THE EFFECT OF VITAMIN A RESTRICTION ON MARBLING AND ADIPOGENIC GENE EXPRESSION IN COMMERCIAL ANGUS AND SIMMENTAL STEERS

A Thesis
Submitted to the Graduate Faculty
of the
North Dakota State University
of Agriculture and Applied Science

By

Evan Elliot Knutson

In Partial Fulfillment of the Requirements
for the Degree of
MASTER OF SCIENCE

Major Department:
Animal Sciences

July 2017

Fargo, North Dakota

North Dakota State University
Graduate School

Title

THE EFFECT OF VITAMIN A RESTRICTION ON MARBLING AND ADIPOGENIC GENE EXPRESSION IN COMMERCIAL ANGUS AND SIMMENTAL STEERS

By

Evan Elliot Knutson

The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

SUPERVISORY COMMITTEE:

Dr. Alison Ward
Chair

Dr. Joel Caton

Dr. Eric Berg

Dr. Kendall Swanson

Dr. Katie Reindl

Approved:

07/06/2017
Date

Dr. Gregory Lardy
Department Chair

ABSTRACT

Vitamin A (VIT-A) restriction has been shown to increase marbling in cattle. Our hypothesis for this study was that a low VIT-A diet would increase marbling and improve meat quality characteristics while having no effect on production characteristics of Angus cross and Simmental steers. We also hypothesize that expression of proadipogenic factors will increase and anti-adipogenic factors will be down regulated in cattle with high marbling scores. Treatments consisted of an unsupplemented VIT-A basal diet and a VIT-A supplemented diet at 2,200 IU/kg DM, arranged in a 2x2-treatment arrangement with breed. There was a significant interaction between breed and treatment on marbling score ($P = 0.008$), Angus cross steers on the low VIT-A had the greatest marbling and resultantly the greatest quality grades ($P = 0.02$). Differences were found in adipogenic gene expression including *FABP4, RXRβ, RXRγ,* and *RALDH1α1* ($P < 0.05$).

ACKNOWLEDGEMENTS

First and foremost, I would like to thank Dr. Alison Ward, for allowing me to delve into the adventures of graduate school. She has allowed me to be independent and take full responsibility for much of my project, yet has lent constant support and advice. She has allowed me to develop my professional writing skills through research proposals and publications, develop formal speaking skills at conferences, mature my scientific mind through experimental design and planning, and develop surgical skills with muscle biopsies. She has been an outstanding role model and I am honored to be her first graduate student.

I would like to thank everyone involved in my project. Billy Ogdahl and the Beef Unit crew, thanks for working, feeding, and caring for my cattle; and Billy for always being a cheerful fun person and a smiling face to brighten the day. Thanks to Trent Gilbury and Sara Underdahl and the BRC crew, for feeding my cattle, monitoring for illness, dealing with the Insentec system, and helping work and load out cattle. Thanks to my lab technicians Courtney Crane, Jena Bjertness, and Jordan Flaten for all your help with lab work, extractions and qPCR would have been a large burden if not for your assistance, and I would have been completely clueless on histology without Jordan's help. Thanks to Ananda Fontoura and James Gaspers for help with bleeding, weighing, and working cattle.

Thanks to my committee, for always having open doors so that I can pick your brains and absorb your knowledge. For being awesome scientists, that serve as models that I can aspire to become. Lastly, for helping with my project and being involved in my research, you have been imperative in sculpting and polishing the mold of my project and my scientific mind.

I have made great friends with many of the graduate students here at NDSU. Thank you for coping with the struggles of graduate school with me, thanks for creating a great atmosphere

that feels like home, and thanks for always creating fun times both at school and outside the workplace.

Finally, thanks to my family, who have always been there for me. My parents Connie and Bill Knutson for instilling in me a drive to learn, a solid work ethic, and passion. To my siblings Valene, Katy, Nick, Zach, and Martina, thanks for being great friends and great people, for having intelligent conversations, for having listening ears, and for creating subtle unspoken competition that drives me to be the best person and scientist that I can. And to Sonia, you're my girl and I'm so blessed. We have weathered the storm of education together, and have endured stress and serenity hand in hand. I am so excited to continue to grow together and build our lives. My family has always been so supportive in everything I do, I feel secure and confident in everything I do because I know I have the best support system possible to fall back on.

DEDICATION

I would like to dedicate this thesis to my parents Dr. Bill and Connie Knutson, who have always provided me with an environment and upbringing that has allowed me to grow, learn, and ultimately succeed at anything I aspire to.

I would also like to dedicate this thesis to my fiancé Sonia Caputo, who supports me in more ways than I can even realize. Thank you for always being there for me, I cannot wait to spend our lives together.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ACTB.....Beta-actin

ADD1.....Adipocyte determination and differentiation factor 1

ADF.....Acid detergent fiber

ADG.....Average daily gain

ADH1C.....Alcohol dehydrogenase 1C

aP2.....Adipocyte protein 2

BW.....Body weight

cAMP.....Cyclic adenosine monophosphate

C.....Celsius

Ca.....Calcium

cc.....Cubic centimeter

cDNA.....Complementary deoxyribonucleic acid

C/EBP.....CCAAR/ enhancer binding protein

CON.....Control

CP.....Crude protein

CSB.....Concentrated separator by-product

CV.....Coefficient of Vit-Ariation

CYG.....Calculated yield grade

DAPI.....4', 6-diamidino-2phenylindole

DDGS.....Dried distillers grains with solutes

DLK1.....Protein delta homolog 1

DM.....Dry matter

DMI.....Dry matter intake

DNA.....Deoxyribonucleic acid

DP1 .....Prostaglandin receptor

FA .....Fatty acid

FABP.....Fatty acid binding protein

GAPDH.....Glyceraldehyde 2-phophate dehydrogenase

G:F .....Gain : Feed

GH.....Growth hormone (somatotropin)

GHR .....Growth hormone receptor

GLUT4.....Insulin Dependent Glucose Transporter

h.....Hour

HCW .....Hot carcass weight

HLH .....Helix loop helix

HPLC .....High performance liquid chromatography

HPRT1 .....Hypoxanthine-guanine phophoibosyltransferase

HVIT-A.....High vitamin A

IGF .....Insulin like growth factor

IGF1R .....Insulin like growth factor 1 receptor

IGFBP .....Insulin like growth factor binding protein

IMF .....Intramuscular fat

IR.....Insulin receptor

IRS .....Insulin receptor substrate

IU .....International Unit

JAK .....Janus kinase

kDa.....kilo Dalton

KPH.....Kidney, pelvic, heart fat

LMA.....Longissimus muscle area

LR .....Long restriction

LSMEANS.....Least squared means

LVA .....Low vitamin A

LXR.....Liver X receptor

mRNA.....Messenger ribonucleic acid

NAD.....nicotinamide adenine dinucleotide

NDF.....Neutral detergent fiber

NDSU.....North Dakota State University

NRC .....National Research Council

P .....Phosphorus

PI-3.....Phosphatidylinositol 3

PPAR.....Peroxisome proliferator-activated receptor

PPRE.....Peroxisome proliferator response element

Pref-1.....Pre-adipocyte factor 1

QG.....Quality grade

qPCR.....Quantitative polymerase chain reaction

RA.....Retinoic acid

RAR .....Retinoic acid receptor

RARE.....Retinoic acid response element

RALDH.....Retinaldehyde dehydrogenase

RBP4.....Retinol binding protein 4

RNA .....Ribonucleic acid

RIA.....Radioimmunoassay

RXR .....Retinoid X receptor

S.C. .....Sub cutaneous

SNP .....Single nucleotide polymorphism

SR.....Short restriction

SREBP-1C .....Sterol responsive element-binding protein 1C

STAT1.....Signal transducer and activator of transcription 1

STRA6 .....Stimulated by retinoic acid 6

TACE .....Tumor necrosis factor converting enzyme

TMR.....Total mixed ration

TNF .....Tumor necrosis factor

UBC .....Ubiquitin C

US .....United States

USDA.....United States department of agriculture

VIT-A.....Vitamin A

VDR .....Vitamin D receptor

YG.....Yield grade

YWHAZ.....14-3-3 protein zeta/delta

LIST OF SYMBOLS

Δ ....Delta

CHAPTER 1: LITERATURE REVIEW

Marbling

In the United States and many countries around the world, marbling in cattle increases the value of a cut of meat. Marbling is the visible flecks of fat found within muscle and is measured between the 12th and 13th rib of a beef carcass (Hale et al., 2013). Marbling is one of the most influential factors that affect consumer preference in meat. Generally, palatability increases with increased marbling through factors such as tenderness, juiciness, and taste (Smith, 2014; Want et al., 2014). Recent studies show that consumers prefer USDA top choice *longissimus* and *gluteus medius* muscles (ribeye and sirloin, respectively), to USDA select graded cuts. Consumers stated that top choice cuts were more tender, juicy, and flavorful (Hunt et al., 2014)

In the United States there are nine classifications of marbling. They include abundant, moderately abundant, slightly abundant, moderate, modest, small, slight, traces, and practically devoid (North Dakota State University, 2010). These classifications correspond to numbers that are used as part of the quality grade calculation. The numbers used are 100-900, with 100 corresponding to practically devoid and 900 corresponding to abundant, with each increase of 100 representing the subsequent marbling classification. The other factor in quality grade is maturity, which range from A to E, youngest to oldest respectively. The majority of steers slaughtered in the US are slaughtered before 30 months of age, and therefor are classified maturity A. Assuming a maturity grade of A, cattle achieving marbling scores of $\geq$700 are classified as prime, 400-699 are classified as choice, 200-399 are classified as select, and <200 are classified as standard (Hale et al., 2013).

Marbling is also known as interfascicular or intramuscular adipose tissue, and is different than other types of fat because it is deposited alongside muscle fibers. Marbling is made up of clusters of individual adipocytes (fat cells) that are located within the perimysial connective tissue, between myofiber bundles (Moody and Cassens, 1968). In cattle with extremely high marbling scores (e.g. Japanese Black cattle grading A5), adipocytes and lipid drops have been identified within muscle bundles (Smith, 2000). However, it is unlikely that this fat depot contributes substantially to marbling score because they are generally invisible to the naked eye (Pethick et al., 2004). Intramuscular fat is usually the last place fat begins to accumulate, and its development is much slower than in other depots, about 5 to 10% than that of subcutaneous adipose tissue (Hood and Allen, 1978). Intramuscular fat deposition is more complex than that of subcutaneous fat in beef cattle. It is affected by the genetic propensity to marble, nutritional plane throughout life, and environmental factors (Smith and Johnson, 2014).

Several strategies can be employed to increase marbling in cattle, including genetic selection, castration, feeding a high energy grain finishing ration, as well as vitamin and mineral manipulation. Genetic propensity to marble is perhaps the most important factor in increasing marbling scores in cattle. Japanese black cattle have perhaps the greatest potential for marbling. Gotoh et al. (2009) showed that at 24 months of age Japanese black cattle had 23.3% IMF, Holsteins had 4.7%, German Angus had 4.4%, and finally Belgian Blue had 0.6% within the *longissimus* muscle. Knutson et al. (2017) reported IMF content of the *longissimus* muscle of commercial Angus and purebred Simmental cattle as 7.2% and 4.3, respectively. Ward et al. (2012) reported that Angus cross steers averaged an intramuscular fat (IMF) content of 5.3%. From the data presented above and information in “Encyclopedia of Animal Science” (2005), Japanese black cattle such as Waygu have the highest marbling potential, British breeds of cattle

such as Angus and Jersey have an intermediate marbling potential, and Continental breeds such as Limousin and Simmental as well as *bos indicus* cattle have the lowest potential for marbling.

Castration is a technique commonly used in the beef industry to control temperament of animals as well as enhance quality grades of cattle. A review by R. A. Field in 1971 concluded that steers had improved quality grade than bulls in all five of the studies included in the review (Field, 1971). Grain based diets are often used to promote marbling, these diets also improve the healthfulness of beef due to a repartitioning of saturated fatty acids to monounsaturated fatty acids (Pethick et al., 2004; Smith, 2014). Several studies have compared a high grain diet to forage based diets, and the effect on marbling. In most cases, grain finished cattle had significantly higher marbling scores than grass finished cattle. Crouse et al. (1983) reported significantly different marbling scores of 3.6 (360) and 4.3 (430) for grass and grain finished cattle, respectively. This is in agreement with Bowling et al. (1978), who reported quality grades of 7.6 and 11.2 for grass and grain finished cattle, respectively.

Several vitamins and minerals have been shown to have effects on marbling in cattle. Method of zinc supplementation was shown to have an effect on marbling. Angus steers supplemented with zinc methionine had higher marbling scores than those that were supplemented with zinc oxide, with scores of 4.4 and 4.0 respectively (Greene et al., 1988). However, steers supplemented with zinc methionine also had higher amounts of subcutaneous fat and KPH fat. Zinc has also been shown to have proadipogenic effects *in vitro* (Tanaka et al., 2001). The micronutrient that has been associated most with marbling score is VIT-A (Harper and Pethick, 2004).

