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# Chemical Characterization of Meat Related to Animal Diet

Rossarin Tansawat
*Utah State University*

# CHEMICAL CHARACTERIZATION OF MEAT RELATED TO ANIMAL DIET

by

Rossarin Tansawat

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Approved:

| | |
|---|---|
| Daren P. Cornforth<br>Major Professor | Robert E. Ward<br>Committee Member |
| Silvana Martini<br>Committee Member | Korry J. Hintze<br>Committee Member |
| Jennifer W. MacAdam<br>Committee Member | Mark R. McLellan<br>Vice President for Research and<br>Dean of the School of Graduate Studies |

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# ABSTRACT

Chemical Characterization of Meat Related to Animal Diet

by

Rossarin Tansawat, Doctor of Philosophy

Utah State University, 2012

Major Professor: Dr. Daren P. Cornforth
Department: Nutrition, Dietetics, and Food Sciences

There is currently much interest in the comparative health benefits of various meat products, including pasture-fed beef. However, little is known about the specific pasture-finishing diets (mixed forages, alfalfa, or sainfoin, compared to grain) on meat quality, consumer preferences, and human health. Thus, additional information is needed to better understand and develop new animal feeding regimes for optimum animal growth, meat flavor, and meat nutritional quality. The objective of the current study was to examine how animal diets, including secondary metabolites in the diet, affect meat chemical characteristics, meat quality, and nutritional value. In study 1 (Chapter 3), grain- vs. pasture-fed beef rib steaks were evaluated. Ribs from pasture-fed animals had a much lower fat content ($P < 0.01$), which was its main positive nutritional attribute. Pasture-fed beef had more ($P < 0.05$) omega-3 polyunsaturated fatty acids (PUFAs) and conjugated linoleic acid (CLA) than grain-fed beef, but was only a moderately good source of PUFA, compared to salmon. Pasture-fed beef had higher antioxidant capacity

and lower measures of oxidation ($P < 0.05$). Pasture and grain diets influenced the volatile profile of cooked meat. Flavor descriptors barny, gamey, and grassy were associated with pasture feeding, and were uniquely shown in this study to be positively correlated with specific aroma volatiles benzaldehyde, toluene, dimethyl sulfone, 3-heptanone, 2-ethyl-1-hexanol, and hexadecanoic acid methyl ester ($P < 0.05$). In study 2 (Chapter 4), the effects of legume pasture-finishing of beef cattle on meat quality were evaluated, comparing alfalfa pasture (containing saponins) versus sainfoin pasture (containing tannins). No strong differences ($P > 0.05$) were found between the two legume diets in all meat characteristics, indicating that sainfoin was similar to alfalfa as a cattle forage. Similar ($P > 0.05$) low TBA values after 12 d of storage at 2 °C were obtained from both diets, comparable to pasture-fed beef from study 1. This verified the prolonged retail shelf life benefit of forage-fed beef, compared to grain-fed beef.

In study 3 (Chapter 5), lambs fed four different diets, plain/control (P), tannins-rich diet (T), saponins-rich diet (S), or choice of them (C), were evaluated on metabolomics profiles using GC/MS technique. Forty metabolites were detected (30 named and 10 unknown). A principal component analysis (PCA) plot showed a clear separation of P, T, and S diet treatments while the C diet was overlapped with S and P diets, indicating that S or P diets were preferred while the T diet was avoided. In summary, the effects of ruminant diets on meat characteristics depended on the type and concentration of plant secondary compounds (PSC), especially the PSC levels contained in the pastures.

(171 pages)

# PUBLIC ABSTRACT

## Chemical Characterization of Meat Related to Animal Diet

Rossarin Tansawat

There is currently much interest in increasing health benefits from consuming nutritious food, including beef. Plant secondary compounds (PSC) such as tannins or saponins in various forages have an influence on animal nutrition and health, depending on the type of PSC and the amount consumed. However, relatively little is known about effects of PSC on meat color, flavor, and nutritional value. Thus, additional information is needed to better understand and to develop new animal feeding regimes for optimum animal growth, meat flavor, and meat nutritional quality.

In the first study, grain- vs. pasture-fed beef rib steaks were evaluated. The objective was to examine meat characteristics as affected by cattle diet; and to examine the relationship between meat volatiles during heating with meat sensory profile, as determined in a separate study. Ribs from pasture-fed animals had much lower fat content, more omega-3 polyunsaturated fatty acids and conjugated linoleic acid than grain-fed beef. Pasture-fed beef also had lower measures of oxidation during retail storage and higher antioxidant capacity. Both diets also influenced the chemical volatile profiles of cooked meat and were distinctively associated with consumer sensory descriptors. Grain beef had higher levels of hexanal, 1-octen-3-ol, 2,3-octandione, and 2,6-bis (1,1-dimethylethyl)-4-ethyl-phenol, uniquely associated with umami and juicy flavors. In the second study, beef finished with two pasture-finishing models, tall fescue-alfalfa (containing PSC saponins) vs. tall fescue-sainfoin (containing PSC tannins), were compared. Meat characteristics were not different between the two legume diets, indicating that sainfoin was comparable to alfalfa as a cattle forage. However, more information is needed regarding rate of weight gain and other production factors for cattle finished on sainfoin pastures.

Metabolomics is the study of the complete set of small molecules produced in a tissue such as muscle during metabolism of carbohydrates, lipids, peptides, or nucleotides. There is limited information about metabolomics of meat animals, i.e., how diet affects the genetic machinery and meat chemistry. In a third study, lambs (infected with red stomach worm larvae) were fed different purified PSC's to determine possible anti-parasitic effects (companion study) and metabolomics profile in lamb loin muscle using a gas chromatography/mass spectroscopy technique. Diet treatments included dried beet pulp supplemented with tannins or saponins, given in single ration or as choice of them. Carbohydrate metabolites were higher in animals fed tannin diets. Cholesterol levels were lower in saponin groups, in agreement with many previous studies reporting cholesterol lowering activity of saponins in mammals.

# ACKNOWLEDGMENTS

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| | |
|---|---|
| %KPH | % Kidney, pelvic, and heart fat |
| a* | Redness |
| AA | Arachidonic acid |
| AMDIS | Automated Mass Spectral Deconvolution and Identification System |
| ANOVA | Analysis of variance |
| AOAC | The Association of Official Agricultural Chemists |
| AUC | Area under curve |
| b* | Yellowness |
| BF | Back fat thickness |
| C | Choice diet |
| CAT | Catalase |
| CLA | Conjugated linoleic acid |
| COX | Cyclooxygenase |
| CT | Condensed tannin |
| DHA | Docosahexaenoic acid |
| dMb | Deoxymyoglobin |
| DPPH | 2,2-diphenyl-1- picrylhydrazyl radical scavenging capacity assay |
| EEF | Ether-extract fat |
| EFA | Essential fatty acids |
| EPA | Eicosapentaenoic acid |
| FA | Fatty acid |
| FAMEs | Fatty acid methyl esters |
| FEC | Faecal egg counts |
| FRAP | Ferric reducing antioxidant power |
| GC | Gas chromatography |
| GC-FID | Gas chromatography with flame ionization detection |
| GC-MS | Gas chromatography - Mass spectrometry |

| | |
|---|---|
| GPx | Glutathione peroxidase |
| GSH | Reduced glutathione |
| GSSG | Oxidized glutathione |
| GT | Glutathione |
| HW | Hanging weight |
| HydrORAC | Hydrophilic ORAC |
| L* | Lightness |
| L3 | Third larval stage |
| LC-PUFA | long chain polyunsaturates |
| LD | Longissimus dorsi |
| LipORAC | Lipophilic ORAC |
| LOX | Lipoxygenase |
| LRI | Linear retention indexes |
| LSD | Least significant different |
| LT | Leukotriene |
| Mb | Myoglobin |
| $MbO_2$ | Oxymyoglobin |
| MDA | Malondialdehyde |
| MetMb/MMb | Metmyoglobin |
| MRA | Metmyoglobin reducing activity |
| MS | Mass spectrometry |
| MSTFA | N-methyl-N-trimethylsilyl-trifluoroacetamide |
| MUFA | Monounsaturated fatty acid |
| NIST | The National Institute of Standards and Technology |
| NMR | Nuclear magnetic resonance |
| ORAC | Oxygen radical absorbance capacity |
| P | Plain/Control diet |
| PC | Principal component |
| PCA | Principal component analysis |

| | |
|---|---|
| PG | Prostaglandin |
| ppm | mg /1000 g |
| PSC | Plant secondary compounds |
| PTFE | Polytetrafluoroethylene |
| PUFA | Polyunsaturated fatty acid |
| PVC | Polyvinyl chloride |
| r | Pearson correlation coefficients |
| RDA | Recommended Dietary Allowance |
| RDI | Recommended Daily Intake |
| REA | Ribeye area |
| ROOH | Lipid peroxides |
| S | Saponin-rich diet |
| SAS | Statistical Analysis Software |
| SFA | Saturated fatty acid |
| SOD | Superoxide dismutase |
| SPME | Solid phase micro-extraction |
| T | Tannin-rich diet |
| TBA | Thiobarbituric acid assay |
| TBARS | Thiobarbituric acid reacting substances |
| TE | Trolox equivalents |
| TEAC | Trolox equivalent antioxidant capacity |
| TMS | Trimethylsilyl |
| TVA | trans-Vaccenic acid |
| TX | Thromboxane |
| USDA | The United States Department of Agriculture |
| ω-3 | Omega-3 fatty acid |
| ω-6 | Omega-6 fatty acid |

# CHAPTER 1

# INTRODUCTION

Good health starts with good food. But, where does good food begin, and what exactly is good food? Most people would agree that in addition to good taste, good food is nutritious. Currently, there is much interest in increasing the health benefits of meat from pasture-fed ruminants. Several studies have revealed health benefits of pasture-fed beef over grain-fed beef (McCluskey *et al*., 2005; Knight *et al*., 2003). These reports have demonstrated a relationship between the animal diet, meat nutritional properties, and human health.

In spite of the attention given to pasture versus grain feeding of livestock, more information is needed regarding effects of plant secondary compounds (PSC) in various forages related to meat quality as well as human health. Plant secondary compounds include toxins produced by plants as deterrents to attack by insects or grazing animals. However, consumption of toxic PSC's can sometimes have positive impact on animal nutrition and health, depending on the type of PSC and the amount consumed (Vasta *et al*., 2008). For example, some tannins enhance animal nutrition by their capability to link to dietary proteins, thus protecting the protein from degradation by rumen bacteria (Lisonbee *et al*., 2009), and saponins reportedly have cholesterol lowering activity in mammals (Guclu-Ustundag & Mazza, 2007). Therefore, it would be of high interest to evaluate characteristics of meat obtained from the enrichment these PSCs in the feed.

Realini *et al*. (2005) were the first to detect positive effect of ergot-alkaloid containing in tall-fescue in carcass subcutaneous adipose tissues from beef cattle fed with

wild-type tall fescue. In their study, sensory panel evaluations show higher chewiness and lower juiciness of 14-day aged steaks from cattle fed nil-ergot as opposed to endophyte-infected tall fescue. Overall, however, there is limited data about the linkage among plants, herbivore diets, meat quality, consumer preferences, and human health. Thus, additional information as regards these relationships is needed to understand and develop new animal feeding regimes for optimum animal growth, meat flavor, and meat nutritional quality.

**Hypothesis**

Animal diets, including secondary metabolites in the diet, have an effect on meat chemical characteristics, meat quality, and nutritional value of meat as a food.

**Objectives**

1. To examine how meat characteristics are affected by cattle diet; specifically the effect of pasture- versus grain-finishing on beef rib steak composition, and the relationship between meat volatiles (chemical assay) with meat flavor profiles (sensory evaluation).
2. To determine the effects on meat quality of a cattle finishing regime consisting of alfalfa-grass mix (bloating legume containing secondary metabolite saponins) versus sainfoin-grass mix (non-bloating legume containing secondary metabolite tannins).
3. To evaluate the effects of a confinement diet of dried beet pulp supplemented with tannins (purified extract of Quebracho), or saponins (extract of *Quillaja*

*saponaria*), when given in single ration or as choice of them, on lamb rib muscle metabolomics profile, compared to a control diet (beet pulp only).

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# CHAPTER 2

# LITERATURE REVIEW

## Animal Diets

In the United States, cattle are typically raised on pasture from birth in the spring until autumn (7 - 9 months). During the winter months, cattle are fed hay, then finished for 90 - 120 days with a high energy grain-based diet (i.e. corn, barley, and soy) supplemented with small amounts of hay until slaughter. Cattle finished with grain ("grain-fed" or "corn-fed" beef) produce high-fat carcasses with a high degree of marbling, associated with high palatability, and preferred by the majority of consumers.

Currently, however, there is much interest in meat from pasture-finished ruminants, which appeals to health-conscious consumers (McCluskey *et al*., 2005) because it is leaner (lower fat content) and has lower caloric levels as compared to grain-fed beef. Pasture-fed beef also has higher levels of polyunsaturated fatty acids (PUFA) including the omega-3 fatty acids (Eriksson & Pickova, 2007; Ponnampalam *et al*., 2006; Gatellier *et al*., 2005; French *et al*., 2000), and conjugated linoleic acid (CLA; Poulson *et al*., 2004; French *et al*., 2000). Conversely, diet affects the flavor of the resultant meat, and off-flavors were related to the meat derived from pasture-fed animals (Mandell *et al*., 1998; Larick *et al*., 1987; Melton *et al*., 1982).

Pastures used for livestock feeding include grass such as tall fescue, and legumes. Legumes are highly nutritious (high protein) due to their ability of nitrogen fixation. However, some legumes, for example alfalfa and clover, can sometimes cause frothy bloat in ruminants (Berg *et al*., 2000; Majak *et al*., 1995), leading to animals' death. As a

result, non-bloating legumes, i.e. cicer milkvetch, sainfoin and bird's foot trefoil, or legume-grass mixtures system are used for livestock feeding to manage the bloating problem.

**Plant Secondary Compounds**

Plant secondary compounds (PSC), also called as plant secondary metabolites, are a diverse group of molecules that constitute the "plant defense system" and are not involved in the primary biochemical pathways of cell growth and reproduction (Wallace, 2004). PSC are increasingly recognized as important in animal health, welfare, and nutrition (Villalba *et al*., 2011). The effective dose of PSC depends on their concentrations, which differs among plant species and parts of the plants. Forages with low concentrations are beneficial, but excessive consumption can detrimentally affect herbivores health. The classification of PSC based on their chemical structure (with and without nitrogen) and the estimated numbers of PSC from natural products that have been found are shown in Table 2-1. In this study, we mainly focused on the characteristics of meat obtained from animals fed with two PSCs, tannins and saponins.

Tannins

Tannins are non-nitrogen containing phenolic PSC. According to their structures, tannins can be divided in two groups; 1) hydrolyzable tannins and 2) non-hydrolyzable tannins or condensed tannins (Haslam, 1989). Hydrolyzable tannins are the low molecular weight (ranging from 500 to over 3,000) phenolic compounds occurring universally in various plants. Chemically, they are composed of the esters of phenolic

**Table 2-1** Classification of an estimated range of plant secondary compounds

| Plant secondary compounds | No. of natural products |
|---|---|
| **With Nitrogen** | |
| Alkaloids | 12,000 |
| Non-protein amino acids | 600 |
| Amines | 100 |
| Cyanogenic glycosides | 100 |
| Glucosinolates | 100 |
| **Without Nitrogen** | |
| **Terpenoids** | |
| Monoterpenes | 1,000 |
| Sesquiterpenes | 3,000 |
| Diterpenes | 2,000 |
| Triterpenes, Tetraterpenes, Saponins, Steroids | 4,000 |
| **Phenolics** (including tannins) | |
| Flavonoids | 2,000 |
| Polyacetylenes | 1,000 |
| Polyketides | 750 |
| Phenylpropanes | 1,000 |

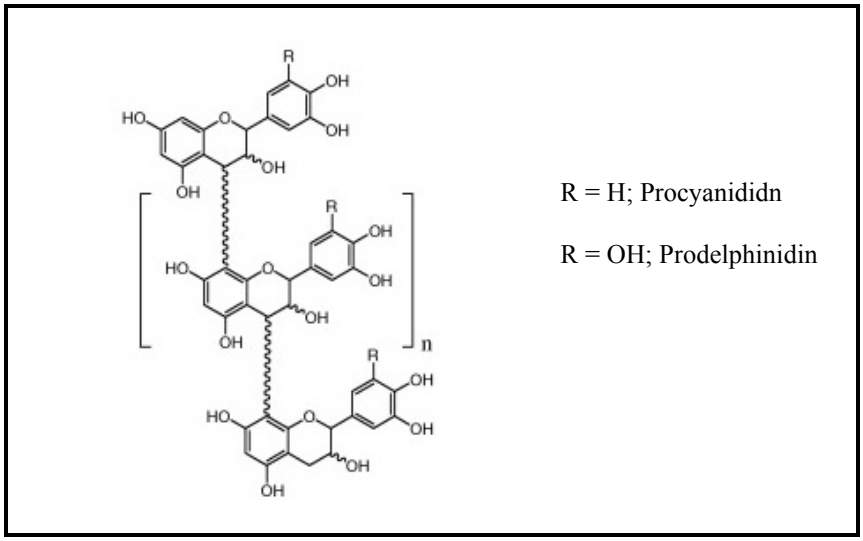
(Adapted from: Acamovic & Brooker, 2005)

acids and a polyol, which is usually glucose. When heating hydrolysable tannins with hydrochloric or sulfuric acids, the yields are gallic or ellagic acids (polyphenolic tannic acid derivatives). Condensed tannins are polymers formed by the condensation of flavans that do not contain sugar residues. There are different types of condensed tannins such as the proanthocyanidins, prodelphinidins, profisetinidins, proguibourtinidins or prorobinetidins. Condensed tannins have high molecular weight (up to 20,000 for

proanthocyanidins) and are the most abundant in woody plants. The structure of condensed tannins is shown in Fig. 2-1.

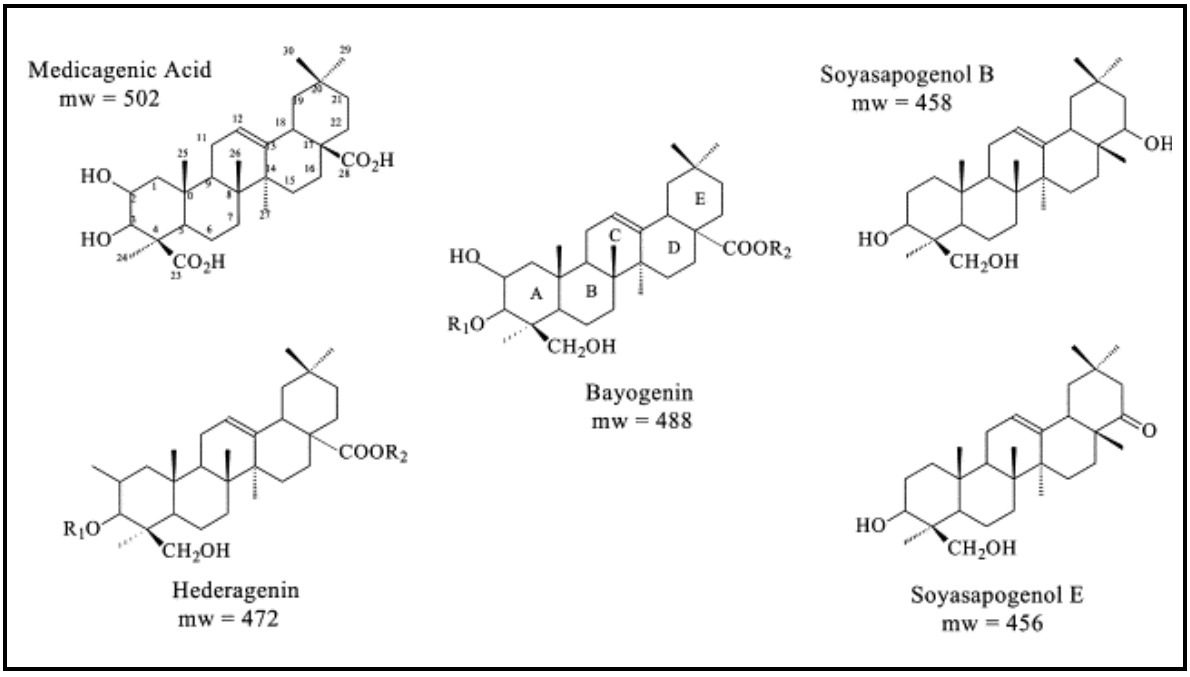
Saponins

Saponins are PSC consisting of one or more hydrophilic glycoside moieties combined with a polycyclic aglycone (Hostettmann & Manton, 1995; http://www.ansci.cornell.edu/plants/toxicagents/saponin.html). The aglycone part (glycoside-free portion), which is also called sapogenins, can be either a steroid (C27) or triterpene (C30). The common saponin aglycones found in legumes are shown in Fig. 2-2. Saponins have a soapy characteristic due to their surfactant properties. The foaming ability of saponins is caused by the combination of a fat-soluble (nonpolar) sapogenin and a water-soluble sugar side chain.




(Source: Barbehenn & Constabel, 2011)

**Figure 2-1** Condensed tannin polymer.




(Source: Huhman & Sumner, 2002)

**Figure 2-2** Chemical structures of common saponin aglycones found in legumes.

**Essential Fatty Acids**

Essential fatty acids (EFA) are fatty acids that humans must acquire by dietary intake because they are vital for normal metabolism but we cannot synthesize them (Goodhart & Shils, 1980). There are two EFAs needed for humans, α-linolenic acid (omega-3 fatty acid) and linoleic acid (omega-6 fatty acid).

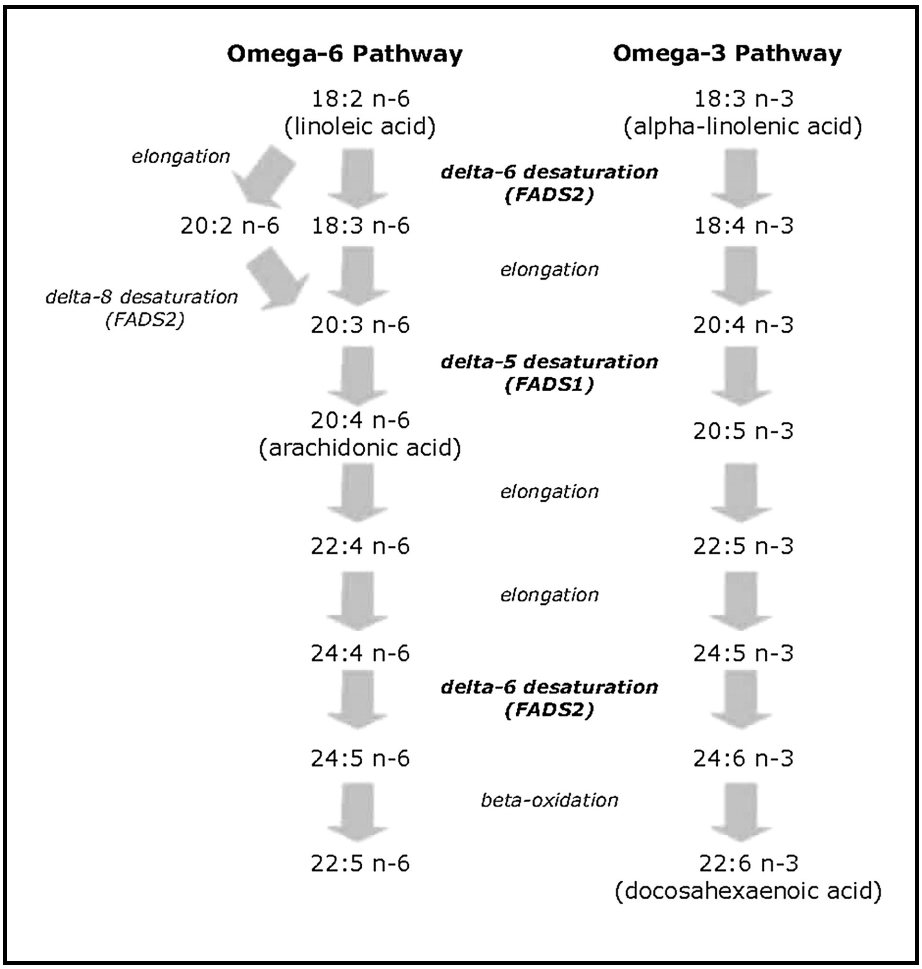
Omega-3 polyunsaturated fatty acids (ω-3 PUFAs) are commonly found in marine fish and seed oils such as flax seeds. Omega-3 fatty acids are essential for normal growth and development and may play an important role in the prevention and treatment of coronary artery disease, hypertension, arthritis, other inflammatory and autoimmune disorders, and cancer (Simopoulos, 1991). After consumption of ω-3 PUFAs, mammals

have an ability to synthesize long-chain ω-3 fatty acids including eicosapentaenoic acid (EPA; C20:5) and docosahexaenoic acid (DHA; C22:6). The biosynthesis pathway of long-chain fatty acids from omega-3 and omega-6 fatty acids is shown in Fig. 2-3.

Dietary omega-6 (ω-6) PUFAs are obtained mostly from vegetable oils, i.e. corn, soybean, olive, and sunflower oils. A large amount of literature suggests that high intake of ω-6 PUFAs reduce risk for coronary heart disease (Harris *et al*., 2009). Figure 2-3 shows that ω-6 PUFAs can be converted to arachidonic acid (AA; C20:4), the substrate for the production of a wide variety of eicosanoids (20-carbon AA metabolites). Arachidonic acid is a polyunsaturated fatty acid that is present in the membrane phospholipids abundant in brain, muscles, and liver. AA can be released from phospholipids by nervous stimulation pathways. Then, two families of enzymes, cyclooxygenase (COX) and lipoxygenase (LOX), catalyze fatty acid oxygenation to produce the eicosanoids (Fig. 2-4). Some eicosanoids such as prostaglandin E2 (PGE2) and thromboxane A2 (TXA2) derived from the COX pathway, and leukotriene B4 (LTB4) derived from the LOX pathway, are considered as pro-inflammatory, vasoconstrictive, and/or pro-aggregatory.

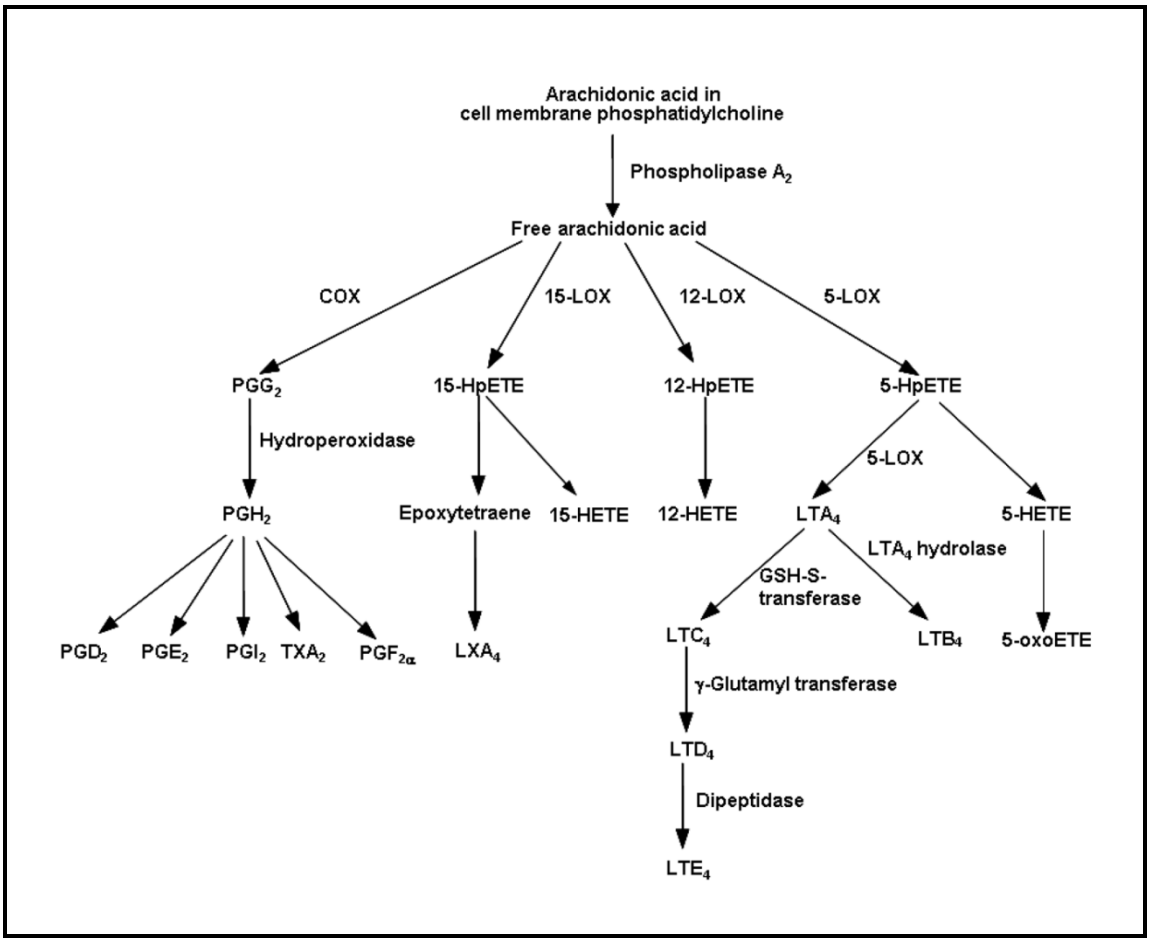
Competition between ω-6 and ω-3 PUFAs occurs in eicosanoid formation. EPA competes with AA for PG and LT synthesis at the COX and LOX levels. Thus, increasing dietary ω-3 intake leads to a decrease in the formation of "bad" or pro-inflammatory eicosanoids. The ratio of ω-6 to ω-3 fatty acids is an important determinant of health (Simopoulos, 2003). Several clinical studies indicated that excessive amounts of ω-6 PUFA and a very high ω-6/ω-3 ratio promote the pathogenesis of many diseases,

including cardiovascular disease, cancer, and inflammatory and autoimmune diseases, whereas increased levels of ω-3 PUFA (a lower ω-6/ω-3 ratio), exert suppressive effects (Simopoulos, 2004; de Lorgeril & Salen, 2003).




