon dioxide, and enhance land biodiversity (Teague et al., 2016). Additionally, consumers perceive grass-finished beef as healthier than conventionally-fed beef. Studies consistently show that when slaughtered at comparable age, grass-finished beef is lower in fat than conventionally finished beef (Descalzo et al., 2005;

Duckett et al., 2013; Leheska et al., 2008). Moreover, consumers are willing to pay a premium price for grass-fed beef because of its perceived health benefits, specifically because of the lower fat content, as well as it being higher in *n*-3 fatty acids (Gwin et al., 2012; McCluskey et al., 2005).

Producers similarly indicate that human health, animal welfare, and the environment are primary reasons that they finish cattle on pasture (Steinberg & Comerford, 2009). In response to the growing consumer interest in grass-fed beef, producers are continually searching for innovations in production strategies that meet the needs of the consumer. The shift toward producing grass-fed beef is mainly centered in small-scale production, as seen by the number of grass-fed cattle marketed per farm in Table 1.3.

Table 1.3. Number of cattle marketed as grass-finished per farm.

Author, Publication Year	Mean	Median	*n*=
Gillespie, et al., 2016	40	16	384
Winrock International, 2012	22[1]	15	78
Steinberg & Comerford, 2009	25	-	26

[1]Reported as number of cattle marketed as grass-finished, while the average number of cattle actually finished on pasture was reported at 63.

Moreover, 96% of producers market their product directly to consumers (Gillespie, 2016). The recent development of grass-fed beef branded programs offers another path for producers to market their animals. These branded programs have existing infrastructure that allow them to process up to 500 cattle weekly and distribute products to the supply chain (Fisk, et al., 2012). This allows producers to capture some of the economies of scale that make large scale beef production efficient.

1.7. Fatty acid profile of beef

Meat and dairy products contribute the majority of SFA to the human diet (Kris-Etherton & Innis, 2007). Table 1.4 summarizes the fatty acid composition of beef finished in both GFB

and conventional finishing systems. In general, the fatty acid profile of beef is approximately 45% SFA, 50% MUFA, and <10% PUFA (Daley et al., 2010). Palmitic and stearic acid make up the majority of SFAs in beef, along with myristic acid. Oleic acid is the predominant MUFA in beef, with other MUFA such as myristoleic and palmitoleic making up a much smaller proportion. Polyunsaturated fats are present in low amounts, and include LA, ALA, ARA, DPA, and DHA (Rhee, 2000; Daley et al., 2010). A recent trend in research has been to investigate ways to alter the fatty acid profile in order to better fit dietary recommendations that suggest limiting saturated fat intake.

Table 1.4. Fatty acid composition of *longissimus thoracis* expressed in mg/100 g of beef.

Author, Publication Year, Finishing system	SFA	MUFA	PUFA	*n*-6	*n*-3	*n*-6:*n*-3
Chail, et al., 2016						
Grass	1466.0*	1207.0*	167.0	n/a	n/a	3.44*
Grain	2732.0*	2824.0*	204.0	n/a	n/a	5.74*
De la Fuente, et al., 2009						
Grass	511.7	487.6	142.7*	n/a	n/a	1.37*
Conventional	487.6	523.6	188.7*	n/a	n/a	14.84*
Ponnampalam, et al., 2006						
Grass	900.0*	930.0*	n/a	191.6	97.6*	1.96*
Conventional	1568.0*	1729.0*	n/a	253.8	63.3*	3.57*

*Indicates a significant difference ($P < 0.05$) was reported between beef from different finishing systems within the same study. "n/a" signifies that the value was not reported in the original study.

1.8. Factors influencing the fatty acid profile of beef

1.8.1. *Nutrition*

A variety of factors affect the FA profile of beef: age at slaughter, breed, and genetics, but perhaps most influential is the animal's diet. Consistent evidence shows that cattle fed a forage-only diet produce meat that differs greatly in its FA profile compared to cattle supplemented with grain; in a review of seven studies comparing beef from cattle fed these different diets, Daley et al. (2010) reported that grass-finished beef has a higher proportion of C18:0, and less C14:0 and C16:0, resulting in a healthier SFA profile, as well as a lower, more

desirable *n*-6:*n*-3 ratio. Furthermore, there is also emerging evidence that different species of forages can affect the FA profile. Duckett et al. (2013) found differences in the percentage of ALA in beef from cattle grazing three different forage species, but indicated no differences in other fatty acids. Beef from cattle grazed on birdsfoot trefoil showed a similar fatty acid composition to beef from cattle grazed on grasses of tall fescue and meadow brome, but had significantly greater amounts of the *n*-3 FA ALA and EPA (0.52 vs 0.27 mg/g tissue and 0.09 vs 0.07 mg/g tissue, respectively) (Chail et al., 2016). Other research has shown that feeding red clover silage versus grass silage results in meat with higher proportions of ALA and CLA in beef and lamb (Lee et al., 2009; Lourenço et al., 2007). While forages have high concentrations of PUFA, saturation of these fatty acids occurs through microbial biohydrogenation in the rumen, resulting in meat and milk products that have a higher SFA and lower PUFA concentration relative to the forages consumed and compared to meat from monogastric livestock. However, Lee et al. (2003) determined that the ruminal outflow of long chain PUFAs LA and ALA were greater for cattle fed red clover silage than those fed grass silage. This lower apparent biohydrogenation is hypothesized to be due to polyphenol oxidase content of red clover protecting lipids from degradation (Van Ranst et al., 2010).

1.8.2. *Level of fatness*

Another influential factor affecting the FA profile of beef is the level of fatness of the animal. PUFA are generally found in phospholipids (PL), while SFA and MUFA are mainly found in triacylglycerol (TG). Phospholipids are components of cell membranes and the content of PL in muscle is largely independent of fatness levels; conversely triacylglycerol is strongly correlated to total fat content. Thus, as fatness levels increase, the ratio of PUFA to SFA (P:S) decreases. In the same way, the LA:ALA ratio is affected by fatness, as LA is preferentially

deposited in PL, while ALA is deposited more equally between PL and TG. While the overall *n*-6:*n*-3 ratio is more dependent on diet than on fatness levels, for a given diet, the LA:ALA ratio will be higher for leaner beef (DeSmet et al., 2004).

1.8.3. *Age at harvest*

Similarly, age at slaughter influences the FA profile in beef. Warren et al. (2008a) indicated that as slaughter age increased from 14 to 24 mos., the P:S ratio declined. Duckett et al. (1993) similarly found that as time on feed and age of cattle increased, the proportion of MUFAs increased, while the proportion of PUFAs decreased. In a time-on-pasture study, Duckett et al., (2014) reported that the proportion of MUFA and SFA increased as cattle aged, while the proportion of PUFA declined from 9% to 5%. As cattle finished on grass are typically slaughtered at an older age than conventionally fed cattle (Leheska et al., 2008; Steinburg and Comerford, 2005), one strategy for producing beef with a more ideal FA profile would be to decrease the age at slaughter.

1.8.4. *Genetics*

In addition to age, Warren et al. (2008a) stated that breed of cattle also influences the nutrient composition, reporting differences in total lipid content, as well as percentages of SFA, MUFA, and PUFA between Aberdeen Angus and Holstein-Friesian cattle. De Smet et al. (2004) concluded that while the effect of genetics is much less than the more widely recognized role that nutrition plays, breed differences and genotypes are involved in synthesis and incorporation of fatty acids. The Japanese beef breed Wagyu and the Korean breed of Hanwoo cattle have risen in popularity, known for their highly marbled beef. These breeds of cattle produce beef with higher concentrations of MUFA than beef breeds traditionally raised in the United States (Gotoh & Joo,

2016). However, breed type differences are generally small, and Smith et al. (2009) argue that diet and time on feed are much more important factors in the composition of the beef FA profile.

1.8.5. *Region, climate, and season*

There are inherent differences in production strategies from region to region, such as longer grazing seasons in the southern U.S. and variation in forage species available to graze (Mathews Jr & Johnson, 2011). Northern regions are more suitable for cool season forages. Warm season forages flourish in the southern climate during the summer, but during the winter, cool season forages are more productive. Slaughter season can also impact the nutritional composition of beef. Sobczuk-Szul et al. (2013) noted that cattle harvested in the summer in Poland had greater concentrations of LA, CLA, ARA and DPA than those harvested in the winter, likely due to finishing the cattle on fresh and harvested forages respectively. Pestana et al. (2012) found that beef harvested in the spring and fall in Portugal from cattle grazing the same forages showed variations in the FA profile. The spring harvested beef contained greater amounts of stearic acid, ALA, and overall *n*-3 FA, and the autumn samples had more palmitic acid, DHA, and more overall *n*-6 FA. Additionally, the beef harvested in the spring had a lower *n*-6:*n*-3 ratio. These differences were attributed to more abundant growth of forage in the spring pastures.

1.8.6. *Summary: Impact of variable production strategies*

Because of the range in production practices of grass-finished beef, a standard nutritional label does not necessarily reflect the variability of the finished product. These emerging fields of study within grass-finished beef allow for greater exploration into how management practices within grass-fed beef production affect the nutritional profile of beef.

1.9. Other nutritional aspects of beef

1.9.1. *Beta-carotene*

β-carotene is a precursor to the fat-soluble vitamin A. It functions as an antioxidant, blocking the action of reactive oxygen species, which can damage cells (Johnson and Russell, 2010). The primary action of β-carotene in humans is the conversion to vitamin A, which is important for vision, immune function, and reproduction (Ross, 2010). Numerous studies have found that grass-finished beef has a higher content of β-carotene than grain-finished beef, as summarized in Table 1.5. This is thought to be due to the high β-carotene content in fresh grass compared to grain, which also lends to the yellow colored fat found in grass-finished cattle (Daley et al., 2010). Forage species also influences β-carotene levels in beef. Duckett et al. (2013) found that beef from steers finished on mixed pasture had a higher β-carotene content than those finished on alfalfa or pearl millet. Simonne et al. (1996) hypothesized that the differing β-carotene content within grass-finished beef is due to the variation of β-carotene in different forage species.

Table 1.5. β-carotene levels in beef *longissimus thoracis* expressed in µg/100 g of beef.

	Finishing System	
Author, Publication Year	**Grass**	**Conventional**
Duckett, et al., 2013	57.8	5.7
Descalzo, et al., 2007	45.0	6.0
Yang, et al., 2002a	16.0	1.0

Harvest method also affects the β-carotene content of forages; maturity of forages at harvest, as well as storage conditions and time in storage can influence the amount of β-carotene (Preston, 2008). Noziére et al. (2006) found that milk from cows fed grass silage had a higher β-carotene levels than milk from cows fed dry hay.

