EFFECT OF BREED, TISSUE TYPE, AND TIME OF WEANING

ON FATTY ACID COMPOSITION AND GENE EXPRESSION IN

ANGUS AND CHAROLAIS FINISHING STEERS

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Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
DOCTOR OF PHILOSOPHY
July, 2010

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ACKNOWLEDGMENTS

The following dissertation, while an individual work, benefited from the insights and direction of several people.

First, I would like to thank my advisors, Dr. Clint R. Krehbiel and Dr. Raluca G. Mateescu for believing in me and giving me the wonderful opportunity to work with them. Dr. Krehbiel, without your direction and advice I may not have even made the big move away from home to Stillwater. Thank you so much for taking the chance on me and getting me started down the right path three years ago. I will always be grateful for the many doors you have opened for my future. Dr. Mateescu, thank you for helping make the transition from being at home to living in Stillwater an easy one. The many dinners and kind words of encouragement will not soon be forgotten. The guidance you have both provided throughout my graduate program has helped me to grow both personally and professionally.

Special thanks go to Dr. Jack W. Dillwith and Dr. Udaya E. Desilva for serving as members of my committee. Dr. Dillwith, you have excited me about fatty acid metabolism and I have learned so much from the time I have spent in discussion in your office. Thank you so much for always taking the time to answer my questions and listen, you have been a great mentor. Dr. Desilva, thank you for always being there when I needed help with my data or had questions.

I would also like to thank Dr. Step, a.k.a. “DL”, for being the most positive person I know. There aren’t too many people who are “two-thumbs up, full throttle forward” at 5am. I feel blessed to have met you and I am very appreciative of the time we got to spend together, the skills and lessons you have taught me will be very beneficial in my future endeavors.

I could not have completed my project without two very important people, Robin Madden and Dr. Dan R. Stein, who helped guide me through the technical details of the laboratory work for this project. I cannot thank each of you enough for the countless hours you have spent making my job easier. You have become close friends and advisors.

I was very fortunate to have wonderful co-workers and friends who helped me by providing daily support and input. I am especially thankful to Dr. Ben Holland, you taught me the ropes at Sparks and have provided much needed advice on countless occasions. Casey McMurphy, you were one of the first people I met upon starting at Oklahoma State University. You have become a great friend, thanks isn’t enough for all study sessions, statistics questions, presentations (the list could go on and on) that you have helped me with. I have to also thank Thomas “Tom Tom” Walraven for keeping me up to date on the happenings in politics and sports, always being willing to listen, and especially for playing an important role in getting my now husband to Stillwater. There are too many names to mention all of them here, but to all of my friends who have provided an encouraging word or helped to lighten my load, I am thankful.

I will always be appreciative to Dr. Donald G. Ely, my Master's advisor at the University of Kentucky. Dr. Ely, I would like to thank you for continuing to be a great mentor and friend. I would not be the person I am today without all of your help along the way. Also, I would like to thank Dr. Fred A. Thrift, another professor from the University of Kentucky who has always been there to provide advice, and occasionally take me to Mom's for breakfast when he is town. I am very privileged to have had the opportunity to work with such wonderful educators.

Finally, I would like to thank my family who has supported me in everything I have done. My parents, Terry and Georgia Lunsford, and my brother, Terry Logan, you have been my foundation to lean on throughout life. Mom and Dad, even when you were nervous about your daughter moving to Oklahoma, you supported me unconditionally. You have instilled in me the desire to set goals and you have taught me how to achieve them. The encouragement, support, and many prayers have helped me to succeed. To my husband, Austin, thank you for believing in me. This first year of marriage has been filled with a lot of changes in our lives, I know I wouldn't be where I am today without you by my side every step of the way. I am truly appreciative of all the sacrifices that you have made for our future. Thank you for being so patient and supportive, you have always been there to encourage me to keep going. I would not have been able to achieve my goal of completing this dissertation and earning my Ph.D. without your continual love and support.

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ABBREVIATIONS

AA	arachidonic acid
ADF	acid detergent fiber
ADG	average daily gain
aa-aRNA	aminoallyl-aRNA
aRNA	antisense RNA
BGA	bovine genome assembly
BW	body weight
Ca	calcium
CLA	conjugated linoleic acid
DDGS	distiller's dried grains plus solubles
ddH_2O	double distilled water
DHA	docosahexaenic acid
DM	dry matter
DNA	deoxyribonucleic acid
DP	dressing percentage
EPA	eicosapentaenoic acid
EST	expressed sequence tags

FAMES	fatty acid methyl esters
GFINDer	Genome Functional Integrated Discoverer
GO	gene ontology
GPAP 3.2	GenePix Auto Processor
GPR	GenePix Pro Results
IPA	Ingenuity Pathways Analysis
KEGG	Kyoto Encyclopedia of Genes and Genomes
KPH	kidney, pelvic, and heart fat
LDL	low-density lipoprotein
LM	longissimus muscle
LW	late weaned
mRNA	messenger RNA
MUFA	monounsaturated fatty acids
n-3	omega-3 fatty acids
n-6	omega-6 fatty acids
NCBI	National Center for Biotechnology Information
NDF	neutral detergent fiber
NW	normal weaned
P	phosphorus
PCR	polymerase chain reaction
PUFA	polyunsaturated fatty acids
REA	ribeye area

RNA	ribonucleic acid
RT	room temperature
SDS	sodium dodecyl sulfate
SFA	saturated fatty acids
ST	semitendinosus muscle
TVA	transvaccenic acid
UFA	unsaturated fatty acids
VFA	volatile fatty acids
VLDL	very-low-density lipoprotein
YG	yield grade

CHAPTER I

INTRODUCTION

Beef is a highly nutritious and valued food. Beef is a rich source of high biological protein and many micronutrients including: vitamins A, B_6, B_{12}, D, E, iron, zinc, and selenium (Biesalski, 2005; Williamson et al., 2005). However, beef is perceived as having high fat content with undesirable composition, i.e., high percentage of saturated fatty acids (**SFA**). Per capita consumption of beef has decreased over the last 20 years. According to the Economic Research Service (USDA), from 1970 to 1999, red meat consumption decreased 11% per capita. Similarly, the American Meat Institute reported a 15% decrease in beef consumption from 1980 to 2000. This is in part due to the perception that beef contains high amounts of fat which is rich in SFA, although beef has been shown to contain lower or similar levels of cholesterol relative to other animal protein sources (Feeley et al., 1972). One major factor affecting beef intake has been the nutritional recommendations of dieticians and health professionals. The consumer has been told that it is desirable to decrease the consumption of foods rich in SFA. Often, it is recommended that beef be excluded from the diet because it is rich in SFA

and atherosclerosis and other vascular diseases are positively correlated with SFA intake. Much of the beef Americans consume (43.2%) is eaten as ground beef (NCBA, 2001), ranging from 5 to 30% fat by weight (uncooked product). It should be noted that, in contrast to the negative effect of some SFA to human health, beef has some fatty acids that have beneficial health effects, e.g. polyunsaturated fatty acids (**PUFA**), omega-3 fatty acids (**n-3**), and conjugated linoleic acid (**CLA**).

The challenge of the beef industry is to develop and implement programs aimed at improving healthfulness of beef utilizing existing natural genetic variation in fat composition. Understanding the genetic mechanism responsible for turning undesirable SFA into monounsaturated fatty acids (**MUFA**) and PUFA should lead to identification of deoxyribonucleic acid (**DNA**) markers to be used in marker assisted selection programs. The molecular information generated through studies looking at environmental and genetic interactions could lead to the identification of molecular DNA markers to be incorporated into breeding decisions to enhance the health value of beef by increasing the content of beneficial n-3, PUFA, and CLA and reducing SFA in beef.

The objective of this study was to review the tools available to manipulate the fatty acid profile of beef and to identify the need to understand the underlying genetic mechanisms of fatty acid manipulation in order to improve the healthfulness of beef in the human diet.

RESEARCH OBJECTIVES

1. To identify the phenotypic variations in fatty acid profile of beef due to breed, tissue type, and time of weaning. Differences in the feedlot performance will be compared to the fatty acid profile analysis from the longissimus (more oxidative) and semitendinosus (more glycolytic) muscles from normal and late weaned Angus and Charolais steers.

2. To identify the genetic mechanisms underlying the phenotypic variation in fatty acid composition in beef. Differences in gene expression within longissimus and semitendinosus muscles between Angus and Charolais steers, and differences between muscle types within breed will be assessed.

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CHAPTER II

LITERATURE REVIEW

DIETARY FATS AND HUMAN HEALTH

Consumers are becoming more aware of the relationships between diet and health and this has increased consumer interest in the nutritional value of foods. Beef is higher in fat and is therefore perceived to be associated with an increased risk of a number of health-related problems such as heart disease, stroke, diabetes, and some cancers. However, contrary to popular belief the amount of saturated fatty acids (**SFA**; 38%) in beef is less than the total amount of unsaturated fatty acids (**USF**) in the form of monounsaturated (**MUFA**; 42%) and polyunsaturated (**PUFA**; 3-5%) on a per edible portion basis (Baghurst, 2004; NCBA, 2008). Furthermore, many consumers are unaware that fats are essential to human health. Essential fatty acids are those that cannot be produced in the human body; therefore, they must be obtained from the diet. Essential fats are present in every healthy cell, muscle, nerve, and organ of the body and are necessary for basic functions. Deficiency of essential fatty acids can lead to health problems such as dermatitis,

impaired wound healing, and growth retardation, while adequate intakes of essential fatty acids may protect against heart disease and diabetes (DRI, 2005). It was estimated that 50% of the American population may consume insufficient quantities of essential fatty acids (Hu et al., 1999).

Fat in beef is present as membrane fat (as phospholipid), intermuscular fat (between the muscles), intramuscular fat (marbling), and subcutaneous fat. Marbling is an important meat palatability trait in relation to juiciness, aroma, and tenderness and is the fat depot of most interest in relation to fatty acid composition and human health. It refers to the white flecks or streaks of adipose tissue between the bundles of muscle fibers. Marbling consists of triacylglycerols (source of energy stored in adipocytes) and phospholipids (cell membranes). The total marbling content depends on the amount of triacylglycerols (as the amount of phospholipids is relatively constant) and is dependent on the degree of overall body fatness, breed, and muscle type.

Saturated Fatty Acids

Palmitic acid (C16:0) is a SFA that accounts for about 27% of the fatty acids in beef. There is strong evidence that palmitic acid raises total serum cholesterol concentrations compared with UFA and carbohydrates (Hegsted et al., 1965; Keys et al., 1965; Mattson et al., 1985; Bonanome et al., 1988; Grundy et al., 1988; Grundy et al., 1994). This occurs predominantly by increasing low-density-lipoprotein (**LDL**) cholesterol levels by suppressing the expression of LDL receptors (Spady et al., 1985; Nicolosi et al., 1990). Palmitic acid accounts for most of the cholesterol raising activity

from beef, thereby increasing the risk of atherosclerosis, cardiovascular disease, and stroke (Nicolosi et al., 1998).

Stearic acid (C18:0) is a SFA that accounts for about 18% of the fatty acids in beef. The effect of stearic acid on total cholesterol levels is low and is in no way detrimental to human health (Bonanome et al., 1988; Zock and Katan, 1992; Kris-Etherton et al., 1993; Yu et al., 1995; Judd et al., 2002). It is not clear why stearic acid does not increase serum cholesterol; however, one theory is that it is absorbed so quickly into tissue that the negative effects are not seen (Grundy et al., 1994).

Beef also contains lauric acid (C12:0) and myristic acid (C14:0) that are also responsible for cholesterol raising effects of beef. Lauric and myristic acids seem to have a similar effect on raising LDL cholesterol as palmitic acid (Grundy et al., 1994), and have shown a strong correlation with early heart attack (Kromhout et al., 1995). However, the percentages of lauric acid (less than 1%) and myristic acid (2-3%) in beef are small; therefore, on a practical basis lauric and myristic acids are less of a concern as LDL cholesterol raising fatty acids than palmitic acid.

Monounsaturated Fatty Acids

Oleic acid (C18:1) is the primary MUFA in beef and accounts for about 33% of the fatty acids in beef. Available evidence indicates that while most SFA raise serum cholesterol concentrations, the MUFA oleic acid does not (Denke, 1994). The body synthesizes a large quantity of oleic acid because it has a variety of biological uses (Grundy, 1994). In several studies on the relative carcinogenicity of fatty acids or their

ability to suppress the immune system, oleic acid had the least negative effect (Grundy, 1994). One reason oleic acid may not raise serum cholesterol concentrations is because it is a favored substrate for the liver enzyme, acyl-CoA transferase, that converts cholesterol to an inactive form, cholesterol acyltransferase (Grundy, 1994). Monounsaturated fatty acids do not raise plasma cholesterol and are not typically associated with coronary heart disease (Baghurst, 2004).

Polyunsaturated Fatty Acids

The predominant PUFA are linoleic acid (C18:2n-6; about 3.5%), alpha-linolenic acid (C18:3n-3; 1.5%), arachidonic acid (**AA**; C20:4; about 1%), eicosapentaenoic acid (**EPA**; C20:5; less than 1%), docosanpetaenoic acid (C22:5; less than 1%), and docosahexaenoic acid (C22:6; less than 1%; Enser et al., 1998). The PUFA/SFA (taken as (C18:2 + C18:3) / (C14:0 + C16:0 + C18:0) ratio for beef is typically low at around 0.1 (Dannenberger et al., 2004; Scollan et al., 2001). A healthy diet should consist of one to four times more omega-6 fatty acids (**n-6**) than omega-3 fatty acids (**n-3**; Daley et al., 2010). Several studies have shown that PUFA actually have a negative association with coronary heart disease incidences (Kingsbury et al., 1969; Shekelle et al., 1981; Miettinen et al., 1982; Simon et al., 1995; Hu et al., 1997; Hu et al., 1999).

Omega-6 and omega-3 fatty acids. The n-3 PUFA have been shown to reduce the risk of cardiovascular disease, cancer, type-2 diabetes, and play a critical role in normal brain and vision function (Leaf et al., 2003; Calder, 2004). Omega-6 and n-3 are two main families of essential fatty acids that are important for human health. The n-6

and n-3 cannot be converted from one form to the other. Both have to be present in the diet in a proper balance for good health. The metabolism of these fatty acids in our bodies requires the same enzyme cascades, leading to competition between the two families of fatty acids. Therefore, balance of these two fatty acid families is essential because an excess of one can interfere with the metabolism of the other and alter each of their biological effects (Horrobin and Manku, 1990; Emken, 1995). Linoleic (C18:2n-6) and alpha-linolenic (C18:3n-3) fatty acids are both essential to humans. From these fatty acids, other important long chain fatty acids are produced through metabolism (Figure1.1), such as AA (C20:4n-6), docosahexaenic acid (**DHA**; C22:6n-3), and EPA (C20:5n-3). The DHA and EPA fatty acids work together to regulate blood clotting, immune response, and inflammatory processes. The AA aids in blood vessel constriction and formation of blood clots. Although n-6 (inflammatory) and n-3 (anti-inflammatory) have opposite functions in the body, both are necessary to maintain a healthy balance between excessive bleeding and excessive blood clotting (Clandinin, 2000).

The amount of n-6 consumed has risen with changes in livestock production and human diet, while n-3 consumption has decreased (Grundy et al., 2004). Ideally, intake of n-6 should be no more than 10 times that of n-3 (DRI, 2005). However, some people believe that this ratio should be even lower at 1:4, n-6 to n-3 (Simopoulos, 1999). Today the typical American diet consists of an n-6 intake of 20-30 times that of n-3 intake (Simopoulos, 1999).

Conjugated linoleic acid. Beef is a rich source of conjugated linoleic acid (**CLA**) which has been reported to inhibit cancer cells and limit tumor development (Chin et

al., 1992; Bauman et al., 2001). Conjugated linoleic acid can occur in the rumen as a result of incomplete biohydrogenation of dietary fatty acids or through the action of Δ^9-desaturase enzyme (Figure 1.2; Kepler and Tove, 1967). The PUFA and MUFA are beneficial for human health and there is evidence of beneficial effects of transvaccenic acid (**TVA**). Studies where rats were given supplements of CLA cis-9, trans-11 indicated that TVA (the precursor of CLA in tissue and major *trans* fatty acids in beef) could also add to the beneficial effects of CLA cis-9, trans-11 on cancer (Corl et al., 2003) and atherogenesis (Lock et al., 2005; Valeille et al., 2005). Beef also contains small amounts of the long chain C20/22 PUFA (EPA and DHA) and recent research has demonstrated that red meat is an important source of these fatty acids for man (Howe et al., 2006). Dannenberger et al. (2004) reported 10 isomers of CLA in beef with CLA cis-9, trans-11 representing approximately 70% of the total CLA isomers. The anticarcinogenic and antiatherogenic effects of CLA cis-9, trans-11 and the anti-obesity effects of trans-10, cis-12 have been well documented (Belury, 2002).

It is evident that different fatty acids have different effects on human health and disease prevention. Therefore, to improve the nutritional value and healthfulness of beef the emphasis should be placed on beneficially altering the fatty acid profile and not simply its fat content. Increasing the content of n-3 PUFA and CLA and reducing SFA, particularly C16:0, with the effect of increasing PUFA:SFA and n-3:n-6 ratio.

Trans Fatty Acids

Both MUFA and PUFA can be *trans* fatty acids. In a *trans* fatty acids, the carbon chains are on opposite sides of the double bond forming a straight chain. This is in contrast to the *cis* form where carbon chains are on the same side of the double bond causing fatty acids to bend. *Trans* fatty acids are produced by the process of hydrogenation either by food manufacturers or by the microbes in the rumen. Processed or industrial *trans* fatty acids are used in items such as margarine and processed snack foods. These are the *trans* fatty acids that have been associated with increases in the risk of coronary heart disease by raising the levels of LDL cholesterol and lowering the levels of HDL (Longnecker, 1993; Tzonou et al., 1993; Judd et al., 1994). The trans configuration can also be produced, in smaller amounts, when fat in the rumen is partially biohydrogenated converting *cis* isomers into *trans* isomers. Conjugated linoleic acid is a naturally occurring *trans* fatty acids in beef and milk, which can have beneficial effects on human health as discussed previously. Due to health concerns The Food and Drug Administration requires that all commercial food items be labeled for *trans* fatty acid content. However, they make a distinction between non-conjugated synthetic *trans* fatty acids and naturally occurring *trans* fatty acids, not requiring naturally occurring *trans* fatty acids to be identified.

FAT DEPOSITION IN THE RUMINANT ANIMAL

The rumen is a hydrolytic and reductive environment, lipids ingested by the ruminant animal are extensively metabolized having a major impact on the composition

of fatty acids available for absorption (Byers and Schelling, 1988; Rule, et al., 1995). There are two major processes that affect lipids in the rumen (Figure 1.3), lipolysis causing the hydrolysis of ester linkages and release of free fatty acids and biohydrogenation which reduces the number of double bonds converting UFA to SFA (Jenkins, 1993).

Lipolysis

Hydrolysis of dietary lipids is predominantly due to rumen bacteria with little evidence for a significant role by rumen protozoa and fungi, or salivary lipases (Bauman and Lock, 2006). Lipolysis releases free fatty acids as well as glycerol (Harfoot, 1981). The glycerol from triglycerides, phospholipids, and glycolipids can be fermented to volatile fatty acids (**VFA**) and carbon dioxide. Rumen lipolysis has been characterized in *Anaerovibrio lipolytica* which hydrolyzes triglycerides and *Butyrivibrio fibrisolvens* which hydrolyzes phospholipids and glycolipids (Jenkins, 1993; Harfoot and Hazelwood, 1997).

There are a number of factors that affect the rate and extent of lipolysis by altering the microorganisms in the rumen. Lipolysis is reduced as the dietary level of fat is increased (Beam et al., 2000), so the general recommendation is that total dietary fat should not exceed 6 to 7% of dietary dry matter (Jenkins, 1993; Doreau et al., 1997; NRC, 2001). Unlike the other components of the ruminant diet, fat does not provide an energy source for the microbes and can inhibit digestion of the other nutrients by limiting bacterial activity (Byers and Schelling, 1988). How fat interferes with microbial fermentation is believed to result from either the coating of feed particles preventing

access to the microorganisms or a direct toxic effect on the rumen microorganisms (Jenkins, 1993). Factors such as low rumen pH and ionophores can also alter the rumen microbial population and inhibit lipolysis (Van Nevel and Demeyer, 1995; 1996; Demeyer and Doreau, 1999).

Biohydrogenation

Biohydrogenation of UFA is the second major transformation that dietary lipids can undergo in the rumen. It requires a free fatty acid to proceed and, as a consequence biohydrogenation rates are always lower than those of hydrolysis, and factors that affect hydrolysis also impact biohydrogenation (Bauman et al., 2003). The process of biohydrogenation has likely evolved as a mechanism to protect rumen microorganisms from the toxic effects of UFA (Jenkins, 1993). When fatty acids were added to pure cultures of ruminal bacteria, microbial growth and metabolism were inhibited (Galbraith et al., 1973; Henderson, 1973). Most biohydrogenation occurs in association with the fine food particles and this has been attributed to extracellular enzymes of bacteria either associated with the feed or free in suspension (Harfoot and Hazelwood, 1997). The major substrates are linoleic and linolenic acids and the rate of rumen biohydrogenation of fatty acids is typically faster with increasing unsaturation (Bauman et al., 2003). For most diets linoleic and linolenic acid are hydrogenated to the extent of 70-95% and 85-100%, respectively (Doreau and Ferlay, 1994; Beam at al., 2000). Hardly any fat exits the rumen unaltered by the rumen microbes.

De novo Synthesis

Microbes synthesize fatty acids de novo from carbohydrate precursors. Rumen microorganisms, bacteria as well as protozoa, synthesize a wide range of fatty acids (Byers and Schelling, 1988). However, fatty acids synthesized de novo consist mainly of C18:0 and C16:0 in an approximate ratio of 2:1 (Knight et al., 1978). Demeyer and Doreau (1999) pooled data from five studies and estimated that the microorganisms of the rumen may contribute up to 17% of the lipid flowing from the rumen.

Absorption

The lipid fraction of digesta entering the small intestine has the same composition to that leaving the rumen, thus, there is no significant absorption or modification of lipids in the omasum or abomasum (Noble, 1981). In both ruminants and monogastrics, there is no lipid absorption until the small intestine (Rule et al., 1995). Lipid reaching the small intestine consists of fatty acids from both dietary and microbial origins.

Pancreatic lipase helps degrade fat into free fatty acids. Bile is necessary for fat absorption. In the small intestine, pancreatic lipase helps degrade fat into free fatty acids which then forms an emulsification with bile to form micelles with bile salts (Byers and Schelling, 1988). Micelles allow the fat to be more soluble and can now be absorbed into the intestinal cells. This process is very similar in both ruminants and monogastrics, but in ruminants the acidic environment extends further down the small-

intestine therefore fat absorption occurs in a more acidic environment (Byers and Schelling, 1988).

Lipids are absorbed as micelles (lipids bound with bile salts) into the lymph system (Byers and Schelling, 1988; Davis, 1990). The lymph system packages the lipids into very-low-density lipoproteins (**VLDL**) and chylomicrons which can then be transported in the blood to the liver for further processing (Byers and Schelling, 1988). Ruminants tend to have more VLDL while monogastrics tend to have more chylomicrons, this is due to the difference in fatty acids present in each species. Ruminants will have more SFA acids due to extensive biohydrogenation in the rumen. Also, ruminants will have trans fatty acids which come from de novo synthesis in the rumen by the microbes. The VLDL and chylomicrons can travel in the blood to peripheral tissue for storage (Byers and Schelling, 1988). The fat deposited by ruminant animals is different than the composition fed in the diet due to changes that occurred in the rumen (lipolysis and biohydrogenation) and due to the inclusion of fatty acid synthesized by the microbes (de novo synthesis) that get incorporated into the animal's tissues.

BREED COMPARISONS

No single cattle breed has all attributes necessary to produce beef in all environments and meet the requirements of all markets. Great variation exists between breeds in performance of both productive and adaptive traits. While nutrition has been clearly shown to contribute to the fatty acid profile of subcutaneous fat as well as

marbling, the genetic factors responsible for determining the fatty acid profile of beef are still being defined. Stearoyl-CoA desaturase is the enzyme responsible for the conversion of SFA to MUFA in the tissues of cattle. As previously described, fat is reduced by the microorganisms in the rumen by biohydrogenation; therefore, the fatty acids absorbed by the ruminant animal are largely SFA, primarily stearic and palmitic acids (Kim and Ntambi, 1999). Given the important role of desaturation of MUFA, stearoyl-CoA desaturase was identified as a candidate gene (Enoch et al., 1976).

The Japanese Black cattle breed is known for highly marbled muscling compared to other breeds (Zembayashi et al., 1995). Not only are the Japanese Black cattle more highly marbled, but their fat has a lower melting point indicating a higher percentage of UFA (Yang et al., 1999a). Taniguchi et al. (2004) analyzed the effect of cattle breed on stearoyl-CoA desaturase gene expression between Japanese Black and Holstein cattle. The effects of diet, age, and climate conditions on stearoyl-CoA have been described previously (Cameron et al., 1994; Martin et al., 1999; Yang et al., 1999b; Chung et al., 2000). The MUFA percentage in Japanese Black cattle was higher than that in Holsteins (Taniguchi et al., 2004), and suggest that messenger RNA (**mRNA**) expression level is a contributing factor controlling fatty acid profile.

The Meat Research Corporation conducted a progeny test of 3,250 feedlot steers that had been grain fed for 200 days in Australia. The steers consisted of Shorthorn, Angus, Murray Grey, Polled Hereford, Hereford, and European × British crosses from 237 different sires (Baud et al., 1994). Baud et al. (1994) showed that marbling score was higher for Shorthorn, Angus, and Murray Grey breeds than Hereford, Polled

Herefords, or European × British crosses. Furthermore, breed of sire was shown to influence marbling score (Baud et al., 1994). This study was one of the first showing evidence of genetic variation for marbling within a breed may be as large as the variation that exists between breeds.

HERITABILITY

Genetic variability consists of differences between breeds, differences between animals within breed estimated by heritability, and differences between animals fed different diets or genotype × environment interaction. Knowledge of the genes that control these traits holds promise of contributing to a sustainable and profitable future for the beef industry

Pitchford et al. (2002) evaluated the role of genetics in the control of fatty acid composition of beef, the results of this study indicated that inheritance accounts for up to 45% of the observed variation, in agreement with previously reported medium to high heritabilities for fatty acid composition in beef. For example, myristic acid concentration is normally distributed and has heritability of 0.40 (Pitchford et al., 2002). High heritabilities and genetic correlations reported would indicate that changes in fatty acid composition could be accomplished by direct or indirect selection. For several reasons (e.g., fatty acid composition is not a single trait, measuring fatty acid composition on a large number of animals for breeding value estimation at a reasonable cost is not feasible, etc.) this approach is not available. However, with better understanding of the genetic variability of the biochemical processes determining

differences in fatty acid composition, e.g. genetic basis for differences in elongase and desaturase enzyme activities, a molecular genetics approach for genetic improvement of these traits could be developed and implemented in the future.

NUTRITION

Grain vs. Grass Finished Cattle

The typical ration for finishing beef cattle today is composed of about 85% grain fed for 90 or more days before harvest. Large amounts of grain are fed to attain a choice carcass grade and the grain-fed taste that many customers seem to prefer. Grass-based beef production systems are low input systems that are particularly suitable to meet the demand of meat retailers and consumers for "naturally" produced beef. Studies have described the potential for increasing the contents of n-3 PUFA in beef by grazing on fresh grass or feeding grass silage (French et al., 2000; Scollan et al., 2001; Dannenberger et al., 2007). There is clear evidence for an enhanced portion of n-3 and CLA in meat from grass fed animals compared with meat from animals fed corn silage and concentrate. There is also substantial phenotypic variability among animals fed the same diet. Studies of CLA content in milk fat shows large individual variation within diet (grass-fed vs. concentrate) but also an overlap of the two distributions, indicating a potential genotype x environment interaction.

Due to biohydrogenation of dietary fatty acids by the microorganisms in the rumen, the influence of diet on lipid composition may be to a lesser extent than in monogastrics. Yet, feeding fresh grass or grass silage compared to concentrates can

result in higher concentrations of n-3 PUFA in the triacylglycerol and phospholipid fractions in bovine muscle (Warren et al., 2002; Nuernberg et al., 2005). The dietary concentration and proportion of n-3 PUFA will increase with increasing inclusion of fresh grass and increasing duration of grazing. Despite ruminal biohydrogenation, a proportion of dietary PUFA can bypass the rumen intact and be absorbed and deposited as body fat (Wood and Enser, 1997).

Different Grains and Processing Methods

A study conducted in Western Australia compared marbling in Angus steers fed for 150 days to investigate the effect of either dry rolled or steam flaked grains (Pethick et al., 1997). Of the dry rolled diets, steers fed corn had the highest percentage of marbling (6.86%) followed by barley (5.44%) and sorghum (4.77%). Steam flaking each grain resulted in higher marbling % and presented the same pattern as the dry rolled grains. These results indicated that marbling is derived from glucose absorbed in the small intestine. Corn diets result in higher glucose digestion in the small intestine and steam flaking further increases grain digestibility in the rumen and small intestine. In this study, differences in marbling % were not correlated with subcutaneous fat depth. This could be due to the fact that the different fat depots rely on different substrates. As mentioned glucose is the substrate for marbling while subcutaneous fat relies on acetate as substrate.