Vitamin A

Vitamin A (VIT-A) is a fat soluble vitamin that serves many physiological functions including vision, reproduction, immunity, and gene expression (Blomhoff and Blomhoff, 2005). Vitamin A is found most commonly in the body as retinol, although there are many other retinoids with varying degrees of VIT-A activity. For herbivores, retinol is not consumed in the feed; it is converted from a plethora of other substances, most commonly carotenes and carotenoids. The primary source of retinol precursors in the diet of cattle is green forages, whereas grains contain few compounds with VIT-A activity (NRC, 2016). Vitamin A is stored in the liver (in the form of retinyl esters), which can store enough retinol to maintain serum retinol levels for several months (NRC, 2016). Several synthetic forms of retinoids have been developed; these may have greater biological effects than natural retinoids (Blomhoff and Blomhoff, 2006).

Sources of Vitamin A

Carotenoids are the primary source of VIT-A in livestock diets. Good sources of carotenoids in livestock diets include high quality forages and silages (NRC, 2016). Carotenoids are pigments found in plants and animal tissues. They are lipid soluble and have varying VIT-A activity. Carotenoid pigments include colors that range from yellow through deep red. There are nearly 600 characterized carotenoids, but only approximately 10% of them can be metabolized to VIT-A (Yeum and Russell, 2002). Most carotenoids are derived from a 40 carbon backbone, and have differences in their functional groups, which are often cyclic and can contain oxygen. There are two main groups of carotenoids, the hydrocarbon group called carotenes and their oxygenated counterparts called xanthophyll's. Carotenes are present in plants in its free form which is entrapped within the chloroplast. Yellow, orange and red fruits and vegetables contain

carotene but the free forms are more abundant in green plants and are absorbed more easily (Ullrey, 1972). Xanthophylls are abundant in yellow and orange fruits and vegetables. (Namitha and Negi, 2010).

Carotenoid content within plants is variable depending on maturity, season, soil content, as well as post-harvest factors such as handling, processing, and storage (Namitha and Negi, 2010). Carotenoids are highly unsaturated, therefore they are easily oxidized and isomerized, leading to reduced VIT-A activity. Exposure to light, heat, and oxygen can all have negative effects on the VIT-A activity of carotenoids (Namitha and Negi, 2010).

In order to have VIT-A activity, carotenoids must contain one unsubstituted β-ionone ring (Ullrey, 1972). The carotenoids with the most relevant VIT-A activity in ruminants are β-carotene, α-carotene, γ-carotene, as well as cryptoxantheine (Castenmiller, 1998). β-carotene has the highest VIT-A activity because it is symmetrically cleaved into two retinol molecules. Carotenoids that are able to be converted to VIT-A are known as pro-VIT-A carotenoids (Castenmiller, 1998). Vitamin A can also be obtained from eating animal tissues, which contain retinoids that have already been converted to their active forms, most commonly retinyl esters. Tissues that contain the majority of retinoids include the liver and adipose tissue (Blomhoff and Blomhoff, 2006). Retinyl palmitate is also commonly used in domestic animal supplements (NRC, 1996).

Requirements

The NRC (2016) requirements of VIT-A for growing and finishing beef cattle is suggested to be 2,200 IU/kg DM. It has been reported based on a survey of nutritionists, that finishing beef cattle are often supplemented up to twice the NRC recommendation (Vasconancelos and Galyean, 2007). The NRC recommendation for pregnant beef heifers and

cows is 2,800 IU/kg DM and the suggested requirement for lactating cows and breeding bulls is 3,900 IU/kg. Different sources have differing VIT-A activity; 1 IU is equivalent to 0.300µg of all-*trans* retinol or 0.550 µg of retinyl palmitate (NRC, 1996). When VIT-A is required in the tissues, it is bound to retinol binding protein 4 (RBP4), which is responsible for transporting it in the plasma. Retinol binding protein 4 allows the hydrophobic retinol molecule to be dissolved in the aqueous solution of the blood. Once it reaches its destination, it is transported from RBP4 into the cells by the trans-membrane protein Stimulated By Retinoic Acid 6 (STRA6) (Raghu and Sivakumar, 2004; Wolf, 2007).

Absorption and Metabolism

The extent of ruminal degradation of carotenoids is relatively unclear, as authors have reported no degradation and others up to 55% degradation (Dawson and Hemington, 1974; King et al., 1962). It seems the majority of studies found moderate degradation, generally approximately 20% (Mora et al., 1999; Potkanski et al., 1974). However, it is apparent that degradation is generally higher in purified products than when provided in forages (Noziere et al., 2006). Carotenoid solubility and absorption in the intestine is largely dependent on dietary lipid content and polarity of the carotenoid, as it needs to be incorporated into micelles for absorption (Furr and Clark, 1997). Carotenoids can be converted to retinol or retinal through the action of β-carotene 15,15' monooxygenase, which acts most efficiently on β-carotene but will also cleave other carotenoids; cleaVit-Age can occur in both enterocytes and hepatocytes (Castenmiller, 1998).

Retinoids exist in the body in four forms, most of which have several isoforms. These include retinol (hydroxyl form), retinaldehyde (aldehyde form), and retinoic acid (the carboxylic form) as well as the storage form, retinyl ester. Retinol is converted to retinaldehyde through the

action of alcohol dehydrogenase enzymes, specifically alcohol dehydrogenase 1C (ADH1C). Retinaldehyde is then converted to retinoic acid (RA) thru the action of retinaldehyde dehydrogenase (RALDH) enzymes by NAD dependent oxidation (Duester, 2000). There are also many isomeric forms of VIT-A; most notably the conversion between 11-*cis* and all-*trans* retinaldyehyde in the eye and 9-*cis* and all-*trans* RA as nuclear receptor ligands. The most active form of retinoid in the body is RA, for which the most common isomers are all-*trans*-RA and 9-*cis* RA (Bonet, 2003). When being converted between retinyl esters, retinol, retinal, and RA the isomer form is maintained (Blomhoff and Blomhoff, 2006).

Function

Vitamin A serves many functions in the body; it is important in vision, reproduction, and immunity. When light hits the eye, 11-*cis*-retinaldehyde is converted to all-*trans*-retinaldehyde to trigger phototransduction (Stecher, 1999). The role of retinol in reproduction is the stimulation of production of steroid hormones (notably progesterone). These hormones are imperative for maintenance of pregnancy and fetal development (Chew, 1993). In the male, retinol is necessary for spermatogenesis and RA for production of testosterone; a deficiency in retinol results in cessation of spermatogenesis and atrophy of the testicles (Appling and Chytil, 1981). The role of VIT-A in the immune system is retinol provides an anti-oxidant based protection for epithelial barriers and also stimulates lymphocyte maturation and normal cytokine production (Mora et al., 2008; Ross, 1992).

More recently, VIT-A has been identified as an important ligand for a family of transcription factors, specifically for those in the nuclear hormone receptor superfamily. The two nuclear receptors that are sensitive to RA are the retinoic acid receptors (RAR) and the retinoid X receptors (RXR). The RAR receptors can be activated by either *all*-trans RA or 9-*cis* RA. This

receptor preferentially heterodimerizes with RXR to activate the retinoic acid response element (RARE) of downstream genes. RXR is activated solely by the 9-*cis* isomer; this receptor is a promiscuous receptor, meaning it heterodimerizes with a number of other receptors. There are specific synthetic ligands for the RXR, called rexinoids, and often have a greater affinity for the receptor than the native 9-*cis* RA (Rigas and Dragnev, 2005). The function of these receptors will be further explained in more detail in the section "Adipogenesis" (page 8).

Adipogenesis

The definition of adipogenesis has been evolving for the last three decades. Adipogenesis is now recognized as a dynamic and plastic process, which leads to the phenotype of a mature adipocyte. It follows the development of an adipose cell through differentiation, proliferation, and maturation to a cell that are capable of storing lipid. It is accepted that fat cells are not merely storage of energy, but are specialized and under the influence of many hormones, cytokines, and nutrients (Feve, 2005).

Adipogenesis starts as stem cells proliferate and begin to form preadipocytes. Many transcriptional pathways have been identified once cells commit to preadipocytes, but the pathways and factors that cause determination from mesenchymal precursor cells to preadipocytes are largely unknown. However, studies performed with 10T1/2 cell lines showing that bone morphogenic protein 4 (BMP4) induces near complete commitment to the adipocyte lineage from mesenchymal stem cells (Otto and Lane, 2005). There is no clear marker for initiation of adipogenesis. The first sign of adipogenesis in a region is the growing network of capillaries, where previously there were no capillary beds, on loose connective tissue (Rosen and Spiegelman, 2000). Adipocytes and their precursor cells are affected by many factors such as the availability of macro- and micro- nutrients, adhesion to tissue, growth factors, endocrine

environment, and neural inputs (Rosen and Spiegelman, 2000). Adipogenesis and specifically lipid filling requires a positive energy balance, meaning intake of energy must exceed energy expenditure.

Fat cells tend to accumulate in clusters. One hypothesis for this observation is that mature adipocytes secrete a recruitment factor, which has not been elucidated (Rosen and Spiegelman, 2000). Mature adipocytes can act in an autocrine and endocrine manner and secrete factors and hormones that are important in adipocyte differentiation, possibly the most potent is insulin-like growth factor 1 (IGF-1). Adipocyte differentiation does not occur in the absence of IGF-1 or insulin, which binds to the IGF-1 receptor, but at a much lower affinity (Smith et al., 1988). Hormonal signaling including insulin and glucocorticoids leads to the increase of cyclic adenosine monophosphate (cAMP), which is considered a prerequisite for differentiation of adipocytes (Hausman et al., 2009). Some hormones of specific interest due to their effect on adipogenesis are insulin, IGF-1, GH, and leptin.

Insulin

Insulin is produced in the pancreatic beta cells, and is secreted from the pancreatic islets upon signaling induced by increasing blood glucose. Insulin is the only hormone that is responsible for lowering blood glucose. Insulin regulates the metabolism of all macronutrients but specifically controls the digestion and metabolism of carbohydrates. Insulin plays a key role in glucose transport into the cell, which in turn affects metabolism and growth. The anabolic effect of GH is stunted without insulin. This is likely do to the lack of energy substrates necessary for amino acid uptake and incorporation into muscle. Insulin has been consistently shown to have a growth promoting effect (Norris and Carr, 2013).

Insulin's greatest effects are seen on hepatic, muscle, and adipose cells. In hepatic cells, insulin activates glycogen sythetase enzymes, which convert glucose into its storage form, glycogen. In muscle cells, insulin enhances the transport of amino acids and glucose into the cells, upregulating protein synthesis. This occurrence is pronounced in species with little fluctuation in glucose such as ruminants (Squires, 2010). In adipose tissue, insulin upregulates the catabolism of glucose to glycerol (Cryer, 1993) and increases lipoprotein lipase which function to release fatty acids (FA) from chylomicrons. These fatty acids and glycerol then combine to form triglycerides and add to lipid droplets of adipocytes (Hadley and Levine, 2009).

The insulin receptor is a plasma membrane receptor, which mediates responses using tyrosine kinases. There are two main groups of intracellular substrates affected by the insulin receptor. These include the insulin receptor substrate family proteins (IRS 1-4), which work through activation of an enzyme named phosphatidylinositol-3 kinase (PI-3-kinase), which carries out the more direct metabolic effects of insulin. The second group of effector molecules are the Shc molecules, which regulate the RAS-MAPK pathway and ultimately control gene transcription (Hadley and Levine, 2009). Ultimately, insulin clears blood glucose by activation and translocation of the GLUT 4 glucose transporter to the plasma membrane, allowing glucose to be transported into the cell (Norris and Carr, 2013).

In ruminants, little glucose is absorbed in the gastrointestinal tract, as it is used in the rumen by microbes. Most humoral glucose is derived from gluconeogenic pathways from propionate, lactate, and glycerol. Glucose metabolism in ruminant adipose tissue is subject to insulin resistance because the ability of insulin to stimulate glucose clearance is lower than that in non-ruminants (Sasaki, 1990). The lack of GLUT4 expression in bovine adipose tissue could be a major cause of high insulin resistance in mature cattle (Abe et al., 1997). It was shown in

beef cattle that as time on feed increases, glucose incorporation into fatty acids of both subcutaneous and intramuscular fat is decreased; likely due to insulin resistance. (Smith, 2017; Choi et al., 2014)

Leptin

Leptin is a 167 amino acid peptide hormone produced primarily in white adipose tissue (Hadley and Levine, 2009). Generally, leptin levels increase with adiposity. Leptin acts primarily on the central nervous system where it causes an inhibitory effect on food intake. Leptin is a pleiotropic hormone that can also influence pathways that control energy expenditure, reproductive function, angiogenesis, and immune cell function (Hadley and Levine, 2009).