1.9.2. *Vitamin E and alpha-tocopherol*

Vitamin E is an antioxidant that helps to prevent against damage by free radicals in the

body. Evidence shows that oxidation of LDL in the body may play a role in the development of atherosclerosis and heart disease (Regnstrom, 1992). There is an association between vitamin E supplementation and reduced risk of CHD in both men and women (Rimm et al., 1993; Stampfer et al., 1993). Table 1.6 presents a summary of recent studies measuring α-tocopherol content of GFB and conventionally finished beef. Grass-fed beef contains greater amounts of vitamin E and α-tocopherol than grain-finished beef. Thus, it is of interest to understand the effects of management on the accumulation of vitamin E in beef.

Nutrition is the largest determinant of α-tocopherol content in beef. As stated, cattle on diets consisting only of forages yield beef with greater amounts of α-tocopherol than those consuming grain-based diets. This is likely due to the higher vitamin E content of fresh pasture. Faustman et al. (1998) suggest that if adequate vitamin E exists in the diet to reach the threshold level in muscle, supplementation will have no effect. Additionally, the authors propose that high quality green forage may provide this quantity of vitamin E. This is supported by Yang et al. (2002a), who noted that supplementation of vitamin E in cattle's diet increased the α-tocopherol content of beef in cattle fed grain based diets, while beef from cattle consuming fresh grass did not show a response to supplementation.

Table 1.6. α-tocopherol levels in beef *longissimus thoracis* expressed in µg/100 g of beef.

	Finishing System	
Author, Publication Year	**Grass**	**Conventional**
Duckett, et al., 2013	343	140
De la Fuente, et al., 2009	375	75
Descalzo, et al., 2007	308	150
Yang, et al., 2002a	450	180

However, while there are differences in α-tocopherol content between beef from grain- and grass-finished cattle, there is little evidence of forage species effecting the vitamin E content of beef. In a study by Duckett et al. (2013), where cattle were grazed on different three different

forages (mixed pasture, pearl millet, and alfalfa), no difference was found in the α-tocopherol content of the beef. Moreover, there is little evidence suggesting that the breed of cattle affects vitamin E levels. Warren et al. (2008b) found that while the vitamin E content of plasma was higher in Angus cattle compared to Holstein, there was no difference in the muscle content.

Vitamin E can delay and reduce lipid oxidation in meat, extending its shelf life. Grass-finished beef has higher lipid stability and color stability due to the higher amounts of antioxidants (Insani et al., 2008; Realini et al., 2004). These qualities are important as grass-finished beef can be more susceptible to lipid oxidation due to the higher concentration of unsaturated fatty acids (Yang et al., 2002b).

1.9.3. *Minerals*

In addition to being a source of essential fatty acids and vitamins, beef is also a valuable source of minerals in the human diet. Red meat consumed at even low levels in the diet is enough to meet the nutritional requirements of iron and zinc (McAfee et al., 2010).

Table 1.7. Mineral content of *longissimus thoracis* expressed per 100 g of muscle of GFB.

Mineral	Duckett, et al., 2013	Duckett, et al., 2009	Leheska, et al., 2008
Phosphorus, mg	--	--	211.9
Potassium, mg	--	306.6	342.4
Sodium, mg	38.6	172.2	55.0
Magnesium, mg	--	21.1	23.1
Iron, mg	1.7	1.7	1.9
Zinc, mg	3.3	4.1	3.6
Copper, µg	56.0	--	70.0
Selenium, µg	--	--	21.2

Table 1.7 presents a summary of mineral content of grass-finished beef. Williams et al. (1983) found greater concentrations of Zn, P, Mg, and K in GFB than in conventionally-finished beef. However, Duckett et. al (2009; 2013) found no differences in mineral content between finishing systems. Grass-finished beef varies significantly in its mineral composition (Leheska et al., 2008), likely because the mineral content of the forages they consume is largely dependent

on the mineral status of the soil on which it is grown (Preston, 2008). Additionally, Duckett et al. (1993) found that time on feed increased the content of iron and potassium in beef, seemingly due to the increased fat content of the beef. In another study, Duckett et al. (2013) found no difference in the mineral composition of beef from cattle grazed on different forage diets of pearl millet, alfalfa, and mixed pasture. However, there is little information on how supplementation of minerals in cattle's diets influences the nutritional content of beef.

1.10. Conclusion

This review has summarized the many factors that can impact the nutritional profile of beef. While it is clear that there are numerous differences between conventionally finished and grass finished beef, the variability of the nutritional qualities of GFB has not been fully investigated. Leheska et al. (2008) endeavored to determine a standard nutrient composition of GFB through a survey of 15 producers across the U.S., but still found significant variation in mineral content. Furthermore, many studies investigating the fatty acid profile of beef report values as a percent composition. While this allows for easy comparison between studies, it does not accurately give an idea of the true variation, or an understanding of fatty acids on a per-serving basis.

Consumer interest in the source of their food continues to rise. As the GFB industry grows in response to consumer demand, there is great potential for more variation in the product marketed. Much of this variation can be attributed to the industry being based in small-scale production, with wide-ranging production methods. However, the growth of the industry has ushered in the participation of cooperative groups and branded programs, with larger-scale production and more standardized management practices. Further research is necessary to better

understand the variability of production methods and nutritional composition to give consumers an accurate understanding of the food they are purchasing.

Consumers show a willingness to pay a premium price for labels that purport health benefits, and health recommendations change year after year as the understanding of diet and human health improves. Therefore, identifying the variation in the grass-finishing industry is imperative to understanding the potential of GFB in the market.

LITERATURE CITED

LITERATURE CITED

AGA. (2017). Grassfed Ruminant Standards v1.3. American Grassfed Association.

Calder, P. C. (2008). Polyunsaturated fatty acids, inflammatory processes and inflammatory bowel diseases. *Molecular nutrition & food research*, 52(8), 885-897.

Chail, A., Legako, J. F., Pitcher, L. R., Griggs, T. C., Ward, R. E., Martini, S., & MacAdam, J. W. (2016). Legume finishing provides beef with positive human dietary fatty acid ratios and consumer preference comparable with grain-finished beef. *Journal of Animal Science, 94*(5), 2184-2197.

Cheung, R., McMahon, P., Norell, E., Kissel, R., and Benz, D. (2017). Back to grass: the market potential for grassfed beef. Retrieved from: https://www.stonebarnscenter.org/wp-content/uploads/2017/10/Grassfed_Full_v2.pdf

Daley, C. A., Abbott, A., Doyle, P. S., Nader, G. A., & Larson, S. (2010). A review of fatty acid profiles and antioxidant content in grass-fed and grain-fed beef. *Nutrition Journal, 9*(10), 1-12.

Degirolamo, C., & Rudel, L. L. (2010). Dietary monounsaturated fatty acids appear not to provide cardioprotection. *Current atherosclerosis reports*, *12*(6), 391-396.

De la Fuente, J., Diaz, M. T., Alvarez, I., Oliver, M. A., Font i Furnols, M., Sanudo, C., . . . Caneque, V. (2009). Fatty acid and vitamin E composition of intramuscular fat in cattle reared in different production systems. *Meat Science, 82*(3), 331-337.

Descalzo, A., Insani, E. M., Biolatto, A., Sancho, A. M., Garcia, P. T., & Pensel, N. A. (2005). Influence of pasture or grain-based diets supplemented with vitamin E on antioxidant/oxidative balance of Argentine beef. *Meat Science*, *70*(1), 35-44.

Descalzo, A. M., Rossetti, L., Grigioni, G., Irurueta, M., Sancho, A. M., Carrete, J., & Pensel, N. A. (2007). Antioxidant status and odour profile in fresh beef from pasture or grain-fed cattle. *Meat Science, 75*(2), 299-307.

DeSmet, S., Raes, K., & Demeyer, D. (2004). Meat fatty acid composition as affected by fatness and genetic factors: A review. *Animal Research, 53*(2)*,* 81-98.

Duckett, S. K., Wagner, D. G., Yates, L. D., Dolezal, H. G., & May, S. G. (1993). Effects of time on feed on beef nutrient composition. *Journal of Animal Science, 71*(8), 2079-2088.

Duckett, S. K., Neel, J. P., Fontenot, J. P., & Clapham, W. M. (2009). Effects of winter stocker growth rate and finishing system on: III. Tissue proximate, fatty acid, vitamin, and cholesterol content. *Journal of Animal Science, 87*(9), 2961-2970.

Duckett, S. K., Neel, J. P., Lewis, R. M., Fontenot, J. P., & Clapham, W. M. (2013). Effects of forage species or concentrate finishing on animal performance, carcass and meat quality. *Journal of Animal Science, 91*(3), 1454-1467.

Duckett, S. K., Fernandez Rosso, C., Volpi Lagreca, G., Miller, M. C., Neel, J. P., Lewis, R. M., . . . Fontenot, J. P. (2014). Effect of frame size and time-on-pasture on steer performance, longissimus muscle fatty acid composition, and tenderness in a forage-finishing system. *Journal of Animal Science, 92*(10), 4767-4774.

Faustman, C., Chan, W. K., Schaefer, D. M., & Havens, A. (1998). Beef color update: the role for vitamin E. *Journal of Animal Science, 76*(4), 1019-1026.

Fisk, J., Williams, A., & King, W. (2012). Expanding grass-based animal agriculture in the Midwest: The pasture project (pp. 14-15, Rep.). Arlington, VA: Winrock International. Retrieved from: http://www.wallacecenter.org/resourcelibrary/expanding-grass-based-animal-agriculture-in-the-midwest.html

Gillespie, J., Sitienei, I., Bhandari, B., Scaglia, G. (2016). Grass-fed beef: How is it marketed by US producers? *International Food and Agribusiness Management Review, 19*(2), 171-188.

Gotoh, T., & Joo, S.-T. (2016). Characteristics and health benefit of highly marbled Wagyu and Hanwoo beef. *Korean Journal for Food Science of Animal Resources, 36*(6), 709-718.

Grande, F., Anderson, J. T., & Keys, A. (1970). Comparison of effects of palmitic and stearic acids in the diet on serum cholesterol in man. *The American Journal of Clinical Nutrition, 23*(9), 1184-1193.

Gropper, S. S., Jack L. (2013). *Advanced nutrition and human metabolism* (Sixth ed.). Wadsworth, Belmont, California.

Gwin, L., Durham, C. A., Miller, J. D., & Colonna, A. (2012). Understanding markets for grass-fed beef: taste, price, and purchase preferences. *Journal of Food Distribution Research, 43*(2), 91-111.

Harfoot C.G., & Hazlewood G.P. (1997) Lipid metabolism in the rumen, pp. 382-426. In: Hobson, P.N., Stewart, C.S. (eds.) The Rumen Microbial Ecosystem. Chapman and Hall, London, UK.

Hodson, L. S., C. M.; Chisholm, W-A. H. (2001). The effect of replacing dietary saturated fat with polyunsaturated or monounsaturated fat on plasma lipids in free-living young adults. *European Journal of Clinical Nutrition, 55*(10), 908-915.