(Source: Koletzko *et al*., 2011)

**Figure 2-3** Biosynthesis of long-chain fatty acids from omega-3 and omega-6 fatty acids.




(Source: Calder, 2010)

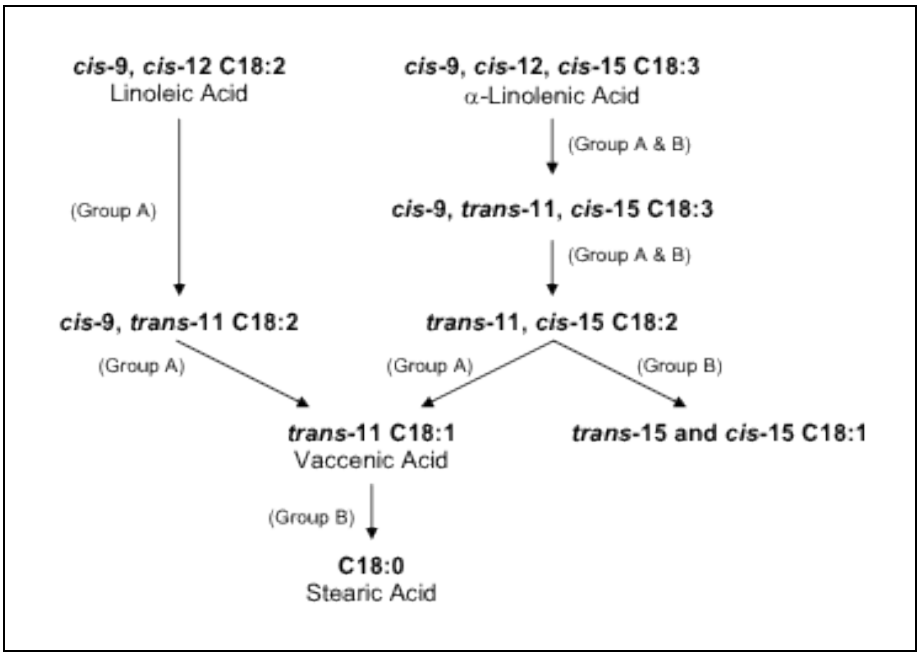
**Figure 2-4** Biosynthesis of eicosanoids from arachidonic acid. COX, cyclooxygenase; HETE, hydroxyeicosatetraenoic acid; HpETE, hydroperoxyeicosatetraenoic acid; LOX, lipoxygenase; LT, leukotriene; LX, lipoxin; oxoETE, oxoeicosatetraenoic acid; PG, prostaglandin; TX, thromboxane.

## Conjugated Linoleic Acids

Conjugated linoleic acids (CLA) are a group of isomers of linoleic acid (C18:2) found mainly in the meat and dairy products derived from ruminants (Daley *et al*., 2010). Of the many isomers identified, the cis-9, trans-11 CLA isomer is the main isomer accounting for up to 80 - 90% of the total CLA in ruminant products (Nuernberg *et al*.,

2002). The positive health effects of CLA are the reduction in body fat accretion and altered nutrient partitioning, anti-diabetic effects, reduction in the development of atherosclerosis, enhanced bone mineralization, and modulation of the immune system (Bauman *et al*., 1999).

CLA can occur from two natural sources; 1) bacterial bio-hydrogenation of PUFAs in the rumen, and 2) desaturation of trans-fatty acids in the adipose tissue and mammary gland of ruminants (Griinari *et al*., 2000; Sehat *et al*., 1999).




(Source: Bauman *et al.*, 2003)

**Figure 2-5** Biochemical pathways for the bio-hydrogenation of linoleic and linolenic acids in the rumen. Rumen bacteria involved in bio-hydrogenation have been classified into two groups, A and B, based on their metabolic pathways, with both groups required to be present in order to obtain complete biohydrogenation of PUFAs.

Linoleic and linolenic acids are the major substrates for microbial bio-hydrogenation (Bauman *et al*., 2003) by an anaerobic rumen bacterium *Butyrivibrio fibrisolvens* (Kepler *et al*., 1966), which is highly dependent on rumen pH (Pariza *et al*., 2000). Figure 2-5 illustrates the biochemical pathways for the bio-hydrogenation of linoleic and linolenic acids in the rumen. De novo synthesis of CLA occurs by the bio-hydrogenation of linoleic acid via trans-vaccenic acid (TVA) as an intermediate. Turpeinen *et al*. (2002) reported a linear relationship between CLA synthesis and TVA content. CLA synthesis increased with the increase of TVA concentration in the diet.

**Lipid Oxidation in Meat**

Lipid oxidation (also termed peroxidation or autoxidation; the spontaneous reaction of a compound with molecular oxygen at room temperature) is a major cause of quality deterioration of stored meat and meat products (Min & Ahn, 2005; Ladikos & Lougovois, 1990). Lipid oxidation is related to flavor deterioration (development of rancidity or warmed-over flavor), loss of color (redness), loss of nutritional value, functional property changes, or the formation of toxic compounds, all of which affect consumer acceptance of the meat (Addis, 1986; Frankel, 1984). Lipid oxidation is a free radical chain reaction or autoxidation that consists of three steps: 1) Initiation, the formation of free radicals; 2) Propagation, the free-radical chain reactions; and 3) Termination, the formation of non-radical products (Fig. 2-6).

Reactive oxygen species (ROS), such as superoxide ($O_2^{\bullet -}$), hydroperoxyl radical ($OH_2\bullet$), hydrogen peroxide ($H_2O_2$), and hydroxyl radical ($\bullet OH$), are the major initiators of the chain reaction. Unsaturated fatty acid moieties are the important lipids involved in

oxidation. The main unsaturated fatty acids comprising the lipids of animal tissues are oleic, linoleic, linolenic, and arachidonic (Ladikos & Lougovois, 1990). The other factors rather than degree of unsaturation that could affect the development of lipid oxidation in meat are the processing and storage conditions of meat, the antioxidants and additives, or the pro-oxidants such as free iron. Soluble (free) iron has been considered a major catalyst for the initiation step of lipid oxidation. In meat, iron sources include hemoglobin & myoglobin, iron-containing enzymes, and transferrin (Min & Ahn, 2005).

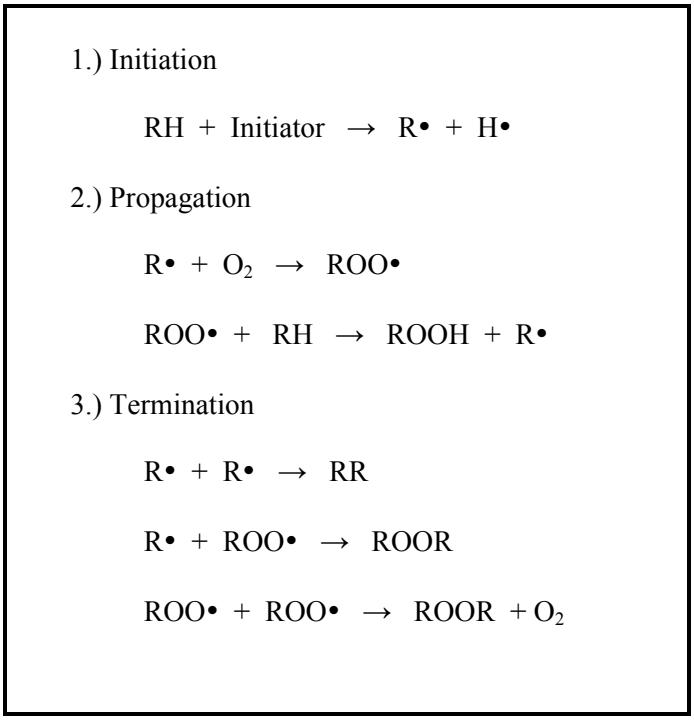



**Figure 2-6** The general schematic diagram for the three steps of autoxidation: Initiation, Propagation, and Termination. RH = unsaturated lipid; R• = lipid free radical; H• = hydrogen free radical; ROO• = peroxy free radical; ROOH = lipid hydroperoxide; RR = lipid dimer; ROOR = lipid peroxide.

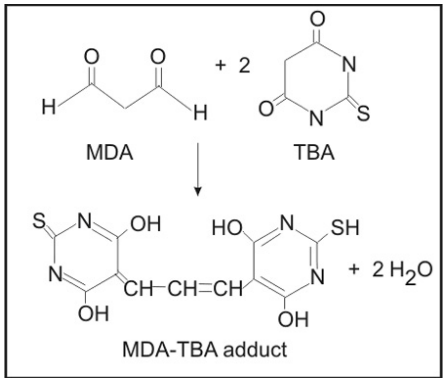
Measurement of lipid oxidation

Lipid oxidation in meat can be measured by either direct or indirect approaches of free radicals. For the direct measurements, free radicals can be detected and characterized by electron spin resonance or spin trapping methods. Markers of free radicals can be indirectly measured by several methods such as iodine value, peroxide value, thiobarbituric acid reacting substances (TBARS), high-performance liquid chromatography with fluorometric detection, or gas chromatography. The thiobarbituric acid (TBA) assay is the most widely employed due to its simplicity for measuring autoxidation of fats and oils in foods. The assay of TBARS measures malondialdehyde (MDA) present in the sample, which is generated from lipid hydroperoxides. The basic principle of this method is the reaction of 1 molecule of MDA and 2 molecules of TBA to form an MDA-TBA complex (pink to red color), which can be quantified by spectrophotometric absorbance at 532 nm (Fig. 2-7).

**Myoglobin Oxidation in Meat**

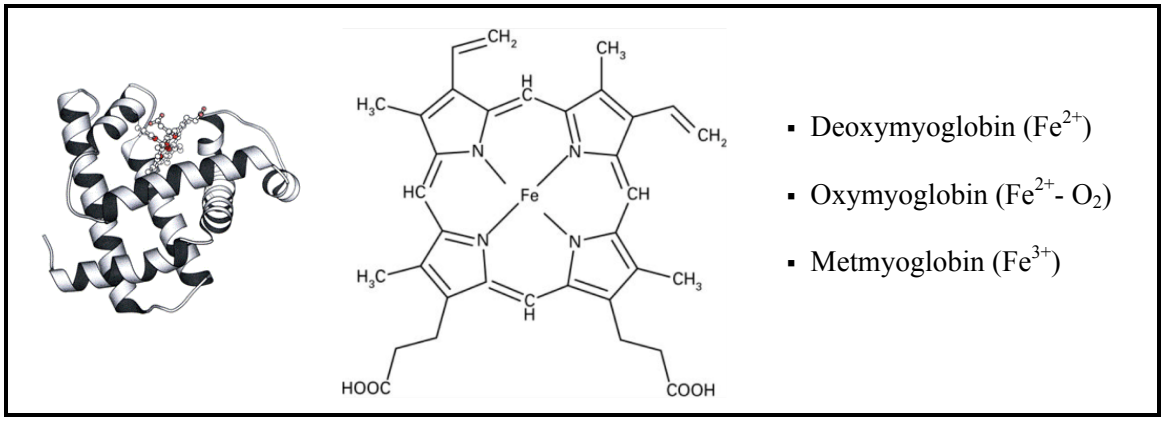
Myoglobin (Mb) is an iron- and oxygen-binding protein found in the muscle tissue. Mb contains the heme porphyrin, which has a single iron molecule at its center (Fig. 2-8). There are three myoglobin pigments important in the fresh meat systems: deoxymyoglobin (dMb), oxymyoglobin ($MbO_2$), and metmyoglobin (MetMb). The redox state of the iron atom determines the color of the meat. Fresh cut beef has the native meat pigment form of dMb (no oxygen bound; purple color). When the fresh beef is exposed to oxygen, oxygen will bind to the heme iron of myoglobin forming $MbO_2$, which has bright red color. The iron of both dMb and $MbO_2$ is in the reduced state (ferrous, $Fe^{2+}$). As meat

ages, it turns brown as the Mb is converted to MetMb and $Fe^{2+}$ is oxidized to $Fe^{3+}$ (ferric), resulting in rejection of fresh retail beef by consumers. MetMb acts as a catalyst of lipid oxidation, and lipid peroxidation increases the rate of MetMb formation, so their levels were closely correlated (Anton *et al.*, 1996).




(Sources: Paul, H. An Introduction to reactive oxygen species: Measurement of ROS in cells, BioTek Instruments, Inc. Available at http://www.biotek.com/assets/tech_resources/ROS_White_Paper.pdf)

**Figure 2-7** TBARS assay reactions. TBA = thiobarbituric acid; MDA = malondialdehyde.




(Sources: http://upload.wikimedia.org/wikipedia/commons/b/be/Heme_b.svg)

**Figure 2-8** From left; myoglobin, heme porphyrin, and myoglobin pigments in fresh meat.

Metmyoglobin reducing activity

Metmyoglobin reducing activity (MRA) is a measurement of the ability of muscle samples to reduce MetMb. Hutchins *et al.* (1967) reported a significant positive correlation between lipid oxidation and MetMb concentration, and a significant negative correlation between MetMb concentration and MRA. Bekhit & Faustman (2005), in their review *"Metmyoglobin Reducing Activity,"* summarized factors affecting MRA into 2 categories: 1) enzymatic, and 2) non-enzymatic systems. Factors affecting enzymatic oxidation/reduction include lipid oxidation, oxygen level, storage time, temperature, pH, light, ions and pro-oxidant chemicals, availability of nucleotides such as NADH/NADPH, or exercise and diet. Factors affecting non-enzymatic metmyoglobin reduction include the presence of EDTA, ascorbic acid, vitamin E, or bacteria.
MRA in meat can be measured by one of these following assays; MetMb reducing activity (Stewart *et al.*, 1965), reduction of nitrite oxide MetMb (Watts *et al.*, 1966), aerobic reducing ability (Ledward, 1972), total reducing activity (Lee *et al.*, 1981), reduction of 2,6-dichlorophenol indophenol (Rossi-Fanelli *et al.*, 1957), methylene blue MetMb reductase activity (Echevarne *et al.*, 1990), or MetMb reductase activity (Reddy & Carpenter, 1991).

**Antioxidants in Meat**

An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Antioxidant compounds can be incorporated in beef muscle through dietary delivery and protect tissues against oxidation from reactive oxygen species, resulting in improved color stability, stabilized fatty acids in meat, and extended storage life.

Antioxidant defenses in meat include non-enzymatic hydrophilic and lipophilic soluble compounds such as vitamin E, vitamin C, carotenoids, ubiquinols, polyphenols, cellular thiols, or antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), which operate to counteract the action of pro-oxidants in muscle tissues (Descalzo & Sancho, 2008). Concentrations of endogenous antioxidants depend on animal species, muscle type and diet. Pasture diet conferred an improved overall antioxidant status in fresh beef when compared to grain finished diets (Descalzo *et al.*, 2005, 2007; Gatellier *et al.*, 2004). Meat obtained from pasture-finished animals is particularly rich in natural antioxidants such as vitamin A, C and E or phytochemicals such as carotenoids and flavonoids (Daley *et al.*, 2010; Wood & Enser, 1997), as well as cancer fighting antioxidants such as glutathione (GT) and SOD (Daley *et al.*, 2010), compared to grain-fed meat.

Antioxidant vitamins

Vitamin E is a fat-soluble vitamin with powerful antioxidant activity. The most bio-potent isoform of vitamin E is α-tocopherol (Pryor, 1996). Vitamin E, α-tocopherol, improves the quality of meat by its pronounced color stabilizing effect, as well as delaying lipid oxidation, thus prolonging retail display life (Descalzo & Sancho, 2008; Faustman *et al.*, 1998; Wood & Enser, 1997). The mechanism of action is the rapid oxidation of α-tocopherol in membranes, in preference to other membrane lipids (about 104 times faster than the propagation of membrane lipids). Therefore, membrane lipids are spared from oxidation, retarding oxymyoglobin oxidation and meat decoloration (Morrisey *et al.*, 2000; Faustman *et al.*, 1998). Alpha-tocopherol also inhibits fatty acid

oxidation in meats, when incorporated into the living muscle, thus protecting the tissue at the onset of the lipid oxidation, and slowing oxidation of membrane phospholipids during meat storage (Descalzo & Sancho, 2008). Dietary vitamin E supplementation of livestock increases concentrations of α-tocopherol within cell membranes (Ashgar *et al.*, 1991; Monahan *et al.*, 1990). Liu *et al.* (1995) reported a review from cumulative experiments that steers fed 500 IU/ daily of vitamin E for 126 day could assuredly benefit the domestic retail market by extending color display life of meat. In addition, many studies report that ruminants finished with forage had higher α-tocopherol levels in their tissues, compared to muscles from concentrated-fed animals (Fuente *et al.*, 2009; Insani *et al.*, 2008; Descalzo *et al.*, 2005; Muramoto *et al.*, 2005; Gatellier *et al.*, 2004; Realini *et al.*, 2004b; Lanari *et al.*, 2002; Yang *et al.*, 2002).

Carotenoids are tetra-terpenoid organic pigments that are naturally occurring in the chloroplasts and chromoplasts of higher plants. β-Carotene is a fat-soluble compound that is thought to act as a biological antioxidant, specifically a quencher of singlet oxygen that protects against lipid oxidation occurring in highly unsaturated fatty acids in cellular membranes (Witt *et al.*, 1992). β-Carotene cooperates with tocopherols in the radical scavenging capacity within the interior of lipid membranes (Tsuchihashi *et al.*, 1995). Muramoto *et al.* (2003) showed that dietary β-carotene supplementation in cattle extended the acceptable color muscle shelf life by 1.5 - 3 days ($P < 0.001$). Incorporation of β-carotene from diet into muscle showed a high variability among experiments, depending on dietary delivery, muscle type, and the individual animal uptake capacity (Descalzo & Sancho, 2008). Cattle produced under extensive grass-based production

systems generally have more yellow carcass fat of derived from dietary carotenoids, compared to concentrate-finished cattle (Daley *et al.*, 2010), associated with a healthier fatty acid profile and a higher antioxidant content (Dunne *et al.*, 2009). Pasture-fed animals were found to incorporate significantly higher amounts of β-carotene into muscle tissues as compared to grain-fed animals (Insani *et al.*, 2008; Descalzo *et al.*, 2005; Muramoto *et al.*, 2003, 2005; Yang *et al.*, 2002).

Vitamin C or L-ascorbic acid is a hydrophilic reducing agent, which inhibits myoglobin oxidation and brown color development in beef (Sanchez-Escalante *et al.*, 2001). Ascorbic acid is commonly added to post-mortem raw ground beef to improve redness retention and extend shelf life during retail display. Descalzo *et al.* (2005) measured the level of vitamin C in fresh beef from pasture- and grain-fed animals and found that pasture-fed animals had higher content of vitamin C when compared to grain-fed beef. There were 21.98 - 25.30 μg/g of ascorbic acid for meat from pasture versus 15.92 - 17.39 μg/g for grain-fed animals, which is lower than the concentrations added to improve meat stability (500 - 1000 μg/g, Realini *et al.*, 2004a). Nevertheless, King *et al.* (1995) reported that meat from broilers finished with L-ascorbic acid for 24 h prior to slaughter did not improve the lipid oxidation status (TBARS values) as compared to control ($P > 0.05$).

Antioxidant enzymes

Antioxidant enzymes constitute the primary mechanism for protecting cells from oxidative damage in vivo (Halliwell & Gutteridge, 1989). The most important antioxidant enzymes in muscle are superoxide dismutase (SOD), catalase (CAT) and glutathione

peroxidase (GPx), which are an intracellular barrier against free radicals in fresh meat (Descalzo & Sancho, 2008). SOD and CAT are coupled enzymes that work together. SOD scavenges superoxide anions by forming hydrogen peroxide ($O_2^{\bullet -} + 2H_2O \rightarrow H_2O_2$) and CAT decomposes the hydrogen peroxide by: $H_2O_2 \rightarrow 2H_2O + O_2$. GPx is an antioxidant enzyme which function is to reduce organic peroxides and free hydrogen peroxide to water. The reduced form of glutathione (GSH) can decompose both $H_2O_2$ or lipid peroxides (ROOH) to oxidized glutathione (GSSG) by GPx catalysis ($2GSH + H_2O_2 \rightarrow GSSG + 2H_2O$; $2GSH + ROOH \rightarrow GSSG + ROH + H_2O$).

Researchers demonstrated diverse results of antioxidant activity in beef as affected by diets. Misra & Fridovich (1972) reported that meat from pasture-fed animals had higher SOD activity than meat from grain-fed animals. The same tendency was also shown by Gatellier *et al.* (2004), Mercier *et al.* (2004), Descalzo *et al.* (2007), and Insani *et al.* (2008). Gatellier *et al.* (2004) found that a pasture diet considerably increased SOD activity in *Longissimus dorsi* muscle of Charolais cows as compared to a mixed diet ($P < 0.001$). However, they also showed that lower activities of CAT and GPx were produced in meat from pasture-fed than meat from mixed-diet cattle ($P < 0.001$). Mercier *et al.* (2004) demonstrated significantly higher ($P < 0.001$) SOD and GPx levels in meat from cattle fed pasture over mixed-diet meat, but the CAT level was not significantly different between diet groups. Descalzo *et al.* (2007) also stated that pasture-fed meat had significantly higher SOD activity than meat from grain-fed animals ($P < 0.05$), but the CAT and GPx activities were similar in both diet treatments. However, Insani *et al.* (2008) reported that SOD activity tended to be higher (but not significantly, $P > 0.10$) in

meat from cattle fed a pasture diet (13.6 units/mg protein) compared to the grain diet (9.8 units/mg protein). Yet, significant difference ($P < 0.05$) in CAT and GPx activities were also reported. CAT levels were significantly higher in meat from forage-fed than meat from animals fed a concentrate diet (11.3 vs. 8.9 units/mg), whereas grain-fed meat had a significantly higher GPx level as compared to pasture-fed meat (12.3 vs. 22.3 units/mg protein).

Measurement of total antioxidant activity in meat

The antioxidant activity is the capability of a compound (composition) to inhibit oxidative degradation, e.g. lipid peroxidation. Several antioxidant assessment methods are available; for example, ferric reducing antioxidant power (FRAP), trolox equivalent antioxidant capacity (TEAC), 2,2-diphenyl-1- picrylhydrazyl radical scavenging capacity assay (DPPH), and oxygen radical absorbance capacity (ORAC) have been used to evaluate antioxidant activities in fresh beef. However, the results varied among different techniques. Gatellier *et al.* (2004) used the benzoate hydroxylation test to measure OH• scavenging activities and found that OH• scavenging activities were more pronounced in meat from mixed diets than from pasture-fed animals ($P < 0.001$). The authors also applied the TEAC test against ABTS•+ [2,2-azin-obis-(3-ethylbenzothiazoline-6-sulphonic acid)] radical cation to the same meat samples and found that there were no significant effects between diet treatments. Descalzo *et al.* (2007) applied FRAP and TEAC assays to measure the antioxidant activities in fresh meat. They reported that beef from pasture-fed animals presented higher reducing potential than meat from grain-fed animals ($P < 0.05$) using the FRAP test, but no differences were found between diet

groups in the TEAC assay. Wu *et al.* (2008) developed the ORAC assay to determine antioxidant activity in meat products and found that hydrophilic ORAC did not differ among forage-finished (alfalfa, pearl millet and naturalize pasture) and high concentrate finished animals ($P < 0.05$). However, higher levels ($P < 0.01$) of lipophilic ORAC were found in beef extract from alfalfa and pearl millet-finished steers, compared to samples from cattle finished on naturalized pasture or high concentrate diets.

**Beef Volatiles**

Flavors and aromas associated with beef are generally those that develop when heat is applied, depended on the amounts and proportions of precursor compounds present. A variety of volatiles occur during heating, i.e., acids, alcohols, aldehydes, aromatic compounds, esters, ethers, furans, hydrocarbons, ketones, lactones, pyrazines, pyridines, pyrroles, sulfides, thiazoles, and thiophenes (Shahidi, 1994). Proteins, carbohydrates, and lipids play primary roles in beef flavor development (Mottram, 1998; Spanier & Miller, 1993). The characteristic flavor of cooked meat derives from thermally induced reactions, principally generated by the Maillard reaction and the degradation of lipids (Mottram, 1998; Bailey, 1983). Maillard reactions occur when carbohydrates (glucose) give off furans, which then react with sulfur-containing amino acid cysteine (Umano *et al.*, 1995), resulting in roasted meat aromas (Brewer, 2006). Amino acids and peptides can produce compounds such as ammonia, aldehydes and amino ketones. Nucleotides produce furanones, which are associated with meaty flavor (Spanier & Miller, 1993). The oxidation of unsaturated fatty acids generates intermediate hydroperoxides that finally results in aldehydes, unsaturated alcohols, ketones, and

lactones (Mottram, 1998). Aldehydes generally possess meaty and tallow odors (Rowe, 2002). Phospholipids in muscle tissue consist of a high proportion of unsaturated fatty acids that are susceptible to oxidation. Example of flavors and aromas associated with volatile compounds in beef are shown in Table 2-2.

Beef aromas could be influenced by heat, animal breed, aging time, muscle type, enhancement such as brine injection, and animal diet. Ruminant diets were reported to have an effect on beef flavors in many studies and positive sensory evaluation is usually higher in meat from animals given a grain-finishing diet in the period before harvest. For instance, Melton's review (1990) found that high-energy grain diets produced more acceptable and intense flavor in meats than low-energy pasture diets. Hedrick *et al*. (1980) demonstrated that meat from steers grazed on fescue pasture has a grassy and bitter flavor.

**Metabolomics**

Metabolomics is the study of the complete set of small-molecule metabolites (metabolome) produced within a biological organism (cells, body fluids, tissues). Stated another way, it is a survey of the unique chemical fingerprints in the body, which are the end products of its gene expression. Metabolites measuring by metabolomic analysis are the intermediates and products of metabolism such as peptides (i.e., cofactors, signaling molecules), nucleotides, carbohydrates, and lipids.

Regarding the "-omics" cascade going from genotype to phenotype, metabolomics is the last -omics among the functional genomics technologies (de Hoog & Mann, 2004; Fig. 2-9). Since the polymorphisms in gene, transcription, and protein levels

**Table 2-2** Flavors and aromas associated with volatile compounds in beef

| Volatile Compounds | Aromas |
|---|---|
| Pentanal | Pungent |
| Hexanal | Green, grassy, fatty |
| Heptanal | Green, fatty, oily |
| Nonanal | Soapy |
| Methional | Cooked potato |
| 12-methyltridecanal | Beefy |
| Nona-2(E)-enal | Tallowy, fatty |
| Deca-2(E), 4(E)-dienal | Fatty, fried potato |
| Butanoic Acid | Rancid |
| Hexanoic Acid | Sweaty |
| Delta-nonalactone | Sweet, dairy, or waxy notes |
| 3-Hydroxy-2-butanone | Buttery |
| 2,3-Octanedione | Warmed over flavor, lipid oxidation |
| 1-Octene-3-ol | Mushroom |
| 2-Pentyl furan | Metallic, green, earthy, beany |
| 2-methyl-3-(methylthio)furan | Meaty, sweet, sulfurous |
| 4-hydroxy-5-methyl-3(2H)-furanone (HMF) | Meaty |
| Methylpyrazine, 2,5- (and 2,6-) dimethylpyrazine | Roasted, nutty |
| Pyrazines | Nutty, cracker- like, bell pepper |
| Amino acids: glycine, alanine, lysine, cysteine, methionine, glutamine, succinic | Sweet |
| Organic acids: lactic, inosinic, ortho- phosphoric, and pyrrolidone carboxylic | Sweet |
| Sugars: glucose, fructose, ribose | Sweet |
| Amino acids: aspartic acid, histidine, asparagines | Sour |
| Organic acids: succinic, lactic, inosinic, ortho-phosphoric, pyrrolidone carboxylic | Sour |
| Hypoxanthine, anserine, carnosine | Bitter |
| Amino acids: arginine, leucine, tryptophan | Bitter |
| Monosodium glutamate (MSG), inosine and guanosine monophosphate (IMP and GMP) | Savory, brothy, beefy |
| Bis (2-methyl-3-furyl) disulfide | Roasted meat |
| 2-methyl-3-furanthiol | Roasted meat |

(Source: Brewer, 2006)

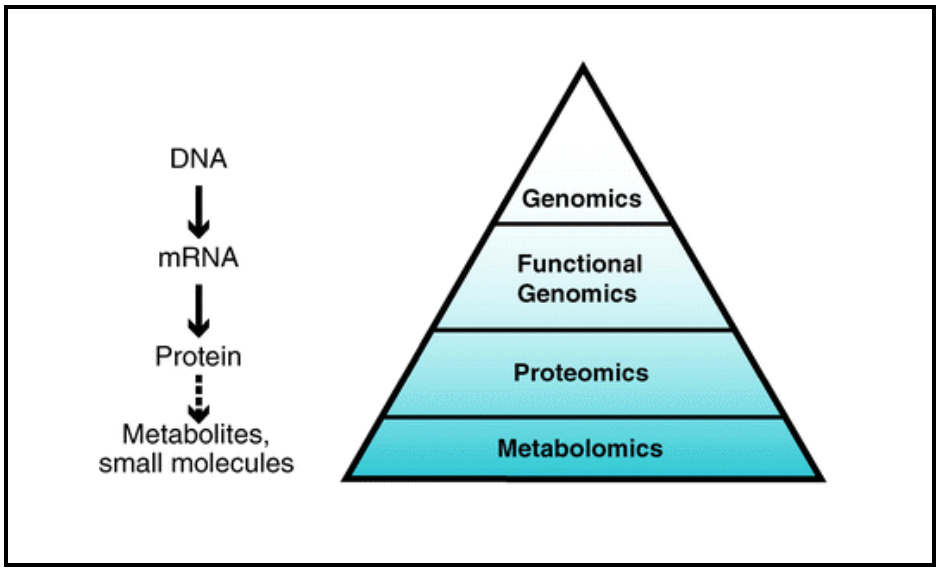
can be influenced by the environment, i.e. diet, which affects the end results of cell metabolism, metabolomics is considered to be a better characterization of the phenotype of an organism than other -omic sciences. The analysis of comprehensive metabolomic profiles will help to achieve an integrated understanding of the genetic capabilities of an organism. Metabolomic techniques contribute to several life sciences including nutrition, pharmacology, and medicine. For example, metabolomics may be used as a tool to understand metabolic disorders such as diabetes and obesity (Griffin & Nicholls, 2006), as a clinical application in oncology (Spratlin *et al*., 2009), as a tool to develop new drugs (Shyur & Yang, 2008; Wishart, 2008a; Kell, 2006), or as a tool for nutraceutical evaluation (Fujimura *et al*., 2011; Balderas *et al*., 2010). Moreover, metabolomics analysis techniques are also applied to individually personalized diets (German *et al*., 2003, 2004) or medicines (Kaddurah-Daouk *et al*., 2008), which are a novel current trend in life sciences.