Lipid Supplementation

Supplementing ruminant diets with different sources of lipids has also proven effective in altering fatty acid composition of beef. Linseed or linseed oil supplementation can increase the concentration of n-3 PUFA with an associated desirable decrease in the n-6:n-3 PUFA ratio (Scollan et al., 2001). Fish oil is rich in both C18:3 n-3 and C18:2 n-3 PUFA and has the potential to increase concentrations of these fatty acids in beef; however, this is dependent on dietary inclusion rate (Scollan et al., 2001; Noci et al., 2005; Noci et al., 2007). Dietary inclusion of ruminally available fatty acids must be limited due to the negative effects on rumen function. Infusion of linseed oil directly into the small intestine has shown to increase the concentration of C18:3 n-3 PUFA in total lipid from 26.3 to 176.5 mg/100g of muscle and also increased the overall UFA:SFA ratio (Scollan et al. , 2006). Research is ongoing in methods to protect dietary lipids from rumen degradation. Supplementation with protected plant oil supplement markedly improved the UFA:SFA ratio from 0.08 to 0.27 in muscle (Scollan et al., 2003). Interestingly, this increase in UFA:SFA ratio was associated with an increase in PUFA but a decrease in intramuscular fat.

It is evident that nutritional value is important to beef quality and nutrition is a major factor influencing fatty acid composition of beef, whereas nutrition and genetics together affect total fat level. Many dietary modifications have been evaluated but it still seems unclear what the nutritional signals are that stimulate the growth of adipocytes in muscle tissue.

IMPLICATIONS

The challenge of the beef industry is to develop and implement a program aimed at improving fatty acid composition of beef utilizing existing natural genetic variation in fat composition. Understanding the genetic mechanism responsible for turning undesirable SFA into MUFA and PUFA should lead to identification of deoxyribonucleic acid (**DNA**) markers to be used in a marker assisted selection program. The molecular information generated from future studies could lead to the identification of molecular DNA markers to be incorporated into breeding decisions. Identifying DNA markers to predict how an animal will respond to each diet should provide an effective management tool as well as selection of breeding stock best suited for conventional or grass-fed production systems.

One of the greatest selling points of beef is that it provides a superior eating experience/taste over other protein sources. Over and above the eating experience, beef is a nutrient rich product. However, it also is considered to have an unhealthful fatty acid composition. Furthermore, there is substantial variation in the concentration of nutrients known to benefit human health. If beef producers could breed and market cattle that had less SFA, higher CLA, or higher n-3 PUFA content, they could enhance the nutritional or health value of beef. This change in fatty acid composition could increase profit to producers because there are an increasing number of consumers that are willing to pay a premium for beef that consistently has a higher nutritional value. In addition, this nutritionally enhanced beef would increase overall demand for beef and insure continued growth of the beef industry.

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Figure 1.1. Metabolism of the essential fatty acids linoleic acid (LA) and alpha-linolenic acid (ALA) to produce arachidonic acid (AA; C20:4n-6), eicosapentaenoic acid (EPA), and docosahexaenic acid (DHA). Adapted from Simopoulos, 1991.

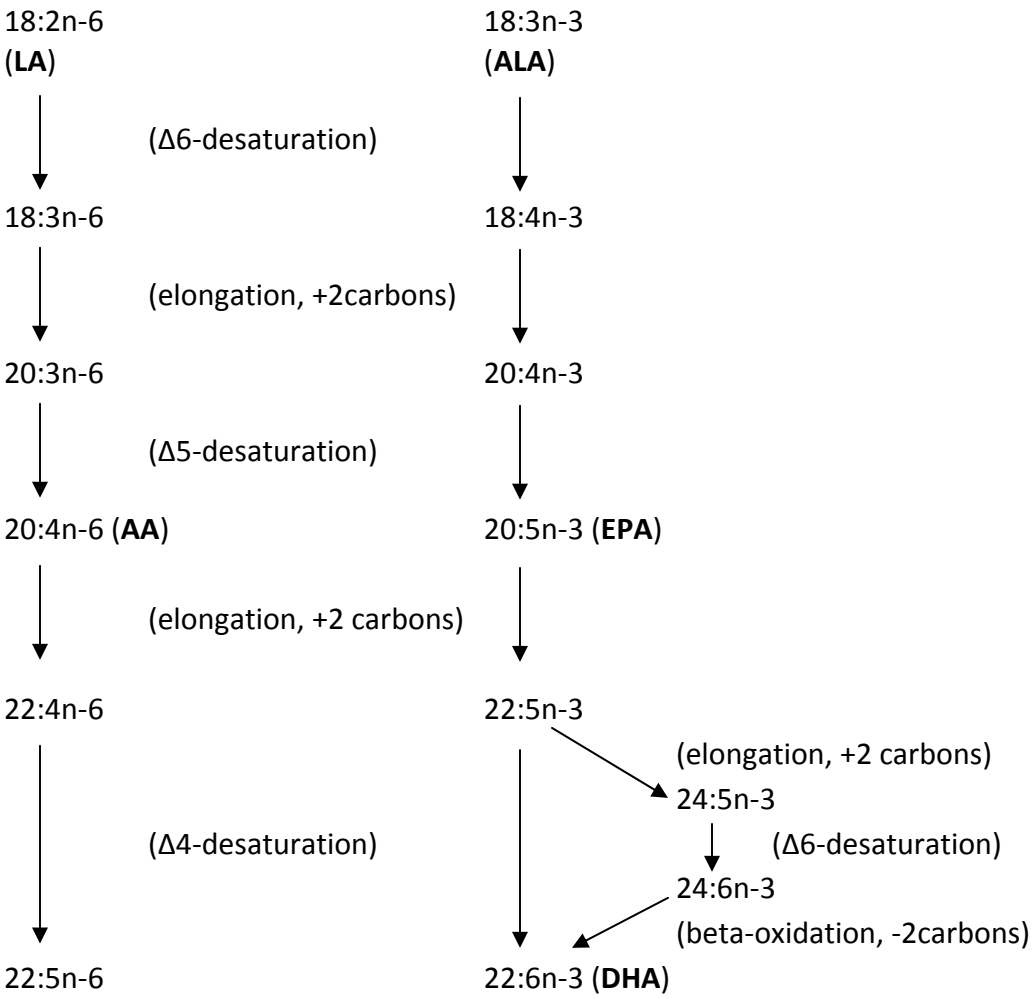


Figure 1.2. Biohydrogenation of linoleic acid (LA) in the rumen and de novo synthesis of conjugated linoleic acid (CLA) in animal tissue. Adapted from Giinari and Bauman, 1999.

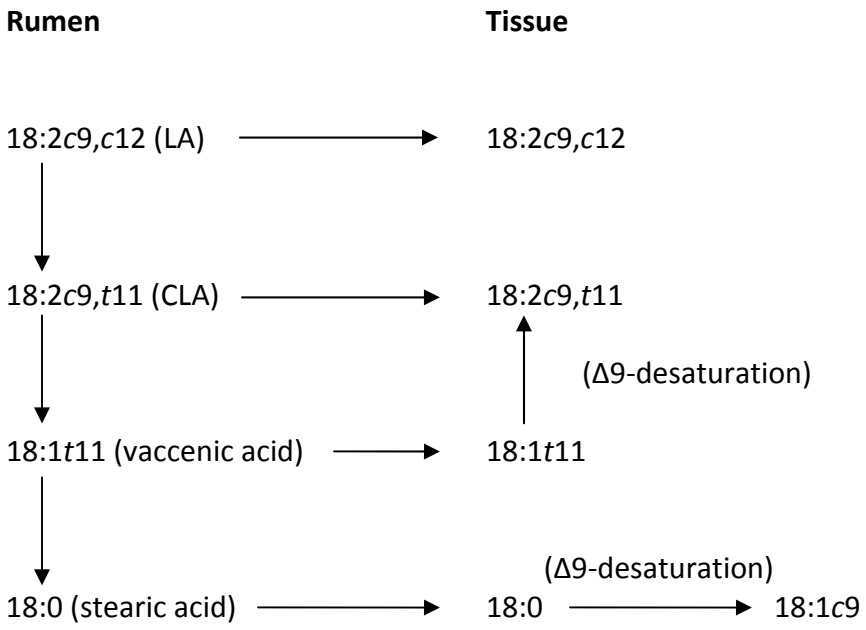
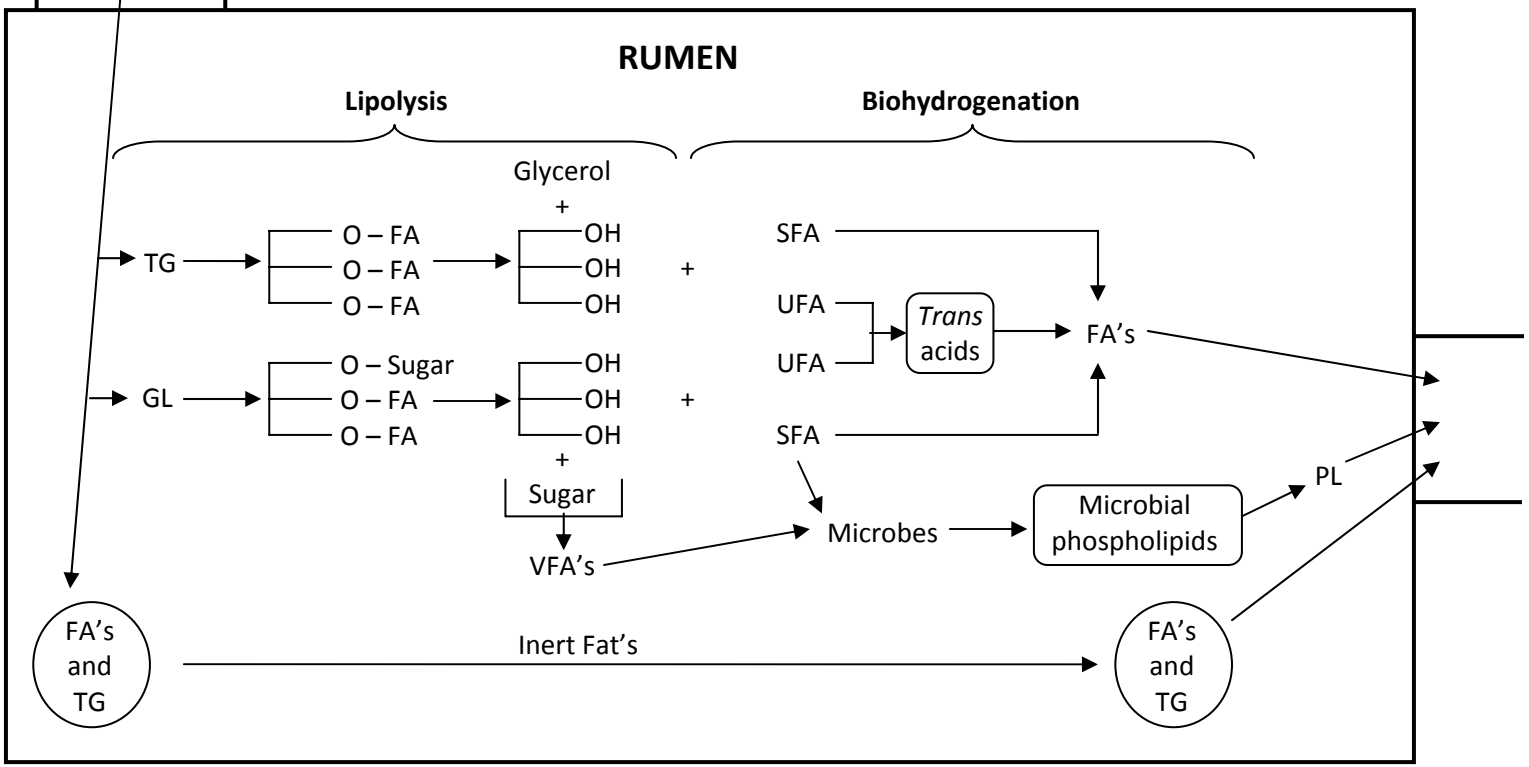


Figure 1.3. Fat digestion in the rumen. Reproduced from Davis (1990). Abbreviations: TG = triglycerides, GL = glycolipids, FA's = Mixture of fatty acids, FA = fatty acid, SFA = saturated fatty acids, UFA = unsaturated fatty acids, *Trans* acids = intermediates in biohydrogenation. Adapted from Bauman et al., 2003.

Fat Source	Cereal Grains	Oil Seeds	Forages	Fat Supplement
Fat Type	TG	TG	GL	FA's and TG



CHAPTER III

EFFECT OF BREED AND TISSUE TYPE ON FATTY ACID COMPOSITION OF LONGISSIMUS AND SEMITENDINOSUS MUSCLES IN NORMAL OR LATE WEANED ANGUS AND CHAROLAIS FINISHING STEERS

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ABSTRACT: Thirty-three steer calves were completely randomized to a 2 × 2 × 2 factorial treatment arrangement, to determine the effect of sire breed (Angus and Charolais), time of weaning (NW = normal weaned at approximately 210 days of age and LW = late weaned at approximately 300 days of age), and tissue type (LM = longissimus muscle, more oxidative and ST = semitendinosus muscle, more glycolytic) on fatty acid composition of beef. Weights were recorded at 28-d intervals to determine animal performance. Biopsies were collected on d 127 and 128 of the finishing phase. All calves were harvested on d 138 and carcass data were collected. Total lipids were

extracted from biopsies, dervitized to their methyl esters, and analyzed using gas chromatography. Angus and Charolais steers had similar BW throughout. LW steers had a heavier ($P \leq 0.05$) initial BW maintained through finishing. Total gain and ADG were greater ($P \leq 0.04$) for Angus than Charolais steers, but did not differ due to weaning. There was no breed x wean interaction effect on feedlot performance. HCW and DP was similar for Angus and Charolais but was greater ($P \leq 0.03$) for LW steers. Angus steers had greater marbling scores ($P = 0.002$), more 12^{th}-rib fat ($P = 0.01$), smaller REA ($P = 0.01$), and greater yield grades ($P = 0.005$) than Charolais steers. There were significant breed × time of weaning × tissue interactions for percent total SFA, UFA, MUFA, PUFA, n-3, and n-6. The n-3:n-6 ratio and percent total CLA differed due to the main effects of time of weaning and tissue. Several individual fatty acids had significant two-way interactions and differences due to time of weaning and tissue; however, there were no differences in fatty acid composition due to breed alone. Beef is a highly nutritious and valued food, providing the essential fatty acids (n-3 and n-6) and several healthy fats (MUFA, PUFA, and CLA) to the human diet. These results provide insight into the challenge of developing and implementing a program to improve the healthfulness of beef utilizing existing variation to manipulate fat composition through management and selection.

Key words: beef, fatty acid, breed, muscle type, weaning

INTRODUCTION

Health professionals recommend a reduction in the overall consumption of saturated fatty acids (**SFA**), while emphasizing the need to increase intake of omega-3 fatty acids (**n-3**; Griel and Kris-Etherton, 2006). Often, it is recommended that beef be excluded from the diet because it is rich in SFA and atherosclerosis and other vascular diseases are positively correlated with SFA intake. However, contrary to popular belief, the amount of SFA (38%) in beef is less than the total amount of unsaturated fatty acids (**UFA**) in the form of monounsaturated (**MUFA**; 42%) and polyunsaturated (**PUFA**; 3-5%) on a per edible portion basis (Baghurst, 2004; NCBA, 2008). In contrast to the negative effect of some SFA to human health, beef has some fatty acids that have beneficial health effects, e.g. PUFA, n-3, and conjugated linoleic acid (**CLA**). Lipids of ruminant origin are among the richest sources of CLA (Chin et al., 1992).

Fat in beef is present as membrane fat (as phospholipid), intermuscular fat (between the muscles), intramuscular fat (marbling), and subcutaneous fat. Beef marbling is an important factor in determining beef quality and carcass value (USDA, 1997). Marbling characteristics are defined by fatty acid composition (Wood et al., 2003; Scollan et al., 2006). Differences in fatty acid composition among breeds and genotypes have been related to carcass fatness; however, De Smet et al. (2004) suggested a possible genetic variation in fatty acid metabolism among breeds that alters fatty acid composition (Harper and Pethick, 2004; Scollan et al., 2006).

The objective of the current study was to identify the phenotypic variations in fatty acid profile of beef due to breed, tissue type, and time of weaning. Differences in

the feedlot performance will be compared to the fatty acid profile analysis from the longissimus (oxidative) and semitendinosus (glycolytic) muscles from normal and late weaned Angus and Charolais steers. If beef producers could breed and market cattle that had less SFA, higher CLA, or higher n-3 PUFA content, they could enhance the nutritional or health value of beef. This change in fatty acid composition could increase profit to producers because increasing the number of consumers that are willing to pay a premium for beef that consistently has a higher nutritional value.

MATERIALS AND METHODS

All research protocols were approved by the Oklahoma State University Institutional Animal Care and Use Committee.

Animals and Diet

Thirty-three steer calves selected from the Oklahoma State University Range Cow Research Center, North Range Unit, approximately 16 km west of Stillwater, OK were used in a completely randomized design with a 2 x 2 factorial arrangement of treatments to evaluate the effects of sire breed and time of weaning on fatty acid composition. Steers are the progeny of predominantly Angus cows bred to one of two sire breeds: Angus or Charolais. Steers were weaned on one of two dates: normal weaned in mid-April at approximately 210 d of age (**NW**) or late weaned in mid-July at approximately 300 d of age (**LW**). Prior to and at weaning, calves were managed according to procedures outlined by Hudson et al. (2010). Post-weaning, all steers were

weighed after a 16-h withdrawal from feed and water, dewormed based on individual body weight (**BW**) with Ivomec Plus® (Merial, Duluth, GA), and implanted with Component E-S with Tylan (200 mg progesterone and 20 mg estradiol benzoate; Vetlife, Overland Park, KS). Steers were then transported to the Oklahoma State University Willard Sparks Beef Research Center (Stillwater, OK), assigned to pens based on arrival BW, and adapted to an 88% concentrate finishing diet (Table 3.1) over an 18-d period. Weights were recorded at 28-d intervals. On d 56, steers were re-implanted with Revalor-S (120 mg trenbolone acetate and 24 mg estradiol benzoate; Intervet/Schering-Plough, Desoto, KS). All steers remained on feed for 138 d before slaughter at Tyson Fresh Meats Inc., Emporia, KS.

Tissue Collection

Biopsies of the longissimus muscle (**LM**) and semitendinosus muscle (**ST**) were collected from all steers on d 127 and d 128 of the finishing phase. The LM sample was collected between the 10^{th} and 13^{th} ribs. Briefly, steers were restrained in a hydraulic squeeze chute, hair was removed from the biopsy site, and a local anesthetic (lidocaine HCL; 20mg/mL; 10mL/biopsy) was administered. Using a scalpel, a 1-cm incision was made and a sterile biopsy needle was used to obtain a LM tissue sample. Semitendinosus muscle was collected according to Snow and Guy (1976). All steers were monitored for swelling during the 24-h post biopsy period.

Fatty Acid Extraction, Derivitization, and Gas Chromatography Analysis

Phenotypic measurements of fatty acids (% composition) were made on thirty-three steers using the chloroform methanol lipid extraction method described by Bligh Dyer (1959). Briefly, tissue samples were homogenized by hand using glass/glass Tenbroeck homogenizers in 3.0 ml of methanol:chloroform (2:1, v:v) solution and lipids were extracted according to Bligh and Dyer (1959). Lipid extracts were stored at -20°C in chloroform containing 0.5% (w/v) butylated hydroxytoluene. Extracted lipids were converted to methyl esters with sodium methylate (0.5 M) to reduce CLA isomerization and boron trifluoride in methanol (14% wt./vol.) to derivitize all free fatty acids, according to the procedures of Nuernberg and others (2002). Fatty acid methyl esters (**FAMES**) were purified on silica gel columns then analyzed by gas chromatography on an Agilent 5890 gas chromatograph with 7673 auto sampler (Agilent Technologies, Wilmington, DE). FAMES were introduced onto a (50% Cyanopropyl)-methylpolysiloxane DB-23 column 30 m x 0.20 mm with a 0.25 µm film thickness (J&W Scientific, Folsom, CA) using a split injector set at 250°C with a 1:25 split ratio. Ultrapure helium was the carrier gas at 1 ml/min and the GC program was as follows: 115°C for 1 min, 20°C/min to 175°C, and 0.8°C/min to 225°C for 3 min for a total run time of 23.50 minutes. A flame ionization detector, operating at 300°C, was used and peak areas were recorded by HP-Chemstation software. Fatty acid methyl esters were identified by comparison of retention times with authentic standards (Nucheck Prep, Inc., Elysian, MN).

Statistical Analysis

Animal performance data were analyzed as a completely randomized design in a 2 × 2 factorial arrangement of treatments (breed = Angus vs. Charolais; time of weaning = NW vs. LW) with the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Steer was considered the experimental unit. The model statement contained fixed effects of breed and time of weaning. The random statement included sire within pen. There were unequal numbers of steers per treatment group (n = 11 for Angus × NW, n = 8 for Angus × LW, n = 6 for Charolais × NW, and n = 8 for Charolais × LW); therefore, the Satterthwaite denominator degrees of freedom were specified. Least squares means were separated using the PDIFF option of SAS. Treatments were determined to be different at $\alpha = 0.05$.

Fatty acid data were analyzed as a completely randomized design in a 2 × 2 × 2 factorial arrangement of treatments (breed = Angus vs. Charolais; time of weaning = NW vs. LW; tissue = LM vs. ST) with the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Individual animal was considered the experimental unit. The model statement contained fixed effects of breed, time of weaning, and tissue. The random statement included sire within pen. There were unequal numbers of steers per treatment group (n = 11 for Angus × NW × LM and Angus × NW × ST; n = 8 for Angus × LW × LM and Angus × LW × ST; n = 6 for Charolais × NW × LM and Charolais × NW × ST; n = 8 for Charolais × LW × LM and Charolais × LW × ST); therefore, the Satterthwaite denominator degrees of freedom were specified. Least squares means were separated using the PDIFF option of SAS. Treatments were determined to be different at $\alpha = 0.05$.

RESULTS

Animal Performance

The effects of breed and time of weaning on feedlot performance and carcass characteristics are shown in Tables 3.2. and 3.3. Animal weights did not differ throughout the finishing period; however, NW steers were lighter than LW steers on d 0 (P = 0.01), 28 (P = 0.01), 56 (P = 0.04), 84 (P = 0.03), and 138 (P = 0.05) of the finishing period. Although BW was not different between breeds, total gain and average daily gain (**ADG**) were greater (P = 0.04) for Angus (269 kg and 1.95 kg/d) versus Charolais (243 kg and 1.76 kg/d) steers regardless of weaning date. Hot carcass weights and dressing percentage (**DP**) were similar (P > 0.10) between breeds but were greater (P = 0.03) for LW versus NW steers. Angus steers had greater marbling scores (482 vs. 408; P = 0.002), 12^{th}-rib fat (1.1 vs. 0.72 cm; P = 0.01), and yield grades (**YG**; 2.94 vs. 2.24; P = 0.05), but smaller ribeye area (REA; 78.4 vs. 86.0 sq. cm; P = 0.01) than Charolais steers. Percentage of kidney, pelvic, and heart fat (**KPH**) did not differ (P > 0.10) due to breed or time of weaning.

Saturated Fatty Acids

The breed × time of weaning × tissue interaction for percent total SFA is shown in Table 3.4 and Figure 3.1. Percent total SFA was similar between all ST treatment groups (Angus × NW × ST, 29.93%; Angus × LW × ST, 30.81%; Charolais × NW × ST, 38.06%; Charolais × LW × ST, 36.99%) regardless of breed or time of weaning. Angus sired steers also had a similar percent SFA within LM treatment groups (Angus × NW × LM, 41.69%

and Angus × LW × LM, 41.56%) regardless of time of weaning. However, Charolais × NW × LM had a lower (42.30 vs. 45.17%; P = 0.02) percent SFA than the Charolais × LW × LM. Overall, percent SFA was higher (P = 0.02) in the LM treatment groups than the ST treatment groups with the exception of the Charolais × NW × LM which was similar to Angus × NW × ST and Angus × LW × ST. Individual FA C10:0, C14:0, C15:0, and C17:0 differed due to the three-way breed × time of weaning × tissue interaction (Table 3.5).

Fatty acid C16:0 differs between treatments due to the two-way interaction of breed × tissue (Figure 3.2). Percent C16:0 higher (P < 0.05) in Angus × LM than in Angus × ST. Neither Angus × LM nor Angus × ST differ from Charolais × LM or Charolais × ST. Percent C12:0 differed due to a time of weaning × tissue interaction (Figure 3.3). The NW × LM had the highest (P < 0.05) percent C12:0, with LW × LM being intermediate, and NW × ST and LW × ST having the lowest percent C12:0.

Percent C18:0 differed by tissue with ST having a higher (P < 0.05) percent C18:0 than LM (Figure 3.4). There was no significant effect on SFA due to the two-way breed × time of weaning interaction or the main effects breed and time of weaning.

Unsaturated Fatty Acids

The breed × time of weaning × tissue interaction for percent total UFA is shown in Table 3.4 and Figure 3.5. Percent total UFA in Charolais × NW × LM differed (P = 0.02) from all other treatment groups. Percent total UFA was similar between all ST treatment groups (Angus × NW × ST, 70.07%; Angus × LW × ST, 69.19%; Charolais × NW × ST, 61.94%; Charolais × LW × ST, 63.01%) regardless of breed or time of weaning.

Angus sired steers also had a similar percent UFA within LM treatment groups (Angus × NW × LM, 58.31% and Angus × LW × LM, 58.44%) regardless of time of weaning. However, the Charolais × NW × LM had a higher (57.70 vs. 54.83%; P = 0.02) percent UFA than the Charolais × LW × LM. In fact, the Charolais × NW × LM also differed from all other treatment groups, having a lower (P = 0.02) percent UFA than Angus × NW × LM, Angus × NW × ST, Angus × LW × LM, Angus × LW × ST, Charolais × NW × ST, and Charolais × LW × ST.

Specific interactions and main effects for individual fatty acids will be discussed in subsequent sections.

Monounsaturated fatty acids. The breed × time of weaning × tissue interaction for percent total MUFA is shown in Table 3.4 and Figure 3.6. Percent total MUFA in the Charolais × NW × LM is similar to Angus × NW × LM and Angus × LW × LM, but lower (P = 0.0002) than Angus × NW × ST, Angus × LW × ST, Charolais × NW × ST, and Charolais × LW × ST, as well as Charolais × LW × LM. Table 3.5 shows the individual fatty acids that differ due to a breed × time of weaning × tissue interaction. The individual MUFA that differ due to the three-way interaction were C17:1, C18:1*c*, C18:1*t*, and C22:1 (Table 3.5). Percent C16:1 differs due to a time of weaning × tissue interaction which is shown in Figure 3.3. The NW × LM had the lowest (P < 0.05) percent C16:1, followed by the LW × LM, and then by NW × ST and LW × ST with the highest percent C16:1. Percent C14:1 differed due to time of weaning with the LW steers having a higher (P < 0.05) percent C14:1 than the NW steers (Figure 3.7). Percent C14:1 also differed between tissues with the ST having a higher percent C14:1 than the LM (Figure 3.4). There were no significant

differences in MUFA due to the two-way interactions breed × time of weaning and breed × tissue or the main effect breed.

Polyunsaturated fatty acids. The breed × time of weaning × tissue interaction for percent total PUFA is shown in Table 3.4 and Figure 3.8. The Charolais × NW × LM had a higher ($P < 0.0001$) percent PUFA than all other treatment groups. The Charolais × LW × LM had a lower ($P < 0.0001$) percent PUFA than the Angus × NW × ST and the Charolais × LW × ST. Three-way interactions for individual PUFA will be discussed in subsequent sections. Percent C18:2*tt* differed due to the two-way interaction of time of weaning × tissue (Figure 3.3). The NW × LM had the lowest ($P < 0.05$) percent C18:2*tt*, with LW × LM intermediate, and NW × ST and LW × ST having the highest (< 0.05) percent C18:2*tt*. Percent C18:2*tt* also differed due to the breed × time of weaning interaction (Figure 3.9). The Angus × NW and Angus × LW had a similar percent C18:2*tt* while the Charolais × NW had a lower ($P < 0.05$) percentage of C18:2*tt* than the Charolais × LW.

Omega-3 fatty acids. The breed × time of weaning × tissue interaction for percent total n-3 is shown in Table 3.4 and Figure 3.10. The Charolais × NW × LM had a similar percent n-3 to both Angus × NW × ST and Charolais × LW × ST, but a higher percent n-3 than all other treatment groups. Two individual n-3 differed due to the three-way breed × time of weaning × tissue interaction (Table 3.5), they were C18:3n-3 and C20:5n-3. There were no two-way interactions or main effects found to be significant ($P < 0.05$) for n-3.

