CAROLINA EVA REALINI

Nutritional effects on carcass traits, meat quality and fatty acid composition of beef, and the role of antioxidants in improving color and lipid stability. (Under the Direction of SUSAN K. DUCKETT)

Research was conducted to evaluate nutritional effects on carcass traits, meat quality and fatty acid composition of beef, and the role of antioxidants in improving color and lipid stability. In experiment 1, thirty Hereford steers were finished either on grass (GRASS, n=10) or concentrates (CONC, n=20). Half of the CONC steers were supplemented with vitamin E (VITE), and postmortem vitamin C (VITC) was added to ground beef. GRASS carcasses had lower weight, conformation, fat depth and ribeye area as well as darker longissimus color and yellower fat than CONC. Initial Warner-Bratzler shear force was similar among treatments, but GRASS had lower values after 7 d postmortem. VITE supplementation of CONC increased lipid stability of ground beef and steaks, but was unable to improve color stability; whereas VITC increased color stability without altering lipid oxidation in ground beef. GRASS enhanced the unsaturated fatty acid (FA) profile of intramuscular fat in beef including conjugated linoleic acid (CLA) and omega-3 (n-3) FAs. In experiment 2, postmortem vitamin C (VITC) was added to ground beef from grass-fed (GRASS) or grain-fed (GRAIN) sources. Near infrared reflectance (NIR) spectroscopy was used to predict FA composition of ground beef and to discriminate samples from GRASS vs. GRAIN. VITC was effective in retarding pigment oxidation in ground beef from both GRAIN and GRASS; however, VITC reduced lipid oxidation in GRAIN samples only, despite higher concentrations of polyunsaturated FAs in GRASS. NIR can be used to predict accurately the content of

total saturated and unsaturated, and stearic, oleic, and linolenic FAs in ground beef; and to discriminate meat samples from GRASS vs. GRAIN. In experiment 3, fourteen Hereford steers were finished on tall fescue infected with either wild-type (TOXIC; n =6) or novel, non-toxic (MAXQ; n = 8) endophyte. Although MAXQ supports higher cattle performance than TOXIC, these results suggest that endophyte type has minimal effects on carcass traits, and meat quality of grass-fed beef. Toxicosis may influence FA metabolism and fat necrosis. Finishing cattle on tall fescue pastures showed potential to enhance the FA profile of intramuscular fat in beef including CLA and n-3 FA. INDEX WORDS: Beef Quality, Pasture-fed, Fatty Acids, Antioxidants

NUTRITIONAL EFFECTS ON CARCASS TRAITS, MEAT QUALITY AND FATTY ACID COMPOSITION OF BEEF, AND THE ROLE OF ANTIOXIDANTS IN IMPROVING COLOR AND LIPID STABILITY

by

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DOCTOR OF PHILOSOPHY

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TABLE OF CONTENTS

ACKNOWLEDGEMENTS iv				
LIST OF TABLES				
LIST OF FIGURES				
CHAPTER				
1 REVIEW OF LITERATURE				
Effect of finishing on grass vs. concentrate on beef fatty acid profiles1				
Nutritional Importance of Omega-3 Fatty Acids and Conjugated Linoleic				
Acid in Human Diets				
Major Fatty Acids in Beef				
Dietary Modifications of Fatty Acid Profiles in Beef5				
Effect of Finishing on Pasture vs. Grain on Beef Fatty Acid Profiles6				
Some Effects of Altering Beef Fat on Meat Quality11				
Conclusions12				
Improvement of Oxidative Stability of Beef with Vitamin E and				
Vitamin C12				
Lipid oxidation13				
Pigment oxidation14				
Vitamin E14				
Vitamin C18				

Page

	Interaction of vitamins E and C	21
	Conclusions	21
	Literature Cited	
,	2 EFFECT OF FINISHING ON GRASS VS. CONCENTRATE AND	
	ANTIOXIDANTS ON FATTY ACID COMPOSITION, COLOR, AND	
	SHELF LIFE OF URUGUAYAN BEEF	41
	Abstract	42
	1. Introduction	43
	2. Materials and Methods	45
	3. Results and Discussion	49
	4. Conclusions	60
	Acknowledgements	61
	References	61
3	EFFECT OF VITAMIN C ADDITION TO GROUND BEEF FROM	
	GRASS-FED OR GRAIN-FED SOURCES ON COLOR AND LIPID	
	STABILITY, AND PREDICTION OF FATTY ACID COMPOSITION	
	BY NEAR INFRARED REFLECTANCE ANALYSIS	79
	Abstract	80
	Introduction	81
	Materials and Methods	82
	Results and Discussion	86
	Conclusions	92
	Literature Cited	93

4	EFFECT OF ENDOPHYTE TYPE ON CARCASS TRAITS, MEAT					
	QUALITY, AND FATTY ACID COMPOSITION OF BEEF CATTLE					
	GRAZING TALL FESCUE	107				
	Abstract	108				
	Introduction	109				
	Materials and Methods	110				
	Results and Discussion	113				
	Implications	122				
	Literature Cited	122				
5	CONCLUSION	139				

LIST OF TABLES

1.1 Fat content, fatty acid composition, and relative distribution of n-6:n-3 fatty acids
in grains and forages
1.2 Conjugated linoleic acid (CLA) concentrations in beef products from different
studies24
2.1 Mean (± S.E.) carcass characteristics of steers
2.2 Lightness (L*), redness (a*), and yellowness (b*) of longissimus and subcutaneous
fat at 24 hr postmortem
2.3 Temperature (means \pm S.E.) change during chilling from grass-fed, and
concentrate-fed carcasses
2.4 Levels of 2-thiobarbituric acid (TBARS, mg malonaldehyde/kg sample) values
during display of ground beef at 2°C74
2.5 Color L*, a*, and b* values during display of ground beef at 2°C
2.6 Intramuscular fatty acid composition (lsmean \pm S.E.) from grass-fed (n=10), and
concentrate-fed (n=20) cattle
3.1 Fatty acid content and composition of ground beef from GRASS- and GRAIN-fed
sources
3.2 Range, mean and standard deviation (SD) of fatty acids (weight percent methyl
ester) for ground beef in the calibration and validation data sets
3.3 Calibration and validation statistics (weight percent methyl ester) for saturated
fatty acids by NIRS spectroscopy100

Calibration and validation statistics (weight percent methyl ester) for saturated	
fatty acids by NIRS spectroscopy10	01
Effect of endophyte type on animal performance and carcass characteristics	30
Effect of endophyte type on muscle pH and subcutaneous fat and longissimus	
muscle color (L* lightness, a* redness, b* yellowness)1	31
Effect of endophyte type on steak and ground beef color (L* lightness, a* redness,	
b* yellowness) at 0 d of display1	32
Effect of endophyte type on fatty acid composition (lsmean \pm S.E.) pooled over	
three fat depots (ground beef, intramuscular, and subcutaneous fat)12	35
Fatty acid composition (lsmean \pm S.E.) of ground beef, intramuscular, and	
subcutaneous fat pooled over two treatments (TOXIC, MAXQ)1	36
Effect of endophyte type on sensory characteristics (lsmean \pm S.E.) of steaks from	
TOXIC and MAXQ cattle	38
	Calibration and validation statistics (weight percent methyl ester) for saturated fatty acids by NIRS spectroscopy

LIST OF FIGURES

1.1 Lipids enclosed in small cells in meat tissue	.25
1.2 Schematic illustration of endogenous (incorporated) vs. exogenous (added) α -	
tocopherol	.26
1.3 Proposed model of oxidation-reduction relationships in beef with emphasis on	
phospholipid and myoglobin oxidation, and vitamins E and C. (PUFA:	
polyunsaturated fatty acids, SOD: superoxide dismutase)	.27
2.1 Effect of postmortem aging on shear force of longissimus steaks from grass (n=10)	
and concentrates (n=20)	.71
2.2 Muscle α -tocopherol concentrations from grass (n=10), concentrates (n=10), and	
concentrates-vitamin E (n=10)	.73
2.3 Levels of 2-thiobarbituric acid (TBARS, mg malonaldehyde/kg sample) values	
during display of steaks at 2°C from grass (n=10), concentrates (n=10), and	
concentrates-vitamin E (n=10)	.75
2.4 Color L*, a*, and b* values during display of longissimus steaks at 2°C from grass	
(n=10), concentrates (n=10), and concentrates-vitamin E (n=10)	.77
3.1 Levels of 2-thiobarbituric acid (TBARS, mg malonaldehyde/kg sample) values	
during display of ground beef at 4°C from (a) grain-fed (n=14), and (b) grass-fed	
(n=18) cattle1	102
3.2 Effect of vitamin C addition to ground beef from grain-fed cattle on color (a) L^*	
lightness and (b) a* redness1	103

3.3	Effect of vitamin C addition to ground beef from grass-fed cattle on color (a) L*
	lightness and (b) a* redness
3.4	PLS regression coefficients from 1550 to 2450 nm for fatty acids in ground beef
	from the chuck. (a) total saturated fatty acid 5-term model; (b) total unsaturated fatty
	acid 5-term model
3.5	Discrimination among ground beef samples from GRASS- and GRAIN-fed sources
	using ISI software
4.1	The effect of endophyte type on lipid oxidation (TBARS) of steaks at 0, 5, 12, and
	21 d of display
4.2	The effect of endophyte type on lipid oxidation (TBARS) of ground beef at 0, 2, 4,
	and 8 d of display
4.3	Effect of endophyte type on tenderness (Warner-Bratzler shear force)

CHAPTER 1

REVIEW OF LITERATURE

Effect of finishing on grass vs. concentrate on beef fatty acid profiles

Dietary recommendations for humans promoting the consumption of less saturated fat have led to an increased interest in meats containing more unsaturated fatty acids. High levels of fat consumption and particularly of saturated fatty acids (SFA) has been associated with increased serum low-density-lipoprotein (LDL) cholesterol concentrations, and increased risk of coronary heart disease (Keys, 1970; Department of Health, 1994). Ruminant edible products are characterized by their highly saturated fat which has decreased consumer interest. Although the effect of dietary cholesterol on heart disease is still under debate, consumers have become concerned about the possible link between cholesterol and heart disease through the mass communications media and health professional advice (Sofos et al. 1996).

This review will discuss briefly the nutritional importance of the omega-3 fatty acids and conjugated linoleic acid, as well as current nutritional recommendations for a healthy diet. The composition of the major fatty acids in beef, and how these fatty acids can be modified by dietary means to more closely match dietary recommendations are considered. Finally, a comparison of the fatty acid profile in beef between grass-fed and grain-fed animals is given, followed by a brief discussion on consequences of modifying lipid composition in beef.

Nutritional Importance of Omega-3 Fatty Acids and Conjugated Linoleic Acid in Human Diets

In recent years the nutritional importance of the omega-3 fatty acids, and the omega-6:omega-3 (n-6:n-3) fatty acid ratio in the human diet has aroused great interest. There is increasing evidence that n-3 polyunsaturated fatty acids (PUFA) play a major role in human health and development. It has been suggested that these fatty acids, specifically eicosapentaenoic acid (EPA, 20:5) and docosahexaenoic acid (DHA, 22:6), are involved in the development of brain and retinal tissues and progression and prevention of human diseases, including heart disease and some cancers (Simopoulos, 1991).

Recent research has also focused on conjugated linoleic acid (CLA) which represents a mixture of positional and geometric isomers of linoleic acid (cis-9, cis-12 octadecadienoic acid). CLA was found to act as a growth factor (Chin et al., 1994), and a fat-to-lean repartitioning agent (Park et al., 1997; Ostrowska et al. 1999), and to show anticarcinogenic (Schulz et al., 1992; Ip, 1997), hypocholesterolaemic and antiatherogenic (Lee et al., 1994; Nicolosi et al., 1997) properties. The predominant CLA isomer is rumenic acid (cis-9, trans-11 octadecadienoic acid) which is produced naturally in the rumen and believed to be the biologically active isomer (Ha et al., 1990; Ip et al., 1991), representing about 90 % of CLA present in milk and 75 % of CLA present in beef fat (Chin et al., 1992).

Current recommendations for a healthy diet are that fat and SFAs should not exceed 35 % and 10 % of calories in the human diet, and that trans unsaturates should not exceed 2% of calories and should be reduced. The ratio of unsaturated to saturated fatty

acids should be around 0.45, and intakes of n-3 PUFAs should be increased relative to n-6 PUFA. A value of 4.0 or less for the diet as a whole is recommended for the n-6:n-3 ratio (Department of Health, 1994).

Major Fatty Acids in Beef

Wood and Enser (1997) pointed out that meat has been identified often wrongly, as a food having a high fat content and an undesirable balance of fatty acids. Ruminant fat tissue differs from that of single-stomached species in containing a higher proportion of SFAs and a lower proportion of PUFAs. This results from the hydrogenating action of the rumen bacteria which convert a high proportion of PUFAs from forage or concentrate diets into SFAs or unsaturated FAs with fewer double bonds (Wood and Enser, 1997).

Linoleic and linolenic acids are essential FAs, which must be supplied in the diet, and are the precursors for the longer chain, higher PUFAs of the n-6 or n-3 families that are formed in the tissues by chain elongation and desaturation. Dietary fats containing n-6 and n-3 fatty acids are hydrolyzed by rumen microorganisms and hydrogenated to mainly stearic acid. However, small amounts of fatty acids escape hydrogenation or are only partially hydrogenated in the rumen, whereby oleic acid and trans, odd chain, branched chain, and conjugated fatty acids are formed. Thus, mainly stearic acid is absorbed and deposited in the tissues, and beef will mostly contain stearic and oleic acid, the latter derived mainly from desaturation in the tissues (Jakobsen, 1999).

The composition of the intramuscular fat content of the beef longissimus muscle is comprised of over 20 individual FAs; however, six FAs contribute over 92 % of the total FA content (Duckett and Andrae, 2000). Duckett et al., (1993) reported that these major FAs in beef i.m. lipid from steers fed on high concentrate diet for 140 d are: oleic (C18:1, 41 %), palmitic (C16:0, 27 %), stearic (C18:0, 15 %), linoleic (C18:2, 3.9 %), palmitoleic (C16:1, 3.7 %), and myristic (C14:0, 3.6 %) acids. This fatty acid composition of beef intramuscular fat corresponds to an average of 44 % SFA, 45 % monounsaturated fatty acids (MUFA), 5 % PUFA, and 5 % odd- and branched-chain FAs (OCFA).

Jakobsen (1999) indicated that fats high in SFAs elevate LDL cholesterol, when compared with MUFAs or PUFAs, but the individual SFAs do not contribute equally to this effect. Palmitic (C16:0) is considered to be one of the major cholesterol-raising saturated fatty acids in the diet of humans. Lauric acid (C12:0), myristic (14:0), and trans fatty acids are also considered cholesterol raising, while stearic acid (18:0) is considered neutral, given that diets high in stearic acid have been shown to lower serum cholesterol compared to other SFAs (Ulbricht and Southgate, 1991; Keys et al., 1965; Denke and Grundy, 1991; Bonanome and Grundy, 1988). Stearic acid is believed to be converted to oleic acid after dietary ingestion which accounts for its different effect on serum cholesterol compared to other saturated fats (Bonanome and Grundy, 1988). Diets containing a high proportion of lipid as MUFA have been shown to be as effective as PUFA at lowering serum cholesterol levels in humans (Mattson and Grundy, 1985), and oleic acid (18:1) is demonstrated to be cholesterol-lowering. However, the cis monounsaturated fatty acids, unlike the PUFAs, do not decrease HDL cholesterol which protects against coronary heart disease (Mattson and Grundy, 1985). Recent epidemiological evidence also suggests that ruminant trans unsaturates, mainly trans vaccenic, are not risk factors for cardiovascular disease (Willett et al., 1993).

Summarizing, based on the FA proportions reported by Duckett et al. (1993) for intramuscular fat (44 % SFA including approximately 15 % stearic acid, 45 % MUFA, 5 % PUFA, and 5 % OCFA), only about 30 % of the fatty acids from beef longissimus muscle would be LDL cholesterol raising. Although ruminant products (meat, milk) are a poor source of n-3 fatty acids due to hydrogenation of PUFAs in the rumen, the ratio n-6:n-3 PUFA is beneficially lower in ruminant meats (Enser et al., 1996). In addition, ruminant fats are among the richest natural sources of CLA isomers, in particular the cis-9, trans-11 isomer (Chin et al., 1992).

Dietary Modifications of Fatty Acid Profiles in Beef

Over the past 40 years extensive research has been dedicated towards changing the fat and cholesterol content and fatty acid composition of animal products involving genetics, feeding strategies and management systems. Jakobsen (1999) pointed out that the purpose of modifying animal fats is to produce high quality products, which meet the dietary recommendations for a reduced intake of fat and cholesterol in the human diet and an optimal ratio between SFA, MUFA and PUFA to minimize the risk for obesity, cancer, and cardiovascular diseases. Although it is the dietary balance of fatty acids in the total diet which is physiologically important, attempts have been made with many individual foods to change them according to new dietary guidelines (Wood and Enser, 1997), and modification of the fatty acid composition of fat in ruminant tissues has not been an exception.

An increase in the consumption of n-3 fatty acids has been recommended to overcome the imbalance in the ratio of n-6:n-3 PUFA in the current diets (10:1) compared to primitive man (1:1, Eaton et al., 1996). Meat, fish, fish oils and eggs are the

only significant sources of n-3 C_{20} PUFA for human diet. Meat has lower concentrations of these fatty acids compared to oily fish. However, since fish consumption is low and beef accounts for an important proportion of the meat in typical USA diets, it is important to enhance the levels of these fatty acids in meat (Scollan et al. 2001). The potential to enrich ruminant tissues with C20 n-3 PUFA was highlighted when it was demonstrated that ruminal microorganisms in vitro did not hydrogenate 20:5 n-3 and 22-6 n-3 to any significant extent (Ashes et al., 1992). Byers and Schelling (1988) also indicated that the modification of dietary C20 and C22 PUFAs in the rumen is partial because of limited hydrolysis of these fats.

Demeyer and Doreau (1999) discussed techniques for decreasing biohydrogenation including coat of some oilseeds, coating of emulsified oils with formaldehyde-treated proteins, protected dry and coated fat grains, Ca salts, fatty acyl amides among others. Some protection occurs when grain-based or grass-based diets are fed normally, leading to relatively more n-6 or n-3 fatty acids respectively. Wood and Enser (1997) indicated that in ruminants the challenge is to increase the P:S ratio while retaining low values for n-6:n-3 similar to those found in cattle and sheep fed on forage diets.

Effect of Finishing on Pasture vs. Grain on Beef Fatty Acid Profiles

The fatty acid compositions of grain- and grass-based diets are quite different and lead to different fatty acid compositions in tissues. Green pasture may contain up to 3 % FAs on a dry matter basis, of which about 90 % will be unsaturated C18 acids (Murphy et al., 1995). Forages are usually lower in fat than most grains fed to cattle, and generally

contain greater proportions of 16:0 and 18:3, and lower proportions of 18:1 and 18:2 (Rule et al., 1995).