Metabolomics analysis techniques

Several analytical techniques are used for metabolomics, including direct-injection mass spectrometry analysis (Dettmer *et al*., 2007; Dunn, 2005) i.e. fourier transform ion cyclotron resonance (FTICR-MS) and time of flight mass spectrometers (TOF-MS), high-performance and ultra-performance liquid chromatography combined with mass spectrometry (HPLC-MS and UPLC-MS; Nordstrom *et al*., 2006), liquid chromatography-mass spectrometry (LC-MS; Lu *et al*., 2008), gas chromatography-mass spectrometry (GC-MS; Styczynski *et al*., 2007; Jonsson *et al*., 2005; Fiehn *et al*., 2000), microfluidic-capillary electrophoresis (Kraly *et al*., 2009), and nuclear magnetic

resonance (NMR) spectroscopy (Wishart, 2008b; Viant *et al*., 2003). Among these procedures, GC-MS is a suitable technique for comprehensive analysis because it combines high separation efficiency with versatile, selective and sensitive mass detection (Koek *et al*., 2011).

Nevertheless, Van der Werf *et al*. (2005) reported that many polar metabolites are thermally labile or are not volatile at all at the temperatures required for their separation by GC-MS. Hence, the analysis of polar metabolites usually requires chemical derivatization of functional groups to reduce polarity and provide volatility and thermal stability prior to analysis (Dettmer *et al*., 2007; Dunn, 2005). Active hydrogens in functional groups, such as -COOH, -OH, -NH, and -SH can be derivatized by alkylation, acylation, or silylation (Dettmer *et al*., 2007). Koek et al. (2011) stated that "silylation reagents are the most versatile and universally applicable derivatization reagents, which are most suitable for comprehensive metabolomics GC-MS analysis." There are two stages of derivatization by silylation. First, carbonyl functional groups of polar metabolites are converted to oximes ($R^1R^2C$=NOH; where $R^1$ and $R^2$ could be hydrogen atoms, alkyl groups, aryl groups, or any combination thereof) with an oximation reagent, followed by the formation of trimethylsilyl (TMS) ethers, TMS esters, TMS sulfides, or TMS amines with silylating reagents (typically N-methyl-N-trimethylsilyl-trifluoroacetamide; MSTFA). Silyl derivatives show a better thermal stability and higher volatility, and they produce more distinct MS spectra than their un-derivatized precursors (Dettmer *et al*., 2007).




(Source: de Hoog & Mann, 2004)

**Figure 2-9** Schematic hierarchy of the relationship between the different "omics", from genomics to functional genomics (transcriptomics), proteomics, and finally to expression of small molecules (metabolomics).

Metabolomics data are typically presented in either quantitative or chemometric schemes. For the chemometric methods, multivariate data analysis such as principal component analysis (PCA) is commonly employed for the data overview to obtain an overall metabolomics pattern of the model.

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# CHAPTER 3

# CHEMICAL CHARACTERIZATION OF PASTURE- AND GRAIN-FED BEEF RELATED TO MEAT QUALITY AND FLAVOR ATTRIBUTES[1]


## Abstract

This study examined pasture and grain feeding effects on meat quality and nutritional attributes, and correlated sensory attributes with cooked meat volatiles. Grain-fed rib steaks had higher fat content ($P < 0.05$), and were lighter, redder and more yellow ($P < 0.05$). Pasture-fed beef contained more ($P < 0.05$) omega-3 fatty acids, despite having lower fat content. Pasture-fed beef had higher antioxidant capacity, and lower oxidation indices ($P < 0.05$). Diets influenced the volatile profile of cooked meat. Flavor descriptors barny, gamey, and grassy were associated with pasture-fed beef, and were uniquely shown here to be positively correlated with aroma volatiles benzaldehyde, toluene, dimethyl sulfone, 3-heptanone, 2-ethyl-1-hexanol, and hexadecanoic acid methyl ester ($P < 0.05$). Grain-fed beef had higher ($P < 0.05$) levels of hexanal, 1-octen-3-ol, 2,3-octandione, and 2,6-bis (1,1-dimethylethyl)-4-ethyl-phenol, uniquely associated with umami and juicy flavors. The main positive nutritional attribute of pasture-fed beef was its low fat content.


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**Introduction**

Current interest in meat from pasture-fed ruminants stems from their appeal to health-conscious consumers (McCluskey *et al*., 2005). Animal feeding plays an important role in ruminant derived muscle foods since it affects the overall quality of meat (Descalzo & Sancho, 2008). Ruminants with high pasture intake result in meat with a higher content of polyunsaturated fatty acids (PUFA; Melton *et al*., 1982; Yang *et al*., 2002), and increased PUFA to saturated fatty acid (SFA) ratio (French *et al*., 2000). PUFAs slow the development of some human diseases such as cancer, obesity and cardiovascular diseases (Riediger *et al*., 2009); so, increasing the proportion of PUFA in meat by means of animal diet is now recommended by some as a healthy alternative to grain-fed beef (Gatellier *et al*., 2004). Ruminant fats have also been found to be the largest natural source of conjugated linoleic acid (CLA; Chin *et al*., 1994). CLA is reported to be a potential anti-carcinogenic and anti-atherogenic substance (French *et al*., 2000). Pasture feeding can raise the concentration of CLA in beef compared with beef from animals fed typical high-grain diets *(*French *et al*., 2000; Poulson *et al*., 2004).

Meat derived from pasture feeding is associated with a higher antioxidant potential (Gatellier *et al*., 2004; Descalzo & Sancho, 2008). Meat from pasture-fed animals is rich in natural antioxidants such as vitamins from group A, C, and E and phytochemicals such as carotenoids and flavonoids (Wood & Enser, 1997; Daly *et al*., 1999), which help protect against lipid oxidation. The antioxidant content of the meat can be increased through dietary delivery and/or endogenous production and therefore protect tissues against oxidation from reactive oxygen species. Lipid oxidation results in the

production of free radicals, which are promoters of myoglobin oxidation and lead to the formation of rancid odors and off-flavors (Greene & Cumuze, 1982). Pasture-finished cattle present some potential advantages over grain-finish due to the fact that antioxidant protection compensates for the high pro-oxidant potential of PUFA's (Mercier *et al*., 2004).

Herbivore diets affect the palatability of meat and off-flavors are associated with the meat of pasture-fed cattle (Larick *et al*., 1987; Mandell *et al*., 1998). The fatty acid composition may affect flavors in meat from cattle finished on pasture (Melton *et al*., 1982). Priolo *et al*. (2001) reported that branched-chain fatty acids and 3-methyl-indole (skatole) were involved in the unpleasant pastoral flavor in sheep; however, it is less important in beef because of the lack of the branched-chain fatty acids. They also reported that the oxidation products of linolenic acid and its derivates, which are derived substantially from pasture, played an important role in the off-flavor of beef. Diterpenoids are the major volatiles in beef samples. Diterpenoids are positively correlated with "gamey/stale" off-flavor in beef fat associated with pasture compared to grain finishing (Larick *et al*., 1987; Maruri & Larick, 1992). The diterpenoid phyt-1-ene in beef subcutaneous fat is positively correlated to "gamey" flavor while negatively correlated with desirable "roasted" flavor (Maruri & Larick, 1992).

There is still relatively little known about animal diet effects on meat headspace volatiles during cooking, and their relationship to meat sensory properties. Thus, the objectives of this study were to examine the effect of diet (pasture or grain) on meat characteristics and headspace volatiles during heating. Measured meat characteristics

included fat content, pH, color change as a function of storage, degree of oxidation (thiobarbituric acid assay; TBA), antioxidant capacity by oxygen radical absorbance capacity (ORAC), and fatty acid composition, including essential fatty acids and CLA. Cooked meat headspace volatile compounds were also measured. Sensory panel evaluation of cooked meat flavor intensity was previously reported on the same samples, using a new beef flavor lexicon of 18 flavor descriptors (Maughan *et al*., 2012). Headspace volatiles data in the present study were then correlated with sensory flavor profile data from the previous study, to evaluate relationships between diet, headspace volatiles, and meat flavor.

**Materials and Methods**

Meat samples

Primal ribs (*Longissimus dorsi* muscles) were used for the analysis. Primal ribs of pasture-fed cattle (n = 3) were purchased from James Ranch, CO; while rib sections of grain-fed cattle (n = 3) were obtained from USU's Animal Science Farm. The grain-fed animals were Black Angus bred, while the pasture-fed animals were Red Angus sired with a mix of Hereford and Angus dams. The pasture-fed animals were 24 - 27 months old and had a hanging weight between 318 - 360 kg. Their diets were supplemented with alfalfa hay during the winter, and they were finished for 110 - 120 d exclusively on irrigated mountain pasture (a variety of plants, including orchard grass, brome, fescue, and clover). The grain-fed animals were 19 - 20 months old, had an *ad libitum* finish diet for 110 - 120 d consisting of 60% corn silage, 30% flaked barley, and 10% alfalfa, and were also 320 - 345 kg in hanging weight. The left and right rib sections were used from

each animal. All of the cattle were steers except the animal labeled Grain #1, which was a heifer. Carcass yield (hanging weight, ribeye area, and backfat thickness at the 12 - 13$^{th}$ rib) and quality grade (marbling score) measurements were recorded for each animal after slaughter. Primal ribs from each animal were vacuum packed after harvest, shipped to the Department of Nutrition, Dietetics, and Food Sciences at USU and immediately frozen at -20 °C until use. The pouches (25 x 35 cm; Koch, Kansas City, MO) used for packaging were of 3-mil thickness (0.75-gauge nylon, 2.25-gauge polyethylene) with an oxygen permeability of 0.6 $cm^3/100\ m^2/24$ h at 0 °C and a water vapor transmission rate of 0.6 $g/100\ m^2/24$ h at 38 °C and 100% relative humidity.

Carcass yield and quality grade measurements were recorded for each animal after slaughter. Carcass yield grade measurements were hanging weight, ribeye area (REA), and backfat thickness (BF) at the 12-13$^{th}$ rib. Carcass quality grade measurements were the marbling score of the ribeye muscle (*Longissimus dorsi*), taken at the 12-13$^{th}$ rib. All carcasses were "A" maturity (<30 months animal age).

## Chemical analyses

### *Fat content*

Ether-extractable fat content of center lean steak (ribs 9-10$^{th}$) was done by AOAC solvent extraction method 24.005 (Williams, 1984), using petroleum ether as the solvent. A 3-4 g sample was added into a pre-weighed small disposable aluminum foil dish. Sample and dish were then accurately weighed. Sand was added, mixed and spread on bottom of dish with a small glass rod. Next, sample (including dish, sand, and glass rod) were left in the oven at 125 °C for 1.5 h. After that, sample (including dish, sand, and

glass rods) were inserted into the extraction thimble and placed in Soxhlet extraction apparatus (Labconco®, Kansas City, MO). Solvent (50 mL petroleum ether) was filled in reclaiming beakers and the beakers were then sealed to the apparatus for extraction. The extraction started by boiling the solvent, at that time, using cold water flowing through the condenser. Extractions were continued for 4 h at a condensation rate of 5-6 drops per sec. Subsequently, thimbles were removed, dried in the oven, cooled in a desiccators, and weighed again. Weigh loss of the samples were determined and calculated as percent fat.

*pH*

Raw steak pH was measured on 10 g of sample that were finely chopped, diluted to 100 ml in distilled water, allowed to equilibrate at room temperature for 30 min and then filtered. Filtrate pH was measured, using a Fisher Accumet pH meter model 610 A (Fisher Scientific Inc, Salt Lake City, UT), equipped with a combination pH electrode calibrated immediately before use to pH 4.0 and 7.0.

*Hunter color measurements*

Frozen primal ribs were cut using a band saw into steaks (1.9 cm thick), placed in a foam tray and over-wrapped with polyvinyl chloride (PVC) and stored in the dark at 2 °C until color readings were taken. Gas permeability of PVC film (Koch, Kansas City, MO, USA) was as follows: $O_2$ permeability = 8400 $cm^3/(24\ h \times m^2 \times atm)$ at 23 °C; water vapor transmission = 83 $g/(24\ h \times m^2)$ at 23 °C and 50% relative humidity.

Meat color was determined instrumentally using a HunterLab Miniscan portable colorimeter (Hunter Associates Laboratory, Inc., Reston, VA) with a 5 mm diameter

aperture, set to use illuminant D-65. The colorimeter was standardized through a single layer of PVC film using both white and black standard tiles. Meat color parameters CIE lightness (L*), redness (a*) and yellowness (b*) were measured. Three Hunter color readings were taken through the PVC film per steak at each storage time (9 readings/treatment). Color measurements were determined at day 0, 1, 2, 4, 7, 9, and 12 of storage. Beef rib steaks typically turn brown within 7 - 8 d retail display at 4 °C. Rate of browning is temperature dependent. Steaks in this study were held longer (12 d) to evaluate rate of browning at the lower temperature of 2 °C used in this study.

*Oxygen radical absorbance capacity (ORAC)*

Hydrophilic and lipophilic oxygen radical absorbance capacity (ORAC) of raw lean beef rib eye steak samples were measured as described by Wu *et al*. (2008). In short, frozen steak samples were partially thawed at 2 °C, and then thin slices (<1 mm thick) were pulverized by mortar and pestle in liquid nitrogen. Pulverized muscle samples (0.5 g) were extracted in a 50 mL centrifuge tube with 10 mL hexane and vortexed for 10 min.

- *Lipophilic ORAC assay:* The hexane layer were removed and evaporated under nitrogen. The dried hexane extract was dissolved in 250 μL of acetone and then diluted with 750 μL of a 7% randomly methylated β-cyclodextrin (RMCD) solution (50% acetone/ 50% water, v/v).
- *Hydrophilic ORAC assay:* The residue remaining after hexane extraction was further treated with 10 mL of 20% ethanol for 1 h on an orbital shaker set at 160 rpm, then centrifuged at 1000 x *g* for 5 min in a Beckman centrifuge (Model

F0850/ Allegra X-22 Series, Palo Alto, CA). The supernatant then was diluted 10 -fold with phosphate buffer (pH 7.4).

An aliquot (25 µL) of hydrophilic and lipophilic extract or Trolox calibration solutions (6.25, 12.5, 25, 50, 100 mM), or blank (phosphate buffer for hydrophilic assay and 7% RMCD for lipophilic assay) were added to each well of the 96-well polypropylene micro-titer plate (Corning Life Sciences, Wilkes Barre, PA), followed by fluorescein (0.004 mM; 150 µL) and then incubated at 37 °C for 30 min before addition of peroxyl generator [25 µL AAPH solution: azo-bis (2-amidinopropane) dihydrochloride; 153 mM]. The microplate reader was programmed to record fluorescence with an excitation reading of 485 nm and an emission wavelength of 520 nm at 1 min intervals for 1 h using a fluorometer (Infinite M200, Tecan®, Durham, NC). The final hydrophilic and lipophilic ORAC values were calculated by using a linear regression model (Y = aX + b) between Trolox concentration (µM) and the net area under the fluorescein decay curve. Data were expressed as micromoles of Trolox equivalents (TE) per g of beef sample. The area under curve (AUC) was calculated using the Magellan 6 program (version 6.5, 2008; Tecan®, Durham, NC). Three replications with duplicate readings were done for each sample.

*Thiobarbituric acid assay (TBA)*

Each steak was placed in a foam tray and over-wrapped with PVC film and stored at 2 °C. Thiobarbituric acid reactive substances (TBARS) were determined at day 0 and 12 of storage in duplicate for each steak (3 steaks per treatment). TBA assay was

performed as described by Buege & Aust (1978). Samples (0.5 g) were mixed with 2.5 mL of 0.375% TBA - 15% TCA - 0.25 N HCl stock solution. The mixture was heated for 10 min in a boiling water bath to develop a pink color, cooled with tap water, then centrifuged at 3200 x *g* for 25 min in a Beckman centrifuge. The supernatant was measured at 532 nm using a UV 2100 U spectrophotometer (Shimadzu Co., Kyoto, Japan). TBA values were calculated as mg of malonaldehyde/1000 g of meat (ppm TBARS).

*Fatty acid chemical composition*

Fatty acid methyl esters (FAMEs) were prepared by direct transesterification using the method of O'Fallon *et al*. (2007) with slight modifications. Frozen steaks were sliced into thin strips (<1.5mm thickness) with a razor blade and approximately 1 g of lean tissue was placed into a screw cap vial along with 100 µL of surrogate standard (C23:0 methyl ester; 5.0 mg/mL in chloroform; Sigma-Aldrich, St. Louis, MO). Next, 700 µL of 10 N KOH in water was added along with 6.3 mL of MeOH. The tubes were subjected to vigorous hand shaking and place in a shaking water bath at 55 °C for 1.5 h. Every 20 min the tubes were shaken by hand for 5 s. Subsequently, the tubes were cooled to room temperature and 580 µL of 24 N $H_2SO_4$ was added. The tubes were shaken vigorously by hand to mix contents, and again placed in the shaking bath for 1.5 h at 55 °C with hand mixing at 20 min intervals. After the incubation the tubes were cooled, 3 mL of hexane was added and vortexed for 5 min. Next, the tubes were centrifuged at 1,000 × g for 5 min and the top layer was transferred to a gas chromatography (GC) vial.

The FAMEs were analyzed by gas chromatography with flame ionization detection (GC-FID) using a Shimadzu GC 2010. The GC was equipped with an HP-88 column (100 m × 0.25 mm × 0.20 μm; Agilent Technologies, Palo Alto, CA). The injector was fitted with a Siltek deactivated split/splitless liner packed with glass wool (Restek, Bellefonte, PA) and held at 250 °C. Hydrogen was used as the carrier gas at a head pressure of 206.7 kPa with a linear column flow rate of 41.0 mL/min. One μL of sample was injected at a 30:1 split ratio and the oven will program as follows: initial column temperature 35 °C hold 2 min, ramp at 40 °C/min to 175 °C hold for 4 min, ramp at 3.5 °C/min to 240 °C hold for 24 min. The FID was operated at 250 °C. Air was supplied at 400 mL/min and hydrogen at 39 mL/min. Fatty acids were identified by similarity to retention time to GC reference standards (Nu-Chek Prep, Inc, Elysian, MN). The standards were also used to generate response factors to adjust for injector and detector discrimination and to insure linearity of detector response. Fatty acid profiles were expressed as percentage in fresh meat.

*Headspace volatile analysis*

Headspace volatiles of beef were analyzed according to the method of Vasta *et al*. (2010). A sample of the frozen *Longissimus dorsi* muscle of each animal was trimmed of external fat and sliced (thickness <1 mm) and three replications per animal were done. Six grams of raw sliced meat was placed in a 20-mL glass vial and capped with a polytetrafluoroethylene (PTFE) septum. For the extraction of headspace volatile compounds a solid phase micro-extraction (SPME) technique was used. A surrogate standard, 1,2 dichlorobenzene (2 μL; 52.7 μmol), was added to the vial containing the

sample. The vial was then placed in a heat bath set at 60 °C for 10 min. A 2 cm-50/30 DVB/CarboxenTM/PDMS fiber (Supelco, Bellefonte, PA) was exposed to the headspace over the sample at 60 °C for 30 min to adsorb the volatiles. The fiber was then removed from the vial and immediately inserted into the GC (GCMS-QP 2010S, Shimadzu Co., Kyoto, Japan). The injector was held at 250 °C and fitted with a 0.75 mm SPME inlet liner (Supelco, Bellefonte, PA). Splitless injection was used with a sampling time of 1 min. Helium was used as carrier gas with a flow rate of 1.0 mL/min. Volatile compounds were separated using an Agilent DB-5ms column (30 m x 0.250 mm x 0.25 mm; Agilent Technologies, Santa Clara, CA). The GC oven temperature program was as follows: 40 °C for 2.4 min; ramp to 325 °C at a rate of 6 °C/min, with a total program time of 43.16 min. The GC/MS interface was heated at 290 °C. The acquisition was performed in electron impact mode (70 eV) at 5 microscans/s, with a mass range 33-350 m/z.

Data files obtained from the GC-MS were exported in the netCDF format and analyzed with the public Automated Mass Spectral Deconvolution and Identification System (AMDIS; version 2.62, 1999-2000) developed by the National Institute of Standards and Technology (NIST). Deconvoluted mass spectra were submitted to the online analysis tool Spectconnect (www.spectconnect.mit.edu) for the systematic detection of analytes that were conserved across samples. Analytes resulting from this analysis were identified by a library search against the NIST Mass Spectral Library (version 2.0, 2005), and by comparison with linear retention indexes (LRI). The LRI were established by injection of standard n-alkanes from 7 to 40 carbons (Supelco,

Bellefonte, PA). Parent peak intensities were normalized to the surrogate standard in each run prior to statistical analysis. Data were expressed as ratio to surrogate.

Sensory evaluation

Sensory evaluation was previously done as described by Maughan *et al*. (2012). In short, frozen ribeye steaks (*L. dorsi* muscles) were cut to a thickness of 2.54 cm and thawed for 24 h before cooking. Samples were prepared following the guidelines from the American Meat Science Association (1995). Steaks were cooked on electric griddles at 163 °C to an internal temperature of 70 °C, measured at the center of the steak by an AquaTuff 35200 digital thermometer (Atkins Technical Inc, Gainesville, FL, USA) equipped with a fast-responding micro-needle probe. Steaks were then cut into 2.54 cm cubes and placed in covered aluminum dishes, and served hot to the panelists. A sensory descriptive panel (n = 12) was recruited and selected from the local community to develop a flavor lexicon for meats. Cooked rib steak samples were evaluated for flavor intensity of 18 attributes (5 basic tastes plus astringent, barny, bloody, brothy, browned, gamey, grassy, juicy, fatty, livery, metallic, oxidized, roast beef), on a 15-point scale, where 1 = no flavor; and 15 = very high intensity, for the attribute under consideration. Panelists tasted and evaluated each sample individually using water and unsalted crackers to clean their palettes between samples. The samples were presented in random order with 3-digit blinding codes under red colored lights to minimize bias. After tasting all samples, panelists waited for 15 min before tasting a replicate of the samples in a new randomized order.

Statistical analysis

The experiment was designed with 2 diet treatments (pasture or grain), and measurements were made on rib steaks from 3 animals per treatment. Sample size was small (n = 3/diet), in consideration of the relatively large number of analyses to conduct. Thus, relatively large treatment differences in measured parameters were required to achieve significance at the 95% level. Nonetheless, finishing diet (pasture or grain) significantly affected numerous meat characteristics in this study.

Measurement of the fat content, raw meat color, ORAC values, fatty acid profile, and headspace volatiles were done in triplicate for each sample. TBA values were done in duplicate for each sample. Sensory evaluation of steaks as affected by diet was previously reported (Maughan *et al*., 2012). Statistical Analysis Software (SAS) version 9.1 (SAS Institute, Inc., Cary, NC) was used for analysis of variance (ANOVA) to identify statistically significant differences between diet treatments at the 95% confidence level. Complete randomized design with the *proc glm* function was used for fat content, ORAC values, fatty acid profile, and headspace volatile experiments. Comparison of the means was made based on *P*-values ($\alpha = 0.05$) using the least significant different (LSD) adjustment to obtain differences of least means squares. Repeated measures design with the *proc mixed* function, using Tukey adjustment to obtain differences of least means squares, was used for raw meat color and TBA experiments. Principle component analysis (PCA) using *proc factor* was used for headspace volatile analysis. Pearson correlation coefficients (r) between mean headspace volatiles (peak intensities normalized to the surrogate standard in each run; n = 6), and sensory attributes (1 - 15

intensity scale, where 15 = highest intensity; n = 6; Maughan *et al*., 2012) were also calculated using SAS *proc corr*.

**Results**

Carcass grade factors and meat characteristics of these animals are shown in Table 3-1. Grain-fed animals had carcass grade ranging from USDA grade low choice to prime, compared to select grade for pasture-fed animals. There were no differences ($P > 0.05$) found in the hanging weight, ribeye area, and back fat thickness between the two diets. Significant differences ($P < 0.05$) were found in pH between the two types of animals, with slightly higher pH in the pasture-fed meat. Ribs from grain-fed animals had higher fat content (12.43%) than pasture-fed animals (3.36%), as expected ($P < 0.05$). Thus, the quality grade was also lower in the pasture-fed compared to grain-fed beef.

**Table 3-1** Characteristics of steaks obtained from grain- and pasture-fed animals

| Samples | HW | REA | BF | Marbling Score | Quality Grade | pH | Fat (%) |
|---|---|---|---|---|---|---|---|
| Grain #1 | 320 | 81.3 | 1.3 | mod abundant | prime | 5.13 | 13.86 |
| Grain #2 | 330 | 80.6 | 0.5 | moderate | high choice | 5.15 | 12.38 |
| Grain #3 | 345 | 87.7 | 1.3 | small | low choice | 5.06 | 11.05 |
| **GF mean** | **332** | **83.2** | **1.0** | **-** | **-** | **5.11** | **12.43** |
| Pasture #1 | 318 | 80.0 | 0.3 | slight 0 | select | 5.28 | 3.03 |
| Pasture #2 | 330 | 78.7 | 0.8 | slight 30 | select | 5.27 | 3.51 |
| Pasture #3 | 360 | 85.8 | 0.5 | slight 30 | select | 5.27 | 3.54 |
| **PF mean** | **336** | **81.5** | **0.5** | **-** | **-** | **5.27** | **3.36** |
| *P*-value[1] | NS | NS | NS | - | - | < 0.05 | < 0.05 |

HW = Hanging weight (kg); REA = Ribeye area ($cm^2$); BF = Back fat thickness (cm);
GF = Grain-fed beef; PF = Pasture-fed beef.
[1] Significantly different between diet treatment means ($P < 0.05$); NS = Not significantly different.

Significant differences were also observed in steak appearance and color. Raw steaks from pasture-fed animals were darker ($P < 0.001$) in color with less red ($P < 0.05$) and yellow hue ($P < 0.01$; Fig. 3-1; see Appendix Table A1-A3 for detailed statistics). Mean lightness values, pooled over storage time were different ($P < 0.001$; 34.03 and 28.77 for grain vs. pasture, respectively). In this study, the interactions of diet treatment and storage time were also significantly different at day 0 time point, as indicated by the asterisk (*; Fig. 3-1A; Appendix Table A4). The main effect of diet treatment (pooled over all time points; days) significantly affected ($P < 0.05$) redness values. Similarly, the main effect of time (pooled among diet treatments) significantly affected ($P < 0.05$) redness values. However, at any given day, there was no significant interaction effect on redness values between diet treatment and storage time (Fig. 3-1B; Appendix Table A5). The main effect of diet treatments and storage time also affected yellowness values ($P < 0.05$). However, the interaction between diet treatment and storage time only significantly affected yellowness values on day 1 of storage ($P < 0.05$; Fig. 3-1C; Appendix Table A6). Rib steaks in retail display typically turn brown after 5-7 days. The longer red color stability of steaks in this study (12 days) was probably due lower storage temperature than typical retail display (2 versus 4 °C, respectively) under dark conditions.

The antioxidant capacity of steaks from the two groups was compared. There was a higher capacity in steaks from the pasture-fed animals in the hydrophilic fraction, but not the lipophilic fraction (Fig. 3-2A; Appendix Table A7-A8). Interestingly, the difference in antioxidant capacity was reflected in the occurrence of lipid oxidation over twelve days of storage. While there were no differences in lipid oxidation on steaks

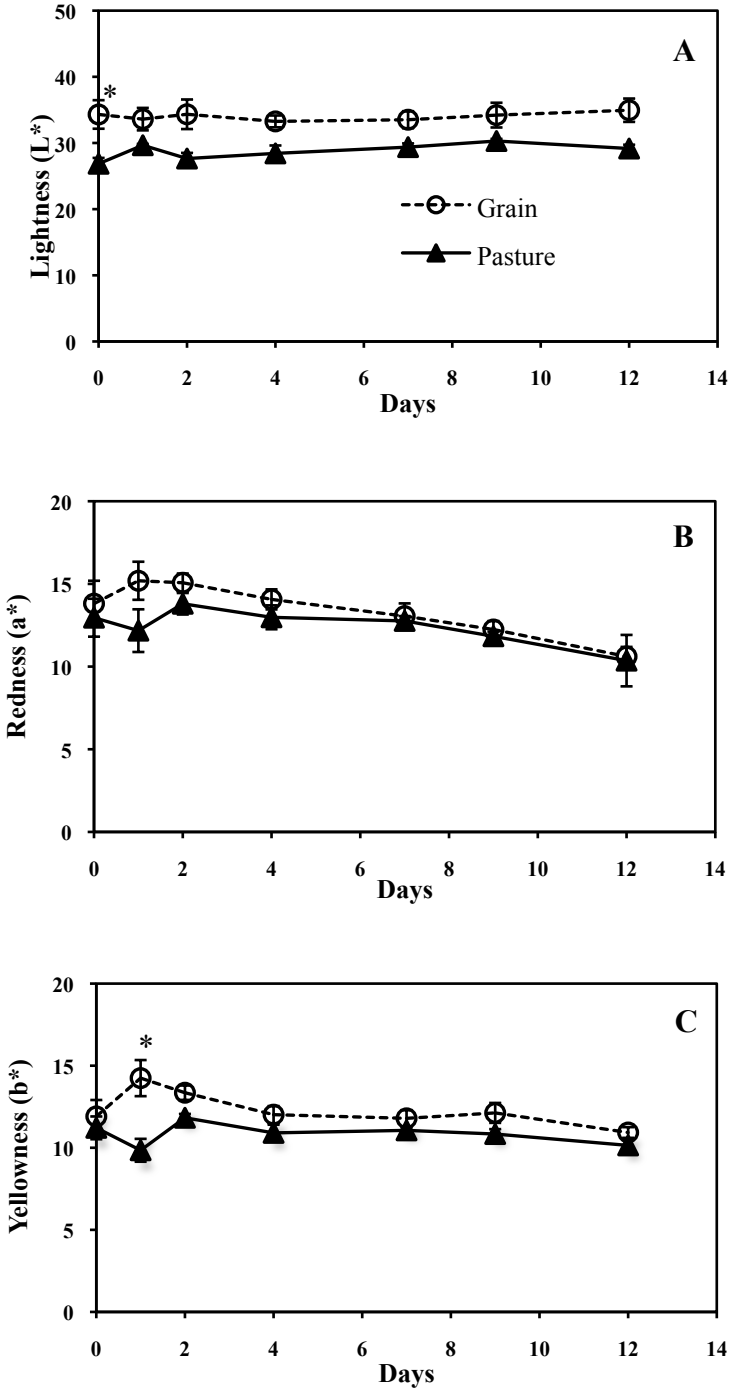


**Figure 3-1** Effect of storage time (day) on grain- and pasture-fed beef color stability at 2 °C. **A:** Lightness (L*); **B:** Redness (a*); **C:** Yellowness (b*). Significant differences ($P < 0.05$) between diet treatments at each storage time point were indicated by an asterisk (*). Error bars = SD.

sampled on day 0, after twelve days of storage the steaks from the grain-fed animals had significantly higher TBA values ($P < 0.05$; Fig. 3-2B; Appendix Table A9-A10).