Omega-6 fatty acids. The breed × time of weaning × tissue interaction for percent total omega-6 fatty acids (**n-6**) is shown in Table 3.4 and Figure 3.11. The Charolais ×

NW × LM had a higher (P < 0.0001) percent n-6 than all other treatment groups. Individual fatty acids 18:2n-6, 20:3n-6, and 20:4n-6 differed due to the three-way breed × time of weaning × tissue interaction (Table 3.5). There were no two-way interactions or main effects found to be significant (P < 0.05) for n-6.

Omega-3 to omega-6 ratio. The n-3:n-6 ratio did not differ due to the three-way or any of the two-way interactions, however, n-3:n-6 ratio did differ between treatments due to both time of weaning and tissue main effects (Table 3.6 and Figure 3.12). The n-3:n-6 ratio was beneficially higher in the LW than the NW steers (P = 0.008) and in ST than LM (P < 0.0001). Breed alone did not affect the n-3:n-6 ratio.

Conjugated linoleic acid. Total percent CLA did not differ due to the three-way interaction or any of the two-way interactions. However, there was a significant breed × time of weaning × tissue interaction for percent C18:2*c*9,*t*11 (Table 3.5). The Angus × LW × ST having higher (P = 0.04) percent C18:2*c*9,*t*11 than all other treatment groups. Also, there was a significant breed × time of weaning interaction for percent C18:2*t*10,*c*12, with Angus × NW and Angus × LW being similar, but Charolais × NW having a lower (P < 0.05) percent C18:2*t*10,*c*12 than the Charolais × LW (Figure 3.9). There were no other significant two-way interactions for percent CLA. Total CLA did differ due to both time of weaning and tissue main effects (Table 3.6 and Figure 3.13). Percent CLA was beneficially higher in the LW than the NW steers (P = 0.0006) and in ST than LM (P < 0.0001). Breed alone did not affect percent CLA.

DISCUSSION

Animal Performance

In a typical beef cattle production system, calves are weaned at approximately 205 d of age. However, there may be advantages to weaning calves later when dams are 4 yrs. or older and high-quality forage is available (Hudson et al., 2010). In this study, the LW steers were heavier than the NW steers when entering the feedlot, which was to be expected. Later weaned calves would have had less stress being older at the time of weaning, and had supplemental nutrition from their dam. The weight advantage of the LW steers was maintained throughout the feeding period, which resulted in a heavier HCW and higher DP than the NW steers. According to Hudson et al. (2010) when high-quality forage is available, later weaned calves could be more profitable than NW calves without a negative effect on cow performance and subsequent reproduction. In this study, sire breed rather than weaning had a larger effect on gain and several carcass characteristics. Angus sired steers gained more BW over the feeding period. Typically Charolais cattle will out gain Angus cattle (Peacock et al., 1982) due to their larger frame size and heavier mature BW. However, the calves used in this study are not pure bred and only sire breed and dam breed is not known. Differences in gain could be coming from superior genetics on the dam side, or hybrid vigor even though this is unlikely since all dams were predominantly Angus. The carcasses of the Angus steers had more fat in marbling and at the 12^{th}-rib, while the Charolais steers produced leaner carcasses with larger REA. Angus cattle are earlier maturing than Charolais and therefore it is typical for Angus cattle to deposit more fat,

while Charolais cattle produce more lean. This data shows that the steer breeds follow the trends for Angus and Charolais cattle seen in current literature (Peacock et al., 1982; Coleman et al., 1993; Arango et al., 2002).

Fatty Acid Profile

Breed, time of weaning, and tissue all played a role in altering the fatty acid composition of beef.

Although there were no differences in fatty acid composition attributed to breed alone, there were significant breed × time of weaning × tissue interactions for percent total SFA, UFA, MUFA, PUFA, n-3, and n-6 (Table 3.4). The Angus sired steers had similar SFA, UFA, MUFA, PUFA, n-3, and n-6 between weaning groups, but differences between tissues. A characteristic of skeletal muscle is its diversity, consisting of different kinds of fibers which moreover, vary within themselves (Pette and Staron, 1990). Schreurs (2008) indicated that muscle type accounts for the largest portion of the variation in muscle characteristics. The two skeletal muscles analyzed in this study vary widely in muscle characteristics including type. Jurie et al. (2006) showed that the ST was more glycolytic and the LM was more oxidative. Muscle fiber types are dynamic, constantly changing in proportion as the animal ages, but muscle type is a reflection of the energy requirements of the muscle (Hocquette et al., 1998; Oddy et al., 2001). Because the ST is involved in animal movement, it may have a greater energy requirement than the LM, which result in the ST having a greater proportion of glycolytic enzymatic activity. Literature has also shown that changes in fatty acid composition within muscle are

associated with changes in proportions of muscle fiber types (Jurie et al., 2005; Von Seggern et al., 2005; Alfaia et al., 2007), and it has been well established that the lipid content is higher in oxidative muscle fibers (Enser et al., 1998). Alfaia et al. (2007) demonstrated that the LM had a greater amount of SFA but a lower proportion of UFA than the ST. This agrees with data from this study which showed the Angus × NW × LM and the Angus × LW × LM had higher ($P = 0.02$) percentages of SFA and lower ($P = 0.02$) percentages of UFA than the Angus × NW × ST, Angus × LW × ST, Charolais × NW × ST, and Charolais × LW × ST (Table 3.4). The n-3:n-6 ratio and percent total CLA also differed between tissues (Table 3.6). The LM had a lower n-3:n-6 ratio and a lower percent total CLA than the ST. These data indicate that the different muscle types preferentially metabolize different classes of fatty acids, with the LM depositing more SFA and the ST depositing more UFA.

In Charolais sired steers differences in SFA, UFA, MUFA, PUFA, n-3, and n-6 appear to be due to time of weaning as well as tissue (Table 3.4). Pre- and post-weaning all animals in this trial were managed according to the same herd protocol as described earlier. Calves were only exposed to different environments and nutrition during the 84-d period between weaning dates. Briefly, the NW calves were separated from their dams and moved into a drylot in mid-April and weaned using a fenceline system (Price et al., 2003). During the 10 d weaning period, calves received a 20% crude protein supplement daily at a rate of 1.81 kg/calf and had ad libitum access to Bermuda grass hay and water. On d 11 the NW calves were moved from the drylot to native range pasture at a stocking rate of approximately 1.22 ha/calf. The LW cow-calf pairs and NW

cows remained on pasture until mid-July when LW steers were weaned following the same procedure described for the NW steers. Both weaning groups entered the feedlot following weaning of the LW steers. Both NW and LW steers would have been consuming pasture during the 84 d period between weaning dates, but pasture was likely a smaller portion of total intake for LW than NW steers. According to Lyford (1988) the longer a calf has access to the dam's milk, the less the calf will supplement the diet with other feed. Several studies have shown an increase in UFA, especially the n-3 and n-6 and CLA (Realini et al., 2004; Descalzo et al., 2005; Nuernberg et al., 2005; Ponnampalam et al., 2006; Garcia et al., 2008; Leheska et al., 2008; Alfaia et al., 2009) in grazing cattle. The Charolais × NW × LM had a lower (P = 0.02) percent total SFA (42.30 vs. 45.17%) and MUFA (32.99 vs. 42.67%), but a higher (P = 0.02) percent total UFA (57.70 vs. 54.83%), PUFA (24.71 vs. 11.10%), n-3 (1.18 vs. 0.58%), and n-6 (23.06 vs. 9.80%) than the Charolais × LW × LM. The n-3:n-6 ratio and percent total CLA also differed between weaning groups (Table 3.6). The LW steers had a larger n-3:n-6 ratio and a greater percentage of CLA. This beneficial increase in n-3:n-6 and CLA in the LW steers could be the result of the longer suckling period. Rumen development in calves depends on the levels of milk consumed by the calf with respect to its growth requirements and the availability and consumption of readily digestible feedstuffs (Lyford, 1988). Stewart (1971) found that calves left to nurse and graze with their dam grew faster and had less rumen development than those grazing alone. This explains the heavier weights seen in the LW steers than the NW steers throughout the finishing phase, but may also explain the differences in fatty acid composition due to weaning

date. The LW steers may have less developed rumen in comparison to the NW steers, which would mean that the nutrients, in particular the fatty acids in the dams milk would be passing directly into the abomasum avoiding biohydrogenation in the rumen. In contrast, the fatty acids from pasture consumed by the NW calves would pass through their developed rumen and be biohydrogenated changing the composition from unsaturated to saturated fatty acids being absorbed in the small intestine.

There may have be more variation within the Charolais sired steers due to the smaller marbling to lean ratio in comparison to the Angus sired steers. Because biopsies may contain differing proportions of muscle tissue and marbling, more variation may be expected when the marbling to lean ratio is small as in the case of the Charolais sired steers. However, these are biological differences inherent in the sire breeds. When consumers eat beef from these breeds, they will be consuming marbling and lean in different ratios. Therefore, measuring fatty acid composition of beef in this way may be a better predictor fatty acid composition of beef and its effects on human health.

CONCLUSIONS

These data show that Angus influenced cattle have the potential to deposit a larger marbling to lean ratio (as indicated by higher marbling scores) than Charolais cattle; however, this difference in total fat does not necessarily translate to a difference in fatty acid composition. On the other hand, time of weaning and thus environmental factors (nutrition), as well as tissue play a key role in determining fatty acid composition in beef.

Beef is a highly nutritious protein source providing healthy fatty acids (MUFA, PUFA, n-3, n-6, and CLA). Overall, improvement of the fatty acids we consume in beef can be altered from management decisions made by the producer to meat cut selections by the consumer.

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Table 3.1. Ingredient and nutrient composition of finishing diet (DM basis)

Item	%
Ingredient	
Dry-rolled corn	67.6
Corn DDGS[1]	15.7
Ground alfalfa hay	6.0
Liquid supplement[2]	5.0
Dry supplement[3]	5.7
Nutrient composition[4]	
DM	86.60
CP	13.98
NDF	16.30
ADF	7.63
Ca	0.70
P	0.43

[1] DDGS = distillers dried grains with soluble.

[2] Synergy 19/14 (Westway Feed Products, New Orleans, LA).

[3] Pelleted supplement contained the following (DM basis): 40.35% ground corn, 19.73% wheat middlings, 21.42% limestone, 4.39% dicalcium phosphate, 5.79% salt, 0.05% manganous oxide, 1.14% available zinc 100, 0.07% zinc sulfate, 4.56% potassium chloride, 1.93% magnesium oxide, 0.06% vitamin A (30,000 IU/g), 0.04% vitamin E (50%), 0.31% Rumensin 80 (Elanco Animal Health, Indianapolis, IN), and 0.17% Tylan 40 (Elanco Animal Health, Indianapolis, IN).

[4] All values are from laboratory analyses and are presented on a 100% DM basis (except DM).

Table 3.2. Effect of breed and time of weaning on feedlot performance of beef steers[1]

	Angus		Charolais			P-value[3]		
Item	NW	LW	NW	LW	SEM[1]	B	W	B × W
n	11	8	6	8				
d 0 wt., kg	259	284	281	312	19	0.32	0.01	0.81
d 28 wt., kg	315	342	322	366	19	0.52	0.01	0.48
d 56 wt., kg	368	388	373	408	20	0.61	0.04	0.57
d 84 wt., kg	429	459	425	468	23	0.93	0.03	0.69
d 112 wt., kg	479	498	475	523	25	0.72	0.06	0.38
d 138 wt., kg	532	549	512	570	23	0.98	0.05	0.29
Total Gain, kg	272	266	231	255	14	0.04	0.46	0.23
Total ADG[4], kg/d	1.97	1.93	1.67	1.85	0.10	0.04	0.46	0.23

[1] NW = normal weaned in mid-April at approximately 210 days of age; LW = late weaned in mid-July at approximately 300 days of age.
[2] SEM of the Least squares means.
[3] Observed significance levels for main effects (B = breed; W = time of weaning) and their interaction (B × W = breed × time of weaning).
[4] ADG = average daily gain.

Table 3.3. Effect of breed and time of weaning on carcass characteristics of finishing beef steers[1]

	Angus		Charolais			P-value[3]		
Item[4]	NW	LW	NW	LW	SEM[2]	B	W	B × W
n	11	8	6	8				
HCW, kg	323	344	317	356	16	0.86	0.03	0.48
Dressing %	60.7	62.5	61.8	62.6	0.8	0.55	0.03	0.35
Marbling[5]	450	513	400	415	24	0.002	0.09	0.28
12th-ribfat, cm	1.04	1.16	0.54	0.90	0.15	0.01	0.09	0.40
REA, sq. cm	78.3	78.4	82.4	89.6	3.2	0.01	0.21	0.23
KPH, %	2.71	2.45	2.33	2.27	0.39	0.55	0.62	0.77
YG	2.80	3.08	2.07	2.40	0.25	0.005	0.19	0.90

[1] NW = normal weaned in mid-April at approximately 210 days of age; LW = late weaned in mid-July at approximately 300 days of age.

[2] SEM of the Least squares means.

[3] Observed significance levels for main effects (B = breed; W = time of weaning) and their interaction (B × W = breed × time of weaning).

[4] HCW = hot carcass weight; REA = ribeye area; KPH = kidney, pelvic, and heart fat; YG = yield grade.

[5] Marbling score units: 400=small^{00}; 500=modest^{00}.

Table 3.4. Breed × time of weaning × tissue interaction for fatty acid composition in finishing beef steers [1,2]

	Angus				Charolais					
	NW		LW		NW		LW			P-value[5]
Item[3]	LM	ST	LM	ST	LM	ST	LM	ST	SEM[4]	B × W × T
SFA, %	41.69[a,c,d]	29.93[b,e]	41.56[a,c,d]	30.81[b,e]	42.30[a,b]	38.06[d,e]	45.17[c]	36.99[d,e]	3.03	0.02
UFA, %	58.31[a,d]	70.07[b]	58.44[a,d]	69.19[b]	57.70[c]	61.94[a,b]	54.83[a]	63.01[b,d]	3.03	0.02
MUFA, %	45.58[a,c,e]	53.32[b,d]	46.15[a,c,e]	56.38[b,d]	32.99[a]	48.25[b,e]	43.67[c,d]	47.95[b,e]	4.39	0.0002
PUFA, %	11.41[a,d]	15.42[b]	11.58[a,d]	12.11[a,b]	24.71[c]	13.69[a,b]	11.10[a]	14.99[b,d]	1.49	<.0001
n-3, %	0.57[a]	0.95[b,d]	0.65[a,c,e]	0.82[b,c]	1.18[d]	0.92[b,e]	0.58[a,c]	1.19[d]	0.09	<.0001
n-6, %	10.27[a]	13.36[b]	10.34[a,b]	10.13[a]	23.06[c]	11.88[a,b]	9.80[a,b]	12.74[a,b]	1.39	<.0001
n-3:n-6	0.06	0.08	0.06	0.14	0.05	0.07	0.06	0.09	0.01	0.10
CLA, %	0.28	0.38	0.33	0.63	0.20	0.35	0.34	0.44	0.08	0.08

[a,b,c,d,e] Within a row, least squares means without common superscript differ (P < 0.05).

[1] LM = longissimus muscle; ST = semitendinosus muscle.

[2] NW = normal weaned in mid-April at approximately 210 days of age; LW = late weaned in mid-July at approximately 300 days of age.

[3] SFA = saturated fatty acids (6:0, 8:0, 10:0, 12:0, 13:0, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 22:0, 23:0, and 24:0); UFA = unsaturated fatty acids (MUFA and PUFA); MUFA = monounsaturated fatty acids (12:1, 14:1, 16:1, 17:1, 18:1*c*, 18:1*t*, 20:1, 22:1, and 24:1); PUFA = polyunsaturated fatty acids (18:2*tt*, 18:2n-6, 18:2*c*9,*t*11, 18:2*t*10,*c*12, 18:3n-3, 20:2, 20:3n-6, 20:3n-3, 20:4n-6, 20:5n-3, 22:2n-6, and 22:6n-3); n-3 = omega 3 fatty acids (18:3n-3, 20:3n-3, 20:5n-3, and 22:6n-3); n-6 = omega 6 fatty acids (18:2n-6, 20:3n-6, 20:4n-6, and 22:2n-6); CLA = conjugated linoleic acids (18:2*c*9,t11 and 18:2*t*10,*c*12).

[4] SEM of the least squares means (n = 11 for Angus × NW × LM and Angus × NW × ST; n = 8 for Angus × LW × LM and Angus × LW × ST; n = 6 for Charolais × NW × LM and Charolais × NW × ST; n = 8 for Charolais × LW × LM and Charolais × LW × ST).

[5] Observed significance levels for the 3-way interaction (B × W × T = breed × time of weaning × tissue).

Table 3.5. Breed × time of weaning × tissue interaction for individual fatty acids in finishing beef steers [1,2,3]

	Angus				Charolais					
	NW		LW		NW		LW			P-value[5]
Item, %	LM	ST	LM	ST	LM	ST	LM	ST	SEM[4]	B × W × T
10:0	0.02[a,d]	0.01[b]	0.02[a,c,e]	0.02[a,b,d]	0.01[b,c]	0.01[a,b,c]	0.02[c,d]	0.01[b,e]	0.004	0.02
12:0	0.20	0.05	0.12	0.07	0.24	0.07	0.13	0.07	0.03	0.99
13:0	0.02	ND	ND	ND	0.01	0.01	0.01	ND	-	-
14:0	2.78[a,b]	2.25[b,c]	2.88[a]	3.07[a]	1.83[c]	2.21[b,c]	3.00[a]	2.18[c]	0.25	0.003
14:1	0.70	1.06	0.86	1.31	0.27	0.85	0.71	0.92	0.26	0.08
15:0	0.52[a,c]	0.63[a,c]	0.57[a,c]	0.84[b,d]	0.41[a]	0.57[c,d,e]	0.51[a,c]	0.49[a,e]	0.09	0.01
16:0	21.37	16.10	20.66	15.71	23.81	24.75	25.70	24.35	4.32	0.28
16:1	3.00	4.94	3.36	5.13	2.05	4.97	2.95	4.82	0.79	0.09
17:0	1.42[a]	1.49[a]	1.43[a]	2.22[b]	1.10[a]	1.35[a]	1.29[a]	1.17	0.26	0.02
17:1	0.96[a,d,f]	1.60[b,e]	0.91[a,d,f]	2.43[c]	0.72[a,b]	1.69[c,d,e]	0.74[a]	1.31[e,f]	0.22	0.005
18:0	13.70	8.29	13.92	7.39	13.31	9.07	14.00	8.71	0.67	0.96
18:1*c*	39.07[a,c]	44.60[b]	38.84[a,c]	45.67[a]	28.58[c]	39.26[a,b]	37.19[a,b]	39.51[a,b]	2.67	0.001
18:1*t*	1.59[a,c]	1.00[b]	1.91[a,c]	1.67[a,c]	1.06[a,b]	1.43[a,b]	1.92[c]	1.38[a,b]	0.29	0.004
18:2*tt*	0.22	0.58	0.28	0.50	0.04	0.34	0.23	0.42	0.15	0.78
18:2n-6	7.88[a,b]	9.27[a]	8.19[a,b]	7.15[b]	17.25[c]	8.53[a,b]	7.62[a,b]	8.84[a,b]	1.01	<.0001
18:2*c*9,*t*11	0.26[a,c]	0.31[a,c]	0.31[a,c]	0.57[b]	0.19[a]	0.31[a,c]	0.32[a,c]	0.38[c]	0.07	0.04
18:2*t*10,*c*12	0.02	0.06	0.02	0.05	0.01	0.04	0.02	0.06	0.01	0.24
18:3n-3	0.30[a,d]	0.41[b,c,e]	0.28[a,d]	0.37[b,e]	0.51[c]	0.41[d,e]	0.30[a,b]	0.44[c,e]	0.04	<.0001
20:0	0.02	ND	0.01	ND	0.03	0.01	0.04	ND	-	-
20:1	0.05	ND	0.02	ND	0.02	0.01	0.07	ND	-	-
20:2	0.11[a,c]	0.19[b,d]	0.09[a,c]	0.16[b,d]	0.23[b]	0.19[b]	0.12[c,d]	0.19[b]	0.02	0.01
20:3n-6	0.53[a,d]	0.80[b]	0.43[a]	0.56[a,d]	1.23[c]	0.64[a,b]	0.50[a]	0.73[b,d]	0.10	<.0001
20:3n-3	ND	0.01	ND	0.01	ND	0.01	ND	0.01	-	-
20:4n-6	1.83[a,d]	3.26[b]	1.58[a,d]	2.29[a,d,e]	4.46[c]	2.71[b,d]	1.69[a]	3.19[b,e]	0.39	<.0001

20:5n-3	0.22[a]	0.52[b,d]	0.31[a,c]	0.43[c,d]	0.50[d]	0.51[d]	0.20[a]	0.70[e]	0.07	0.0001
22:1	0.09[a]	0.01[b]	0.11[a]	0.01[b]	0.26[c]	0.01[b]	0.08[a]	0.01[b]	0.01	<.0001
22:2n-6	ND	ND	0.01	ND	0.11	ND	ND	ND	-	-
22:6n-3	0.07	0.03	0.06	ND	0.17	ND	0.08	0.04	-	-
23:0	ND	ND	0.04	0.01	0.19	ND	ND	0.01	-	-
24:0	0.52	ND	0.49	0.05	1.35	ND	0.48	0.02	-	-

[a,b,c,d,e,f] Within a row, least squares means without a common superscript differ ($P < 0.05$).

[1] LM = longissimus muscle; ST = semitendinosus muscle.

[2] NW = normal weaned in mid-April at approximately 210 days of age; LW = late weaned in mid-July at approximately 300 days of age.

[3] ND = Not detectable

[4] SEM of the least squares means (n = 11 for Angus × NW × LM and Angus × NW × ST; n = 8 for Angus × LW × LM and Angus × LW × ST; n = 6 for Charolais × NW × LM and Charolais × NW × ST; n = 8 for Charolais × LW × LM and Charolais × LW × ST).

[5] Observed significance levels for the 3-way interaction (B × W × T = breed × time of weaning × tissue).

Table 3.6. Time of weaning and tissue main effects for fatty acid composition in finishing beef steers

	Time of weaning[2]			Tissue[4]			P-value[6]	
Item[1]	NW	LW	SEM[3]	LM	ST	SEM[5]	W	T
n-3:n-6	0.07	0.09	0.006	0.06	0.09	0.006	0.008	<.0001
CLA, %	0.30	0.43	0.04	0.29	0.44	0.04	0.0006	<.0001

[1] n-3 = omega 3 fatty acids (18:3n-3, 20:3n-3, 20:5n-3, and 22:6n-3); n-6 = omega 6 fatty acids (18:2n-6, 20:3n-6, 20:4n-6, and 22:2n-6); CLA = conjugated linoleic acids (18:2*c*9,t11 and 18:2*t*10,*c*12).

[2] NW = normal weaned in mid-April at approximately 210 days of age; LW = late weaned in mid-July at approximately 300 days of age.

[3] SEM of the least squares means (n = 17 for NW; n = 16 for LW).

[4] LM = longissimus muscle; ST = semitendinosus muscle.

[5] SEM of the least squares means (n = 33 for LM and ST).

[6] Observed significance levels for the main effects (T = tissue; W = time of weaning).

Figure 3.1. Breed × time of weaning × tissue interaction for percentage of saturated fatty acids (SFA) from longissimus muscle (LM) and semitendinosus muscle (ST) from normal weaned (NW) and late weaned (LW) Angus and Charolais finishing steers (n = 11 for Angus × NW × LM and Angus × NW × ST; n = 8 for Angus × LW × LM and Angus × LW × ST; n = 6 for Charolais × NW × LM and Charolais × NW × ST; n = 8 for Charolais × LW × LM and Charolais × LW × ST). ^{a,b,c,d,e} Least squares means without a common superscript differ ($P < 0.05$; SEM = 3.03).

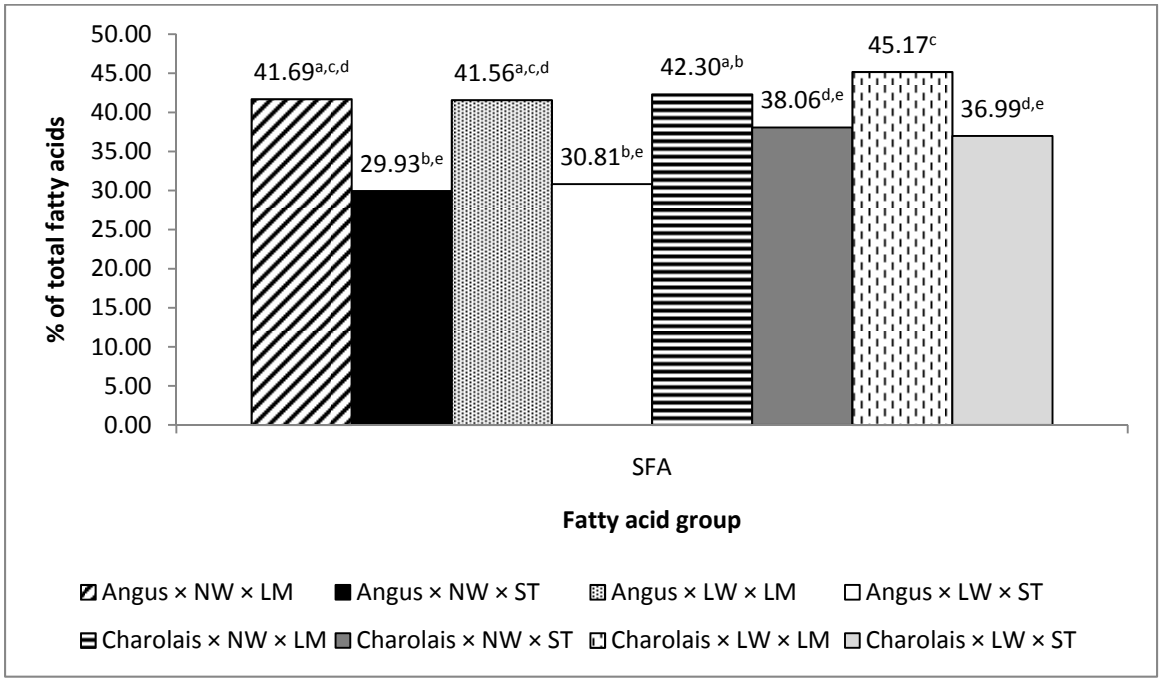


Figure 3.2. Breed × tissue interaction for percentage of C16:0 fatty acid in longissimus muscle (LM) and semitendinosus muscle (ST) of Angus and Charolais finishing steers (n = 19 for Angus x LM and Angus x ST; n = 14 for Charolais × LM and Charolais × ST). [a,b] Least squares means without a common superscript differ (P < 0.05; SEM = 3.79).

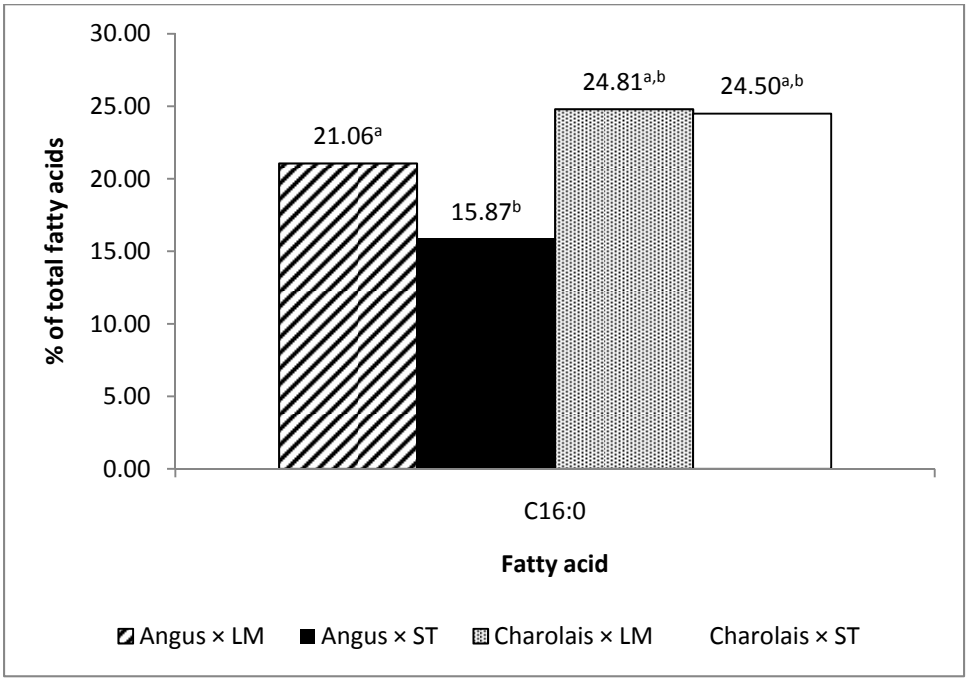


Figure 3.3. Time of weaning × tissue interaction for percentage of C12:0, C16:1, and C18:2*tt* in longissimus muscle (LM) and semitendinosus muscle (ST) from normal weaned (NW; mid-April at approximately 210 days of age) and late weaned (LW; mid-July at approximately 300 days of age) finishing beef steers (n = 17 for NW × LM and NW × ST; n = 14 for LW × LM and LW × ST). ^{a,b,c} Least squares means for C12:0 without a common superscript differ (P < 0.05; SEM = 0.02). ^{l,m,n} Least squares means for C16:1 without a common superscript differ (P < 0.05; SEM = 0.48). ^{x,y,z} Least squares means for C18:2*tt* without a common superscript differ (P < 0.05; SEM = 0.09).