Body (1974) reported that the lipid of white clover leaves and stems was primarily linoleic (C18:2) and linolenic (C18:3) acids. Linolenic acid also was found to be a major constituent of the total lipid content of a mixture of tall fescue, orchardgrass and ladino clover (Melton et al., 1982). In contrast to forages, linoleic acid is the major fatty acid in cereals and oil seeds used in concentrate diets (Wood and Enser, 1997). For instance, the lipid component of sorghum is high in 18:1 (34 %) and 18:2 (50 %) and low in 18:3 (3 %) (Becker, 1992).

Jakobsen (1999) pointed out that the n6:n3 ratio is relatively high in the most commonly used feed ingredients for farm animals (cereal grains, soy oil, corn oil, sunflower oil), except in linseed oil, fish oil, and grass. The fat content, FA composition, and the n6:n3 ratio for commonly fed grains and forages are compared in Table 1.1.

Finishing diet (grass vs grain) has been shown to affect the fatty acid composition of beef. Several reports have shown that in ruminants, grain feeding leads to higher concentrations of unsaturated fatty acids, particularly oleic acid (C18:1) and lower concentrations of saturated fatty acids, particularly stearic acid (C18:0) compared with forage feeding (Wood, 1984). Westerling and Hedrick (1979) analyzed the effects of grass and grain diets on the fatty acid composition of intramuscular and subcutaneous fat of steers and heifers. It was found that both intramuscular and subcutaneous fat from grass-fed animals contained more saturated fatty acids, palmitic and stearic, and less unsaturated fatty acids, primarily oleic, than did fat from grain-fed animals. Further research comparing grass and concentrates for finishing cattle has shown that beef from animals grazed on pasture contained higher concentrations of the fatty acids, branched 15:0 (13-methyl tetradecanoic acid), branched 15:1 (13-methyl tetradecenoic acid), 18:0, 18:3, 20:3, 20:4, and 22:5 and less of 16:0 and 17:0 (Brown et al., 1979; Westerling and Hedrick, 1979; Miller et al., 1981; Melton et al., 1982;) than beef from concentrate-fed animals. Rule et al. (1995) also showed that grass-fed animals typically have lower 18:1, and greater 18:0 and 18:3 proportions than grain-fed animals. Larick and Turner (1989) compared the fatty acid profile of steers fed either in confined (all grain) or grain-on-grass feeding facilities. Muscle from grain-on-grass cattle had greater quantities of PUFAs in the neutral and polar lipids, higher 18:2 and 18:3 in the neutral lipids, and higher 18:2, 18:3, 20:3, 20:4, and 22:5 in the polar lipids than confined animals.

Recent research has focused on the nutritional importance of the P:S ratio, and more specifically the n-6:n-3 fatty acid ratio, as well as the importance of unique fatty acids such as CLA in the human diet. Yang et al. (2002a) reported that grain-fed beef contained the highest percentage of SFAs and MUFAs for longissimus and gluteus medius muscles; while pasture-fed beef had the highest percentage of PUFAs for longissimus, gluteus medius, and semimembranosus muscles. The percentage of polyunsaturation ranged from 12.5 to 17.9 % for grass-fed beef compared to only 5.6 to 12.5 % for grain-fed beef. Duckett et al. (1993) reported a higher P:S ratio (0.26) for muscle from grass-finished steers than that from concentrate-finished animals (0.07). Rule et al. (2002) reported P:S ratios of 0.23 and 0.12 for pasture-fed cows and feedlot steers, respectively; while French et al. (2000) reported ratios of 0.13 and 0.09 for grassfed and concentrate-fed steers.

Mitchell et al. (1991) and Enser et al. (1998) showed that adipose tissues from grass-based diets had higher concentrations of n-3 PUFA in body tissues, while concentrate-based diets had higher concentrations of n-6 PUFA. These differences are a consequence of fatty acid composition of the diet, α -linolenic acid (18:3, the n-3 series precursor) being the major fatty acid in grass lipids, and linoleic acid (18:2, the n-6 series precursor) being a major component in grains (Marmer et al., 1984).

Several reports similarly found a lower n-6:n-3 PUFA ratio in grass-fed cattle than in concentrate-fed cattle. Wood and Enser (1997) reported n-3:n-6 ratios of 0.77 and 0.12 for grass- and concentrate-fed beef, respectively. Grass-fed cattle showed higher longissimus concentrations of the n-3 FAs 18:3, 20:4, EPA, DPA, and DHA, while concentrate-fed cattle had higher concentrations of the n-6 FAs 18:2, 20:3 and 20:4 (arachidonic). Enser et al. (1998) reported a much less desirable range in the n-6:n-3 ratio in muscles from bulls fed concentrates (15.6-20.1) compared with steers fed grass (2.0-2.3). Rule et al. (2002) obtained n-6:n-3 ratios of 1.95 and 6.38 for pasture-fed cows and feedlot steers, respectively; while French et al. (2000) showed ratios of 2.33 and 4.15 for grass-fed and concentrate-fed steers.

French et al. (2000) compared the fatty acid composition of steers offered different proportions of grass and concentrates ranging from 8 kg of concentrate plus 1 kg of hay to 22 kg of grazed grass DM. Results showed that decreasing the proportion of concentrate in the diet, caused a linear decrease in the concentration of intramuscular

9

SFAs, and in the n-6:n-3 ratio; and a linear increase in the P:S ratio and the CLA concentration.

CLA concentrations in beef products from different studies are presented in Table 1.2. Research has shown that including grass in the diet of dairy and beef cattle increased CLA concentration in milk and beef intramuscular fat, respectively (Lawless et al., 1998; French et al., 2000; Yang et al., 2002a; Rule et al., 2002).

French et al. (2000) reported 10.8 and 3.7 mg total CLA/g lipid in longissimus muscle for grass-fed and concentrate supplemented grass-fed beef, respectively. Shantha et al. (1997) reported 7.7 and 5.2 mg total CLA/g lipid in semimembranosus muscle for grass-fed and corn supplemented grass-fed beef, respectively. Rule et al. (2002) reported 4.1 and 2.6 mg CLAc9t11/g lipid in longissimus muscle for pasture-fed cows and feedlot steers, respectively. Sackmann et al. (2002) reported 4.5 mg CLA c9,t11/g lipid in longissimus muscle for grain-fed heifers; while Mir et al., (1999) reported a much lower CLA concentration of 1.7 mg total CLA/g lipid in pars costalis diaphragmatis muscle for cattle fed a barley-based diet.

CLA concentrations have also been reported for ground beef. Chin et al. (1992) reported concentrations of 4.3 mg/g lipid; while Shantha et al. (1994) reported a range in CLA concentrations for ground beef from the chuck between 6.6 and 8.2 mg/g fat. The nutritional background of the animals in both studies is unknown since Chin et al. (1992) and Shantha et al. (1994) obtained samples from retail markets. Sackmann et al. (2002) reported concentrations of 7.0 mg CLA c9,t11/g lipid in ground beef from the chuck for grain-fed heifers.

Some Effects of Altering Beef Fat on Meat Quality

Ruminant edible products are characterized by their highly saturated fat, which has decreased consumer interest. Available information suggest that it is possible to produce beef enriched with specific fatty acids through dietary means (grass vs grain), in order to make meat and meat products more acceptable to consumers from a health perspective. However, altering the PUFA composition in beef may have important implications in other quality characteristics of meat such as shelf life and flavor.

The greatest sensory difference in beef from forage-fed and grain-fed steers appears to be in flavor of the fat (Maruri and Larick, 1992). The less desirable flavor of forage-fed beef has been described as intense milky-oily, sour, fishy, or grassy flavor (Shroeder et al., 1980; Melton et al., 1982). High concentrations of unsaturated fatty acids increase the potential for rancidity and formation of off-flavors often associated with forage-finished beef (Bennett et al., 1995). Altered meat flavors have been linked to higher concentrations of linolenic acid (C18:3; Mandell et al., 1998) and other lipids including diterpenoids (product of ruminal catabolism of chlorophyll) (Griebenow et al., 1997), polar lipids, and lipid-soluble compounds from plants.

Although an increase in the n-3 fatty acid concentration is desirable from a human health perspective, oxidative stability in meat is reduced. Lipid and muscle pigment oxidation are the major problems causing shelf life quality deterioration in meat. It has been shown that PUFAs are extremely reactive and through thermal (Selke et al., 1980) and autoxidative degradation (Lea, 1957; Younathan and Watts, 1960) will yield a number of carbonyl compounds that influence flavor. Shahidi (1992) estimated that linolenic acid is twice as prone to lipid oxidation as linoleic acid. Thus, higher PUFA concentrations may contribute to the more rapid development of oxidative rancidity reported in grass-fed beef (Yang et al., 2002a).

Conclusions

The importance of grain as a feed base for cattle may be declining and the participation of forages in finishing diets increasing in line with the greater need for extensification in agriculture. Griebenow et al. (1997) emphasized that feedlots are coming under close scrutiny due to problems associated with animal waste, and large amounts of grains being fed to cattle rather than direct human consumption. Demeyer and Doreau (1999) highlighted that some technologies available for modification of ruminant fat may be questionable by consumers or governments (feed processing, fatty acyl amides, feed additives, genetic manipulation), and indicated that the role of pasture vs. concentrate feeding in providing PUFA-enriched products should receive more attention.

Current research has shown that animals finished on forages have higher levels of PUFAs, omega-3 fatty acids, and CLA than cattle finished on concentrates. However, altering the PUFA content in beef may have important implications for meat quality, such as shelf life and flavor characteristics of the meat due to their greater susceptibility to oxidation and the production of volatile compounds during cooking. Thus, the greater susceptibility to oxidation and flavor defects of PUFA-enriched beef require further evaluation. The improvement of oxidative stability of beef with antioxidants, vitamin E and vitamin C, is considered next in this review.

Improvement of Oxidative Stability of Beef with Vitamin E and Vitamin C

Quality deterioration in meat occurs because of lipid and muscle-pigment oxidation. These two factors are the most important problems in maintaining a stable display of retail beef. The fine control mechanisms that exist to prevent lipid oxidation reactions in vivo are much less effective in meat and lipid oxidation proceeds in a comparatively uncontrolled manner (Sies, 1986). Some implications of lipid oxidation in muscle foods involve deterioration in flavor, odor, color, nutritive value and even safety of meat products (Buckley et al., 1995).

The oxidative stability of muscle depends upon the balance between antioxidants, such as α -tocopherol and some carotenoids, and pro-oxidants including the concentrations of polyunsaturated fatty acids (PUFA) and free iron in the muscle (Monahan, 2000). Dietary supplementation with α -tocopheryl acetate (vitamin E) is known to be effective in increasing muscle α -tocopherol levels and improving oxidative stability after slaughter. Vitamin C, which is a powerful synergist with vitamin E, also prevents color and lipid oxidation in beef.

This review will discuss briefly general aspects of lipid and pigment oxidation, followed by a discussion of the effects of vitamin E, vitamin C and their interaction on myoglobin and lipid stability of beef.

Lipid oxidation

Of the factors affecting rate and extent of lipid oxidation in muscle, the primary one is the level of polyunsaturated fatty acids present in tissue. Of the muscle lipid fractions, the polar phospholipids contain the highest proportion of unsaturated fatty acids and it has been established that this fraction, as opposed to the neutral lipid fraction, is primarily responsible for lipid oxidation in meat (Igene et al., 1980). Lipid oxidation is the process by which molecular oxygen reacts with unsaturated lipids to form lipid peroxides. Lipid oxidation is known to proceed by a free radical chain reaction mechanism involving initiation, propagation/branching, and termination stages (see Monahan, 2000 for more details).

Pigment oxidation

Color of fresh beef is a primary factor used by consumers to judge meat quality (Cassens et al., 1988). Pigmentation in beef is principally due to myoglobin, which is present in skeletal muscle fibers and bound to the outer membrane of mitochondria and the sarcoplasmic reticulum. The redox state of heme iron and the presence and nature of the ligand bound to myoglobin account for the color imparted to meat. Oxidation of heme iron from the ferrous state in deoxymyoglobin and oxymyoglobin to the ferric state in metmyoglobin results in formation of the brownish-red color that consumers find undesirable. Rancidity is due to oxidation of unsaturated fatty acids in meat phospholipid and is correlated with myoglobin oxidation in fresh beef (Faustman et al., 1989a).

Vitamin E

Vitamin E is a potent and widely studied antioxidant in biological systems. The major naturally-occurring lipid soluble antioxidant in skeletal muscle is tocopherol. Tocopherols consist of four different isomers, α , β , gamma and δ . α -tocopherol, which has the highest vitamin E activity, is a lipophilic antioxidant that is present in all cellular membranes.

The principal antioxidant role of vitamin E is to neutralize free radicals that could initiate a chain reaction, particularly among unsaturated fatty acids in membranes (Burton and Traber, 1990). Cellular and subcellular membranes are susceptible to lipid oxidation because of their relatively high concentration of PUFAs and close proximity to oxygen, transition metals and peroxidases (Vladimirov et al., 1980).

 α -tocopherol has been found to be an effective antioxidant when incorporated into muscle via the diet. However, when used as an additive in meat, its effectiveness is less predictable. Mitsumoto et al. (1993) reported that myoglobin and lipid oxidation were suppressed because of the greater antioxidant efficacy of endogenous rather than exogenous α -tocopherol.

Lipids are enclosed in small cells made of connective tissue, massed together in the familiar honeycomb formation (Figure 1.1). The cell walls consist mainly of collagen, with other proteins, hydrated with about 75% water. Access of any added substance to the lipid inside the cells is hindered mechanically by this cellular structure. For any chemical reaction to take place between the lipids and other substances not normally present in the fat cells, the other substances must first be transported from outside the meat, in the aqueous phase, along and through the fatty tissue cell walls. The reactions with lipid must then take place not in the aqueous phase but in the lipid phase or at the interface. Not many of the substances with the potential to be involved in lipid reactions can easily satisfy these solubility requirements (Ranken, 1994).

 α -tocopherol is positioned with its chromanol ring located among the polar portion of phospholips and its phytol side chain interacting with the unsaturated fatty acyl chains of phospholipids in the interior of the membrane (Fukuzawa and Fujii, 1992). This specific membrane localization of α -tocopherol (Figure 1.2) allows it to efficiently protect the highly oxidizable PUFAs from peroxidation by free radicals produced by the adjacent membrane bound enzymes and other interloping prooxidants (Farrell, 1988).

Cattle cannot synthesize vitamin E and normally obtain it by consuming pasture. Grains are relatively low in vitamin E and an extended grain-feeding period may deplete tissue α -tocopherol levels in cattle (Faustman et al., 1989b; Williams et al., 1992). Research has shown consistently that supranutritional supplementation of vitamin E to finishing cattle causes accumulation of α -tocopherol in muscle tissue, resulting in improved color and lipid stability. Vitamin E supplementation has improved myoglobin and lipid stability of fresh beef (Arnold et al., 1992; Arnold et al., 1993; Faustman et al., 1989a), ground beef (Faustman et al., 1989a; Mitsumoto et al., 1993) and frozen beef (Lanari et al. 1993).

In most published beef studies, dietary α -tocopheryl acetate has been supplemented to animals via grain concentrates, and in combination with forages. In general, the greater the amount of vitamin E and/or the longer the supplementation, the higher the tissue concentration of α -tocopherol (Arnold et al., 1993).

Faustman et al. (1989a) reported that a minimum tissue level of approximately 3.0 mg α -tocopherol/kg muscle was sufficient to have a significant impact on the reduction of pigment and lipid oxidation. Arnold et al. (1993) concluded that the target α -tocopherol level in fresh muscle for optimum protection against discoloration was approximately 3.5 µg α -tocopherol/g meat. However, Liu et al. (1996) concluded that these critical α -tocopherol concentrations reported by Faustman et al. (1989a) and Arnold et al. (1993) may be the minimal critical levels that need to be achieved in order to enhance meat quality.

Extensive research has been conducted using different levels of vitamin E supplementation for different periods of time. Morgan et al. (1993) and Sanders et al. (1993) reported that vitamin E supplementation of 500 IU per day for 84-126 days accomplishes the desired effect on beef case-life. Smith et al. (1996) reviewed results

from different field studies which reported that 500-1000 IU per head per day of vitamin E for 90-100 days prior to harvest is efficacious for beef marketed in both domestic and export trades.

Faustman et al. (1998) suggested that if a nutritional program delivers sufficient vitamin E to obtain the threshold level in muscle, then additional supplementation is unnecessary. It is often assumed that feed ingredients and forage supply a substantial proportion of the vitamin E requirements for animals, but this is not always true and vitamin E supplements should be formulated according to the nature of the other ingredients (e.g. levels of PUFAs) in the diet (Roche Report 1988 cited by Kerry et al., 2000).

 α -tocopherol concentrations in fresh forage can theoretically result in muscle saturation with α -tocopherol, since green forage may be a good dietary source of α tocopherol when pasture quality allows for high levels of α -tocopherol consumption (Faustman et al., 1998). However, α -tocopherol concentration in the muscle may not always be an accurate indicator of oxidative stability. Larick and Turner (1989) reported that diet influences the PUFA composition of phospholipids in M. pectoralis. Access to rye and ryegrass pasture vs. corn/corn silage or wheat/corn silage diets resulted in increased concentrations of C18:2, C18:3, C20:3, C20:4, and C22:5 fatty acids in the phosphlipid fraction of the muscle. Thus, the significance of α -tocopherol could be compromised by a phospholipid fraction that is more susceptible to lipid oxidation (Faustman et al., 1998). It would be interesting to see how TBARS values (lipid oxidation indicator widely reported in literature) compare among different studies if they were expressed as mg/g lipid rather than mg/g tissue, or even more specifically as mg/g of unsaturated lipid.

Gray et al. (1994) reported that the type of diet fed to meat-producing animals can influence meat flavor and lipid oxidation. These authors reported that beef from animals fed high energy diets (grain) was rated higher by sensory panels than beef from animals fed low-energy diets (forage). Research conducted recently in Australia (Yang et al., 2002a) showed that vitamin E supplementation of pasture-fed cattle did not alter muscle tocopherol contents. However, pasture feeding with and without vitamin E supplementation increased lipid oxidation of aged beef compared to supplemented grainfed beef. Results from these studies also confirmed the previously reported interaction between α -tocopherol and β -carotene by Pellett et al. (1994). The α -tocopherol supplementation of pasture-fed cattle reduced tissue concentrations of β -carotene, which would reduce carcass fat yellowness making grass-fed beef more acceptable to some Asian markets (Yang et al., 2002b).

The economic importance of vitamin E supplementation has been evaluated. Smith et al. (1996) calculated that the benefit-cost ratio for supplementing vitamin E to all grain fed steers/heifers would be 8.5:1 (based upon: benefit, \$672.7 million; cost, \$ 79.5 million). Smith et al. (1993) calculated that the cost range in supplementing a single steer/heifer was between \$1.43 and \$3.00 while the profit range to the US beef industry through retail cuts from a single animal was between \$20.29 and \$60.07.