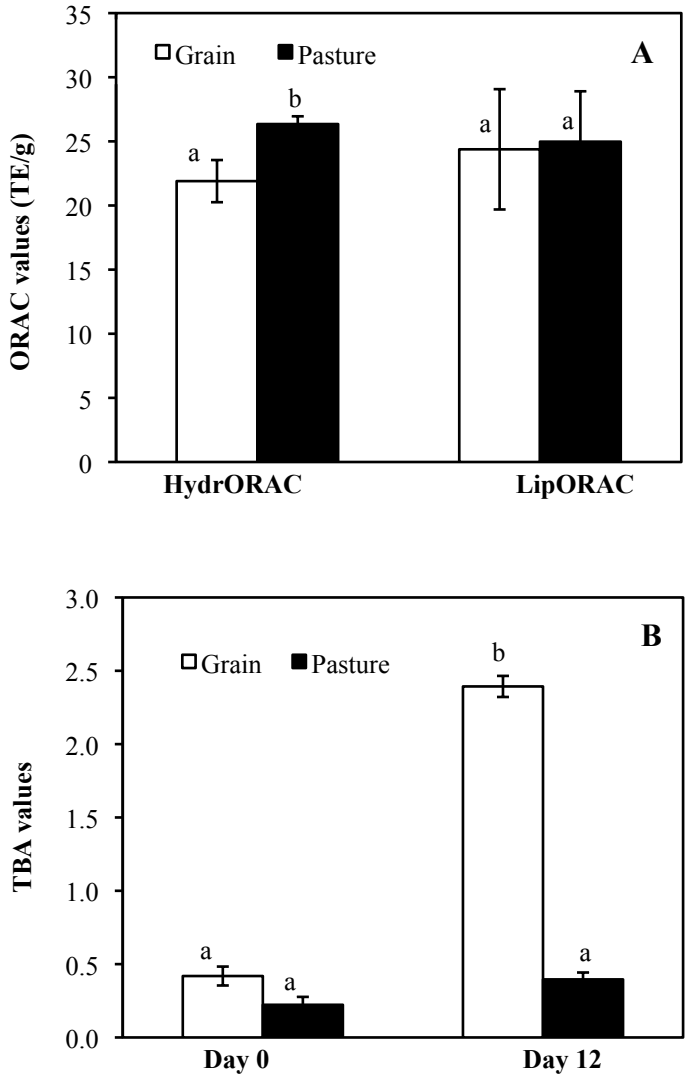


**Figure 3-2** Antioxidant status (ORAC values) and lipid oxidation (TBA values) measurements of beef obtained from grain and pasture-fed animals. **A:** ORAC values. HydrORAC represents the hydrophilic antioxidant and LipORAC represents the lipophilic antioxidant capacity; **B:** TBA values (mg of malonaldehyde/kg meat) at time 0 and after 12 days of storage at 2 °C. Different letters within the same column indicate significantly differences ($P < 0.05$). Error bars = SD.

Twenty-nine fatty acids were detected in the beef samples with a minimum percentage cutoff of 0.02%. The thirteen most abundant fatty acids are shown graphically in Fig. 3-3 (Appendix Table A11), and these make up approximately 96% of the total fatty acids. From the figure it is clear that the pasture-fed beef had significantly higher percentage of polyunsaturated fatty acids (PUFAs; $P < 0.05$), including conjugated linoleic acid (CLA; $P < 0.01$). As a preliminary analysis, individual fatty acids were presented as a percentage of the total fatty acids. On a percentage basis, the pasture-fed beef contained a greater percentage of long chain polyunsaturates (LC-PUFA) than the grain-fed beef including arachadonic acid (20:4n6), eicosapentaentoic acid (20:5n3) and docosapentaenoic acid (22:5n3) where as a significantly lower amount of palmitic (C16:0) and oleic (C18:1n9c) fatty acids were found in these same samples.

To estimate the absolute amount of different fatty acid classes, a value was calculated based on an 85 g (3 oz) serving and the data are presented in Table 3-2. The grain-fed beef had significantly more saturated and monounsaturated fatty acids, but the PUFA levels did not differ. When the two classes of essential fatty acids are considered, values for omega-6 fatty acids were not different, but there were significantly more total omega-3 and LC omega-3 fatty acids in the pasture-fed beef samples. As a result, the omega-6 to omega-3 ratio was significantly lower in the pasture-fed beef.

To investigate how the different animal diets potentially affect sensory properties of the meat, the volatiles were measured after heating the samples to 60 °C. Using SPME, twenty-five different volatile compounds were detected in the headspace of these samples and the data is shown in Table 3-3 (Appendix Table A12). The values are semi-

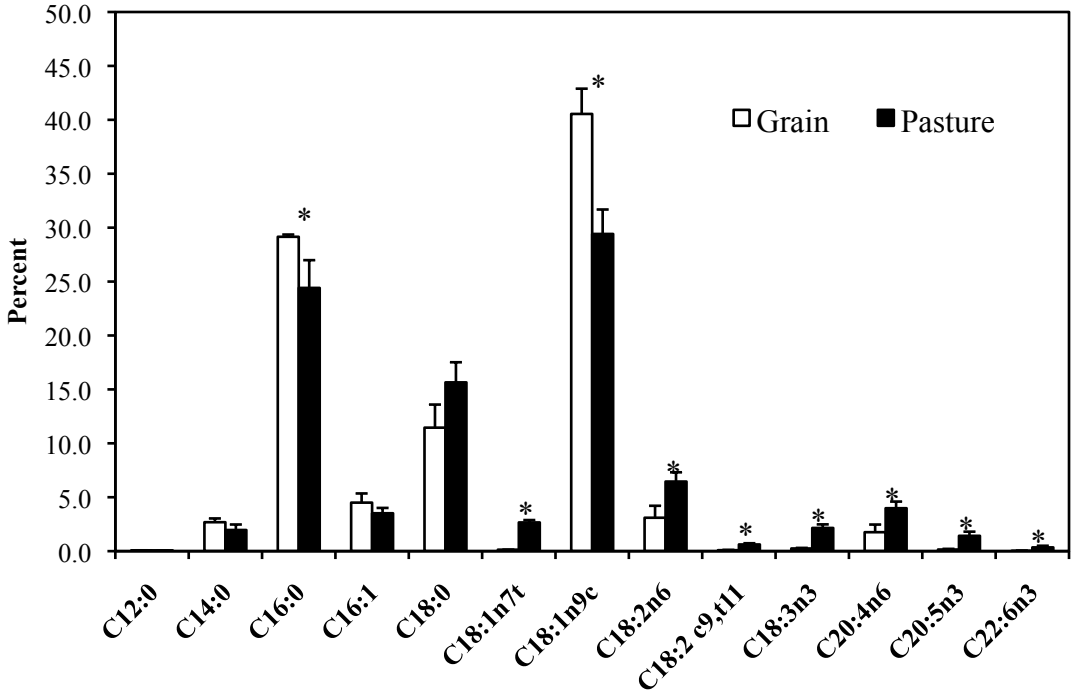


**Figure 3-3** Fatty acid chemical composition for meat samples obtained from animals fed with grain- and pasture-based diet. Asterisk (*) indicates significantly differences in fatty acid percentage between diet treatments ($P < 0.05$). Error bars = SD.

quantitative as they represent the ratio of the area of the dominant ion to that of the surrogate standard.

Across the two diets there were ten volatiles that were different and these are shown according to magnitude in Fig. 3-4. Four of the ten compounds were more abundant in the grain-fed beef including hexenal, 1-octen-3-ol, 2,3-octanedione, and 2,6-bis (1,1-dimethylethyl)-4-ethyl-phenol. Six of the ten compounds were higher in the pasture-fed beef, including dimethyl sulfone, toluene, 3-heptanone, hexadecanoic acid

methyl ester, benzaldehyde and 2-ethyl-1-hexanol. The volatiles that differed between samples are visualized graphically using principle components analysis (PCA) in Fig. 3-5 to better understand the effect of the dietary treatments and to relate them to the sensory descriptors associated with each sample. Principal component 1 accounted for 77.71% of the variability, while the principal component 2 accounted for 11.06% of the variability. It is clear from the graph that samples obtained from grain-fed animals are characterized by a headspace rich in hexanal, 2,6-bis (1,1-dimethylethyl)-4-ethyl-phenol, 2,3-octanedione, and 1-octen-3-ol; while the meat obtained from pasture-fed animals is characterized by a headspace rich in benzaldehyde, toluene, dimethyl sulfone, 3-heptanone, hexadecanoic acid, methyl ester and 2-ethyl-1-hexanol. Using these same samples in a trained descriptive sensory panel, we previously found that animal diets were significantly associated with specific flavor terms (Maughan *et al.*, 2012).

**Table 3-2** Fatty acid composition of beef samples per 85 g (3 oz) serving[1]

| **Fatty acid** | **Grain-fed** (n = 3) | **Pasture-fed** (n = 3) | ***P*-value**[2] |
|---|---|---|---|
| Saturates *g* | 3.6 ± 0.4 | 1.0 ± 0.1 | < 0.01 |
| Monounsaturates[3] *g* | 3.8 ± 0.3 | 0.9 ± 0.1 | < 0.01 |
| Polyunsaturates *g* | 0.5 ± 0.1 | 0.4 ± 0.1 | NS |
| Omega-6 *g* | 0.5 ± 0.1 | 0.3 ± 0.1 | NS |
| Long chain ω-6 *g* | 0.20 ± 0.06 | 0.11 ± 0.02 | NS |
| Omega-3 *mg* | 60 ± 10 | 130 ± 30 | < 0.05 |
| Long chain ω-3 *mg* | 40 ± 10 | 80 ± 20 | < 0.05 |
| ω-6: ω-3 ratio | 6.9 ± 0.5 | 2.0 ± 0.1 | < 0.01 |

[1] Concentration values were calculated for each animal as the product of the total fat content and the percentage of individual fatty acids. The fatty acid portion of the total fat was calculated to be approximately 75.0% for the grain-fed beef and 75.3% for the pasture-fed beef based on the average fatty acid molecular weight.
[2] NS = Not significantly different.
[3] Includes vaccenic acid.

**Table 3-3** Volatile profile of muscle from beef fed with grain or pasture diets

| LRI | Compounds | Grain-fed (n = 3) | Pasture-fed (n = 3) | P-value [1] |
|---|---|---|---|---|
| 707 | 2-Butanone, 3-hydroxy- | 14.13 ± 4.20 | 17.61± 7.90 | NS |
| 770 | Toluene | 0.21 ± 0.04 | 1.11 ± 0.47 | < 0.05 |
| 764 | 1-Pentanol | 0.66 ± 0.19 | 0.48 ± 0.06 | NS |
| 789 | Butanoic acid | 0.47 ± 0.42 | 0.34 ± 0.20 | NS |
| 787 | Hexanal | 2.10 ± 0.31 | 0.68 ± 0.19 | < 0.01 |
| 786 | 2,3-Butanediol | 6.61 ± 1.48 | 8.25 ± 3.31 | NS |
| 867 | Hexanoic acid, methyl ester | 0.54 ± 0.21 | 0.36 ± 0.23 | NS |
| 871 | 1-Hexanol | 0.30 ± 0.09 | 0.34 ± 0.01 | NS |
| 890 | 3-Heptanone | 0.00 ± 0.00 | 0.64 ± 0.18 | < 0.01 |
| 898 | Heptanal | 0.42 ± 0.09 | 0.37 ± 0.12 | NS |
| 918 | Butyrolactone | 2.19 ± 0.85 | 2.07 ± 0.99 | NS |
| 926 | Dimethyl sulfone | 0.42 ± 0.31 | 2.25 ± 0.33 | < 0.01 |
| 960 | Benzaldehyde | 0.18 ± 0.03 | 0.31 ± 0.06 | < 0.05 |
| 966 | Octanal | 0.76 ± 0.19 | 0.63 ± 0.19 | NS |
| 983 | 1-Octen-3-ol | 1.99 ± 0.24 | 1.17 ± 0.10 | < 0.05 |
| 993 | 2,3-Octanedione | 0.73 ± 0.17 | 0.12 ± 0.11 | < 0.01 |
| 1028 | 2-Ethyl-1-hexanol | 0.10 ± 0.09 | 54.23 ± 29.91 | < 0.05 |
| 1069 | 1-Octanol | 0.67 ± 0.25 | 0.83 ± 0.14 | NS |
| 1103 | Nonanal | 2.49 ± 0.37 | 2.40 ± 0.86 | NS |
| 1183 | Octanoic Acid | 0.15 ± 0.01 | 0.11 ± 0.04 | NS |
| 1205 | Decanal | 0.12 ± 0.05 | 0.07 ± 0.03 | NS |
| 1221 | Undecane, 2,8-dimethyl- | 0.21 ± 0.04 | 0.18 ± 0.10 | NS |
| 1651 | 2-Ethylhexyl 2-ethylhexanoate | 0.80 ± 1.23 | 0.37 ± 0.18 | NS |
| 1760 | Phenol, 2,6-bis (1,1-dimethylethyl)-4-ethyl- | 0.56 ± 0.10 | 0.03 ± 0.04 | < 0.01 |
| 1870 | Hexadecanoic acid, methyl ester | 0.12 ± 0.11 | 0.37 ± 0.01 | < 0.05 |

[1] Significantly different between diet treatment means in the same row ($P < 0.05$);
NS = Not significantly different.

In Table 3-4, correlation coefficients are shown for volatile and sensory attributes that were different ($P < 0.05$) in the ANOVA analysis. Toluene, benzaldehyde, and 2-ethyl-1-hexanol were higher in pasture-fed beef (Table 3-3) but there were no significant correlations between these compounds and any specific flavors. Hexanal and 2,6-bis (1,1-dimethylethyl)-4-ethyl-phenol were higher in grain-fed beef, and negatively correlated with barny ($P < 0.01$) and bitter ($P < 0.001$) flavors. 3-heptanone was higher in pasture-fed beef, and positively correlated with barny and bitter flavors ($P < 0.01$). Dimethyl sulfone was higher in the pasture-fed steaks, and positively correlated with barny flavor ($P < 0.001$). 1-Octen-3-ol was higher in grain-fed samples, and positively correlated with umami flavor ($P < 0.01$). 2,3-Octanedione was also higher in grain-fed beef, and negatively correlated with barny flavor ($P < 0.01$). Hexadecanoic acid methyl ester was higher in pasture-fed steaks, and positively correlated with bitter flavor ($P < 0.01$).

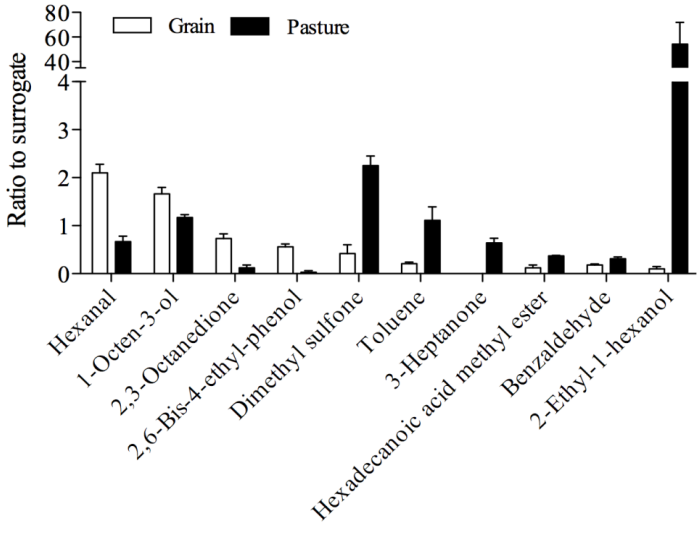


**Figure 3-4** Ratio to surrogate of headspace volatile compounds from beef fed with grain or pasture diets. Error bars = SD.

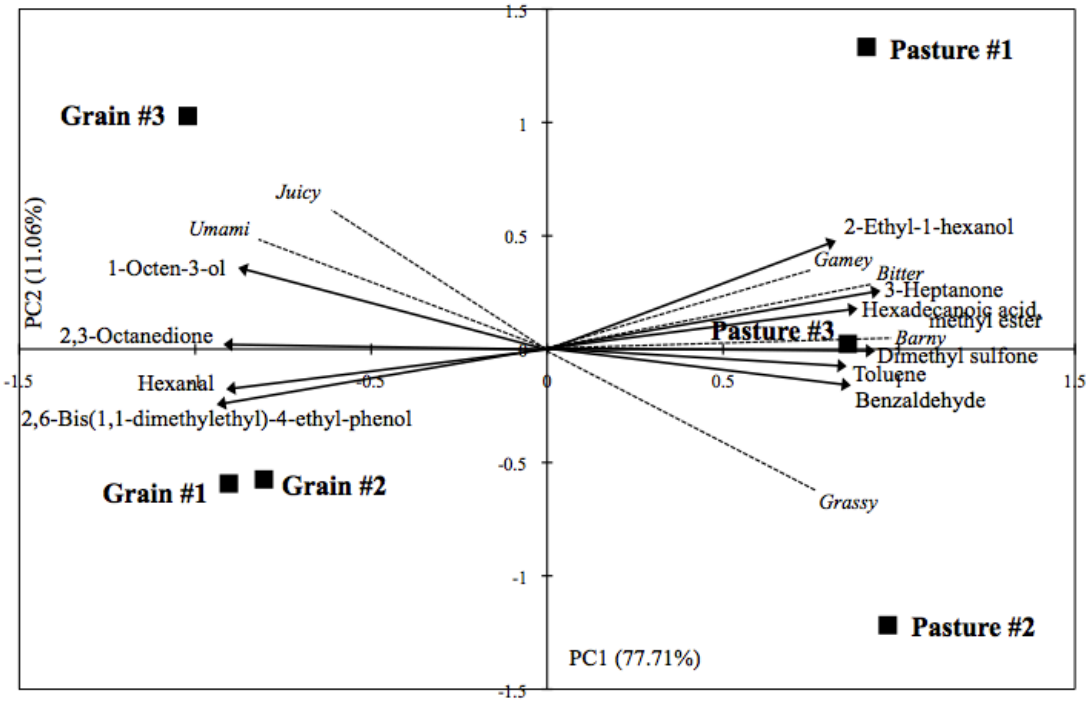


**Figure 3-5** Principal component analysis (PCA) of volatile compounds in grain-fed and pasture-fed beef samples heated to 60 °C for 30 min.

**Table 3-4** Pearson correlation coefficients (r) among means of volatiles with sensory intensity. In this table, only volatile and sensory attributes that were different between diets ($P < 0.05$) in the ANOVA analysis are shown

| | Barny | Bitter | Gamey | Grassy | Juicy | Umami |
|---|---|---|---|---|---|---|
| Toluene | 0.89 | 0.75 | 0.37 | 0.75 | -0.34 | -0.72 |
| Hexanal | -0.95 * | -0.98 ** | -0.80 | -0.61 | 0.56 | 0.69 |
| 3-Heptanone | 0.95 * | 0.94 * | 0.79 | 0.57 | -0.38 | -0.68 |
| Dimethyl sulfone | 0.97 ** | 0.90 | 0.62 | 0.75 | -0.55 | -0.81 |
| Benzaldehyde | 0.89 | 0.71 | 0.39 | 0.80 | -0.41 | -0.80 |
| 1-Octen-3-ol | -0.84 | -0.74 | -0.65 | -0.91 | 0.85 | 0.92 * |
| 2,3-Octanedione | -0.93 * | -0.84 | -0.72 | -0.74 | 0.54 | 0.85 |
| 2-Ethyl-1-hexanol | 0.83 | 0.89 | 0.85 | 0.35 | -0.22 | -0.50 |
| 2,6-bis (1,1-Dimethylethyl)-4-ethyl-phenol | -0.97 * | -0.99 ** | -0.80 | -0.59 | 0.48 | 0.69 |
| Hexadecanoic acid, methyl ester | 0.90 | 0.94 * | 0.67 | 0.61 | -0.48 | -0.57 |

* $P < 0.01$; ** $P < 0.001$.

**Discussion**

In the present study, pasture-fed beef was significantly darker than grain-fed samples (Fig. 3-1). This is in agreement with Hoving-Bolin *et al*. (1999), who reported that pasture-fed beef had darker color and lower L* values than grain-fed beef. The increase in redness of grain-fed beef at day 1 was likely due to bloom development of steaks newly exposed to oxygen in the oxygen permeable PVC film overwrap. Pasture-fed beef meat is typically darker than grain-fed beef, and bloom development was not as pronounced. Panel evaluation of the same steaks (Maughan *et al.*, 2012) showed that grain-fed beef was juicier ($P < 0.05$) and preferred by consumers over pasture-fed beef (7.05 and 6.08, respectively, on a 9-point scale, where 6 = slightly liked and 7 = moderately liked).

It has been demonstrated that PUFAs, which contain double bonds, undergo more rapid oxidation than SFA (Leyton *et al*., 1987). Thus, theoretically, pasture-fed beef should be more easily oxidized than grain-fed beef. Interestingly, lower TBA values ($P < 0.05$) were found in beef from pasture-fed than grain-fed animals after 12 d storage at retail conditions (Fig. 3-2B). This outcome might be associated with significantly higher antioxidant levels (ORAC values) found in pasture-fed beef (Fig. 3-2A) that protect against the oxidation reactions occurring in the meat. These properties would benefit in prolonging meat storage shelf life.

Our results indicated that ribs from pasture-fed animals had lower ($P < 0.05$) fat content than grain-fed animals, yet are higher in the relative percentage of monounsaturated fatty acids (MUFA; includes vaccenic acid) and polyunsaturated fatty

acids (PUFA; Table 3-2, Fig. 3-3). An increase in omega-3 fatty acids in steaks from pasture-fed cattle has previously been reported, resulting in a lower omega-6 to omega-3 ratio in intramuscular fat (French *et al*., 2000; Gatellier *et al*., 2005; Ponnampalam *et al*., 2006). Excessive amounts of omega-6 PUFA and a very high omega-6/omega-3 ratio are thought to promote the development of many diseases, including cardiovascular disease, cancer, and inflammatory and autoimmune diseases, whereas increased levels of omega-3 PUFA (a low omega-6 to omega-3 ratio) exert suppressive effects (Simopoulos, 2002). An omega-6 to omega-3 ratio >4 is a risk factor in cancers and coronary heart disease, especially the formation of blood clots leading to a heart attack (Simopoulos, 2002). Yet, the current ratio in the American diet is estimated to be approximately 10:1 (Blasbalg *et al*., 2011). Therefore, it is important to maintain balance in the diet between omega-3 and omega-6 PUFA since these substances work together to promote health.

There is no official recommended daily allowance (RDA) for LC omega-6 or LC omega-3 fatty acids in the human diet. However, the International Society for the Study of Fatty Acids and Lipids (ISSFAL, 2012) suggests an intake of 500 mg/d omega-3 fatty acids (http://www.mollersomega3.com/c-77-Recommended-omega-3-intake.aspx). Consumption of a serving of pasture-fed beef improves the daily omega-6 to omega-3 ratio (Daley *et al*., 2010), but not as effectively as a serving of fish or flaxseeds. In this study, the amount of omega-3 fatty acids provided by an 85 g serving of pasture or grain fed beef as % of ISSFAL recommendations were calculated (Table 3-5). Pasture-fed beef only supplied a fraction of omega-3 PUFA, compared to oily fish such as salmon. Consumption of an 85 g serving of pasture-fed beef contained 83.3 mg of omega-3 fatty

acids or 16.7% of the ISSFAL recommended level. For comparison, 85 g of cooked salmon contains ~1,830 mg omega-3 fatty acids or ~366% of the ISSFAL recommended level (Table 3-5).

Epidemiological studies of CLA to cancer and heart diseases in humans are very limited and sometimes contradictory (Gebauer *et al.*, 2011). Only one large case-control human study has shown a positive effect of dietary CLA to reduce incidence of breast cancer in postmenopausal women (Aro *et al.*, 2000). CLA intake by the control group (without cancer) in this study was 132 mg CLA/d. Using this value as 100%, an 85 g/d serving of pasture- or grain-fed beef in this study, or salmon had only 9.77%, 5.98%, and 1.14%, respectively, of putative effective CLA level (Table 3-6).

Animal diet also influenced the volatile profile in the meat as demonstrated by the headspace analysis (Table 3-3, Fig. 3-4). Steaks from grain-fed beef had higher ($P < 0.05$) levels of hexanal, 1-octen-3-ol, 2,3-octandione, and 2,6-bis (1,1-dimethylethyl)-4-ethyl-phenol. Hexanal is a dominant volatile aldehyde product of fat oxidation (Brunton

**Table 3-5** Omega-3 fatty acids (FA) in 85 g serving of grain- or pasture-fed beef, as % of recommended by the International Society for the Study of Fatty Acids and Lipids (ISSFAL)*

| Treatment | Ether-Extract Fat (%) | FA (% EEF) | FA (g/100 g meat) | ω-3 FA (% total FA) | ω-3 FA (mg/3 oz. serving) | ω-3 ISSFAL* (mg/d) | % ω-3/ 85 g serving |
|---|---|---|---|---|---|---|---|
| Grain-fed | 12.43 | 75.2 ** | 9.34 | 0.4 | 31.8 | 500 | 6.4 |
| Pasture-fed | 3.36 | 75.2 | 2.52 | 3.9 | 83.8 | 500 | 16.7 |
| Salmon | - | - | - | - | 1,830 *** | 500 | 366.0 |

* ISSFAL recommended level for ω-3 fatty acids = 500 mg/d (http://www.mollersomega3.com/c-77-Recommended-omega-3-intake.aspx).
** Based on fatty class composition of beef *L. dorsi* muscle (Insausti *et al*., 2004).
*** Salmon, Atlantic farmed (Kris *et al*., 2003).

**Table 3-6** Conjugated linoleic acid (CLA) in 85 g serving of grain- or pasture-fed beef, as % of level associated with reduced incidence of breast cancer in postmenopausal women*

| Treatment | Ether-Extract Fat (%) | FA (% EEF) | FA (g/100 g meat) | CLA (% total FA) | CLA (mg/3 oz. serving) | CLA level* (mg/d) | % CLA per 85 g serving |
|---|---|---|---|---|---|---|---|
| Grain-fed | 12.43 | 75.2 | 9.34 | 0.1 | 7.9 | 132 | 5.98 |
| Pasture-fed | 3.36 | 75.2 | 2.52 | 0.6 | 12.9 | 132 | 9.77 |
| Salmon | 5.9 ** | - | - | - | 1.5 | 132 | 1.14 |

* Effective CLA level associated with reduced breast cancer in postmenopausal women = 132 mg CLA/d (Aro *et al*., 2000).
** Salmon (Nutritive Value of Foods, 1981).

*et al*., 1999), which is an important volatile decomposition product of hydroperoxides formed from omega-6 PUFAs (Frankel *et al*., 1989). Hexanal is used as an indicator of meat flavor deterioration and a measure of overall lipid peroxidation (Shahidi & Pegg, 1994). In this study, hexanal was found at higher levels in grain-fed than pasture-fed beef, associated with the higher percent fat levels of grain-fed beef. 1-Octen-3-ol is a volatile alcohol with mushroom-like aroma, found in dry cured ham (Garcia *et al*., 1991) and dry sausage (Berdagué *et al*., 1993). 2,3-Octanedione is a volatile ketone found in warmed-over flavor (WOF) beef and has been positively correlated with sensory evaluation of WOF (r = 0.81) and high TBA values (r = 0.88; St. Angelo *et al*., 1987). Larick *et al*. (1987) also found 2,3-octanedione in the volatile fraction of subcutaneous fat from both grain and pasture fed animals. 2,6-bis (1,1-dimethylethyl)-4-ethyl-phenol is a volatile compound found in deer urine as indicated in the patent of Newman (1996). Previous work by Maughan *et al*. (2012) and the present study indicated that sensory panelists preferred the grain-fed over pasture-fed beef even though grain-fed beef had higher levels of oxidation products including hexanal and 2,3-octanedione.

Dimethyl sulfone, toluene, 3-heptanone, hexadecanoic acid methyl ester, benzaldehyde, and 2-ethyl-1-hexanol were the volatiles found more abundant in the pasture-fed beef ($P < 0.05$). Dimethyl sulfone (methylsulfonylmethane) is a volatile sulfur compound found in roast beef (Mussinan & Katz, 1973). Min *et al*. (1977) confirmed the presence of toluene in roast beef extracts, and they speculated that the occurrence of such compounds could be due to the thermal degradation of amino acids or the breakdown of co-extracted lipid species, such as trans-2-trans-4-decadienal. 3-heptanone is a volatile ketone found in irradiated cooked sausage (Ahn *et al*., 1999) and alligator meat (Baek & Cadwallader, 2006), where it is thought to contribute to the undesirable odor and flavor meat from older alligator. Hexadecanoic acid methyl ester is a volatile component found in dry-cured ham (Berdagué *et al*., 1991) and dry fermented sausage (Ansorena *et al*., 2000). Mottram & Edwards (1983) reported the significantly higher benzaldehyde levels in lean meat than in the fatty triglyceride and phospholipid fractions, explaining some of the changes in roast beef aroma after removal of the lipid fraction. 2-ethyl-1-hexanol is by far the most abundant compound in pasture fed beef samples (Fig. 3-4). It is a fatty alcohol with plasticizer properties. This compound was previously found in duck meat (Wu & Liou, 1992) and pork myofibrillar protein (Benito *et al*., 2005). However, recent research shows that it is a contaminant derived from packaging material (Rivas-Cañedo *et al*., 2009). In the present study, beef primal ribs were vacuum packaged and frozen (-20 °C for 2-3 months prior to analysis). The vacuum bags for the Colorado pasture-fed beef ribs were obtained from a different supplier than

the Utah grain-fed beef. This procedural difference may account for the greater levels of 2-ethyl-1-hexanol derived from packaging in pasture-fed samples.