It was noted by Anubhuti and Arora (2008) that the physiological effects are greater when reducing leptin levels rather than increasing them. They state that there are several pathways by which leptin inhibits food intake, including PI-3-kinase as well as the insulin signal transduction pathway, which allow insulin and leptin to act as peripheral signals to the CNS for energy homeostasis. Leptin can inhibit intramyocellular lipid accumulation by increasing β-oxidation through the activation of AMPK, which then inhibits acetyl-CoA carboxylase (ACC), allowing fatty acid transport to the mitochondria for oxidation (Anubhuti and Arora, 2008). In Angus and Limousin steers, it has been reported that intramuscular leptin levels were greater in Angus, which also had more intramuscular fat; greater leptin levels were attributed to the greater fat content (Bonnet et al., 2007). A breed difference between British and continental breeds has been discovered. A SNP that caused an arginine (encoded by the C allele) to cysteine (encoded by the T allele) AA transition occurs in exon 2 of the leptin gene. The T allele results in fatter carcasses, whereas the C allele results in leaner carcasses; British breeds have a higher frequency

of the T allele. Animals homozygous for the T allele also exhibited higher leptin levels (Buchanan et al., 2002).

IGF-1

There have been several studies showing anecdotal evidence that IGF-1 plays a critical role in the differentiation of pre-adipocytes. One such research project was performed by Smith et al. (1988), where they showed that AXC serum (which was devoid of IGF-1) did not allow the conversion of preadipocytes into adipocytes. When IGF-1 was added at physiological concentration, the preadipocytes began to mature and gain the morphology of the mature adipocyte and began to accumulate triglycerides. Insulin mimicked the results of IGF-1 in this experiment, but only when added at supraphysiological amounts. It is believed that both IGF-1 and insulin carry out their effect on adipogenesis through the IGF-1 receptor. This would explain why much more insulin was required to mimic the action of IGF-1, because insulin binds the IGF-1 receptor with a 70-500 (Smith, 1988; Christoffersen, 1998) fold lower affinity than IGF-1 itself (Smith et al., 1988). Another, more recent study, reaffirmed that IGF-1 and insulin are imperative for adipocyte differentiation by performing a double knockout of the IGF-1 receptor and insulin receptor (IR), no lipid accumulation occurred (Boucher et al., 2012).

It has been recognized that pre-adipocytes have many more receptors for IGF-1 than for insulin. Zezulak and Green (1986) suggested that GH actually initiates initial differentiation and sensitizes cells to IGF-1, possibly by upregulating IGF-1 receptors, causing a greater effect. After differentiation, the IGF-1 receptors have been shown to remain constant, whereas insulin receptors begin to increase. Insulin receptors have been shown to increase 13 fold from day 0 to 8 of differentiation, meaning that insulin may play a larger role in the final stages of adipocyte maturation (Christoffersen, 1998).

There have been several pathways proposed as to how IGF-1 affects transcription of adipogenic genes. Peroxisome proliferater activated receptor γ (PPARγ) and CCAAR/ enhancer binding protein α (C/EBPα) are two key transcription factors in the adipogenic pathway. Boucher et al. (2012) showed that during adipocyte differentiation PPARγ and C/EBPα mRNA increased >20 fold. However, in insulin receptor and IGF-1 receptor (IGF1R) knockout mice, there was no increase in PPARγ and C/EBPα mRNA. This shows that IGF-1 and insulin are upstream activators of the transcription factors of PPAR γ and C/EBPα.

Another proposed pathway for how IGF-1 induces differentiation of adipocytes is through the RAS pathway. Benito et al. (1991) performed an experiment where they introduced functionally active *RAS* genes into 3T3-L1 cells. They noted that the expression of N-ras^{Lys61} alone was responsible for the adipocyte differentiation observed. They made the connection to the IGF-1 and insulin receptors when they exposed non-transfected 3T3-L1 cells to insulin, and this stimulated the formation of the biologically active Ras*GTP complex. Though they used insulin for the stimulation of adipogenesis, it is implicated that the effect is carried out through the IGF-1 receptor tyrosine-kinase pathway (MacDougald and Lane, 1995).

A final pathway that IGF-1 may affect adipogenesis is through the regulation of protein kinases. There are several protein kinases, including MAPK, that have been suspected to be affected by IGF-1, and that also play a role in the activation of the transcription factors of PPARγ and C/EBPα. It has been shown that IGF-1 stimulation of MAPK is maximal at the proliferation stage of adipogenesis (Boney et al., 1998). Once the preadipocytes began to differentiate, the stimulation of MAPK from IGF-1 decreased rapidly. This suggests that the uncoupling of the MAPK from its ligand induced receptor is a key event in the transition from proliferation to differentiation phases within adipogenesis.

Somatotropin

Somatotropin, also commonly called growth hormone (GH), is produced by the anterior pituitary and accounts for 4 to 10% of the mass of the anterior pituitary. The main function of GH is to stimulate skeletal and skeletal muscle growth, and works to repartition energy from fat deposition to muscle accretion. GH also stimulates lipolysis, milk production, and has a positive effect on IGF-1 secretion. Many of GH functions are carried out through the stimulation of IGF's, which are secreted from the liver; the liver is the primary target endocrine gland for GH, having a plethora of GH receptors (Norris and Carr, 2013).

Glucocorticoids can down regulate the expression of preadipocyte factor 1 (PREF-1), an inhibitor of adipogenesis, as well as promote the dimerization of C/EBPα and C/EBPβ (Smas et al., 1999; Wu et al., 1996). The factors that affect adipocytes and their precursor cells either inhibit or promote a series of transcriptional changes that dictate the differentiation process (Feve, 2005). The transcriptional beginning of differentiation is the expression of C/EBPβ and γ. PREF-1 is a potent inhibitor of adipogenesis through its inhibition of the expression of C/EBPα and C/EBPβ (Sul et al., 2000). The expression of C/EBPδ and C/EBPβ in turn, appears to be required for the appearance of the two downstream transcription factors C/EBPα and PPARγ (peroxisome proliferator-activated receptor gamma), which reciprocally regulate each other and are regarded as the master control genes of adipogenesis (Farmer, 2005; Hausman et al., 2009). These genes maintain the differentiated state of adipocytes (Rosen, 2005). Another family of transcription factors that is important in adipogenesis is the helix-loop-helix family (HLH), specifically ADD1/SREBP-1c (adipocyte determination and differentiation factor-1/sterol responsive element-binding protein-1c). This transcription factor has been proposed to alter adipogenesis by controlling the production of ligands that bind to the PPAR-γ receptor,

upregulating its activity (Kim, 1998). PPARγ must dimerize with the retinoid x receptor (RXR) to activate its response element; RXR requires the ligand 9-*cis* retinoic acid (RA) to activate it (Zhang et al., 2002).

The different oxidation states of vitamin A have differing effects on adipogenesis. Retinoic acid (RA) has both a positive and negative effect on adipogenesis, dependent on the stage of adipocyte development. During the commitment stage, when progenitor cells are committed to preadipocytes, RA increases the activation of the extracellular signal-regulated kinases (ERK) pathway, in turn inducing adipogenic commitment (Bost et al., 2002). During the differentiation stage, RA has an inhibitory effect; this affect has been attributed to the inhibition of C/EBPβ (Schwarz et al., 1997). After differentiation, low levels of RA are necessary for, and stimulatory of, the maturation of adipocytes through induction of PPARγ and fatty acid binding protein 4 (FABP4) (Reichert et al., 2011). This necessity for RA is based upon the need of RAR and RXR for ligands to activate and mediate the activity of PPARγ and C/EBPα and β (Xue et al., 1996) At higher levels, RA reduces lipid accumulation due to the activation of PPARα and PPARβ/δ, which induce fatty acid oxidation and lipid catabolism (Wang et al., 2016). Retinaldehyde solely has an antagonistic affect on adipogenesis. Retinaldehyde inhibits adipogenesis by binding to retinol binding proteins (CRBP1 and RBP4) and well as suppressing the activity of PPARγ and RXR (Ziouzenkova et al., 2007). There are several genes that are known to affect adipogenesis, but when and how they change and elicit change within the ruminant are vastly unknown. Some of the key genes for adipogenesis are reviewed here.

PPARγ

Peroxisome proliferator activated receptor γ is a nuclear hormone receptor that is activated by a number of substances including unsaturated long chain fatty acids, eicosanoids,

and prostaglandins (Fajas, 1998). Peroxisome proliferator activated receptor γ is currently recognized as the master coordinator of the adipogenic differentiation process Loss of function studies have demonstrated that PPARγ is conditionally necessary for adipogenesis both *in vivo* and *in vitro* (Barak et al., 1999). It is a positive regulator of adipogeneis, meaning that while it is present on the peroxisome proliferator response element (PPRE) of DNA it increases adipocyte development. In order to activate the PPRE, PPARγ must dimerize with RXR (Tontonoz et al., 1994). Peroxisome proliferator activated receptor γ has been shown to act as a factor to promote preadipocyte determination, but also appears to stimulate the process of terminal differentiation. The function of this receptor is dependent on a functioning DNA binding domain, this serves as proof that it carries out its effect through the transcription of genes relevant to adipocyte development (Tontonoz et al., 1994). Many factors that inhibit adipogenesis target and reduce PPARγ expression, such as tumor necrosis factor α (TNFα) and interleukin-1 (IL-1) (Suzawa, 2003).

C/EBPα

CCAAT-enhancer binding protein (C/EBPα) is a leucine zipper transcription factor that is known to enhance the differentiation and maturation of adipocytes. CCAAT-enhancer binding protein works synergistically with PPARγ to increase adipocyte differentiation and maturation (Feve, 2005). The requirement for C/EBPα during adipocyte formation was validated using C/EBPα knockout mice, which resulted in embryonic lethality and/or failure to accumulate lipid in the adipocyte. Although this event can be counteracted by the overexpression of PPARγ, the opposite is not true (Wang et al., 1995). One of the actions of C/EBPα that promotes adipocyte differentiation is the repression of the E2F/DP1 complex, which when active blocks differentiation and causes programmed cell death (Porse, 2001). Another role of C/EBPα is to

bind and activate the (FABP4) promotor, increasing its production; FABP4 is a protein carrier for fatty acids, important for filling adipocytes with lipids (Herrera, 1989). Additionally, C/EBPα maintains the insulin receptor upon the cell membrane, thereby allowing the sufficient uptake of glucose into the adipocyte (El-Jack et al., 1999).

RXR

The retinoid X receptor (RXR) is a part of group of receptors called nuclear orphan receptors. This group of receptors is defined by related protein makeup and having small ligands that bind to DNA to alter transcription (Bugge, 1992). The RXR receptor is one of the most common receptors in the body, with at least one of its three forms (α, β, and γ) being expressed in every cell in the body. The primary ligand for the RXR receptor is 9-*cis* RA (Mangelsdorf, 1992). The RXR receptor can bind DNA as a homodimer, or it can bind as a promiscuous heterodimer with several other receptors such as the PPAR, liver X receptor (LXR), vitamin D receptor (VDR), and retinoic acid receptor (RAR). Many of these receptors can bind to their response elements as homodimers, but DNA binding and translational regulation is increased when bound heterodimerically with RXR (Bugge, 1992). These nuclear receptor heterodimers fall into two classes, permissive and non-permissive. Permissive dimers can be activated by RXR or its heterodimer, whereas non-permissive dimers can only be activated by RXR's partners' ligand making it much more sensitive to the concentration of that ligand (Leblanc and Stunnenberg, 1995).

RAR

The retinoic acid receptor (RAR) falls into the superfamily of nuclear hormone receptors. The RAR (α,β,γ) function as heterodimers with the three RXR (α,β,γ). These receptors are ligand dependent, and bind to a retinoic acid response element (RARE) to carry out their transcriptional

effect (Mangelsdorf and Evans, 1995). When RAR ligands are not present, the heterodimer recruits corepressor proteins that lead to condensed chromatin and inactive transcription. When the ligand binds, the RAR repressor proteins are released and a different set of coactivator proteins are recruited that activate transcription (Germain et al., 2006).