Hu, F. B., Stampfer, M. J., Manson, J. E., Rimm, E., Colditz, G. A., Rosner, B. A., . . . Willett, W. C. (1997). Dietary fat intake and the risk of coronary heart disease in women. New *England Journal of Medicine, 337*, 1491-1499.

IARC. (2015). Monographs evaluate consumption of red meat and processed meat. International Agency for Research on Cancer, Press release No. 240. World Health Organization. Retrieved from: https://www.iarc.fr/en/media-centre/pr/2015/pdfs/pr240_E.pdf

Insani, E. M., Eyherabide, A., Grigioni, G., Sancho, A. M., Pensel, N. A., & Descalzo, A. M. (2008). Oxidative stability and its relationship with natural antioxidants during refrigerated retail display of beef produced in Argentina. *Meat Science, 79*(3), 444-452.

Jackson, R. L., Taunton, O. D., Morrisett, J. D., & Gotto, A. M. (1978). The role of dietary polyunsaturated fat in lowering blood cholesterol in man. *Circulation research, 42*(4), 447-453.

Jenkins, T. C., Wallace, R. J., Moate, P. J., & Mosley, E. E. (2008). BOARD-INVITED REVIEW: Recent advances in biohydrogenation of unsaturated fatty acids within the rumen microbial ecosystem1. *Journal of Animal Science, 86*(2), 397-412.

Johnson, E. J. & Russell, R. M. (2010). Beta-Carotene, pp. 115-120 In: Coates PM, Betz JM, Blackman MR, et al., (eds.), Encyclopedia of Dietary Supplements. 2nd ed. Informa Healthcare, London and New York.

Kremmyda, L.-S., Tvrzicka, E., Stankova, B., & Ales, Z. (2011). Fatty acids as biocompounds: their role in human metabolism, health and disease - a review. Part 2: fatty acid physiological roles and applications in human health and disease. *Biomedical papers, 155*(3), 195-218.

Kris-Etherton, P. M., Harris, W. S., Appel, L. J., & Nutrition, C. (2003). Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Arteriosclerosis, Thrombosis, and Vascular Biology, 23*(2), e20-30.

Kris-Etherton, P. M., & Innis, S. (2007). Position of the American Dietetic Association and Dietitians of Canada: Dietary fatty acids. *Journal of the American Dietetic Association, 107*(9), 1599-1611.

Lee, M. R. F., Harris, L. J., Dewhurst, R. J., Merry, R. J., & Scollan, N. D. (2003). The effect of clover silages on long chain fatty acid rumen transformations and digestion in beef steers. *Journal of Animal Science, 76*(3), 491-501.

Lee, M. R. F., Evans, P. R., Nute, G. R., Richardson, R. I., & Scollan, N. D. (2009). A comparison between red clover silage and grass silage feeding on fatty acid composition, meat stability and sensory quality of the M. Longissimus muscle of dairy cull cows. *Meat Science, 81*(4), 738-744.

Leheska, J. M., Thompson, L. D., Howe, J. C., Hentges, E., Boyce, J., Brooks, J. C., . . . Miller, M. F. (2008). Effects of conventional and grass-feeding systems on the nutrient composition of beef. *Journal of Animal Science, 86*(12), 3575-3585.

Lourenço, M., De Smet, S., Raes, K., & Fievez, V. (2007). Effect of botanical composition of silages on rumen fatty acid metabolism and fatty acid composition in longissimus muscle and subcutaneous fat of lambs. *animal, 1*(6), 911-921.

Lozier, J., Rayburn, E., & Shaw, J. (2005). Growing and selling pasture-finished beef: Results of a nationwide survey. *Journal of Sustainable Agriculture, 25*(2), 93-112.

Mathews Jr, K. H., & Johnson, R. J. (2011). Cattle sector production practices and regional price differences. *Journal of Current Issues in Globalization, 5*(4), 383.

McAfee, A. J., McSorley, E. M., Cuskelly, G. J., Moss, B. W., Wallace, J. M. W., Bonham, M. P., & Fearon, A. M. (2010). Red meat consumption: An overview of the risks and benefits. *Meat Science, 84*(1), 1-13.

McCluskey, J. J., Wahl, T. I., Quan, L., & Wandschneider, P. R. (2005). U.S. grass-fed beef: Marketing health benefits. *Journal of Food Distribution Research, 36*(3), 1-8.

Mozaffarian, D., Micha, R., & Wallace, S. (2010). Effects on coronary heart disease of increasing polyunsaturated fat in place of saturated fat: A systematic review and meta-analysis of randomized controlled trials. *PLoS Medicine, 7*(3), 1-10.

Mozaffarian, D., & Ludwig, D. S. (2015). The 2015 us dietary guidelines: Lifting the ban on total dietary fat. *Journal of the American Medical Association, 313*(24), 2421-2422.

Nozière, P., Grolier, P., Durand, D., Ferlay, A., Pradel, P., & Martin, B. (2006). Variations in carotenoids, fat-soluble micronutrients, and color in cows' plasma and milk following changes in forage and feeding level. *Journal of Dairy Science, 89*(7), 2634-2648.

Pestana, J. M., Costa, A. S. H., Martins, S. V., Alfaia, C. M., Alves, S. P., Lopes, P. A., . . . Prates, J. A. M. (2012). Effect of slaughter season and muscle type on the fatty acid composition, including conjugated linoleic acid isomers, and nutritional value of intramuscular fat in organic beef. *Journal of the Science of Food and Agriculture, 92*(12), 2428-2435.

Ponnampalam, E. N., Mann, N. J., & Sinclair, A. J. (2006). Effect of feeding systems on omega-3 fatty acids, conjugated linoleic acid and trans fatty acids in Australian beef cuts, potential impact on human health. *Asia Pacific Journal of Clinical Nutrition, 15*(1), 21-29.

Preston, R. L. (2008). Typical composition of feeds for cattle and sheep (Vol. 44, pp. 76). Minneapolis: Penton Media, Inc.

Realini, C. E., Duckett, S. K., Brito, G. W., Rizza, M. D., & De Mattos, D. (2004). Effect of pasture vs. concentrate feeding with or without antioxidants on carcass characteristics, fatty acid composition, and quality of Uruguayan beef. *Meat Science, 66*(3), 567-577.

Regnstrom, J., Nilsson, J., Tornvall, P., Hamsten, A., & Landou, C. (1992). Susceptibility to low-density lipoprotein oxidation and coronary atherosclerosis in man. *The Lancet, 339*(8803), 1183-1186.

Rhee, K. S. (2000). Fatty acids in meat and meat products, pp. 83–108. In: Chow, C. K. (ed.), Fatty Acids in Foods and Their Health Implications. Marcel Dekker, Inc., New York.

Rimm, E. B., Stampfer, M. J., Ascherio, A., Giovannucci, E., Colditz, G. A., & Willett, W. C. (1993). Vitamin E consumption and the risk of coronary heart disease in men. *New England Journal of Medicine, 328*(20), 1450-1456.

Ross, A. C. (2010). Vitamin A, pp. 778-791. In: Coates PM, Betz JM, Blackman MR, et al., (eds.), Encyclopedia of Dietary Supplements. 2nd ed. Informa Healthcare, London and New York.

Scollan, N., Hocquette, J.-F., Nuernberg, K., Dannenberger, D., Richardson, I., & Moloney, A. (2006). Innovations in beef production systems that enhance the nutritional and health value of beef lipids and their relationship with meat quality. *Meat Science, 74*(1), 17-33.

Shingfield, K., Bonnet, M., & Scollan, N. (2013). Recent developments in altering the fatty acid composition of ruminant-derived foods. *animal, 7*(Suppl. 1), 132-162.

Simonne, A. H., Green, N. R., & Bransby, D. I. (1996). Consumer acceptability and beta-carotene content of beef as related to cattle finishing diets. *Journal of Food Science, 61*(6), 1254-1257.

Simopoulos, A. (1991). Omega-3 fatty acids in health and disease and in growth and development. *American Journal of Clinical Nutrition, 54*(3), 438-463.

Simopoulos, A. P. (2002). The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomedicine & Pharmacotherapy, 56*(8), 365-379.

Sobczuk-Szul, M., Wroński, M., Wielgosz-Groth, Z., Mochol, M., Rzemieniewski, A., Nogalski, Z., . . . Purwin, C. (2013). The effect of slaughter season on the Fatty Acid profile in four types of fat deposits in crossbred beef bulls. *Asian-Australasian Journal of Animal Sciences, 26*(2), 275-281.

Stampfer, M. J., Hennekens, C. H., Manson, J. E., Colditz, G. A., Rosner, B., & Willett, W. C. (1993). Vitamin E consumption and the risk of coronary disease in women. *New England Journal of Medicine,* 328, 1444-1449.

Steinberg, E. L., & Comerford, J. W. (2009). Case study: A survey of pasture-finished beef producers in the northeastern United States. *The Professional Animal Scientist, 25*(1), 104-108.

Teague, W. R., Apfelbaum, S., Lal, R., Kreuter, U. P., Rowntree, J., Davies, C. A., . . . Byck, P. (2016). The role of ruminants in reducing agriculture's carbon footprint in North America. *Journal of Soil and Water Conservation, 71*(2), 156-164.

Umberger, W. J., Boxall, P. C., & Lacy, R. C. (2009). Role of credence and health information in determining US consumers' willingness-to-pay for grass-finished beef. Australian *Journal of Agricultural and Resource Economics, 53*(4), 603-623.

USDA, AMS. (2016a). Withdrawal of United States Standards for Livestock and Meat Marketing Claims. Retrieved from: https://www.federalregister.gov/documents/2016/01/12/2016-00440/withdrawal-of-united-states-standards-for-livestock-and-meat-marketing-claims#h-8.

USDA, ARS. (2016b). USDA Nutrient Database for Standard Reference, Release 28. Nutrient Data Laboratory. Retrieved from: http://www.ars.usda.gov/ba/bhnrc/ndl

USDA, NASS. (2017). Livestock slaughter; 2016 summary. Retrieved from: http://usda.mannlib.cornell.edu/usda/current/LiveSlauSu/LiveSlauSu-04-19-2017.pdf

USDHHS & USDA (2015). 2015-2020 Dietary guidelines for Americans. 8th Edition. Retrieved from: https://health.gov/dietaryguidelines/2015/guidelines/

Van Ranst, G., Lee, M. R. F., & Fievez, V. (2010). Red clover polyphenol oxidase and lipid metabolism. *animal, 5*(4), 512-521.

Warren, H. E., Scollan, N. D., Enser, M., Hughes, S. I., Richardson, R. I., & Wood, J. D. (2008a). Effects of breed and a concentrate or grass silage diet on beef quality in cattle of 3 ages. I: Animal performance, carcass quality and muscle fatty acid composition. *Meat Science, 78*(3), 256-269.

Warren, H. E., Scollan, N. D., Nute, G. R., Hughes, S. I., Wood, J. D., & Richardson, R. I. (2008b). Effects of breed and a concentrate or grass silage diet on beef quality in cattle of 3 ages. II: Meat stability and flavour. *Meat Science, 78*(3), 270-278.