Comparison between the headspace volatiles and flavor profiles shown in the PCA graph (Fig. 3-5) and the correlation coefficients (Table 3-4) indicated that negative attributes such as barny and bitter were higher in pasture-fed beef ($P < 0.05$), and were associated with higher levels of 3-heptanone or dimethyl sulfone. On the other hand, the positive attributes such as umami in grain-fed steaks was correlated with 1-octen-3-ol. St. Angelo *et al*. (1988) also identified hexanal, and 2,3-octanedione in meats, among other compounds. Brewer *et al*. (2008) also identified hexanal, 3-hydroxy-2-butanone, 1-octen-3-ol, butanoic acid, and nonanal. These authors also suggested that the livery off-flavor in the meat was positively correlated with pentanal, hexanal, 3-hydroxy-2-butanone, and hexanoic acid while rancid off-flavor was correlated with pentanal and 2-phenyl furan and not correlated with hexanal. However, further research needs to be done in this area to confirm these relationships.

**Conclusions**

Animal diet affected many chemical characteristics of beef, including the volatile profiles in cooked meat. Rib steaks from pasture-fed animals had darker color ($P < 0.05$) and lower fat content ($P < 0.05$), with higher MUFA and PUFA ratio ($P < 0.05$), compared to grain-fed beef. Rib steaks from pasture-fed beef also had higher hydrophilic ORAC values ($P < 0.05$), and lower TBA values ($P < 0.05$) after 12 d refrigerated storage, indicating higher resistance to lipid oxidation during storage, compared to grain-fed beef. Although sample size was small (n = 3 animals/diet), mean differences between

diet treatments were relatively large, and statistically different between diets at the 95 - 99% confidence level.

Pasture and grain diets influenced the volatile profile of cooked meat. Flavor descriptors barny, gamey, and grassy were associated with pasture feeding, and were uniquely shown in this study to be positively correlated with aroma volatiles benzaldehyde, toluene, dimethyl sulfone, 3-heptanone, 2-ethyl-1-hexanol, and hexadecanoic acid methyl ester ($P < 0.05$). Grain-fed beef had higher ($P < 0.05$) levels of hexanal, 1-octen-3-ol, 2,3-octandione, and 2,6-bis (1,1-dimethylethyl)-4-ethyl-phenol, uniquely associated with umami and juicy flavors. Pasture-fed beef had higher levels of omega-3 fatty acids than grain-fed beef, but was only a moderate source of omega-3 fatty acids, compared to salmon. The main positive nutritional attribute of pasture-fed beef was the ~75% reduction in fat calories per serving, compared to grain-fed beef. Hence, pasture-fed beef would have health benefits in people at risk for chronic diseases, i.e., cardiovascular disease or anyone who wants to lose weight.

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# CHAPTER 4

# COMPARISON OF ALFALFA- VERSUS SAINFOIN-FINISHING DIET ON BEEF CHEMICAL CHARACTERISTICS AND HEADSPACE VOLATILES[1]


## Abstract

In the western U.S., alfalfa (legume) and mixed grasses are the two predominant livestock forages. Unlike alfalfa, sainfoin is a non-bloating, drought tolerant legume with high nutritional value for ruminant feeding. Additionally, among the grasses, tall fescue is fast growing and nutritious to livestock, but produces an ergot alkaloid toxin when infected by fungi, slowing cattle growth. Sainfoin is a legume containing tannins, which can bind and inactivate ergotamine when both plants are consumed by ruminants. Our collaborators are examining the possibility that mixed fescue-sainfoin pasture allows cattle to grow faster than on fescue alone. The objective of this study is to determine the effects of sainfoin versus alfalfa pasture on meat quality and aroma profiles. The meat quality parameters included quality grade, % fat, color stability, MRA (resistance to browning), TBA (resistance to lipid oxidation), FRAP (antioxidant capacity), fatty acid composition, and aroma profile in the headspace of heated meat. Beef carcasses from both diet treatments (n = 3/treatment) were very lean (select or standard quality grade), with 4 – 6% fat content. There were no differences between two legume-fed diets in lightness and redness ($P > 0.05$). Similarly, no diet differences were found in muscle MRA, TBA, FRAP, or fatty acid analyses ($P > 0.05$). For headspace analysis, there were 45 compounds detected. However, nonanoic and decanoic acids were the only 2 out of 45


[1] Coauthored by Tansawat, R., Ward, R.E., Martini, S. & Cornforth, D.P.

compounds different between diet treatments ($P < 0.05$). In terms of meat chemical characteristics and volatile profiles, sainfoin pasture was comparable to alfalfa as a cattle forage. Thus, further studies are justified to evaluate the economics of sainfoin in cattle finishing diets compared to alfalfa.

**Introduction**

Currently, there are several studies showing nutritional health benefits of pasture-fed beef over grain-fed beef (Daley *et al*., 2010), which has stimulated much interest among health-conscious consumers. Still, there is much to learn about the effect of different type of pasture feedings on meat quality. Tall fescue [*Schedonorus phoenix* (Scop.) Holub] is one of the most important cool-season forage grasses due to its fast growth rate and nutritional value to livestock, occupying approximately 5.5 million acres in the United States (Lacefield *et al*., 2003). Tall fescue is well-adapted to a wide range of soil and climatic conditions, so it is a versatile plant used for animal feed. However, excessive consumption of tall fescue can be toxic to livestock. Tall fescues can be infected with an endophytic fungus (*Neotyphodium coenophialum*), which produces secondary metabolites (alkaloids) that help protect the plant against insects and parasitic soil nematodes. Some ergopeptine alkaloids, especially ergovaline, are toxic for cattle because they act as a vasoconstrictor (Schnitzius *et al*., 2001).

Legumes are plants in the family Fabaceae (or Leguminosae) with nitrogen fixation ability in a symbiotic relationship with Rhizobium bacteria found in the soil. Within nodules formed on legume roots, nitrogen gas from the atmosphere is converted into ammonia, which is then assimilated into amino acids (the building blocks of

proteins). Hence, legume seed and foliage have higher protein content than non-legume crops, which is desirable for livestock feed. Nonetheless, legumes can sometimes cause frothy bloat in cattle, possibly due to the toxic effects of secondary metabolite saponins (Lindahl *et al*., 1957), particularly from legumes such as alfalfa (*Medicago sativa*) and clover (*Trifolium*). Thick foam is developed on top of the rumen liquid, preventing cattle from exhaling fermentation gases, which may lead to abdominal distension, inhibiting respiration and heart function, sometimes causing the animal's death.

Non-bloating legumes such as sainfoin (*Onobrychis viciifolia*), birdsfoot trefoil (*Lotus corniculatus*), and cicer milkvetch (*Astragalus cicer*) are legume forages that do not cause bloat in cattle. Sainfoin and birdsfoot trefoil contain the secondary metabolites tannins in the form of condensed tannins (CT) structure. Tannins are generally considered anti-quality factors for livestock because they bind irreversibly with proteins and inhibit intake and impede protein utilization. On the other hand, some CT can enhance nutrition by providing high-quality protein to the small intestine by binding to degradable protein in the rumen, making the protein unavailable for digestion and absorption until it reaches the small intestine (Lisonbee *et al*., 2009). The association between plant proteins and CT from these two plants is stable and insoluble at rumen pH (6.5 to 7.0). At the pH of the abomasum (2.5 to 3.0), some CT-protein complexes are unstable, allowing plant amino acids to become available for absorption in the higher pH (8.0 - 9.0) of the small intestine of the cattle (Mangan, 1988). MacAdam *et al*. (2011), Waghorn (2008), and Min *et al*. (2003) reported the potential of birdsfoot trefoil to produce high average daily gain in cattle; however, Waghorn (2008) and Min *et al*. (2003) gave details that, unlike CT in

birdsfoot trefoil, CT in sainfoin has not been shown to be beneficial to improve livestock production by this pathway. Yet, Theodoridou *et al*. (2010) showed that feeding sainfoin to ruminants can be used to alter the form of excreted N; therefore, potentially reduce environmental N pollution without negatively affect the amount of N retention, which is beneficial in ruminant nutrition.

Legume-grass mixtures are also used to improve animal and pasture productivity as well as managing bloat. Tannins have the potential to interact with other plant secondary compounds such as alkaloids and saponins, neutralizing their negative effects. Lisonbee *et al*. (2009) showed that lambs receiving intraruminal infusions of tannins increased their consumption of the high-saponin variety of alfalfa and the high-alkaloid variety of tall fescue relative to lambs not infused with tannins (controls). Owens *et al*. (2012) demonstrated that sheep fed a tannin-containing legume (birdsfoot trefoil) for 30 min subsequently consumed greater amounts of endophyte-infected tall fescue than sheep supplemented with high-saponin alfalfa. The objective of this study is to determine the effects of alfalfa (a bloat-causing legume containing the secondary metabolite saponins) versus sainfoin (a non-bloat-causing legume containing the secondary metabolite tannins) on meat quality including color stability, and metmyoglobin reducing activity (MRA), lipid oxidation and antioxidant status, fatty acid composition, and headspace volatile profiles. Legume-grass mixtures (alfalfa or sainfoin mixed with tall fescue) were used in this study.

**Materials and Methods**

Meat samples

The study was conducted at the Utah State University Intermountain Irrigated Pasture Project farm in Lewiston, UT, as part of a collaborative project with Dr. Juan Villalba. Mr. Brody Maughan was our collaborator responsible for animal management. In short, three blocks, each 9 acres in size, were divided into two 4.5-acre plots containing three 1.5-acre strips. Pastures, including alfalfa (*Medicago sativa* variety Vernal), sainfoin (*Onobrychis viciifolia* variety Shoshone), and tall fescue (*Festuca arundinacea* variety Kentucky-31) were seeded in these three 1.5-acre strips as shown in Figure 4-1.

Eight cattle were assigned to each plot, and animals within each plot had free access to the grass, legume and grass-legume mix throughout the day. All plots provided *ad libitum* forage to cattle (usually about 20 - 25 lbs of feed per head per day) for 90 - 110 days before animal harvest. One animal was randomly selected from each plot for chemical analysis of muscle; thus, six animals in total were used in this study (3 animals/diet treatment). At slaughter, cattle were 22 - 24 months old with 220 - 277 kg hanging carcass weights. All animals were steers, with the exception of one alfalfa-fed heifer.

Animals were slaughtered at the USU South Farm abattoir (Wellsville, UT). The ribs of each animal were then vacuum packaged after harvest and shipped to the Department of Nutrition, Dietetics, and Food Sciences at USU. Muscle was immediately frozen at -20 °C until use. Primal ribs (*Longissimus dorsi* muscles) of three alfalfa- and

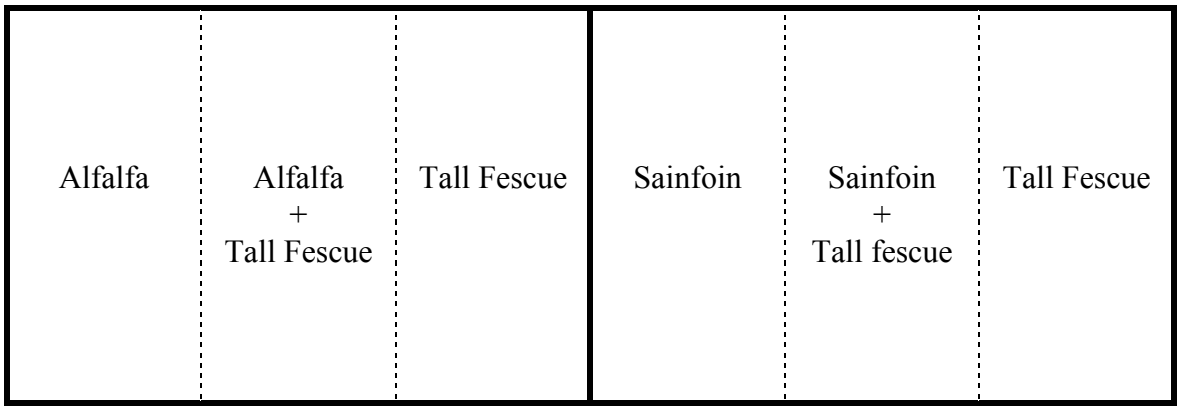


**Figure 4-1** Pasture design.

three sainfoin-fed cattle were used for the analyses. Carcass quality and yield grade measurements were obtained for each animal after harvest. Carcass quality grade factors included marbling score of the ribeye muscle (*Longissimus dorsi*), taken at the 12 - 13$^{th}$ rib, and carcass maturity score, indicated by degree of ossification the ventral processes of the thoracic vertebrae and ribs. Carcass yield grade measurements included hot carcass weight, back fat thickness (BF) and ribeye area (REA) at the 12 - 13$^{th}$ rib, and internal fat (% kidney, pelvic, and heart fat; %KPH) as a percent of carcass weight.

Chemical analyses

*Fat content*

Ether-extractable fat content of uncooked rib steaks was done by the solvent extraction method (Williams, 1984), using petroleum ether as the solvent. See details in Materials and Methods, Chapter 3.

*pH*

Raw beef pH was measured on 10 g finely chopped meat, diluted to 100 mL in distilled water, allowed to equilibrate at room temperature for 30 min and then filtered. Filtrate pH was measured, using a Fisher Accumet pH meter model 610 A (Fisher Scientific Inc, Salt Lake City, UT), equipped with a combination pH electrode calibrated immediately before use to pH 4.0 and 7.0.

*Hunter color measurements*

Each ribeye steak (1.9 cm thick) was placed in a Styrofoam tray and over-wrapped with oxygen-permeable polyvinyl chloride (PVC) and stored at 2 °C. Color measurements were determined at days 0, 1, 2, 4, 7, 9, and 12 of storage. Meat color was determined instrumentally using a HunterLab Miniscan portable colorimeter (Hunter Associates Laboratory, Inc., Reston, VA) with a 5 mm diameter aperture, set to use illuminant D-65. The colorimeter was standardized through a single layer of PVC film using both white and black standard tiles. Meat color parameters lightness (L*), redness (a*) and yellowness (b*) were measured. Triplicate readings were made on non-overlapping areas of the sample and values were then averaged.

*Metmyoglobin Reducing Activity (MRA) test*

MRA was measured as described by Mancini *et al*. (2008). A 3 cm x 3 cm x 2 cm sample of muscle tissue that did not contain any visible fat or connective tissue was removed. Beef samples were submerged in 0.3% $NaNO_2$ solution for 20 min at room temperature to induce metmyoglobin (MMb) formation. Beef samples were then removed

from the beaker and blotted to remove excess solution. The desired surface of beef was then placed in an impermeable bag and vacuum packaged. Reflectance spectra were recorded at the meat surface using a HunterLab Miniscan portable colorimeter (Reston, VA) with a 5 mm diameter aperture, set to use illuminant D-65. The initial amount of MMb formed on the surface was instrumentally determined. Percentage of MMb formed after the oxidizing treatment was determined by calculating the $(K/S)_{572} \div (K/S)_{525}$ ratio. After that, beef samples were placed in an impermeable bag, vacuum packaged, and incubated at 30 °C to allow MMb reduction. Samples were rescanned after 2 h to determine the amount of MMb remaining. The metmyoglobin reducing activity (% of MMb reduced) was calculated by the following equation:

MRA (% of MMb reduced) = [(Initial %MMb − Final %MMb) ÷ Initial %MMb] × 100.

*Thiobarbituric acid assay (TBA)*

Each steak was placed in a foam tray and over-wrapped with PVC film and stored at 2 °C. Thiobarbituric acid reactive substances (TBARS) were determined at days 0 and 12 of storage. The TBA assay was performed as described by Buege & Aust (1978). See details in Materials and Methods, Chapter 3.

*Ferric reducing antioxidant power (FRAP) test*

FRAP was measured as described by Benzie & Strain (1996). FRAP reagent was prepared by adding 200 mL acetate buffer (300 mM; pH 3.6), 20 mL TPTZ solution (10 mM of 2,4,6-tri [2-pyridyl]-s-triazine), 20 mL $FeCl_3$ solution (20 mM), and 24 mL distilled water together. In a cuvette, 30 μL distilled water and 1 mL of FRAP reagent

were mixed thoroughly. The solution was then incubated in the 37 °C water bath for 4 min and measured at absorbance 593 nm using a UV 2100 U spectrophotometer (Shimadzu Co., Kyoto, Japan). A blank solution was also run along with the standards. Each sample and standard value was corrected by subtraction of the absorbance of the water blank. Linear regression for the standards was constructed (absorbance against concentration). The FRAP values were calculated by using the regression equation. Data were expressed as mM Fe(II)/L of sample.

*Fatty acid chemical composition*

Fatty acids were analyzed using the method developed by O'Fallon *et al*. (2007). Steaks were sliced into thin strips (< 1.5 mm thickness) with a razor blade and approximately 1 g of lean tissue was placed into 16 x 125 mm screw-cap Pyrex culture tubes, using 1 mL of C17:1 as an internal standard (0.2826 mg/mL of C17:1 per 1 mL of MeOH). Fatty acid methyl esters (FAMEs) were synthesized by adding a 0.7 mL of 10 N KOH in water, followed by a 5.3 mL of MeOH into the tubes. After that, the tubes were incubated in a 55 °C water bath for 1.5 h, with vigorous hand-shaking for 5 s every 20 min to dissolve and hydrolyze the tissues. Tubes were then cooled in a cold water bath and 0.58 mL of aqueous 24 N $H_2SO_4$ was added. Consequently, samples were incubated again in a water bath at 55 °C for 1.5 h, with vigorous hand-shaking for 5 s every 20 min. After FAMEs synthesis, tubes were cooled again in a cold water bath. Two milliliters of hexane was added and tubes were centrifuged at 500 rpm, 4 °C, for 5 min. The upper supernatant, containing FAMEs, was placed into GC vials and store at -20°C until analysis. The FAMEs were analyzed by gas chromatography with flame ionization

detection (GC-FID) as described in Chapter 3. Fatty acid profiles were expressed as mg/100 g of fresh meat.

*Headspace volatile analysis*

The volatile profile of heated meat was determined as described by Vasta *et al*. (2010). Meat samples (6 g) were sliced and placed in closed rubber-capped vials. Samples were then heated at 70 °C for 10 min and fiber (2 cm-50/30 DVB/CarboxenTM/PDMS; Supelco, Bellefonte, PA) was exposed to the headspace over the sample at 70 °C for 30 min to adsorb the volatiles. A 2 μL aliquot of 1,2 dichlorobenzene (52.7 μmol) was added as a surrogate to each vial containing a beef sample. Data were expressed as the ratio of volatile to surrogate. See details in Materials and Methods, Chapter 3.

Statistical analysis

The experiment was designed with 2 diet treatments (alfalfa- vs. sainfoin-fed) and measurements were made on steaks from 3 animals per treatment. The measurements of fat content, pH, TBA, MRA, FRAP, fatty acid composition, and headspace volatile analyses were done in triplicate for each treatment. Hunter color measurement was done in duplicate for each sample. Statistical Analysis Software (SAS) version 9.1 (SAS Institute, Inc., Cary, NC) was used for analysis of variance (ANOVA) to identify statistically significant differences between samples at the 95% confidence level. Comparison of the means was made based on *P*-values ($\alpha = 0.05$) using the least significant different (LSD) adjustment to obtain differences of least means squares.

Complete randomized design with the *proc glm* function was used for MRA, FRAP, fatty acid profiles, and headspace volatile experiments. Repeated measures design with the *proc mixed* function, using Tukey adjustment to obtain differences of least means squares, was used for raw meat color and TBA experiments.

**Results**

Carcass characteristics including yield and quality grade are shown in Table 4-1. There was no different ($P > 0.05$) in HW, REA, BF, and % KPH between beef rib samples from cattle on the sainfoin treatment and animals finished on alfalfa pasture. The marbling score was "slight" in all of the alfalfa-raised animals, corresponding to USDA "select" quality grade. Two cattle on the sainfoin treatment had lower marbling score of "traces", corresponding to USDA "standard" quality grade.

**Table 4-1** Characteristics of steaks obtained from alfalfa- and sainfoin-fed animals

| Samples | HW | REA | BF | KPH | Marbling Score | USDA Grade | Sex |
|---|---|---|---|---|---|---|---|
| Alfalfa #1 | 99.7 | 56.1 | 0.5 | 2.0 | Slight 90 | Select + | Heifer |
| Alfalfa #3 | 111.1 | 70.3 | 0.4 | 2.0 | Slight 40 | Select - | Steer |
| Alfalfa #5 | 114.3 | 66.5 | 0.3 | 2.0 | Slight 40 | Select - | Steer |
| **Alfafla mean** | **108.4** | **67.3** | **0.4** | **2.0** | **-** | **-** | **-** |
| Sainfoin #2 | 104.8 | 55.5 | 0.4 | 2.0 | Slight 10 | Select - | Steer |
| Sainfoin #4 | 125.6 | 70.3 | 0.3 | 1.5 | Traces | Standard + | Steer |
| Sainfoin #6 | 115.2 | 72.3 | 0.1 | 1.5 | Traces | Standard + | Steer |
| **Sainfoin mean** | **115.2** | **66.0** | **0.3** | **1.7** | **-** | **-** | **-** |
| *P*-value[1] | NS | NS | NS | NS | - | - | - |

HW = Hanging Weight (kg); REA = Rib Eye Area ($cm^2$); BF = Back Fat thickness (cm); KPH = kidney, pelvic, and heart fat (% of carcass weight).
[1] Significantly different between diet treatment means ($P < 0.05$); NS = Not significantly different.

Figure 4-2 shows that meat color stability was strongly affected by time of storage. Typically, fresh beef steaks in PVC package (80% oxygen) have bright red color with a* ~ 16 and L* ~ 45 (0 - 100 scale; 0 = completely black, 100 = completely white), while b* is low (~15). Yellowness (b*) is the component of brownness that can change in the aged beef, which can go up to >25 (John *et al*., 2005). In this study, lightness (L*) was increased ($P < 0.001$) while redness (a*) and yellowness (b*) values were decreased over 12 d storage at 2 °C ($P < 0.0001$ and $P = 0.001$, respectively). There were no differences between the two legume-fed diets in lightness ($P < 0.05$) and redness ($P < 0.05$) descriptors. Yellowness was significantly lower in alfalfa-fed treatment ($P < 0.01$), but there was no significant interaction effect on b* values between diet treatment and storage time at any given day (see Appendix Table B1-B6 for detailed statistics).

Fat content of rib steaks were not different ($P > 0.05$) between alfalfa and sainfoin diet treatments (Table 4-2; Appendix Table B7). Mean rib muscle pH was also similar between forage treatments ($P > 0.05$; Appendix Table B8). MRA is another parameter that was conducted to measure meat discoloration by measuring the resistance of myoglobin to nitrite-induced oxidation. There were not any changes in % of MMb reduced in meat samples between diet treatments ($P < 0.05$; Appendix Table B9). Rate of lipid oxidation in ribs was determined by TBA reactivity. TBA values, compared between diet treatments, were similar ($P > 0.05$) in both initial and final-storage time points (day 0 and day 12; Appendix Table B10-B11). Moreover, TBA values of meat from the two diets were the same after 12 d storage at 2 °C ($P > 0.05$), which was comparable to the TBA results from grain- vs. grass fed beef experiment that was

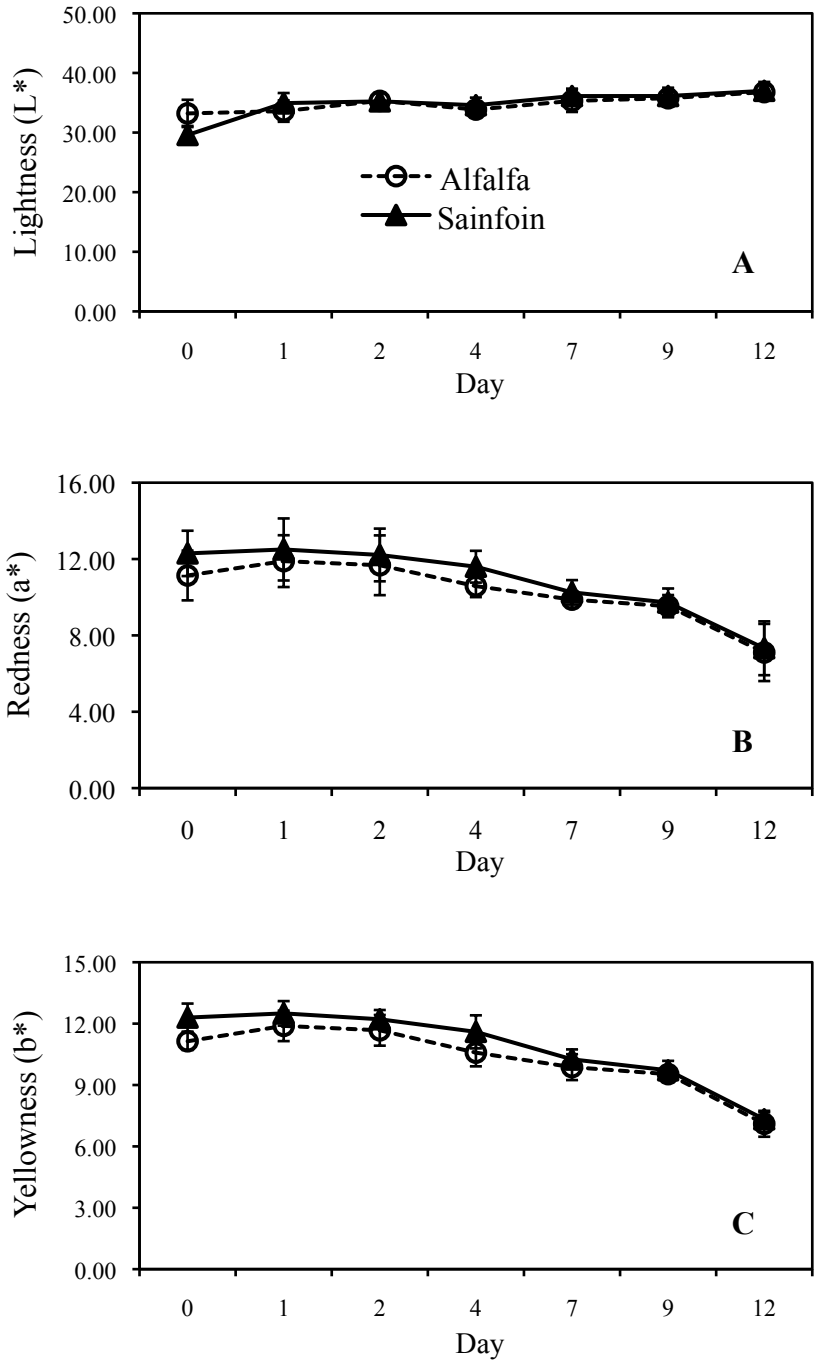


**Figure 4-2** Effect of storage time (day) on alfalfa- and sainfoin-fed beef color stability at 2 °C. **A:** Lightness (L*); **B:** Redness (a*); **C:** Yellowness (b*). Error bars = SEM.

**Table 4-2** Fat content, pH, metmyoglobin reducing activity (MRA), thiobarbituric acid assay (TBA), and ferric reducing antioxidant power (FRAP) of alfalfa- and sainfoin-fed beef

| Treatment | Fat (%) | pH | MRA (%MMb[1]) | TBA | | FRAP [Mm Fe(II)] |
|---|---|---|---|---|---|---|
| | | | | Day 0 | Day 12 | |
| **Alfalfa-fed** (n=3) | 6.07 ± 1.54 | 5.14 ± 0.14 | 75.47 ± 1.30 | 0.15 ± 0.04 | 0.24 ± 0.05 | 0.0755 ± 0.0075 |
| **Sainfoin-fed** (n=3) | 4.43 ± 1.47 | 5.10 ± 0.06 | 72.85 ± 1.32 | 0.23 ± 0.25 | 0.33 ± 0.09 | 0.0677 ± 0.0065 |
| *P*-value | 0.25 | 0.62 | 0.07 | 0.87 | 0.08 | 0.23 |

[1] % MMb = % of Metmyoglobin reduced.

discussed in Chapter 3. Antioxidant capacity of steaks was also evaluated, using FRAP test. There were no significant differences found between the two pasture treatments ($P > 0.05$; Appendix Table B12).

With regards to fatty acid composition, the thirteen most abundant fatty acids are shown graphically in Figure 4-3. The amount of myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), vaccenic acid (C18:1n7t), and linolenic acid (C18:3n3) were numerically higher in alfalfa-fed than in sainfoin-fed animals, but not different at a significance level of $\alpha = 0.05$ (Appendix Table B13).

Volatile compounds were measured above the headspace of heated meat at 70 °C to study the favorable and unfavorable effects of diet treatments on sensory properties of the meat. Feeding different legumes did not have a strong effect on the meat volatiles. There were 45 volatiles detected across the two diet treatments, but only 2 volatiles that were significantly affected by diets ($P < 0.05$; Figure 4-4; Appendix Table B14). Ratio of the area of nonanoic and decanoic acids to surrogate standard were significantly higher in meat from alfalfa-fed than from sainfoin-fed animals ($P < 0.05$).