Vitamin C

Ascorbic acid (vitamin C) is another nutrient with reducing capacity, which accounts for its ability to delay oxymyoglobin oxidation. Vitamin C is water-soluble and

functions by contributing either one or two electrons to more oxidized neighboring species. Ascorbate can reduce metal ions, scavenge singlet oxygen, and it can also reduce the α -tocopheryl radical (Parker, 1989).

Ascorbic acid either promotes or inhibits lipid oxidation reactions depending upon its concentration. At low concentrations, ascorbate accelerates lipid oxidation through its ability to reduce iron into the active ferrous state. Low concentrations (0.02 to 0.03%) of ascorbate have been reported to be either ineffective or prooxidative in beef (Roozen, 1987 cited by Decker and Mei, 1996; St. Angelo et al., 1988), whereas high concentrations (0.5%) can exhibit antioxidant activity in beef (St. Angelo et al., 1988; Shantha et al., 1995). At high concentrations, ascorbate inhibits lipid oxidation by inactivating free radicals and lipid oxidation catalysts such as ferryl myoglobin.

It has been shown that the antioxidant/prooxidant activity of ascorbate is influenced by iron concentrations, showing more pronounced antioxidant activity at lower concentrations. Thus, the antioxidant activity or ascorbic acid may be improved by using it in combination with chelators which control the iron activity (Decker and Mei, 1996).

Pre- and post-mortem application of vitamin C have been evaluated as a means for improving lipid and color stability of beef. Hood (1975) extended color display life by 5, 2 and 1-2 d for psoas, gluteus medius and semimembranosus, respectively, by infusing 1.4 mol ascorbic acid (2.8 mol/l) into the jugular vein of beef heifers 10 min. before slaughter. Liu et al. (1994) reported that intravenous infusion of sodium ascorbate caused transient accumulation of ascorbate in plasma and muscles, and that ascorbate improved color stability in muscles. Schaefer et al. (1995) suggested that intravenous infusion of ascorbate may not be practical for large-scale industry implementation because of its time requirement.

Mitsumoto et al. (1991a) studied the effects of vitamin C dip treatment on color and lipid stability in longissimus muscle from Holstein and crossbred beef steers. Dip treatment in vitamin C solution was effective in retarding oxidation of beef color and lipid in comparison with undipped control. Vitamin C dip treatment showed lower metmyoglobin percentages from day 7 and TBARS values from day 1 than the undipped control. Mitsumoto et al. (1991b) also studied the effect of spreading a vitamin C solution on the surface of fresh beef longissimus muscle. Vitamin C was a useful treatment to retard metmyogobin formation for at least 3 days compared to the control. Other researchers reported that the addition of vitamin C maintained a good color in ground beef (Mitsumoto et al., 1991c; Demos et al., 1996; Rhee et al., 1997).

When meat, including its fat, is minced or chopped, the cellular tissue is more or less broken down so that the lipids are exposed to chemical attack from which they were previously protected. At the same time the muscle tissue is also broken down and its constituents made more readily available for chemical or microbial interactions. Chemical changes including rancidity are therefore more likely to occur, or to proceed more rapidly, in ground meat than in the same meat without comminution. In the intact fat cell there may be natural antioxidant systems present. Disruption of the cells not only exposes the internal lipids to chemical attack but may also inactivate any protective antioxidants which may have beef present (Ranken, 1994). The economic importance of vitamin C treatment has not been evaluated. Liu et al. (1995) estimated that reduction of discoloration discounts at the retail level could result in added value to the U.S. beef industry in excess of \$700 million per year.

Finally, it should be mentioned that the use of ascorbic acid is limited in many meat products due to its ability to maintain the reduced state of myoglobin, thus potentially misleading consumer perception of meat freshness (Decker and Mei, 1996).

Interaction of vitamins E and C

Schaefer et al. (1995) proposed a model for the redox relationships between myoglobin and phospholipid in beef with emphasis on vitamins E and C (Figure 1.3). It seems that myoglobin and lipid are less prone to lipid oxidation, provided α -tocopherol is present with ascorbic acid (synergistic relationship). Once the tocopheryl radical is formed, the molecule is no longer an active antioxidant. However, ascorbic acid can reduce the tocopheryl radical, thus regenerating its antioxidant activity (Parker, 1989).

Mitsumoto et al. (1991a) showed that steaks from α -tocopherol acetatesupplemented LD had even greater color and lipid stability when dipped in L-ascorbic acid solutions, indicating that tissue α -tocopherol may interact with other compounds to provide an enhanced antioxidative effect. Mitsumoto et al. (1991c) added vitamin E, vitamin C or both to ground beef loin and found the combination to be the most effective antioxidant treatment.

Conclusions

Product shelf life is an increasing concern for the beef industry, which is becoming more consumer than production driven by both domestic and international markets. Thus, product quality and consistency are the main current objectives for the beef industry, and appearance and stability of the product are an important component of meat quality and consistency. Lipid and color stability are significant factors in developing convenience beef products. The beef industry is well behind its competitors (white meat and pork) in product development, and this is one of the problems associated with the consistent decrease in beef consumption over the years.

The literature clearly points out that vitamin E supplementation in the feedlot phase of beef production has important benefits on the shelf life of beef. However, these results have been poorly implemented in the industry so far, due to the segmented nature of the beef production system. Feeding cattle with vitamin E benefits the industry, and it is difficult for the producer to recover the investment. If this technology is to be adopted, a rapid and non-destructive method to detect and quantify vitamin E in beef is needed in order to implement a payment mechanism from the industry back to the producer.

Table 1.1

Fat content, fatty acid composition, and relative distribution of n-6:n-3 fatty acids in

		FatFatty acid (% of total fatty acids)							
		(%)	16:0	18:0	18:1	18:2	18:3	Others	n-6:n-3
Grains	Corn ^a	4.5	13	2	33	50	2	-	25
	Barley ^a	2.6	20	1	12	58	9	-	6.4
	Wheat ^a	2.2	21	2	14	58	5	-	11.6
	Oats ^a	4.4	19	1	33	44	3	-	14.7
	Concentrates ^b	2.6	33	20	25	17	2	3	-
Forages	Grass ^b	2.9	21	3	6	14	49	7	-
	Grass Mixture ^a	2.5	18	2	4	20	55	1	0.4
	Tall fescue ^c	2.1	20	5	4	11	55	5	-
	Perennial ryegrass ^c	2.3	22	4	5	13	51	5	-

grains and forages.

^a Jakobsen, 1999; ^b French et al., 2000 (concentrates: ground barley 46 %, sugar beet pulp 42 %, soybean meal 8 %, and tallow 1 %); ^c Dewhurst et al., 2001 (Tall fescue: *Festuca arundinacea*, Perennial ryegrass: *Lolium perenne*).
Table 1.2

Conjugated linoleic acid (CLA) concentrations in beef products from different studies.

Reference	Type of beef product	Total CLA concentration (mg/g lipid)
Chin et al., 1992	Round	2.9 (79 % c9,t11)
	Fresh ground round	3.8 (84 % c9,t11)
	Fresh ground beef	4.3 (85 % c9,t11)
Mir et al., 1999	Barley-fed (par costalis diaphragmatis)	1.7
Shantha et al., 1997	Grass-fed (semimembranosus)	7.7 (7.4 c9,t11)
	Corn on grass-fed (semimembranosus)	5.2 (5.1 c9,t11)
Shantha et al., 1994	Ground chuck	6.6-8.2
	Raw steaks (ribeye, round, T-bone, sirloin)	3.1-8.5
Sackmann et al., 2002	Grain-fed (longissimus)	4.5 c9,t11
	Grain-fed (ground chuck)	7.0 c9,t11
French et al., 2000	Grass-fed (longissimus)	10.8
	Concentrate-fed (longissimus)	3.7
Rule et al., 2002	Grass-fed (longissimus)	4.1
	Feedlot (longissimus)	2.6
Fritsche et al., 1998	Beef fat from German foods	1.2-12.0
Fogerty et al., 1988	Lean Australian beef	2.3-12.5



Figure 1.1. Lipids enclosed in small cells in meat tissue.^a

^aAdapted from Ranken, 1994.



Figure 1.2. Schematic illustration of endogenous (incorporated) vs. exogenous (added)

 α -tocopherol.^a

^aAdapted from Mitsumoto, 2000





polyunsaturated fatty acids, SOD: superoxide dismutase).^a

^a Adapted from Schaefer et al., 1995

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CHAPTER 2

EFFECT OF FINISHING ON PASTURE VS. CONCENTRATE AND ANTIOXIDANTS ON FATTY ACID COMPOSITION, COLOR, AND SHELF LIFE OF URUGUAYAN BEEF ¹

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Abstract

Thirty Hereford steers were finished either on pasture (PASTURE, n=10) or concentrate (CONC, n=20) to determine dietary and antioxidant treatment effects on fatty acid composition, shelf life, and meat quality of Uruguayan beef. Half of the steers finished on concentrate were supplemented with 1000 I.U. vitamin E head⁻¹d⁻¹ (VITE). Postmortem vitamin C (VITC) was added to ground beef (1% w/v:) displayed for 8 d at 2° C. CONC carcasses had greater (P < 0.05) carcass weight, conformation, degree of finishing, fat depth, and ribeye area than PASTURE. PASTURE carcasses showed darker (P < 0.05) longissimus color and yellower (P < 0.05) fat at 24 h postmortem than CONC. Initial Warner-Bratzler shear force (WBSF) values were similar (P > 0.05) between PASTURE and CONC beef. However, PASTURE beef had lower (P < 0.05) WBSF values at 7 and 14 d postmortem. Longissimus α -tocopherol levels were greater (P < 0.01) for PASTURE and CONC-VITE compared to CONC. Ground beef from VITE had the lowest TBARS values, while samples from PASTURE had the lowest lipid stability with numerically higher TBARS levels than other treatments. VITC treatment did not (P > 0.05) reduce lipid oxidation of ground beef. Steaks from PASTURE and VITE had similar (P > 0.05) TBARS values, which were lower (P < 0.05) than steaks from CONC during 21 d of display. VITE supplementation of CONC cattle had no effect (P > 0.05) on color stability of ground beef or steaks. The a* (redness) values were higher when VITC was added to ground beef. Longissimus fatty acid content of CONC was twofold greater (P < 0.01) than PASTURE. The percentages of 14:0, 14:1, 16:0, 16:1 and 18:1 fatty acids were higher (P < 0.01) in the intramuscular fat of CONC, while PASTURE showed greater (P < 0.01) proportions of 18:0, 18:2, 18:3, 20:4, 20:5, and 22:5. Total

conjugated linoleic acid (CLA) and CLA isomer c9t11 were higher (P < 0.01) for PASTURE than CONC. VITE supplementation of CONC increased lipid stability of ground beef and steaks, but was unable to improve color stability; whereas VITC addition to ground beef increased color stability without altering lipid oxidation. Finishing cattle on pasture enhanced the unsaturated fatty acid profile of intramuscular fat in beef including CLA and omega-3 fatty acids.

Key Words: Beef, Pasture, CLA, Antioxidants

1. Introduction

Beef cattle production systems in Uruguay rely almost exclusively on grazed pastures. However, more recently intensive beef production systems have gained increased interest by some beef producers. The focus is to produce a differentiated product in a vertically integrated manner to target both domestic, but particularly international markets. This production system, however, differs from a typical feedlot grain-based diet in the United States, in that the rations are formulated with 50 % maize silage, and 50 % grain.

Dietary recommendations for humans promoting the consumption of less saturated fat have led to an increased interest in meats containing more unsaturated fatty acids. Consumption of saturated fatty acids (SFA) has been associated with increased serum low-density-lipoprotein cholesterol concentrations, and increased risk of coronary heart disease (Keys, 1970). Ruminant fat has a higher SFA and a lower polyunsaturated:saturated fatty acid (PUFA:SFA) ratio than non-ruminant fat, due to hydrogenation of dietary unsaturated fatty acids in the rumen (French et al., 2000a). However, the nutritional background of meat-producing animals may alter the fatty acid composition of ruminant tissue fat.

Recent research has focused on the nutritional importance of the n-6:n-3 fatty acid ratio in the human diet, and on conjugated linoleic acid (CLA) isomers because of their anticarcinogenic properties (Ha et al., 1990, Ip et al., 1994). The nutritional value of n-3 PUFAs is well recognized, and increased consumption of these fatty acids has been recommended (Department of Heath, 1994). Ruminant fats are among the richest natural sources of CLA, in particular the cis-9, trans-11 isomer, which arises from microbial hydrogenation of dietary linoleic acid in the rumen (Ha et al., 1990). Previous research has shown that including grass in the diet of dairy and beef cattle increased CLA concentration in milk and beef intramuscular fat, respectively (Lawless et al., 1998; French et al., 2000a; Yang et al., 2002a).

Although an increase in the n-3 fatty acid concentration is desirable from a human health perspective, oxidative stability of meat is reduced. Lipid and muscle pigment oxidation are the major problems causing quality deterioration in meat. Thus, enrichment with antioxidants is necessary in order to prevent the risk of oxidative damage (Jakobsen, 1999). The objectives of this study were to compare carcass characteristics, beef quality, and longissimus fatty acid composition from cattle finished on pasture or on a concentrate-based diet; and to evaluate the effect of antioxidants, antemortem vitamin E and postmortem vitamin C, on product shelf life.

2. Materials and Methods

2.1. Animals and diets

Thirty Hereford steers backgrounded on pasture were finished either on pasture (n=10) or concentrate (n=20) during summer (November 2001-February 2002). Pastureand concentrate-fed steers were fed in commercial operations run by the National Institute of Agricultural Research of Uruguay in conjunction with the Uruguayan Hereford Breeders Association, and the Uruguayan Association of Natural Intensive Beef Producers. The pasture consisted predominantly of perennial ryegrass (*Lolium perenne*), birdsfoot trefoil (*Lotus corniculatus*), white clover (*Trifolium repens*), and tall fescue (*Festuca arundinacea*) with presence of weeds. The concentrate ration consisted of 50 % maize silage, 28 % wheat hulls, 18 % corn, and 5 % supplement (predominantly wheat hulls, Rumensin[®], and urea). Half of the steers finished on concentrate were supplemented with 1000 I.U vitamin E head⁻¹ d⁻¹. Concentrate-fed steers were slaughtered in a commercial meat plant according to normal procedures after 100 d of finishing. Pasture-fed steers were slaughtered in the same commercial meat plant after a grazing period of 130 d.

2.2. Slaughter and sampling procedures

Carcasses were graded after slaughter using the Uruguayan grading system as specified by the National Meats Institute (I.N.A.C., 1997), and carcass data recorded (conformation, age, degree of finishing, dentition). At 24 h postmortem, carcasses were cut between the 12th and 13th ribs and additional carcass data collected (ribeye area, fat depth, pH, subcutaneous fat and ribeye area color). The pistola cut was weighed and the ribeye roll (IMPS 112) and clod (IMPS 114) were removed from each carcass, vacuumpackaged and transported to a meat laboratory.

The ribeye roll was fabricated into steaks for fatty acid analysis, vitamin E determination, tenderness, and lipid and color stability measurements. Steaks for fatty acid analysis, vitamin E and shear force measurements were individually vacuum packaged and frozen for subsequent analysis. Steaks for lipid and color stability measurements were individually placed on Styrofoam trays, over-wrapped with oxygen permeable film, and displayed for 21 d at 2°C in a lighted cooler. The clod was stored in a cooler at 2°C for one week. After storage the clod was trimmed and ground (0.635 cm). Each ground beef sample was divided into two equal sub-samples. Sodium ascorbate (1% w/v; VITC) was added to one sub-sample, mixed, and formed into 114 g patties. The remaining sub-sample did not receive a postmortem VITC treatment and was formed into 114 g patties. Patties samples were placed on Styrofoam trays, overwrapped with oxygen permeable film and stored for 8 d in a 2°C lighted cooler.

2.3. Lipid oxidation analysis

Lipid stability was evaluated in the ground beef and steaks that were displayed for instrumental color. Lipid oxidation was determined by measuring 2-thiobarbituric acid reactive substances (TBARS, Jo and Ahn, 1998) at 0, 5, 13 and 21 d of display for steaks, and at 0, 3, and 8 d of display for ground beef.

2.4. Instrumental color

Instrumental color measurements were recorded for L* (lightness; 0: black, 100: white), a* (redness/greenness; positive values: red, negative values: green), and b* (yellowness/blueness; positive values: yellow, negative values: blue) using a Minolta

chromameter (CR-210, Minolta Inc., Osaka, Japan). Color readings were determined on subcutaneous fat and ribeye area at 24 h postmortem. Ground beef and steak color measurements were obtained at 0, 3, 6, and 8 d, and 0, 5, 13, and 21 d of display for each sample, respectively. Values were recorded from three locations of the upper surface of each patty and steak randomly selected to obtain a representative reading of the surface color.

2.5. Fatty acid composition

Steaks were submerged in liquid nitrogen (-196°C), pulverized and stored at -20°C. Total lipid was determined following the chloroform-methanol procedure of Folch et al. (1957), modified by using a 10:1 ratio of chloroform-methanol to sample. Extract containing approximately 25 mg of lipid was converted to fatty acid methyl esters (FAME) following the method of Park and Goins (1994). The FAME were analyzed using a HP6890 (Hewlett-Packard) gas chromatograph, and separated using a 100-m SP 2560 capillary column (0.25 mm i.d. and 0.20 µm film thickness, Supelco, Bellefonte, PA). Column oven temperature was programmed at 150 to 165°C at 1°C/min, 165 to 167°C at 0.2°C/min, 167 to 225°C at 1.5°C/min and held at 225°C for 15 min with 1:100 split. The injector and detector were maintained at 250°C. Hydrogen was the carrier gas at a flow rate of 1 mL/min. Individual fatty acids were identified by comparison of retention times with standards (Sigma, St. Louis, MO; Supelco, Bellefonte, PA; Matreya, Pleasant Gap, PA).

2.6. Tenderness (Warner-Bratzler Shear Force)

Steaks (2.5 cm) were vacuum packaged, stored in a cooler at 2°C and frozen after 0, 7 and 14 d of aging for subsequent Warner-Bratzler shear force determination. Steaks

were thawed for 24 h at 2°C, and boiled in a water bath to an internal temperature of 71°C (AMSA, 1995). Steaks were allowed to come to room temperature before six 1.27cm cores were removed per steak parallel to the longitudinal orientation of the muscle fibers. All cores were sheared perpendicular to the long axis of the core using a Warner-Bratzler meat shear machine (Standard Shear Model 2000 D, G-R Manufacturing Co; Manhattan, Kansas).

2.7. Vitamin E analysis

Muscle α -tocopherol concentrations were measured according to Koprivnjak et al. (1996) with modifications, using reverse phase high performance liquid chromatography (HPLC AKTA Purified System, Amersham Pharmacia Biotech) with fluorescence detection (Shimadzu RF-10A XL). Briefly, duplicate 25 mg muscle samples were weighed, combined with 400 µL of methanol, and mixed vigorously using a pestle for 15 sec. The homogenate was then vortexed for 30 sec, and centrifuged for 10 min at 14000 rpm (microcentrifuge Eppendorf 5415C). An aliquot of the supernatant was transferred into a vial and 30 µL were injected into the HPLC. Wavelength settings were 295 nm and 325 nm for excitation and emission, respectively. The mobile phase consisted of methanol with a flow rate of 1.0 mL min⁻¹. The column used was Sephasil Peptide C18 5u ST 4.6/100. The α -tocopherol concentrations were calculated based on peak area of external standards (Sigma, St. Louis, MO).