Whelan, J., & Rust, C. (2006). Innovative dietary sources of n-3 fatty acids. *Annual Review of Nutrition, 26*(1), 75-103.

Williams, J. E., Wagner, D. G., Walters, L. E., Horn, G. W., Waller, G. R., Sims, P. L., & Guenther, J. J. (1983). Effect of production systems on performance, body composition and lipid and mineral profiles of soft tissue in cattle. *Journal of Animal Science, 57*(4), 1020.

Williams, P. G. (2007). Nutritional composition of red meat. *Nutrition & Dietetics, 64*(Suppl 4), S113-S119.

Williamson, C. S., Foster, R. K., Stanner, S. A., & Buttriss, J. L. (2005). Red meat in the diet. *Nutrition Bulletin, 30*(4), 323-355.

Yang, A., Brewster, M. J., Lanari, M. C., & Tume, R. K. (2002a). Effect of vitamin E supplementation on α-tocopherol and β-carotene concentrations in tissues from pasture- and grain-fed cattle. *Meat Science, 60*(1), 35-40.

Yang, A., Lanari, M. C., Brewster, M., & Tume, R. K. (2002b). Lipid stability and meat colour of beef from pasture- and grain-fed cattle with or without vitamin E supplement. *Meat Science, 60*(1), 41-50.

CHAPTER 2

A NUTRITIONAL SURVEY OF COMMERCIALLY AVAILABLE GRASS-FINISHED BEEF

2.1. 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 stimulating the popularity of GFB, including perceptions surrounding its healthfulness, environmental impact, and animal welfare. Regarding the nutritional qualities of GFB, numerous studies have outlined the differences between GFB and conventionally-finished beef (CFB), indicating that GFB has higher proportions of nutrients potentially 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 has been limited to controlled research trials. Although 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 who has only consumed forages from birth to harvest. Producers use a wide range of management strategies to raise and market cattle under a GFB label. Thus, we expect to find variation in the nutritional composition of GFB. 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) Confusion can arise regarding the distinction between these similar labels. There is a need for clear understanding of the nutritional qualities of the product sold to consumers today associated with the grass-fed label. Thus, 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 a product that consumers are willing to pay a premium for (Umberger et al., 2009; McCluskey et al., 2005). To do this, a

nationwide survey of beef producers, all who market cattle under a GFB label, was conducted to tie production practice to the fatty acid (FA), mineral, and fat-soluble vitamin content of the commercially available GFB produced.

2.2. Materials and Methods

2.2.1. *Sample collection*

A number of GFB producers nationwide were identified to participate in a confidential survey regarding their production methods, in addition to submitting samples of beef for nutritional analysis. Beef samples were collected from ten states and represented a broad area across the United States. Two sample collection periods were established; Fall (September 2016 to February 2017), and Spring (June to August 2017). The sampling periods served to account for the geographical and climatic variability across the regions where beef was sampled. One set of fall samples were submitted past the targeted end date for sample collection and were processed with the spring samples, but included in the fall results due to harvest date. Over the two sampling periods, samples (n = 750) were collected in fall (n = 390) and spring (n = 360). We excluded any producers that submitted less than 7 samples from the statistical analysis, resulting in a sample size of 385 for fall and 355 for spring. Samples were collected at fabrication, and carcass hanging times varied (24-96 hrs). A collection protocol was sent to all processors participating in the study. We requested two 56 g samples per animal, cut from the anterior portion of the strip loin (IMPS/NAMP 180 Beef Loin, Strip Loin). Samples were individually bagged, labeled with date, producer, and slaughter facility, frozen and shipped once all samples were collected. The time samples spent in freezer storage averaged 31 d before shipping and freezer temperatures were not disclosed by the processors. Samples were packed in an insulated container on dry ice or ice packs, shipped overnight, and verified frozen on arrival.

Samples were stored at -30°C for 24 h, then transferred to a -80°C freezer and stored until sample analysis occurred.

2.2.2. *Survey*

In order to gain a greater understanding of the management and processing associated with the collected beef samples, a 27 and 5 question confidential survey was developed for participating producers and processors, respectively (see Appendix). The surveys were administered in an online format using Qualtrics Version 2016 (Provo, UT, USA), and emailed to participants (n = 17) 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. From a processing perspective, aging, capacity and other processing strategies were surveyed. From the original producers, 71% (n = 12) provided greater than seven samples for project inclusion. From these twelve producers, we had a 75% response rate to the survey (n = 9). The producers partnered with processors (n = 8) across the United States to harvest cattle. Of the eight processors, 63% (n = 5) responded to the survey.

2.2.3. *Fatty acid analysis*

2.2.3.1. *Reagents and standards*

Analytical-grade reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA). 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, USA). All other FA standard curves were created using Supelco 37 Component FAME Mix (Sigma-Aldrich; St. Louis, MO).

2.2.3.2. *Microwave assisted extraction*

A modified version of the microwave assisted extraction (MAE) method 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, USA). Strip loin samples were transferred to a freezer at -20°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 (BHT) was added to the vessels, and FAs were extracted using the following microwave parameters: 55°C for 15 mins with initial ramp of 2 mins at 400W maximum power. Vessel contents were then filtered using Whatman lipid free filters (Weber Scientific; Hamilton, NJ, USA) into a test tube containing 3.5 mL HPLC water. Samples were centrifuged at 2500 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, USA) at 40°C to obtain extracted FAs.

2.2.3.3. *Fatty acid methylation*

Fatty acids were methylated using a modified version of Ichihara and Fukubyashi (2010). Two mg of oil were transferred into a test tube containing internal standard. After this, 500 µL toluene and 1 mL 1.09M methanolic HCl was added. Test tubes were purged with nitrogen, capped using Teflon lined caps, and sealed with parafilm. Samples were heated at 100°C for 1.5 h in heating blocks. Samples were removed from heat and 2 mL of saturated sodium bicarbonate solution and 2 mL hexane were added and then centrifuged. The upper organic phase was

removed to a new test tube and dried to obtain fatty acid methyl esters (FAMEs). The FAMEs were suspended in isooctane and transferred to GC vials containing glass inserts. Samples were stored at -20°C until GC analysis.

2.2.3.4. *Gas chromatography-mass spectrometry analysis*

The Perkin Elmer (Waltham, MA, USA) 680/600S gas chromatography-mass spectrometer (GC-MS) was used for analysis. An Agilent Technologies (Santa Clara, CA, USA) DB-23, 30-m column was used for FAME quantification. The GC temperature parameters were as followed: initial temperature at 100°C for 0.5 min; ramp 7.0°C/min to 245°C; hold 2 min. A standard curve was created using Supelco 37 FAME mix. Due to its abundance in the beef samples, a second curve was created with a higher concentration of oleic acid methyl ester. Additionally, FAMEs of CLA and DPA were included in this curve due to their absence in the FAME mix. Seven point curves were created for all standards with duplicate injection. Data analysis was conducted using MassLynx V4.1 SCN 714 (Waters Corporation; Milford, MA, USA). Concentrations were normalized based on starting beef sample weight.

2.2.4. ***Mineral analysis***

Beef samples were sectioned; a 2 g sample was dried overnight in a 75° C oven to determine the dried sample weight to calculate the dry matter fraction, and a 1 g sample digested overnight in an oven at 95° C with 2 mL of nitric acid. The digested samples were diluted with water to approximately 100 times the dried tissue mass. Analysis was conducted following the method of Wahlen et al. (2005) using an Agilent 7900 Inductively Coupled Plasma - Mass Spectrometer (Agilent Technologies Inc., Santa Clara, CA, USA). 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, USA) and bovine

muscle standards used as a control (National Institute of Standards and Technology, Gaithersburg, MD, USA).

2.2.5. *Alpha-tocopherol and beta-carotene analysis*

The antioxidants β-carotene and α-tocopherol were analyzed based on the method of Rettenmaier et al., (1992). Briefly, 0.5 g tissue samples were mechanically homogenized in 2 mL of water, then frozen to aid in the lysing of cells. After thawing, ethanol was added to an aliquot of the solution to precipitate proteins. Hexane was added to extract the vitamins, and vortexed and centrifuged to separate and remove the hexane layer. 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 sampler vials. A five-point calibration curve was created using β-carotene and α-tocopherol standards (Sigma-Aldrich; St. Louis, MO) 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, Milford, MA). 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, 2.1x50 mm analytical column (Sigma-Aldrich; St. Louis, MO). The flow rate was 0.5 mL/min and detection was by UV absorption at 450 nm for β-carotene and 292 nm for α-tocopherol. Peak integration was done by the ApexTrack method of Empower Pro 5.

2.2.6. *Statistical analysis*

Statistical analysis was conducted using Prism v7.0d for Mac OS X (GraphPad Software., La Jolla, CA, USA). Fall and spring data was analyzed with an unpaired t-test, with a ROUT outlier test removing outliers at Q = 1%. Analysis of seasonal differences for individual producers was conducted using Kruskal-Wallis and uncorrected Dunn's test for preplanned

pairwise comparisons with outliers removed as stated previously. Producer comparisons were made using Kruskal-Wallis, correcting for multiple comparisons by controlling the false discovery rate and reported with a q-value. Spearman correlation was computed using R v3.3.3.

All fatty acid values expressed are in mg/100g beef tissue. Macrominerals are expressed in mg/100g tissue, while microminerals and antioxidants are expressed in μg/100g tissue.

2.3. Results and Discussion

2.3.1. *Survey*

2.3.1.1. *Production methods*

There was great variation in the size of farms that participated in the survey. The number of cattle marketed annually by respondent ranged from 25-5000 cattle (mean = 942, median = 600; Table 2.1). This is considerably greater than Gillespie et al. (2016) and Steinberg et al. (2009) who indicated survey respondents annually marketed a mean of 40 and 25 hd, respectively. 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 sample varying in production capacity and identified branded programs, cooperatives, and even small producers with an established criterion of >7 samples necessary for study participation. The mean cattle age at harvest was 26.8 ± 2.3 mo. This number was higher than expected and surpassed those reported by Steinberg and Comerford (2009) and Lozier et al. (2005) who reported mean cattle slaughter ages of 20.7 ± 4.7 and 20.8 ± 6.8 mo, respectively. As expected, survey respondents indicated their breed of cattle as Angus or Angus cross, with one producer listing "British."

Finishing diets (defined as the diet cattle were fed for the last 60 days of finishing) for fall and spring are listed in Table 2.1. A wide variety of finishing strategies were indicated, with

some producers relying solely on perennial pastures, while others finished 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. With this, there is a broader description by label of defining grass-fed. As an 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 promotants were excluded from use by all producers. Similarly, Lozier et al. (2005) indicated that 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. Gillespie et al. (2016) indicated that 97% of respondents would classify their beef as "hormone-free" and 93% as "antibiotic-free."