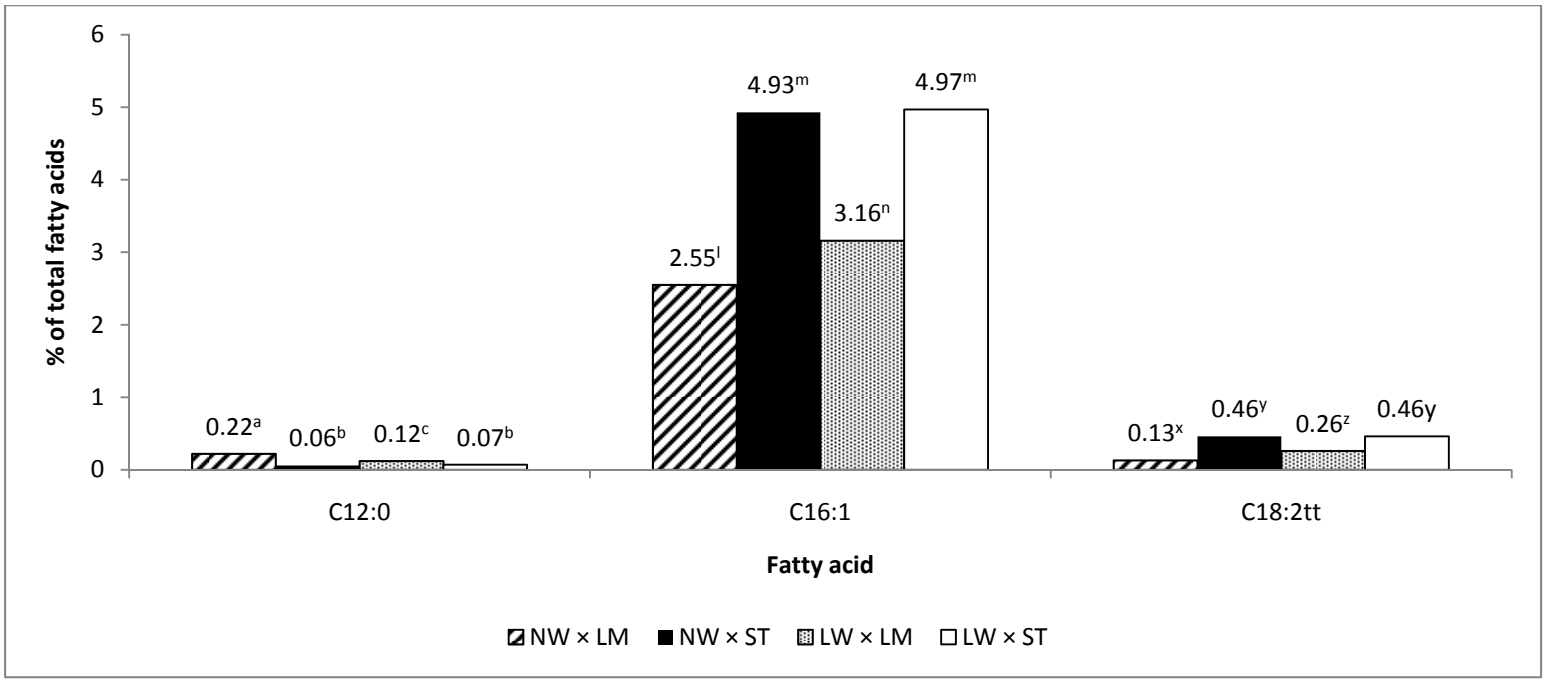


Figure 3.4. Tissue main effect for percentage of C14:1 and C18:0 in longissimus muscle (LM) and semitendinosus muscle (ST) from finishing beef steers (n = 33 for LM and ST). [a,b] Least squares means for C14:1 without a common superscript differ (P < 0.05; SEM = 0.13). [x,y] Least squares means for C18:0 without a common superscript differ (P < 0.05; SEM = 0.35).

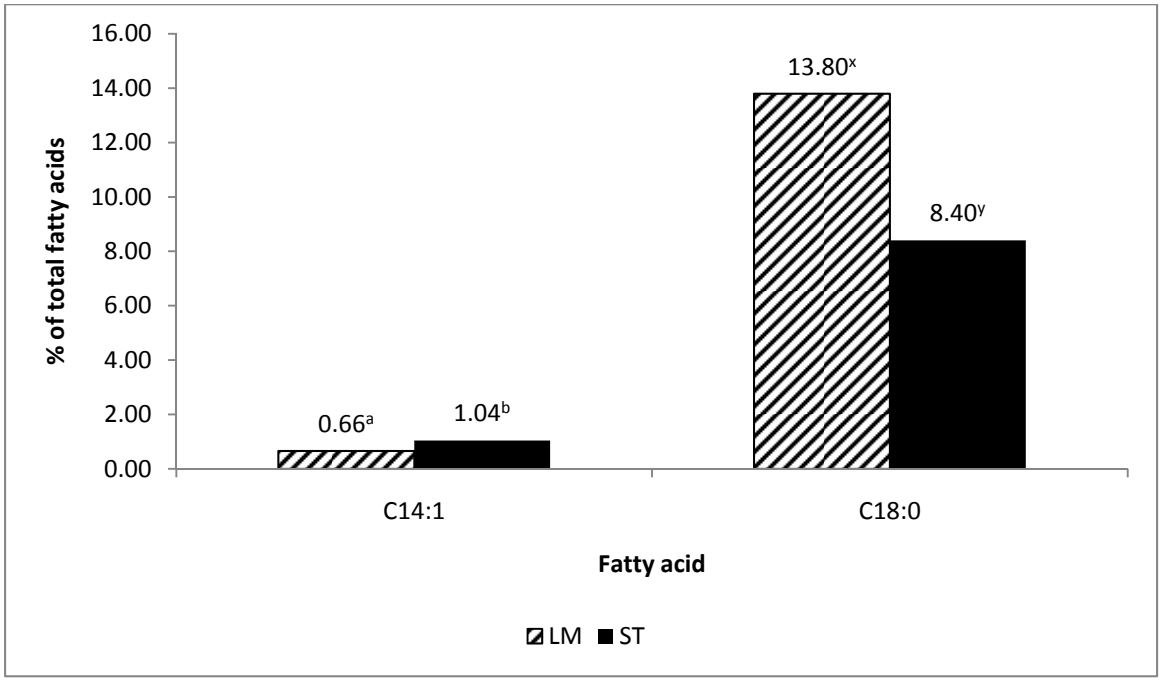


Figure 3.5. Breed × time of weaning × tissue interaction for percentage of unsaturated fatty acids (UFA) from longissimus muscle (LM) and semitendinosus muscle (ST) from normal weaned (NW) and late weaned (LW) Angus and Charolais finishing steers (n = 11 for Angus × NW × LM and Angus × NW × ST; n = 8 for Angus × LW × LM and Angus × LW × ST; n = 6 for Charolais × NW × LM and Charolais × NW × ST; n = 8 for Charolais × LW × LM and Charolais × LW × ST). [a,b,c,d] Least squares means without a common superscript differ ($P < 0.05$; SEM = 3.03).

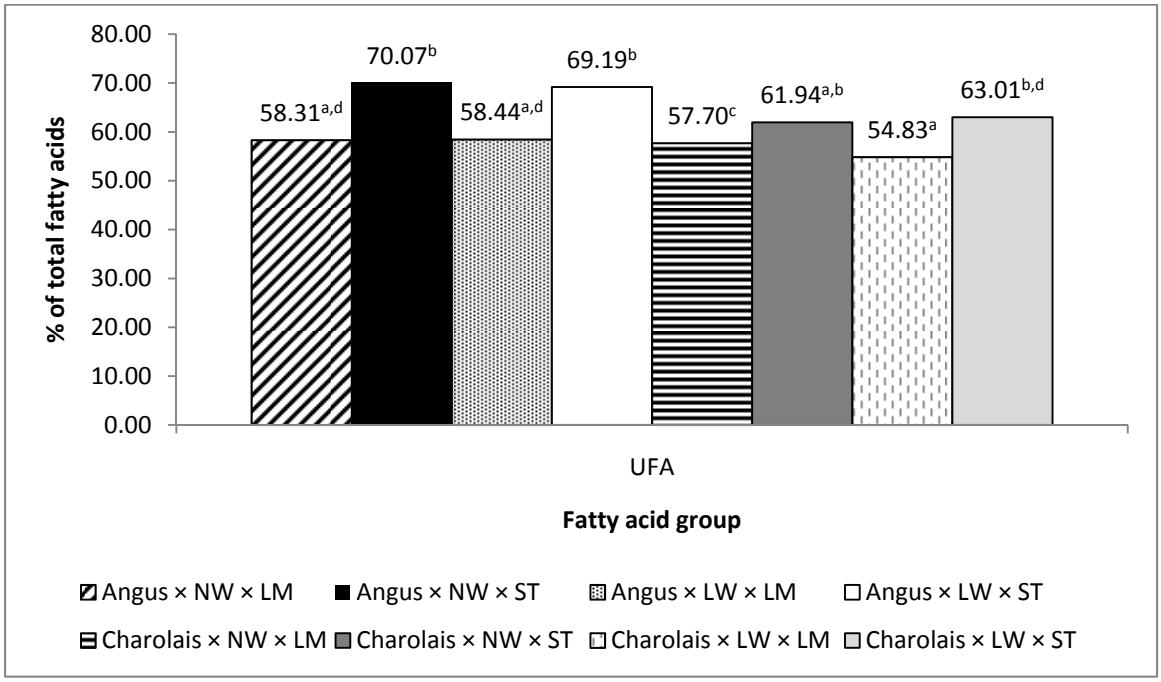


Figure 3.6. Breed × time of weaning × tissue interaction for percentage of monounsaturated fatty acids (MUFA) from longissimus muscle (LM) and semitendinosus muscle (ST) from normal weaned (NW) and late weaned (LW) Angus and Charolais finishing steers (n = 11 for Angus × NW × LM and Angus × NW × ST; n = 8 for Angus × LW × LM and Angus × LW × ST; n = 6 for Charolais × NW × LM and Charolais × NW × ST; n = 8 for Charolais × LW × LM and Charolais × LW × ST). ^{a,b,c,d,e} Least squares means without a common superscript differ ($P < 0.05$; SEM = 4.39).

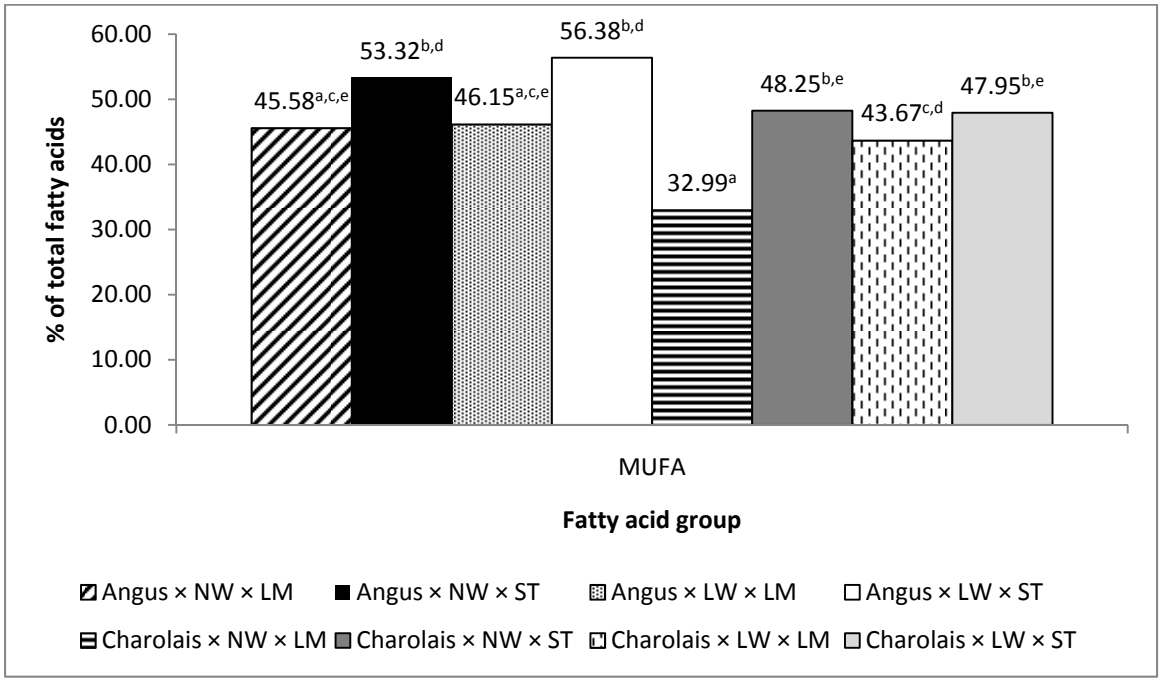


Figure 3.7. Time of weaning main effect for percentage of C14:1 in normal weaned (NW; mid-April at approximately 210 days of age) and late weaned (LW; mid-July at approximately 300 days of age) finishing beef steers (n = 17 for NW; n = 14 for LW). [a,b] Least squares means without a common superscript differ (P < 0.05; SEM = 0.14).

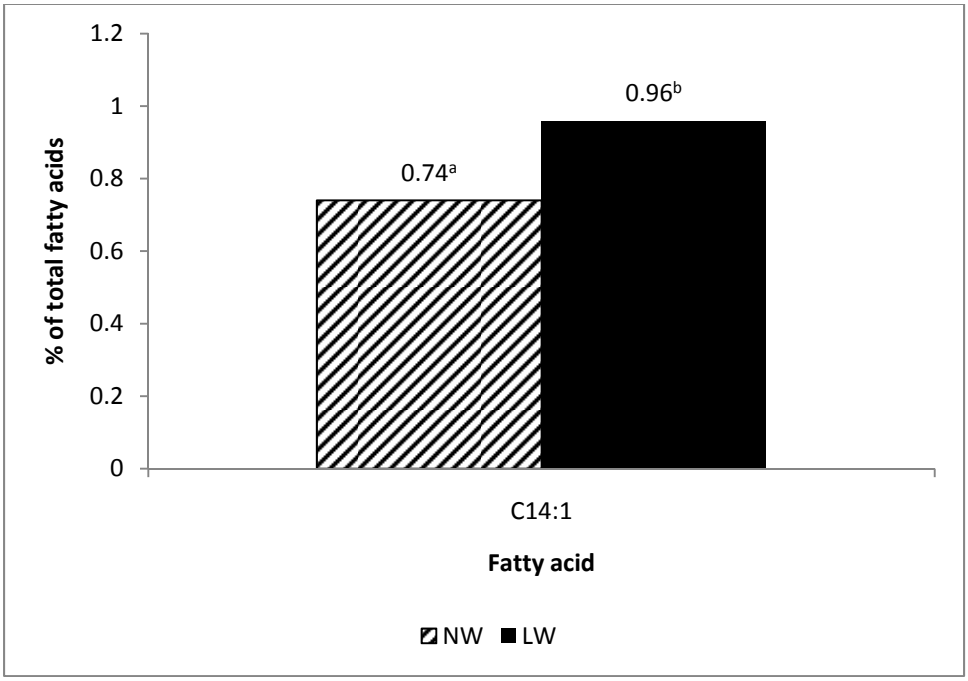


Figure 3.8. Breed × time of weaning × tissue interaction for percentage of polyunsaturated fatty acids (PUFA) from longissimus muscle (LM) and semitendinosus muscle (ST) from normal weaned (NW) and late weaned (LW) Angus and Charolais finishing steers (n = 11 for Angus × NW × LM and Angus × NW × ST; n = 8 for Angus × LW × LM and Angus × LW × ST; n = 6 for Charolais × NW × LM and Charolais × NW × ST; n = 8 for Charolais × LW × LM and Charolais × LW × ST). [a,b,c,d] Least squares means without a common superscript differ ($P < 0.05$; SEM = 1.49).

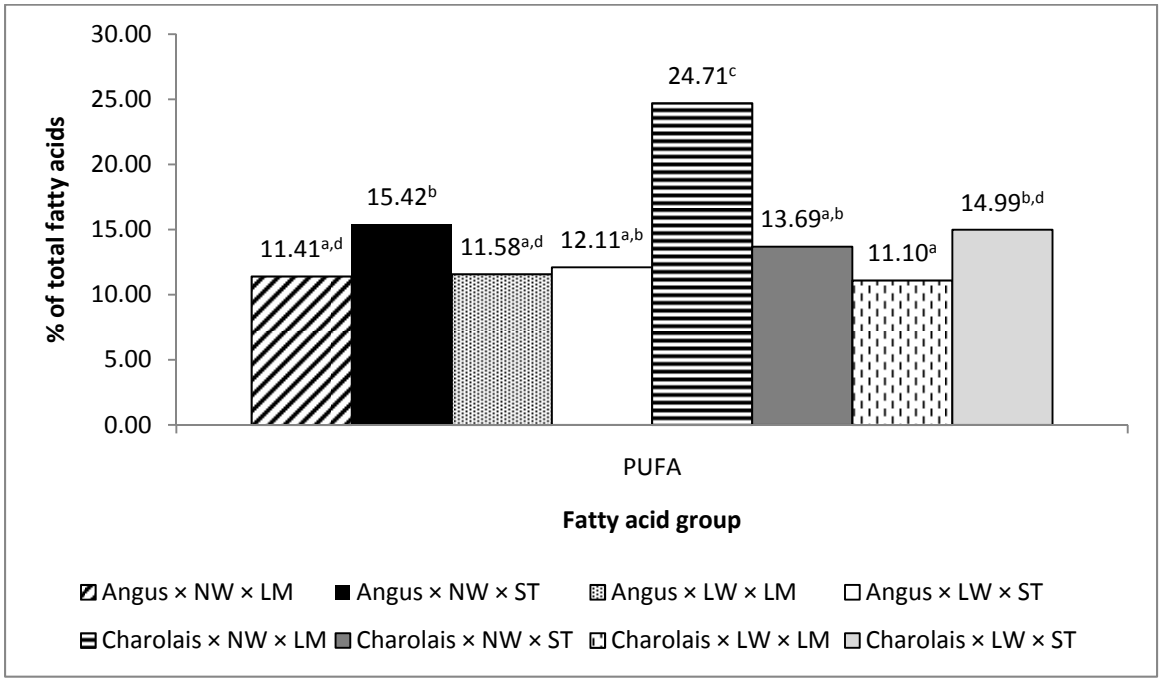


Figure 3.9. Breed × time of weaning interaction for percentage of C18:2*tt* and C18:2*t*10,*c*12 in normal weaned (NW; mid-April at approximately 210 days of age) and late weaned (LW; mid-July at approximately 300 days of age) Angus and Charolais finishing steers (n = 11 for Angus × NW; n = 8 for Angus × LW; n = 6 for Charolais × NW; n = 8 for Charolais × LW). ^{a,b} Least squares means for C18:2*tt* without a common superscript differ (P < 0.05; SEM = 0.15). ^{x,y} Least squares means for C18:2*tt* without a common superscript differ (P < 0.05; SEM = 0.01).

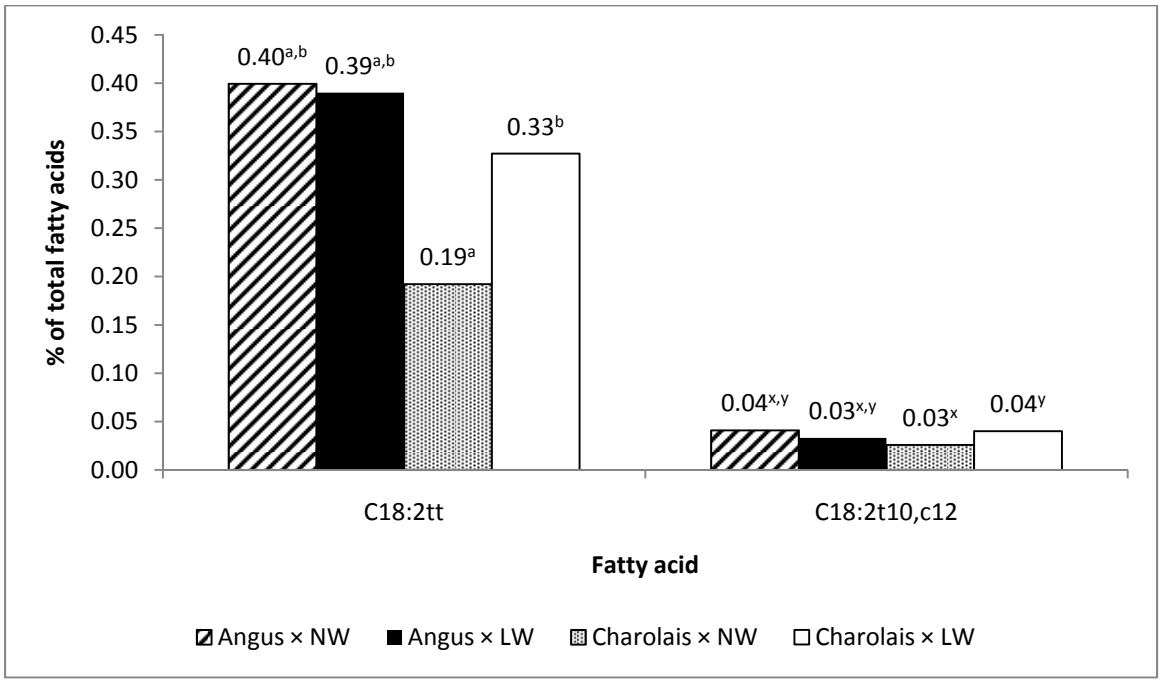


Figure 3.10. Breed × time of weaning × tissue interaction for percentage of omega-3 fatty acids (n-3) from longissimus muscle (LM) and semitendinosus muscle (ST) from normal weaned (NW) and late weaned (LW) Angus and Charolais finishing steers (n = 11 for Angus × NW × LM and Angus × NW × ST; n = 8 for Angus × LW × LM and Angus × LW × ST; n = 6 for Charolais × NW × LM and Charolais × NW × ST; n = 8 for Charolais × LW × LM and Charolais × LW × ST). ^{a,b,c,d,e} Least squares means without a common superscript differ ($P < 0.05$; SEM = 0.09).

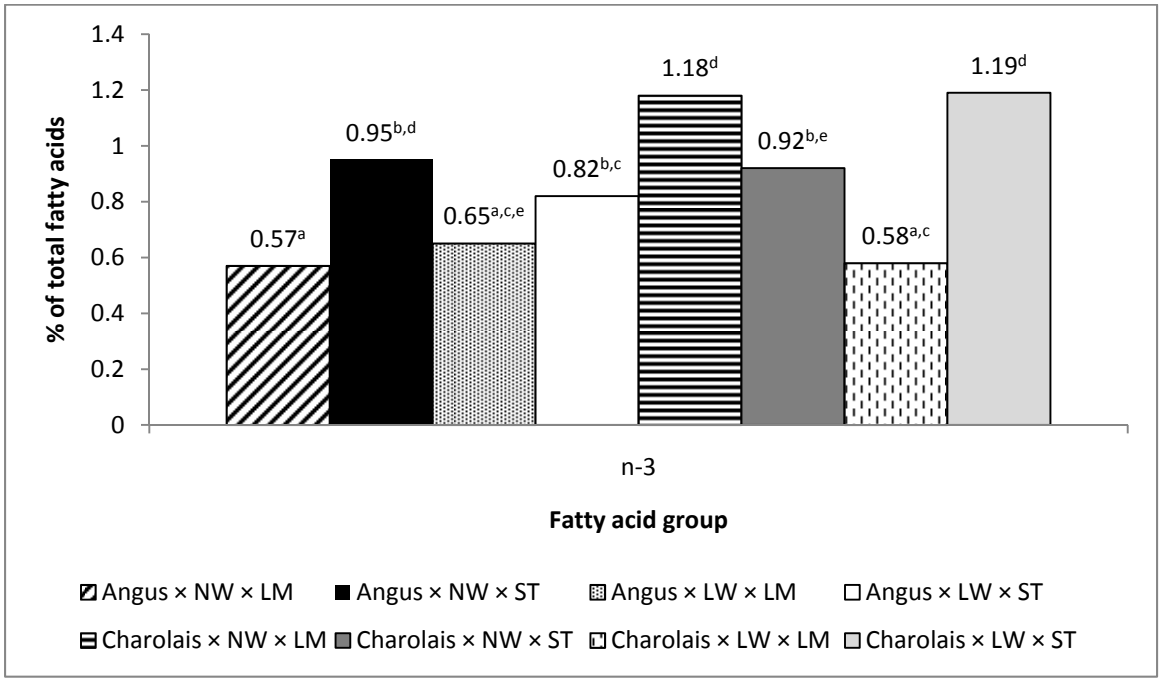


Figure 3.11. Breed × time of weaning × tissue interaction for percentage of omega-6 fatty acids (n-6) from longissimus muscle (LM) and semitendinosus muscle (ST) from normal weaned (NW) and late weaned (LW) Angus and Charolais finishing steers (n = 11 for Angus × NW × LM and Angus × NW × ST; n = 8 for Angus × LW × LM and Angus × LW × ST; n = 6 for Charolais × NW × LM and Charolais × NW × ST; n = 8 for Charolais × LW × LM and Charolais × LW × ST). ^{a,b,c} Least squares means without a common superscript differ ($P < 0.05$; SEM = 1.39).

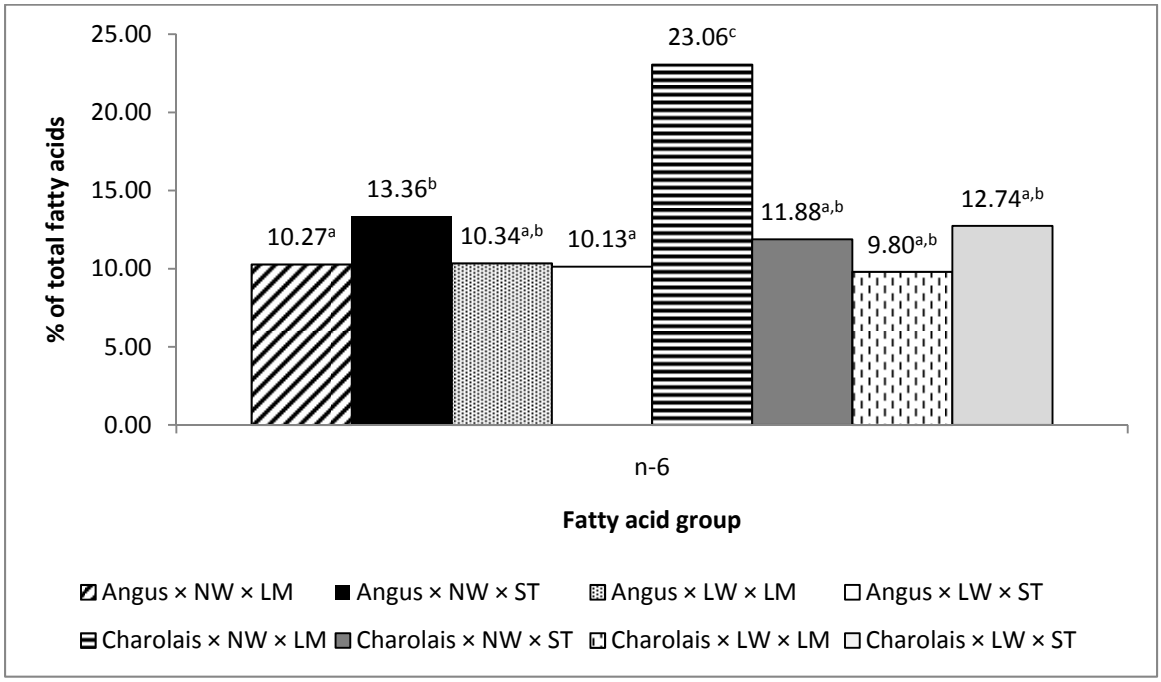


Figure 3.12. Time of weaning and tissue main effects for ratio of omega-3 fatty acids (n-3) to omega-6 fatty acids (n-6) from longissimus muscle (LM) and semitendinosus muscle (ST) from normal weaned (NW) and late weaned (LW) finishing beef steers (n = 17 for NW; n = 16 for LW; n = 33 for LM; n = 33 for ST). ^{a,b} Least squares means for time of weaning without a common superscript differ (P < 0.05; SEM = 0.006). ^{x,y} Least squares means for tissue without a common superscript differ (P < 0.05; SEM = 0.006).