2.8. Statistical analysis

Results were analyzed by analysis of variance using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). Pre-planned, non-orthogonal contrasts were used to compare means from cattle finished on pasture and concentrate with or without vitamin E

supplemention. Variables measured over time were analyzed as repeated measures. Vitamin E supplementation did not alter any carcass characteristic, Warner-Bratzler shear force, or fatty acid composition, and data were pooled across vitamin E treatment for concentrate-fed cattle.

3. Results and Discussion

3.1. Carcass characteristics

Vitamin E supplementation did not alter (P > 0.05) any of the carcass characteristic measured. The effect of finishing beef cattle on pasture or concentrate on carcass characteristics is shown in Table 2.1. Carcasses from cattle finished on concentrate were heavier (P < 0.05) than those finished on pasture. Conformation scores tended to be lower (P < 0.10) indicating better conformation from carcasses finished on concentrate than pasture. Carcasses from concentrate-finished animals had greater (P <0.05) fat depth, ribeye area, and degree of finishing than pasture-finished animals. Dietary treatment did not alter (P > 0.05) age at slaughter, pistola cut weight, or longissimus muscle pH values. Similarly, Bidner et al. (1986) and Morris et al. (1997) observed no change in muscle pH between pasture- and concentrate-fed cattle.

3.2. Instrumental longissimus and fat color

Objective color measurements of longissimus muscle and subcutaneous fat at the $12/13^{\text{th}}$ rib interface taken at 24 h postmortem are shown in Table 2.2. Longissimus muscle of animals finished on pasture had lower (P < 0.05) L* values indicating a darker colored lean than concentrate-finished animals. Others (McCaughey & Cliplef, 1996; Bidner et al., 1986; Schroeder et al., 1980) have shown darker muscle color in pasture-fed

than grain-fed beef. There were no differences (P > 0.05) in longissimus a* or b* among treatments.

For subcutaneous fat, carcasses from pasture-fed cattle had higher (P < 0.05) L* values than carcasses from animals fed concentrate and supplemented with vitamin E. Finishing cattle on pasture increased (P < 0.05) subcutaneous fat b* values indicating more yellowness compared to fat from concentrate finished animals not supplemented with vitamin E. However, subcutaneous fat b* values did not differ (P > 0.05) among pasturefed cattle and concentrate-fed animals that were supplemented with vitamin E. Numerous studies have consistently shown that feedlot-finished cattle have whiter fat color scores than pasture-fed animals (Schaake et al, 1993; Bennett et al., 1995; Simonne et al., 1996). Fat color is largely dependent on its carotenoid content derived from plants. Green, fresh pastures usually contain high quantities of carotenoids (up to 500 ppm of dry matter); dry or cut hay may have considerably less (less than 50 ppm), whereas most grains contain only small concentrations of carotenoids (usually less than 5 ppm, Tume & Yang, 1996). More recently, Yang et al. (2002b) studied the effect of vitamin E supplementation on α to copherol and β -carotene concentrations in tissues from pasture- and grain-fed cattle. It was shown that α -tocopherol supplementation of pasture-fed cattle reduced tissue concentrations of β -carotene, which would reduce carcass fat yellowness. Pellett et al. (1994) also reported that high concentrations of α -tocopherol appear to reduce the concentrations of β -carotene. Although the carotene concentration in subcutaneous fat from concentrate-fed animals in this study would be expected to be much lower than that from pasture-fed steers, vitamin E supplementation did not decrease subcutaneous b* values in concentrate-fed cattle.

3.3. Beef tenderness (Warner-Bratzler Shear Force)

Warner-Bratzler shear force (WBSF) values over 14 d of aging are presented in Figure 2.1. Initial tenderness did not differ (P > 0.05) between pasture- and grain-fed cattle (4.7 vs. 4.5 kg, respectively). Studies comparing forage vs. concentrate finishing of beef have produced mixed results on palatability attributes. Some studies found a negative effect of forage finishing on meat tenderness (Mitchell et al., 1991; Smith, 1990), while others showed that pasture-fed beef can be produced with no deleterious effects on meat quality including tenderness (Mandell et al., 1998; French et al., 2001). In many experiments, dietary effects are confounded with animal age, growth rate or carcass weight and fatness at slaughter. In this study, initial shear force values were similar between steaks from pasture- and concenctrate-fed steers despite differences (P < 0.05) in carcass weight (226 vs. 240 kg), fatness (fat depth: 3.8 vs. 6.1 mm), and temperature during chilling (Table 2.3). More extensive aging was evident in steaks from pasture-fed cattle, which had WBSF values approximately 1 kg (P < 0.01) and 0.6 kg (P < 0.05) lower at 7 and 14 d of aging respectively than steaks from concentrate-fed animals. French et al. (2001) found that supplementing grass with low levels of concentrate produced the most tender and acceptable meat at two days postmortem, but that further aging eliminated all treatment effects on eating quality of beef. Huffman et al. (1996) proposed that a WBSF of 4.1 kg could be used as a threshold to indicate that 98 % of restaurant and home consumers would find a longissimus steak acceptable in tenderness. Based on this threshold, longissimus steaks from both dietary treatments in this study would be acceptable in tenderness (WBSF < 4.1 kg) after 7 d of aging.

3.4. Muscle a-tocopherol concentrations

Longissimus concentrations of α -tocopherol were greater (P < 0.01, Figure 2.2) for pasture- and concentrate-fed cattle supplemented with vitamin E (3.91 and 3.74 μ g/g, respectively), compared to concentrate-fed control animals (2.92 μ g/g). Faustman et al. (1989) reported minimum tissue levels of 3.0 μ g α -tocopherol/g muscle, while Arnold et al. (1993) proposed 3.5 μ g/g as the target concentration to have a significant impact on the reduction of pigment and lipid oxidation. Liu et al. (1996) concluded that these critical α -tocopherol concentrations might be the minimal critical levels that need to be achieved in order to enhance meat quality. Results from different field studies reported that 500-1,000 IU.animal⁻¹.d⁻¹ of vitamin E for 90-100 days prior to harvest is efficacious for beef marketed in both domestic and export trades (Smith et al., 1996). Roeber et al. (2001) evaluated the effect of three supplementation levels with α -tocopherol on product shelf life, and concluded that 1,000 IU.animal⁻¹.d⁻¹ of α -tocopherol for at least 100 d can be used to increase retail caselife and to improve the overall color acceptability of steaks and ground beef products. In the present study, supplementation of α -tocopherol to concentrate-fed cattle with 1,000 IU.animal⁻¹.d⁻¹ for 100 d was sufficient to achieve similar (P > 0.05) muscle α -tocopherol content to grass-fed cattle, at levels beyond the proposed critical concentrations for improving shelf life.

Vitamin E supplementation of pasture-fed cattle was not considered in this research. Faustman et al. (1998) suggested that if a nutritional program delivers sufficient vitamin E to obtain the threshold level in muscle, then additional supplementation is unnecessary. α -tocopherol concentrations in fresh forage can theoretically result in muscle saturation with α -tocopherol, since green forage may be a good dietary source of

 α -tocopherol when pasture quality allows for high levels of α -tocopherol consumption (Faustman et al., 1998). Research conducted recently in Australia (Yang et al., 2002a) showed that vitamin E supplementation of pasture-fed cattle did not alter muscle tocopherol contents.

3.5. Lipid oxidation

Table 2.4 shows lipid oxidation values, as determined by TBARS, for ground beef samples displayed during 8 d at 2°C. Ground beef from cattle finished on concentrate showed higher (P < 0.05, 0 d) initial lipid oxidation values than pasture-fed. At 3 d of display, there were no differences (P > 0.05) among dietary treatments (pasture, concentrate, and concentrate-vitamin E) in lipid oxidation. However, TBARS values for ground beef from pasture-fed animals were numerically higher (P > 0.05) than samples from concentrate-fed cattle, with the exception of vitamin C treated ground beef from cattle supplemented with vitamin E. Ground beef from vitamin E supplemented cattle finished on concentrate had lower numerical (P > 0.05) TBARS than control samples without addition of vitamin C. However, TBARS were higher (P < 0.05) when vitamin C was added. At 8 d of display, ground beef from vitamin E supplemented cattle had lower (P < 0.05) lipid oxidation values, exhibiting greater lipid stability as a consequence of the vitamin E antioxidant activity, than ground beef treated with vitamin C from concentratefed cattle, and pasture-fed cattle regardless of vitamin C addition. However, lipid oxidation of ground beef from vitamin E supplemented cattle did not differ (P > 0.05) from samples treated with vitamin C or from ground beef from concentrate-fed cattle without vitamin C. Ground beef from pasture-fed steers had the lowest lipid stability with numerically higher TBARS levels than other treatments. There was no evidence of

vitamin C treatment effect or a synergistic effect between vitamin E and C on lipid oxidation during 8 d of lighted display at 2°C. There was no interaction (P > 0.05) between dietary treatments and vitamin C. These results are in disagreement with Schaefer et al. (1995) who pointed out that myoglobin and lipid are less prone to lipid oxidation, provided α -tocopherol is present with ascorbic acid. Parker et al. (1989) proposed that once the tocopheryl radical is formed, the molecule is no longer an active antioxidant, but ascorbic acid can reduce the radical regenerating its antioxidant activity. Previous studies also showed that steaks and ground beef from α -tocopherol acetatesupplemented LD had even greater color and lipid stability when dipped in L-ascobic acid solution (Mitsumoto et al., 1991a,b).

Figure 2.3 shows the TBARS values for longissimus steaks at different storage times during 21 d of lighted display at 2°C. Steaks from pasture-fed animals had lower (P < 0.01) initial TBARS values than steaks from concentrate-fed cattle. With increasing display time, lipid oxidation was lower (P < 0.05) for steaks from pasture-fed and vitamin E supplemented concentrate-fed cattle than for control concentrate-fed steers. This is in contrast to the results of Yang et al. (2002a) who found that pasture feeding increased lipid oxidation of aged beef compared to vitamin E supplemented grain-fed beef, despite similar muscle α -tocopherol concentrations. In the present study, pasture-fed beef achieved similar muscle α -tocopherol concentrations as well as similar lipid stability to steaks from concentrate-fed cattle supplemented with vitamin E. The results for concentrate-fed cattle are consistent with previous research findings which showed that supplementing feedlot cattle with vitamin E improved lipid stability of beef during retail display (Houben et al., 2000; Roeber et al., 2001; Stubbs et al., 2002).

3.6. Instrumental color of longissimus steaks and ground beef

The changes in ground beef color (L*, a*, and b*) over 8 d of illuminated display at 2°C are shown in Table 2.5. Ground beef from pasture-fed cattle had numerically higher (P > 0.05) L* values than concentrate-fed animals, indicating a lighter product after 6 d of display. Dietary treatment did not alter (P > 0.05) a* or b* values during display. Supplementation of concentrate-fed cattle with vitamin E had no effect (P > 0.05) on color L*, a*, or b* of ground beef samples. Vitamin C treatment did not alter (P > 0.05) L* values of ground beef. In contrast to vitamin E, vitamin C addition to ground beef showed a clear effect on redness for all dietary treatments (pasture, concentrate, and concentrate-vitamin E), by delaying metmyoglobin formation and retaining a redder (P < 0.05) color during display. Addition of vitamin C also had a clear effect on yellowness (b* value) of ground beef. Vitamin C treated ground beef was yellower (P < 0.05) than control ground beef from pasture-fed, concentrate-fed and vitamin E supplemented concentrate-fed cattle.

The changes in longissimus steak color (L*, a*, b*) over 21 d of display at 2°C are presented in Figure 2.4. Steaks from pasture-fed cattle had higher (P < 0.05) L* values than concentrate-fed cattle during 21 d of display. This is in contrast to the results from longissimus color recorded at 24 hr postmortem, which showed that pasture-fed steers had lower L* values indicating a darker color. Pasture-fed beef was redder (P < 0.05) and yellower (P < 0.05) than concentrate-fed beef after 5 d of display regardless of vitamin E supplementation. Vitamin E supplementation of concentrate-fed cattle had no effect (P > 0.05) on L*, a*, or b* lean color values. The lack of response to vitamin E supplementation in color stability of ground beef as well as whole muscle is in agreement

to Yang et al. (2002a), who reported that supra-nutritional supplementation of grain-fed cattle with vitamin E did not affect meat redness or stability compared with nonsupplemented cattle. However, these results were only evaluated over a 7 d period of aerobic storage. In contrast to the results in this study, previous publications have shown important responses in meat color stability to vitamin E supplementation of grain-fed cattle (Houben et al., 2000; Roeber et al., 2001; Stubbs et al., 2002). Yang et al. (2002a) attributed the absence of response to vitamin E supplementation in color stability to the relatively high levels of muscle α -tocopherol (1.8-2.4 µg/g) present in non-supplemented grain-fed animals. Eikelenboom et al. (2000) suggested that α -tocopherol concentrations between 2.1 and 4.4 μ g/g in unsupplemented muscle may have reduced the response of the LD muscle to the vitamin E treatment. Results from this research showed that the mean α -tocopherol level for longissimus muscle of unsupplemented concentrate-fed cattle (2.92 μ g/g) was within this concentration range. The steers used in this research were backgrounded on good quality pastures before the differential dietary treatments. Hill & Williams (1993) and Yang et al. (2002a) reported limited benefit from vitamin E supplementation on color stability of fresh beef from cattle fed good quality pasture immediately before grain feeding. The composition of the concentrate ration fed to cattle in the present study, is different from the corn- or sorghum-based feedlot rations used in the finishing production systems of USA or Australia. The depletion of α -tocopherol in muscle as a consequence of a feedlot finishing period would be slower when the ration is predominantly corn silage with corn and wheat hulls compared to a grain-based ration.

3.7. Intramuscular fatty acid composition

The lipid content and fatty acid composition of longissimus intramuscular fat for pasture- and concentrate-fed cattle are presented in Table 2.6. Vitamin E supplementation had no effect (P > 0.05) on the fatty acid content or composition of intramuscular fat. Longissimus fatty acid content of concentrate-fed steers was twofold greater (P < 0.01) than pasture-fed animals (3.18 vs. 1.68 %, respectively). These values are similar to the mean fat contents reported by Yang et al. (2002a) in LD from concentrate- and pasture-fed animals (3.63 vs. 1.71 %, respectively). The main fatty acids in the intramuscular fat from pasture- and concentrate-fed cattle were oleic (18:1), palmitic (16:0) and stearic (18:0), which acounted for 71 % and 77 % of the total fatty acids, respectively.

The percentages of myristic (14:0), myristoleic (14:1), palmitic (16:0), palmitoleic (16:1) and oleic (18:1) acids were higher (P < 0.01) in the intramuscular fat of concentrate finished cattle than pasture-fed animals. Pasture-fed cattle had higher (P < 0.01) concentrations of stearic (18:0), linoleic (18:2) linolenic (18:3), arachidonic (20:4), eicosapentaenoic (20:5, EPA), and docosapentaenoic (22:5, DPA) acids than concentrate-fed cattle. Dietary treatment did not alter (P > 0.05) the concentration of docosahexaenoic acid (22:6, DHA). Similarly, others (Brown et al., 1979; Melton et al., 1982) have shown greater concentrations of stearic, linolenic and arachidonic acids in pasture-fed vs. concentrate-fed animals.

More lipid oxidation would be expected from the higher content of PUFAs in the fat of longissimus steaks from pasture-fed compared to concentrate-fed animals. However, longissimus α -tocopherol content from pasture-fed cattle was higher than the proposed critical concentrations for improving shelf life leading to greater (P < 0.05) lipid stability than steaks from concentrate-fed animals that were not supplemented with vitamin E. Mincing muscle tissue disrupts cellular integrity and exposes more of the lipids to the oxidative catalysis; it also dilutes the antioxidants and increases the exposure of the tissue to oxygen (Hultin, 1988). This may explain the greater lipid oxidation observed in ground beef from pasture-fed animals compared to ground beef from concentrate-fed cattle regardless of vitamin E supplementation at 8 d of display. However, muscle α -tocopherol concentrations or fatty acid composition of ground beef from different dietary treatments were not measured in this study.

Intramuscular fat from pasture-fed cattle had greater (P < 0.05) concentrations of total CLA and CLA isomer cis-9, trans-11 than concentrate-fed (5.3 vs. 2.5 and 4.1 vs. 2.3 mg CLA/g lipid, respectively). Previous research has shown that including pasture in the diet of dairy and beef cattle increased CLA concentration in milk and beef intramuscular fat, respectively (Lawless et al., 1998; French et al., 2000a; Yang et al., 2002a). French et al. (2000a) reported 10.8 and 3.7 mg total CLA/g lipid in longissimus muscle for grass-fed and concentrate supplemented grass-fed beef, respectively. Shantha et al. (1997) reported 7.7 and 5.2 mg total CLA/g lipid in semimembranosus muscle for grass-fed and corn supplemented grass-fed beef, respectively. Rule et al., (2002) reported 4.1 and 2.6 mg CLAc9t11/g lipid in longissimus muscle for pasture-fed cows and feedlot steers, respectively.

Grass-fed beef contained a similar (P > 0.05) proportion of saturated fatty acids (SFA), a lower (P < 0.05) concentration of monounsaturated fatty acids (MUFA) and a higher (P < 0.01) percentage of polyunsaturated fatty acids (PUFA) than concentrate-fed cattle. Current recommendations are that the PUFA:SFA (P:S) ratio should be around

0.45 (Department of Health, 1994). The P:S ratios in this study were lower than the recommended ratio, being 0.20 for pasture-fed and 0.13 for concentrate-fed cattle. Duckett et al. (1993) also reported a higher P:S ratio (0.26) for muscle from grass-finished steers than for that from concentrate-finished animals (0.07). Similar P:S ratios to concentrate-fed cattle from this study have been reported for typical retail beef in the UK (0.11, Enser et al., 1996) and fish-meal-supplemented Charolais steers (0.11-0.13, Mandell et al., 1997).