PREF-1

Preadipocyte factor 1 (PREF-1), otherwise known as protein delta homolog 1 (DLK1), is a transmembrane protein that is encoded by the *PREF-1* (*DLK1*) gene. In order to be activated the PREF-1 protein must be cleaved by the TNF-α converting enzyme (TACE). The active form is a soluble protein of about 50kDa (Wang and Sul, 2006). PREF-1 has been shown to inhibit adipocyte differentiation by upregulating Sox9 expression, through the activation of the ERK pathway. The Sox9 protein then binds to the promotor of C/EBPβ and C/EBPδ, reducing their expression (Wang and Sul, 2009). C/EBPβ and C/EBPδ work to activate PPARγ and C/EBPα, which are required and sufficient to cause adipocyte differentiation (Wu et al. 1996). It has been shown that PREF-1 suppresses adipocyte differentiation is through its action on fibronectin, by binding to the C-terminal region of fibronectin and causing the activation of the α5β1 integrin. The activation of this integrin causes a cascade of activation and ultimately the activation of the MEK/ERK pathway (Wang et al. 2010).

FABP4

Fatty acid binding proteins (FABP) fall into the superfamily of lipid-binding proteins (LBP), which are carrier proteins for fatty acids that are expressed in many tissues throughout the body. There are a total of nine different FABP that have different origins and tissue specific distribution throughout the body. The function of all of these proteins is the uptake and intracellular transport of fatty acids. FABP4 is also known by A-FABP or adipocyte protein 2

(aP2) (reviewed by Chmurzynska, 2006). Besides being a transporter for fatty acids, which serve as ligands for PPAR, it has also been shown that FABP4 itself specifically enhances the activity of PPAR (Tan et al. 2002). FABP4 directly associates with the PPAR to enhance the ligation of this receptor, serving to increase the transcriptional activity of the PPAR. This effect is not apparent at high ligand concentrations, but becomes apparently essential at low ligand concentrations. *FABP4* gene expression has been shown to be associated with marbling score and back fat depth in Waygu × Limousin F2 cattle (Michal et al., 2006). *FABP4* has been proposed as a potential candidate gene for obesity, because it is located within the quantitative trait loci for serum leptin levels in mice (Ogino et al., 2003).

ADH1C

Alcohol dehydrogenase 1C (ADH1C) is an enzyme that plays an important role in the metabolism of vitamin A. ADH1C is responsible for the oxidation of retinol to retinaldehyde (Molotkov et al., 2002). The genotype of cattle at the loci for the ADH1C enzyme has an effect on intramuscular fat deposition when vitamin A is limited in the diet (Ward et al., 2015).

RALDH1a1

Retinaldehyde dehydrogenases (RALDH) are found in the superfamily of enzymes that function to oxidize aldehydes, the oxidation of retinaldehyde to retinoic acid is of importance to this study. This oxidation can occur to both the 9-*cis* conformation as well as the all-*trans* conformation of retinal, and the conformation is conserved in the product; which can alter the function of retinoic acid (Patterson et al., 2013). Retinoic acid has been shown to lower RBP4 and increase insulin sensitivity as well as increasing lipid oxidation capacity in skeletal muscle; thereby decreasing adiposity (Amengual et al., 2008; Manolescu et al., 2010). In a study by Ziouzenkova (2007), mice lacking the RALDH1 gene had suppressed adipogenesis

(approximately 75% less white fat accumulation), weight gain, and reduced adipocyte size in vivo. This was coupled with a substantially higher metabolic rate, respiratory quotient, and body temperature of mice lacking RALDH1. This reiterates that retinaldehyde is an anti-adipogenic substance, and that RA can have adipogenic stimulating properties.

The Effect of Vitamin A on Marbling

There have been multiple studies performed looking at the influence of a low VIT-A diet on marbling scores. In general, the findings have been that low vitamin A diets are correlated with higher marbling scores. It was first realized that VIT-A affected intramuscular fat deposition by Oka et al. (1998), who discovered that in Tajima Japanese black cattle serum VIT-A was negatively correlated with marbling score. In a series of three following studies, steers were on treatment for 16, 10, and 6 months for their respective experiment. These cattle were supplemented with 20 ml retinyl palmitate intramuscularly every 2 months in every experiment. The second experiment lasting 16 months began when the steers were approximately 15 months of age, the third and fourth experiments began when steers were approximately 23 and 25 months old, respectively. The only differences in marbling scores were seen in experiment two. The author suggested that the adipogenic inhibitory effect of VIT-A was occurring before 23 months of age (Oka et al., 1998). This was supported by another study using Japanese black steers, which looked at several biochemical parameters and associated them with marbling score. Vitamin A level was negatively correlated with marbling, but only during the 21-25 months of age period (Adachi et al., 1999). Nade et al. (2003) aimed to finish Waygu cattle faster (24 months), while testing the effect of VIT-A restriction. Vitamin A restriction was implemented on the same steers from 7-12 months of age, and 22-24 months of age, and significantly increased marbling. This may suggest that one of the key periods for effecting intramuscular adipogenesis

is within one of these time periods (Nade et al., 2003). The production system in Japan typically finishes cattle at approximately 32 months of age, which is beneficial for marbling development; but in the United States, cattle are typically finished within 18 months of age (USDA, 2016).

After the research in Japan, researchers began to investigate this relationship within the United States (US) production system. One of the first studies examined the effect of both restricted intake and a high and low VIT-A treatment (Pyatt et al., 2005). The VIT-A treatment diets contained 2,300 and 7,250 IU/kg DM for low and high respectively, both of which are greater than the NRC recommended 2,200 IU/kg DM. This study included three experiments all of which used Angus × Simmental cattle, one using heifers, and two using steers. No differences in marbling were seen in any of the three experiments. Steers consuming 2,300 IU/kg DM VIT-A had higher and tended to have improved gain to feed (G:F) compared to steers consuming 7,250 IU/kg DM VIT-A. This research likely yielded no differences in marbling score because the low VIT-A treatment was not actual VIT-A restriction, according to NRC (2016) recommendations of 2,200 IU/kg DM VIT-A for finishing cattle. A subsequent study examined VIT-A restriction on purebred Angus cattle. After 12 months on pasture, steers were moved to a feedlot for 10 months and fed a high grain diet (which was very low in VIT-A) with either no supplemental VIT-A (LVA) or 6,000 IU VIT-A/cwt per day (HVA) (Siebert et al., 2006). Marbling score was not measured, but VIT-A supplementation resulted in a 26% reduction in ether extractable intramuscular fat (IMF) content. Ether extractable IMF is highly correlated with marbling score ($R^2 = 0.86$; Dow et al., 2010). Siebert et al. (2006) speculated that the propensity to marble is genetic and influenced by environmental factors, and added that breeds such as Angus, Hereford, Wagyu, and Jersey's intramuscular fat matures much earlier than other European and tropical breeds.

Gorocica-Buenfil et al. (2007) tested the explanation of breed propensity to marble, and its interaction to VIT-A supplementation level. A group of 70 Holstein steers were used to determine the amount of time needed to observe an effect of VIT-A supplementation on marbling scores; VIT-A was either not restricted at all (CON), restricted for 131 d (SR), or restricted for 243 d (LR). The control diet contained approximately 950 IU VIT-A/kg DM, CON steers received diet and 2,200IU/kg DM VIT-A supplement, SR were fed the same diet until they reached 131 d from slaughter, and LR spent all of finishing without VIT-A supplementation. There was no significant effect of VIT-A supplementation level on marbling or quality grade. Numerically LR had greater marbling scores than both other groups, but the SR had lower marbling scores than the control group. Another study that examined the effect of VIT-A restriction on marbling in cattle with lower propensity used Limousin × Luxi cross steers over three experiments (Wang et al., 2007). Steers were fed for 6, 3, and 3 months for experiment one, two, and three, respectively. Steers were fed a 40% concentrate and 60% roughage diet that was low in VIT-A and formulated to result in 0.5 kg average daily gain (ADG). Experiment one and two began at 12 months of age and experiment three began at 24 months of age. Vitamin A was supplemented at 4 levels, unsupplemented basal diet (CON), 1,100, 2,200, and 4,400 IU VIT-A/kg DM for treatments VIT-A1, VIT-A2, and VIT-A3, respectively. There was no effect of VIT-A supplementation on marbling score or quality grade, the author noted that the ADG was significantly higher when cattle were supplemented at the NRC recommended 2,200 IU/kg DM. These studies show that the effect of VIT-A restriction may not be as pronounced in cattle that have a low propensity to marble. We may also conclude that in cattle that are becoming deficient in VIT-A, the first signs are decreased dry matter intake (DMI) and ADG.

A more recent study sought to test the hypothesis that commercial Angus cattle are often over-supplemented vitamins A and D, without clear benefit (Pickworth et al., 2012). One hundred sixty four Angus cross steers were used in a 2x2 factorial with vitamin A and D either supplemented at 3,750 and 1,860 IU/kg DM for A and D, respectively; or unsupplemented. Treatments began when steers weighed approximately 284 kg, and lasted until steers weighed approximately 584 kg. Vitamin D had minimal effects on carcass adiposity, and had no effect on marbling. Steers lacking VIT-A supplementation graded higher, and approximately 12% more steers brought premiums at slaughter. The marbling scores of non-supplemented steers averaged 582, and the marbling scores of supplemented steers averaged 550.

Ward et al. (2012) investigated the relationship of VIT-A restriction and the genotype for the *ADH1C* gene. This gene has two alleles, assigned T and C alleles. A total of 130 Angus cross steers were backgrounded on a low VIT-A diet for three months, at which time they were assigned to treatment; which were unsupplemented or supplemented with 750,000 IU/month, which equates to near the NRC recommended 2,200 IU/kg DM. Steers were maintained on the finishing diet for 5 months. Marbling was significantly greater in unsupplemented steers than supplemented steers, with scores of 570 and 540, respectively. There was no effect of genotype or genotype x supplementation on marbling scores, but there was an interaction of genotype x supplementation on IMF, with unsupplemented TT steers having 24.4% more IMF than supplemented TT steers and within the unsupplemented group TT steers had 22.9% more IMF than CC steers. The authors concluded that VIT-A restriction increases IMF in Angus steers and when VIT-A is restricted the TT genotype results in greater IMF than the CC genotype. The explanation for the differences seen between genotypes is that TT steers have a greater ability to convert retinol to retinal when retinol is limiting, and retinaldehyde is decreased at the cost of

maintenance of RA when VIT-A is limiting; RA has a stimulatory effect on adipogenesis, whereas retinal an inhibitory effect (Ward et al., 2012). There have been many studies examining the effect and mechanism of retinol, retinal, and RA *in vitro*, which were addressed in the adipogenesis section of this review, but the realm of *in vivo* mechanistic studies is just being unveiled.

Hypothesis and Objectives

Our hypothesis for this study is that a low Vitamin A diet will increase marbling, improve meat quality characteristics and increase adipogenic gene expression, while having no effect on production characteristics in Angus cross and Simmental steers.

Our objectives include discovering the differential gene expression between Simmental and commercial Angus cattle, as well as between the nutritional treatments, to discover the key time period for nutritional regulation of adipogenesis, to increase marbling in both breeds of cattle, and ultimately to provide a nutritional option to increase profits for producers without negative repercussions.

Literature Cited

Amengual, J., J. Ribot, M. L. Bonet, and A. Palou. 2008. Retinoic acid treatment increases lipid oxidation capacity in skeletal muscle of mice. Obesity. 16:585–591. doi:10.1038/oby.2007.104

Anubhuti, V. and S. Arora. 2008. Leptin and its metabolic interactions – an update. Diabetes Obes. Metab. 10:973-993. doi:10.1111/j.1463-1326.2008.00852.x

Appling, D. R. and F. Chytil. 1981. Evidence of a role for retinoic acid (vitamin A-acid) in the maintenance of testosterone production in male rats. Endocrinology. 108:2120-2123

Bardot, T. C., N. Aldridge, N. Latruffe, and S. Green. 1993. PPAR-RXR heterodimer activates a peroxisome proliferator response element upstream of the bifunctional gene. Biochem. Biophys. Res. Commun. 192:37-45.

Bell, E. C., M. John, R. J. Hughes, T. Pham. 2014. Ultra-performance liquid chromatographic determination of tocopherols and retinol in human plasma. J. Chromatogr. Sci. 52:1065-1070. doi:10.1093/chromsci/bmt161

Benito, M., A. Porras, A. R. Nebreda, and E. Santos. 1991. Differentiation of 3T3-L1 fibroblasts to adipocytes induced by transfection of ras oncogenes. Science. 253:565-568.

Blomhoff, R. and H. K. Blomhoff. 2006. Overview of retinoid metabolism and function. Dev. Neurobiol. 66:7:606-630. doi:10.1002/neu.20242

Bonet, M. L., J. Ribot, F. Felipe, and A. Palou. 2003. Vitamin A and the regulation of fat reserves. Cell. Mol. Life Sci. 60:1311-1321. doi:10.1007/s00018-003-2290-x

Boney, C. M., R. M. Smith, and P. A. Gruppuso. 1998. Modulation of insulin-like growth factor 1 mitogenic signaling in 3T3-L1 preadipocyte differentiation. Endocrinology. 139:1638-1644.