2.3.1.2. *Processing methods*

Weekly processing capacity for the facilities ranged from 25-1000 head of cattle (data not shown). None of the respondents indicated they implemented the technique of dry-aging the beef. This is in contrast to surveys done by Steinberg and Comerford (2009) and Lozier et al. (2005) who indicated that the practice of dry aging beef for two weeks was nearly universal amongst GFB producers. This may be attributed to the size of the processing facilities - due to the relatively large scale of production, and aging carcasses for two weeks may not be practical. Only one processor indicated the use of electrical stimulation of the carcass during processing. Three processors reported marketing their beef to restaurants, distributors, and grocery stores, while two indicated they sold directly to consumers or to health food stores.

Table 2.1. Producer-reported data on farm capacity, age of cattle, and finishing diets.

Producer	# of cattle marketed annually	Age at harvest (mos)	Sample size (n =)	Fall finishing diet	Spring finishing diet
1	300	NA[1]	25	Perennial pasture	--
2	600	23-24 (f)[2] 26 (sp)	75	BMR[3] 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	--
4	800	23-24	107	Summer annuals & warm season perennial pasture, plus cool season baleage *OR* cool season annuals & 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

[1]NA indicates producer did not disclose information
[2]Producer 2 indicated a difference in age between cattle harvested in the fall (f) vs spring (sp)
[3]BMR: Brown midrib

2.3.2. *Fatty acids*

Table 2.2. Fatty acid content of grass-finished beef.

Fatty Acid (mg/100g)	Mean	SEM	Min	Max
Total Lipid (g/100g)	2.95	0.03	1.66	6.24
Total FA	723	17.7	84.4	3610
SFA	321	8.23	29.4	1790
C14:0	15.1	0.51	1.13	97.2
C16:0	203	5.45	13.4	1100
C17:0	7.91	0.24	0.90	55.3
C18:0	94.3	2.16	14.7	554
MUFA	320	8.81	15.2	1710
C14:1	4.21	0.14	0.32	30.9
C16:1	23.8	0.71	0.86	139
C18:1	292	8.03	13.6	1620
PUFA	80.8	1.10	25.2	224
C18:2*n*-6	46.7	0.94	11.9	168
C18:3*n*-3	5.93	0.15	0.28	29.6
C20:4*n*-6	16.8	0.25	4.4	50.2
C20:5*n*-3	3.56	0.09	0.21	13.8
C22:5*n*-3	4.01	0.07	0.41	10.4
C22:6*n*-3	0.33	0.01	0.05	0.96
n-6 PUFA	67.2	1.21	17.1	220
n-3 PUFA	13.6	0.29	0.95	48.4
n-6:*n*-3	9.92	0.47	1.16	96.1
CLA	1.53	0.06	0.05	23.1

Total FA: Total fatty acids (SFA+MUFA+PUFA)
SFA: Saturated fatty acids (C14:0+C16:0+C17:0+C18:0+C23:0+C24:0)
MUFA: Monounsaturated fatty acids (C14:1+C16:1+C18:1)
PUFA: Polyunsaturated fatty acids (C18:2+C18:3+C20:3+C20:4+C20:5+C22:5+C22:6)
n-6: Omega-6 fatty acids (C18:2+C20:3+C20:4)
n-3: Omega-3 fatty acids (C18:3+C20:5+C22:5+C22:6)
CLA: Conjugated linoleic acid

Total FA content in the beef samples was highly variable and ranged from 84.4-3610.5 mg/100g beef (Table 2.2). Variation was expected, due the framework of the survey with both large and small producers with broad geographical representation. Mean total FA content was 723.4 mg/100g beef, which was lower than expected. For instance, de la Fuente et al. (2009) and Chail et al. (2016) reported total FA values in GFB 37% and 76% greater respectively. We hypothesize that the cattle harvested for the current study may have been slaughtered at a leaner finish. Pethwick et al. (2004) indicated that the primary factor influencing marbling

accumulation is net energy for fat synthesis; age is also a factor, but the correlation is not linear. Therefore, the greater values for total FA in the literature could be attributed to a more controlled environment, including careful monitoring of dietary energy. Within the samples collected for this study, there was great variation amongst samples and finishing protocols. Furthermore, sample preparation methods could also play a role in recovery of FA. Traditionally, whole samples are homogenized before extraction, but due to the quantity of samples in this study, an alternative method of taking a core from the sample was used.

2.3.2.1. *Fatty acids of interest*

Stearic acid (C18:0), a saturated fatty acid (SFA), which is generally recognized to have a net neutral effect on serum cholesterol (Grande et al., 1970) accounted for 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 (MUFA) and SFA were both 44% of total; previous studies have reported that GFB has significantly less MUFA than SFA (Duckett et al., 2013; Descalzo et al., 2005; Realini et al., 2004) The most prevalent polyunsaturated fatty acid (PUFA) in beef, linoleic acid (C18:2*n*-6), an omega-6 (*n*-6) FA, was 6.5% of total FA. This is greater than reported for by Duckett et al. (2013) for either GFB or CFB (2.6 and 2.7%, respectively). Descalzo et al. (2005) reported C18:2*n*-6 concentrations as 5.4% of total FA for GFB, and 4.7% for CFB. Linolenic acid (C18:3*n*-3) is the most prevalent omega-3 (*n*-3) FA in beef, and was 0.81% of total FA. This FA is typically found in greater concentrations in GFB than CFB, due to the *n*-3 content of the diet (Daley et al., 2010). Duckett et al. (2013) reported C18:3*n*-3 as 1.2% of total FA for GFB, and 0.24% for CFB. Both C18:2*n*-6 and C18:3*n*-3 are essential fatty acids for humans, and are necessary for the synthesis of long chain PUFA (LCPUFA) in the body.

However, the efficiency of this conversion is low, and thus it is important to obtain LCPUFA in the diet as well. Eicosapentaenoic acid (EPA; C20:5*n*-3), docosapentaenoic acid (DPA; C22:5*n*-3), and docosahexaenoic acid (DHA; C22:6*n*-3) were present in this sample set at concentrations of 0.49, 0.55 and 0.04% of total FA respectively. Duckett et al. (2013) reported concentrations of 0.55, 0.85, and 0.09% for these same FA.

The ratio of *n*-6:*n*-3 FA (mean = 9.9, median = 4.1) was greater than expected for GFB. The mean ratios for individual producers ranged from 1.8 to 28.3, and due to the non-normal distribution of the data, the median value could be a better indicator of the overall ratio. Research has consistently shown that cattle finished solely on grass have a lower *n*-6:*n*-3 ratio than conventionally finished beef, with GFB typically having a ratio below 2, and conventionally finished beef 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). Duckett et al. (2009) proposed that the *n*-6:*n*-3 ratio could be the best indicator of feedstuffs used in a finishing system. The *n*-6:*n*-3 ratio differences in GFB and conventionally-fed beef are generally attributed to the greater amounts of C18:2*n*-6 in concentrates than in 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 C18:2*n*-6 content could result in a *n*-6:*n*-3 ratio that more similar to conventionally finished beef. Baublits et al. (2006) found that beef from cattle grazing orchardgrass 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). However, Duckett et al. (2009) reported that beef from cattle fed varying levels of SH before a 150 d finishing phase on forages had no differences in *n*-6:*n*-3 ratio. Feeding SH during the finishing phase can improve

performance. Pugh (2003) indicated that forage finished cattle supplemented with SH had a greater average daily gain and finished with a higher yield grade (2.8 vs 1.4) and quality grade (USDA Choice vs USDA Standard) than those finished solely on grass. Kiesling (2013) hypothesized that cattle supplemented with SH had a greater apparent biohydrogenation than those supplemented with corn, resulting in a higher content of C18:0 and CLA in the beef. Importantly, many grass-finished beef programs, such as the American Grassfed Association (AGA) prohibit the supplementation of soy products to cattle under their label (AGA Grassfed Ruminant Standards, 2017).

2.3.2.2. *Seasonal effect*

Five producers submitted beef samples during both the fall (n = 180) and spring (n = 268) collection periods, and the seasonal comparisons are in Figures 2.1 and 2.2. No differences by season were found in SFA, MUFA, or PUFA. However, *n*-3 PUFA was greater in the spring than the fall (14.8 vs 11.4 mg/100g beef; $P<0.0001$) and correspondingly, the *n*-6 to *n*-3 ratio was lower in the spring (10.3 vs 6.4; $P<0.0001$). Similarly, CLA was greater in the spring than the fall (1.49 vs 1.28 mg/100g, $P<0.05$). Pestana et al. (2012) reported that Portuguese cattle grazing the same forages showed greater amounts of *n*-3 PUFA in cattle harvested from spring pastures than those harvested from fall pastures at a similar age, attributing the difference to the greater abundance of forage available in the spring season. Of the four producers who reported cattle finishing diets, only one indicated that cattle were not fed fresh forages (Table 2.1). Thus, seasonal differences in forage abundance and quality could play into the *n*-3 PUFA and CLA content of the beef in this study.

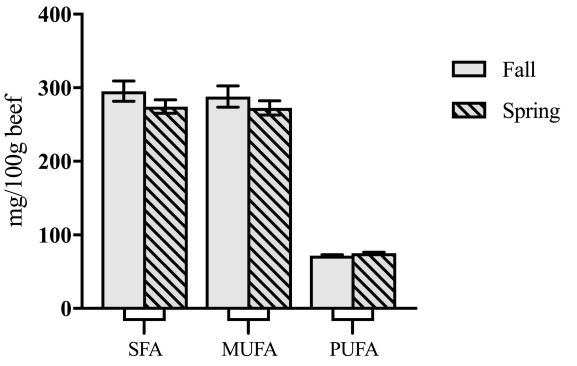

Figure 2.1. Seasonal comparison of fatty acids. SFA: Saturated fatty acids (C14:0 + C16:0 + C17:0 + C18:0 + C23:0 + C24:0); MUFA: Monounsaturated fatty acids (C14:1 + C16:1 + C18:1); PUFA: Polyunsaturated fatty acids (C18:2*n*-6 + C18:3*n*-3 + C20:3*n*-6 + C20:4*n*-6 + C20:5*n*-3 + C22:5*n*-3 + C22:6*n*-3)

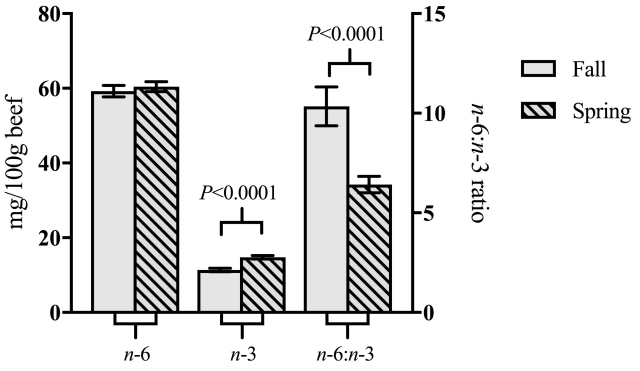

Figure 2.2. Seasonal comparison of PUFA. *n*-6: Omega-6 fatty acids (C18:2*n*-6 + C20:3*n*-6 + C20:4*n*-6); *n*-3: Omega-3 fatty acids (C18:3*n*-3 + C20:5*n*-3 + C22:5*n*-3 + C22:6*n*-3)