Intramuscular fat from pasture-fed cattle had a more favorable n-6:n-3 fatty acids ratio than concentrate-fed animals (1.4 vs. 3.0, respectively). An increase in the consumption of n-3 fatty acids is recommended (Department of Health, 1994) to overcome the imbalance in the ratio of n-6:n-3 PUFA in the current diets (10:1) compared to primitive man (1:1, Eaton et al. 1996). Mitchell et al. (1991) and Enser et al. (1998) reported that adipose tissues from pasture-based diets had higher concentrations of n-3 PUFA in body tissues, while concentrate-based diets had higher concentrations of n-6 PUFA. These differences are a consequence of fatty acid composition of the diet, α -linolenic acid (18:3, the n-3 series precursor) being the major fatty acid in grass lipids, and linoleic acid (18:2, the n-6 series precursor) being a major component in grains (Marmer et al., 1984). Previous research similarly found a lower n-6:n-3 PUFA ratio in pasture-fed cattle than in concentrate-fed cattle. Rule et al. (2002) reported n-6:n-3 ratios of 1.95 and 6.38 for pasture-fed cows and feedlot steers, respectively; while French et al. (2000) reported ratios of 2.33 and 4.15 for grass-fed and concentrate-fed steers.
4. Conclusions

Pasture-fed carcasses showed darker ribeye area color and yellower fat at grading than concentrate-fed. Altough pasture-fed carcasses were lighter and leaner than concentrate-fed, there were no differences in initial tenderness between the groups. Moreover, pasture-fed beef showed a greater potential for postmortem tenderization through aging, becoming more tender than concentrate-fed beef after 7 d of storage.

Supplementation of α -tocopherol to cattle finished on concentrate with 1,000 IU animal⁻¹ d⁻¹ for 100 d was sufficient to achieve similar muscle α -tocopherol content to pasture-fed cattle, at levels beyond the proposed critical concentrations for improving shelf life. Ground beef displayed for 8 d from supplemented cattle showed greater lipid stability as a consequence of the vitamin E antioxidant activity, while pasture-fed ground beef was the least stable. However, similar lipid stability was found in steaks displayed during 21 d between pasture-fed and supplemented concentrate-fed cattle. Vitamin E supplementation of concentrate-fed animals improved lipid stability of beef steaks. There was no evidence of a vitamin C treatment effect or a synergistic effect between vitamin E and C on ground beef lipid stability. Supplementation of cattle finished on concentrate with vitamin E had no effect on color stability of ground beef or steaks. However, vitamin C addition to ground beef was effective in delaying metmyoglobin formation and retaining a redder color during display.

Beef from pasture-fed cattle resulted in a higher P:S ratio and a lower n-6:n-3 ratio in intramuscular fat of steers compared to concentrate-fed beef. Finishing on pasture was an effective way to obtain CLA-enriched beef and thus the potential health benefits of CLA consumption in humans. Results from this study suggest that the negative image

of beef attributed to its highly saturated nature may be overcome by enhancing the fatty acid profile of intramuscular fat in beef through pasture feeding from a human health perspective.

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Table 2.1

Mean (\pm S.E.) carcass characteristics of steers.

Characteristic	Pasture (n=10)	Concentrate (n=20)
Hot carcass wt, kg	$225.6^{a} \pm 4.405$	$240.1^{b} \pm 3.115$
Age ^e	1.9 ± 0.121	$1.8~\pm~0.086$
Conformation ^f	$3.0^{\rm c} \pm 0.123$	$2.7^{d} \pm 0.087$
Degree of finishing ^g	$1.5^{a} \pm 0.095$	$2.0^{b} \pm 0.067$
Pistola cut wt, kg ^h	46.5 ± 0.911	47.4 ± 0.645
Fat depth, mm	$3.8^{a} \pm 0.614$	$6.1^{b} \pm 0.434$
Ribeye area, cm ²	$55.2^{a} \pm 2.168$	$62.9^{b} \pm 1.533$
PH	$5.7~\pm~0.039$	$5.7~\pm~0.028$

^{e,f,g} Carcass characteristics were evaluated according to the procedures of the Uruguayan National Meats Institute (INAC, 1997). ^{a,b}Means within the same row with uncommon superscripts differ (P<0.05).

^{a,o}Means within the same row with uncommon superscripts differ (P<0.05). ^{c,d}Means within the same row with uncommon superscripts differ (P<0.10). ^eBased on dentition, a lower number indicates younger animal (1: baby teeth to 4:full teeth).

^fA lower number indicates better conformation (1 to 6).

^gA lower number indicates lack of finishing (0 to 4).

^hPistola cut (round and loin).

Table 2.2

Diet	Pasture (n=10)	Concentrate (n=10)	Concentrate (n=10)	
Vitamin E (I.U.head ⁻¹ d ⁻¹)	0	0	1000	S.E.
REA color				
L*	33.80 ^b	35.56 ^a	36.34 ^a	0.692
a*	20.45	20.42	20.95	0.520
b*	8.77	8.44	9.22	0.313
Fat color				
L*	72.44 ^a	71.81 ^{ab}	69.78 ^b	0.858
a*	5.94	5.15	5.51	0.302
b*	15.23 ^a	13.53 ^b	14.48^{ab}	0.540

Lightness (L*), redness (a*), and yellowness (b*) of longissimus and subcutaneous fat at

24 hr postmortem.

^{a,b}Means within the same row with uncommon superscripts differ P < 0.05.



Fig. 2.1. Effect of postmortem aging on shear force of longissimus steaks from pasture (n=10) and concentrate (n=20).

	Temperature, °C			
Chilling time	Pasture (n=10)	Concentrates (n=20)		
1 h	$36.63^{a} \pm 0.422$	$39.25^{b} \pm 0.298$		
3 h	$22.14^a\pm0.599$	$26.65^{b} \pm 0.423$		
22 h	$0.73^{a} \pm 0.154$	$1.77^{\rm b} \pm 0.109$		

Table 2.3. Temperature (means \pm S.E.) change during chilling from pasture-fed, and concentrate-fed carcasses.

^{a,b} Means within the same raw with uncommon superscripts differ P < 0.01.



Fig. 2.2. Muscle α -tocopherol concentrations from grass (n=10), concentrates (n=10), and concentrates-vitamin E (n=10).

Table 2.4

Levels of 2-thiobarbituric acid (TBARS, mg malonaldehyde/kg sample) values during display of ground beef at 2°C.

Diet	Concentrate				Pas	Pasture	
Vitamin E (I.U.head ⁻¹ d ⁻¹)	0	0	1000	1000	0	0	
Vitamin C (1 % w/v)	0	1	0	1	0	1	S.E.
0 d	0.245 ^a	0.245 ^a	0.264 ^a	0.264 ^a	0.155 ^b	0.155 ^b	0.019
3 d	0.493^{ab}	0.368 ^b	0.406 ^{ab}	0.552^{a}	0.561 ^a	0.527^{ab}	0.065
8 d	0.457^{ab}	0.580 ^a	0.357 ^b	0.466 ^{ab}	0.646 ^a	0.587^{a}	0.079

^{a,b}Means within the same row with uncommon superscripts differ P < 0.05.



Fig. 2.3. Levels of 2-thiobarbituric acid (TBARS, mg malonaldehyde/kg sample) values during display of steaks at 2°C from pasture (n=10), concentrate (n=10), and concentrate-vitamin E (n=10).

Diet			Concentrate				ture	
Vitamin E (IU/he	ad/d)	0	0	1000	1000			
Vitamin C (1 % v	w/v)	0	1	0	1	0	1	S.E.
Lightness, L*	0 d	36.884	36.884	36.887	36.887	37.450	37.450	0.567
	3 d	35.953	38.110	36.557	37.147	36.567	37.410	0.735
	6 d	36.133 ^b	37.113 ^{ab}	36.477 ^b	36.977 ^{ab}	37.257 ^{ab}	38.360 ^a	0.501
	8 d	35.490 ^c	37.053 ^{ab}	36.460 ^{bc}	36.177 ^{bc}	37.510 ^{ab}	38.267 ^a	0.521
Redness, a*	0 d	27.457	27.457	27.430	27.430	29.240	29.240	0.861
	3 d	21.263 ^a	25.127 ^b	20.870 ^a	26.133 ^b	21.910 ^a	25.510 ^b	0.665
	6 d	19.933 ^c	25.117 ^b	20.127 ^c	25.257 ^b	19.800 ^c	28.153 ^a	0.620
	8 d	18.013 ^d	21.727 ^c	18.103 ^d	23.710 ^b	19.927 ^{cd}	26.983 ^a	0.081
Yellowness, b*	0 d	13.773	13.773	13.963	13.963	14.500	14.500	0.470
	3 d	11.763 ^a	13.727 ^b	11.903 ^a	13.623 ^b	12.283 ^a	13.593 ^b	0.371
	6 d	11.100 ^c	12.884 ^b	11.410 °	13.113 ^b	11.750 °	14.620 ^a	0.308
	8 d	10.847 ^e	12.433 ^{bc}	11.177 ^{de}	12.497 ^b	11.737 ^{cd}	14.083 ^a	0.315

Color L*, a*, and b* values during display of ground beef at 2°C.

a,b,c,d,e Means within the same row with uncommon superscripts differ P < 0.05.



Fig. 2.4. Color L*, a*, and b* values during display of longissimus steaks at 2° C from pasture (n=10), concentrate (n=10), and concentrate-vitamin E (n=10).

Table 2.6

Intramuscular fatty acid composition (lsmean \pm S.E.) from pasture-fed (n=10), and

Fatty acid, %	Pasture (n=10)	Concentrate (n=20)
Total lipid	$1.68^{a} \pm 0.245$	$3.18^{b} \pm 0.173$
•		
14:0, myristic	$1.64^{a} \pm 0.104$	$2.17^{b} \pm 0.073$
14:1, myristoleic	$0.23^{a} \pm 0.025$	$0.41^{b} \pm 0.017$
16:0, <i>palmitic</i>	$21.61^{a} \pm 0.530$	$24.26^{b} \pm 0.375$
16:1, palmitoleic	$2.50^{a} \pm 0.140$	$3.38^{b} \pm 0.099$
18:0, <i>stearic</i>	$17.74^{a} \pm 0.507$	$15.77^{b} \pm 0.358$
18:1, <i>n-9 oleic</i>	$31.54^{a} \pm 0.771$	$37.28^{b} \pm 0.545$
18:2, n-6 linoleic	$3.29^{\circ} \pm 0.217$	$2.84^{d} \pm 0.154$
18:3, <i>n-3 linolenic</i>	$1.34^{a} \pm 0.055$	$0.35^{b} \pm 0.039$
CLA [*] c9t11	$0.41^{a} \pm 0.023$	$0.23^{b} \pm 0.016$
Total CLA ^{**}	$0.53^{a} \pm 0.031$	$0.25^{b} \pm 0.022$
20:4, n-6 arachidonic	$1.28^{a} \pm 0.097$	$0.95^{\rm b}~\pm~0.069$
20:5, <i>n-3</i> EPA [*]	$0.69^{a} \pm 0.053$	$0.30^{ m b}~\pm~0.037$
22:5, <i>n-3 DPA</i> [*]	$1.04^{a} \pm 0.070$	$0.56^{\rm b} \pm 0.047$
22:6, <i>n-3 DHA</i> *	0.09 ± 0.016	0.09 ± 0.012
Unidentified	$16.49^{a} \pm 0.603$	$11.41^{b} \pm 0.426$
*		
SFA	49.08 ± 0.723	47.62 ± 0.511
MUFA [*]	$40.96^{a} \pm 0.796$	$46.36^{b} \pm 0.563$
PUFA [*]	$9.96^{a} \pm 0.607$	$6.02^{b} \pm 0.429$
PUFA:SFA	$0.20^{a} \pm 0.013$	$0.13^{b} \pm 0.009$
<i>n-6:n-3</i> ratio	$1.44^{a} \pm 0.109$	$3.00^{\rm b} \pm 0.077$

concentrate-fed (n=20) cattle.

^{a,b}Means within the same column with uncommon superscripts differ P < 0.01. ^{c,d}Means within the same column with uncommon superscripts differ P < 0.10. ^{*}CLA: conjugated linoleic acid, EPA: eicosapentaenoic acid, DPA:

docosapentaenoic acid, DHA: docosahexaenoic acid, SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids.

^{**} Total CLA includes: c9t11, t10c12, t9t11, and other isomers that were unable to be identified specifically.

CHAPTER 3

EFFECT OF VITAMIN C ADDITION TO GROUND BEEF FROM GRASS-FED OR GRAIN-FED SOURCES ON COLOR AND LIPID STABILITY, AND PREDICTION OF FATTY ACID COMPOSITION BY NEAR INFRARED REFLECTANCE ANALYSIS¹

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Abstract

Research was conducted to determine the effect of postmortem vitamin C addition (VITC) versus no VITC (CONTROL) to ground beef from grass-fed (GRASS) or grainfed (GRAIN) sources on color and lipid stability during 8 d of illuminated display at 4°C. The use of near infrared reflectance (NIR) spectroscopy to predict the fatty acid composition of ground beef and its potential to discriminate samples from different nutritional backgrounds were also evaluated. Total fatty acid content of ground beef was 53% lower (P < 0.05) for GRASS than GRAIN. Ground beef from GRASS had greater (P < 0.01) percentages of saturated (SFA) and polyunsaturated (PUFA) fatty acids, and lower (P < 0.01) percentages of monounsaturated (MUFA) fatty acids than GRAIN. For GRAIN, VITC reduced (P < 0.01) lipid oxidation, and resulted in darker (P < 0.01) and redder (P < 0.01) color of the ground beef from 2 to 8 d of display compared to CONTROL. For GRASS, lipid oxidation did not differ (P > 0.05) for VITC and CONTROL. VITC improved (P < 0.01) color stability by prolonging more red color in GRASS during 8 d of display. Results from partial least squares modeling showed accurate predictions using NIR for total saturated [standard error of performance (SEP = 1.16 %), coefficient of determination on the validation set $(r^2 = 0.87)$] and unsaturated (SEP = 1.18 % and r^2 = 0.90) fatty acid contents of ground beef, as well as the composition of stearic, oleic, and linolenic (SEP = 1.2, 1.27, and 0.07 %; $r^2 = 0.91, 0.92$, and 0.93, respectively). However, the composition of other individual fatty acids was poorly predicted. VITC was effective in retarding pigment oxidation in ground beef from both GRAIN and GRASS; however, VITC reduced lipid oxidation in GRAIN samples only, despite higher PUFA percentages in GRASS. NIR can be used to predict accurately

the content of total saturated and unsaturated, and stearic, oleic, and linolenic fatty acids in ground beef. NIRS showed potential to discriminate meat samples originating from different feeding production systems.

Keywords: beef, vitamin C, fatty acids, near infrared spectroscopy

Introduction

Product shelf life is an increasing concern for the beef industry, which has become more consumer than production driven in both domestic and international markets. Lipid and muscle pigment oxidation are the major problems causing shelf life quality deterioration in meat. Lipid oxidation is responsible for the development of offodors and off-flavors; and muscle pigment oxidation affects lean color, which is of critical importance in consumer purchase decisions for fresh meat. Liu et al. (1995) estimated that reduction of discoloration discounts at the retail level could add value to the U.S. beef industry in excess of \$700 million per year. Addition of antioxidants to meat products is known to be effective in reducing pigment and lipid oxidation and extending the product shelf life. Pre- and post-mortem application of vitamin C has been evaluated as a means for improving lipid and color stability of beef (Mitsumoto et al., 1991; Schaefer et al., 1995; Wheeler et al., 1996; Rhee et al., 1997).

Dietary recommendations for humans promoting the consumption of less saturated fat have led to an increased interest in meats containing more unsaturated fatty acids. Consumption of saturated fatty acids (SFA) has been associated with increased serum low-density-lipoprotein cholesterol concentrations, and increased risk of coronary heart disease (Keys, 1970). The nutritional background of meat-producing animals can influence the fatty acid composition of ruminant tissue fat. Meat from pasture-fed cattle may have increased concentrations of polyunsaturated fatty acids (PUFA) compared with beef from grain-fed cattle. Meats containing greater contents of the highly unsaturated lipids may be more prone to lipid oxidation than those more saturated, and consequently may respond differently to antioxidants (Yang et al., 2002).

Near infrared (NIR) spectroscopy has been developed as a rapid and accurate technical tool for estimating chemical compositions of foods, and it has been applied to determination of total fat and fatty acids in beef muscle without prior fat extraction (Windham and Morrison, 1998). In recent years the importance of the 'green image' of animal products, and traceability of production systems has aroused great consumer interest (Prache and Theriez, 1999).

This study was designed to evaluate the effect of postmortem vitamin C addition to ground beef from grass-fed or grain-fed sources on color and lipid stability during 8 d of illuminated display at 4°C. The use of NIR to predict the fatty acid composition of ground beef and its potential to discriminate samples from different nutritional backgrounds were also evaluated.

Materials and Methods

2.1. Meat samples

Ground beef samples were obtained from two sources: 1) 18 carcasses harvested after finishing on a high concentrate diet for 112 d (GRAIN) or 2) 14 carcasses harvested after finishing on tall fescue (*Festuca arundinacea*) pastures for 135 d (GRASS). After harvest, the clod (IMPS 114) was removed from each carcass, trimmed and ground (0.635 cm). Each ground beef sample was divided into two equal sub-samples. Sodium ascorbate (1% w/v; VITC) was added to one sub-sample, mixed, and formed into 114 g patties. The remaining sub-sample did not receive a postmortem treatment (CONTROL) and was formed into 114 g patties. Patties were placed on Styrofoam trays, overwrapped with oxygen permeable film, and stored in a 4°C lighted cooler for each display time (0, 2, 4, and 8 d) and treatment (CONTROL vs. VITC).

2.2. Lipid oxidation analysis

Lipid stability was evaluated in the ground beef samples that were displayed for instrumental color. Lipid oxidation was determined by measuring 2-thiobarbituric acid reactive substances (TBARS, Jo and Ahn, 1998) at 0, 2, 4 and 8 d of display, and was expressed as mg of malonaldehyde produced per kg of sample.

2.3. Instrumental color

Instrumental color measurements were recorded for L* (lightness; 0: black, 100: white), a* (redness/greenness; positive values: red, negative values: green), and b* (yellowness/blueness; positive values: yellow, negative values: blue) using a Minolta chromameter (CR-210, Minolta Inc., Osaka, Japan). Color readings were obtained at 0, 2, 4, and 8 d, of display from three locations of the upper surface of each patty randomly selected to obtain a representative reading of the surface color.

2.4. Sensory analysis

Triangle test for difference was conducted on ground beef from VITC and CONTROL samples to determine whether there was a significant difference between the treatments in aroma and flavor. The ground beef samples were formed into patties, broiled to an internal temperature of 71°C, and served warm to a six-member trained sensory panel following AMSA guidelines for cookery and sensory evaluation of meat (AMSA, 1978). Panelists were seated in individual booths with pass through doors for serving samples under red lights to eliminate the influence of cooked meat color.

2.5. Fatty acid analysis

Ground beef samples were submerged in liquid nitrogen (-196°C), pulverized and stored at -20°C. Total lipid was determined following the chloroform-methanol procedure of Folch et al. (1957), modified by using a 10:1 ratio of chloroform-methanol to sample. Extract containing approximately 25 mg of lipid was converted to fatty acid methyl esters (FAME) following the method of Park and Goins (1994). The FAME were analyzed using a HP6890 (Hewlett-Packard) gas chromatograph, and separated using a 100-m SP 2560 capillary column (0.25 mm i.d. and 0.20 µm film thickness, Supelco, Bellefonte, PA). Column oven temperature was programmed at 150 to 165°C at 1°C/min, 165 to 167°C at 0.2°C/min, 167 to 225°C at 1.5°C/min and held at 225°C for 15 min with 1:100 split. The injector and detector were maintained at 250°C. Hydrogen was the carrier gas at a flow rate of 1 mL/min. Individual fatty acids were identified by comparison of retention times with standards (Sigma, St. Louis, MO; Supelco, Bellefonte, PA; Matreya, Pleasant Gap, PA, USA).