Bonnet, M., Y. Faulconnier, C. Leroux, C. Jurie, I. Cassar-Malek, D. Bauchart, P. Boulesteix, D. Pethick, J. F. Hocquette, and Y. Chilliard. 2007. Glucose-6-phosphate dehydrogenase and leptin are related to marbling differences among Limousin and Angus or Japanese Black × Angus steers. J. Anim. Sci. 85:2882-2894. doi:10.2527/jas.2007-0062

Bost, F., L. Caron, I. Marchetti, C. Dani, Y. Le Marchand-Brustel, and B. Binetruy. 2002. Retinoic acid activation of the ERK pathway is required for embryonic stem cell commitment into the adipocyte lineage. Biochem. J. 361:621-627. doi**:**10.1042/bj3610621

Boucher, J., M. Mori, K. Lee, G. Smyth, C. W. Liew, Y. Macotela, M. Rourk. M. Bluher, S. J. Russell, and C. R. Kahn. 2012. Impaired thermogenesis and adipose tissue development in mice with fat-specific disruption of insulin and IGF-1 signaling. Nature. 3:902. doi:10.1038/ncomms1905

Bowling, R. A., J. K. Riggs, G. C. Smith, Z. L. Carpenter, R. L. Reddish, and O. D. Butler. 1978. Production, carcass and palatability characteristics of steers produced by different banagement systems. J. Anim. Sci. 46:333-340. doi:10.2527/jas1978.462333x

Buchanan, F. C., C. J. Fitzsimmons, A. G. Vit-An Kessel, T. D. Thue, D. C. Winkelman-Sim, S. M. Schmutz. 2002. Association of a missense mutation in the bovine leptin gene with carcass fat content and leptin mRNA levels. Genet. Sel. Evol. 34:105-116. doi:10.1051/gse:2001006

Bugge T. H., J. Pohl, O. Lonnoy, and H. G. Stunnenberg. 1992. RXR alpha, a promiscuous partner of retinoic acid and thyroid hormone receptors. EMBO J. 11:1409–1418.

Castenmiller, J. J. M and C. E. West. 1998. Bioavailability and bioconversion of carotenoids. Annu. Rev. Nutr. 18:19-38.

Chew, B. P. 1993. Effects of supplemental β-carotene and vitamin A on reproduction in swine. J. Anim. Sci. 71:247-252.

Choi, S. H., D. T. Silvey, B. J. Johnson, M. E. Doumit, K. Y. Chung,J. E. Sawyer, G. W. Go, and S. B. Smith. 2014. Conjugated linoleic acid (t-10, c-12) reduces fatty acid synthesis de novo, but not expression of genes for lipid metabolism in bovine adipose tissue ex vivo. Lipids. 49:15-24. doi:10.1007/s11745-013-3869-0

Christoffersen, C. T., H. Tornqvist, C. J. Vlahos, D. Bucchini, J. Jami, P. D. Meyts, and R. L. Joshi. 1998. Insulin and insulin-like growth factor-1 receptor mediated differentiation of

3T3-F442A cells into adipocytes: effect of PI 3-kinase inhibition. Biochem. Biophys. Res. Comm. 246(2):426-430. doi:10.1006/bbrc.1998.8637

Clemmons, D. R., W. Busby, T. Arai, T. J. Nam, J. B. Clarke, J. I. Jones, and D. K. Ankrapp. 1995. Role of insulin-like growth factor binding proteins in the control of IGF actions. Prog. Growth Factor Res. 6:357-366.

Crouse, J. D., H. R. Cross, and S. C. Seideman. 1984. Effects of a grass or grain diet on the quality of three beef muscles. J. Anim. Sci. 58:619-625. doi:10.2527/jas1984.583619x

Cryer, P. E. 1993. Glucose counterregulation: prevention and correction of hypoglycemia in humans. Am. J. Physiol. 264: E149-55.

Dawson, R. M. and N. Hemington. 1974. Digestion of grass lipids and pigments in the sheep rumen. Br. J. Nutr. 32:327-340.

Dow, D. L., B. R. Wiegand, M. R. Ellersieck, and C. L. Lorenzen. 2011. Prediction of fat percentage within marbling score on beef longissimus muscle using 3 different fat determination methods1. J. Anim. Sci. 89:1173-1179. doi:10.2527/jas.2010-3382

Duester, G. 2000. Families of retinoid dehydrogenases regulation vitamin A function: production of visual pigment and retinoic acid. Eur. J. Biochem. 267:4315-4324. doi:10.1046/j.1432-1327.2000.01497.x

El-Jack A. K., J. K. Hamm, P. F. Pilch, and S. R. Farmer. 1999. Reconstitution of insulin-sensitive glucose transport in fibroblasts requires expression of both PPARγ and C/EBPα. J. Bio.l Chem. 274:7946–7951. doi:10.1074/jbc.274.12.7946

Fajas, L., J. Fruchart, and J. Auwerx. 1998. Transcriptional control of adipogenesis. Curr. Opin. Cell Biol. 10:165-173.

Farmer, S. R. 2005. Regulation of PPARgamma activity during adipogenesis. Int. J. Obes. 29(Suppl. 1):S13–S16. doi:10.1038/sj.ijo.0802907

Feve, B. 2005. Adipogenesis: cellular and molecular aspects. Best Pract. Res. Clin. Endocrionol. Metab. 19:483-499. doi:10.1016/j.beem.2005.07.007

Field, R. A. 1971. Effect of castration on meat quality and quantity. J. Anim. Sci. 32:849-858. doi:10.2527/jas1971.325849x

Furr, H. C. and R. M. Clark. 1997. Intestinal absorption and tissue distribution of carotenoids. J. Nutr. Biochem. 8:364-377.

Gotoh, T., E. Albrecht, F. Teuscher, K. Kawabata, K. Sakashita, H. Iwamoto, and J. Wegner. 2009. Differences in muscle and fat accretion in Japanese black and European cattle. Meat Sci. 82:300-308. doi:10.1093/j.meatsci.2009.01.026

Greene, L. W., D. K. Lunt, F. M. Byers, N. K. Chirase, C. E. Richmond, R. E. Knutson, and G. T. Schelling. 1988. Performance and carcass quality of steers supplemented with zinc oxide or zinc methionine. J. Anim. Sci. 66:1818-1823. doi:10.2527/jas1988.6671818x

Hadley, M. E. and J. E. Levine. 2009. Endocrinology. 6th ed. Pearson Education, Inc. Noida, India. 11-12:241-273

Hale, D. S., K. Goodson, and J. W. Savell. 2013. USDA beef quality and yield grades. Texas A&M AgriLife Extention Service. College Station, TX.

Harper G. S. and D. W. Pethick. 2004. How might marbling begin?. Aust. J. Anim. Sci. 44:653-662. doi:10.1071/EA02114

Hausman, G. J., M. V. Dodson, K. Ajuwon, M. Azain, K. M. Barnes, L. L. Guan, Z. Jiang, S. P. Poulos, R. D. Sainz, S. Smith, M. Spurlock, J. NoVit-Akofski, M. E. Fernyhough, and W.

G. Bergen. 2009. Board-Invited Review: The biology and regulation of preadipocytes and adipocytes in meat animals. J. Anim. Sci. 87:1218-1246. doi:10.2527/jas.2008-1427

Herrera, R., S. R. Ro, G. S. Robinson, K. G. Xanthopoulos, and B. M. Spiegelman. 1989. A direct role for C/EBPα and the AP-1 binding site in gene expression linked to adipocyte differentiation. Mol. Cell. Biol. 9:5331-5339.

Hood, R. L. and C. E. Allen. 1978. Lipogenesis in isolated intramuscular adipose tissue from four bovine muscles. J. Anim. Sci. 46:1626–1633.

Hunt, M. R., A. J. Garmyn, T. G. O'Quinn, C. H. Corbin, J. F. Legako, R. J. Rathmann, J. C. Brooks, and M. F. Miller. 2014. Consumer assessment of beef palatability from four beef muscles from USDA Choice and Select graded carcasses. Meat Sci. 98:1-8. doi:10.1016/j.meatsci.2014.04.004

Kim, J. B., H. M. Wright, M. Wright, and B. M. Speigelman. 1998. ADD1/SREBP1 activates PPAR-gamma through the production of endogenous ligand. PNAS. 95:4333–4337.

King, T. B., T. G. Lohman, and G. S. Smith. 1962. Evidence of rumeno-reticular losses of vitamin A and carotene. J. Anim. Sci. 21:1002

Koder, K. R., and R. M. Evans. 1999. PPAR gamma is required for placental, cardiac, and adipose tissue development. Mol. Cell. 4:585–595.

Laron, Z. 2001. Insulin-like growth factor 1 (IGF-1): a growth hormone. J. Clin. Pathol. 54:311-316

Leblanc B.P. and H. G. Stunnenberg. 1995. 9-cis retinoic acid signaling: changing partners causes some excitement. Genes Dev. 9:1811-6.

MacDougald, O. A. and M. D. Lane. 1995. Transcriptional regulation of gene expression during adipocyte differentiation. Annu. Rev. Biochem. 64:645-73.

Mangelsdorf, D. J. and R. M. Evans. 1995. The RXR heterodimers and orphan receptors. Cell. 83:841– 850.

Mangelsdorf, D. J., U. Borgmeyer, R. A. Heyman, Y. J. Zhou, E. S. Ong, A. E. Oro, A. Kakizuka, and R. M. EVit-Ans. 1992. Characterization of three RXR genes that mediate the action of 9-cis retinoic acid. Genes Dev. 6:329-44. doi:10.1101/gad.6.3.329

Manolescu, D. C., A. Sima, and P. V. Bhat. 2010. All-trans retinoic acid lowers serum retinol-binding protein 4 concentrations and increases insulin sensitivity in diabetic mice. J. Nutr. 140:311-6. doi:10.3945/jn.109.115147

Michal, J. J., Z. W. Zhang, C. T. Gaskins, and Z. Jiang. 2006. The bovine fatty acid binding protein 4 gene is significantly associated with marbling and subcutaneous fat depth in Wagyu x Limousin F2 crosses. Anim. Genet.37:400–402. doi:10.1111/j.1365-2052.2006.01464.x

Molotkov, A., X. Fan, and G. Duester. 2002. Excessive vitamin A toxicity in mice genetically deficient in ether alcohol dehydrogenase Adh1 or Adh3. Eur. J. Biochem. 269:2607–2612. doi:10.1046/j.1432-1033.2002.02935.x

Mora, J. R., M. Iwata, and U. H. Andrian. 2008. Vitamin effects on the immune system: vitamins A and D take centre stage. Nat. Rev. Immunol. 8:685-698. doi:10.1038/nri2378

Mora, O., J. L. Romano, E. Gonzalez, F. J. Ruiz, A. Shimada. 1999. In vitro and in situ disappearance of beta-carotene and lutein from Lucerne (Medicago sativa) hay in bovine and caprine ruminal fluids. J. Sci. Food Agric. 79:273-276. doi:10.1002/(SICI)1097-0010(199902)79:2<273::AID-JSFA191>3.0.CO;2-V

Nade, T., S. Hirabara, T. Okumura, and K. Fujita. 2003. Effects of vitamin A on carcass composition concerning younger steer fattening of Wagyu cattle. Asian-Aust. J. Anim. Sci. 16:353. doi:10.5713/ajas.2003.353

Namitha, K. K. and P. S. Negi. 2010. Chemistry and biotechnology of carotenoids. Crit. Rev. Food Sci. Nutr. 50:728-760. doi:10.1080/10408398.2010.499811

Noziere, P., B. Graulet, A. Lucas, B. Martin, P. Grolier, and M. Doreau. 2006. Carotenoids for ruminants: From forages to dairy products. Anim. Feed Sci. Tech. 131:418-450. doi:10.1016/j.anifeedsci.2006.06.018

NRC. 1996. Nutrient requirements of beef cattle. 7th rev. ed. Natl. Acad. Press, Washington, DC.

NRC. 2016. Nutrient requirements of beef cattle. 8th rev. ed. Natl. Acad. Press, Washington, DC.

North Dakota State University. 2010. Quality grades of beef carcasses. North Dakota Beef Quality Assurance. Fargo, ND

Ogden C. L., M. D. Carroll, B. K. Kit, and K. M. Flegal. 2014. Prevalence of childhood and adult obesity in the United States, 2011-2012. JAMA. 311:806-814. doi:10.1001/jama.2014.732.

Ogino T., D. H. Moralejo, H. Kose, T. Yamada, and K. Matsumoto. 2003. Serum leptin concentration is linked to chromosomes 2 and 6 in the OLETF rat, an animal model of type 2 diabetes with mild obesity. Mamm. Genome. 14:839–44. doi:10.1007/s00335-003-2295-7

Oka, A., Y. Maruo, T. Miki, M. Yamasaki, and T. Saito. 1998. Influence of vitamin A on the quality of beef from the Tijima strain of Japanese Black cattle. Meat Sci. 48:159-167.