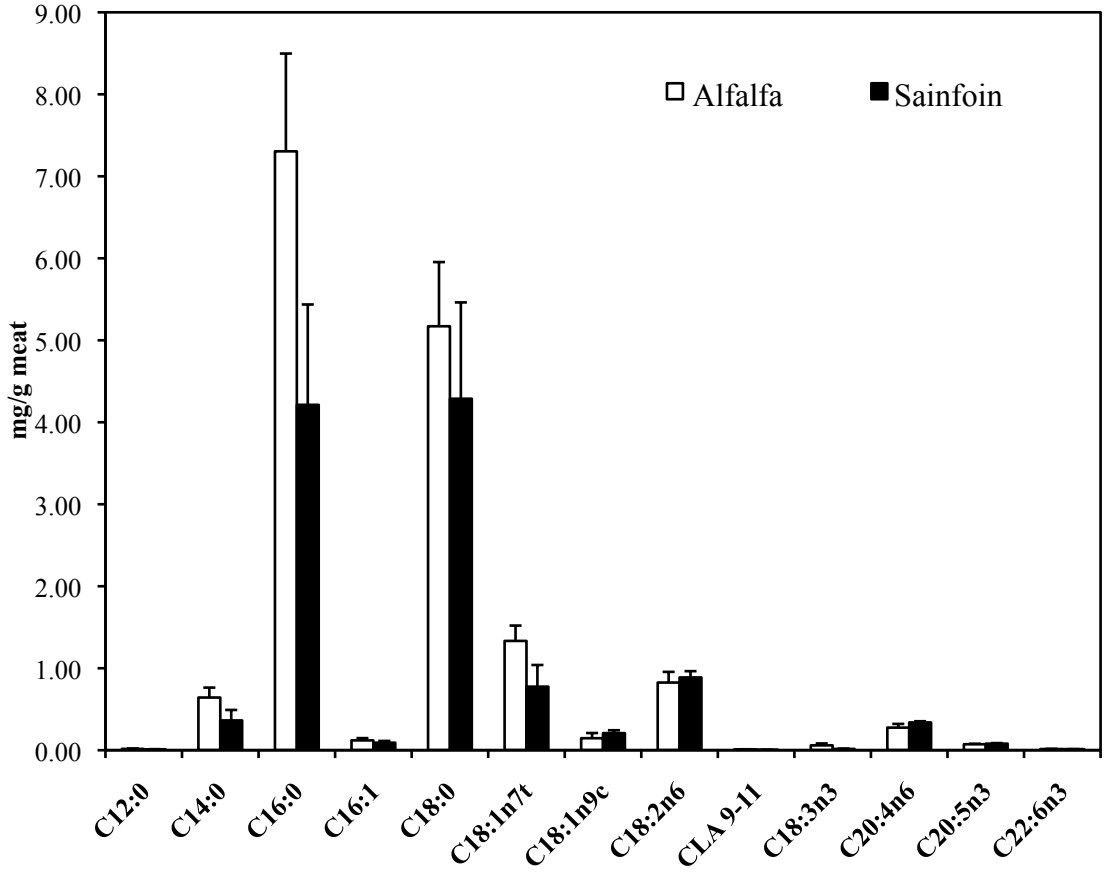


**Figure 4-3** Fatty acid chemical composition (mg/g meat) for meat samples obtained from animals fed with alfalfa- and sainfoin-based diet. Error bars = SEM.

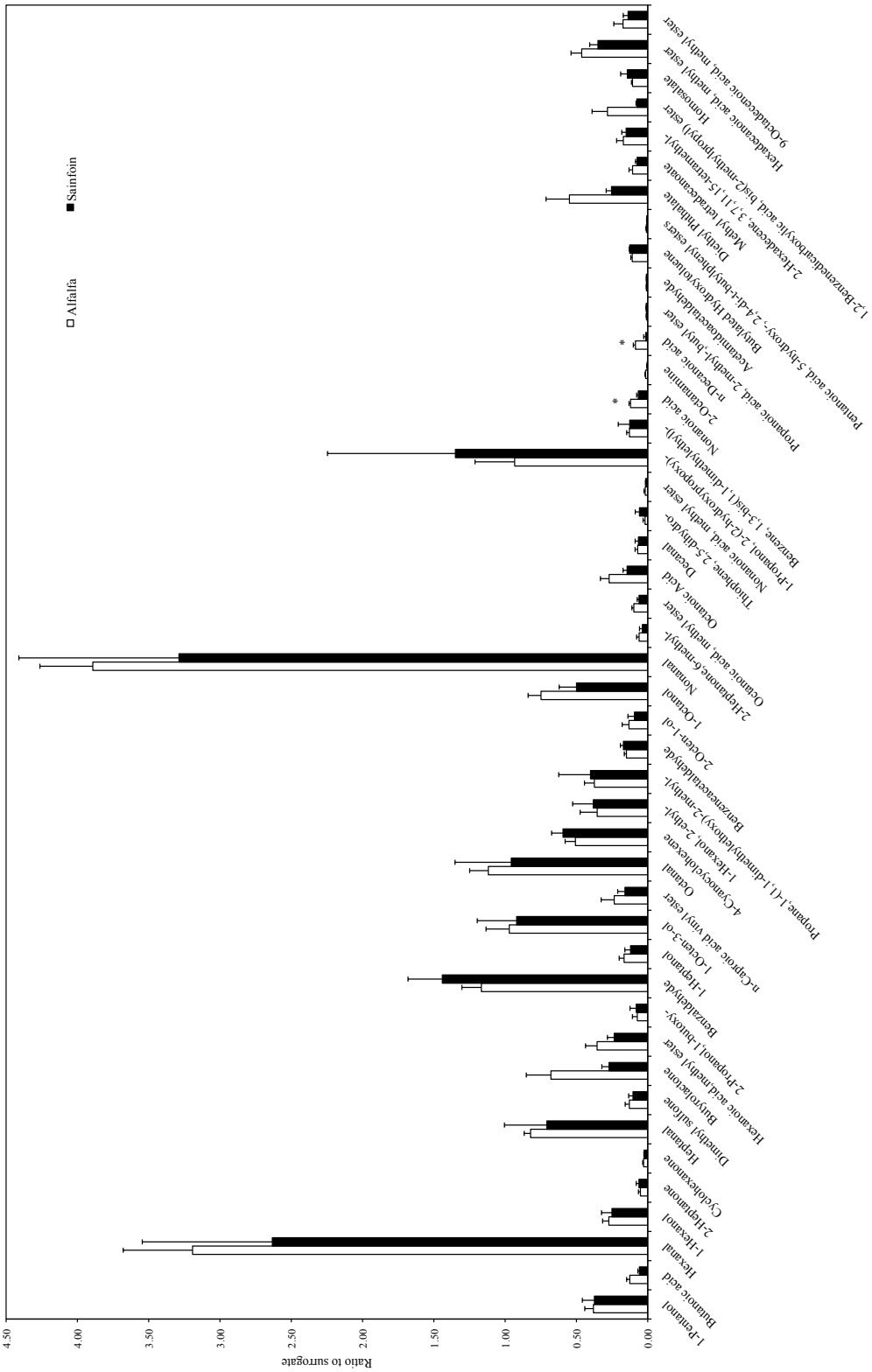


**Figure 4-4** Ratio to surrogate of headspace volatile compounds from beef fed with alfalfa- or sainfoin-based diets. * = Ratio to surrogate is significantly different between diet treatments ($P < 0.05$). Error bars = SEM.

**Discussion**

Sainfoin is a non-bloating legume with high nutritional value for livestock feeding. In this study, chemical analyses of meat from alfalfa- versus sainfoin-fed cattle were generally not significant different ($P > 0.05$), in terms of color stability, resistance to browning (MRA), resistance to lipid oxidation (TBA), antioxidant capacity (FRAP), fatty acid composition, and volatile profile. Hence, it can be suggested that tannin-containing sainfoin was equivalent to saponin-containing alfalfa pasture as a cattle forage in a legume-grass mixed system. According to data from Chapter 3, similar low TBA values after 12 d of storage at retail conditions (2 °C) were obtained from ribs from cattle fed a pasture-mixed diet (a variety of plants, including orchard grass, brome, fescue, and clover) as compared to animals fed alfalfa-tall fescue and sainfoin-tall fescue fed in this study. There were no differences ($P > 0.05$) in lipid oxidation on steaks sampled at day 0 and after 12 d storage in both experiments (pasture-fed: day 0 = 0.22 vs. day 12 = 0.60; alfalfa-fed: day 0 = 0.15 vs. day 12 = 0.24; sainfoin-fed: day 0 = 0.23 vs. day 12 = 0.33). TBA numbers greater than 1.00 are commonly associated with rancid flavor/odor (Greene & Cumuze, 1981), but all TBA values from both experiments were less than 1 even after 12 d storage. Therefore, this study confirmed that pasture diets, including legumes and grasses, have longer storage shelf life than beef from typical grain-finishing diet.

In the assay for headspace volatiles of heated meat, only 2 out of 45 detected volatiles were significantly different between diet treatments. Nonanoic and decanoic acids were higher in alfalfa-fed beef ($P < 0.05$). Nonanoic and decanoic acids are

volatiles found in charbroiling and frying hamburger meat (Rogge *et al*., 1991) and dry-cured ham (Martín *et al*., 2006; Andrés *et al*., 2002; Berdagué *et al*., 1991; Garcia *et al*., 1991). Several volatiles that were associated with gamey, bitter, barny, and grassy flavors in Chapter 3 were also found in this study (in both alfalfa- and sainfoin-fed) including dimethyl sulfone, benzaldehyde, 2-ethyl-1-hexanol, and hexadecanoic acid.

Maughan (2011) conducted descriptive sensory profiling and consumer test for meat obtained from the same animals (ribeye steak, *L. dorsi* muscles). For descriptive analysis, 12 panelists were recruited from the local community and trained to identify and quantify the flavor characteristics of the meat obtained from cattle fed alfalfa or sainfoin diets [Panelists' screening and training was performed as described in Maughan *et al*. (2012)], and a lexicon of 18 meat flavor descriptors (astringent, barny, bitter, bloody, brothy, browned, gamey, grassy, juicy, fatty, livery, metallic, oxidized/warmed-over flavor, roast beef, salty, sour, sweet, and umami) was used on a 15-point scale (1 = no flavor and 15 = very high flavor intensity) for of each attribute. No significant differences ($\alpha = 0.05$) were found in the flavor characteristics of meats obtained from cattle fed different legume diets for all flavor descriptors. A consumer test was also performed on the same rib steak samples, with 120 panelists and utilizing a 9-point hedonic scale (1 = dislike extremely, 9 = like extremely). No significant differences were also obtained in the acceptability of meat from both diets with values of 6.71 for alfalfa treatment and 6.96 for sainfoin treatment, where a score of 6 = like slightly, and 7 = like moderately.

A contributing factor to the similarity in chemical characterization and sensory evaluation of rib steaks in this study might be due to an unexpected problem with live

animal grazing management during the finishing period; specifically, the cattle in the sainfoin pasture treatment probably did not accumulate as much sainfoin-derived tannins as called for in the experimental design, because over-grazing of sainfoin in period 1, resulted in less sainfoin being available as forage later in the summer.

There were three grazing periods (Period 1: from May 20$^{th}$ to June 21$^{st}$; Period 2: from July 8$^{th}$ to August 5$^{th}$; Period 3: from August 17$^{th}$ to September 7$^{th}$), with 12 animals/pasture in Period 1. However, the yields of sainfoin in Periods 2 and 3 decreased substantially from the beginning to the end of each Period, probably for the reason that animals grazed more heavily on sainfoin than grass, probably because of its greater nutritional quality and flavor, compared to grass. Consequently, 4 animals/plot had to be removed before the start of Period 3, in order to make more forage available per animal. With this change in animal number, average daily gains of cattle increased during Period 3, but were still below the gains achieved during Period 1 (the period with the greatest forage yields). Nevertheless, sainfoin consumption increased during Period 3 compared to Period 2 in the sainfoin pasture treatment, and meat from these animals was found to be not different in chemical characteristics or flavor, compared to meat from alfalfa-based pasture.

**Conclusions**

Meat quality and acceptability of pasture-finished cattle on sainfoin appears comparable to meat from animals finished on alfalfa-based pasture. Meat quality, in terms of color stability, resistance to browning, resistance to lipid oxidation, antioxidant status, fatty acid composition, and aroma profiles was similar between the two diet

treatments, indicating that sainfoin pasture was comparable to alfalfa as a cattle forage. The benefits of sainfoin is that it is drought-tolerant, high in protein, does not cause bloat in cattle, and its tannins can inactivate ergotamine toxicity when sainfoin and tall fescue are consumed together by ruminants. However, in this study, sainfoin forage yield was decreased during the latter stages of the finishing period. Thus, more information is needed regarding rate of weight gain and other production factors for cattle finished on sainfoin pastures compared to alfalfa.

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# CHAPTER 5

# METABOLOMIC ANALYSIS OF LAMB MUSCLE AS AFFECTED BY TANNIN OR SAPONIN SUPPLEMENTED DIET OF ANIMALS INFECTED WITH RED STOMACH WORM LARVAE (*HAEMONCHUS CONTORTUS*)[1]

**Abstract**

Parasite infection is a major cause for reduced productivity in livestock. Currently, plant secondary compounds (PSC), especially tannins and saponins, are increasingly used as chemical feed additives for anti-parasitic properties. However, more information is needed about how diets drive the genetic machinery and affect the body chemistry of herbivores. The objective of this study was to evaluate the effects of a diet containing tannins (T) or a diet containing saponins (S) when given in single ration or as choice of them (C), on lamb metabolomics profile, using a GC/MS technique, compared to a plain/control diet (P). There were 40 metabolites detected in total (30 named and 10 unknown). Principal component analysis showed a clear separation of the P, T, and S treatments, while C diets were separated in to two groups related to the P and S diets, respectively. Carbohydrate metabolites were mostly increased in the T diet ($P < 0.05$). T and S diets significantly reduced C10:0 and C17:0 fatty acids in muscle. Cholesterol levels were lower ($P < 0.05$) in the S than T diet. No differences were found among amino acids metabolites ($P > 0.05$). Vitamin E and phosphoric acid were higher ($P < 0.05$) in the P and T diets, respectively. Differences were also detected in other small molecules such as cresol, isocarbostyril, phthalate, and acetamide.

[1] Coauthored by Tansawat, R., Cornforth, D.P. & Ward, R.E.

**Introduction**

Helminth (parasitic worm) infections are a major cause of reduced productivity in livestock (Githiori *et al.*, 2006). Currently, increasing awareness of hazards associated with the use of antibiotic and chemical feed additives has accelerated investigations into plants and their extracts as feed additives (Wallace, 2004). Plant secondary compounds (PSC), a diverse group of molecules that constitute the plant defense system, are not required for the primary biochemical pathways of cell growth and reproduction (Wallace, 2004), and are increasingly recognized as important for animal health, welfare, and nutrition (Villalba *et al.*, 2011). Research has revealed animal acceptability of some PSC as well as pros and cons of these compounds. The effective dose of PSC depends on their biochemistry. Forages with low concentrations can be beneficial, but excessive consumption can detrimentally affect herbivores intake or health. Many of the studies demonstrated an anti-parasitic effect of PSC in mammals. Tannins and saponins are classes of PSC that have been identified and used in livestock productivity and health as alternatives to chemical feed additives (Provenza & Villalba, 2010; Rochfort *et al.*, 2008).

Tannins are naturally occurring water-soluble plant polyphenols with the capability to bind and precipitate proteins (Spencer *et al.*, 1988). At appropriate concentrations, some tannins improve nutrient utilization (Barry *et al.*, 2001), alleviate bloat (Min & Hart, 2003), and reduce internal parasites (Athanasiadou *et al.*, 2000; Scalbert, 1991). Tannin-rich plants have attracted most attention for their effect on internal nematodes in ruminants (Hoste *et al.*, 2006; Nguyen *et al.*, 2005). Condensed

tannins extracted from various forages can markedly decrease the viability of the larval stages of several nematodes in sheep and goats by interfering with parasite egg hatching and development to infective stage larvae (Brunet & Hoste, 2006; Min & Hart, 2003).

Saponins are a class of PSC found in natural sources, particularly in some plant species. Their structure includes one or more hydrophilic glycoside moieties combined with a lipophilic triterpene derivative (Hostettmann & Manton, 1995). Saponins have been widely used in animal nutrition to reduce methanogenesis in the rumen (Patra & Saxena, 2010). Moreover, saponins have pronounced anti-protozoal activity due to their ability to bond to cholesterol present in protozoa membranes (Makkar *et al.*, 1998), thus reducing the predation of protozoa on rumen bacteria. Saponin-containing plants were reported to have toxic effects on protozoa *in vitro* (Makkar *et al.*, 1998; Newbold *et al.*, 1997). Also, they were evaluated for effects against rodent nematode L3 (infective larvae; Fakae *et al.*, 2000) and were suspected to be an active compound against gastrointestinal parasite nematodes (Ibrahim, 1992) and cestodes (Julien *et al.*, 1985).

A previous study, done by our collaborators (Copani *et al.*, 2012), with use of the same animals as the present study, verified anti-parasitic properties of tannins and saponins in lambs by the reduction of faecal egg counts (FEC). Lambs fed with a diet containing 8% quebracho tannins and 1.5% of saponins from quillaja bark had a reduction in FEC of 51.6% and 38,8%, respectively, relative to animals receiving a diet without PSC. However, more information is needed about how diets containing PSC's drive genetic machinery and affect body chemistry. This information can be obtained by the study of metabolomics. Few metabolomic studies have evaluated the effect of PSC

consumption in ruminants, especially when they were infected with parasites. Iason & Villalba (2006) suggested that since foraging choices are limited by the ability of animals to experience the consequences of their behaviors and associate particular cues in foods with their specific effects in the body, animals must at least "sample" plants that contain PSC. Thus, the objective of this study was to evaluate the effects of a diet containing Quebracho tannins, or a diet containing *Quillaja saponaria* saponins, when given in single ration or as choice, on animal metabolomics profiles. Metabolomics analysis was conducted using GC/MS techniques combined with multivariate data analysis to distinguish four different diet treatments; 1) control diet, 2) tannin-rich diet, 3) saponin-rich diet, and 4) choice diet.

**Materials and Methods**

Animal and dietary treatment

The study was conducted at the Utah State University Intermountain Irrigated Pasture Project farm, at Lewiston, UT, as part of a collaborative project with Dr. Juan Villalba and Daniela Brogna. Twenty-eight 2-month-old commercial Finn-Columbia-Polypay-Suffolk crossbred lambs were placed randomly in individual pens and assigned to four dietary groups (7 animals per treatment) as follows:

1) Control/Plain diet (P) consisted of beet pulp + 1.5% vegetable oil.
2) Tannin-rich diet (T) consisted of P diet + 8% tannins (27 kg beet pulp + 2.4 kg tannins).
3) Saponin-rich diet (S) consisted of P diet + 1.5% saponins (29 kg beet pulp + 0.45 kg saponins).

4) Choice diet (C); animals had free access for a choice of T or S diets. All lambs had access to fresh potable water and trace mineral salt blocks.

Extracted Quebracho tannins were supplied by Industria Argentina ATO, UNITAN SAICA, Buenos Aires, Argentina. Saponins were obtained from Sigma Chemical Co. (St. Louis, MO USA; product no. S-7900), extracted from *Quillaja saponaria* bark. Experimental diets were prepared every 2 days in a batch of 30 kg.

Animals were handled according to the following experimental design: at day 0, lambs were weighed and drenched with a combination of anti-parasitic agents of Pyrantel Pamoate (Stronid® T) 25 mg/kg + Albendazole (Valbazen®) 7.5 mg/kg; from day 0 to 10, animals were fed a diet consisting of alfalfa pellets *ad libitum* and 300 g of rolled barley per head per day; at day 10, faecal samples of each animal were taken to assess FEC. From day 10 to day 22 lambs were familiarized with the experimental diets (adaption period); at day 23 each animal was infected orally with a normal syringe containing 30 ml of water solution with a single dose of 5,000 *Haemonchus contortus* L3 (third larval stage); from day 23 to 49, the animals were kept on a diet of *ad libitum* alfalfa pellets. At day 49 FEC was assessed again but the level of infestation was inappropriate because the number of eggs in faeces was lower than the expected level for an infection. Thus, animals were re-infested again at day 50 by similar procedure but using a higher single dose of 8,000 *Haemonchus contortus* L3. After the second larval infestation, animals were kept on an *ad libitum* of alfalfa pellets diet until day 73. From day 73 to 85, animals received the experimental diets. After the experimental period, lambs received *ad libitum* alfalfa pellets again until slaughter.

Lambs were slaughtered at the USU South Farm abattoir (Wellsville, UT) by Dick Whittier. After harvest, carcasses were refrigerated at 4°C for 24 h before being shipped to the meat lab, Department of Nutrition, Dietetics, and Food Sciences at Utah State University (Logan, UT, USA). The *longissimus dorsi* muscle was taken, immediately vacuum-packaged, and frozen at -20 °C until the analysis (~ 4 weeks).

Metabolomics measurements

Metabololites of lamb were analyzed using the method developed by Shakya *et al.* (2009), with modification. Lamb muscle samples (~2 g) were snap-frozen in liquid nitrogen and pulverized with mortar and pestle to preserve the metabolic state. Then 100 mg tissue powder was transferred to a test tube with a screw cap and extracted with 900 µL methanol. Samples were vortexed for 1 min and sonicated in a heated water bath ultrasonicator set to 70 °C (Fisher Scientific Model FS60, Pittsburgh, PA) for 5 min. Debris was removed by centrifugation at 5000 *g* (IEC Multi RF, Thermo Electron Corporation, Asheville, NC) and the supernatant was then dried in a vacuum oven (Thermo Electron Corporation, Marietta, OH). After that, samples were re-suspended in 100 µL pyridine containing 20 mg/mL of O-methoxyamine hydrochloride. Tricosane (C23:0; 5 mg/mL in chloroform) was added as an internal standard. The solution was vortexed for 1 min and incubated at 30 °C for 1.5 h. Next, a 50 µL of N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) containing 1% trimethylchlorosilane was added for silylation. The mixture was again vortexed for 1 min and incubated at 37 °C for 30 min. Subsequently, samples were clarified by quick centrifugation and transferred to 100 µL-vial inserts (Agilent Technologies Inc, Santa Clara, CA, catalog no. 5183-2085).

Separation was performed by GC/MS (GCMS-QP 2010S, Shimadzu Co., Kyoto, Japan) with use of an RXI-17SilMS column (35 m x 0.25 mm, film thickness = 0.25 μm). One microliter of the derivatized sample was injected splitless by an AOC-5000 Auto Injector (Shimadzu Co., Kyoto, Japan) with a sampling time of 1 min. Inlet temperature was 270 °C. Helium was used as the carrier gas (1 mL/min). The initial GC oven temperature program was 60 °C and held for 5 min. Then, temperature was ramped at the rate of 10 °C/min to final temperature of 300 °C, which was held again for 5 min. The total program time was 34 min. The GC/MS interface was set at 250 °C, the ion source temperature being 200 °C. The GC/MS interface was heated at 290 °C. The acquisition was performed in electron impact mode (70 eV) at the rate of 0.5 $s^{-1}$, with a mass range of 50-600 m/z. Data files obtained from the GC-MS were exported in the netCDF format. Peak picking and deconvolution were performed using the public Automated Mass Spectral Deconvolution and Identification System (AMDIS; version 2.62, 1999-2000) developed by the National Institute of Standards and Technology (NIST). Deconvoluted mass spectra were submitted to the online analysis tool Spectconnect (www.spectconnect.mit.edu) for the systematic detection of possible metabolites that were conserved across samples. Metabolites resulting from this analysis were identified by a database search against the NIST Mass Spectral (version 2.0, 2005) and Fiehn (Agilent Technologies Inc, Santa Clara, CA) libraries, and by comparison with linear retention indices (LRI). The LRI were established by injection of standard n-alkanes from 7 to 40 carbons (Supelco, Bellefonte, PA). Parent peak intensities were normalized to the

surrogate standard in each run prior to statistical analysis. Data were expressed as ratio to surrogate.

Statistical analysis

The experiment was designed with 4 diet treatments (P, T, S, or C) and measurements were made on 7 animals per treatment. Individual animals were considered experimental units. Statistical Analysis Software (SAS) version 9.3 (SAS Institute, Inc., Cary, NC) was used for multivariate data analysis. First, principal components analysis (PCA) was performed using *proc factor*. All named metabolites were examined by the PCA. Next, the same metabolites detected with different LRI were pooled together. Analysis of variance (ANOVA), using a complete randomized design with *proc glm* to identify statistically significant differences between diet treatments at the 95% confidence level. For significance testing, multiple comparisons were calculated by the least significant different (LSD) test to obtain differences among mean values based on *P*-values at $\alpha = 0.05$.

**Results and Discussion**

PCA was conducted to examine a possible separation and overview the metabolomics pattern of the four different diet treatments using all metabolites detected by the GC/MS technique. Fig. 5-1 shows a plot of the principal component (PC) scores for the most important PCs (PC1 vs. PC2). The first two PCs account for 42.14% of the total variation (PC1 = 28.72% and PC2 = 13.42%). The PCA plot shows a clear separation of P, T, and S treatments. Part of the separation based on the PC1 is not

obvious, but fairly distinct between T and C treatments. The PC2 is separating according to whether lambs received PSC or not. The metabolites with positive coefficients for the second principal component are from the P diet, whereas metabolites with negative coefficients are from the T and S diets. The C diet is separated into 2 groups; one group is related to the P diet and another group is placed close to the S diet. Accordingly, it may be that the animals showed a preference for saponins over tannins when they had free access for a choice of T or S diets. This is in an agreement with the previous study by Copani *et al.* (2012), using the same animals, which reported that lambs preferred saponins. Among the C diet treatments, animals ate significantly more saponin-containing food (58 g/kg) compared to 18 g/kg of tannin-containing food ($P < 0.05$) during parasitic infection, even the S diet showed less effect on parasitic lowering activity than the T diet in this study.

There were 40 metabolites in total detected by GC/MS (30 named and 10 unknown). Thirty metabolites were group together for carbohydrates, lipids, amino acids, vitamins and minerals, nucleotides, and other small molecules as shown in Table 5-1. Fifteen out of thirty metabolites were found to be significantly different among diet treatments ($P < 0.05$; see more data of graphically statistics in Appendix C).

Carbohydrate metabolites including sugars and sugar alcohols tended to be significantly higher in tannin-rich diet than other diet treatments. Ribose, fructose, glucose, as well as sorbitol were highest in T ($P < 0.05$). It could possibly be explained by the structure of condensed tannins (CT). According to their structure as shown in Chapter 2 (Fig. 2-1), there are more hydroxy groups (-OH) in CT structures as compared

to saponin structures (Fig. 2-2); and hydroxyl groups are the binding sites for sugar molecules, by formation of glycosidic condensation links with sugar hydroxyls. Pentitol (ribitol) is a pentose alcohol formed by the reduction of ribose. At the present time, there is no possible explanation for the observation why ribitol was significantly higher in the T diet than other diets. *Myo*-Inositol is another sugar alcohol, which plays an important role as the structural basis for a number of secondary messengers in eukaryotic cells, including inositol phosphates, phosphatidylinositol (PI) and phosphatidylinositol phosphate (PIP) lipids. In this study, *myo*-inositol levels were significantly lower in C diet and there was no obvious explanation for this result.

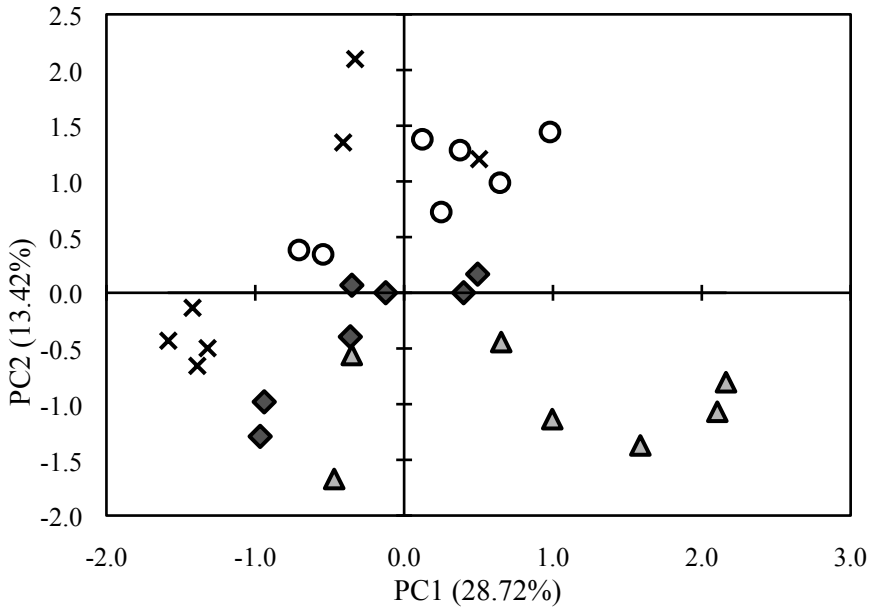


**Figure 5-1** Principle component analysis of animals arranged by metabolites found in lamb muscle fed various diets (Open circle = Plain/Control diet; Triangle = Tannin-rich diet; Diamond = Saponin-rich diet; Cross = Choice diet).