2.6. Statistical analysis

Fatty acid content and composition data were analyzed using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC) with feed source (GRASS or GRAIN) in the model. Lipid oxidation and color data were analyzed as repeated measures.

2.7. Spectroscopic analysis

Duplicate sub-samples of approximately 100 g of ground beef were placed in a rectangular-shaped cell (44 x 114 x 12 mm), and scanned with the NIRSystem 6500

monochromator (NIRSystems, Silver Spring, MD, USA) and the transport attachment. The spectrum of each sub-sample was the average of 16 scans obtained as the cell made one pass by the detector. The duplicate scans of each sample were examined visually for consistency and then averaged.

ISI software (Infrasoft International Inc., Port Matilda, PA, USA) was used to process NIR data and develop chemometric models, covering a wavelength range of 400-2500 nm. A total of 256 ((18 GRAIN + 14 GRASS) x 4 display times (0, 2, 4 and 8 d) x 2 CONTROL vs. VITC) ground beef samples were scanned. Ground beef samples were chosen for calibration and validation data with a procedure titled SELECT. SELECT is an algorithm that compares the Mahalanobis (H) distance of one sample spectrum to that of all other samples. If the difference in the H distance between individual samples is less than a specified cutoff value (0.5), those samples statistically have the same spectra and only one sample from that "neighborhood" is needed for the calibration data set. Thus, using SELECT, 68 samples were selected. The SELECT ground beef samples were randomly then divided into calibration (n = 48) and validation (n = 20) sets. The method of partial least squares (PLS) was used for all chemometric models. A second central difference with gap=20 nm and a smoothing interval=10 nm was performed on the spectra to reduce sample-to-sample baseline variation and to enhance absorption peaks.

Cross validation was performed during model development, whereby one-fourth of the calibration samples at a time were temporarily removed from the calibration set. Performance statistics were accumulated for each group of removed samples. The optimal number of factors for a fatty acid model was that which produced a minimum in overall error between modeled and reference values (standard error of cross validation). On completion of a calibration for a fatty acid, the model was applied to the validation samples. Model performance was reported as the coefficient of determination (r^2) , the standard error of performance (SEP), and the average difference between modeled and reference values (bias).

Results and Discussion

3.1. Lipid content and fatty acid composition

The lipid content and fatty acid composition of ground beef samples from GRAIN and GRASS sources are presented in Table 3.1. Total fatty acid content of the samples was 53 % lower (P < 0.05) for ground beef from GRASS than GRAIN. The main fatty acids from GRASS and GRAIN were oleic (18:1), palmitic (16:0) and stearic (18:0), which accounted for approximately 79 and 80 % of the total fatty acids, respectively. The percentages of myristic (14:0), myristoleic (14:1), palmitoleic (16:1), oleic (18:1), and arachidonic (20:4) fatty acids were higher (P < 0.05) in ground beef from GRAIN than GRASS. There were no differences (P > 0.05) in palmitic (16:0) percentage between the groups. The proportions of stearic (18:0), linoleic (18:2), and linolenic (18:3) were higher (P < 0.01) in GRASS compared to GRAIN. The fatty acid compositions of grain- and grass-based diets are quite different and lead to different fatty acid compositions in tissues. Forages are usually lower in fat than most grains fed to cattle, and generally contain greater proportions of 16:0 and 18:3, and lower proportions of 18:1 and 18:2. Linolenic acid is the major fatty acid in grass lipids whereas cereals and oil seeds used in concentrate diets are major sources of linoleic acid (Wood & Enser, 1997). Results from this study are similar to those obtained by Rule, Smith & Romans (1995) which showed

that grass-fed animals typically have lower 18:1, and greater 18:0 and 18:3 proportions than grain-fed animals.

Conjugated linoleic acids (CLA) represent a mixture of positional and geometric isomers of linoleic acid, which have recently gained importance due to metabolic and anticarcinogenic properties in experimental animals. Ruminant fats are among the richest natural sources of CLA, in particular the cis-9, trans-11 isomer (Chin et al., 1992), which arises from microbial hydrogenation of dietary linoleic acid in the rumen (Kepler & Tove, 1967). Concentrations of total CLA and CLA isomer cis-9, trans-11 were 33 and 27 % higher for ground beef fat from GRASS compared to GRAIN. Previous research has shown that including grass in the diet of dairy and beef cattle increased CLA concentration in milk and beef intramuscular fat, respectively (Lawless et al., 1998; French et al., 2000; Yang et al., 2002). The CLA concentrations reported for ground beef in this study (12.6 and 8.4 mg total CLA/g lipid for GRASS and GRAIN, respectively) are higher than previously published values. Chin et al. (1992) reported CLA concentrations in ground beef of 4.3 mg/g lipid; while Shantha et al. (1994) reported a range in CLA concentrations for ground beef from the chuck between 6.6 and 8.2 mg/g fat. Both Chin et al. (1992) and Shantha et al. (1994) obtained samples from retail markets; thus, the different nutritional background of the animals in this study would likely explain the higher levels observed.

Ground beef samples from GRASS had greater (P < 0.01) percentages of saturated (SFA) and polyunsaturated (PUFA) fatty acids, and lower (P < 0.01) percentages of monounsaturated (MUFA) fatty acids than GRAIN. Current recommendations for a healthy diet are that the PUFA:SFA (P:S) ratio should be around 0.45 (Department of Health, 1994). The P:S ratios in this study were lower than the recommended ratio, being 0.09 for GRASS and 0.06 for GRAIN. Duckett et al. (1993) also reported a higher P:S ratio (0.26) for muscle from grass-finished steers than for that from concentrate-finished animals (0.07). Similar P:S ratios to grain-fed cattle from this study have been reported for typical retail beef in the UK (0.11, Enser et al., 1996) and fish-meal-supplemented Charolais steers (0.11-0.13, Mandell et al., 1998).

3.2. Lipid stability (TBARS)

Figure 3.1 shows lipid oxidation values, as determined by TBARS, for ground beef samples displayed for 8 d at 4°C. VITC addition reduced (P < 0.01) lipid oxidation in ground beef from GRAIN, but did not alter (P > 0.05) TBARS values in ground beef from GRASS. TBARS reductions in ground beef by vitamin C addition have been reported (Shivas et al., 1984; Mitsumoto et al., 1991; Kulshrestha and Rhee, 1996; Rhee et al., 1997). The oxidative stability of meat depends upon the balance between antioxidants, such as α -tocopherol and some carotenoids, and pro-oxidants including the concentrations of PUFA and free iron in muscle (Monahan, 2000). Meats containing greater contents of the highly unsaturated lipids may be more prone to lipid oxidation than those more saturated. However, results from this experiment showed that ground beef from GRASS was more stable than GRAIN over 8 d of display, and did not respond to vitamin C addition in retarding lipid oxidation despite higher PUFA percentages in GRASS. These differences in lipid stability may be attributed to a greater antioxidant capacity of ground beef from GRASS compared to GRAIN. The α -tocopherol concentrations in fresh forage can theoretically result in muscle saturation with α tocopherol, since green forage may be a good source when pasture quality allows for high levels of α -tocopherol consumption (Faustman et al., 1998). Yang et al. (2002) reported similar longissimus α -tocopherol content for grain-fed steers that were supplemented with 2500 IU dl- α -tocopheryl acetate/head/day, and grass-fed steers that were not supplemented with vitamin E (4.3 vs. 4.5 µg/g tissue, respectively).

3.3. Instrumental color

The changes in meat lightness (L*), and redness (a*) over 8 d of illuminated display at 4°C are shown in Figures 3.2 and 3.3, respectively. VITC addition had no effect (P > 0.05) on yellowness (b*) of ground beef from GRAIN and GRASS sources (data not presented). For GRAIN, VITC addition resulted in darker (P < 0.01) and redder (P < 0.01) color of the ground beef from 2 to 8 d of display compared to CONTROL. For GRASS (Figure 3.3), VITC improved (P < 0.01) color stability by prolonging more red color in ground beef during 8 d of display. However, VITC addition did not influence (P > 0.05) lightness of ground beef from GRASS, except at day 4 of display where VITC treated samples were lighter than CONTROL. Other workers also observed that the addition of vitamin C maintained a good color in ground beef (Shivas et al., 1984; Mitsumoto et al., 1991; Demos et al., 1996; Rhee et al., 1997).

3.4. Sensory panel evaluation

The percent of correct sample identification (VITC vs. CONTROL) by sensory panelists for aroma and flavor, was 23 % and 24 % for GRASS, and 44 % and 38 % for GRAIN, respectively. The lack of response to vitamin C addition in lipid oxidation from GRASS samples may explain the lower percentage of correct identification by panelists compared to GRAIN samples in sensory evaluation. However, sensory analysis indicated that vitamin C addition to ground beef may not have a profound effect on aroma and flavor of meat.

3.5. Near infrared spectroscopy

The range, mean and standard deviation of fatty acids (FA) from ground beef in the calibration and validation data sets are presented in Table 3.2. The range and mean values for each FA in the calibration set were similar to those in the validation data set.

Separate PLS NIR calibration equations were obtained for individual FAs, total saturated FAs and total unsaturated FAs. The total saturated FA model contained 5 factors, with scores from factors 1, 2, and 4 explaining 36, 17, and 16 % of the spectral variation, respectively. The total unsaturated FA model contained 5 factors, with scores from factors 1, 2, and 4 explaining 54, 15, and 9 % of the spectral variation, respectively.

The PLS regression coefficients as a function of wavelength (1550-2450 nm) for the total saturated and unsaturated FA models are shown in Figure 3.4 (a) and (b), respectively. This region of the spectra is primarily related to oil. The wavelengths 1724 and 1756 nm are related to CH_2 stretch first overtone (different lipid content), and at 2300 and 2348 nm related to CH combinations (Murray, 1986).

Tables 3.3 and 3.4 show the calibration and validation statistics for the saturated and unsaturated FA NIR equations, respectively. The standard errors of cross validation (SECV) ranged between 0.05 for linolenic and 1.22 for total saturated FAs. The multiple coefficients of determination (R^2) ranged from 0.37 for myristic to 0.95 for linolenic. The R^2 was higher for 18:0, 18:1, 18:2, 18:3 and total unsaturated FAs, and lower for 14:0, 16:0, and total saturated FAs than those reported by Windham and Morrison (1998) for beef neck muscle.

Samples were predicted for stearic acid and total saturated FA with an SEP of 1.2 and 1.16 % and r^2 of 0.91 and 0.87, respectively. Oleic, linolenic and total unsaturated FAs were accurately predicted with SEP and r^2 of 1.27, 0.07 and 1.18 %, and 0.92, 0.93 and 0.90, respectively. Validation statistics on all other individual FAs were not accurate. These results are in agreement with Windham and Morrison (1998) who suggested that the failure to determine accurately some individual FAs was due to similarities in their NIR absorption pattern, because different FAs have the same absorbing molecular group (-CH₂-), and the narrow standard deviation of the data sets. In the present study, similar standard deviation values of the data sets were found compared to those reported by Windham and Morrison (1998).

To determine the potential of NIR to discriminate between ground beef samples from different nutritional backgrounds, the calibration and validation spectral sample sets were combined and reduced with principal component analysis (PCA). PCA is a projection method for extraction of the systematic variations in a data set resulting in principal component models. Translated into principal components (PC), the new coordinate system has fewer dimensions than the original NIR data set, and the directions of the new coordinate axes (called principal components) were calculated to describe the largest variations. The localization of the samples in the new system, i.e. their coordinates related to the PCs, are called scores. Scores calculated from these components were plotted as shown in Figure 3.5.

The plot shows two distinctive clusters of data that correspond to the ground beef samples that derived from different animal feeding backgrounds (GRASS vs. GRAIN). Cozzolino et al. (2002) also reported the potential application of NIR as a rapid screening tool that will differentiate between meat samples that originate from different feeding systems. Cozzolino et al. (2002) proposed that moisture, pigments and fat could explain the discrimination between the contrasting groups of feeding systems. In this study, PC1 explained 47 % of the total variance with the highest loadings observed around 458 and 586 nm. These absorption bands are related with carotenoid and myoglobin meat pigments, respectively. Prache and Theriez (1999) reported that carotenoid pigments present in fat tissue absorb light between 450 and 510 nm. Liu and Chen (2000) reported at least 7 absorption bands at 430, 440, 455, 545, 560, 575, and 585 nm associated with the changes in the oxidation and denaturation of myoglobin due to cooking and cold storage. Greater tissue concentration of carotenoids (mainly β-carotene and lutein) in grass-fed compared to grain-fed cattle has been reported (Knight et al., 2001; Yang et al., 2002). PC2 explained 35 % of the total variance with the highest loadings found at 1726, 1766, 2310, and 2350 nm. These spectral regions are related to the fat content of the ground beef samples (Murray and Williams, 1987). Finally, PC3 explained 9 % of the total variance with the highest loadings at 1390, and 1878 nm which are characteristic of water absorption (OH overtones; Murray, 1986) and related to the moisture content of the beef samples.

Conclusions

Postmortem vitamin C addition was effective in retarding pigment oxidation in ground beef from both grain-fed and grass-fed sources; however, vitamin C addition reduced lipid oxidation in grain-fed ground beef samples only, despite higher PUFA percentages in grass-fed sources. A possible explanation for the observed difference in the effectiveness of vitamin C on lipid oxidation between ground beef sources, may be due to a higher antioxidant status in beef finished on pasture. Results from sensory analysis suggest that vitamin C addition to ground beef may have minimal effects on aroma and flavor of meat. This study showed that NIRS can be used to accurately predict the total saturated and unsaturated fatty acid contents of ground beef, as well as the composition of stearic, oleic, and linolenic. However, the composition of other individual fatty acids was poorly predicted. NIRS showed potential as a rapid and easily implemented method to discriminate ground beef samples originating from different nutritional systems.

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Fatty acid content and composition of ground beef from GRASS- and GRAIN-fed sources.

Fatty acid, %	GRASS (n=14)	GRAIN (n=18)
Total lipid	$11.43^{a} \pm 1.203$	$24.57^{b} \pm 1.061$
14:0, myristic	$2.91^{\circ} \pm 0.114$	$3.22^{d} \pm 0.101$
14:1, palmitoleic	$0.43^a\pm0.039$	$0.84^{b} \pm 0.034$
16:0, palmitic	23.86 ± 0.278	24.21 ± 0.245
16:1, myristoleic	$2.35^a\pm0.102$	$3.09^{b} \pm 0.090$
18:0, <i>stearic</i>	$19.44^{a} \pm 0.446$	$14.64^{b} \pm 0.393$
18:1, <i>oleic</i>	$35.41^{a}\pm0.538$	$41.58^b\pm0.474$
18:2, linoleic	$1.77^{c} \pm 0.064$	$1.49^{d}\pm0.057$
18:3, linolenic	$0.62^a\pm0.014$	$0.18^b\pm0.012$
CLA [*] c9t11	$0.98^a\pm0.052$	$0.72^{\rm b}\pm0.046$
Total CLA [*]	$1.26^{a} \pm 0.056$	$0.84^{b}\pm0.049$
20:4, arachidonic	$0.08^{a}\pm0.006$	$0.11^{b} \pm 0.006$
Unidentified	9.35 ± 0.091	6.46 ± 0.080
SFA^*	$48.11^{a} \pm 0.533$	$43.95^b\pm0.470$
MUFA [*]	$38.19^{a} \pm 0.558$	$45.51^{b} \pm 0.492$
PUFA [*]	$4.35^{a} \pm 0.091$	$2.63^{b} \pm 0.080$
PUFA:SFA	$0.09^{\rm a}\pm0.002$	$0.06^{\mathrm{b}}\pm0.002$

^{a,b}Means within the same column with uncommon superscripts differ P < 0.01 $^{\rm c,d}$ Means within the same column with uncommon superscripts differ P < 0.05

*CLA: conjugated linoleic acid; Total CLA: c9t11, t10c12, t9t11, other; SFA: percent of total identified saturated fatty acids; MUFA: percent of total identified monounsaturated fatty acids; PUFA: percent of total identified polyunsaturated fatty acids.

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Range, mean and standard deviation (SD) of fatty acids (weight percent methyl ester) for ground beef in the calibration and validation data sets.

Fatty acid	Calibration $(n=48)$		Validation $(n = 20)$		
	Range	Mean	SD	Range Mean	SD
14:0, myristic	1.50-4.13	3.06	0.50	2.41-4.15 3.01	0.45
16:0, palmitic	21.70-26.37	24.12	1.13	21.70-25.92 23.74	1.01
18:0, stearic	11.65-23.64	17.19	3.11	14.26-23.63 17.74	2.68
Total saturated [*]	40.84-52.04	46.05	3.05	42.27-51.74 46.20	2.95
18:1, <i>oleic</i>	31.95-43.79	38.27	3.85	32.15-45.58 38.54	3.96
18:2, linoleic	0.64-2.14	1.23	0.46	0.87-2.10 1.61	0.33
18:3, linolenic	0.14-0.72	0.43	0.23	0.13-0.72 0.41	0.23
Total unsaturated**	37.58-49.47	44.52	3.90	38.81-50.52 44.95	3.48

* Percentage of total identified saturated fatty acids ** Percentage of total identified unsaturated fatty acids

Calibration and validation statistics (weight percent methyl ester) for saturated fatty acids by NIRS spectroscopy.

	Calibration $(n = 48)$		Val	Validation $(n = 20)$		
Fatty acid	Factors	SECV ^a	R^2	SEP ^b	Bias	r^2
14:0, myristic	1	0.36	0.37	0.43	- 0.18	0.23
16:0, palmitic	6	0.69	0.61	0.76	- 0.29	0.10
18:0, stearic	7	0.99	0.87	1.2	0.70	0.91
Total saturated	5	1.22	0.82	1.16	0.16	0.87

^a SECV = Standard error of cross-validation.

^b SEP = Standard error of performance

Calibration and validation statistics (weight percent methyl ester) for saturated fatty acids by NIRS spectroscopy.

	Calibration $(n = 48)$		Validation $(n = 20)$			
Fatty acid	Factors	SECV	R^2	SEP	Bias	r^2
18:1, <i>oleic</i>	5	0.94	0.94	1.27	0.18	0.92
18:2, linoleic	2	0.26	0.65	0.60	0.34	0.04
18:3, linolenic	5	0.05	0.95	0.07	0.01	0.93
Total unsaturated	5	1.09	0.91	1.18	0.11	0.90



Fig. 3.1. Levels of 2-thiobarbituric acid (TBARS, mg malonaldehyde/kg sample) values during display of ground beef at 4°C from (a) grain-fed (n=14), and (b) grass-fed (n=18) cattle.



Fig. 3.2. Effect of vitamin C addition to ground beef from grain-fed cattle on color (a) L* lightness and (b) a* redness.