Otto, T. C. and M. D. Lane. 2005. Adipose development: From stem cell to adipocyte. Crit. Rev. Biochem. Mol. Biol. 40:229–242. doi:10.1080/10409230591008189

Paterson, E. K., H. Ho, R. Kapadia, and A. K. Ganesan. 2013. 9-*cis* retinoic acid is the ALDH1A1 product that stimulates melanogenesis. Exp. Dermatol. 22:202–209. doi:10.1111/exd.12099

Pethick, D. W., G. S. Harper, and V. H. Oddy. 2004. Growth, development and nutritional manipulation of marbling in cattle: a review. Aust. J. Exp. Agr. 44:705-715. doi:10.1071/EA02165

Porse, B. T., T. A. Pederson, X. Xu, B. Lindberg, U. M. Wewer, L. Friis-Hansen, and C. Nerlov. 2001. E2F repression by C/EBPα is required for adipogenesis and granulopoiesis in vivo. Cell. 107:247-258. doi:10.1016/S0092-8674(01)00516-5

Potkanski, A. A., R. E. Tucker, and G. E. Mitchell. 1974. Pre-intestinal carotene losses in sheep fed alfalfa or isolated carotene beadlets. Int. J. Vitam. Nutr. Res. 44:3-7.

Pyatt, N. A., L. L. Berger, and T.G. Nash. 2005. Effects of vitamin a and restricted intake on performance, carcass characteristics, and serum retinol status in Angus x Simmental feedlot cattle. Prof. Anim. Sci. 21:318-331. doi:10.15232/S1080-7446(15)31223-7

Raghu, P. and B. Sivakumar. 2004. Interactions amongst plasma retinol-binding protein, transthyretin and their ligands: implications in vitamin A homeostasis and transthyretin amyloidosis. Biochem. Biophys. Acta. 1703:1-9. doi:doi.org/10.1016/j.bbapap.2004.09.023

Reichert, B., R. Yasmeen, S. M. Jeyakumar, F. Yang, T. Thomou, H. Alder, and O. Ziouzenkova. 2011. Concerted action of aldehyde dehydrogenases influences depot-specific fat formation. Mol. Endocrinol. 25:799-809. doi:10.1210/me.2010-0465

Riga J. R. and K. H. Dragnev. 2005. Emerging role of rexinoids in non-small cell lung cancer: focus on bexrotene. Oncologist. 10:22-33. doi:10.1634/theoncologist.10-1-22

Romero, G. and J. Larner. 1993. Insulin mediators and the mechanism of insulin action. Adv. Pharmacol. 24:21-50.

Rosen, E. D. and B. M. Spiegelman. 2000. Molecular regulation of adipogenesis. Annu. Rev. Cell Dev. Biol. 16:145-71. doi:10.1146/annurev.cellbio.16.1.145

Rosen, E. D. 2005. The transcriptional basis of adipocyte development. Prostaglandins Leukot. Essent. Fatty Acids. 73:31.–34. doi:10.1016/j.plefa.2005.04.004

Ross, C. 1992. Vitamin A status: relationship to immunity and the antibody response. Proc. Soc. Exp. Biol. Med. 200:303-320.

Salmon W. D. and W. H. Daughaday. 1957. A hormonally controlled serum factor which stimulates sulfate incorporation by cartilage in vitro. J. Lab. Clin. Med. 49:825–36.

Schwarz, E. J., M. J. Reginato, D. Shao, S. L. Krakow, and M. A. Lazar. 1997. Retinoic acid blocks adipogenesis by inhibiting C/EBPbeta-mediated transcription. Mol. Cell. Biol. 17:1552-1561.

Siebert, B. D., Z. A. Kruk, J. Davis, W. S. Pitchford, G. S. Harper, and C. D. K. Bottema. 2006. Effect of low vitamin A status on fat deposition and fatty acid desaturation in beef cattle. Lipids. 41:365-370. doi:10.1007/s11745-006-5107-5

Smas, C. M., L. Chen, L. Zhao, M. J. Latasa, and H. S. Sul. 1999. Transcriptional repression of pref-1 by glucocorticoids promotes 3T3–L1 adipocyte differentiation. J. Biol. Chem. 274:12632.–12641. doi: 10.1074/jbc.274.18.12632

Smith, S. B. 2017. Practical application of the basic aspects of GLUT4 membrane trafficking and insulin signaling on issues related to animal agriculture. J. Anim. Sci. 2017:95: 2185-2197. doi:10.2527/jas2016.0984

Smith, S. B. and B. J. Johnson. 2014. Marling: Management of cattle to maximize the deposition of intramuscular adipose tissue. Nat. Cat. Beef Ass. URL:http://www.beefresearch.org/CMDocs/BeefResearch/PE_White_%20Papers/Marbling%20%20Management%20of%20cattle%20to%20maximize%20the%20deposition%20of%20intramuscular%20adipose%20tissue.pdf

Smith, P.J., L.S. Wise, R. Berkowitz, C. Wan, and C.S. Rubin. 1988. Insulin-like growth factor-I is an essential regulator of 3T3-L1 adipocytes. J. Biol. Chem. 263:9402-9406.

Stecher, H., M. H. Gelb, J. C. Saari, and K. Palczewski. 1999. Preferential release of 11-cis-retinol from retinal pigment epithelial cells in the presence of cellular retinaldehyde-binding protein. J. Biol. Chem. 274:8577-8585.

Sul, H. S., C. Smas, B. Mei, and L. Zhou. 2000. Function of pref-1 as an inhibitor of adipocyte differentiation. Int. J. Obes. Relat. Metab. Disord. 24(Suppl. 4):S15-S19. doi:10.1038/sj.ijo.0801494

Suzawa, M., I. Takeda, J. Yanagisawa, F. Ohtake, S. Ogawa, T. Yamauchi, T. Kadawaki, Y. Takeuchi, H. Shibuya, Y. Gotoh, K. Matsumoto, and S. Kato. 2003. Cytokines suppress adipogenesis and PPARγ function through the TAK1/TAB1/NIK cascade. Nat. Cell Biol. 5:224-230. doi:10.1038/ncb942

Tan N. S., N. S. Shaw, N. Vinckenbosch, P. Liu, R. Yasmin, and B. Desvergne. 2002. Selective cooperation between fatty acid binding proteins and peroxisome proliferator-activated receptors in regulating transcription. Mol. Cell Biol. 22:5114–5127. doi:10.1128/MCB.22.14.5114-5127.2002

Tanaka, S., E. Takahashi, T. Matsui, and H. Yano. 2001. Zinc promotes adipocyte differentiation in vitro. Asian-Australas. J. Anim. Sci. 7:966-969.

Tontonoz, P., E. Hu, R. A. Graves, A. I. Budavari, and B. M. Spiegelman. 1994. mPPARy2: tissue-specific regulator of an adipocyte enhancer. Genes Dev. 8:1224-1234.

Tontonoz, P., E. Hu, and B. M. Spiegelman. 1994. Stimulation of Adipogenesis in Fibroblasts by PPARγ2, a lipid-activated transcription factor. Cell. 79:1147-1156.

United States Department of Agriculture. 2016. Overview of the United States cattle industry. National Agricultural Statistics Service. http://usda.mannlib.cornell.edu/usda/current/USCatSup/USCatSup-06-24-2016.pdf

Vasconcelos, J. T. and M. L. Galyean. 2007. Nutritional recommendations of feedlot consulting nutritionists: The 2007 Texas Tech University survey. J. Anim. Sci. 85:2772-2781. doi:10.2527/jas.2007-0261

Wang, B., Q. Yang, C. L. Harris, M. L. Nelson, J. R. Busboom, M. Zhu, and M. Du. 2016. Nutrigenomic regulation of adipose tissue development – the role of retinoic acid: A review. Meat Sci. 120:100-106. doi:10.1016/j.meatsci.2016.04.003

Wang, N. D., M. J. Finegold, A. Bradley, C. N. Ou, S. V. Abdelsayed, M. D. Wilde, L. R. Taylor, D. R. Wilson, and G. J. Darlington. 1995. Impaired energy homeostasis in C/EBP-alpha knockout mice. Science. 269:1108–1112.

Wang, W. J., S. P. Wang, Y. S. Gong, J. Q. Wang, and Z. L. Tan. 2007. Effects of vitamin A supplementation on growth performance, carcass characteristics and meat quality in Limosin x Luxi crossbreed steers fed a wheat straw-based diet. Meat Sci. 77:450-458. doi:10.1016/j.meatsci.2007.04.019

Wang, Y. and H. S. Sul. 2006. Ectodomain shedding of preadipocyte factor 1 (Pref-1) by tumor necrosis factor alpha converting enzyme (TACE) and inhibition of adipocyte differentiation. Mol. Cell Biol, 26:5421–35. doi:10.1128/MCB.02437-05

Wang Y., L. Zhao, C. Smas, and H. S. Sul. 2010. Pref-1 interacts with fibronectin to inhibit adipocyte differentiation. Mol. Cell Biol. 30:3480–92. doi:10.1128/ MCB.00057-10

Ward, A. K., J. J. McKinnon, S. Hendrick, and F. C. Buchanan. 2012. The impact of vitamin A restriction and ADH1C genotype on marbling in feedlot steers1. J. Anim. Sci. 90:2476-2483. doi:10.2527/jas.2011-4404

Wolf, G. 2007. Identification of a membrane receptor for retinol-binding protein functioning in the cellular uptake of retinol. Nutr. Rev. 65:385-388. doi:10.1111/j.1753-4887.2007.tb00316.x

Wu, Z., N. L. Bucher, and S. R. Farmer. 1996. Induction of peroxisome proliferator-activated receptor gamma during the conversion of 3T3 fibroblasts into adipocytes is mediated by C/EBPbeta, C/EBPdelta, and glucocorticoids. Mol. Cell. Biol. 16:4128.–4136. doi:10.1128/MCB.16.8.4128

Yeum, K and R. M. Russell. 2002. Carotenoid bioavailability and bioconversion. Annu. Rev. Nutr. 22:483-504. doi:10.1146/annurev.nutr.22.010402.102834

Zezulak K. M. and H. Green. 1986. The generation of insulin-like growth factor-1 sensitive cells by growth hormone action. Science. 233:551-553

Zhang, Y., M. Matheny, S. Zolotukhin, N. Tumer, and P. J. Scarpace. 2002. Regulation of adiponectin and leptin gene expression in white and brown adipose tissues: Influence of β_3-adrenergic agonists, retinoic acid, leptin and fasting. Biochem. Biophys. Acta. 1584:115-122. doi:10.1016/S1388-1981(02)00298-6

Xue, J. C., E. J. Schwarz, A. Chawla, and M. A. Lazar. 1996. Distinct stages in adipogenesis revealed by retinoid inhibition of differentiation after induction of PPARγ. Mol. Cell. Biol. 16:1567−1575.

Ziouzenkova, O., G. Orasanu, M. Sharlach, T. E. Akiyama, J. P. Berger, J. Viereck, J. A Hamilton, G. Tang, G. G. Dolnikowski, S. Vogel, G. Duester, and J. Plutzky. 2007. Retinaldehyde represses adipogenesis and diet-induced obesity. Nat. Med. 13:695−702. doi:10.1038/nm1587

CHAPTER 2: EFFECT OF FEEDING A LOW VITAMIN A DIET ON CARCASS AND PRODUCTION CHARACTERISTICS OF STEERS WITH A HIGH AND LOW PROPENSITY FOR MARBLING[1]

Abstract

Commercial Angus steers consisting of a minimum of 75% Angus genetics (Angus x Simmental) and purebred Simmental steers were used to evaluate the effect of a low vitamin A diet on growth and meat quality characteristics. Breed and vitamin A treatments were arranged in a 2x2 factorial design. After a 3-month growing period on a low vitamin diet (1017 IU/kg), 64 steers (32 commercial Angus, 32 Simmental) were allocated to one of two dietary treatments for finishing. One treatment consisted of the basal diet with no supplemental vitamin A (LVIT-A; 723 IU vitamin A/kg DM), the other (CON) was supplemented with vitamin A at the NRC recommended concentration of 2200 IU/kg DM. At the completion of finishing, steers were slaughtered at a commercial abattoir. Two strip loin steaks were collected from each steer to analyze meat quality characteristics including intramuscular fat content, color (L*, a*, b*), Warner-Bratzler sheer force, cook loss, subjective marbling score, and pH. Blood samples were collected at three time points throughout the trial to analyze serum retinol concentration. Camera image analysis was used for analysis of marbling, 12th rib s.c. fat, longissimus muscle area, HCW, KPH, and quality and yield grades. During finishing, steers in the LVIT-A treatment had lower serum retinol (P = 0.02). There was an interaction of breed and vitamin A treatment on

[1]The material in this chapter was co-authored by E. E. Knutson, X. Sun, A. B. P. Fontoura, J. Gaspers, J. H. Liu, K. R. Carlin, M. Bauer, K. C. Swanson, and A. K. Ward. It has been submitted for publication to the Journal of Animal Science. E. E. Knutson and A. K. Ward had the responsibilities of collecting samples. E. E. Knutson is the author that developed the conclusions, did the original and corrections drafting, and developed the statistical modelling. A. K. Ward served as proofreader and checked the statistical analysis conducted by E. E. Knutson.

marbling (P=0.008). Within the commercial Angus cattle, the LVA treatment resulted in 16% greater marbling scores, and 27% of cattle grading higher (choice vs. prime) than their CON counterparts. Intramuscular fat analysis yielded a breed and treatment effect ($P < 0.01$), but no interaction. Simmentals had greater ($P < 0.01$) LM area and loin pH, whereas commercial Angus steers had greater s.c. fat and KPH ($P < 0.01$) and subsequently greater ($P <.01$) yield grade. In conclusion, feeding a LVA diet during finishing increased marbling in commercial Angus but not in Simmental steers. Commercial Angus steers had greater s.c. fat and KPH, whereas Simmental cattle had larger LM area. By removing vitamin A supplementation from the finishing rations fed to commercial Angus steers, the value of a carcass can be potentially increased, increasing profits for producers and the industry as a whole.