**Table 5-1** Metabolites from lamb muscles fed four different diets

| Metabolites | Ratio to Surrogate | | | | P-value |
|---|---|---|---|---|---|
| | P | T | S | C | |
| **Carbohydrate** | | | | | |
| **Sugar** | | | | | |
| Ribose | 0.0029 b | 0.0326 a | 0.0015 b | 0.0019 b | <0.01 |
| Fructose | 0.3201 ab | 0.5797 a | 0.1752 b | 0.1436 b | 0.02 |
| Glucose | 3.6080 ab | 4.7198 a | 2.1202 bc | 1.3991 c | 0.02 |
| Mannose | 0.7645 | 0.7073 | 0.4790 | 0.3366 | NS |
| Sorbose | 0.0568 | 0.0774 | 0.0313 | 0.0191 | NS |
| Galactose | 0.2208 | 0.1408 | 0.1094 | 0.1366 | NS |
| **Sugar Alcohol** | | | | | |
| Sorbitol | 0.0177 b | 0.0600 a | 0.0050 b | 0.0025 b | 0.01 |
| Pentitol (Ribitol) | 0.2534 a | 0.1109 b | 0.0924 b | 0.1094 b | 0.03 |
| *myo*-Inositol | 2.7766 a | 2.6926 a | 2.3029 a | 1.1029 b | <0.01 |
| **Lipid** | | | | | |
| **Fatty acid** | | | | | |
| Acetic acid (C2:0) | 0.0202 | 0.0150 | 0.0172 | 0.0113 | NS |
| Propanoic acid (Propionic, C3:0) | 0.0612 | 0.0458 | 0.0358 | 0.0334 | NS |
| Butyric acid (Butanoic acid, C4:0) | 0.1543 | 0.1002 | 0.1137 | 0.1013 | NS |
| Capric acid (Decanoic acid, C10:0) | 0.0940 ab | 0.0528 b | 0.0791 b | 0.1226 a | 0.02 |
| Lauric acid (Dodecanoic acid, C12:0) | 0.0175 | 0.0173 | 0.0163 | 0.0188 | NS |
| Palmitic acid (Hexadecanoic acid, C16:0) | 0.0855 | 0.0947 | 0.1093 | 0.0970 | NS |
| Margaric acid (Heptadecanoic acid, C17:0) | 0.0085 b | 0.0006 c | 0.0026 c | 0.0126 a | <0.01 |
| Stearic acid (Octadecanoic acid, C18:0) | 0.1538 | 0.1480 | 0.1603 | 0.1582 | NS |
| Behenic acid (Docosanoic acid, C22:0) | 0.0007 a | 0.0007 a | 0.0008 a | 0.0001 b | <0.01 |
| **Glycerolipid** | | | | | |
| Glycerol | 6.4810 | 4.6089 | 5.2215 | 4.5998 | NS |
| **Sterol** | | | | | |
| Cholesterol | 1.7582 ab | 1.9482 a | 1.6879 b | 1.4013 c | <0.01 |
| **Amino Acid** | | | | | |
| Glycine | 0.0065 | 0.0055 | 0.0064 | 0.0064 | NS |
| Phenylalanine | 0.0013 | 0.0014 | 0.0016 | 0.0013 | NS |
| Tryptophan | 0.0025 | 0.0027 | 0.0029 | 0.0032 | NS |
| **Vitamin and Mineral** | | | | | |
| **Vitamin** | | | | | |
| Nicotinamide (Vitamin B3) | 0.0854 | 0.0408 | 0.0723 | 0.0646 | NS |
| Vitamin E | 0.0013 a | 0.0003 b | 0.0005 b | 0.0007 ab | 0.02 |
| **Mineral** | | | | | |
| Phosphoric acid | 0.8549 bc | 2.7964 a | 1.6968 ab | 0.1509 c | 0.01 |
| **Nucleotide** | | | | | |
| Pyrimidine | 0.0002 | 0.0002 | 0.0004 | 0.0004 | NS |
| **Other small molecules** | | | | | |
| Hydroxy toluene (Cresol) | 0.0220 b | 0.0268 a | 0.0273 a | 0.0211 b | <0.01 |
| Isocarbostyril | 0.0012 ab | 0.0007 b | 0.0014 a | 0.0017 a | 0.01 |
| Phthalate | 0.0495 a | 0.0199 b | 0.0202 b | 0.0589 a | <0.01 |
| Acetamide | 0.0054 a | 0.0029 b | 0.0054 a | 0.0064 a | <0.01 |

P = Plain/Control diet; T = Tannin-rich diet; S = Saponin-rich diet; C = Choice diet.
Values in rows with different letters are significantly different ($P < 0.05$); NS = Not significant different.

Fatty acids in ruminants come from fat or muscle tissues, de novo synthesize in the body, or absorption from the diet with help of rumen bacteria (bio-hydrogenation). Oil, and perhaps excess glucose from beet pulp, fed in this study was able to be stored as fat in the body. Decanoic acid (C10:0), heptadecanoic acid (C17:0), and docosanoic acid (C22:0) were the fatty acid metabolites found significantly different among diets ($P < 0.05$). Tannin and saponin diets significantly reduced C10:0 and C17:0 fatty acids in muscle tissue. Cholesterol levels in muscle were significantly lower in the S diet, compared to the T diet (1.69 versus 1.95 ratio relative to surrogate). There are a large number of studies that also reported the cholesterol lowering activity of saponins in mammals (Guclu-Ustundag & Mazza, 2007; Gurfinkel & Rao, 2003; Kim *et al.*, 2003; Potter *et al.*, 1993; Sidhu & Oakenfull, 1986). Sidhu & Oakenfull (1986) stated that the cholesterol-reducing effect was attributed to the ability of saponins to form insoluble complexes (micelles) with sterols such as cholesterol and bile acids.

There were no differences ($P > 0.05$) among amino acid metabolites found in lamb muscle, as affected by diet treatments. For vitamins and minerals, significant differences were obtained among diet treatments for vitamin E and phosphoric acid. However, there is no obvious explanation for higher α-tocopherol and phosphoric acid ($P < 0.05$) in muscles from lambs fed P and T diets, respectively. However, since both cholesterol and vitamin E are fat-soluble, perhaps saponins formed mixed micelles with both cholesterol and vitamin E, causing lower tissue levels of both cholesterol and vitamin E.

Several other small molecules were also detected in lamb muscle. One interesting marker that was significantly increased ($P < 0.05$) was hydroxytoluene or cresol from animals in the T and S diet treatments. Cresol is an aromatic phenolic compound found in many foods and in wood. Further work is needed to confirm and explain the higher levels of cresol found in animals fed T and S diets in this study. Isocarbostyril is a plant alkaloid with reported anti-tumor properties (Evidente & Kornienko, 2009), which was higher ($P < 0.05$) in S and C diet treatments. Again, further work is needed to confirm and explain this observation. Phthalate levels were significantly different among diets. Phthalate is an environmental contamination metabolite found in plastics and cosmetic products (Hoppin *et al.*, 2002), and also found in the urine of mammals, including humans (Blount *et al.*, 2000). No explanation is apparent for the observation that phthalate levels were lower ($P < 0.05$) in muscle of animals fed T and S diets in this study. Finally, acetamide metabolites were found to be significantly different among diets, and were lower in muscle from lambs fed the T diet. Acetamide was probably a residue of the silylating reagent MSTFA; but it is discussed here because acetamide is also an anti-helminthic drug metabolite (Koch *et al.*, 1979). Thus, acetamide was possibly a metabolite of Albendazole that was applied to the lambs earlier in the experiment.

**Conclusions**

Metabolomics is a powerful tool to measure many of the low molecular weight, water-soluble metabolites in the same sample, which is very useful in life sciences. Using the metabolomics approach, the effects of tannin- and saponin-containing diets versus control (plain) diet in lamb infected with red stomach worm larvae *Haemonchus*

*contortus* could be differentiated by the use of GC/MS combined with multivariate data analysis (PCA). This technique helped us to determine animal feeding behavior, i.e., the type of diet lambs chose to heal themselves when infected with nematode parasites. However, further study with use of more than one metabolomics technique is necessary to verify the results reported here, especially the possible effect of saponins diet to lower lamb muscle cholesterol levels, compared to animals on the tannins diet.

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# CHAPTER 6

# OVERALL SUMMARY

Animal diets affected many chemical characteristics of meat including fat content, color stability, antioxidant status and resistance to lipid oxidation, fatty acid composition, volatile profiles, as well as specific metabolites in muscle tissues. In this study, the main nutritional health benefits of meat obtained from pasture-finished cattle, including grass or legume-based pastures, was its low fat content. Fatty acid composition of pasture-finished beef was improved, with higher MUFA and PUFA ratio as compared to traditional grain-fed beef. But is the pasture-fed beef a good source of PUFA and CLA or not, when compared to other foods? A new aspect of this study was to evaluate PUFA and CLA levels of pasture-fed beef compared to salmon. Salmon and other fatty fishes were found to be very rich PUFA sources. By comparison to salmon, pasture-fed beef is just a moderate source of omega-3 fatty acids (16.7% compared to 366% of recommended level ω-3 fatty acids in an 85 g serving). There is no consensus among nutritionists regarding CLA levels that provide human health benefits. However, one Finnish case-control epidemiological study reported that a CLA level of 132 mg/d was associated with reduced breast cancer in postmenopausal women, compared to 126 mg CLA/d in patients with breast cancer. If 132 mg/d is used as the benchmark CLA level, then pasture- or grain-fed beef in this study provided only 9.77% and 5.98% of recommended CLA levels in an 85 g serving, respectively.

Another new and valuable aspect of this research compared to previous studies was to use principal component analysis to link beef flavor attributes with specific

headspace volatile compounds. Six volatiles were higher in the headspace of heated pasture-fed beef, including dimethyl sulfone, toluene, 3-heptanone, hexadecanoic acid methyl ester, benzaldehyde, and 2-ethyl-1-hexanol, and they were uniquely associated with gamey, barny, bitter, and grassy flavors. In terms of retail sales, pasture-finished beef had a prolonged shelf life (at least 14 d at retail storage conditions) due to resistance to lipid oxidation, and these results were consistent for many experimental trials.

In the second experiment of this study, no strong differences found between the two legume diet treatments, sainfoin-tall fescue or alfalfa tall-fescue, in various meat characteristics (color stability, resistance to browning, resistance to lipid oxidation and antioxidation status, fatty acid composition, and headspace volatile profiles). Nonanoic and decanoic acids were the only 2 out of 45 compounds different between legume diets. Thus, meat quality was similar between the two diet treatments indicating that sainfoin-tall fescue mixtures were comparable to alfalfa-tall fescue mixtures as a pasture-finishing diet before cattle harvest. However, a contributing factor to the similarity in chemical characterization and sensory evaluation of rib steaks in this study was an unexpected problem with live animal grazing management during the finishing period. Animals in the sainfoin pasture treatment probably did not accumulate as much sainfoin-derived tannins due to over-grazing of sainfoin in early summer, resulting in less sainfoin available as forage later in the summer. Therefore, the practical aspects (pasture yield, growth rate) of finishing cattle on sainfoin versus alfalfa pasture needs further study before any firm conclusions can be drawn regarding feasibility of finishing cattle on sainfoin pastures.

In the third experiment of this study, forty metabolites (30 named and 10 unknown) were detected by metabolomics analysis with use of a GC/MS technique to characterize lamb meat as affected by a beet pulp diet containing tannins (T) or saponins (S) when given in single ration or as choice (C) as compared to a plain/control diet (P). This is the first study to use metabolomic techniques to evaluate possible dietary effects on small molecule composition of meat from domestic ruminants. The identified metabolites consisted of carbohydrates (sugar and sugar alcohols), lipids including glycerolipids and cholesterol, amino acids, vitamins and phosphoric acid, and other small molecules such as cresol and phthalate. Fifteen metabolites were found to be significantly different among diet treatments. Cholesterol levels in muscle were significantly lower in the S diet, compared to the T diet. Principal component analysis plot clearly showed a separation pattern of P, T, and S diet treatments. For animals in the C group that had free access to all diets, P and S diets were preferred while the T diet was avoided.

In summary, the effects of ruminant diets on meat characteristic depended on the type and concentration of plant secondary compounds (PSC), especially the levels of PSC contained in the pastures. In feeding experiment 2 (legume pasture-finishing with sainfoin (tannins) or alfalfa (saponins,) the concentration of PSC's were apparently not high enough to affect meat characteristics. However, in experiment 3, where lambs were confinement-fed a beet pulp diet supplemented with purified tannin and saponin extracts, meat characteristics were significantly affected. Between experiments 2 and 3, several factors were different, including species (beef versus lamb), feeding regime (pasture versus confinement), and analysis methods (metabolomics assay in the lamb confinement

feeding study. Hence, further metabolomic studies are recommended for pasture-feeding trials, to better understand effects of plant secondary compounds on meat characteristics and quality.

# APPENDICES

# APPENDIX A

Statistics for Chapter 3

**Table A1** Type 3 tests of fixed effects (ANOVA) for Hunter color measurements (Lightness, L*)

| Effect | Num DF | Den DF | F Value | Pr > F |
|---|---|---|---|---|
| Diet | 1 | 28 | 53.44 | <.0001 |
| Day | 6 | 28 | 0.43 | 0.8494 |
| Diet*Day | 6 | 28 | 0.56 | 0.7602 |

**Table A2** Type 3 tests of fixed effects (ANOVA) for Hunter color measurements (Redness, a*)

| Effect | Num DF | Den DF | F Value | Pr > F |
|---|---|---|---|---|
| Diet | 1 | 28 | 4.43 | 0.0444 |
| Day | 6 | 28 | 4.08 | 0.0046 |
| Diet*Day | 6 | 28 | 0.56 | 0.7583 |

**Table A3** Type 3 tests of fixed effects (ANOVA) for Hunter color measurements (Yellowness, b*)

| Effect | Num DF | Den DF | F Value | Pr > F |
|---|---|---|---|---|
| Diet | 1 | 28 | 22.48 | <.0001 |
| Day | 6 | 28 | 2.24 | 0.0684 |
| Diet*Day | 6 | 28 | 2.44 | 0.0504 |

**Table A4** Differences of least squares means for Hunter color measurements (Lightness, L*)

| Effect | Diet | Day | Diet | Day | Estimate | SE | DF | t Value | Pr > \|t\| | Adj | Adj P |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Diet*Day | grain | 0 | pasture | 0 | 7.4467 | 1.9007 | 28 | 3.92 | 0.0005 | Tukey | 0.0275 |
| Diet*Day | grain | 1 | pasture | 1 | 3.9467 | 1.9007 | 28 | 2.08 | 0.0471 | Tukey | 0.7098 |
| Diet*Day | grain | 2 | pasture | 2 | 6.6900 | 1.9007 | 28 | 3.52 | 0.0015 | Tukey | 0.0684 |
| Diet*Day | grain | 4 | pasture | 4 | 4.8333 | 1.9007 | 28 | 2.54 | 0.0168 | Tukey | 0.4162 |
| Diet*Day | grain | 7 | pasture | 7 | 4.1400 | 1.9007 | 28 | 2.18 | 0.0380 | Tukey | 0.6462 |
| Diet*Day | grain | 12 | pasture | 12 | 5.7900 | 1.9007 | 28 | 3.05 | 0.0050 | Tukey | 0.1803 |

**Table A5** Differences of least squares means for Hunter color measurements (Redness, a*)

| Effect | Diet | Day | Diet | Day | Estimate | SE | DF | t Value | Pr > \|t\| | Adj | Adj P |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Diet*Day | grain | 0 | pasture | 0 | 0.8500 | 1.2882 | 28 | 0.66 | 0.5148 | Tukey | 1.0000 |
| Diet*Day | grain | 1 | pasture | 1 | 3.0167 | 1.2882 | 28 | 2.34 | 0.0265 | Tukey | 0.5409 |
| Diet*Day | grain | 2 | pasture | 2 | 1.2700 | 1.2882 | 28 | 0.99 | 0.3326 | Tukey | 0.9989 |
| Diet*Day | grain | 4 | pasture | 4 | 1.0833 | 1.2882 | 28 | 0.84 | 0.4075 | Tukey | 0.9998 |
| Diet*Day | grain | 7 | pasture | 7 | 0.2933 | 1.2882 | 28 | 0.23 | 0.8215 | Tukey | 1.0000 |
| Diet*Day | grain | 12 | pasture | 12 | 0.2500 | 1.2882 | 28 | 0.19 | 0.8475 | Tukey | 1.0000 |

**Table A6** Differences of least squares means for Hunter color measurements (Yellowness, b*)

| Effect | Diet | Day | Diet | Day | Estimate | SE | DF | t Value | Pr > \|t\| | Adj | Adj P |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Diet*Day | grain | 0 | pasture | 0 | 0.6967 | 0.8400 | 28 | 0.83 | 0.4139 | Tukey | 0.9998 |
| Diet*Day | grain | 1 | pasture | 1 | 4.3967 | 0.8400 | 28 | 5.23 | <.0001 | Tukey | 0.0010 |
| Diet*Day | grain | 2 | pasture | 2 | 1.5267 | 0.8400 | 28 | 1.82 | 0.0799 | Tukey | 0.8505 |
| Diet*Day | grain | 4 | pasture | 4 | 1.1100 | 0.8400 | 28 | 1.32 | 0.1971 | Tukey | 0.9837 |
| Diet*Day | grain | 7 | pasture | 7 | 0.7333 | 0.8400 | 28 | 0.87 | 0.3901 | Tukey | 0.9997 |
| Diet*Day | grain | 12 | pasture | 12 | 0.7867 | 0.8400 | 28 | 0.94 | 0.3570 | Tukey | 0.9993 |

**Table A7** ANOVA for hydrophilic ORAC

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 1 | 89.0222722 | 89.0222722 | 9.75 | 0.0066 |
| Error | 16 | 146.1417778 | 9.1338611 | | |
| Corrected Total | 17 | 235.1640500 | | | |

**Table A8** ANOVA for lipophilic ORAC

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 1 | 1.596089 | 1.596089 | 0.02 | 0.8785 |
| Error | 16 | 1057.986022 | 66.124126 | | |
| Corrected Total | 17 | 1059.582111 | | | |

**Table A9** Type 3 tests of fixed effects (ANOVA) for TBA

| Effect | Num DF | Den DF | F Value | Pr > F |
|---|---|---|---|---|
| Diet | 1 | 28 | 53.44 | <.0001 |
| Day | 6 | 28 | 0.43 | 0.8494 |
| Diet*Day | 6 | 28 | 0.56 | 0.7602 |

**Table A10** Differences of least squares means for TBA

| Effect | Diet | Day | Diet | Day | Estimate | SE | DF | t Value | Pr > \|t\| | Adj | Adj P |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Diet*Day | grain | 0 | pasture | 0 | 7.4467 | 1.9007 | 28 | 3.92 | 0.0005 | Tukey | 0.0275 |
| Diet*Day | grain | 12 | pasture | 12 | 5.7900 | 1.9007 | 28 | 3.05 | 0.0050 | Tukey | 0.1803 |

**Table A11** Statistics for fatty acid composition of muscle from beef fed with grain or pasture diets

| Fatty acid | % Composition | | | | | | P-Value * |
|---|---|---|---|---|---|---|---|
| | Grain #1 | Grain #2 | Grain #3 | Pasture #1 | Pasture #2 | Pasture #3 | |
| Lauric acid (C12:0) | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.0 | 0.3739 |
| Myristic acid (C14:0) | 2.3 | 2.8 | 3.0 | 2.4 | 2.1 | 1.4 | 0.1128 |
| Palmitic acid (C16:0) | 29.1 | 29.0 | 29.4 | 26.4 | 25.3 | 21.5 | 0.0329 * |
| Palmitoleic acid (C16:1) | 3.6 | 4.6 | 5.3 | 3.0 | 4.0 | 3.6 | 0.1666 |
| Stearic acid (C18:0) | 13.7 | 11.2 | 9.4 | 17.7 | 14.1 | 15.2 | 0.0612 |
| Vaccenic acid (C18:1n7t) | 0.1 | 0.1 | 0.1 | 2.9 | 2.6 | 2.5 | <0.0001 * |
| Oleic acid (C18:1n9c) | 38.7 | 43.2 | 39.7 | 26.8 | 31.1 | 30.3 | 0.0042 * |
| Linoleic acid (C18:2n6) | 3.8 | 1.8 | 3.7 | 6.4 | 5.6 | 7.4 | 0.0156 * |
| CLA (C18:2 c9,t11) | 0.1 | 0.1 | 0.1 | 0.5 | 0.6 | 0.7 | 0.0010 * |
| Linolenic acid (C18:3n3) | 0.3 | 0.2 | 0.3 | 2.1 | 1.8 | 2.5 | 0.0008 * |
| Arachidonic acid (C20:4n6) | 2.1 | 0.9 | 2.2 | 3.5 | 3.8 | 4.7 | 0.0148 * |
| Eicosapentaenoic acid (C20:5n3) | 0.2 | 0.1 | 0.2 | 1.2 | 1.2 | 1.9 | 0.0058 * |
| Docosahexaenoic acid (C22:6n3) | 0.0 | 0.0 | 0.1 | 0.2 | 0.3 | 0.5 | 0.0335 * |

* = Significantly different between within diet treatment means in the same row ($P < 0.05$).

**Table A12** Statistics for headspace volatiles of muscle from beef fed with grain or pasture diets

| LRI | Volatile Compounds | Grain #1 | Grain #2 | Grain #3 | Pasture #1 | Pasture #2 | Pasture #3 | P-value[1] |
|---|---|---|---|---|---|---|---|---|
| 707 | 2-Butanone, 3-hydroxy- | 18.00 | 14.74 | 9.66 | 26.45 | 11.27 | 15.10 | 0.5380 |
| 770 | Toluene | 0.25 | 0.16 | 0.21 | 1.26 | 1.49 | 0.58 | 0.0302 * |
| 764 | 1-Pentanol | 0.57 | 0.53 | 0.88 | 0.48 | 0.54 | 0.42 | 0.1954 |
| 789 | Butanoic acid | 0.79 | 0.00 | 0.62 | 0.58 | 0.22 | 0.22 | 0.6535 |
| 787 | Hexanal | 2.36 | 2.19 | 1.76 | 0.78 | 0.79 | 0.45 | 0.0025 * |
| 786 | 2,3-Butanediol | 6.86 | 5.02 | 7.96 | 11.68 | 5.07 | 7.99 | 0.4793 |
| 867 | Hexanoic acid, methyl ester | 0.37 | 0.47 | 0.77 | 0.56 | 0.12 | 0.40 | 0.3723 |
| 871 | 1-Hexanol | 0.21 | 0.31 | 0.38 | 0.33 | 0.34 | 0.35 | 0.4657 |
| 890 | 3-Heptanone | 0.00 | 0.00 | 0.00 | 0.84 | 0.52 | 0.55 | 0.0034 * |
| 898 | Heptanal | 0.36 | 0.38 | 0.52 | 0.34 | 0.49 | 0.27 | 0.5515 |
| 918 | Butyrolactone | 2.53 | 1.22 | 2.82 | 3.21 | 1.61 | 1.40 | 0.8846 |
| 926 | Dimethyl sulfone | 0.45 | 0.09 | 0.71 | 2.00 | 2.63 | 2.12 | 0.0023 * |
| 960 | Benzaldehyde | 0.21 | 0.17 | 0.16 | 0.32 | 0.36 | 0.24 | 0.0301 * |
| 966 | Octanal | 0.57 | 0.77 | 0.94 | 0.73 | 0.75 | 0.42 | 0.4492 |
| 983 | 1-Octen-3-ol | 1.63 | 1.44 | 1.91 | 1.28 | 1.10 | 1.12 | 0.0290 * |
| 993 | 2,3-Octanedione | 0.58 | 0.69 | 0.91 | 0.00 | 0.14 | 0.21 | 0.0061 * |
| 1028 | 1-Hexanol, 2-ethyl- | 0.00 | 0.17 | 0.12 | 85.92 | 26.49 | 50.28 | 0.0350 * |
| 1069 | 1-Octanol | 0.39 | 0.72 | 0.89 | 0.83 | 0.70 | 0.97 | 0.3727 |
| 1103 | Nonanal | 2.15 | 2.42 | 2.89 | 3.14 | 2.60 | 1.46 | 0.8803 |
| 1183 | Octanoic Acid | 0.16 | 0.16 | 0.14 | 0.15 | 0.08 | 0.09 | 0.1107 |
| 1205 | Decanal | 0.17 | 0.08 | 0.11 | 0.07 | 0.11 | 0.04 | 0.2341 |
| 1221 | Undecane, 2,8-dimethyl- | 0.21 | 0.17 | 0.25 | 0.30 | 0.13 | 0.13 | 0.7224 |
| 1651 | 2-Ethylhexyl 2-ethylhexanoate | 0.01 | 2.23 | 0.17 | 0.38 | 0.54 | 0.18 | 0.5781 |
| 1760 | Phenol, 2,6-bis (1,1-dimethylethyl)-4-ethyl- | 0.62 | 0.61 | 0.45 | 0.00 | 0.08 | 0.00 | 0.0010 * |
| 1870 | Hexadecanoic acid, methyl ester | 0.00 | 0.19 | 0.18 | 0.38 | 0.37 | 0.35 | 0.0175 * |

* = Significantly different between diet treatment means in the same row ($P < 0.05$).

# APPENDIX B

Statistics for Chapter 4

**Table B1** Type 3 tests of fixed effects (ANOVA) for Hunter color measurements (Lightness, L*)

| Effect | Num DF | Den DF | F Value | Pr > F |
|---|---|---|---|---|
| Diet | 1 | 70 | 1.88 | 0.1748 |
| Day | 6 | 70 | 4.69 | 0.0005 |
| Diet*Day | 6 | 70 | 1.78 | 0.1161 |

**Table B2** Type 3 tests of fixed effects (ANOVA) for Hunter color measurements (Redness, a*)

| Effect | Num DF | Den DF | F Value | Pr > F |
|---|---|---|---|---|
| Diet | 1 | 70 | 1.80 | 0.1835 |
| Day | 6 | 70 | 9.22 | <.0001 |
| Diet*Day | 6 | 70 | 0.11 | 0.9955 |

**Table B3** Type 3 tests of fixed effects (ANOVA) for Hunter color measurements (Yellowness, b*)

| Effect | Num DF | Den DF | F Value | Pr > F |
|---|---|---|---|---|
| Diet | 1 | 70 | 10.31 | 0.0020 |
| Day | 6 | 70 | 4.56 | 0.0006 |
| Diet*Day | 6 | 70 | 0.31 | 0.9288 |

**Table B4** Differences of least squares means for Hunter color measurements (Lightness, L*)

| Effect | Diet | Day | Diet | Day | Estimate | SE | DF | t Value | Pr > \|t\| | Adj | Adj P |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Diet*Day | alfalfa | 0 | sainfoin | 0 | 5.2667 | 1.5195 | 70 | 3.47 | 0.0009 | Tukey | 0.0511 |
| Diet*Day | alfalfa | 1 | sainfoin | 1 | -0.7067 | 1.5195 | 70 | -0.47 | 0.6433 | Tukey | 1.0000 |
| Diet*Day | alfalfa | 2 | sainfoin | 2 | 0.4883 | 1.5195 | 70 | 0.32 | 0.7489 | Tukey | 1.0000 |
| Diet*Day | alfalfa | 4 | sainfoin | 4 | -0.3917 | 1.5195 | 70 | -0.26 | 0.7973 | Tukey | 1.0000 |
| Diet*Day | alfalfa | 7 | sainfoin | 7 | 0.3283 | 1.5195 | 70 | 0.22 | 0.8296 | Tukey | 1.0000 |
| Diet*Day | alfalfa | 9 | sainfoin | 9 | 0.03667 | 1.5195 | 70 | 0.02 | 0.9808 | Tukey | 1.0000 |
| Diet*Day | alfalfa | 12 | sainfoin | 12 | 0.4900 | 1.5195 | 70 | 0.32 | 0.7481 | Tukey | 1.0000 |

**Table B5** Differences of least squares means for Hunter color measurements (Redness, a*)

| Effect | Diet | Day | Diet | Day | Estimate | SE | DF | t Value | Pr > \|t\| | Adj | Adj P |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Diet*Day | alfalfa | 0 | sainfoin | 0 | -1.1567 | 1.1573 | 70 | -1.00 | 0.3210 | Tukey | 0.9991 |
| Diet*Day | alfalfa | 1 | sainfoin | 1 | -0.6117 | 1.1573 | 70 | -0.53 | 0.5988 | Tukey | 1.0000 |
| Diet*Day | alfalfa | 2 | sainfoin | 2 | -0.5400 | 1.1573 | 70 | -0.47 | 0.6422 | Tukey | 1.0000 |
| Diet*Day | alfalfa | 4 | sainfoin | 4 | -1.0167 | 1.1573 | 70 | -0.88 | 0.3827 | Tukey | 0.9998 |
| Diet*Day | alfalfa | 7 | sainfoin | 7 | -0.3817 | 1.1573 | 70 | -0.33 | 0.7425 | Tukey | 1.0000 |
| Diet*Day | alfalfa | 9 | sainfoin | 9 | -0.1867 | 1.1573 | 70 | -0.16 | 0.8723 | Tukey | 1.0000 |
| Diet*Day | alfalfa | 12 | sainfoin | 12 | -0.2200 | 1.1573 | 70 | -0.19 | 0.8498 | Tukey | 1.0000 |

**Table B6** Differences of least squares means for Hunter color measurements (Yellowness, b*)

| Effect | Diet | Day | Diet | Day | Estimate | SE | DF | t Value | Pr > \|t\| | Adj | Adj P |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Diet*Day | alfalfa | 0 | sainfoin | 0 | -0.9267 | 0.5944 | 70 | -1.56 | 0.1235 | Tukey | 0.9506 |
| Diet*Day | alfalfa | 1 | sainfoin | 1 | -0.5550 | 0.5944 | 70 | -0.93 | 0.3536 | Tukey | 0.9996 |
| Diet*Day | alfalfa | 2 | sainfoin | 2 | -0.2200 | 0.5944 | 70 | -0.37 | 0.7124 | Tukey | 1.0000 |
| Diet*Day | alfalfa | 4 | sainfoin | 4 | -1.0017 | 0.5944 | 70 | -1.69 | 0.0964 | Tukey | 0.9142 |
| Diet*Day | alfalfa | 7 | sainfoin | 7 | -0.5867 | 0.5944 | 70 | -0.99 | 0.3270 | Tukey | 0.9992 |
| Diet*Day | alfalfa | 9 | sainfoin | 9 | -0.5683 | 0.5944 | 70 | -0.96 | 0.3423 | Tukey | 0.9994 |
| Diet*Day | alfalfa | 12 | sainfoin | 12 | -1.1900 | 0.5944 | 70 | -2.00 | 0.0491 | Tukey | 0.7609 |

**Table B7** ANOVA for fat content

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 1 | 4.05081667 | 4.05081667 | 1.79 | 0.2519 |
| Error | 4 | 9.04986667 | 2.26246667 | | |
| Corrected Total | 5 | 13.10068333 | | | |

**Table B8** ANOVA for pH

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 1 | 0.00326667 | 0.00326667 | 0.30 | 0.61 |
| Error | 4 | 0.04393333 | 0.01098333 | | |
| Corrected Total | 5 | 0.04720000 | | | |

**Table B9** ANOVA for MRA

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 1 | 10.32281667 | 10.32281667 | 6.01 | 0.0704 |
| Error | 4 | 6.87466667 | 1.71866667 | | |
| Corrected Total | 5 | 17.19748333 | | | |

**Table B10** Type 3 tests of fixed effects (ANOVA) for TBA

| Effect | Num DF | Den DF | F Value | Pr > F |
|---|---|---|---|---|
| Diet | 1 | 8 | 1.31 | 0.2852 |
| Day | 1 | 8 | 1.41 | 0.2690 |
| Diet*Day | 1 | 8 | 0.01 | 0.0504 |