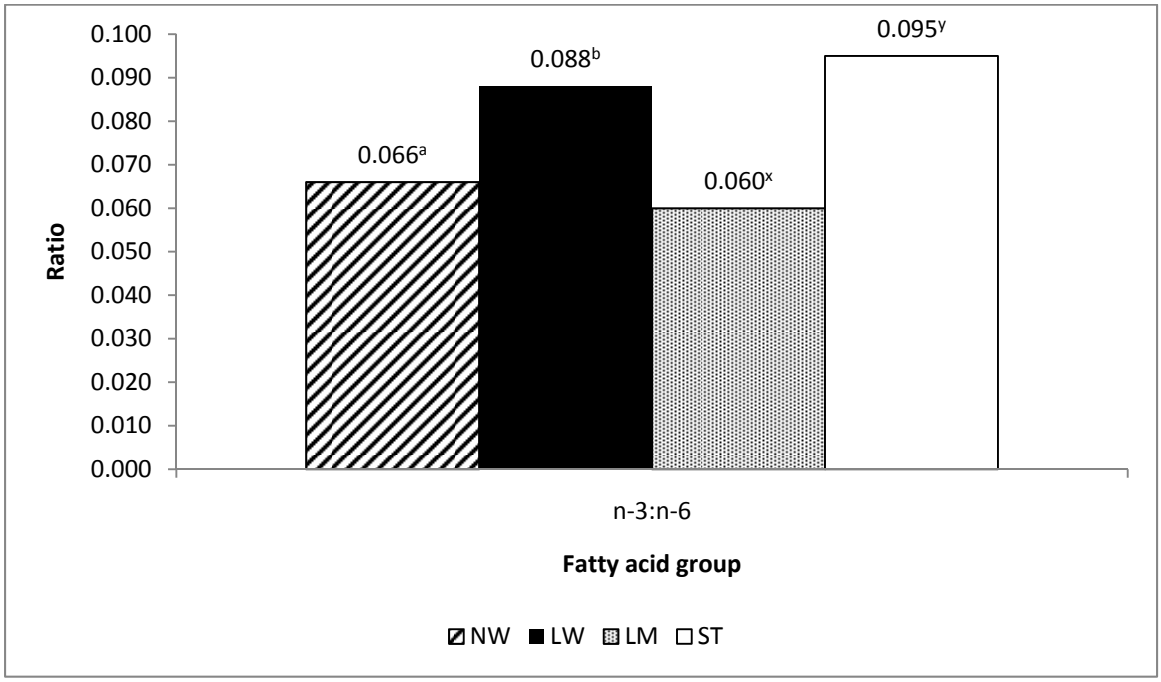
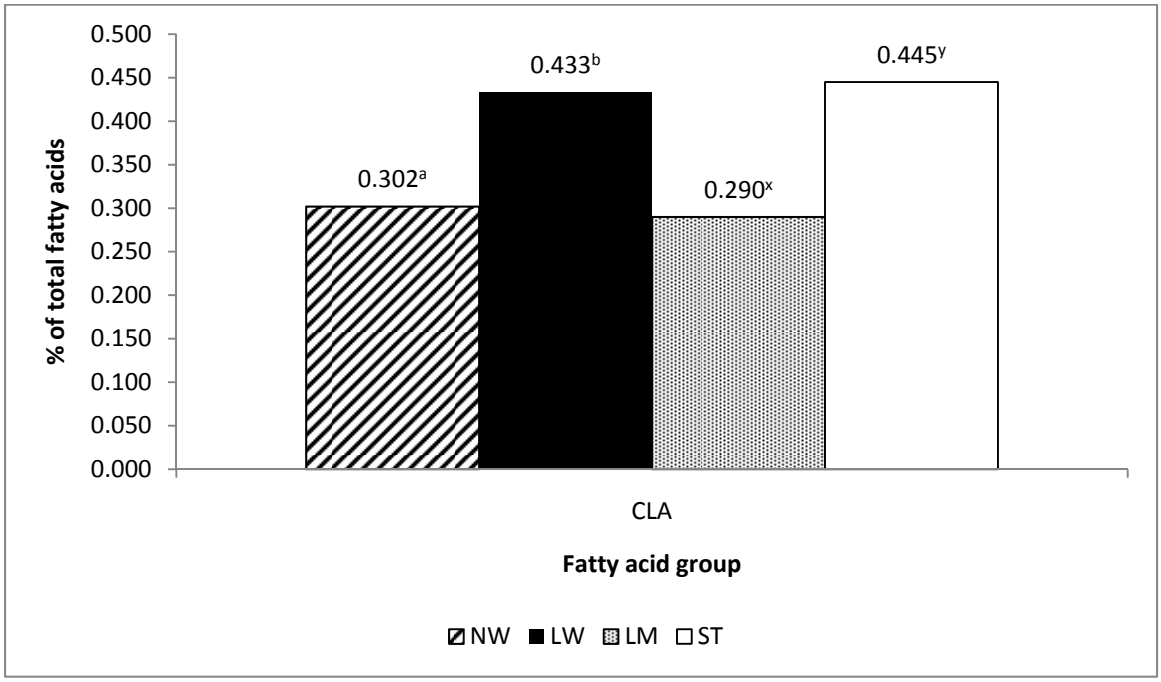


Figure 3.13. Time of weaning and tissue main effects for percentage of conjugated linoleic acid (CLA) in longissimus muscle (LM) and semitendinosus muscle (ST) from normal weaned (NW) and late weaned (LW) finishing beef steers (n = 17 for NW; n = 16 for LW; n = 33 for LM; n = 33 for ST). [a,b] Least squares means for time of weaning without a common superscript differ (P < 0.05; SEM = 0.04). [x,y] Least squares means for tissue without a common superscript differ (P < 0.05; SEM = 0.04).



CHAPTER IV

EFFECT OF BREED AND TISSUE TYPE ON GENE EXPRESSION OF LONGISSIMUS AND SEMITENDINOSUS MUSCLES IN NORMAL OR LATE WEANED ANGUS AND CHAROLAIS FINISHING STEERS

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ABSTRACT: Twelve steer calves were used in a completely randomized design to evaluate the effects of sire breed and tissue type on gene expression related to fatty acid composition. Tissue biopsies were taken from the longissimus muscle (LM; more oxidative) and the semitendinosus muscle (ST; more glycolytic) of Angus and Charolais sired steers. Biopsies were collected on d 127 and 128 of the finishing phase. All calves were harvested on d 138 and carcass data were collected. A bovine whole-genome 70-mer oligo array containing 24,000 long oligonucleotide probes was used for this

experiment. Total RNA was extracted, labeled with Alexa Fluor™ 555 and 647 dye, and hybridized to arrays. Hybridization signals were captured, processed using GenePix™ Pro 4.0, and uploaded to the GenePix Auto Processor. Ingenuity Pathways Analysis (**IPA**; Ingenuity® Systems, http://www.ingenuity.com) was used to identify the most relevant biological mechanisms, pathways, and functions of differently expressed genes. The LM had almost two times more (55 vs. 30) differentially expressed genes than the ST, but double (41 vs. 21) the number of genes

were annotated in the LM than the ST. Five networks were presented for LM and four networks were presented for ST. The LM and ST shared the common function of cell cycle in their most significant network. Angus steers had almost 4 times fewer (35 vs. 136) differentially expressed genes than the Charolais steers, with more than 4 times fewer (22 vs. 96) genes having annotations in the Angus steers than the Charolais steers. Three networks were presented for Angus steers and 11 networks were presented for Charolais steers. The Angus and Charolais steers had no common functions in their top network. Inconsistencies in these results may be due to bias in the biopsy samples in that the lean to fat ratio and the proportion of glycolytic to oxidative muscle fiber types leads to an unbalanced comparison between the sire breeds and tissue types. Without being able to separate the contribution of muscle vs. lipid and the proportion of glycolytic vs. oxidative muscle fiber types an accurate interpretation of this data or correlation with the fatty acid profile are not possible. This data set is limited by the fact that biopsy samples were only taken at one point in time. The desire to understand the genetic mechanisms underlying changes in fatty acid composition of beef remains,

future research will need to be addressed before correlations between phenotype (fatty acid profile) and gene expression can be made.

Key words: beef, gene expression, microarray, fatty acids

INTRODUCTION

Health professionals recommend a reduction in the overall consumption of saturated fatty acids (**SFA**), while emphasizing the need to increase intake of omega-3 fatty acids (**n-3**; Griel and Kris-Etherton, 2006). Often, it is recommended that beef be excluded from the diet because it is rich in SFA and atherosclerosis and other vascular diseases are positively correlated with SFA intake. However, contrary to popular belief the amount of SFA (38%) in beef is less than the total amount of unsaturated fatty acids (**UFA**) in the form of monounsaturated fatty acids (**MUFA**; 42%) and polyunsaturated fatty acids (**PUFA**; 3-5%) on a per edible portion basis (Baghurst, 2004; NCBA, 2008). In contrast to the negative effect of some SFA to human health, beef has some fatty acids that have beneficial health effects, e.g. PUFA, n-3, and conjugated linoleic acid (**CLA**). Lipids of ruminant origin are among the richest sources of CLA (Chin et al., 1992).

Differences in fatty acid composition among breeds and genotypes have been related to carcass fatness; however, De Smet et al. (2004) suggested a possible genetic variation in fatty acid metabolism among breeds that alters fatty acid composition (Harper and Pethick, 2004; Scollan et al., 2006). The challenge of the beef industry is to develop and implement programs aimed at improving healthfulness of beef utilizing existing natural genetic variation in fat composition. Understanding the genetic mechanism responsible for turning undesirable SFA into MUFA and PUFA should lead to identification of deoxyribonucleic acid (**DNA**) markers to be used in marker assisted selection programs. The molecular information generated through studies looking at environmental and genetic interactions could lead to the identification of molecular

DNA markers to be incorporated into breeding decisions to enhance the health value of beef by increasing the content of beneficial n-3, PUFA, and CLA and reducing SFA in beef.

The objective of the current study was to identify the genetic mechanisms underlying the phenotypic variation in fatty acid composition in beef. Differences in gene expression within longissimus and semitendinosus muscles between Angus and Charolais steers, and differences between muscle types within breed will be assessed.

MATERIALS AND METHODS

All research protocols were approved by the Oklahoma State University Institutional Animal Care and Use Committee.

Animals and Diet

Twelve steer calves selected from the Oklahoma State University Range Cow Research Center, North Range Unit, approximately 16 km west of Stillwater, OK were used in a completely randomized design to evaluate the effects of sire breed and tissue type on gene expression related to fatty acid composition. Steers are the progeny of predominantly Angus cows bred to one of two sire breeds: Angus or Charolais. Steers were late weaned in mid-July at approximately 300 d of age (**LW**). Prior to and at weaning, calves were managed according to procedures outlined by Hudson et al. (2010). Post-weaning, all steers were weighed after a 16-h withdrawal from feed and water, dewormed based on individual body weight (**BW**) with Ivomec Plus® (Merial,

Duluth, GA), and implanted with Component E-S with Tylan (200 mg progesterone and 20 mg estradiol benzoate; Vetlife, Overland Park, KS). Steers were then transported to the Oklahoma State University Willard Sparks Beef Research Center (Stillwater, OK), assigned to pens based on arrival BW, and adapted to an 88% concentrate finishing diet (Table 4.1) over an 18-d period. Weights were recorded at 28-d intervals. On d 56, steers were re-implanted with Revalor-S (120 mg trenbolone acetate and 24 mg estradiol benzoate; Intervet/Schering-Plough, Millsboro, DE). All steers remained on feed for 138 d before slaughter at Tyson Fresh Meats Inc., Emporia, KS.

Animal performance data were analyzed as a completely randomized design in a 2 × 2 factorial arrangement of treatments (breed = Angus vs. Charolais; time of weaning = NW vs. LW) with the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Steer was considered the experimental unit. The model statement contained fixed effects of breed and time of weaning. The random statement included sire within pen. There were unequal numbers of steers per treatment group (n = 11 for Angus × NW, n = 8 for Angus × LW, n = 6 for Charolais × NW, and n = 8 for Charolais × LW); therefore, the Satterthwaite denominator degrees of freedom were specified. Least squares means were separated using the PDIFF option of SAS. Treatments were determined to be different at $\alpha = 0.05$. Data presented in previous chapter.

Tissue Collection

Biopsies of the longissimus muscle (**LM**) and semitendinosus muscle (**ST**) were collected from all steers on d 127 and d 128 of the finishing phase. The LM sample was

collected between the 10th and 13th ribs. Briefly, steers were restrained in a hydraulic squeeze chute, hair was removed from the biopsy site, and a local anesthetic (lidocaine HCL; 20mg/mL; 10mL/biopsy) was administered. Using a scalpel, a 1-cm incision was made and a sterile biopsy needle was used to obtain a LM tissue sample. Semitendinosus muscle was collected according to Snow and Guy (1976). All steers were monitored for swelling during the 24-h post biopsy period.

Microarray

Total RNA extraction, purification, and quantification. Total RNA was extracted by an acidic phenol-chloroform extraction using TRIzol Reagent® (4 ml TRIzol per 0.5 g of muscle tissue; Invitrogen, Carlsbad, CA). Tissue samples in TRIzol were homogenized and centrifuged at 3,500 x g for 15 min at 4°C to separate insoluble material and excess fat. The lower aqueous (TRIzol) phase was transferred to a fresh tube. Chloroform (0.2 ml/ml TRIzol) was added to each homogenate sample and vortexed. After 3 min incubation at room temperature, samples were centrifuged at 3,500 x g for 30 min at 4°C. The aqueous phase containing RNA was transferred to a fresh tube. Isopropyl alcohol was added and mixed with the aqueous phase in a 1:1 ratio to precipitate the RNA. Samples were allowed to incubate at room temperature for 10 min and at -20°C overnight. Samples were thawed on ice and centrifuged at 12,000 x g for 10 min at 4°C. The supernatant was removed and the remaining RNA pellet was gently washed with chilled 75% ethanol then centrifuged at 7,500 x g for 5 min at 4°C. The supernatant was removed as before and tubes were inverted to allow the RNA pellets to air dry for 5 min

then suspended in DNase-RNase-free water (200 µL). A subsequent phenol:chloroform:isoamyl alcohol (25:24:1; pH 5.2) extraction of the RNA (1:1 ratio, respectively) was carried out. The RNA/phenol:chloroform:isoamyl alcohol mixture was centrifuged at 10,600 x g for 5 min at 4°C and the upper aqueous phase was transferred to a fresh tube and precipitated with 0.01 volumes of sodium acetate (3M; pH 5.2) and 2.5 volumes of chilled 98% ethanol. The samples were then maintained at -20°C overnight and centrifuged at 10,600 x g for 30 min at 4°C. The supernatant was removed and the RNA pellet was washed with 70% chilled ethanol and suspended in DNase/RNase free water and stored at -80°C. The integrity of the RNA was analyzed using gel electrophoresis (1.5%) and the RNA was quantified using a NanoDrop® ND-100 Spectrophotometer (NanoDrop Technologies, Willmington, DE).

Array specifications. A bovine whole-genome 70 base oligo array designed by the Bovine Microarray Consortium (Elsik et al., 2006) containing 24,000 long oligonucleotide probes was used for this experiment. The array included 16,846 probes designed from expressed sequence tags (**EST**) that were aligned to homologous vertebrate proteins and to the 6X bovine genome assembly (**BGA**). The probe set was supplemented with oligos designed from 703 predicted RefSeq genes, 5,943 reproductive tissue ESTs with a BGA but no protein alignment, and 504 positive and negative controls. The following criteria were used in EST selection and probe design: 1) predicted constitutive exon, 2) polymorphism avoidance, 3) minimal distance to 3' end of protein coding region, and 4) optimal Tm and specificity. Probe sequences and annotations are available at http://www.bovineoligo.org.

Sample labeling and hybridization. Slides were pre-hybridized by rinsing in 0.1% sodium dodecyl sulfate (**SDS**) for 5 min, followed by rinsing in sterile double distilled water (**ddH_2O**) for 15 s, and immediately dried by spinning 2 min on a slide centrifuge. The slides were placed in a 50 ml conical vial containing warmed (48°C) commercial pre-hybridization buffer (BlockIT Microarray Blocking Solution, TeleChem Int., Sunnyvale, CA) and incubated at 48°C for 4 h. The slides were removed and then rinsed in sterile ddH_2O for 15 s and dried on a slide centrifuge for 2 min. The RNA extracted from LM and ST tissue biopsy samples of six LW steers from each breed were selected for the microarray experiment to compare gene expression between breeds for each tissue and between tissues within breed. Total RNA (500 ng) was used for antisense RNA (**aRNA**) amplification in a TargetAmp™ 1-Round Aminoallyl-aRNA Amplification Kit 101 (Epicentre Biotechnologies, Madison, WI), with modifications to the manufacturer protocol. Six microliters of aminoallyl-UTP, rather than 2.4 µl of aminoallyl-UTP, was added to each reaction for in vitro transcription of aminoallyl-aRNA (**aa-aRNA**). The aa-aRNA was purified using the Qiagen MinElute PCR Purification Kit (Qiagen Inc., Valencia, CA). Fluorescent labeling was performed on 12.5 µl of aa-aRNA using Alexa Fluor™ 555 and Alexa Fluor™ 647. The dye-labeled aa-aRNA was gain purified using the Qiagen MinElute PCR Purification Kit (Qiagen Inc., Valencia, CA). Yield was determined using a NanoDrop® ND-100 Spectrophotometer (NanoDrop Technologies, Willmington, DE). For each sample, the fold amplification and total picomoles of Alexa Fluor™ 555 and 647 dye incorporation was calculated. To hybridize the purified dye-coupled aa-aRNA to the microarray, the entire hybridization solution (80 µl) containing 20 µl Alexa Fluor™ 555-

labeled aa-aRNA, 20 µl Alexa Fluor™ 647-labeled aa-aRNA, and 40 µl of 2x formamide-based hybridization buffer was denatured for 65°C for 10 min and then loaded on a warm (42°C) pre-hybridized microarray slide under a 24 x 60 mm lifterslip (Erie Scientific, Portsmouth, NH). The slides were then incubated at 42°C for 20 h in a humidified hybridization chamber. Following hybridization, the lifter slip was removed by immersing each slide into a prewarmed (42°C) 2x Sodium Chloride/Sodium Citrate (SSC)/0.2% sodium dodecyl sulfate solution. Each microarray was then washed in three post-hybridization solutions for 15 min with gentle agitation. First slides were washed in 2x SSC/0.2% SDS (42°C), followed by 0.2x SSC (**RT**), and finally 0.2x SSC (RT). The microarray was then immediately dried for 2 min on a slide centrifuge and stored in a light proof vacuum sealed desiccator until image acquisition.

Microarray data acquisition and pre-processing. Hybridization signals were captured by scanning the microarray slides with lasers at two wavelengths, 543 and 633 nm, using a ScanArray ™ Express confocal laser scanner (PerkinsElmer Life Sciences Inc., Boston, MA) at a pixel size resolution of 10 microns with the resulting images saved as 16 bit TIFF images. The images were then placed in GenePix™ Pro 4.0 (Axon Instruments Inc., Union City, CA) to acquire intensity values for Alexa Fluor™ 555 and Alexa Fluor™ 647 channels for foreground and background. The GenePix Pro Results (**GPR**) files generated from the image analysis were uploaded to the GenePix Auto Processor (**GPAP 3.2**) website from the Oklahoma State University Department of Biochemistry and Molecular Biology (http://darwin.biochem.okstate.edu/gpap32/). Preprocessing and normalization of data was accomplished utilizing the R-project

statistical environment (http://www.r-project.org) with the bioconductor and LIMMA packages (http://bioconductor.org) through the GPAP 3.2 website (http://darwin.biochem.okstate.edu/gpap32/) (Weng and Ayoubi, 2004). Background correction was performed using Robust Multi-array Average algorithm (Allison et al., 2006) available in the Bioconductor/LIMMA package. Two different normalization methods were utilized in the analysis to adjust and balance for the technical or systematic variation between the features within an array and between arrays for differences not caused by treatment: 1) Global Loess Normalization – Global intensity dependent normalization to balance the variation associated with dye bias within each array (Yang et al., 2001; 2002); and 2) Quantile Normalization to balance the effect of the Alexa Fluor™ 555 and Alexa Fluor™ 647 dye bias intensity between arrays (Bolstad et al., 2003). The expression ratio for each feature was calculated using the following formula where two-fold change is represented by $\log_2$ ratio > 1.0 (up-regulation) or < -1.0 (down-regulation) with M value ($\log_2$ ratio) = $\log_2$ (F647-B647$_{intensity}$/F55-B555$_{intensity}$). A moderated T-test was used to identify the differentially expressed features along with a M value and P-value, obtained from the moderated t-statistic after false discovery rate adjustment using the Benjamini and Hochberg method (Benjamini and Hochberg, 1995). Features expressed on at least 4 out the 6 microarrays with $|t| > 2.0$, M-value > 1.0 or < -1.0 (representing a fold change of > 2.0 or < -2.0), and a P-value < 0.01 were considered significantly differentially expressed.

Gene ontology and pathway analysis. Oligo features considered significantly differentially expressed were further characterized using the Genome Functional

Integrated Discoverer (**GFINDer**) (http://www.medinfopoli.polimi.it/GFINDer/) and Entrez Gene (http://www.ncbi.nlm.nih.gov/sites/enterez?db=gene). Ingenuity Pathways Analysis (Ingenuity® Systems, http://www.ingenuity.com) was used to identify the most relevant biological mechanisms, pathways, and functions of differently expressed genes. IPA enables the visualization and exploration of gene interactions and relies on the most currently known relationships among human, mouse, and rat genes and proteins. All differentially expressed genes and associated M-values were uploaded to the program. Genes were overlaid onto a global molecular network developed from information contained in Ingenuity's Knowledge Base. Networks of genes were then algorithmically generated based on their connectivity. The Functional Analysis identified the biological functions that were most significant to the data set. Right-tailed Fisher's exact test was used to calculate a p-value determining the probability that each biological function assigned to that data set is due to chance alone.

RESULTS

Microarray - Breed Comparisons within Tissue

Up-regulated indicates that the gene had a greater expression in Charolais than Angus steers, and conversely, down-regulated indicates the gene had a lower expression in Charolais than Angus steers. The number of differentially expressed up- and down-regulated genes that were annotated and used for IPA analysis for breed and tissue comparisons are shown in Table 4.1.

Longissimus muscle. Fifty-five array elements were found to be differentially ($P < 0.01$) expressed in LM with at least a 2-fold change in expression, with 39 elements up-regulated and 16 down-regulated. Of the 55 differentially expressed genes, 41 had known gene annotations in the National Center for Biotechnology Information (**NCBI**) database, and were used for subsequent gene ontology (**GO**) analysis. Fourteen of the differentially expressed genes did not have known gene data annotations on the NCBI database. Gene symbol, gene name, Entrez gene ID, M-value, fold-change, GO/KEGG ID, and GO term/KEGG pathway for the differentially expressed genes is presented in Table 4.2.

Of the 41 array elements shown to significantly correspond to specific annotated genes, 37 were eligible for network and function/pathway analysis using IPA. The networks generated by IPA for LM, including number of focus genes, network score, and top three network molecular/cellular functions, disease/disorder, or physiological system development and function are presented in Table 4.3. Networks were ranked by how relevant they are to the genes of the dataset with a greater score indicating a lower probability of finding the observed number of genes in a given network by random chance. Five networks were presented for LM, the top 2 networks for LM are diagrammed in Figures 4.1 and 4.2. Network 1 had an IPA score of 39 and included 16 focus genes (12 up-regulated and 4 down-regulated) with functions related to the cell cycle, connective tissue development and function, and lipid metabolism. Network 2 had an IPA score of 33 and included 14 focus genes (11 up-regulated and 3 down-

regulated) with functions related to cellular movement, hematological system development and function, and immune cell trafficking.

Figure 4.3 shows the molecular and cellular functions associated with all generated networks for LM. Functions are ranked by significance which is expressed as the negative $\log_{10}$ of the p-value for each function, which is represented on the y-axis. Twenty-two molecular and cellular functions were associated with the networks generated for LM. The top functions included cell cycle (9 focus genes; P = 0.0009 to 0.05), cell-to-cell signaling and interaction (5 focus genes; P = 0.004 to 0.04), cellular development (8 focus genes; P = 0.004 to 0.05), and cell death (5 focus genes; P = 0.03 to 0.05).

Semitendinosus muscle. Thirty array elements were found to be differentially (P < 0.01) expressed in ST with at least a 2-fold change in expression, with 26 elements up-regulated and 4 down-regulated. Of the 30 differentially expressed genes, 21 had known gene annotations in the NCBI database, and were used for subsequent GO analysis. Nine of the differentially expressed genes did not have known gene data annotations on NCBI database. Gene symbol, gene name, Entrez gene ID, M-value, fold-change, GO/KEGG ID, and GO term/KEGG pathway for the differentially expressed genes is presented in Table 4.4.

All of the 21 array elements shown to significantly correspond to specific annotated genes were eligible for network and function/pathway analysis using IPA. The networks generated by IPA for ST, including number of focus genes, network score, and top three network molecular/cellular functions, disease/disorder, or physiological

system development and function are presented in Table 4.5. Networks were ranked by how relevant they are to the genes of the dataset with a greater score indicating a lower probability of finding the observed number of genes in a given network by random chance. Four networks were presented for ST, the top network for ST is diagrammed in Figure 4.4. Network 1 had an IPA score of 32 and included 13 focus genes (11 up-regulated and 2 down-regulated) with functions related to the cell cycle, cell death, and connective tissue disorders.

Figure 4.5 shows the molecular and cellular functions associated with all generated networks for ST. Functions are ranked by significance which is expressed as the negative $\log_{10}$ of the p-value for each function, which is represented on the y-axis. Twenty-one molecular and cellular functions were associated with the networks generated for ST. The top functions included cellular assembly and organization (7 focus genes; P = 0.0002 to 0.04), molecular transportation (6 focus genes; P = 0.001 to 0.03), and protein trafficking (5 focus genes; P = 0.001 to 0.03).

Microarray – Tissue Comparisons within Breed

Up-regulated indicates that the gene had a greater expression in semitendinosus muscle than LM, and conversely, down-regulated indicates the gene had a lower expression in semitendinosus muscle than LM. The number of differentially expressed up- and down-regulated genes that were annotated and used for IPA analysis for breed and tissue comparisons are shown in Table 4.1.

Angus. Thirty-five array elements were found to be differentially ($P < 0.01$) expressed in Angus steers with at least a 2-fold change in expression, with 32 elements up-regulated and 3 down-regulated. Of the 35 differentially expressed genes, 22 had known gene annotations in the NCBI database, and were used for subsequent GO analysis. Thirteen of the differentially expressed genes did not have known gene data annotations on NCBI database. Gene symbol, gene name, Entrez gene ID, M-value, fold-change, GO/KEGG ID, and GO term/KEGG pathway for the differentially expressed genes is presented in Table 4.6.

Of the 22 array elements shown to significantly correspond to specific annotated genes, 20 were eligible for network and function/pathway analysis using IPA. The networks generated by IPA for Angus steers, including number of focus genes, network score, and top three network molecular/cellular functions, disease/disorder, or physiological system development and function are presented in Table 4.7. Networks were ranked by how relevant they are to the genes of the dataset with a greater score indicating a lower probability of finding the observed number of genes in a given network by random chance. Three networks were presented for Angus steers, the top network for Angus steers is diagrammed in Figure 4.6. Network 1 had an IPA score of 22 and included 9 focus genes (7 up-regulated and 2 down-regulated) with functions related to the cardiovascular system development and function, cell signaling, and molecular transport.

Figure 4.7 shows the molecular and cellular functions associated with all generated networks for Angus steers. Functions are ranked by significance which is

expressed as the negative $\log_{10}$ of the p-value for each function, which is represented on the y-axis. Thirty molecular and cellular functions were associated with the networks generated for Angus steers. The top functions included cell morphology (6 focus genes; P = 0.0003 to 0.05), cellular development (6 focus genes; P = 0.0003 to 0.05), cell death (7 focus genes; P = 0.001 to 0.05), cellular assembly and organization (8 focus genes; P = 0.001 to 0.05), cellular function and maintenance (7 focus genes; P = 0.001 to 0.04), and gene expression (6 focus genes; P = 0.002 to 0.05).

Charolais. One hundred thirty-six array elements were found to be differentially ($P < 0.01$) expressed in Charolais steers with at least a 2-fold change in expression, with 128 elements up-regulated and 8 down-regulated. Of the 136 differentially expressed genes, 96 had known gene annotations in the NCBI database, and were used for subsequent GO analysis. Forty of the differentially expressed genes did not have known gene data annotations on NCBI database. Gene symbol, gene name, Entrez gene ID, M-value, fold-change, GO/KEGG ID, and GO term/KEGG pathway for the differentially expressed genes is presented in Table 4.8.

Of the 96 array elements shown to significantly correspond to specific annotated genes, 80 were eligible for network and function/pathway analysis using IPA. The networks generated by IPA for Charolais steers, including number of focus genes, network score, and top three network molecular/cellular functions, disease/disorder, or physiological system development and function are presented in Table 4.9. Networks were ranked by how relevant they are to the genes of the dataset with a greater score indicating a lower probability of finding the observed number of genes in a given

network by random chance. Eleven networks were presented for Charolais steers, the top 3 networks for Charolais steers are diagrammed in Figures 4.8, 4.9, and 4.10. Network 1 had an IPA score of 25 and included 13 focus genes (13 up-regulated and 0 down-regulated) with functions related to the cancer, skeletal muscular disorders, and cellular development. Network 2 also had an IPA score of 25 and included 13 focus genes (13 up-regulated and 0 down-regulated) with functions related to the cell cycle, cardiac output, cardiovascular system development and function.