Key words: marbling, vitamin a, Angus, Simmental, meat quality, adipogenesis

Introduction

Marbling is one of the most influential factors that affect consumer preference in meat; generally, palatability increases with increased marbling through factors such as tenderness, juiciness, and taste (Smith, 2014; Wang et al., 2009). Feeding a low vitamin A diet has been shown to increase marbling without increasing s.c. fat deposition (Gorocia-Buenfil et al., 2007 a,b). Other studies have found no difference in marbling with a vitamin A restricted-diet (Bryant et al., 2004; Pyatt et al., 2005). As different levels of vitamin A at different time points have yielded varied effects on marbling, it is clear that the mechanisms controlling the deposition of intramuscular fat are not well understood. Intramuscular fat deposition is more complex than that of subcutaneous fat in beef cattle, as it is affected by the genetic propensity to marble, nutritional plane throughout life, and environmental factors (Pethick et al., 2004). Vitamin A restriction has been shown previously to enhance marbling in cattle that genetically have a high propensity to

marble such as Angus and Tijama cattle (Adachi et al., 1999; Oka et al., 1998). In contrast, in Limosin × Luxi cattle (genetically lower propensity to marble), no difference in marbling was observed between vitamin A treatments (Wang et al., 2007). In Angus-cross steers vitamin A restriction had no effect on ADG, DMI, or feed efficiency (Gorocia-Buenfil et al., 2007 a,b, 2008). Restricting dietary vitamin A may be a strategy to increase marbling without negatively impacting other production characteristics. The effect of vitamin A restriction on cattle with high or low propensity to marble has yet to be compared, and may be an important aspect to consider when determining vitamin A supplementation. The hypothesis of this study is that a low vitamin A diet will increase marbling and improve meat quality characteristics while having no effect on production characteristics of Angus cross and Simmental steers.

Materials and Methods

Protocols described herein were approved by the North Dakota State University Animal Care and Use Committee (protocol number A16004).

Experimental Design and Treatments

Pure-bred Simmental and commercial Angus (consisting of at least 75% Angus genetics) calves at approximately 7 months of age (32 per breed) were obtained from the North Dakota State University beef unit. Prior to the beginning of the trial, the steers had been grazing on summer pasture. Following a 2-wk acclimation period, steers were fed a growing diet containing low concentrations of vitamin A (1017 IU/kg DM) for 95 d. This diet was designed to begin to deplete the liver vitamin A stores, which can sustain serum retinol for 2 to 4 mo (NRC, 1996).

Steers were allocated to treatment on day 96 of the trail. A total of 16 steers per breed were stratified by weight and allocated to 2 pens and 2 nutritional treatments. The low vitamin A (LVA) treatment was the basal diet that contained 723 IU vitamin A/kg of DM (Table 1). The

control (CON) treatment was the LVA diet supplemented with an additional 2,200 IU of vitamin A/kg of DM, based on NRC (2016) recommendations, for a total of 2,723 IU vitamin A/kg DM. The finishing phase spanned 156 or 184 d depending on slaughter date. Steers were weighed monthly and individual feed intake was recorded using the RIC feeding system (Insentec, Markness, The Netherlands), these measures were used to monitor for signs of vitamin A deficiency.

Table 2.1. Ingredient and nutrient composition (DM basis) of TMR growing and finishing diets fed

Item	Backgrounding, %	Finishing, %
Ingredient		
Brome hay	15.0	---
Wheat straw	30.0	10.0
Barley	10.0	---
Corn	20.0	60.0
CSB[2]	---	5.0
DDGS[3]	20.0	20.0
Supplement[1]	5.0	5.0
Nutrient composition		
CP	15.0	14.7
NDF	48.2	25.5
ADF	26.0	10.3
Ca	0.69	1.01
P	0.32	0.53

[1]Supplement contained ground corn, limestone, urea, salt, monensin (176.4g/kg premix, Elanco, Greenfield, IN), tylosin (88.2g/kg premix, Elanco), vitamin D (3,000 IU/kg), and a trace mineral premix.
[2]Concentrated separator by-product (partially de-sugared beet molasses).
[3]Dried distiller's grains with solubles.

Serum Retinol and Hormone Analysis

Serum was collected at the beginning of the growing period (day 0), beginning of finishing (day 96), and end of finishing (day 268). These samples were used to monitor for serum retinol and hormone concentrations. Blood was collected using a 10 mL blood collection tube with no additives via jugular venipuncture. Blood was protected from light, allowed to clot, and stored on ice for 2 hours, then centrifuged for 10 minutes at 2,000 × *g* and serum collected. Serum was stored at -80°C, until retinol extraction was performed. Extraction and HPLC preparation was performed under dim red light to limit retinol degradation. Retinol extraction followed a procedure outlined previously (Gorocia-Buenfil et al., 2007b). Retinol quantification was performed via HPLC in the North Dakota State University Core Synthesis and Analytical Services laboratory.

Serum concentrations of leptin, insulin, IGF-1, and GH were measured in samples collected at the beginning and end of finishing via radioimmunoassay analysis at South Dakota State University Department of Animal Science (Brookings, SD). Serum concentrations of insulin were determined in duplicate using a Porcine Insulin RIA kit (PI-12K, EMD Millipore Corporation, St. Charles, MO). The antibody used in the kit cross-reacts with bovine insulin with 90% efficiency. Serum concentrations of leptin were determined in duplicate using the Multi-Species Leptin kit (XL-85K, EMD Millipore Corporation, St. Charles, MO). Serum concentrations of IGF-1 were determined in duplicate using a protocol previously described (Daughaday et al., 1980). Recombinant human IGF-I (GF-050; Austral Biological, San Ramon, CA) was used as the standard and radioiodinated antigen. Antiserum (UB2-495; National Hormone and Peptide Program, NIDDK) was used at a dilution of 1:62,500. Serum concentrations of GH were determined in duplicate using procedures described by Klindt et al.

(1983). Bovine GH (AFP-9884C, National Hormone and Pituitary Program) was used as the radio-iodinated antigen and standard. Growth hormone antiserum (AFPB55, National Hormone and Pituitary Program) was used at an initial dilution of 1:200,000.

Slaughter and Meat Quality Analysis

Steers were slaughtered in two groups after 156 (24 steers) and 184 d (37 steers) of feeding the finishing diet, when they reached approximately 610 kg of BW. Steers were slaughtered at a commercial abattoir (Tyson Fresh Meats., Dakota City, NE) where carcass data was collected by trained personnel. This data included hot carcass weight (HCW), USDA quality grade (QG), calculated yield grade (YG), calculated yield grade (CYG), and kidney pelvic heart fat (KPH) percentage. *Longissimus* muscle area (LMA), marbling score, and back fat depth were assessed by computer image analysis at the abattoir.

A boneless strip loin (IMPS 180) was collected from each carcass. The strip loin was transported to the North Dakota State University meat laboratory, where they were stored for 2 weeks at 37°C. After aging, two 2.6 cm steaks were cut from the center section of each loin. Steaks were analyzed for meat quality characteristics including subjective marbling score, marbling texture score, intramuscular fat, Minolta color score, pH, drip loss, and Warner-Bratzler shear force. Subjective marbling score was assessed by an experienced grader. Intramuscular fat was analyzed by ether extract following procedures described by the AOAC (2010). Minolta color score was measured with a Chroma Meter CR-410 (Konica Minolta, Tokyo, Japan). Meat pH was measured with a meat pH meter (Hanna HI99163, Hanna Instruments, Woonsocket, RI). Steak weights were recorded, steaks were cooked to a final temperature of 71°C with a clamshell-style grill (GR30Spectrum Brands Inc., Madison, WI), and cooked weights were recorded to calculate drip-loss. After cooking, steaks were placed on a

metal tray to allow cooling to room temperature. After cooling to room temperature, six 1.3-cm cores were removed from each steak parallel to the muscle fiber orientation. These cores were sheared perpendicular to the muscle fibers using a Mecmesin BFG500N force gauge (Mecmesin, Slinfold, West Sussex, UK).

Statistical Analysis

The experiment was a completely randomized block (slaughter date) design with a 2 x 2 factorial treatment arrangement, comparing breed (commercial Angus vs. Simmental) with vitamin A treatment (LVA vs. CON). Data were analyzed using the mixed procedure of SAS (v. 9.4; SAS Inst., Cary, NC). Breed, treatment, and their interaction were used as fixed effects of interest; slaughter date was used as a fixed effect as the block. Individual animal served as the experimental unit. When an interaction was observed ($P \leq 0.05$), means were separated using the LSMEANS procedure, and P-values ≤ 0.05 were considered significant.

Hormone and vitamin A data were also analyzed using the mixed procedure of SAS (v. 9.4; SAS Inst., Cary, NC). Fixed effects of interest included breed, treatment, date, and the interaction between each and between all three. If the P-value for an interaction was ≥ 0.05, it was removed from the model. Date was used as a repeated measure using an unstructured covariance structure, with animal as the subject. Means were separated using the LSMEANS procedure, and P-values ≤ 0.05 were considered significant.

Results

Serum Retinol Concentration

At the beginning of the growing period (d 0), steers in both treatments showed no difference ($P = 0.23$) in serum retinol concentration (Figure 1). Throughout growing all steers had been on similar diets, yet steers that would be assigned to the control diet ended the growing

phase with greater (P = 0.01) serum retinol than steers that would be allocated to the LVA diet. After allocation to treatment for the finishing phase, the CON treatment serum retinol levels increased (P < 0.01) to the end of finishing and were greater (P < 0.01) than the serum retinol of the LVA treatment, which had also increased (P < 0.01) since the beginning of backgrounding.

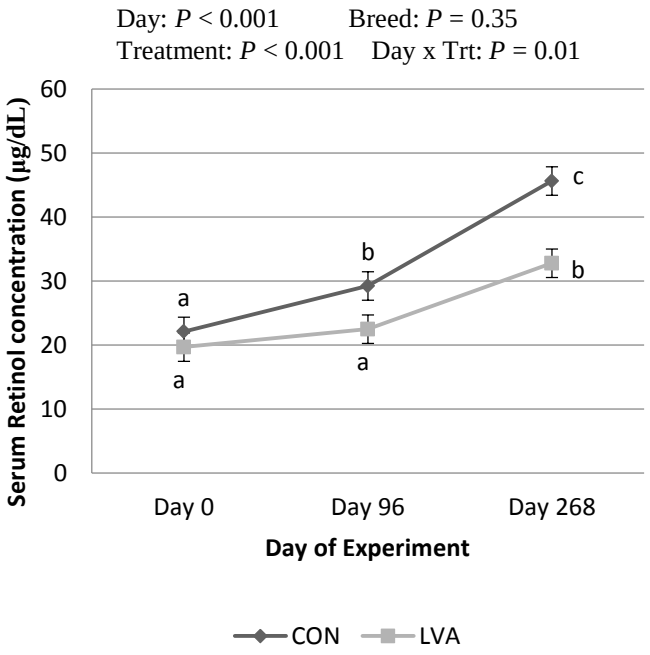

Figure 2.1. Serum retinol concentration increased more over time in the CON treatment than in the LVA treatment. Day 0 corresponds to the beginning of backgrounding, d 96 corresponds to the beginning of finishing, and d 268 corresponds to the end of finishing. LVA = basal diet containing 1,017 IU vitamin A/kg DM, CON = basal diet supplemented at 2,200 IU vitamin A/kg DM

Hormone Concentrations

The serum concentration of leptin resulted in a three way interaction ($P < 0.001$) between breed, treatment, and time (Table 2, Figure 2). Simmental cattle fed the LVA treatment had greater serum leptin concentrations than all other groups ($P < 0.05$). At the end of finishing, both treatments of commercial Angus cattle had greater concentrations of leptin than both treatments of Simmental cattle ($P < 0.05$).