**Table B11** Differences of least squares means for TBA

| Effect | Diet | Day | Diet | Day | Estimate | SE | DF | t Value | Pr > \|t\| | Adj | Adj P |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Diet*Day | alfalfa | 0 | sainfoin | 0 | -0.08333 | 0.1111 | 8 | -0.75 | 0.4748 | Tukey | 0.8743 |
| Diet*Day | alfalfa | 12 | sainfoin | 12 | -0.09667 | 0.1111 | 8 | -0.87 | 0.4097 | Tukey | 0.8202 |

**Table B12** ANOVA for FRAP

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 1 | 0.00009841 | 0.00009841 | 2.01 | 0.2296 |
| Error | 4 | 0.00019625 | 0.00004906 | | |
| Corrected Total | 5 | 0.00029467 | | | |

**Table B13** Statistics for fatty acid composition of muscle from beef fed with alfalfa or sainfoin diets

| Fatty acid | mg Fatty Acid/ g Meat sample | | | | | | P-value |
|---|---|---|---|---|---|---|---|
| | Alfalfa #1 | Alfalfa #3 | Alfalfa #5 | Sainfoin #1 | Sainfoin #2 | Sainfoin #3 | |
| Lauric acid (C12:0) | 0.0087 | 0.0144 | 0.0257 | 0.0116 | 0.0138 | 0.0030 | 0.3193 |
| Myristic acid (C14:0) | 0.4134 | 0.6887 | 0.8257 | 0.5161 | 0.46448 | 0.1092 | 0.1883 |
| Palmitic acid (C16:0) | 4.9330 | 8.7082 | 8.2725 | 5.6638 | 5.1942 | 1.7802 | 0.1446 |
| Palmitoleic acid (C16:1) | 0.0854 | 0.0998 | 0.1744 | 0.0938 | 0.1279 | 0.0537 | 0.4668 |
| Stearic acid (C18:0) | 3.9510 | 4.9304 | 6.6313 | 5.6776 | 5.2322 | 1.9513 | 0.5653 |
| Vaccenic acid (C18:1n7t) | 1.0303 | 1.2939 | 1.6750 | 1.1408 | 0.9231 | 0.2619 | 0.1601 |
| Oleic acid (C18:1n9c) | 0.0309 | 0.2496 | 0.1588 | 0.2627 | 0.2194 | 0.1432 | 0.4402 |
| Linoleic acid (C18:2n6) | 0.7414 | 0.6538 | 1.0807 | 0.9819 | 0.9450 | 0.7362 | 0.7006 |
| CLA (C18:2 c9,t11) | 0.0043 | 0.0092 | 0.0134 | 0.0053 | 0.0066 | 0.0025 | 0.2234 |
| Linolenic acid (C18:3n3) | 0.0324 | 0.1058 | 0.0395 | 0.0200 | 0.0194 | 0.0079 | 0.1406 |
| Arachidonic acid (C20:4n6) | 0.3180 | 0.1848 | 0.3238 | 0.3592 | 0.3467 | 0.3106 | 0.2550 |
| Eicosapentaenoic acid (C20:5n3) | 0.0653 | 0.0695 | 0.0842 | 0.0749 | 0.0931 | 0.0722 | 0.4628 |
| Docosahexaenoic acid (C22:6n3) | 0.0130 | 0.0108 | 0.0190 | 0.0137 | 0.0135 | 0.0125 | 0.6981 |

**Table B14** Statistics for headspace volatiles of muscle from beef fed with alfalfa or sainfoin diets

| LRI | Volatile Compounds | Alfalfa #1 | Alfalfa #3 | Alfalfa #5 | Sainfoin #2 | Sainfoin #4 | Sainfoin #6 | P-value |
|---|---|---|---|---|---|---|---|---|
| 765.6 | 1-Pentanol | 0.40 | 0.27 | 0.47 | 0.35 | 0.53 | 0.24 | 0.95 |
| 789.7 | Butanoic acid | 0.17 | 0.09 | 0.12 | 0.05 | 0.05 | 0.08 | 0.05 |
| 792.8 | Hexanal | 2.94 | 2.50 | 4.13 | 4.22 | 2.62 | 1.06 | 0.62 |
| 864.0 | 1-Hexanol | 0.25 | 0.21 | 0.36 | 0.26 | 0.37 | 0.12 | 0.81 |
| 885.0 | 2-Heptanone | 0.05 | 0.03 | 0.08 | 0.06 | 0.09 | 0.03 | 0.68 |
| 888.6 | Cyclohexanone | 0.03 | 0.04 | 0.02 | 0.02 | 0.02 | 0.03 | 0.60 |
| 897.6 | Heptanal | 0.87 | 0.73 | 0.86 | 1.19 | 0.78 | 0.16 | 0.72 |
| 911.3 | Dimethyl sulfone | 0.09 | 0.18 | 0.12 | 0.09 | 0.16 | 0.06 | 0.58 |
| 916.1 | Butyrolactone | 0.57 | 0.45 | 1.02 | 0.25 | 0.37 | 0.20 | 0.09 |
| 921.4 | Hexanoic acid,methyl ester | 0.24 | 0.32 | 0.51 | 0.23 | 0.32 | 0.16 | 0.27 |
| 938.7 | 2-Propanol,1-butoxy- | 0.08 | 0.01 | 0.13 | 0.16 | 0.01 | 0.08 | 0.90 |
| 957.9 | Benzaldehyde | 1.35 | 1.25 | 0.90 | 1.73 | 1.63 | 0.96 | 0.38 |
| 969.2 | 1-Heptanol | 0.15 | 0.11 | 0.23 | 0.16 | 0.16 | 0.04 | 0.44 |
| 978.2 | 1-Octen-3-ol | 0.83 | 0.78 | 1.30 | 0.84 | 1.43 | 0.49 | 0.88 |
| 982.5 | n-Caproic acid vinyl ester | 0.20 | 0.10 | 0.41 | 0.20 | 0.22 | 0.06 | 0.51 |
| 1001.0 | Octanal | 1.37 | 0.93 | 1.06 | 1.50 | 1.18 | 0.19 | 0.72 |
| 1015.2 | 4-Cyanocyclohexene | 0.46 | 0.41 | 0.65 | 0.44 | 0.62 | 0.72 | 0.46 |
| 1027.8 | 1-Hexanol, 2-ethyl- | 0.59 | 0.19 | 0.29 | 0.66 | 0.18 | 0.30 | 0.89 |
| 1029.5 | Propane,1-(1,1-dimethylethoxy)-2-methyl- | 0.50 | 0.36 | 0.26 | 0.82 | 0.32 | 0.06 | 0.91 |
| 1041.8 | Benzeneacetaldehyde | 0.13 | 0.18 | 0.13 | 0.20 | 0.13 | 0.18 | 0.45 |
| 1066.4 | 2-Octen-1-ol | 0.09 | 0.07 | 0.23 | 0.07 | 0.18 | 0.03 | 0.59 |
| 1069.7 | 1-Octanol | 0.91 | 0.60 | 0.73 | 0.64 | 0.60 | 0.26 | 0.18 |
| 1103.1 | Nonanal | 4.30 | 3.15 | 4.22 | 4.72 | 4.07 | 1.07 | 0.64 |
| 1109.4 | 2-Heptanone,6-methyl- | 0.04 | 0.04 | 0.10 | 0.07 | 0.00 | 0.04 | 0.44 |
| 1122.0 | Octanoic acid, methyl ester | 0.08 | 0.09 | 0.12 | 0.07 | 0.08 | 0.04 | 0.12 |
| 1167.5 | Octanoic Acid | 0.17 | 0.26 | 0.38 | 0.11 | 0.20 | 0.12 | 0.13 |
| 1205.5 | Decanal | 0.04 | 0.07 | 0.10 | 0.08 | 0.10 | 0.02 | 0.88 |
| 1212.5 | Thiophene, 2,5-dihydro- | 0.01 | 0.00 | 0.05 | 0.04 | 0.02 | 0.12 | 0.30 |
| 1222.0 | Nonanoic acid, methyl ester | 0.03 | 0.00 | 0.02 | 0.02 | 0.01 | 0.02 | 0.75 |
| 1238.0 | 1-Propanol, 2-(2-hydroxypropoxy)- | 1.13 | 0.38 | 1.29 | 3.11 | 0.19 | 0.74 | 0.68 |
| 1250.0 | Benzene, 1,3-bis(1,1-dimethylethyl)- | 0.13 | 0.16 | 0.09 | 0.28 | 0.09 | 0.01 | 0.98 |
| 1263.2 | Nonanoic acid | 0.10 | 0.12 | 0.14 | 0.08 | 0.04 | 0.08 | 0.03 * |
| 1301.4 | 2-Octanamine | 0.02 | 0.01 | 0.01 | 0.01 | 0.00 | 0.00 | 0.10 |
| 1360.2 | n-Decanoic acid | 0.07 | 0.08 | 0.12 | 0.00 | 0.04 | 0.00 | 0.03 * |
| 1374.4 | Propanoic acid, 2-methyl-,butyl ester | 0.01 | 0.01 | 0.00 | 0.01 | 0.01 | 0.01 | 0.49 |
| 1418.7 | Acetamidoacetaldehyde | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.78 |
| 1505.0 | Butylated Hydroxytoluene | 0.13 | 0.09 | 0.10 | 0.13 | 0.12 | 0.13 | 0.17 |
| 1506.1 | Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl esters | 0.01 | 0.00 | 0.01 | 0.01 | 0.01 | 0.01 | 0.51 |
| 1586.7 | Diethyl Phthalate | 0.83 | 0.55 | 0.26 | 0.33 | 0.24 | 0.20 | 0.15 |
| 1723.2 | Methyl tetradecanoate | 0.14 | 0.06 | 0.12 | 0.07 | 0.06 | 0.10 | 0.31 |
| 1843.4 | 2-Hexadecene, 3,7,11,15-tetramethyl- | 0.09 | 0.25 | 0.18 | 0.10 | 0.21 | 0.14 | 0.75 |
| 1859.6 | 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester | 0.46 | 0.30 | 0.09 | 0.09 | 0.08 | 0.07 | 0.13 |
| 1893.5 | Homosalate | 0.11 | 0.09 | 0.12 | 0.05 | 0.20 | 0.18 | 0.48 |
| 1924.3 | Hexadecanoic acid, methyl ester | 0.59 | 0.33 | 0.46 | 0.28 | 0.30 | 0.46 | 0.30 |
| 2095.1 | 9-Octadecenoic acid, methyl ester | 0.28 | 0.05 | 0.19 | 0.12 | 0.09 | 0.20 | 0.65 |

* = Significantly different between diet treatment means in the same row ($P < 0.05$).

# APPENDIX C

## Statistics for Chapter 5

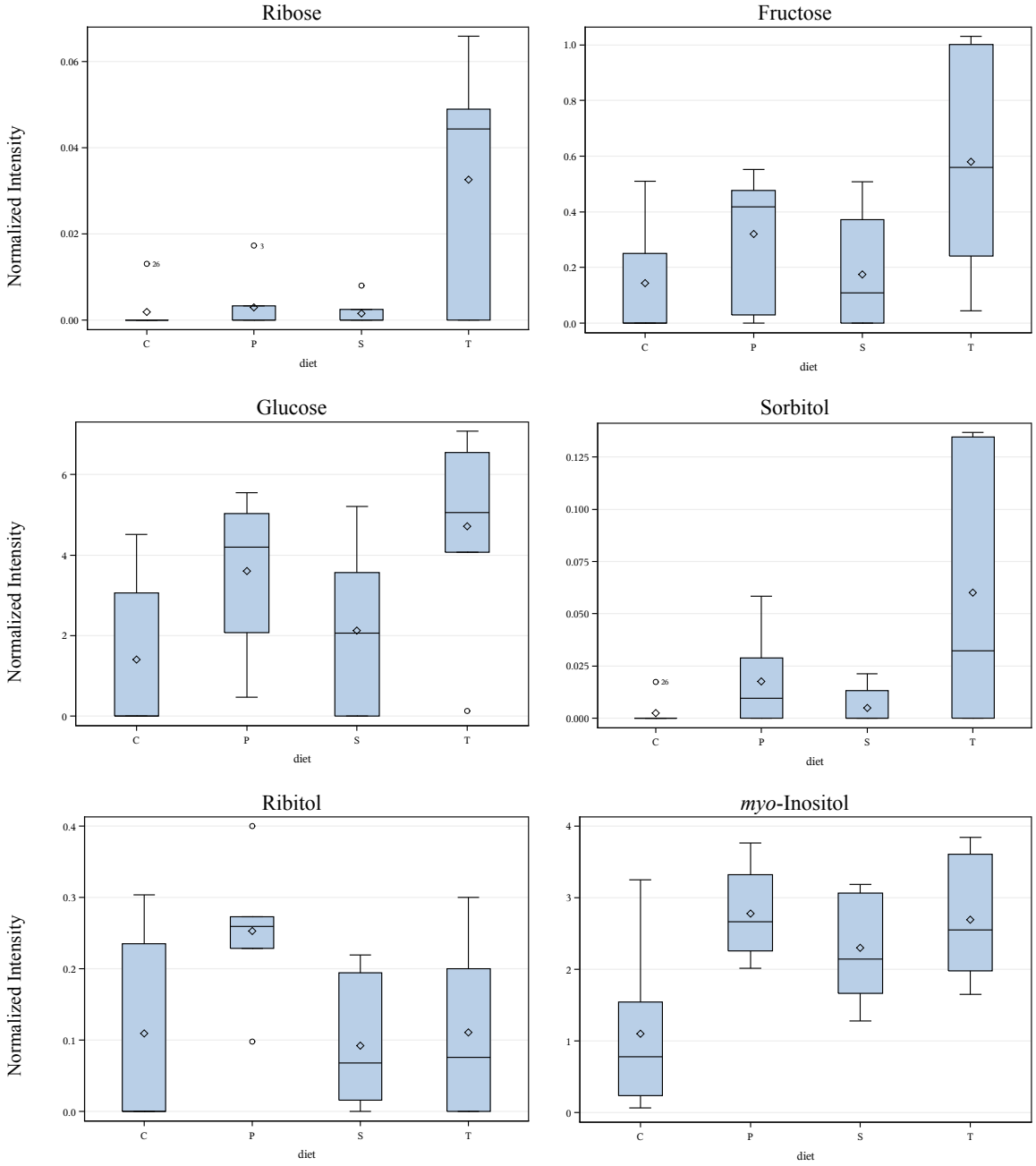


**Figure C1** Box and whisker plots of normalization levels of carbohydrates (sugar and sugar alcohols) of lambs fed different diets. The top and bottom of the box represent the 75$^{th}$ and 25$^{th}$ percentiles. The whiskers indicate the maximum and minimum points. P = Plain/Control diet; T = Tannin-rich diet; S = Saponin-rich diet; C = Choice diet.

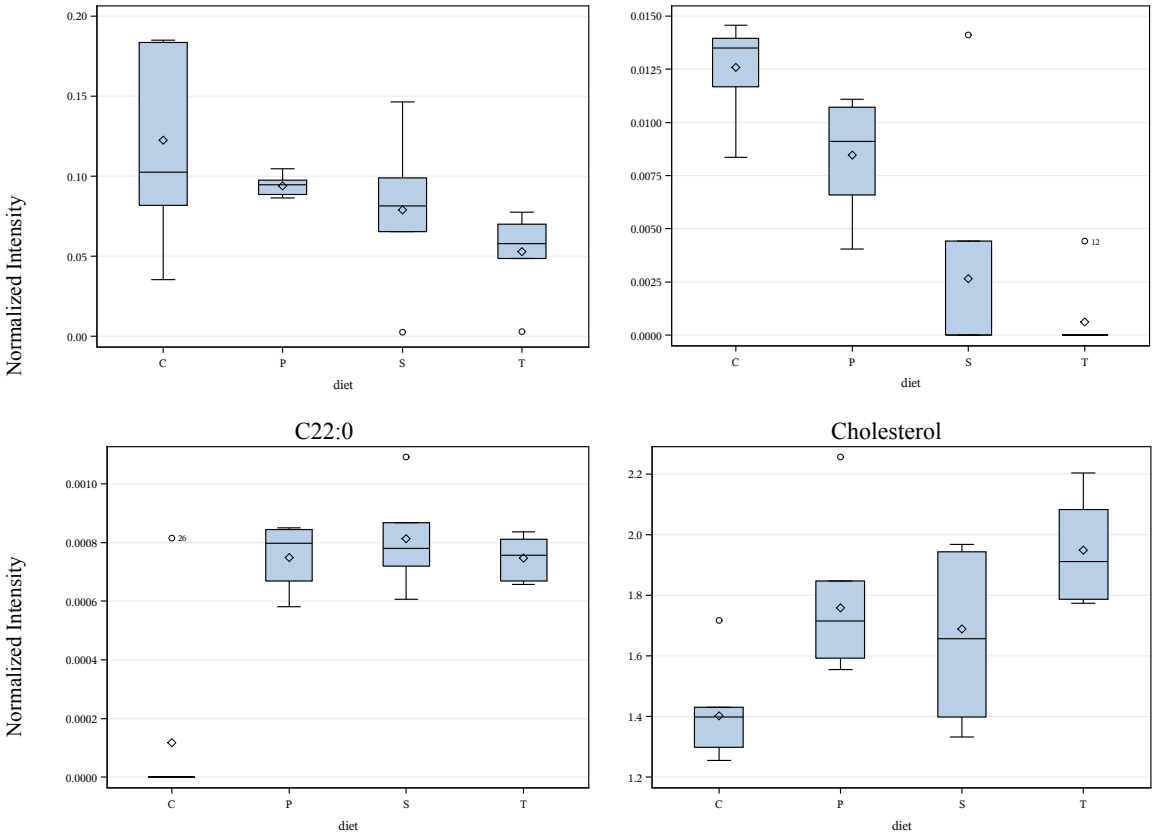


**Figure C2** Box and whisker plots of normalization levels of lipids of lambs fed different diets. The top and bottom of the box represent the 75$^{th}$ and 25$^{th}$ percentiles. The whiskers indicate the maximum and minimum points. P = Plain/Control diet; T = Tannin-rich diet; S = Saponin-rich diet; C = Choice diet.

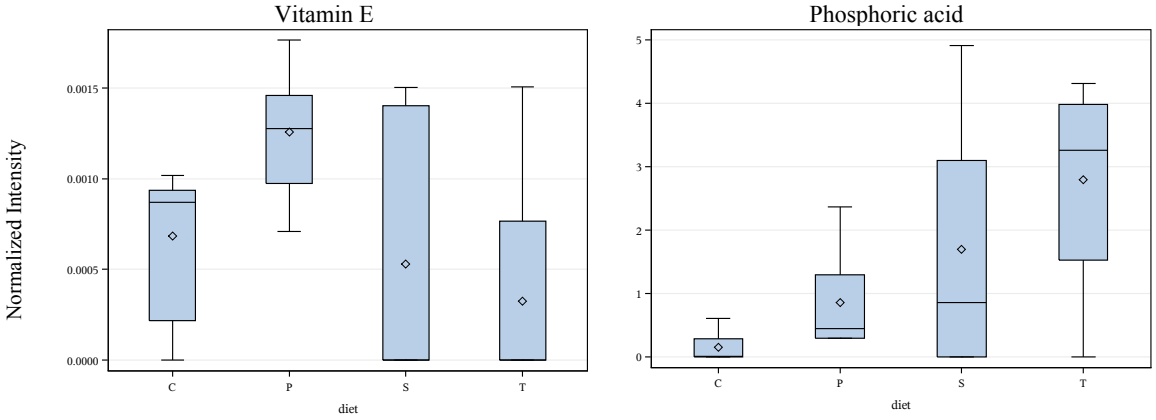


**Figure C3** Box and whisker plots of normalization levels of vitamin and mineral of lambs fed different diets. The top and bottom of the box represent the 75$^{th}$ and 25$^{th}$ percentiles. The whiskers indicate the maximum and minimum points. P = Plain/Control diet; T = Tannin-rich diet; S = Saponin-rich diet; C = Choice diet.

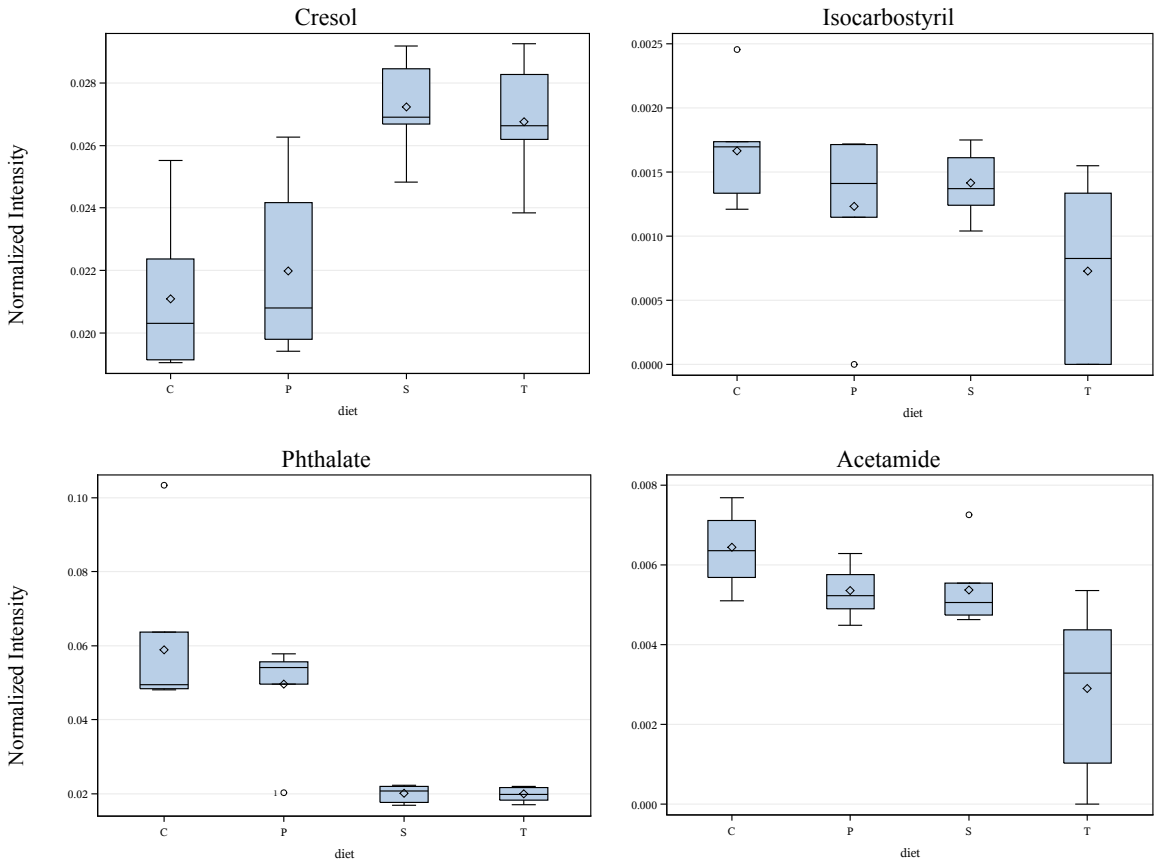


**Figure C4** Box and whisker plots of normalization levels of other small molecules of lambs fed different diets. The top and bottom of the box represent the 75$^{th}$ and 25$^{th}$ percentiles. The whiskers indicate the maximum and minimum points. P = Plain/Control diet; T = Tannin-rich diet; S = Saponin-rich diet; C = Choice diet.

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# CURRICULUM VITAE

ROSSARIN TANSAWAT
(August 2012)

**EDUCATION:**

Ph.D. in Nutrition, Dietetics, and Food Sciences 8/2012
Utah State University, Logan, UT

M.S. in Nutrition and Food Sciences 4/2009
Utah State University, Logan, UT

B.S. in Pharmaceutical Sciences, with honors 3/2005
Chulalongkorn University, Bangkok, Thailand

**RESEARCH INTEREST:**

- Food chemistry, food analysis, metabolomics, nutrition
- Study of the relationship between food chemical characteristics and their nutritional attributes to improve and develop novel functional foods as well as dietary supplements in the future

**EXPERIENCES:**

**Graduate Research Assistant**, Dept. of Nutrition and Food Sciences, USU, Logan, UT, 2006 – 2012

**Teaching Assistant**, Dept. of Nutrition and Food Sciences, USU, Logan, UT, Spring 2012

**Lab Instructor**, Dept. of Nutrition and Food Sciences, USU, Logan, UT, Fall 2008 – 2011

**Medical journal editor**, MIMs, Bangkok, Thailand, 1/2006-7/2006

**Pharmacist**, Boots Ltd., Bangkok, Thailand, 2005 – 2006

**Internship**, Food Chemistry R&D Trainee, Nutrition Division, Dept. of Health, Ministry of Public Health, Bangkok, Thailand, 8/2004; Pharmacist trainee, Vibavadee Hospital, Bangkok, Thailand, 4/2004; Pharmacist trainee, Prachomklao Hospital, Pechburi, Thailand, 5/2004; Healthcare Assistant, Boots Ltd., Bangkok, Thailand, 8/2002 – 2/2003

**CERTIFICATION & AWARDS:**

Thai Professional Pharmacist License (No.19491)

USU Intermountain Graduate Research Symposium Poster competition, Logan, UT

- 1st place (*Chemical characterization of grass- and grain-fed beef related to meat quality and flavor attributes*), 2011
- 3rd place (*Antioxidant status and thiobarbituric acid value of raw turkey muscle from birds fed Camelina meal, a high ω-3 fatty acid supplement*), 2010

Institute of Food Technologists (IFT) Research Poster Competition, Bonneville section, Salt Lake City, UT

- 2nd place (*Decomposition of milk mineral and sodium tripolyphosphate by bacterial growth in ground beef*), 2009

Certificates

- International Teaching Assistance Workshop, USU, Logan, UT, 2010
- Laboratory Safety Initial Training, USU, Logan, UT, 2007

- Australian National Chemistry Quiz Award, Bangkok, Thailand, 1999 – 2000

Gandhi scholarship recipient ($4,000), USU, Logan, UT, 2008

## PUBLICATIONS:

Maughan, C., Tansawat, R., Cornforth, D.P., Ward, R.E. & Martini, S. (2012). Development of a beef flavor lexicon and its application to compare the flavor profile and consumer acceptance of rib steaks from grass- or grain-fed cattle. *Meat Science*, 90:1, 116-121.

Tansawat, R., Maughan, C., Ward, R.E., Martini, S. & Cornforth, D.P. (2012). Chemical characterization of pasture- and grain-fed beef related to meat quality and flavor attributes. *International Journal of Food Science and Technology*, Accepted.

Brogna, D.M.R., Tansawat, R., Cornforth, D.P., Ward, R.E., Vasta, M.V., Luciano, G., Priolo, A. & Villalba, J.J. (2012). Effect of beet pulp diet containing tannin or saponin extract offered singly or together (free choice) on lamb meat quality. *Journal of Agricultural and Food Chemistry*, Submitted.

Maughan, B., Tansawat, R., Maughan, C., Provenza, F.D., Villalbe, J.J., Ward, R.E, & Martini, S. & Cornforth, D.P. (2012). Importance of sainfoin or alfalfa chemical diversity on grazing behavior and meat characteristics. *Journal of Animal Science*, Submitted.

## PRESENTATIONS & ABSTRACTS:

Tansawat, R., Ward, R.E., Martini, S. & Cornforth, D.P. (2012). Sainfoin is equivalent to alfalfa as a beef cattle forage. Intermountain Student Poster Competition, Utah State University, Logan, UT, April 5.

Tansawat, R., Ward, R.E., Martini, S. & Cornforth, D.P. (2011). Chemical characterization of grass- and grain-fed beef related to meat quality and flavor attributes. Reciprocal Meat Conference, American Meat Science Association, Kansas State University, Manhattan, KS, June 19.

Tansawat, R., Ward, R.E., Martini, S. & Cornforth, D.P. (2011). Chemical characterization of grass- and grain-fed beef related to meat quality and flavor attributes. IFT Bonneville Section Suppliers Night Poster Competition, Salt Lake City, UT, April 5.

Tansawat, R., Ward, R.E., Martini, S. & Cornforth, D.P. (2011). Chemical characterization of grass- and grain-fed beef related to meat quality and flavor attributes. Intermountain Student Poster Competition, Utah State University, Logan, UT, March 31. ($1^{st}$ place)

Tansawat, R., Cornforth, D.P., Ward, R.E. & Frame, D.P. (2010). Antioxidant status and thiobarbituric acid value of raw turkey muscle from birds fed Camelina meal, a high ω-3 fatty acid supplement. Institute of Food Technologists National Meeting, Chicago, IL, July 19.

Tansawat, R., Cornforth, D.P., Ward, R.E. & Frame, D.P. (2010). Antioxidant status and thiobarbituric acid value of raw turkey muscle from birds fed Camelina meal, a high ω-3 fatty acid supplement. Intermountain Student Poster Competition, Utah State University, Logan, UT, March 30. ($3^{rd}$ place)

Tansawat, R. & Cornforth, D.P. (2009). Iron binding by milk mineral - A possible antioxidant and anti-microbial mechanism. Reciprocal Meat Conference, American Meat Science Association, University of Arkansas, Rogers, AK, June 23.

Tansawat, R. & Cornforth, D.P. (2009). Decomposition of milk mineral and sodium tripolyphosphate by bacterial growth in ground beef. Institute of Food Technologists National Meeting, Anaheim, CA, June 9.

Tansawat, R. & Cornforth, D.P. (2009). Decomposition of milk mineral and sodium tripolyphosphate by bacterial growth in ground beef. IFT Bonneville Section Suppliers Night Poster Competition, Salt Lake City, UT, April 7. ($2^{nd}$ place)

## TECHNICAL SKILLS:

**Laboratory**: GC-MS/GC-FID based analysis, Spectrophotometer, Fluorometer, Aseptic & basic microbial techniques

**Computer**: Microsoft Offices (Excel, Word, PowerPoint), SAS, SPSS

**Statistics**: Experimental design, ANOVA, ANCOVA, General Linear Models, Regression, Factor analysis

## MEMBERSHIPS & ACTIVITIES:

Utah State University (USU) Alumni Association, 2009 – present

Institute of Food Technologists (IFT), 2007 – present

USU student representative, IFT College Bowl Competition, 2007 – 2009

USU Food Science Club, 2006 – present

Thai Pharmacy Council, 2005 – present