Figure 4.11 shows the molecular and cellular functions associated with all generated networks for Charolais steers. Functions are ranked by significance which is expressed as the negative $\log_{10}$ of the p-value for each function, which is represented on the y-axis. Twenty-six molecular and cellular functions were associated with the networks generated for Charolais steers. The top functions included cellular assembly and organization (6 focus genes; P = 0.004 to 0.03), cell death (8 focus genes; P = 0.007 to 0.05), and small molecular biochemistry (7 focus genes; P = 0.008 to 0.03).

DISCUSSION

The objective of IPA network generation is to hypothesize, using the most pertinent information available in the literature, how network eligible genes in a data set interact in a biological system. In this study, networks were generated through IPA using the dataset of differentially expressed genes identified through GPAP 3.2 described previously. IPA lists 32 functions for the heading of “molecular and cellular functions” which are subdivided into lower level secondary function “categories”.

Functional comparison, as well as metabolic signaling pathway comparison between treatments, is a tool that allows for further determination if whether and to what extent a given function or pathway is affected by a treatment.

In the breed comparisons (Angus vs. Charolais), the LM had almost two times more (55 vs. 30) differentially expressed genes than the ST, but double (41 vs. 21) the number of genes were annotated in the LM than the ST (Table 4.1). The LM and ST shared the common function of cell cycle in their most significant network.

In tissue comparisons (longissimus vs. semitendinosus), the Angus steers had almost 4 times fewer (35 vs. 136) differentially expressed genes than the Charolais steers, with more than 4 times fewer (22 vs. 96) genes having annotations in the Angus steers than the Charolais steers. The Angus and Charolais steers had no common functions in their top network.

Only the comparison of the LM between the Angus and Charolais steers had a significant function in lipid metabolism, however, this functional group only used two of the differentially expressed genes (focus genes). Reasons for inconsistencies in these results and why there is only very limited associations with lipid metabolism may lie in the characteristics of the biopsy samples. The biopsy samples are confounded in that they contain both muscle tissue as well as lipid in the form of marbling, and the ratio of muscle to lipid is likely biased by the animal breed and tissue type. Angus cattle are earlier maturing than Charolais and therefore it is typical for Angus cattle to deposit more fat, while Charolais cattle produce more lean (Peacock et al., 1982; Coleman et al., 1993; Arango et al., 2002). Therefore, the tissue biopsies used in this analysis may be

biased in that the lean to fat ratio would be expected to be lower in the fatter Angus steers than in the leaner Charolais steers.

Jurie et al. (2006) showed that the ST was more glycolytic and the LM was more oxidative. Muscle fiber types are dynamic, constantly changing in proportion as the animal ages, but muscle type is a reflection of the energy requirements of the muscle (Hocquette et al., 1998; Oddy et al., 2001). Because the ST is involved in animal movement, it may have a greater energy requirement than the LM, which result in the ST having a greater proportion of glycolytic enzymatic activity. Because Angus cattle are earlier maturing than Charolais, the proportion of glycolytic and oxidative muscle fiber types in the LM and ST in the two breeds may differ greatly. Literature has also shown that changes in fatty acid composition within muscle are associated with changes in proportions of muscle fiber types (Jurie et al., 2005; Von Seggern et al., 2005; Alfaia et al., 2007), and it has been well established that the lipid content is higher in oxidative muscle fibers (Enser et al., 1998). These factors will need to be considered in future research before correlations between phenotype (fatty acid profile) and gene expression can be made.

Also, this data set is limited by the fact that biopsy samples were only taken at one point in time. The fatty acids present in the tissues may or may not be affected by the genes being expressed at that same time point. Quantifying the change in gene expression and the change in fatty acid profile over time might result in gene expression changes that correlate with changes in fatty acid composition. Pyatt and Berger (2005) have demonstrated that adipogenesis in animals is a complex physiological process

influenced by different factors such as genetics, environment, breed, age, sex, nutrition, and management practices.

In conclusion, it is not possible to correlate the gene expression data from this microarray analysis with the fatty acid profile data. More research is needed to be able to eliminate some of the inherent bias in the biopsy samples in the current study. None the less, the gene lists generated from this study may help future researchers. The desire to understanding the genetic mechanisms underlying changes in fatty acid composition of beef will continue to be driven by the fact that increasing the healthfulness of beef and improving meat quality could lead to increased product value.

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Table 4.1. Ingredient and nutrient composition of finishing diet (DM basis)

Item	%
Ingredient	
Dry-rolled corn	67.6
Corn DDGS[1]	15.7
Ground alfalfa hay	6.0
Liquid supplement[2]	5.0
Dry supplement[3]	5.7
Nutrient composition[4]	
DM	86.60
CP	13.98
NDF	16.30
ADF	7.63
Ca	0.70
P	0.43

[1]DDGS = distillers dried grains with soluble.
[2]Synergy 19/14 (Westway Feed Products, New Orleans, LA).
[3]Pelleted supplement contained the following (DM basis): 40.35% ground corn, 19.73% wheat middlings, 21.42% limestone, 4.39% dicalcium phosphate, 5.79% salt, 0.05% manganous oxide, 1.14% available zinc 100, 0.07% zinc sulfate, 4.56% potassium chloride, 1.93% magnesium oxide, 0.06% vitamin A (30,000 IU/g), 0.04% vitamin E (50%), 0.31% Rumensin 80 (Elanco Animal Health, Indianapolis, IN), and 0.17% Tylan 40 (Elanco Animal Health, Indianapolis, IN).
[4]All values are from laboratory analyses and are presented on a 100% DM basis (except DM).

Table 4.2. The number of differentially expressed up- and down-regulated genes that were annotated and used for IPA analysis for breed and tissue type comparisons

	Angus x Charolais		Longissimus x Semitendinosus	
Item, no. of genes	LM	ST	Angus	Charolais
Differentially expressed	55	30	35	136
Up-regulated	39	26	32	128
Down-regulated	16	4	3	8
Annotated	41	21	22	96
IPA Analysis Eligible	37	21	20	80

Table 4.3. Gene symbol, gene name, Entrez gene ID, M-value, fold-change, GO/KEGG ID, and GO term/KEGG pathway for the differentially expressed genes in longissimus muscle from Angus and Charolais steers

Gene Symbol	Gene Name	Entrez Gene ID	M	Fold Change	(GO)/KEGG Pathway	Term/Pathway
Biological Regulation						
LUC7L	LUC7-like (S. cerevisiae) [Bos taurus]	535131	1.78	3.44		
Cellular Process						
CA2	carbonic anhydrase II [Bos taurus]	280740	1.15	2.22	00910	Nitrogen metabolism
HPRT1	hypoxanthine phosphoribosyltransferase 1 [Bos taurus]	281229	1.45	2.74	00983, 01100, 00230	Drug metabolism - other enzymes, Metabolic pathways, Purine metabolism
QKI	quaking homolog, KH domain RNA binding (mouse) [Bos taurus]	493722	1.00	2.00		
BCAR3	breast cancer anti-estrogen resistance 3 [Bos taurus]	506231	-1.12	-2.17		
USP4	ubiquitin specific peptidase 4 (proto-oncogene) [Bos taurus]	508042	1.66	3.17		
RARRES 2	retinoic acid receptor responder (tazarotene induced) 2 [Bos taurus]	508990	1.46	2.75		
DDIT4L	DNA-damage-inducible transcript 4-like [Bos taurus]	510906	1.07	2.11		
POLR2E	polymerase (RNA) II (DNA directed) polypeptide E, 25kDa [Bos taurus]	512971	-1.17	-2.25	05016, 01100, 00230, 00240, 03020	Huntington's disease, Metabolic pathways, Purine metabolism, Pyrimidine metabolism, RNA polymerase
ING1	inhibitor of growth family, member 1 [Bos taurus]	513047	-1.05	-2.07		
PGD	phosphogluconate dehydrogenase [Bos taurus]	514939	2.71	6.57	00480, 01100, 00030	Glutathione metabolism, Metabolic pathways, Pentose phosphate pathway
PRMT5	protein arginine methyltransferase 5 [Bos taurus]	515594	-1.03	-2.05		
TERF1	telomeric repeat binding factor (NIMA-interacting) 1 [Bos	519474	-1.50	-2.84		

	taurus]					
TBC1D8	TBC1 domain family, member 8 (with GRAM domain) [Bos taurus]	527162	1.28	2.42		
MEOX1	mesenchyme homeobox 1 [Bos taurus]	529059	1.37	2.59		
CAMK2A	calcium/calmodulin-dependent protein kinase II alpha [Bos taurus]	530719	1.10	2.15	04020, 04012, 05214, 04912, 04720, 04916, 04722, 04740, 04114, 04310	Calcium signaling pathway, ErbB signaling pathway, Glioma, GnRH signaling pathway, Long-term potentiation, Melanogenesis, Neurotrophin signaling pathway, Olfactory transduction, Oocyte meiosis, Wnt signaling pathway
UBQLN2	ubiquilin 2 [Bos taurus]	539529	1.12	2.18		
DYNLL2	dynein light chain LC8-type 2 [Bos taurus]	540369	1.39	2.61	04962	Vasopressin-regulated water reabsorption
EIF3G	eukaryotic translation initiation factor 3, subunit G [Bos taurus]	615695	1.05	2.07		
TMBIM6	transmembrane BAX inhibitor motif containing 6 [Bos taurus]	616210	1.09	2.14		
PTOV1	similar to Prostate tumor over expressed gene 1 [Bos taurus]	617775	-1.06	-2.08		
ITGB1BP3	integrin beta 1 binding protein 3 [Bos taurus]	780788	1.18	2.26		
Metabolic Process						
METTL7A	methyltransferase like 7A [Bos taurus]	613844	1.42	2.68		
METTL7A	methyltransferase like 7A [Bos taurus]	613844	1.65	3.14		
Response to Stimulus						
BOLA-N	MHC class I antigen [Bos taurus]	100126048	1.13	2.19	05330,	Allograft rejection,

					04612, 05320, 04514, 04144, 05332, 04650, 04940, 05416	Antigen processing and presentation, Autoimmune thyroid disease, Cell adhesion molecules (CAMs), Endocytosis, Graft-versus-host disease, Natural killer cell mediated cytotoxicity, Type I diabetes mellitus, Viral myocarditis
DERL1	Der1-like domain family, member 1 [Bos taurus]	404121	1.11	2.16	05014	Amyotrophic lateral sclerosis (ALS)
Other						
C21H14orf142	chromosome 14 open reading frame 142 ortholog [Bos taurus]	505131	1.75	3.36		
ARL13A	13A ADP-ribosylation factor-like 13A [Bos taurus]	512780	-1.00	-2.00		
JAM3	junctional adhesion molecule 3 [Bos taurus]	513412	1.12	2.17	04514, 04670, 04530	Cell adhesion molecules (CMAs), Leukocyte transendothelial migration, Tight junction
TOPORS	topoisomerase I binding, arginine/serine-rich [Bos taurus]	523321	1.34	2.53		
NEURL4	neuralized homolog 4 (Drosophila) [Bos taurus]	528485	-1.09	-2.12		
SEMA4F	sema domain, immunoglobulin domain (Ig), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 4F [Bos taurus]	530018	-1.16	-2.24	04360	Axon guidance
PPP2R5A	protein phosphatase 2, regulatory subunit B', alpha [Bos taurus]	533788	1.17	2.25	04114, 04310	Oocyte meiosis, Wnt signaling pathway
SIAH1	seven in absentia homolog 1 (Drosophila) [Bos taurus]	534655	1.77	3.41	04120, 04310, 04115	Ubiquitin mediated proteolysis, Wnt signaling pathway, p53 signaling pathway
CPEB3	cytoplasmic polyadenylation element binding protein 3	537016	1.24	2.36		

	[Bos taurus]					
MORF4L2	mortality factor 4 like 2 [Bos taurus]	538442	1.29	2.44		
LOC539314	baculoviral IAP repeat-containing-like [Bos taurus]	539314	-1.13	-2.19	04210, 04510, 04621, 05200, 05222, 04120	Apoptosis, Focal adhesion, NOD-like receptor signaling pathway, Pathways in cancer, Small cell lung cancer, Ubiquitin mediated proteolysis
C5H12orf23	chromosome 12 open reading frame 23 ortholog [Bos taurus]	539347	1.30	2.46		
KDM5B	lysine (K)-specific demethylase 5B [Bos taurus]	540633	1.14	2.21		
C8H9orf21	orf21 chromosome 9 open reading frame 21 ortholog [Bos taurus]	616897	-1.13	-2.20		
ZFP106	zinc finger protein 106 homolog (mouse) [Bos taurus]	788468	-1.07	-2.10		

Table 4.4. Networks generated for longissimus muscle from Angus and Charolais steers using Ingenuity Pathways Analysis (Ingenuity® Systems, www.ingenuity.com)[1,2]

Network ID	Molecules in Network	Score	Focus Molecules	Top Functions
1	ALB, beta-estradiol, ↑**C12ORF23**, C19ORF2, ↑**CA2**, ↑**CAMK2A**, CDKN1A, ↑**CPEB3**, ↑**DERL1**, ↑**EIF3G**, GADD45G, GH1, IL4, ↓**ING1**, INS1, ↑**ITGB1BP3**, JAM, JAM2, ↑**JAM3**, KIF2C, ↑**LUC7L**, PEG3, PLK1, ↓**POLR2E**, RNA polymerase II, ↑**SIAH1**, SSTR4, ↑**TBC1D8**, ↓**TERF1**, TJP1, TNKS, ↑**TOPORS**, TTK, Vegf, ↓**ZFP106**	39	16	Cell Cycle, Connective Tissue Development and Function, Lipid Metabolism
2	AHCY, ↓**BCAR3**, BRP44L, C10ORF10, CAMK2N2, CCND1, ↑**DDIT4L**, EHD1, EIF2A, EIF2AK2, GSPT1, ↑**HPRT1**, ↑**KDM5B**, KHSRP, ↑**MEOX1**, ↑**MORF4L2**, MRFAP1, MT1E, PFAS, ↑**PPP2R5A**, ↓**PRMT5**, ↓**PTOV1**, ↑**QKI**, ↑**RARRES2**, RB1, SNRPD1, SNRPG, TARS, ↑**TMBIM6**, TNF, TRAF6, TSN, TTK, ↑**UBQLN2**, ↑**USP4**	33	14	Cellular Movement, Hematological System Development and Function, Immune Cell Trafficking
3	DBNL, ↓**NEURL4**	3	1	Cell Morphology, Nervous System Development and Function, Cellular Compromise
4	↑**METTL7A**, MME	3	1	Cancer, Cell Death, Neurological Disease
5	MIR183 (includes EG:406959), SEMA4, ↓**SEMA4F**	2	1	Cancer, Genetic Disorder, Respiratory Disease

[1]

[2]Differentially expressed genes are highlighted in bold text with (↑) indicating up-regulation and (↓) indicating down-regulation.

Table 4.5. Gene symbol, gene name, Entrez gene ID, M-value, fold-change, GO/KEGG ID, and GO term/KEGG pathway for the differentially expressed genes in semitendinosus muscle from Angus and Charolais steers

Gene Symbol	Gene Name	Entrez Gene ID	M	Fold Change	(GO)/KEGG Pathway	Term/Pathway
Cellular Process						
GNG2	guanine nucleotide binding protein (G protein), gamma 2 [Bos taurus]	281203	1.15	-2.23	04062	Chemokine signaling pathway
PDE6C	phosphodiesterase 6C, cGMP-specific, cone, alpha prime [Bos taurus]	281975	-1.21	-2.31	00230	Purine metabolism
RAB3A	RAB3A, member RAS oncogene family [Bos taurus]	282029	1.37	-2.59		
RIMKLA	ribosomal modification protein rimK-like family member A [Bos taurus]	507323	1.67	-3.18		
ADRM1	adhesion regulating molecule 1 [Bos taurus]	519729	1.27	-2.40		
Metabolic Process						
P4HA1	prolyl 4-hydroxylase, alpha polypeptide I [Bos taurus]	518288	2.11	-4.31	00330, 01100	Arginine and proline metabolism, Metabolic pathways
Other						
BLZF1	basic leucine zipper nuclear factor 1 [Bos taurus]	504820	1.11	-2.16		
CDK5RAP2	CDK5 regulatory subunit associated protein 2 [Bos taurus]	508100	-1.07	-2.09		
AMICA1	adhesion molecule, interacts with CXADR antigen 1 [Bos taurus]	509575	-1.17	-2.25		
PROSC	proline synthetase co-transcribed homolog (bacterial) [Bos taurus]	509643	1.71	-3.27	go:0005622	cellular component
DCTN1	dynactin 1 (p150, glued homolog, Drosophila) [Bos taurus]	511045	1.88	-3.68	05016, 04962	Huntington's disease, Vasopressin-regulated water reabsorption
JTB	jumping translocation breakpoint [Bos taurus]	513970	1.76	-3.38		
IGBP1	immunoglobulin (CD79A) binding protein 1 [Bos taurus]	534155	1.24	-2.36		
CHD2	chromodomain helicase DNA binding protein 2 [Bos taurus]	535026	-1.36	-2.57		

PVALB	parvalbumin [Bos taurus]	538603	1.04	-2.06		
ERLIN1	ER lipid raft associated 1 [Bos taurus]	617074	1.17	-2.25		
DUSP16	dual specificity phosphatase 16 [Bos taurus]	618644	1.21	-2.31	04010	MAPK signaling pathway
ANG2	angiogenin 2 [Bos taurus]	783907	1.58	-2.99		
GPRASP1	G protein-coupled receptor associated sorting protein 1 [Bos taurus]	100124524	1.08	-2.12		
KRI1, ATG4D	KRI1 homolog (S. cerevisiae) [Bos taurus], ATG4 autophagy related 4 homolog D (S. cerevisiae) [Bos taurus]	511427, 615657	1.46	-2.75	04140	Regulation of autophagy
LOC782598, KIF20B	M-phase phosphoprotein 1-like [Bos taurus], kinesin family member 20B [Bos taurus]	782598, 514679	1.30	-2.47		

Table 4.6. Networks generated for semitendinosus muscle from Angus and Charolais steers using Ingenuity Pathways Analysis (Ingenuity® Systems, www.ingenuity.com)[1,2]

Network ID	Molecules in Network	Score	Focus Molecules	Top Functions
1	ABCA2, ↑**ADRM1**, ↑**ATG4D (includes EG:84971),** beta-estradiol, ↑**BLZF1**, CDK5R1, ↓**CDK5RAP2**, ↑**DUSP16**, EPO, ↑**ERLIN1**, HNF4A, IL4, ↑**JTB**, ↑**KIF20B**, L-proline, ↑**P4HA1**, ↓**PDE6C**, PIN1, PRC1, ↑**PROSC**, ↑**PVALB**, RAB2A, ↑**RAB3A**, RABAC1, RPN2, SEC23A, SEC24D, SEMA7A, SYTL4, TIMM13, TP53, UBE2S, UCHL5, USMG5, XBP1	32	13	Cell Cycle, Cell Death, Connective Tissue Disorders
2	Actin, ACTR1A, AKTIP, ↑**ANG**, CEACAM1, CLIP1, CXCL9, ↑**DCTN1**, DCTN2, DCTN3, DCTN4, DCTN6, GNAI2, GNB1, ↑**GNG2,** ↑**GPRASP1**, GSN, HAP1, Histone H1, ↑**IGBP1**, ITK, KSR1, MAP2K3, MAP2K6, MYH11, OPRD1, PFDN1, PLA2, Plc beta, PLCB2, RAC1, RASGRF1, Rho gdi, TDGF1	10	5	Cellular Assembly and Organization, Cell Signaling, Nucleic Acid Metabolism
3	↑**KRI1**, RPLP0 (includes EG:6175)	3	1	Cancer, Gastrointestinal Disease, Cell Death
4	MIRLET7B (includes EG:406884), MIRLET7C, ↑**RIMKLA**, STAT5A	3	1	Cancer, Gastrointestinal Disease, Cellular Assembly and Organization

[1]

[2]Differentially expressed genes are highlighted in bold text with (↑) indicating up-regulation and (↓) indicating down-regulation.

Table 4.7. Gene symbol, gene name, Entrez gene ID, M-value, fold-change, GO/KEGG ID, and GO term/KEGG pathway for the differentially expressed genes in longissimus and semitendinosus muscles from Angus steers

Gene Symbol	Gene Name	Entrez Gene ID	M	Fold Change	(GO)/KEGG Pathway	Term/Pathway
Biological Regulation						
TKT	transketolase [Bos taurus]	445425	1.97	3.92	01100, 00030	Metabolic pathways, Pentose phosphate pathway
Cellular Process						
PTH	parathyroid hormone [Bos taurus]	280903	-1.80	-3.47	04080	Neuroactive ligand-receptor interaction
NEDD8	neural precursor cell expressed, developmentally down-regulated 8 [Bos taurus]	286796	2.17	4.49		
ANPEP	alanyl (membrane) aminopeptidase [Bos taurus]	404191	-1.25	-2.38	00480, 04640, 01100, 04614	Glutathione metabolism, hematopoietic cell lineage, metabolic pathways, Renin-angiotensin system
SRGN	serglycin [Bos taurus]	509501	1.06	2.08		
EIF2B4	eukaryotic translation initiation factor 2B, subunit 4 delta, 67kDa [Bos taurus]	521926	1.02	2.03		
NRBP1	nuclear receptor binding protein 1 [Bos taurus]	532919	1.02	2.03		
TAF9	TAF9 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 32kDa [Bos taurus]	532936	1.04	2.06	03022	Basal transcription factors
DPYSL2	dihydropyrimidinase-like 2 [Bos taurus]	533746	1.12	2.18	04360	Axon guidance
CCNDBP1	cyclin D-type binding-protein 1 [Bos taurus]	617512	1.06	2.09		
Immune System Process						
PSME1	proteasome (prosome, macropain) activator subunit 1 (PA28 alpha) [Bos taurus]	510041	1.02	2.02	04612, 03050	Antigen processing and presentation, Proteasome
Other						

COL1A2	collagen, type I, alpha 2 [Bos taurus]	282188	1.01	2.01	04512, 04510	ECM-receptor interaction, Focal adhesion
COL1A2	collagen, type I, alpha 2 [Bos taurus]	282188	1.40	2.64	04512, 04510	ECM-receptor interaction, Focal adhesion
COL1A2	collagen, type I, alpha 2 [Bos taurus]	282188	1.67	3.18	04512, 04510	ECM-receptor interaction, Focal adhesion
COL18A1	collagen, type XVIII, alpha 1 [Bos taurus]	508076	1.83	3.56		
LOC516723	tripartite motif-containing 39-like [Bos taurus]	516723	1.61	3.05		
PRUNE2	prune homolog 2 (Drosophila) [Bos taurus]	518308	1.43	2.69		
MAPK14	mitogen-activated protein kinase 14 [Bos taurus]	534492	1.02	2.03	05014, 05142, 04664, 04912, 05140, 04670, 04010, 04621, 04722, 04914, 04622, 04660, 04620, 04370	Amyotrophic lateral sclerosis (ALS), Chagas disease, Fc epsilon RI signaling pathway, GnRH signaling pathway, Leishmaniasis, Leukocyte transendothelial migration, MAPK signaling pathway, NOD-like receptor signaling pathway, Neurotrophin signaling pathway, Progesterone-mediated oocyte maturation, RIG-I-like receptor signaling pathway, T cell receptor signaling pathway, Toll-like receptor signaling

						pathway, VEGF signaling pathway
DTNA	dystrobrevin, alpha [Bos taurus]	541153	1.32	2.49		
RYBP	RING1 and YY1 binding protein [Bos taurus]	616217	1.08	2.11		
GSTM1	glutathione S-transferase mu 1 [Bos taurus]	783879	1.53	2.88	00982, 00480, 00980	Drug metabolism-cytochrome P450, Glutathione metabolism, Metabolism of xenobiotics by cytochrome P450
LOC511937/ ANXA1	annexin A1-like [Bos taurus], annexin A1 [Bos taurus]	511937, 327662	1.09	2.12		

Table 4.8. Networks generated for longissimus and semitendinosus muscles from Angus steers using Ingenuity Pathways Analysis (Ingenuity® Systems, www.ingenuity.com)[1,2]

Network ID	Molecules in Network	Score	Focus Molecules	Top Functions
1	Angiotensin II receptor type 1, ↓**ANPEP**, ↑**ANXA1**, Ap1, APLP1, ↑**CCNDBP1**, chondroitin sulfate, ↑**COL18A1**, ↑**COL1A2**, Collagen type I, ↑**DPYSL2**, EFNA1, EGLN1, ERK, F11, HIF1AN, HPSE, Integrin alpha 2 beta 1, ITGB1BP1, LGALS7, Lh, ↑**MAPK14**, MT3, NFkB (complex), NID2, PECAM1, Pkc(s), PMCH, ↑**PRUNE2**, ↓**PTH**, RGS1, TFPI2, THBS4, Vegf, ZBTB7B	22	9	Cardiovascular System Development and Function, Cell Signaling, Molecular Transport
2	CEBPZ, CLEC2A, CUL9, ↑**DTNA**, ↑**EIF2B4**, GSTA5, ↑**GSTM1**, GSTM2, IL6, MYC, NAE1, ↑**NEDD8**, P4HA1, ↑**PSME1**, retinoic acid, RPL5, RPL6, RPL7, RPL11, RPL13, RPL23, RPL26, RPL27, RPL21 (includes EG:6144), RPL7A (includes EG:6130), RPS7, RPS13, RPS16, RPS20, RPS23, RPS4X, TADA1, ↑**TAF9**, ↑**TKT**, TP53	16	7	Protein Synthesis, RNA Post-Transcriptional Modification, Cellular Assembly and Organization
3	C1ORF103, ERBB2, MBP, MLF1, ↑**NRBP1**, RAC3, RIBC2, RIF1, TGFB1, TSC22D1, TSC22D4	2	1	Cellular Development, Respiratory System Development and Function, Immunological Disease

[1]

[2]Differentially expressed genes are highlighted in bold text with (↑) indicating up-regulation and (↓) indicating down-regulation.