Fig. 3.3. Effect of vitamin C addition to ground beef from grass-fed cattle on color (a) L* lightness and (b) a* redness.



Fig. 3.4. PLS regression coefficients from 1550 to 2450 nm for fatty acids in ground beef from the chuck. (a) total saturated fatty acid 5-term model; (b) total unsaturated fatty acid 5-term model.



Fig. 3.5. Discrimination among ground beef samples from GRASS- and GRAIN-fed sources using ISI software.

CHAPTER 4

EFFECT OF ENDOPHYTE TYPE ON CARCASS TRAITS, MEAT QUALITY, AND FATTY ACID COMPOSITION OF BEEF CATTLE GRAZING TALL FESCUE¹

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Abstract

Fourteen Hereford steers were used to compare carcass traits, meat quality, and fatty acid composition of beef from cattle grazing tall fescue infected with either wildtype (TOXIC; n = 6) or novel, non-toxic (MAXQ; n = 8) endophyte. At the end of the grazing period (135 d), steers were harvested at a commercial meat plant and carcass data collected. Average daily gain, liveweight, and hot carcass weight were greater (P < 0.05); while dressing percentage, fat depth, ribeye area, and kidney, pelvic and heart fat were numerically higher (P > 0.05) for MAXQ cattle than for TOXIC. No differences (P > 0.05) 0.05) in longissimus color (L* lightness, a* redness, and b* yellowness) or pH were observed between MAXQ and TOXIC. TOXIC subcutaneous fat was lighter (P < 0.01) than MAXQ, but no differences (P > 0.05) were observed in a* or b* values. Steaks from TOXIC cattle tended (P < 0.10) to have higher L* and b* than MAXQ, but no difference in a* was observed at 0 d of display. Ground beef from TOXIC cattle also had higher (P < 0.05) L* than MAXQ with no differences in a* or b* at 0 d. Color changes over time were similar (P > 0.05) among the treatments for both steaks and ground beef. TBARS values were higher (P < 0.05) for MAXQ than TOXIC only at 12 d of display for steaks, and numerically higher (P > 0.05) during 8 d of display for ground beef. Adipose tissues from TOXIC cattle had a higher (P < 0.05) percent of saturated fatty acids (SFA), and a lower (P < 0.05) percent of monounsaturated fatty acids (MUFA) than MAXQ. The fatty acid composition of intramuscular fat more closely resembled that of ground beef than subcutaneous fat. Ground beef and intramuscular fat had higher concentrations of SFA, MUFA, and CLA c9t11, and lower concentrations of polyunsaturated fatty acids (PUFA) and PUFA:SFA ratio than subcutaneous fat. The omega-6:omega-3 fatty acid ratio was

similar among fat depots. Warner-Bratzler shear force was higher (P < 0.01) for MAXQ than for TOXIC steaks at 2, 4, and 8 d postmortem; but did not differ (P > 0.05) after 14 d. However, sensory panel evaluation detected greater (P < 0.01) chewiness, and lower (P < 0.05) juiciness for MAXQ than for TOXIC steaks aged for 14 d. Although tall fescue pastures infected with non-toxic endophyte support a higher cattle performance than toxic endophyte, these results suggest that endophyte type has minimal effects on carcass traits, and meat quality of grass-fed beef. Fescue toxicosis may influence fatty acid metabolism and may be involved in the occurrence of fat. Finishing cattle on tall fescue pastures showed potential to enhance the fatty acid profile of intramuscular fat in beef including CLA and omega-3 fatty acids from a human health perspective.

Key Words: Tall fescue, MaxQTM, Beef quality

Introduction

Tall fescue (*Festuca arundinacea*), a major component of pasture systems in the United States (over 14 million ha), is generally infected with the fungal endophyte *Neotyphodium coenophialum*. The fungal endophyte shares a symbiotic relationship with tall fescue protecting the host plant against disease, insects, and drought. It has also been identified as the causative agent of fescue toxicosis. Some of the symptoms associated with toxicosis include increased respiration rates, rectal temperatures, salivation, and nervousness, rough hair coats, reduced weight gains, and decreased overall performance in beef steers (Hoveland et al., 1983; Stuedemann and Hoveland, 1988). Management and grazing recommendations have been suggested for alleviating tall fescue toxicosis in beef cattle. More recently, non-toxic endophytes from New Zealand have been incorporated into tall fescue cultivars grown in the United States. Current research has shown that tall fescue pastures infected with non-toxic endophyte, MAXQTM (Pennington Seed), are a promising alternative for combating toxicosis and greatly improving beef productivity (Bondurant et al., 2001; Duckett et al., 2001; Watson et al., 2001). However, no data is available on how endophyte type may alter carcass characteristics and beef quality in cattle finished on tall fescue pastures. The objective of this study was to determine the effect of endophyte type (novel, non-toxic **MAXQ** vs. wild-type **TOXIC**) in beef cattle grazing tall fescue on carcass traits, meat quality, and fatty acid composition.

Materials and Methods

Animals and Sampling Procedures. Fourteen Hereford steers grazed replicated (n = 2) tall fescue pastures infected with either wild-type (TOXIC; n=6) or novel, non-toxic (MAXQ; n=8) endophyte for 135 d during spring-early summer 2001 at the Central Georgia Experiment Station, Eatonton, GA. At the end of the grazing period, steers were harvested at a commercial meat plant and carcass data collected including adjusted fat thickness, ribeye area, marbling score, percentage of kidney, pelvic and heart fat, skeletal maturity, and USDA yield and quality grade. Carcass value was calculated based on current value based marketing grids (AMS, 2001). The ribeye roll (IMPS 112) and the clod (IMPS 114) were removed from each carcass, vacuum-packaged and transported to the University of Georgia Meat Science Laboratory. The ribeye roll was fabricated into steaks for fatty acid analysis, tenderness, sensory panel evaluation and lipid and color stability measurements. Steaks for fatty acid analysis, shear force measurements, and sensory panel evaluation were individually vacuum packaged and frozen for subsequent analysis. The clod was ground (0.635 cm), and prepared into patties. Steaks and patties,

for lipid and color stability measurements, were individually placed on Styrofoam trays, over-wrapped with oxygen permeable film, and displayed at 4°C in a lighted cooler.

Instrumental Color. Instrumental color measurements were recorded for L* (lightness; 0: black, 100: white), a* (redness/greenness; positive values: red, negative values: green), and b* (yellowness/blueness; positive values: yellow, negative values: blue) using a Minolta chromameter (CR-210, Minolta Inc., Osaka, Japan). Color readings were determined at 24 h postmortem on subcutaneous fat and the exposed longissimus muscle after ribbing between the 12th and the 13th ribs. Steak and ground beef color measurements were obtained at 0, 5, 12, and 21 d, and 0, 2, 4, and 8 d of display, respectively. Values were recorded from three locations of the upper surface of each steak and ground beef sample randomly selected to obtain a representative reading of the surface color.

Lipid Oxidation Analysis (TBARS). Lipid stability was evaluated in the same steaks and ground beef that were displayed for instrumental color. Lipid oxidation was determined by measuring 2-thiobarbituric acid reactive substances (TBARS; Jo and Ahn, 1998) at 0, 5, 12 and 21 d of display for steaks, and at 0, 2, 4, and 8 d of display for ground beef.

Warner-Bratzler Shear Force. Steaks (2.5 cm) were vacuum packaged, stored in a cooler at 4°C and frozen after 2, 4, 8, 14 and 21 d of aging for subsequent Warner-Bratzler shear force determination. Steaks were thawed for 24 h at 4°C, and broiled to an internal temperature of 71°C (AMSA, 1995). Steaks were allowed to cool to room temperature before six 1.27-cm cores were removed per steak parallel to the longitudinal

orientation of the muscle fibers. All cores were sheared perpendicular to the long axis of the core using a Texture Analyzer TA-XT2 with a Warner-Bratzler shear attachment.

Sensory Panel Evaluation. Steaks were broiled to an internal temperature of 71°C, cut into 2.54 x 1.27 x 1.27 cm cubes, and served warm to a nine-member trained sensory panel according to AMSA (1995). Each panelist evaluated two cubes from each sample for juiciness (amount of juice perceived in the mouth), and chewiness (amount of work it takes to get the samples ready to swallow) using a five-point scale (1: not at all juicy/chewy to 5: extremely juicy/chewy).

Fatty Acid Composition. Longissimus steaks, ground beef, and subcutaneous fat samples were submerged in liquid nitrogen (-196°C), pulverized and stored at -20°C. Total lipid was determined following the chloroform-methanol procedure of Folch et al. (1957), modified by using a 10:1 ratio of chloroform-methanol to sample. Extract containing approximately 25 mg of lipid was converted to fatty acid methyl esters (FAME) following the method of Park and Goins (1994). The FAME were analyzed by gas chromatography (Agilent 6850, Wilmington, DE), and separated using a 100-m SP 2560 capillary column (0.25 mm i.d. and 0.20 μm film thickness, Supelco, Bellefonte, PA). Column oven temperature was programmed at 150 to 165°C at 1°C/min, 165 to 167°C at 0.2°C/min, 167 to 225°C at 1.5°C/min and held at 225°C for 15 min with 1:100 split. The injector and detector were maintained at 250°C. Hydrogen was the carrier gas at a flow rate of 1 mL/min. Individual fatty acids were identified by comparison of retention times with standards (Sigma, St. Louis, MO; Supelco, Bellefonte, PA). *Statistical Analysis.* Data were analyzed using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC) with treatment and replicate in the model. Variables measured over time were analyzed as repeated measures. Treatment means were separated using the least square means procedure. There were no interactions between treatments (TOXIC and MAXQ) and fat depots (ground beef from the chuck, intramuscular, and subcutaneous fat from the rib) for lipid content and fatty acid profile of the samples with the exception of myristoleic acid (14:1). Thus, the fatty acid data was presented by treatment and fat depot. Simple correlations between growth rate, fat depth, hot carcass weight, and longissimus color with Warner-Bratzler Shear force values, were computed using the CORR procedure of SAS.

Results and Discussion

The effect of endophyte type on animal performance and carcass characteristics is presented in Table 4.1. Average daily gain, liveweight, and hot carcass weight were greater (P < 0.05) for steers grazing MAXQ compared to TOXIC infected tall fescue. Similarly, previous research has shown that tall fescue pastures infected with non-toxic endophyte, MAXQ, are a promising alternative for combating toxicosis and greatly improve growth rates and beef productivity (McCann et al., 2000; Bondurant et al., 2001; Duckett et al., 2001; Watson et al., 2001). Altough steers grazing different pastures did not differ (P > 0.05) in other carcass characteristics, dressing percentage, fat depth, ribeye area, and kidney, pelvic and heart fat were numerically higher for MAXQ than for TOXIC cattle. All TOXIC carcasses and 75% of MAXQ carcasses graded Select. No differences were observed for USDA yield grade. Carcass value was numerically higher for MAXQ with about \$40 more per head than TOXIC due to greater carcass weight. The greater economic advantage of finishing cattle on non-toxic compared to endophyte infected pastures may be carried over a feedlot finishing period. Duckett et al. (2000) reported a greater carcass value (\$161 more per head) from steers finished in feedlot (112 d) after grazing non-toxic compared to endophyte-infected pastures due to advantages in carcass weight and quality grade. In contrast, others (Coffey et al., 1990; Beconi et al., 1995) have shown that deleterious effects of consumption of endophyte-infected tall fescue did not negatively affect subsequent feedlot performance.

Table 4.2 shows the effect of endophyte type on muscle pH, subcutaneous fat color, and longissimus muscle color. Longissimus pH at 24 h postmortem did not differ (P > 0.05) among treatments. Page et al. (2001) analyzed the relationship among color and pH in 1,000 carcasses selected to represent the US beef carcass population as reported by the 1995 National Beef Quality Audit. The authors proposed a threshold fat thickness at approximately 0.76 cm, below which carcasses have higher muscle pH values and darker-colored muscle. In addition, a longissimus muscle pH of 5.87 was reported as the approximate cut-off between normal and dark-cutting carcasses. In this study, fat depth was below the proposed threshold suggesting a potential for high incidence of dark-cutting carcasses. Although pH was below the cut-off value of 5.87 for both treatments, it was higher than the average value of 5.51 reported for the 680 steer carcasses analyzed by Page et al. (2001). No differences (P > 0.05) in color were (L* lightness, a* redness, and b* yellowness) observed for longissimus muscle between MAXQ and TOXIC cattle. The average L* value for longissimus muscle of steers reported by Page et al. (2001) was 39.62 compared to 35.72 and 34.54 for TOXIC and MAXQ, respectively. Compared with concentrate-fed cattle, darker lean color has been

detected in many studies with forage-finishing programs (McCaughey and Cliplef, 1996; Bennett et al., 1995; Bidner et al., 1986; Schroeder et al., 1980). TOXIC subcutaneous fat was lighter (P < 0.01, higher L*) in color than MAXQ, but no differences were found in fat redness or yellowness.

The effect of endophyte type on steak and ground beef color at 0 d of display is presented in Table 4.3. Steaks from TOXIC cattle tended (P < 0.10) to have higher L* and b* values than MAXQ. There were no differences (P > 0.05) in steak redness between the groups. Ground beef from TOXIC cattle was lighter (P < 0.05, higher L*) compared to MAXQ. Other than lightness, no effect (P > 0.05) of endophyte type was observed on ground beef color. There were no differences (P > 0.05) in steak or ground beef color between the treatments measured at 5, 12, and 21 d, and 2, 4, and 8 d of display, respectively (data not shown).

The effect of endophyte type on lipid oxidation (TBARS) in steaks and ground beef is presented in Figures 4.1 and 4.2, respectively. Lipid oxidation was higher (P < 0.05) in steaks from MAXQ than TOXIC cattle only at 12 d of display. In ground beef, lipid oxidation was numerically higher from MAXQ compared to TOXIC cattle; however, TBARS means did not differ (P > 0.05) among treatments. The numerically higher TBARS values of steaks and ground beef from MAXQ compared to TOXIC may be related to differences in the fatty acid composition between the treatments.

The effect of endophyte type on the lipid content and fatty acid composition pooled over three fat depots (ground beef from the chuck, intramuscular, and subcutaneous fat from the rib) is presented in Table 4.4. No interactions (P > 0.05) between fat depots and treatments for lipid content and fatty acid composition were found with the exception of myristoleic (P < 0.05, 14:1). There were no differences between TOXIC and MAXQ (P > 0.05) in the 14:1 content of ground beef and subcutaneous fat (0.42 vs. 0.44 and 0.30 vs. 0.38 %, respectively), whereas 14:1 was higher (P < 0.05) in the intramuscular fat from MAXQ than TOXIC (0.87 vs. 0.47 %, respectively).

Total lipid content was unaffected (P > 0.05) by endophyte type. Endophyte type did not alter (P > 0.05) the proportions of myristic (14:0), palmitic (16:0), linoleic (18:2), linolenic (18:3), arachidonic (20:4), eicosapentaenoic (20:5, EPA), docosapentaenoic (22:5, DPA), and docosahexaenoic (22:6, DHA) acids, or the percentage of total polyunsaturated fatty acids (PUFA). There were no differences (P > 0.05) between the fat depots from MAXQ and TOXIC in the proportion of total conjugated linoleic acid (11.8 and 10.4 mg CLA/g lipid, respectively). However, the adipose tissue from MAXQ tended (P < 0.10) to have a higher proportion of CLA isomer cis-9, trans-11 than TOXIC (9.8 vs. 8.4 mg CLA/g lipid).

Adipose tissues from TOXIC cattle had higher (P < 0.05) proportions of stearic (18:0) acid, and lower (P < 0.05) proportions of myristoleic (14:1, intramuscular fat only), palmitoleic (16:1) and oleic (18:1) acids than MAXQ. Consequently, the percentage of saturated fatty acids (SFA) was higher (P < 0.05) and the percentage of monounsaturated fatty acids (MUFA) was lower (P < 0.05) in the adipose tissues of TOXIC as compared to MAXQ steers. The greater degree of lipid saturation in the fat of longissimus steaks and ground beef samples from TOXIC cattle may explain the greater (lower numerical TBARS) lipid stability detected in TOXIC samples compared to MAXQ.

Numerous disorders occur in livestock grazing infected tall fescue, and several clinical signs of toxicosis are consistent with the development of bovine fat necrosis. These include increased body temperature, reduced performance, and rough hair coat (Stuedemann et al., 1975; Hoveland et al., 1983; Stuedemann and Hoveland, 1988). Steers grazing TOXIC endophyte infected tall fescue in this study showed these clinical signs of toxicosis. Stuedemann and Hoveland (1988) proposed that toxic tall fescue influences lipid metabolism and that there may be a link between poor animal performance on tall fescue and occurrence of fat necrosis. Increased body temperature caused by heat stress or administration of a pyrogenic substance has been shown to alter lipid metabolism, resulting in lower blood cholesterol concentrations (Noble et al., 1973; O'Kelly and Reich, 1975). Stuedemann et al. (1985) reported that necrotic fat contains more crude protein and ash, with less ether-extractable material, and 3 to 4 times higher cholesterol content than does normal fat. Rumsey et al. (1979) studied the chemical composition of necrotic fat lesions in beef cows grazing fertilized toxic tall fescue. The molar proportion of stearic acid was greater and the proportions of oleic and palmitoleic lower in the necrotic fat residue than in normal fat residue. Although the occurrence of necrotic fat was not evaluated in the present study, results from the fatty acid composition analysis (higher 18:0 and lower 14:1, 16:1, and 18:1 in TOXIC fat) suggest that fescue toxicosis may influence lipid metabolism and may be involved in the occurrence of fat necrosis. Rumsey et al. (1979) reported that necrotic fat lesions were found in abdominal fat, but not in subcutaneous fat or pericardial fat depots. In this study, the fatty acid composition of intramuscular and subcutaneous fat from the rib, and ground beef fat from the chuck were evaluated, but not abdominal fat or other fat depots.

Current recommendations are that the PUFA:SFA (P:S) ratio should be around 0.45, and that consumption of *n*-3 fatty acids should be increased (Department of Health, 1994) to overcome the imbalance in the ratio of *n*-6:*n*-3 PUFA in the current diets (10:1) compared to primitive man (1:1, Eaton et al. 1996). The P:S ratios in this study were lower than the recommended ratio, being 0.11 for both treatments. The *n*-6:*n*-3 ratio was similar (P > 0.05) for TOXIC and MAXQ (1.40 vs. 1.44, respectively), and beneficial from a human health perspective compared to higher ratios reported in the literature for feedlot cattle. Rule et al. (2002) reported *n*-6:*n*-3 ratios of 1.95 and 6.38 for pasture-fed cows and feedlot steers, respectively. French et al. (2000) reported ratios of 2.33 and 4.15 for grass-fed and concentrate-fed steers.