On an individual producer basis, only beef submitted by producer eight had a seasonal effect (data not shown). The amount of *n*-3 PUFA were greater in the spring than the fall (17.8 vs 13.9 mg/100g beef; $P<0.001$) and the beef had a *n*-6:*n*-3 ratio of 3.7 and 4.2 ($P<0.05$) for spring and fall respectively (data not shown). Producer 8 indicated differing feeding strategies for cattle in the finished in the spring vs. fall, while the other producers reported finishing diets that were the same or similar, regardless of finishing season (Table 2.1). Differences in forage-finishing plant species have been shown to affect *n*-3 PUFA levels in beef. Duckett et al. (2013) reported that different forage finishing strategies affected the percentage of C18:3*n*-3, with beef from cattle finished on alfalfa showing a greater concentration than those finished on mixed pasture or pearl millet (1.32% vs. 1.17 and 1.06%, respectively). Schmidt et al. (2013) reported that forage species influenced the concentrations of both C18:2*n*-6 and C18:3*n*-3, resulting in a lower *n*-6:*n*-3 ratio in beef from cattle finished on alfalfa, bermudagrass, and cowpea compared to those finished on chicory or pearl millet (1.88, 1.90 and 1.80 vs 2.11 and 2.26%, respectively). Additionally, Chail et al. (2016) reported that cattle finished on birdsfoot trefoil, a perennial legume, exhibited a greater amount of C18:3*n*-3 and C20:5*n*-3 in beef compared to beef from

cattle finished on grass (52 vs 27 mg/100g and 9 vs 7 mg/100g, respectively). While the information provided by the producers regarding finishing diets were limited, the reversal of the *n*-3 PUFA in beef produced by producer 8 could be attributed to forage species, as well as abundance and quality of forage available in the two seasons.

2.3.2.3. *Omega-6 and omega-3 FA content of GFB*

The amount of *n*-6 and *n*-3, and the *n*-6:*n*-3 ratio were highly variable across producers ($P<0.0001$; Figure 2.3). Beef from producers 4, 5 and 6 had the least *n*-3, and correspondingly had the greatest *n*-6:*n*-3 ratio. Similarly to the overall mean *n*-6:*n*-3 ratio, the ratios for these producers was greater than values previously reported for GFB. Chail et al. (2016), Duckett et al. (2013), Realini et al. (2004), reported *n*-6:*n*-3 ratios of 3.44, 1.33, and 1.44 for GFB respectively. Producers 1, 3, 9, 10, & 11 had the greatest amounts of n-3 and the lowest n-6:n-3 (range = 1.8 to 2.2), which is consistent with other reported results for GFB. These producers reported that cattle finishing diets consisted only of fresh forages. Producers 2 and 8 had mean *n*-6:*n*-3 ratios of 4.4 and 3.9 respectively, and indicated supplementation of various harvested feedstuffs (Table 2.1), including forage silage. The results of the current study are consistent with the results presented by French et al. (2000) who reported 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.

Beef from producers 4, 5, and 6 had significantly greater *n*-6:*n*-3 ratios than the remaining producers ($P<0.0001$), and were higher than values for beef from feedlot finished cattle reported by Duckett et al. (2013) and Duckett et al. (2009) (6.01 and 4.84, respectively). Chail et al. (2016) reported a *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 15.21. 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, it appears that the results of the current study indicate that cattle finished on fresh forages can 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.

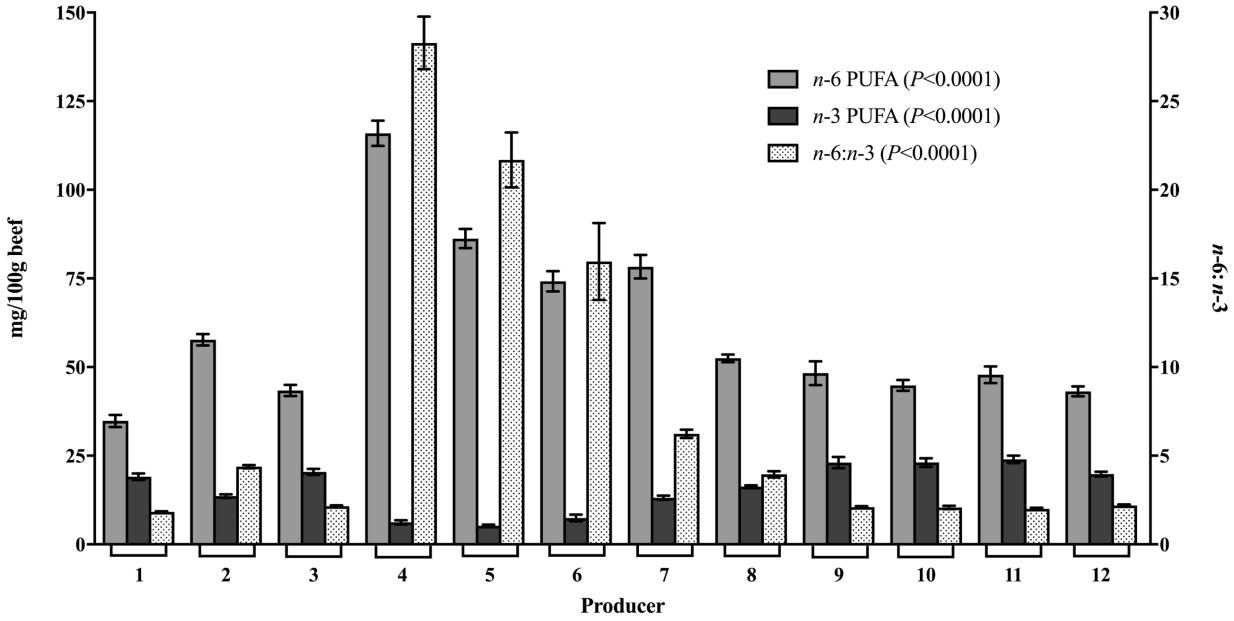

Figure 2.3. Producer comparison of *n*-6, *n*-3, and *n*-6:*n*-3.

Table 2.3 reflects selected correlation values of different response variables obtained in the study. SFA and MUFA were both highly correlated with total fatty acid (r^2 = 0.996 and 0.993, respectively), while PUFA had a lower correlation (r^2 = 0.599). This is consistent with results from Warren et al. (2008) that found that as total FA increased, the proportion of SFA increased, while PUFA decreased. Duckett et al. (1993) similarly found that increased time on feed lowered the PUFA:SFA ratio. One hypothesis for increasing the amount of beneficial *n*-3 FA in a serving of grass-fed beef is to increase the overall fat content. However, when looking at PUFAs in terms of the *n*-6:*n*-3 ratio, there is a moderate correlation (r^2 = 0.402) between the total

amount of FA and the *n*-6:*n*-3 ratio, indicating that as animals fatten, the *n*-6:*n*-3 ratio increases, limiting the benefit gained from any additional *n*-3 FA. This is in contrast to DeSmet et al. (2004) who hypothesized that for a given diet, the *n*-6:*n*-3 ratio would decrease as animals fatten. Our results may be due to the variation in diets fed to the animals, but as a survey of commercially available GFB, it indicates that GFB with a higher fat content does not necessarily imply greater *n*-3 FA.

Table 2.3. Spearman correlation (r^2) between fatty acid classes and antioxidants ($P<0.05$).

	SFA	**MUFA**	**PUFA**	***n*-6**	***n*-3**	***n*-6:*n*-3**	**β-c**	**α-t**
Total FA	0.996	0.993	0.599	0.600	-0.191	0.402	-0.305	-0.240
SFA		0.985	0.569	0.573	-0.193	0.390	-0.300	-0.232
MUFA			0.531	0.536	-0.175	0.363	-0.279	-0.231
PUFA				0.939	-0.218	0.551	-0.386	-0.240
***n*-6**					-0.496	0.786	-0.491	-0.312
***n*-3**						-0.908	0.495	0.276
***n*-6:*n*-3**							-0.564	-0.340
β-c								0.756

Total FA: Total fatty acids; SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids; *n*-6: Omega-6 fatty acids; *n*-3: Omega-3 fatty acids; β-c: beta-carotene; α-t: alpha-tocopherol

2.3.3. *Minerals*

Beef mineral content is presented in Table 2.4. Mineral content was similar to the levels reported for GFB by Duckett et al. (2009) and Leheska et al. (2008), though in general, K, Mg, and Fe were greater, and Na was lower. Copper and Se were lower than reported by Leheska (2008) (70.0 and 21.2 µg/100g, respectively). Copper was below the limit of quantification for a number of samples, so the mean includes only those samples that were quantifiable (n=435). Table 2.5 shows the effect of season on mineral content of beef. Potassium was not affected by season, while S ($P<0.01$), P ($P<0.0001$), and Na ($P<0.001$) were greater in the spring vs. fall and

Mg was lower in the spring ($P<0.0001$). Iron was the only micromineral with a seasonal effect, with greater amounts found in the spring samples ($P<0.001$). The nutrient composition of forages is dependent on numerous factors, including soil mineral content, soil moisture, weather, and climate, as well as plant species, maturity, and leaf to stem ratio (Kilcher, 1981). Thus, without further study, seasonal differences found in this survey cannot be attributed to any specific variable.

Table 2.4. Mineral content of grass-finished beef.

Macrominerals (mg/100g)	**Mean**	**SEM**	**Min**	**Max**
Sodium	41.7	0.29	25.0	93.0
Magnesium	25.9	0.06	22.0	33.0
Phosphorus	210	0.43	171	277
Sulfur	206	0.50	173	274
Potassium	423	0.84	330	526
Microminerals (µg/100g)				
Iron	2130	19.6	1170	9830
Zinc	4080	27.8	2200	6840
Copper	63.3	0.65	49.0	250
Selenium	17.6	0.26	6.0	68.0

Furthermore, great variation ($P<0.001$) existed between producers for all beef mineral content (data not shown). Due to the national scope of our study and that mineral content of forages fed to cattle is greatly dependent on the soils on which the feed was grown (Preston, 2008), the high level of variation we observed should be expected. Furthermore, Schmidt et al. (2013) 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. However, Duckett et al. (2009) found no difference in mineral content between GFB and CFB. Preston (2008) suggested supplementing minerals to ruminants in locations where there are deficiencies in soils. However, regardless of location, all of the producers in this study indicated that they supplemented minerals in their finishing programs. This survey study is

unable to provide a clear explanation for the variation in mineral content, due to the inherent variation of soil mineral status by location, and the wide variation in forages fed to the cattle. Despite variation in mineral composition, GFB remains a good source of Fe and Zn (Williamson et al., 2005).