Table 4.9. Gene symbol, gene name, Entrez gene ID, M-value, fold-change, GO/KEGG ID, and GO term/KEGG pathway for the differentially expressed genes in longissimus and semitendinosus muscles from Charolais steers

Gene Symbol	Gene Name	Entrez Gene ID	M	Fold Change	(GO)/KEGG Pathway	Term/Pathway
Cellular Process						
QPCT	glutaminyl-peptide cyclotransferase [Bos taurus]	281437	1.18	2.26		
CPSF3	cleavage and polyadenylation specific factor 3, 73kDa [Bos taurus]	281712	1.03	2.04		
PAFAH1B1	platelet-activating factor acetylhydrolase 1b, regulatory subunit 1 (45kDa) [Bos taurus]	282513	1.48	2.79	00565, 01100	Ether lipid metabolism, Metabolic pathways
ASB15	ankyrin repeat and SOCS box-containing 15 [Bos taurus]	282591	1.32	2.50		
NDUFS3	NADH dehydrogenase (ubiquinone) Fe-S protein 3, 30kDa (NADH-coenzyme Q reductase) [Bos taurus]	287327	1.30	2.46	05010, 05016, 01100, 00190, 05012	Alzhemier's disease, Huntington's disease, Metabolic pathways, Oxidative phosphorylation, Parkinson's disease
HDAC2	histone deacetylase 2 [Bos taurus]	407223	1.06	2.09	04110, 05220, 05016, 04330, 05200	Cell cycle, Chronic myeloid leukemia, Huntington's disease, Notch signaling pathway, Pathways in cancer
RPA1	replication protein A1, 70kDa [Bos taurus]	504844	1.05	2.07	03030, 03440, 03430, 03420	DNA replication, Homologous recombination, Mismatch repair, Nucleotide excision repair
BCKDK	branched chain ketoacid dehydrogenase kinase [Bos taurus]	505005	-1.01	-2.02		
CCT2	chaperonin containing TCP1, subunit 2 (beta) [Bos taurus]	505313	1.27	2.41		
MLX	MAX-like protein X [Bos taurus]	508531	1.08	2.11		

PALMD	palmdelphin [Bos taurus]	509823	1.07	2.10		
NEDD4L	neural precursor cell expressed, developmentally down-regulated 4-like [Bos taurus]	510003	1.23	2.35	04960, 04144, 04120	Aldosterone-regulated sodium reabsorption, Endocytosis, Ubiquitin mediated proteolysis
GNS	glucosamine (N-acetyl)-6-sulfatase [Bos taurus]	512444	1.14	2.20	00531, 04142, 01100	glycosaminoglycan degredation, Lysosome, Metabolic pathways
WWP1	WW domain containing E3 ubiquitin protein ligase 1 [Bos taurus]	513789	1.28	2.42	04144, 04120	Endocytosis, Ubiquitin mediated proteolysis
RPL17	ribosomal protein L17 [Bos taurus]	514046	1.15	2.21	03010	Ribosome
AFG3L2	AFG3 ATPase family gene 3-like 2 (yeast) [Bos taurus]	515757	1.01	2.01		
ADPRHL1	ADP-ribosylhydrolase like 1 [Bos taurus]	519627	2.38	5.22		
HIST1H2BD	histone 1, H2bi [Bos taurus]	519935	1.19	2.29	05322	Systemic lupus erythematosus
TIMM9	translocase of inner mitochondrial membrane 9 homolog (yeast) [Bos taurus]	521073	1.15	2.22		
RNF11	ring finger protein 11 [Bos taurus]	522791	1.82	3.52		
PSPH	phosphoserine phosphatase [Bos taurus]	533630	1.60	3.03	00260, 01100	Glycine, serine and thronine metabolism, Metabolic pathways
HMBOX1	homeobox containing 1 [Bos taurus]	539099	1.22	2.32		
TXNDC9	thioredoxin domain containing 9 [Bos taurus]	539143	1.84	3.59		
CHMP5	chromatin modifying protein 5 [Bos taurus]	539781	1.34	2.54	04144	Endocytosis
ZNF148	zinc finger protein 148 [Bos taurus]	613265	1.66	3.17		
GTF2A2	general transcription factor IIA, 2, 12kDa [Bos taurus]	613479	1.06	2.08	03022	Basal transcription factors
CXCL12	chemokine (C-X-C motif) ligand 12 [Bos taurus]	613811	1.40	2.65	04062, 04060, 04672, 04670	Chemokine signaling pathway, Cytokine-cytokine receptor interaction, Intestinal immune network for

						Iga production, Leukocyte transendothelial migration
ENY2	enhancer of yellow 2 homolog (Drosophila) [Bos taurus]	614069	1.38	2.61		
RPP30	ribonuclease P/MRP 30kDa subunit [Bos taurus]	615098	1.18	2.26		
ADI1	acireductone dioxygenase 1 [Bos taurus]	615764	1.12	2.17	00270, 01100	Cysteine and methionine metabolism, Metabolic pathways
CISD2	CDGSH iron sulfur domain 2 [Bos taurus]	781260	1.02	2.03		
Establishment of Localization						
ATP6V1C1	ATPase, H+ transporting, lysosomal 42kDa, V1 subunit C1 [Bos taurus]	338089	1.27	2.42	04966, 01100, 00190	Collecting duct acid secretion, Metabolic pathways, Oxidative phosphorylation
ATP1B3	ATPase, Na+/K+ transporting, beta 3 polypeptide [Bos taurus]	532844	1.10	2.14	04960, 04260, 04964	Aldosterone-regulated sodium reabsorption, Cardiac muscle contraction, Proximal tubule bicarbonate reclamation
SEC62	SEC62 homolog (S. cerevisiae) [Bos taurus]	538938	1.39	2.62	03060	Protein export
Immune System Process						
ILF2	interleukin enhancer binding factor 2, 45kDa [Bos taurus]	539760	1.00	2.01		
Metabolic Process						
DHRS12	dehydrogenase/reductase (SDR family) member 12 [Bos taurus]	507276	1.07	2.10		
AADAT	aminoadipate aminotransferase [Bos taurus]	508929	1.35	2.54	00310, 01100, 00380	Lysine degredation, Metabolic pathways, Tryptophan metabolism
METTL7A	methyltransferase like 7A [Bos taurus]	613844	1.18	2.26		

Other						
PLP2	proteolipid protein 2 (colonic epithelium-enriched) [Bos taurus]	399683	1.07	2.10		
PPP1R7	protein phosphatase 1, regulatory (inhibitor) subunit 7 [Bos taurus]	505297	1.12	2.17		
OAT	ornithine aminotransferase [Bos taurus]	505323	1.94	3.83	00330, 01100	Arginine and proline metabolism, Metabolic pathways
BPHL	biphenyl hydrolase-like (serine hydrolase) [Bos taurus]	505457	1.11	2.16		
COL9A2	collagen, type IX, alpha 2 [Bos taurus]	505942	1.21	2.31		
WBP4	WW domain binding protein 4 (formin binding protein 21) [Bos taurus]	506275	1.27	2.42		
MFF	mitochondrial fission factor [Bos taurus]	506291	1.12	2.17		
COL16A1	collagen, type XVI, alpha 1 [Bos taurus]	507010	1.24	2.36		
USP9X	ubiquitin specific peptidase 9, X-linked [Bos taurus]	507307	1.09	2.13		
EMP2	epithelial membrane protein 2 [Bos taurus]	507667	1.19	2.28		
TM2D2	TM2 domain containing 2 [Bos taurus]	507906	1.09	2.13		
C22H3ORF64	chromosome 3 open reading frame 64 ortholog [Bos taurus]	508782	1.24	2.37		
LOC508963	hypothetical LOC508963 [Bos taurus]	508963	-1.24	-2.36		
HAGH	hydroxyacylglutathione hydrolase [Bos taurus]	509274	1.20	2.30	00620	Pyruvate metaboilism
WRB	tryptophan rich basic protein [Bos taurus]	509739	1.50	2.82		
ZNF419	zinc finger protein 419 [Bos taurus]	510702	1.15	2.22		
TNFRSF10A	tumor necrosis factor receptor superfamily, member 10a [Bos taurus]	510798	1.12	2.18		
DDX1	DEAD (Asp-Glu-Ala-Asp) box polypeptide 1 [Bos taurus]	510816	1.01	2.01		
FAM114A1	family with sequence similarity 114, member A1 [Bos taurus]	511198	1.45	2.74		
LOC515452	hypothetical protein LOC515452 [Bos taurus]	515452	1.25	2.37		

LOC516723	tripartite motif-containing 39-like [Bos taurus]	516723	1.04	2.05		
WDR13	WD repeat domain 13 [Bos taurus]	523881	1.53	2.89		
DKC1	dyskeratosis congenita 1, dyskerin [Bos taurus]	525619	-1.13	-2.19		
BBS5	Bardet-Biedl syndrome 5 [Bos taurus]	528191	1.16	2.24		
CETN3	centrin, EF-hand protein, 3 (CDC31 homolog, yeast) [Bos taurus]	532953	1.32	2.50		
GBP6	guanylate binding protein family, member 6 [Bos taurus]	533657	1.06	2.08		
SNX14	sorting nexin 14 [Bos taurus]	533801	1.78	3.43		
SLIT2	slit homolog 2 (Drosophila) [Bos taurus]	534164	-1.30	-2.46	04360	Axon guidance
MRPS31	mitochondrial ribosomal protein S31 [Bos taurus]	534185	1.03	2.04		
NHLRC2	NHL repeat containing 2 [Bos taurus]	534327	-1.66	-3.17		
INSIG2	insulin induced gene 2 [Bos taurus]	534440	1.15	2.22		
TPD52L2	tumor protein D52-like 2 [Bos taurus]	534704	1.07	2.09		
GALNT11	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 11 (GalNAc-T11) [Bos taurus]	534904	1.57	2.96	01100, 00512	Metabolic pathways, O-Glycan biosynthesis
AIFM1	apoptosis-inducing factor, mitochondrion-associated, 1 [Bos taurus]	535714	1.03	2.04	04210	Apoptosis
MGC159911	UPF0636 protein C4orf41 homolog [Bos taurus]	537211	1.15	2.22		
DOCK11	dedicator of cytokinesis 11 [Bos taurus]	537297	1.20	2.30		
LOC538590	collagen alpha-1(II) chain-like [Bos taurus]	538590	1.56	2.95		
PVALB	parvalbumin [Bos taurus]	538603	1.20	2.30		
C5H12orf23	chromosome 12 open reading frame 23 ortholog [Bos taurus]	539347	1.14	2.20		
ABRA	actin-binding Rho activating protein [Bos taurus]	539379	1.02	2.03		
ANGEL2	angel homolog 2 (Drosophila) [Bos taurus]	540507	1.12	2.17		
CRTAP	cartilage associated protein [Bos taurus]	540924	1.16	2.23		

CGGBP1	CGG triplet repeat binding protein 1 [Bos taurus]	613824	1.44	2.72		
LOC615412	BAI1-associated protein 2-like 1-like [Bos taurus]	615412	1.05	2.08		
C10H15orf24	chromosome 15 open reading frame 24 ortholog [Bos taurus]	615423	1.14	2.20		
LSM6	LSM6 homolog, U6 small nuclear RNA associated (S. cerevisiae) [Bos taurus]	615916	1.26	2.40	03018, 03040	RNA degredation, Splicesome
LOC616206	NEZHA-like isoform 2 [Bos taurus]	616206	1.50	2.83		
LOC616557	chromosome 10 open reading frame 76-like [Bos taurus]	616557	1.00	2.00		
DUSP16	dual specificity phosphatase 16 [Bos taurus]	618644	1.33	2.51	04010	MAPK signaling pathway
C29H11orf10	chromosome 11 open reading frame 10 ortholog [Bos taurus]	767958	1.29	2.44		
BEX2	brain expressed X-linked 2 [Bos taurus]	768028	1.69	3.23		
CSTF2T	cleavage stimulation factor, 3' pre-RNA, subunit 2, 64kDa, tau variant [Bos taurus]	783569	1.39	2.61		
YPEL3	yippee-like 3 (Drosophila) [Bos taurus]	787498	1.10	2.14		
FARSB	henylalanyl-tRNA synthetase, beta subunit [Bos taurus]	788792	1.12	2.18	00970	Aminoacyl-tRNA biosynthesis
LIX1L	Lix1 homolog (mouse)-like [Bos taurus]	788858	-1.42	-2.67		
WHSC2	Wolf-Hirschhorn syndrome candidate 2 [Bos taurus]	789389	1.05	2.07		
LOC100190887	hypothetical protein LOC100190887 [Bos taurus]	1E+08	1.52	2.88		
LOC100302586	hypothetical protein LOC100302586 [Bos taurus]	1E+08	1.55	2.93		

Table 4.10. Networks generated for longissimus and semitendinosus muscles from Charolais steers using Ingenuity Pathways Analysis (Ingenuity® Systems, www.ingenuity.com)[1,2]

Network ID	Molecules in Network	Score	Focus Molecules	Top Functions
1	↑**ADPRHL1**, ↑**ANGEL2**, ARRB2, ↑**C11ORF10**, CCT8, CDKN1A, ↑**CETN3**, ↑**CRTAP**, ↑**CSTF2T**, CYTH2, DLG4, FXC1, GH1, ↑**GNS**, GRB2, ↑**HIST1H2BD**, HNF4A, IMMT, Insulin, KHDRBS1, ↑**KIAA1543**, MAP3K3, NCK1, ↑**PALMD**, SETDB1, SRC, ↑**TIMM9**, TOE1, TRAF6, TUBB3, TUBB4, ↑**TXNDC9**, UBQLN1, ↑**WDR13**	25	13	Cancer, Skeletal and Muscular Disorders, Cellular Development
2	↑**ADI1**, beta-estradiol, ↑**BPHL**, ↑**C12ORF23**, CAP2, CDKN1A, ↑**CISD2**, COBRA1, COMP, ↑**CPSF2**, CPSF3, ERH, GH1, ↑**GNS**, GREM1, GTF2A1, ↑**GTF2A2**, GTF2B, ↑**HMBOX1**, HNRPDL, ↑**INSIG2**, MIR290 (includes EG:100049710), ↑**NEDD4L**, OSTF1, ↑**PSPH**, RDBP, STC2, SYMPK, TBPL1, TFIIE, TFIIF, TGFB1, ↑**WHSC2**, WWOX, ↑**ZNF148**	25	13	Cell Cycle, Cardiac Output, Cardiovascular System Development and Function
3	APLF, ATP, ↑**ATP1B3**, BCKDHB, ↓**BCKDK**, Ck2, ↑**COL9A2**, CPE, Cytoplasmic Dynein, DBT, ↑**DDX1**, DHX15, ↓**DKC1**, ↑**DUSP16**, Dynein, ↑**GALNT11**, KATNA1, MYC, Na-k-atpase, NOLC1, NR3C1, NUDC, ↑**OAT**, OPA1, ↑**PAFAH1B1**, PTP4A3, retinoic acid, Rfc, ↑**RPA1**, ↑**RPP30**, SSR1, ↑**TM2D2**, TOP2B, TPD52, ZFP36	22	12	Cancer, Cellular Assembly and Organization, DNA Replication, Recombination, and Repair
4	↑**ABRA**, ↑**AFG3L2**, ↑**AIFM1**, APPBP2, ↑**ATP6V1C1**, BCAP31, BIRC6, BUB3, Calcineurin protein(s), CASP3, Cathepsin, ↑**CHMP5**, cholesterol, cyanide, DHX15, ↑**DOCK11**, EPN1, GNLY, HIP1, HLTF, HTT, IFT57, ↓**LIX1L**, MIR206 (includes EG:406989), MLF2, NDUFA9 (includes EG:4704), NDUFS1, ↑**NDUFS3**, NDUFS5, NDUFV2, ↑**PVALB**, SNRNP70, TNFRSF1A, ↑**USP9X**, ↑**WBP4**	20	11	Cell Death, Cellular Compromise, Embryonic Development
5	APPL2, CHI3L1, ↑**COL16A1**, CTNNB1, DEK, EIF4E, ↑**EMP2**, ↓**FAM105B**, Groucho, ↑**HAGH**, ↑**HDAC2**, IGF2BP1, IKBKE, IL1B, ↑**ILF2 (includes EG:3608),** LSM2, LSM4, LSM5, ↑**LSM6**, LSM7, MT1E, OGG1, ↑**PLP2**, POLD2, PPP1CB, ↑**PPP1R7**, PPP1R8, ↑**QPCT (includes EG:25797),** SF1, TFAP4, TNF, TNIP1, TP73, TPD52, ↑**TPD52L2**	19	11	Molecular Transport, Gene Expression, Cell Death
6	ACTB, ALB, beta-estradiol, ↑**BEX2**, ↑**C4ORF41**, CAP2, ↑**CCT2**, ↑**ENY2**, GPC1, GREM1, Hat, LAPTM5, ↑**MRPS31**, MYO6, ↑**NEDD4L**, NFkB (complex), OPTN, PACRG, PPP2R2B, PPP2R2C, ↑**RNF11**, RNF216, ↑**RPL17**, RPS27, RPS27A (includes EG:6233), SCN10A, SCN5A, SFTPC, ↓**SLIT2**, TAX1BP1, TNIP1, TRRAP, USP22, ↑**WWP1**, ZNF638	18	10	Cell-To-Cell Signaling and Interaction, Tissue Development, Cardiovascular Disease
7	↑**C11ORF83**, PHLDA3	2	1	DNA Replication, Recombination, and Repair
8	↑**METTL7A**, MME	2	1	Cancer, Cell Death, Neurological Disease
9	SLC4A1, ↑**WRB**	2	1	Cell Morphology, Cellular Compromise, Molecular Transport
10	↑**CGGBP1**, FMR1, SUV39H2	2	1	Connective Tissue Development and Function, Nervous System Development and Function, Skeletal and Muscular System Development and Function
11	CIC, MIRN101B, RHOJ, ↑**SEC62**	2	1	Connective Tissue Disorders, Inflammatory Disease, Skeletal and Muscular Disorders

[1]

[2]Differentially expressed genes are highlighted in bold text with (↑) indicating up-regulation and (↓) indicating down-regulation.

Figure 4.1. Network 1 generated for longissimus muscle from Angus and Charolais steers using Ingenuity Pathways Analysis (Ingenuity® Systems, www.ingenuity.com). Depicted genes are involved in: Cell Cycle, Connective Tissue Development and Function, and Lipid Metabolism. Node color indicates expression level of gene: red = up-regulated in Charolais vs. Angus, green = down-regulated in Charolais vs. Angus.

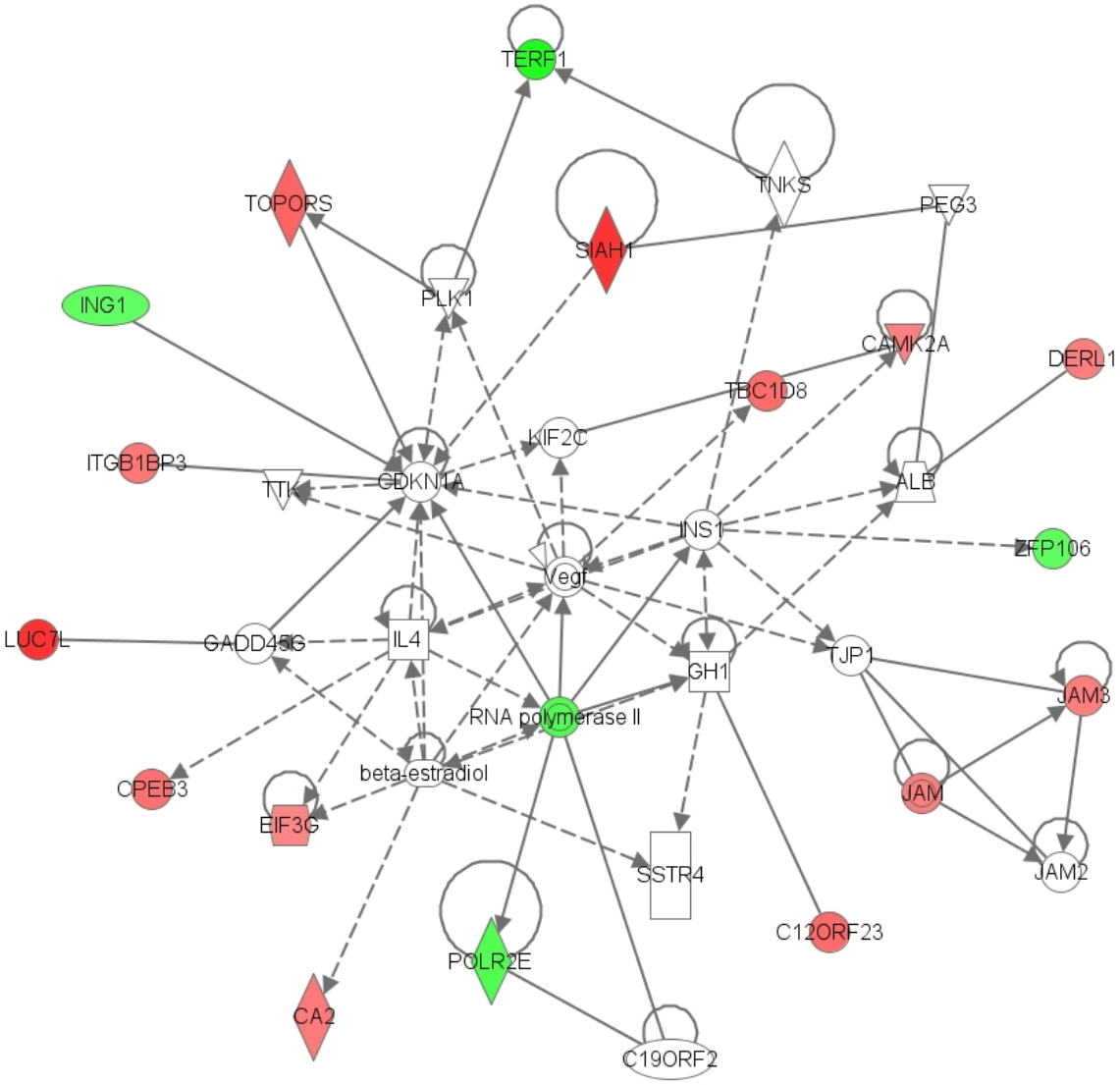


Figure 4.2. Network 2 generated for longissimus muscle from Angus and Charolais steers using Ingenuity Pathways Analysis (Ingenuity® Systems, www.ingenuity.com). Depicted genes are involved in: Cellular Movement, Hematological System Development and Function, Immune Cell Trafficking. Node color indicates expression level of gene: red = up-regulated in Charolais vs. Angus, green = down-regulated in Charolais vs. Angus.

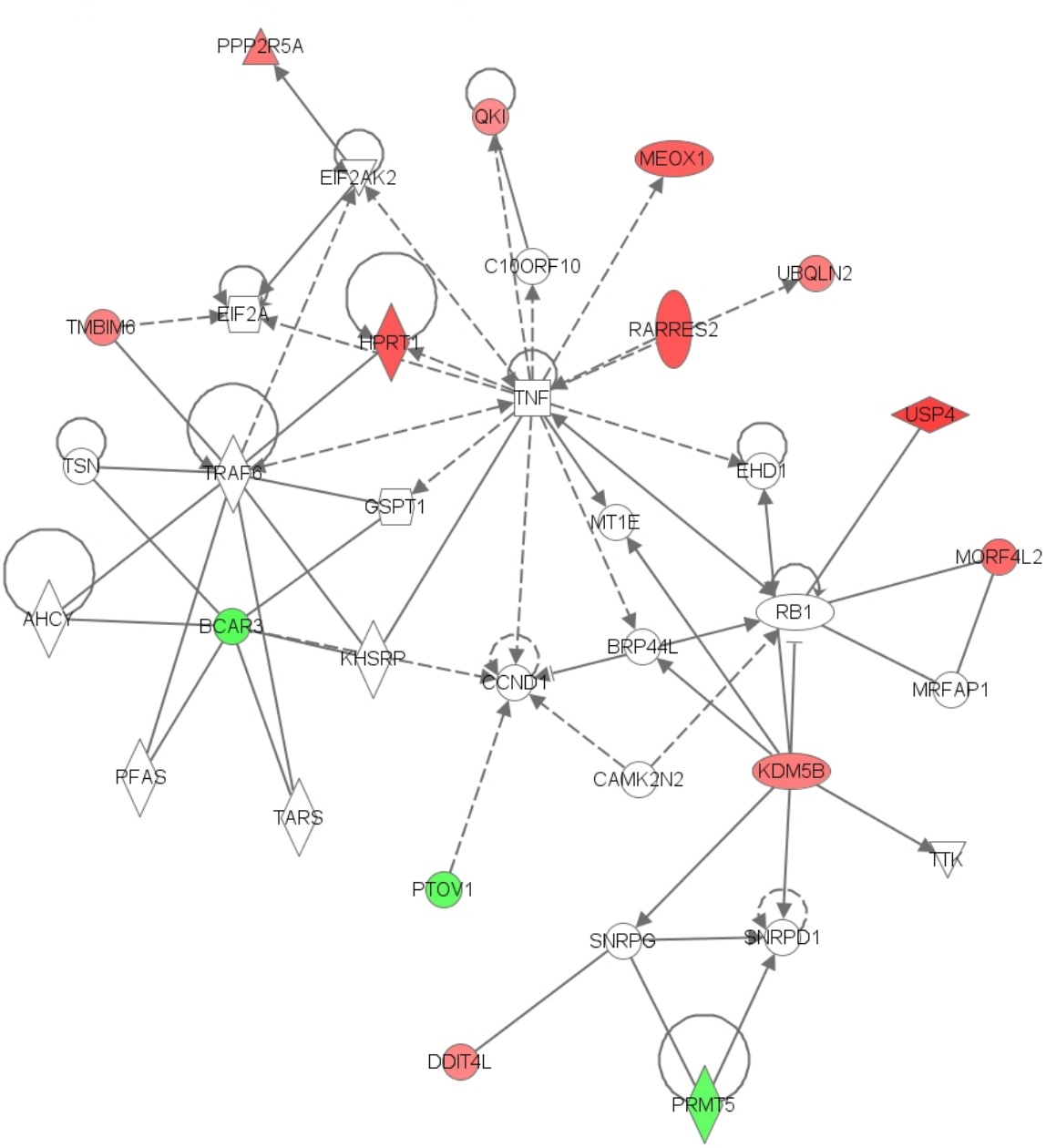


Figure 4.3. Molecular and cellular functions associated with all generated networks for longissimus muscle from Angus and Charolais steers using Ingenuity Pathways Analysis (Ingenuity® Systems, www.ingenuity.com). Threshold significance ($P < 0.05$) expressed as the $-\log_{10}$ of the P-value calculated using the right-tailed Fischer's exact test, and is represented by the orange horizontal line. Numbers above each bar are the number of genes associated with that function.

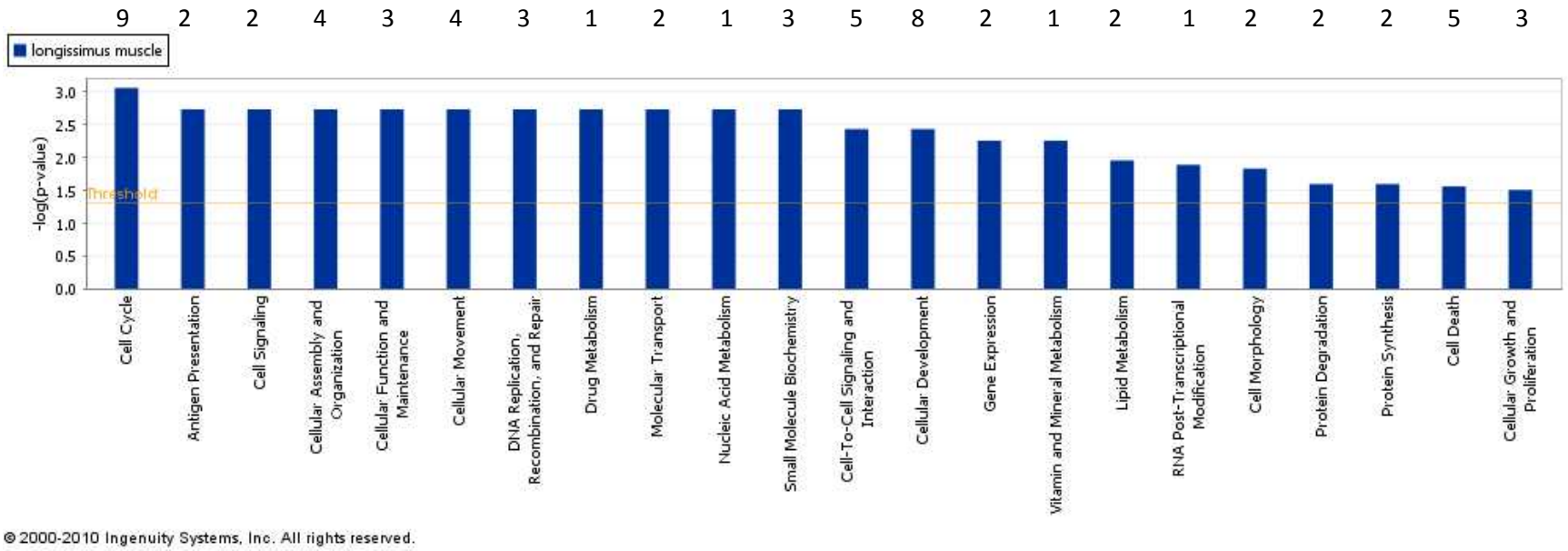


Figure 4.4. Network 1 generated for semitendinosus muscle from Angus and Charolais steers using Ingenuity Pathways Analysis (Ingenuity® Systems, www.ingenuity.com). Depicted genes are involved in: Cell Cycle, Cell Death, Connective Tissue Disorders. Node color indicates expression level of gene: red = up-regulated in Charolais vs. Angus, green = down-regulated in Charolais vs. Angus.