Total lipid and fatty acid composition of ground beef, intramuscular, and subcutaneous fat pooled over two treatments (TOXIC v. MAXQ) are shown in Table 4.5. There were considerable variations in the percent of total lipid between the 3 fat depots evaluated. The fatty acid profile shows that the lipid composition of intramuscular fat more closely resembled that of ground beef than subcutaneous fat. The proportions of myristic, palmitic, stearic, and oleic acids were similar (P > 0.05) among ground beef and intramuscular fat, but higher (P < 0.05) than subcutaneous fat. Consequently, the percent of total SFA was lower (P < 0.05) in subcutaneous fat compared to either ground beef or intramuscular fat. The concentration of palmitoleic and total MUFA were lower (P < 0.05) in subcutaneous than intramuscular and ground beef fat, which were similar (P > 0.05).

The proportions of the PUFAs including linoleic, linolenic, EPA, DPA, and DHA were highest (P < 0.05) in subcutaneous fat and lowest (P < 0.05) in ground beef. Arachidonic acid concentrations were higher (P < 0.05) in subcutaneous than intramuscular and ground beef fat. As a result, the percentage of total PUFA and the P:S ratio differed among the three fat depots. The proportion of total PUFA and the P:S ratio were higher (P < 0.05) for subcutaneous than intramuscular fat with ground beef fat being intermediate. Other studies also found higher contents of polyunsaturated fatty acids in triceps brachii (Enser et al., 1998 and Terrell et al., 1967) and supraspinatus (Rule et al., 2002) compared to longissimus dorsi muscle.

Differences between fat depots were not observed (P > 0.05) for the *n*-6:*n*-3 fatty acid ratio. The concentrations of total CLA and CLA isomer cis-9, trans-11 were lower (P < 0.05) in subcutaneous fat (8.5 and 7.1 mg/g fat, respectively) compared to either intramuscular (12.4 and 10.4 mg/g fat, respectively) or ground beef fat (12.4 and 9.7 mg/g fat, respectively). French et al. (2000) reported similar longissimus CLA concentrations for grass-fed beef (10.8 mg CLA/g lipid). Previous research has shown that including grass in the diet of dairy and beef cattle increased CLA concentration in milk and beef intramuscular fat, respectively (Lawless, 1998; French et al., 2000; Yang et al., 2002). Shantha et al. (1997) reported 7.7 and 5.2 mg total CLA/g lipid in semimembranosus muscle for grass-fed and corn supplemented grass-fed beef, respectively. Rule et al., (2002) reported 4.1 and 2.6 mg CLAc9t11/g lipid in longissimus muscle for pasture-fed cows and feedlot steers, respectively. The CLA concentrations reported in this study are higher than values reported for longissimus muscle from feedlot cattle. French et al. (2000) reported 3.7 mg total CLA/g lipid for beef cattle fed

concentrates; while Mir et al., (1999) reported 1.7 mg total CLA/g lipid for cattle fed a barley-based diet.

The CLA concentrations reported for ground beef in this study are higher than previously published values. Chin et al. (1992) reported CLA concentrations in ground beef of 4.3 mg/g lipid; while Shantha et al. (1994) reported a range in CLA concentrations for ground beef from the chuck between 6.6 and 8.2 mg/g fat. Both Chin et al. (1992) and Shantha et al. (1994) obtained samples from retail markets; thus, the different nutritional background of the animals in this study would likely explain the higher levels observed.

The effect of endopyte type on tenderness of longissimus steaks (Warner-Bratzler shear force: WBSF) is shown in Figure 4.3. Shear force values were higher (P < 0.05) for MAXQ than for TOXIC steaks during the first week of aging at 2, 4, and 8 d postmortem. However, shear force did not differ (P > 0.05) between endophyte types at 14 d. At 21 d postmortem, steaks from MAXQ tended (P < 0.10) to have lower shear force values than TOXIC. The effect of endophyte type on sensory characteristics of longissimus steaks from TOXIC and MAXQ cattle is presented in Table 4.6. Sensory panel evaluation based on a five point scale showed greater (P < 0.01) chewiness, and lower (P < 0.05) juiciness for MAXQ than for TOXIC longissimus steaks aged for 14 d. The initial greater toughness observed in steaks from MAXQ cattle, may be associated with greater muscle growth and larger muscle fiber diameter than steers grazing TOXIC endophyte-infected tall fescue. Steaks from MAXQ cattle had lower (P < 0.10) numerical L* values than TOXIC, indicating a darker color measured at 2 d postmortem. L* tended to be negatively correlated with WBSF measured at 2 d (r = -0.48, P < 0.10).

Studies comparing different beef finishing production systems have produced mixed results on palatability attributes. Some studies found a negative effect of forage finishing on meat tenderness (Mitchell et al., 1991; Smith, 1990); while others showed that grass-fed beef can be produced with no deleterious effects on meat quality including tenderness (Mandell et al., 1998; French et al., 2001). In many experiments, dietary effects are confounded with animal age, growth rate or carcass weight and fatness at slaughter. In this study, initial shear force values were higher for MAXQ than TOXIC despite heavier (P < 0.05) carcass weight (246 vs. 223 kg) and numerically greater fatness (fat depth: 5.3 vs. 4.4 mm) in MAXQ. Numerous studies (Bowling et al., 1977; Bowling et al., 1978; Lochner et al., 1980; Dolezal et al., 1982) have reported a positive correlation between muscle tenderness, carcass weight and fatness. However, other researchers have reported no correlation (P > 0.05) between carcass weight or carcass fat score and WBSF (French et al., 2001). In this study, there was no correlation between growth rate (average daily gain) or fat depth and WBSF; whereas hot carcass weight was positively correlated with WBSF (r = 0.68, P < 0.05) measured at 2 d postmortem.

More extensive aging was evident for longissimus steaks from MAXQ cattle, which showed a decrease in WBSF values of approximately 7 kg compared to 4 kg for steaks from TOXIC cattle in 21 d. French et al. (2001) found that supplementing grass with low levels of concentrates produced the most tender and acceptable meat at two days postmortem, but that further aging (14 d) eliminated all treatment effects on eating quality of beef. Previous studies have shown that cattle grown rapidly prior to slaughter produced more tender meat than their slower growing counterparts (Aberle et al., 1981; Fishell et al., 1985). Cattle which grow more rapidly pre-slaughter have increased rates of protein turnover, resulting in higher concentrations of proteolytic enzymes in carcass tissues at slaughter which, in turn, may affect collagen solubility and/or myofibril fragmentation (Aberle et al., 1981; Hall and Hunt, 1982; Miller et al., 1983). Huffman et al. (1996) proposed that a WBSF of 4.1 kg could be used as a threshold to indicate that 98 % of restaurant and home consumers would find a longissimus steak acceptable in tenderness. Based on this threshold, longissimus steaks from this study were acceptable in tenderness (WBSF < 4.1 kg) only after 21 d of aging.

Implications

Although significant economic losses in the beef cattle industry are associated with fescue toxicosis, tall fescue will continue to represent a major pasture component in the United States production systems. Current research has demonstrated that non-toxic (MAXQ) endophyte-infected tall fescue is a promising alternative for combating toxicosis, and greatly improve steer daily gains, finishing and carcass weights. However, results from this study suggest that endophyte type has minimal effects on carcass traits, and meat quality of grass-fed beef. Fescue toxicosis may influence fatty acid metabolism and may be involved in the occurrence of fat. Finishing cattle on tall fescue pastures showed potential to enhance the fatty acid profile of intramuscular fat in beef including CLA and omega-3 fatty acids from a human health perspective.

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	End	ophyte type
Characteristic	TOXIC (n=6)	MAXQ (n=8)
Average Daily Gain, kg/d	$0.33^a\pm0.060$	$0.68^{\text{b}}\pm0.052$
Liveweight, kg	$428.58^{a} \pm 11.844$	$468.98^{b} \pm 10.257$
Hot Carcass Weight, kg	$223.07^{a} \pm 7.679$	$246.07^{b}\pm 6.650$
Dressing %	54.19 ± 0.672	54.64 ± 0.582
Fat depth, cm	0.44 ± 0.054	0.53 ± 0.047
Ribeye Area, cm ²	51.75 ± 3.192	58.12 ± 2.765
Kidney, pelvic, heart fat %	0.92 ± 0.200	1.06 ± 0.173
Overall maturity ^a	143.33 ± 5.716	148.75 ± 4.950
Marbling score ^b	430.83 ± 10.771	411.25 ± 9.328
USDA quality grade ^c	3.00 ± 0.151	2.75 ± 0.131
USDA yield grade	2.41 ± 0.125	2.41 ± 0.108
Carcass value, \$/hd	505.33 ± 19.612	546.38 ± 16.984

Table 4.1. Effect of endophyte type on animal performance and carcass characteristics.

^{a,b} Means within the same row with uncommon superscripts differ P < 0.05.

^a Overall maturity: A = 100, B = 200.

^b Marbling score: Slight = 400, Small = 500.

^c Quality grade score: 2 = Standard+, 3 = Select.

	Endophyte type		
Characteristic	TOXIC (n=6)	MAXQ (n=8)	
Muscle pH at 24 h postmortem	5.71 ± 0.023	5.71 ± 0.020	
Subcutaneous fat color			
L*	$79.02^a\pm0.385$	$77.64^{b} \pm 0.333$	
a*	2.20 ± 0.382	2.68 ± 0.330	
b*	11.74 ± 0.576	12.22 ± 0.500	
Longissimus muscle color			
L^*	35.72 ± 0.559	34.54 ± 0.484	
a*	18.17 ± 0.455	17.66 ± 0.394	
b*	6.01 ± 0.250	5.48 ± 0.216	

Table 4.2. Effect of endophyte type on muscle pH and subcutaneous fat and longissimus muscle color (L* lightness, a* redness, b* yellowness).
	Endophyte type		
Instrumental color	TOXIC (n=6)	MAXQ (n=8)	
Steaks			
L^*	$38.25^{c} \pm 0.717$	$36.59^{d} \pm 0.621$	
a*	14.34 ± 0.968	13.08 ± 0.838	
b*	$5.08^{\circ} \pm 0.250$	$4.45^{d}\pm0.217$	
Ground beef			
L^*	$47.35^{a} \pm 0.748$	$45.01^{b} \pm 0.648$	
a*	21.98 ± 0.532	22.06 ± 0.461	
b*	10.92 ± 0.424	10.12 ± 0.368	

Table 4.3. Effect of endophyte type on steak and ground beef color (L* lightness, a* redness, b* yellowness) at 0 d of display.

^{a,b} Means within the same row with uncommon superscripts differ P < 0.05.

^{c,d} Means within the same row with uncommon superscripts differ P < 0.10.



Figure 4.1. The effect of endophyte type on lipid oxidation (TBARS) of steaks at 0, 5, 12, and 21 d of display.



Figure 4.2. The effect of endophyte type on lipid oxidation (TBARS) of ground beef at 0, 2, 4, and 8 d of display.

	Endonhyte type		
Fatty acid, %	TOXIC	MAXQ	
Total lipid	22.77 ± 0.948	23.73 ± 0.821	
14:0, <i>myristic</i>	2.66 ± 0.079	2.75 ± 0.068	
14:1, myristoleic	$0.40^{a} \pm 0.036$	$0.56^{b} \pm 0.031$	
16:0, <i>palmitic</i>	24.07 ± 0.235	23.60 ± 0.203	
16:1, palmitoleic	$2.10^{a} \pm 0.098$	$2.55^{b} \pm 0.085$	
18:0, <i>stearic</i>	$19.01^{a} \pm 0.552$	$17.22^{b} \pm 0.478$	
18:1, <i>n-9 oleic</i>	$33.06^{a} \pm 0.582$	$35.06^{b} \pm 0.504$	
18:2, <i>n-6 linoleic</i>	1.83 ± 0.082	1.69 ± 0.071	
18:3, <i>n-3 linolenic</i>	0.81 ± 0.039	0.74 ± 0.034	
CLA [*] c9t11	$0.84^{\rm c} \pm 0.060$	$0.98^{\rm d} \pm 0.052$	
Total CLA ^{**}	1.04 ± 0.064	1.18 ± 0.055	
20:4, n-6 arachidonic	0.40 ± 0.042	0.43 ± 0.036	
20:5, <i>n-3 EPA</i> *	0.37 ± 0.043	0.34 ± 0.037	
22:5, <i>n-3 DPA</i> *	0.41 ± 0.041	0.44 ± 0.035	
22:6, <i>n-3 DHA</i> *	0.04 ± 0.005	0.04 ± 0.005	
Unidentified	13.81 ± 0.366	13.39 ± 0.317	
SFA [*]	$53.36^{a} \pm 0.747$	$50.64^{b} \pm 0.647$	
MUFA [*]	$40.93^{a} \pm 0.733$	$43.74^{b} \pm 0.635$	
PUFA [*]	5.71 ± 0.245	5.62 ± 0.212	
PUFA:SFA	0.11 ± 0.005	0.11 ± 0.005	
<i>n-6:n-3</i> ratio	1.40 ± 0.075	1.44 ± 0.065	

Table 4.4. Effect of endophyte type on fatty acid composition (lsmean \pm S.E.) pooled over three fat depots (ground beef, intramuscular, and subcutaneous fat).

^{a,b}Means within the same row with uncommon superscripts differ P < 0.05

^{c,d}Means within the same row with uncommon superscripts differ P < 0.10

*CLA: conjugated linoleic acid, EPA: eicosapentaenoic acid, DPA: docosapentaenoic acid, DHA: docosahexaenoic acid, SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids.

** Total CLA includes: c911, t10c12, t9t11, and other isomers that were unable to be identified specifically.

	Fat depot			
Fatty acid. %	Ground beef	Intramuscular	Subcutaneous	S.E.
Total lipid	11.59 ^a	1.46 ^b	56.70 ^c	1.087
r F				
14:0, myristic	2.90^{a}	3.14 ^a	2.07^{b}	0.090
14:1, myristoleic	0.43 ^a	0.67 ^b	0.34 ^a	0.041
16:0, palmitic	23.90 ^a	24.65 ^a	22.96 ^b	0.269
16:1, palmitoleic	2.33 ^{ab}	2.64 ^a	2.02^{b}	0.112
18:0, <i>stearic</i>	19.54 ^a	18.55 ^a	16.26 ^b	0.632
18:1, <i>n-9 oleic</i>	35.30 ^a	34.90 ^a	31.97 ^b	0.666
18:2, <i>n-6 linoleic</i>	1.78^{a}	0.77 ^b	2.75 [°]	0.093
18:3, n-3 linolenic	0.63 ^a	0.49 ^b	1.21 ^c	0.044
$CLA^* c9t11$	0.97^{a}	1.04^{a}	0.71 ^b	0.068
Total CLA ^{**}	1.24^{a}	1.24^{a}	0.85 ^b	0.073
20:4, n-6 arachidonic	0.08^{a}	0.03 ^a	1.14 ^b	0.048
20:5, <i>n-3 EPA</i> [*]	0.35 ^a	0.03 ^b	0.68°	0.049
22:5, <i>n-3 DPA</i> [*]	0.25^{a}	0.08 ^b	0.94 ^c	0.046
22:6, <i>n-3 DHA</i> *	0.03^{a}	0.01 ^b	0.09 ^c	0.006
Unidentified	11.25 ^a	12.83 ^b	16.73 ^c	0.420
SFA [*]	53.21 ^a	53.18 ^a	49.62 ^b	0.856
MUFA [*]	41.99 ^{ab}	43.81 ^a	41.21 ^b	0.840
PUFA [*]	4.81 ^a	3.02 ^b	9.18 ^c	0.280
PUFA:SFA	0.09^{a}	0.06 ^b	0.19 ^c	0.006
<i>n-6:n-3</i> ratio	1.55	1.35	1.36	0.086

Table 4.5. Fatty acid composition (lsmean \pm S.E.) of ground beef, intramuscular, and subcutaneous fat pooled over two treatments (TOXIC, MAXQ).

^{a,b,c} Means within the same column with uncommon superscripts differ P < 0.05

*CLA: conjugated linoleic acid, EPA: eicosapentaenoic acid, DPA: docosapentaenoic acid, DHA: docosahexaenoic acid, SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids.

** Total CLA includes: c9t11, t10c12, t9t11, and other isomers that were unable to be identified specifically.



Figure 4.3. Effect of endophyte type on tenderness (Warner-Bratzler shear force).

Table 4.6. Effect of endophyte type on sensory characteristics (lsmeans \pm S.E.) of steaks from TOXIC and MAXQ cattle.

	Endophyte type	
Characteristic	TOXIC (n=6)	MAXQ (n=8)
Chewiness*	$2.80^{a} \pm 0.134$	$3.65^{b} \pm 0.114$
Juiciness*	$2.65^{\circ} \pm 0.097$	$2.43^d\pm0.083$

^{a,b} Means within the same row with uncommon superscripts differ P < 0.01.

 $^{\rm c,d}$ Means within the same row with uncommon superscripts differ P < 0.05.

* 1: not at all chewy/juicy, 5: extremely chewy/juicy.

CHAPTER 5

CONCLUSION

In experiment 1, pasture-fed carcasses had lower weight, conformation, fat depth and ribeye area as well as darker longissimus color and yellower fat than concentrate-fed carcasses. Initial Warner-Bratzler shear force was similar among treatments, but longissimus steaks from pasture-fed cattle had lower shear force values after 7 d postmortem. Supplementation of α -tocopherol to cattle finished on concentrate with 1,000 IU animal⁻¹d⁻¹ for 100 d was sufficient to achieve similar muscle α -tocopherol content to pasture-fed cattle, at levels beyond the proposed critical concentrations for improving shelf life. Vitamin E supplementation of concentrate-fed steers increased lipid stability of ground beef and steaks, but was unable to improve color stability; whereas vitamin C addition to ground beef increased color stability without altering lipid oxidation. Finishing cattle on pasture enhanced the unsaturated fatty acid profile of intramuscular fat in beef including conjugated linoleic acid and omega-3 fatty acids.

In experiment 2, postmortem vitamin C addition was effective in retarding pigment oxidation in ground beef from both grain-fed and grass-fed sources; however, vitamin C addition reduced lipid oxidation in grain-fed ground beef samples only, despite higher concentrations of polyunsaturated fatty acids in grass-fed sources. A possible explanation for the difference in the effectiveness of vitamin C on lipid oxidation between ground beef sources, may be a higher antioxidant status in beef finished on pasture. Results from sensory analysis suggest that vitamin C addition to ground beef may have minimal effects on aroma and flavor of meat. This study showed that near infrared reflectance spectroscopy can be used to predict accurately the content of total saturated and unsaturated, and stearic, oleic, and linolenic fatty acids in ground beef; and to discriminate meat samples originating from different nutritional systems.

Current research has demonstrated that non-toxic (MAXQ) endophyte-infected tall fescue is a promising alternative for combating toxicosis, greatly improving steer daily gains, finishing and carcass weights. However, results from experiment 3 suggest that endophyte type has minimal effects on carcass traits, and meat quality of grass-fed beef. Fescue toxicosis may influence fatty acid metabolism and may be involved in the occurrence of fat necrosis. Further research is required in this area to evaluate the effect of toxicosis in fatty acid metabolism and its implications. Finishing cattle on tall fescue pastures showed potential to enhance the fatty acid profile of intramuscular fat in beef including CLA and omega-3 fatty acids from a human health perspective.