Table 2.5. The effect of harvest season on mineral content of grass-finished beef.

Macrominerals (mg/100g)	Fall	Spring	SEM[1]	p-value
Sodium	42.0[a]	39.8[b]	0.43	0.0003
Magnesium	25.4[b]	26.2[a]	0.14	<0.0001
Phosphorus	216[a]	205[b]	0.82	<0.0001
Sulfur	208[a]	204[b]	0.93	0.0033
Potassium	422	424	1.83	ns
Microminerals (µg/100g)				
Iron	1990[b]	2140[a]	25.6	0.0001
Zinc	3990	4010	44.8	ns
Copper	62.4	61.0	0.94	ns
Selenium	18.0	18.8	0.46	ns

[1]Pooled (largest) SE of means
[a,b]Means in the same row with differing superscripts differ

2.3.4. *Alpha-tocopherol and beta-carotene*

Two fat soluble vitamins present in beef are of interest for their antioxidant properties: vitamin A and E. These are measured in the current study directly for vitamin E as α-tocopherol and indirectly for vitamin A as its precursor, β-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). Survey results for α-tocopherol and β-carotene are indicated in Table 2.6. The mean beef α-tocopherol content was 611 µg/100g of tissue. Only 358 samples had β-carotene content above the limit of quantification, and the mean reflects those samples (n = 358; Table 2.6). The α-tocopherol content in this study was greater than reported for GFB by Duckett et al. (2013) (343 µg/100g) and De la Fuente et al. (2009) (375 µg/100g). Duckett et al. (2013) reported β-carotene content for beef from cattle grazing mixed pasture at 57.8 µg/100g. Both α-tocopherol and β-carotene showed a seasonal effect - both were greater

(P<0.0001 and P<0.001, respectively) in the fall than the spring. However, on an individual basis, only beef from producer 8 showed a significant difference in α-tocopherol content between fall and spring (data not shown). This could again be attributed to differences in seasonal feeding strategies that producer 8 implemented, compared to the finishing strategies of the other producers that did not change much between spring and fall. Preston (2008) indicated that the 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.

Table 2.6. Antioxidant content of grass-finished beef.

Antioxidants (µg/100g)	Mean	SEM	Min	Max
α-tocopherol	611	9.90	161	1680
β-carotene	32.2	0.56	11.0	103

The α-tocopherol and β-carotene content was highly variable between producer beef samples (P<0.0001; Figures 2.4 and 2.5). Beef from producers 2, 5, 6 and 7 showed lower amounts of α-tocopherol than all other producers (P<0.0001), though still within the range 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 β-carotene content below the limit of quantification (LOQ <11 µg/100g) for nearly all samples. In a comprehensive review, Ballet et al. (2000) indicated that α-tocopherol content is greater in fresh forages than in harvested hays. Duckett et al. (2013) reported no difference in α-tocopherol in beef from cattle finished on mixed pasture, alfalfa, and pearl millet, but found that β-carotene was lower in beef from cattle finished on alfalfa (0.405 vs. 0.578 and 0.519 µg/100g). Conversely, Schmidt et al. (2013) found no difference in α-tocopherol

content in beef finished on five different forage species, but found that β-carotene varied significantly (38-160 μg/100g). These results indicate that finishing with fresh forages can achieve greater antioxidant content in beef, which is widely promoted as a health benefit of GFB, compared to finishing on harvested forages.

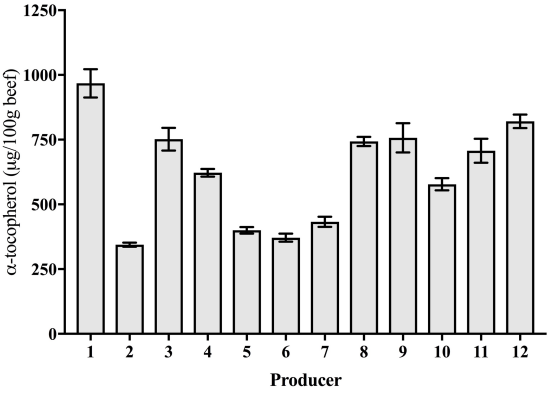

Figure 2.4. Producer comparison of α-tocopherol content in grass-finished beef.

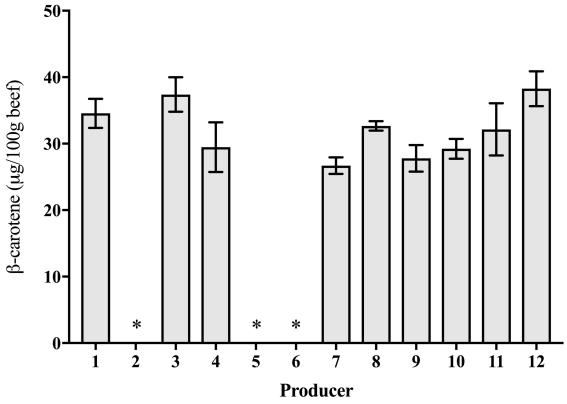

Figure 2.5. Producer comparison of β-carotene content in grass-finished beef. *β-carotene in nearly all beef samples from producers 2, 5, and 6 were below the LOQ

2.4. Summary

This survey of commercially available GFB indicates that producers were using a wide variety of feeding strategies to finish cattle, including both 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. A seasonal effect was identified for *n*-3 FA and the *n*-6:*n*-3 ratio, and the ratio was 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 α-tocopherol and β-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 served to gain a greater understanding of the trends and variation amongst GFB produced in the United States.

APPENDIX

APPENDIX

A.1. Producer Survey

1. Total acres of farm/ranch:

2. Total grazeable acres:

3. Enter grazeable acres of each forage type:
   Warm season perennials (acres):
   Warm season annuals (acres):
   Cool season perennials (acres):
   Cool season annuals (acres):

4. Do you use cover crops in your pasture rotation?
❍ Yes
❍ No

5.Typical cover crop(s) or mixes used?

6. Do you graze your cover crop?
❍ Yes
❍ No

7. Which cover crops do you graze?

8. What livestock enterprises do you currently operate? (check all that apply)
❑ Cow/calf
❑ Stocker
❑ Grass finishing
❑ Sheep
❑ Goat
❑ Pastured Pig
❑ Pastured Poultry

9. Are your livestock enterprises integrated (stacked)?
❍ Yes
❍ No

10. If you submitted samples for analysis in the fall of 2016, please indicate:
   a. Breed of cattle
   b. The diet cattle were finished on
   c. Age of cattle at slaughter (months)

11. If you are participating for the first time in this trial this spring, or if there are differences between the samples submitted in fall and those to be submitted this spring, please provide:
    a. Breed of cattle
    b. The diet cattle were finished on (Spring 2017)
    c. Age of cattle at slaughter (months)

12. How many grass fed cattle do you market annually?

13. What is the typical length of your grazing season (days)?

14. What is your primary forage mix? (check all that apply)
- ❑ Orchard Grass
- ❑ Fescue
- ❑ Perennial Rye
- ❑ Big Bluestem
- ❑ Smooth Bromegrass
- ❑ Timothy
- ❑ Bermudagrass
- ❑ Clover
- ❑ Annuals
- ❑ Alfalfa
- ❑ Small Grains (oat, rye, triticale, wheat)
- ❑ Sorghum

15. How would you describe your pasture management technique? (check all that apply)
- ❑ Continuous Grazing
- ❑ Management Intensive Grazing
- ❑ Holistic Grazing
- ❑ Mob Grazing

16. Typical grazing rotation? (check all that apply)
- ❑ No rotation (continuous)
- ❑ No more than once a month
- ❑ Every 2-3 weeks
- ❑ Once a week
- ❑ Every 2-5 days
- ❑ Every day
- ❑ Multiple times daily

17. Average rest time per paddock/pasture before re-grazing?

18. Do your cattle receive any supplemental feed?
- ❍ Yes
- ❍ No

19. If so, what do you supplement? (check all that apply)
- ❑ Hay
- ❑ Alfalfa
- ❑ Sorghum silage
- ❑ Corn Silage
- ❑ Spent brewers grains
- ❑ Wheat middlings
- ❑ Soy hulls
- ❑ Other _____

20. How often do you supplement feed? (check all that apply)
- ❑ No more than once a month
- ❑ Every 2-3 weeks
- ❑ Once a week
- ❑ Every 2-5 days
- ❑ Every day
- ❑ Only when no grass/forage available
- ❑ When pastures/paddocks need extra rest
- ❑ Only during the winter
- ❑ Only during emergencies

21. Do you utilize a mineral program?
- ❍ Yes
- ❍ No

22. What minerals do you use? (check all that apply)
- ❑ Synthetic
- ❑ Natural
- ❑ Mixed Mineral
- ❑ Calcium
- ❑ Cobalt
- ❑ Copper
- ❑ Iodine
- ❑ Iron
- ❑ Manganese
- ❑ Molybdenum
- ❑ Nickel
- ❑ Selenium
- ❑ Sulfur
- ❑ Zinc
- ❑ Other _____

23. Do you use apple cider vinegar in the cattle's water?
- ❍ Yes
- ❍ No

24. Do you have city water or well water?
- ❍ City water
- ❍ Well water

25. How often do you use antibiotics in your herd?
- ❍ Routinely
- ❍ Yearly
- ❍ Only as needed
- ❍ Never

26. Do you use ionophores?
- ❍ Yes
- ❍ No

27. Do you use any type of growth promotant? (check all that apply)
- ❑ None
- ❑ Zilmax
- ❑ Testosterone
- ❑ Estradiol
- ❑ Progesterone
- ❑ Melengestrol acetate
- ❑ Trenbolone acetate
- ❑ Zeranol

A.2. Processor Survey

1. How many grassfed cattle do you slaughter weekly?

2. What is your aging process?

- ❍ Dry aging
- ❍ Wet aging

2b. Do you dry age beef before wet aging? If so, for how long?

3. How long is your aging process?

4. Do you electrically stimulate beef carcasses?

- ❍ Yes
- ❍ No

5. Where do you market your grassfed beef? (check all that apply)

- ❑ Directly to consumers
- ❑ Restaurants
- ❑ Distributors
- ❑ Health food stores (Whole Foods, etc.)
- ❑ Grocery stores (Kroger, Wal-Mart, Costco, Albertsons, etc.)

LITERATURE CITED

LITERATURE CITED

AGA (2017). Grassfed Ruminant Standards v1.3. American Grassfed Association.

Ballet, N., Robert, J. C., & Williams, P. E. V. (2000). Vitamins in Forages. In: Givens, D. I., Owen, E., Axeford, R. F. E., Omed, H. M. (Eds.), *Forage evaluation in ruminant nutrition* (pp. 399-431). Wallingford, UK: CABI Publishing

Baublits, R. T., Brown, A. H., Pohlman, F. W., Rule, D. C., Johnson, Z. B., Onks, D. O., . . . Pugh, R. B. (2006). Fatty acid and sensory characteristics of beef from three biological types of cattle grazing cool-season forages supplemented with soyhulls. *Meat Science, 72*(1), 100-107.