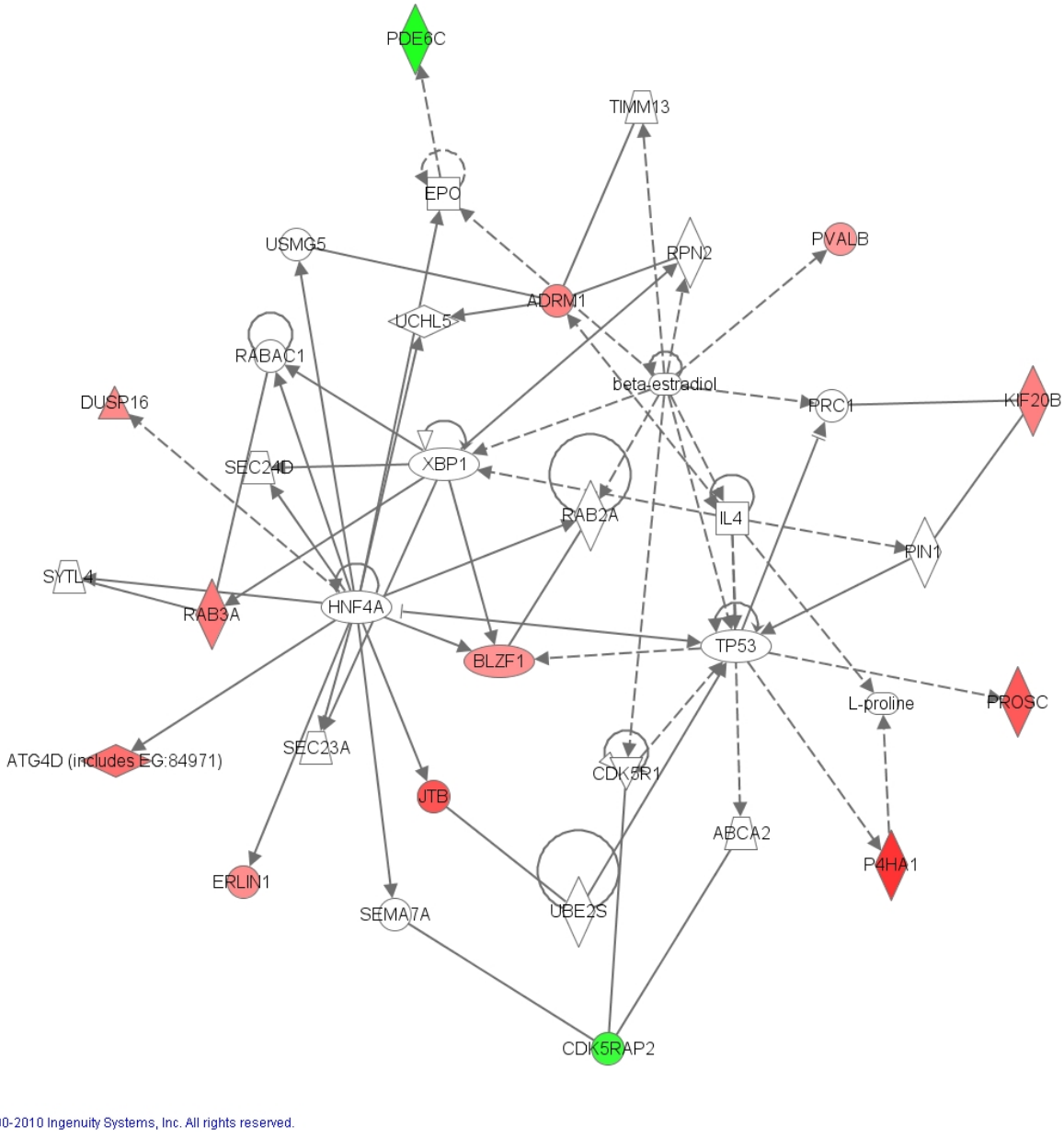


Figure 4.5. Molecular and cellular functions associated with all generated networks for semitendinosus muscle from Angus and Charolais steers using Ingenuity Pathways Analysis (Ingenuity® Systems, www.ingenuity.com). Threshold significance ($P < 0.05$) expressed as the $-\log_{10}$ of the P-value calculated using the right-tailed Fischer's exact test, and is represented by the orange horizontal line. Numbers above each bar are the number of genes associated with that function.

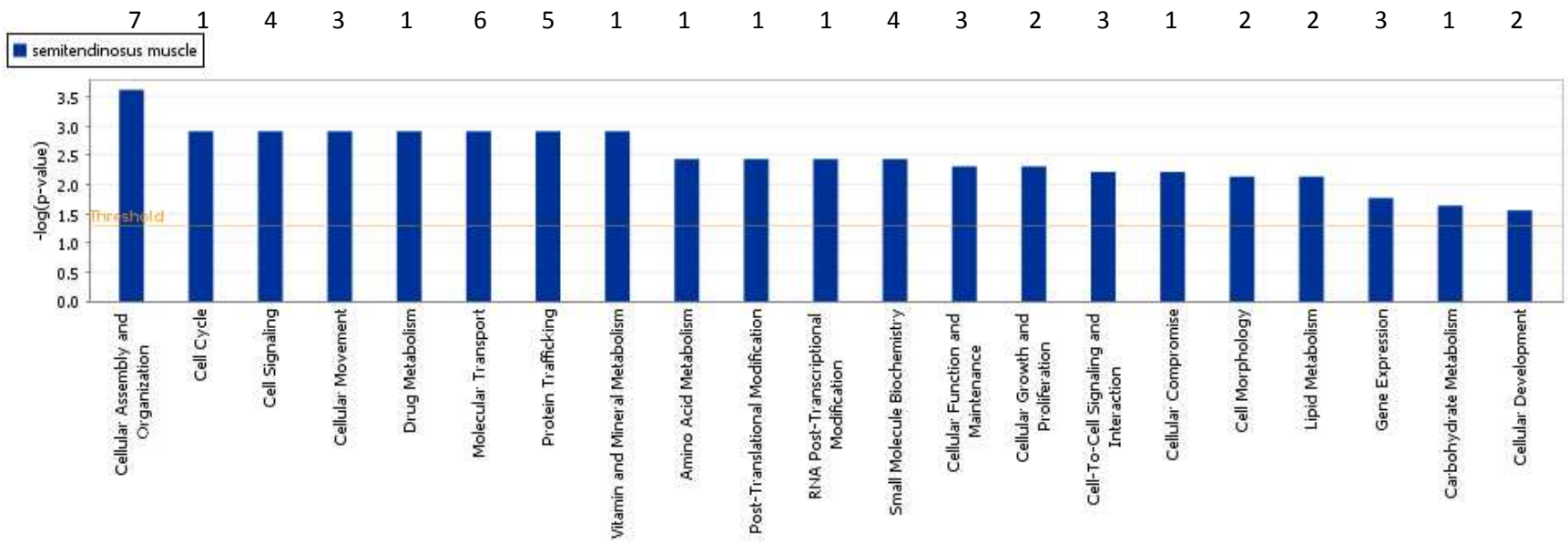


Figure 4.6. Network 1 generated for longissimus and semitendinosus muscles from Angus steers using Ingenuity Pathways Analysis (Ingenuity® Systems, www.ingenuity.com). Depicted genes are involved in: Cardiovascular System Development and Function, Cell Signaling, Molecular Transport. Node color indicates expression level of gene: red = up-regulated in longissimus muscle vs. semitendinosus muscle, green = down-regulated in longissimus muscle vs. semitendinosus muscle.

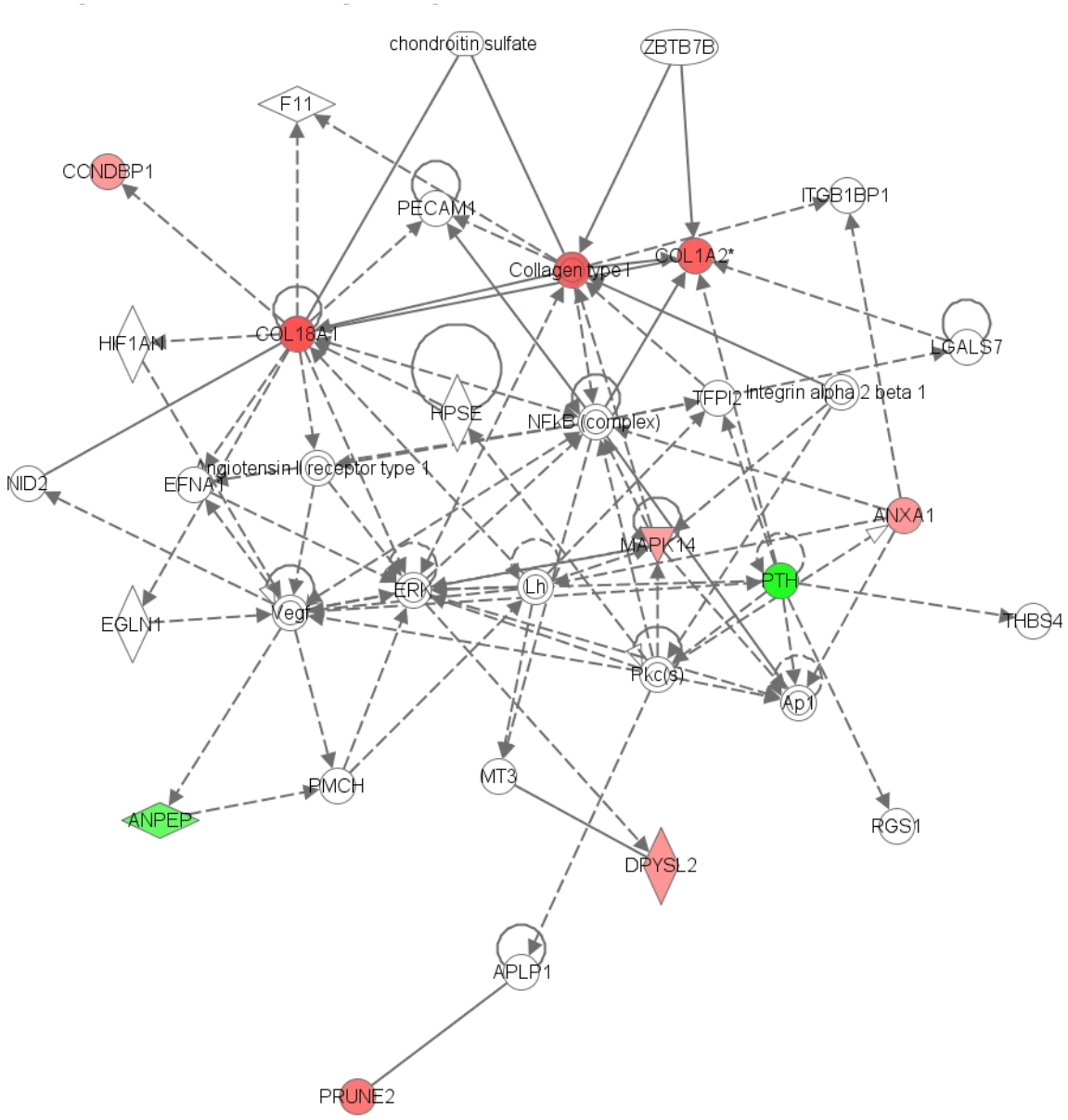


Figure 4.7. Molecular and cellular functions associated with all generated networks for longissimus and semitendinosus muscles from Angus steers using Ingenuity Pathways Analysis (Ingenuity® Systems, www.ingenuity.com). Threshold significance ($P < 0.05$) expressed as the $-\log_{10}$ of the P-value calculated using the right-tailed Fischer's exact test, and is represented by the orange horizontal line. Numbers above each bar are the number of genes associated with that function.

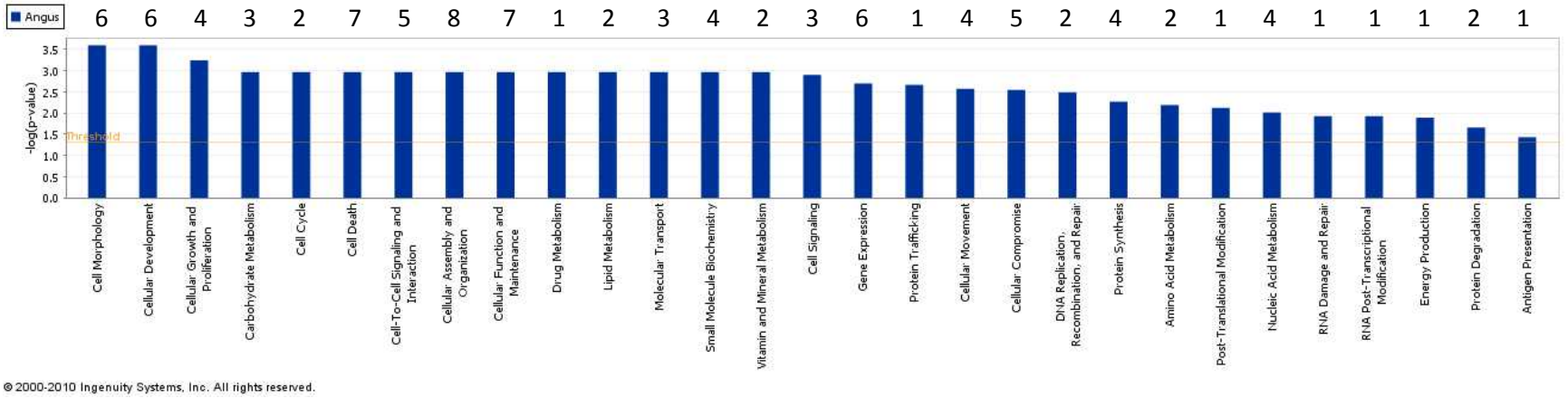


Figure 4.8. Network 1 generated for longissimus and semitendinosus muscles from Charolais steers using Ingenuity Pathways Analysis (Ingenuity® Systems, www.ingenuity.com). Depicted genes are involved in: Cancer, Skeletal and Muscular Disorders, Cellular Development. Node color indicates expression level of gene: red = up-regulated in longissimus muscle vs. semitendinosus muscle, green = down-regulated in longissimus muscle vs. semitendinosus muscle.

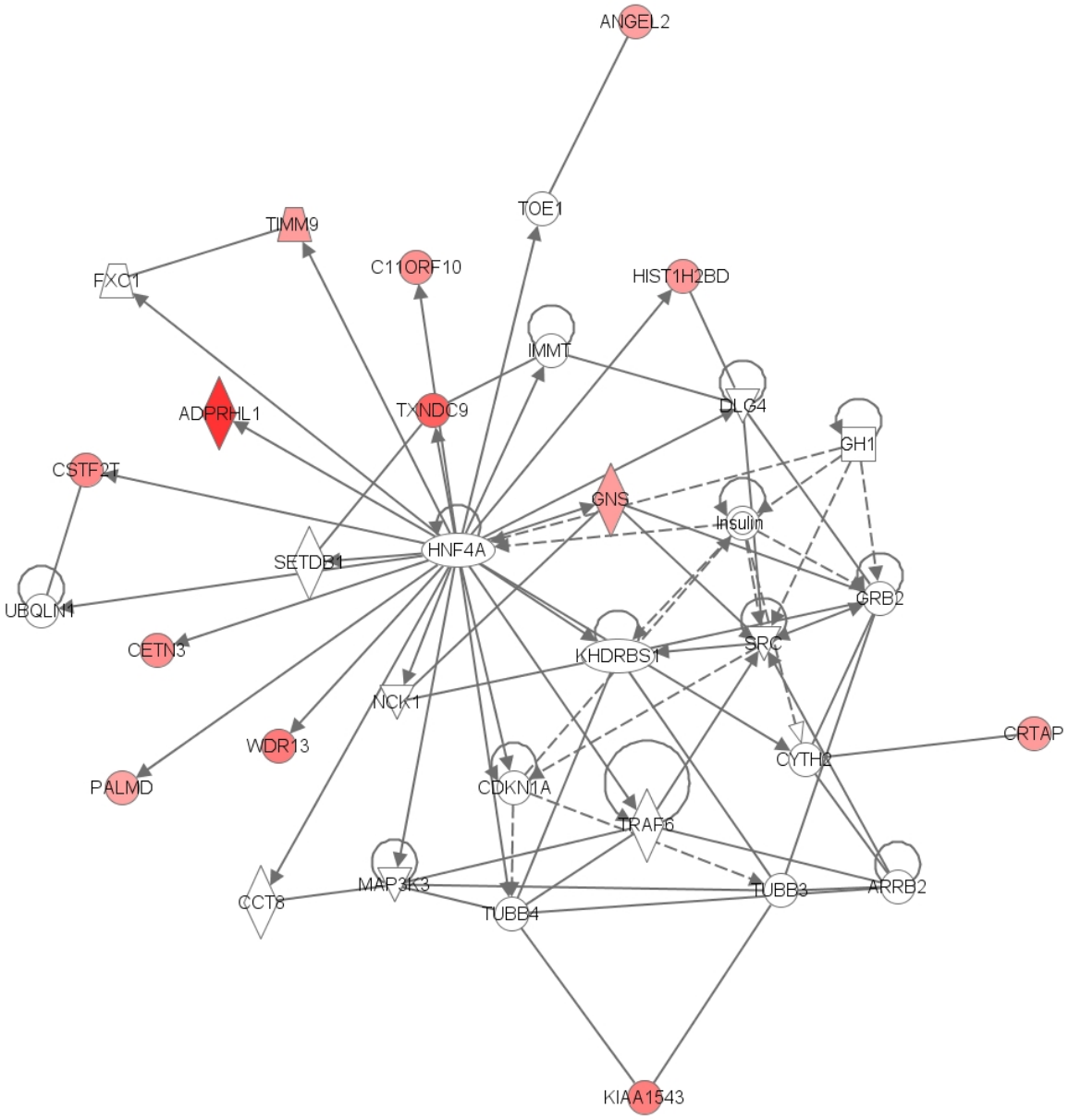


Figure 4.9. Network 2 generated for longissimus and semitendinosus muscles from Charolais steers using Ingenuity Pathways Analysis (Ingenuity® Systems, www.ingenuity.com). Depicted genes are involved in: Cell Cycle, Cardiac Output, Cardiovascular System Development and Function. Node color indicates expression level of gene: red = up-regulated in longissimus muscle vs. semitendinosus muscle, green = down-regulated in longissimus muscle vs. semitendinosus muscle.

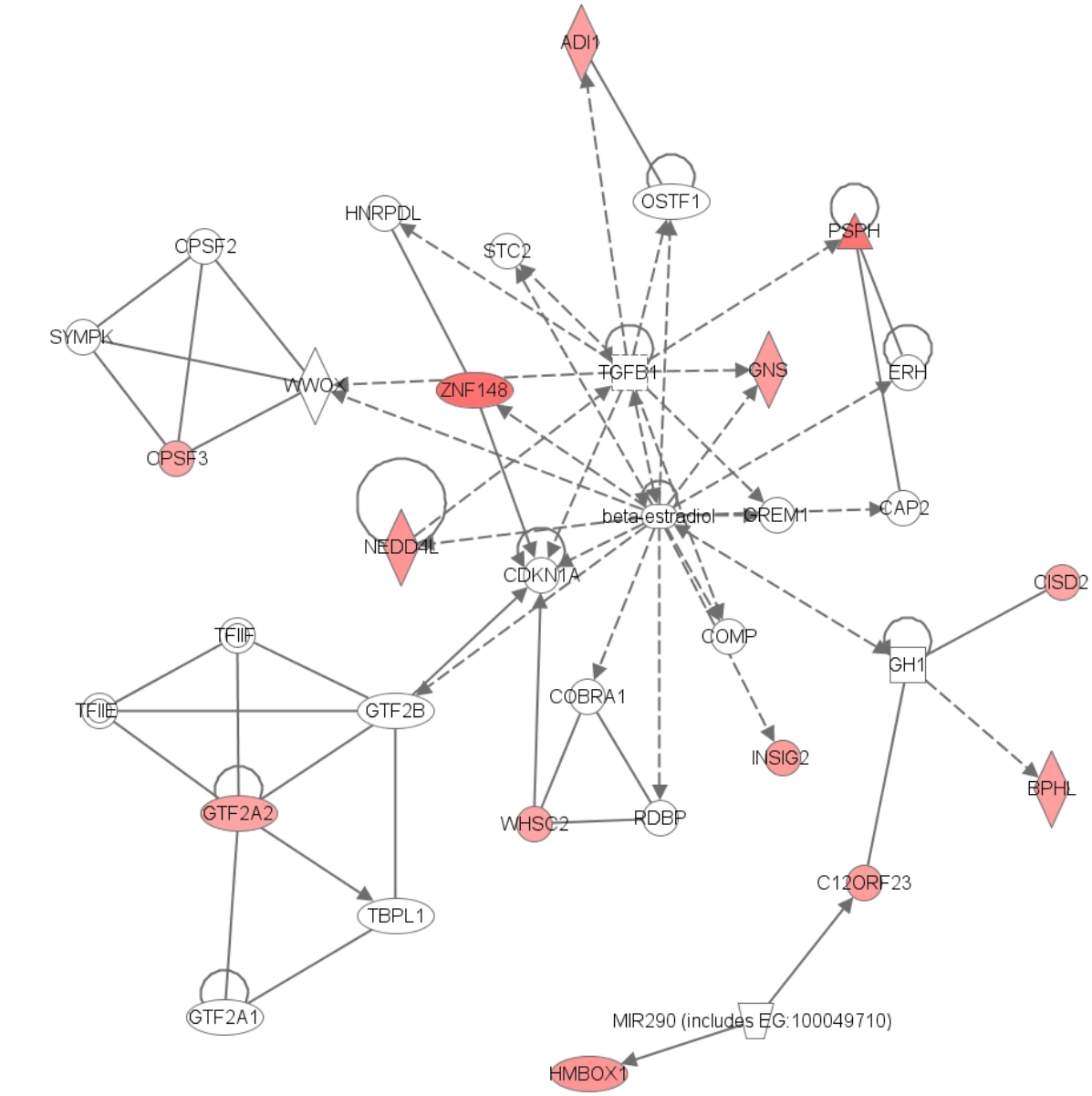


Figure 4.10. Network 3 generated for longissimus and semitendinosus muscles from Charolais steers using Ingenuity Pathways Analysis (Ingenuity® Systems, www.ingenuity.com). Depicted genes are involved in: Cancer, Cellular Assembly and Organization, DNA Replication, Recombination, and Repair. Node color indicates expression level of gene: red = up-regulated in longissimus muscle vs. semitendinosus muscle, green = down-regulated in longissimus muscle vs. semitendinosus muscle.

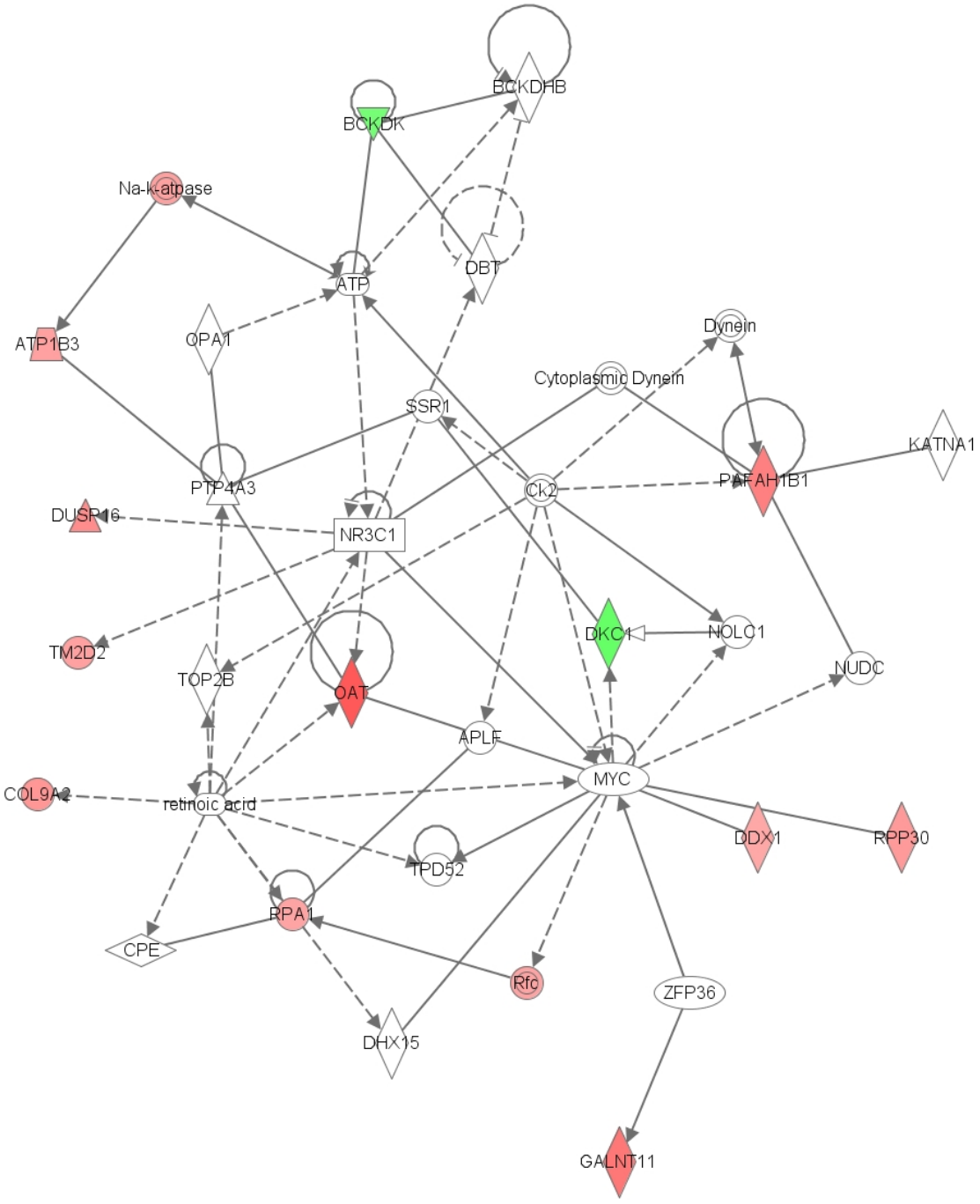
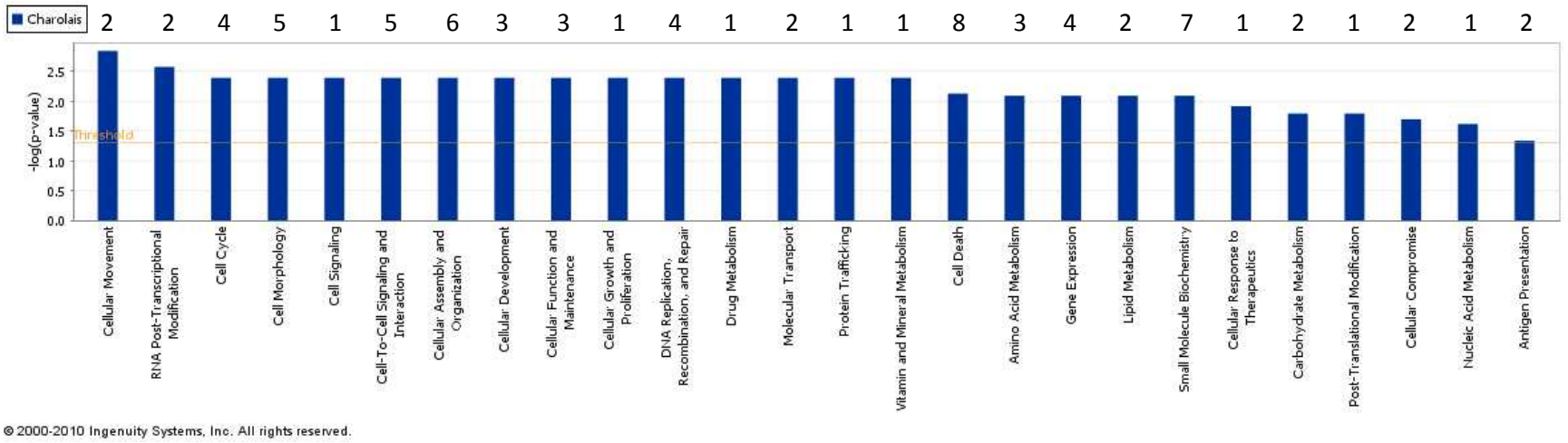


Figure 4.11. Molecular and cellular functions associated with all generated networks for longissimus and semitendinosus muscles from Charolais steers using Ingenuity Pathways Analysis (Ingenuity® Systems, www.ingenuity.com). Threshold significance ($P < 0.05$) expressed as the $-\log_{10}$ of the P-value calculated using the right-tailed Fischer's exact test, and is represented by the orange horizontal line. Numbers above each bar are the number of genes associated with that function.



VITA

Andrea Karen Sexten

Candidate for the Degree of Doctor of Philosophy

Thesis: EFFECT OF BREED, TISSUE TYPE, AND TIME OF WEANING ON FATTY ACID COMPOSITION AND GENE EXPRESSION IN ANGUS AND CHAROLAIS FINISHING STEERS

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The effect of breed and tissue type on fatty acid composition and gene expression was evaluated using 33 steer calves. Calves were completely randomized to a 2 x 2 x 2 factorial treatment arrangement: sire breeds (Angus and Charolais), weaning dates (NW = normal weaned at approx. 210 days of age and LW = late weaned at approx. 300 days of age), and tissues (LM = longissimus muscle and ST = semitendinosus muscle). Weights were recorded at 28 d intervals to determine animal performance. Tissue biopsies were taken on d 127 and 128 of the finishing phase. All calves were harvested on d 138 and carcass data were collected. Total lipids were extracted from biopsies for fatty acid profile analysis using gas chromatography. Total RNA was extracted and hybridized to a bovine whole-genome 70-mer oligo array containing 24,000 long oligonucleotide probes used for gene expression analysis. Angus steers had greater weight gains (P=0.04), greater marbling scores (P = 0.002), more 12^{th}-rib fat (P = 0.01), smaller REA (P = 0.01), and greater yield grades (P = 0.005) than Charolais steers. There were significant breed × time of weaning × tissue interactions for percent total SFA, UFA, MUFA, PUFA, n-3, and n-6. The n-3:n-6 ratio and percent total CLA differed due to the main effects of time of weaning and tissue. Several individual fatty acids had significant two-way interactions and differences due to time of weaning and tissue. The LM had almost two times more (55 vs. 30) differentially expressed genes than the ST. The LM and ST shared the common function of cell cycle in their most significant network. Angus steers had almost 4 times fewer (35 vs. 136) differentially expressed genes than the Charolais steers, and had no common functions in their top network. The desire to understanding the genetic mechanisms underlying changes in fatty acid composition of beef remains, and future research will need to be conducted before correlations between phenotype (fatty acid profile) and gene expression can be made. Beef is a highly nutritious and valued food, providing the essential fatty acids (n-3 and n-6) and several healthy fats (MUFA, PUFA, and CLA) to the human diet. These results provide insight into the challenge of developing and implementing a program to improve the healthfulness of beef utilizing existing variation to manipulate fat composition through management and selection.

ADVISER'S APPROVAL: Dr. Clint R. Krehbiel