Chail, A., Legako, J. F., Pitcher, L. R., Griggs, T. C., Ward, R. E., Martini, S., & MacAdam, J. W. (2016). Legume finishing provides beef with positive human dietary fatty acid ratios and consumer preference comparable with grain-finished beef. *Journal of Animal Science, 94*(5), 2184-2197.

Cheung, R., McMahon, P., Norell, E., Kissel, R., and Benz, D. (2017). Back to grass: the market potential for grassfed beef. Retrieved from: https://www.stonebarnscenter.org/wp-content/uploads/2017/10/Grassfed_Full_v2.pdf

Daley, C. A., Abbott, A., Doyle, P. S., Nader, G. A., & Larson, S. (2010). A review of fatty acid profiles and antioxidant content in grass-fed and grain-fed beef. *Nutrition Journal, 9*(10), 1-12.

De la Fuente, J., Diaz, M. T., Alvarez, I., Oliver, M. A., Font i Furnols, M., Sanudo, C., . . . Caneque, V. (2009). Fatty acid and vitamin E composition of intramuscular fat in cattle reared in different production systems. *Meat Science, 82*(3), 331-337.

Descalzo, A., Insani, E. M., Biolatto, A., Sancho, A. M., Garcia, P. T., & Pensel, N. A. (2005). Influence of pasture or grain-based diets supplemented with vitamin E on antioxidant/oxidative balance of Argentine beef. *Meat Science*, *70*(1), 35-44.

Descalzo, A. M., Rossetti, L., Grigioni, G., Irurueta, M., Sancho, A. M., Carrete, J., & Pensel, N. A. (2007). Antioxidant status and odour profile in fresh beef from pasture or grain-fed cattle. *Meat Science, 75*(2), 299-307.

DeSmet, S., Raes, K., & Demeyer, D. (2004). Meat fatty acid composition as affected by fatness and genetic factors: A review. *Animal Research, 53*(2)*,* 81-98

Duckett, S. K., Wagner, D. G., Yates, L. D., Dolezal, H. G., & May, S. G. (1993). Effects of time on feed on beef nutrient composition. *Journal of Animal Science, 71*(8), 2079-2088.

Duckett, S. K., Neel, J. P., Fontenot, J. P., & Clapham, W. M. (2009). Effects of winter stocker growth rate and finishing system on: III. Tissue proximate, fatty acid, vitamin, and cholesterol content. *Journal of Animal Science, 87*(9), 2961-2970.

Duckett, S. K., Neel, J. P., Lewis, R. M., Fontenot, J. P., & Clapham, W. M. (2013). Effects of forage species or concentrate finishing on animal performance, carcass and meat quality. *Journal of Animal Science, 91*(3), 1454-1467.

French, P., Stanton, C., Lawless, F., O'Riordan, E. G., Monahan, F. J., Caffery, P. J., & Moloney, A. P. (2000). Fatty acid composition, including conjugated linoleic acid of intramuscular fat from steers offered grazed grass, grass silage or concentrate-based diets. *Journal of Animal Science, 78*(11), 2849-2855.

Garcia, P. T., Pensel, N. A., Sancho, A. M., Latimori, N. J., Kloster, A. M., Amigone, M. A., & Casal, J. J. (2008). Beef lipids in relation to animal breed and nutrition in Argentina. *Meat Science, 79*(3), 500-508.

Gillespie, J., Sitienei, I., Bhandari, B., Scaglia, G. (2016). Grass-fed beef: How is it marketed by US producers? *International Food and Agribusiness Management Review, 19*(2), 171-188.

Grande, F., Anderson, J. T., & Keys, A. (1970). Comparison of effects of palmitic and stearic acids in the diet on serum cholesterol in man. *The American Journal of Clinical Nutrition, 23*(9), 1184-1193.

Ichihara, K. I., & Fukubayashi, Y. (2010). Preparation of fatty acid methyl esters for gas-liquid chromatography. *Journal of Lipid Research, 51*(3), 635-640.

Kilcher, M. R. (1981). Plant development, stage of maturity and nutrient composition. *Journal of Range Management 34*(5), 363-364.

Kiesling, D. D. (2013) Performance, carcass traits and fatty acid profiles of yearling beef cattle supplemented with self-fed byproducts on pasture. Graduate Theses and Dissertations. 13121. http://lib.dr.iastate.edu/etd/13121

Leheska, J. M., Thompson, L. D., Howe, J. C., Hentges, E., Boyce, J., Brooks, J. C., . . . Miller, M. F. (2008). Effects of conventional and grass-feeding systems on the nutrient composition of beef. *Journal of Animal Science, 86*(12), 3575-3585.

Lozier, J., Rayburn, E., & Shaw, J. (2005). Growing and selling pasture-finished beef: Results of a nationwide survey. *Journal of Sustainable Agriculture, 25*(2), 93-112.

McCluskey, J. J., Wahl, T. I., Quan, L., & Wandschneider, P. R. (2005). U.S. grass-fed beef: Marketing health benefits. *Journal of Food Distribution Research, 36*(3), 1-8.

Medina, A. L., da Silva, M. A. O., de Sousa Barbosa, H., Arruda, M. A. Z., Marsaioli, A., & Bragagnolo, N. (2015). Rapid microwave assisted extraction of meat lipids. *Food Research International, 78*, 124-130.

Pestana, J. M., Costa, A. S. H., Martins, S. V., Alfaia, C. M., Alves, S. P., Lopes, P. A., . . . Prates, J. A. M. (2012). Effect of slaughter season and muscle type on the fatty acid composition, including conjugated linoleic acid isomers, and nutritional value of intramuscular fat in organic beef. *Journal of the Science of Food and Agriculture, 92*(12), 2428-2435.

Ponnampalam, E. N., Mann, N. J., & Sinclair, A. J. (2006). Effect of feeding systems on omega-3 fatty acids, conjugated linoleic acid and trans fatty acids in Australian beef cuts, potential impact on human health. *Asia Pacific Journal of Clinical Nutrition, 15*(1), 21-29.

Preston, R. L. (2008). Typical composition of feeds for cattle and sheep (Vol. 44, pp. 76). Minneapolis: Penton Media, Inc.

Pugh, R. B. (2003) Soybean hull supplementation to pasture-based beef cattle finishing diets. Master's Thesis, University of Tennessee. http://trace.tennessee.edu/utk_gradthes/2151

Realini, C. E., Duckett, S. K., Brito, G. W., Rizza, M. D., & De Mattos, D. (2004). Effect of pasture vs. concentrate feeding with or without antioxidants on carcass characteristics, fatty acid composition, and quality of Uruguayan beef. *Meat Science, 66*(3), 567-577.

Rettenmaier, R., & Schuep, W. (1992). Determination of vitamins A and E in liver tissue. *International Journal for Vitamin and Nutrition Research (62)* 312-317.

Schmidt, J. R., Miller, M. C., Andrae, J. G., Ellis, S. E., & Duckett, S. K. (2013). Effect of summer forage species grazed during finishing on animal performance, carcass quality, and meat quality. *Journal of Animal Science, 91*(9), 4451-4461.

Steinberg, E. L., & Comerford, J. W. (2009). Case study: A survey of pasture-finished beef producers in the northeastern United States. *The Professional Animal Scientist, 25*(1), 104-108.

Umberger, W. J., Boxall, P. C., & Lacy, R. C. (2009). Role of credence and health information in determining US consumers' willingness-to-pay for grass-finished beef. Australian *Journal of Agricultural and Resource Economics, 53*(4), 603-623.

USDA, AMS. (2016). Withdrawal of United States Standards for Livestock and Meat Marketing Claims. Retrieved from: https://www.federalregister.gov/documents/2016/01/12/2016-00440/withdrawal-of-united-states-standards-for-livestock-and-meat-marketing-claims#h-8.

Warren, H. E., Scollan, N. D., Enser, M., Hughes, S. I., Richardson, R. I., & Wood, J. D. (2008). Effects of breed and a concentrate or grass silage diet on beef quality in cattle of 3 ages. I: Animal performance, carcass quality and muscle fatty acid composition. *Meat Science, 78*(3), 256-269.

Wahlen R., Evans L., Turner J., & Hearn R. (2005). The use of collision/reaction cell ICP-MS for the determination of elements in blood and serum samples. *Spectroscopy 20*(12), 84-89.

Williamson, C. S., Foster, R. K., Stanner, S. A., & Buttriss, J. L. (2005). Red meat in the diet. *Nutrition Bulletin, 30*(4), 323-355.

Yang, A., Brewster, M. J., Lanari, M. C., & Tume, R. K. (2002). Effect of vitamin E supplementation on α-tocopherol and β-carotene concentrations in tissues from pasture- and grain-fed cattle. *Meat Science, 60*(1), 35-40.

CHAPTER 3

CONCLUSIONS AND FUTURE RESEARCH

The results of this study indicate that the nutritional aspects of grass-finished beef (GFB) can be as variable as the methods used to produce it. The size of farms that participated in this study ranged from individual producers with a small, direct-marketing focus, to cooperative structures that pool resources to meet demand, to branded programs that use proven economies of scale to match the efficiency of conventionally raised beef. This allowed us to gain a representative understanding of GFB that is currently available to consumers.

One reason conventional beef production systems can continually provide tasty and efficient portions of beef is the consistent source of dietary energy fed to the animals through high-starch diets. The GFB industry has made great strides to make its products more efficient and consistent to meet consumer demand by continuing to improve their ability to deliver high-quality fresh and harvested forages to cattle year-round. One area of further study would be to evaluate the effect of available dietary energy on the partitioning of *n*-6 and *n*-3 FA in grass-finished beef cattle. In our study, the ratio of *n*-6 to *n*-3 FA in beef samples averaged by producer ranged from 1.8 to 28.3, with a median of 4.1 for the overall sample set. Intuitively, a common industry comment is to recommend increasing the fattening of GFB to raise the *n*-3 FA content. However, our correlations indicate this may not be as straightforward as thought. Generally, as there was greater total FA, *n*-3 FA comprised a smaller percentage of the overall total FA content. Overall, these challenges demonstrate the need to continue to use research to more aptly understand the influences of diet, management and even genetics on beef healthfulness.

Another important area of research would be to address the impact of feeding harvested forages vs fresh forages during the finishing period on the nutritional composition of GFB. In many regions of the United States, fresh forage is not available year-round, and producers must supplement their cattle with stored feed. There is no doubt that that forage source and potential

form of storage greatly influenced our results. To properly represent the nutritional qualities of GFB to the consumer market, GFB producers must understand the impacts of their production strategies. Consumer demand for high quality, healthy food with little-to-no negative impact on the environment continues to push the popularity of specialty foods such as GFB, and it is vital that the product presented to them is accurately represented.