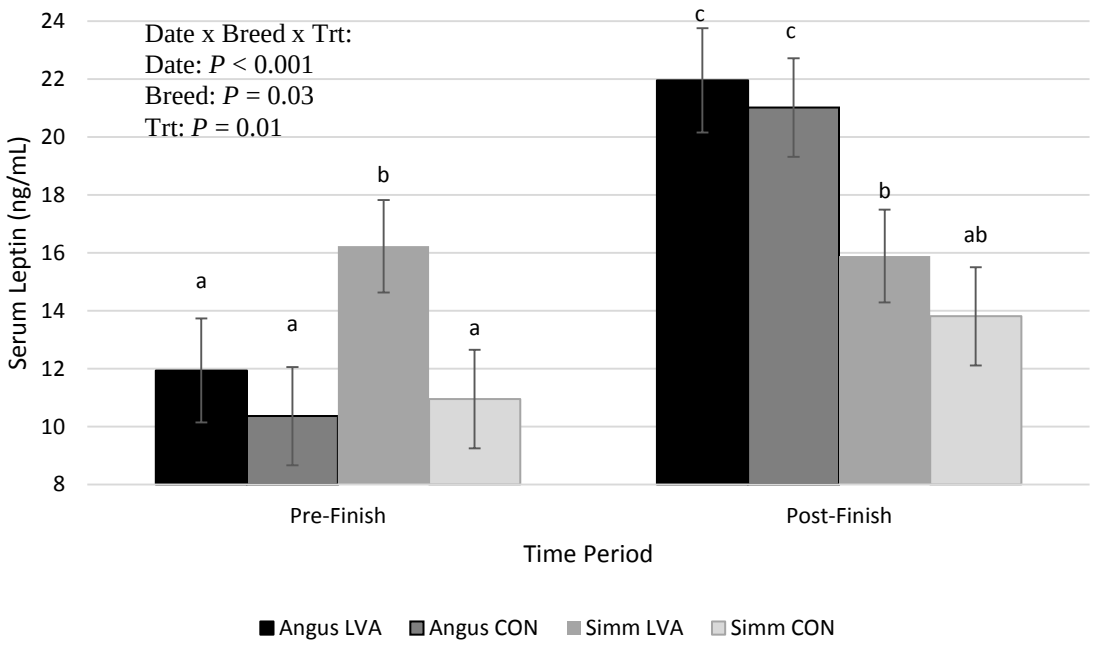

Figure 2.2. The effect of dietary vitamin A treatment (Trt) and breed on serum leptin concentration at the beginning (Pre-Finish, d 96) and end of the finishing period (Post-Finish, d 268). Dietary treatments consisted of low vitamin A (LVA) which contained no supplemental vitamin A, and control (CON) which contained supplemental 2,200 IU vitamin A/kg DM. The breeds were commercial Angus (Angus; minimum 75% Angus genetics) and pure bred Simmental (Simm). [abc]Means not sharing a common superscript are significantly different ($P < 0.05$)

Growth hormone was greater ($P = 0.01$) in Simmental cattle than in commercial Angus cattle but there was no effect of treatment or time. There was a tendency ($P = 0.09$) for IGF-1 to be greater in the CON treatment than the LVA treatment, with no breed effect. There was no effect ($P = 0.61$ and $P = 0.13$, respectively) of breed or treatment on insulin concentration.

Table 2.2. Effect of dietary vitamin A concentration on serum hormone level

Item	Trt[1]		Breed[2]		Time[3]		SEM	*P*-value[4]				
	LVA	CON	Simm	Angus	Pre	Post		Trt	Breed	Time	Breed ×Time	Trt × Time
Insulin[7]	22.1	24.1	23.4	22.8	9.2	37.0	3.0	0.13	0.61	<0.001	---	---
Leptin[5,6]	16.3	14.0	14.2	16.3	12.4	18.2	1.3	0.01	0.03	<0.001	---	---
GH[6]	9.4	8.8	11.0	7.1	11.8	6.3	2.3	0.71	0.02	<0.001	---	0.02
IGF-1[6]	140	151	143	148	140	151	12	0.08	0.48	<0.001	<0.001	---

[1]Trt = treatment, LVIT-A = basal diet containing 723 IU/kg DM vitamin A, CON = basal diet supplemented at 2,200IU/kg DM

[2]Simm = Pure bred Simmental steers, Angus = Commercial Angus steers containing > 75% Angus

[3]Pre = sampling pre-finishing (d= 96), Post = sampling at the end of finishing (d= 268)

[4]VIT-A=vitamin A level, Breed = Angus or Simmental; breed x treatment interaction was removed as it showed no significance

[5]VIT-A × breed × time interaction presented in Figure 3.

[6]Expressed in ng/mL

[7]Expressed in μU/mL

Production characteristics

Average daily gain of steers fed the LVA treatment was greater ($P = 0.03$) than the CON treatment, however, there was no effect of treatment on final BW or DMI (Table 2.3). There was no effect of VIT-A treatment on any other production measurement.

Final BW was greater ($P < 0.0001$) for the commercial Angus than Simmental steers. Commercial Angus steers exhibited greater DMI ($P < 0.01$) than Simmental steers. Simmental steers had a greater ($P = 0.02$) gain to feed ratio than the commercial Angus steers. Interestingly, there was no effect of breed or treatment on hot carcass weight ($P = 0.28$), as there was with final BW.

Table 2.3. Effects of dietary vitamin A concentration and breed on production characteristics

	Trt[1]		Breed[2]			*P*-value[3]		
Item	LVA[1]	CON[1]	Simm[2]	Angus[2]	SEM	Trt	Breed	Trt × Breed
BW, kg	610	598	585	624	12	0.44	0.02	0.51
ADG, kg/d	1.52	1.44	1.45	1.51	0.03	0.03	0.08	0.29
DMI, kg/d	11.10	10.58	10.29	11.39	0.25	0.12	0.002	0.20
G:F, kg/kg	0.138	0.137	0.142	0.134	0.003	0.75	0.02	0.59

[1]Trt = treatment, LVA = basal diet containing 723 IU/kg DM vitamin A, CON = basal diet supplemented with 2,200IU/kg DM for a concentration of 2,923
[2]Simm = Purebred Simmental steers, Angus = commercial Angus steers containing > 75% Angus
[3]Trt = vitamin A level, Breed = Angus or Simmental

Meat Quality Characteristics

There was an interaction between breed and treatment on marbling score ($P = 0.008$). Within the commercial Angus steers, the LVA treatment had 16% greater marbling scores than CON commercial Angus steers (Figure 3). There was no difference ($P = 0.98$) in marbling score between LVA and CON Simmental steers. Subjective marbling score yielded similar results, though the interaction between breed and treatment was not significant ($P > 0.05$). Treatment and breed both resulted in differences ($P = 0.003$ and $P < 0.001$, respectively).

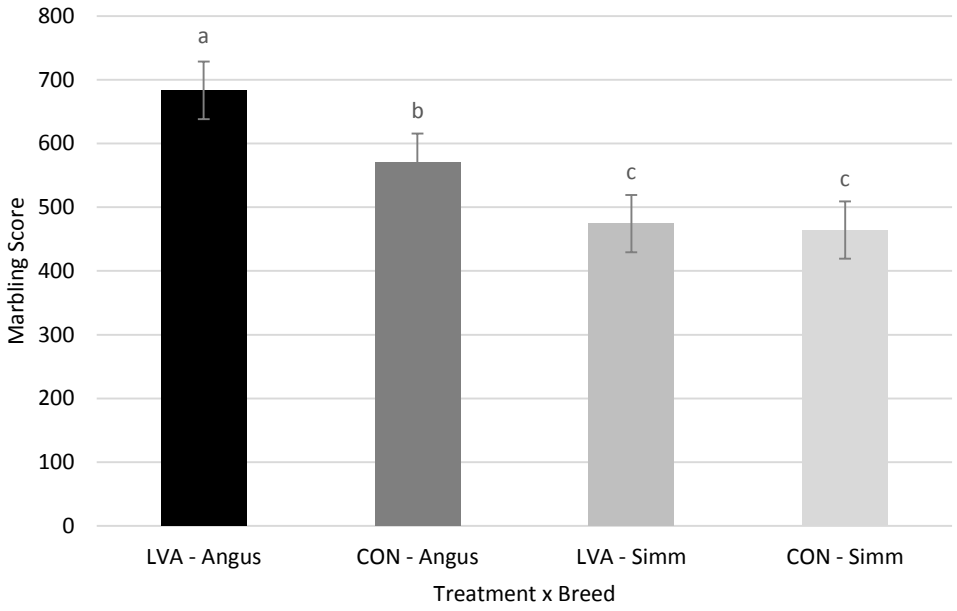

Figure 2.3. Marbling score in the longissimus of Angus-cross (Angus) or Simmental (Simm) steers fed a finishing diet with low vitamin A (LVA) or control (CON; supplemented with 2200 IU vitamin A/kg DM). Marbling was assessed using camera image data. [abc]Means not sharing a common superscript are different ($P < 0.05$).

The difference in marbling score was associated with QG differences. A greater proportion ($P = 0.02$) of commercial Angus cattle graded choice than Simmental steers (Table 4; Figure 2.4). When examining prime carcasses, there was a significant interaction of breed and treatment (Figure 2.4), with more LVA commercial Angus cattle grading prime than any other group ($P = 0.02$).

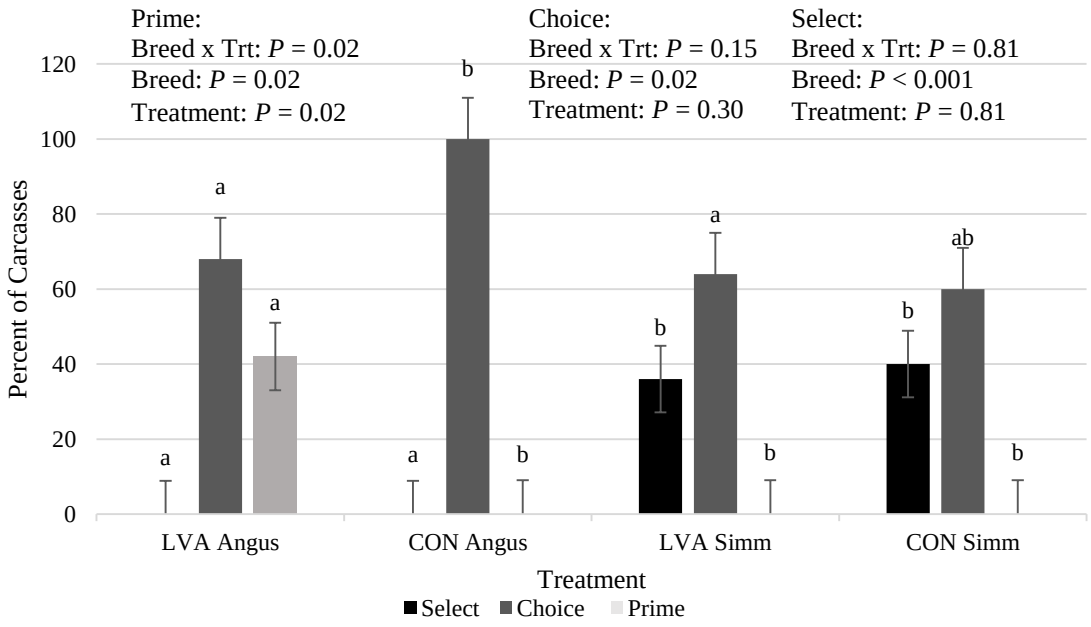

Figure 2.4. Quality grades compared across treatment and breed. LVA Angus cross cattle were the only group to result in prime grading carcasses. Angus cross steers in the CON treatment resulted in 100% choice carcasses. Simmental cattle resulted in more select carcasses than either treatment of Angus cross cattle. [abc]Treatments not sharing a common superscript within each quality grade are different ($P < 0.05$).

The intramuscular fat analysis is in agreement with marbling score, with observed main effects of treatment and breed ($P = 0.04$ and $P < 0.001$, respectively) but no interaction. Commercial Angus steers had greater ($P < 0.0001$) backfat and KPH fat than Simmental steers but lower LMA ($P = 0.0002$; Table 2.4). Subsequently, Commercial Angus cattle exhibited greater YG ($P < 0.0001$), having a lower percentage of closely trimmed retail cuts.

Cook loss was higher in the LVA treatment than the CON treatment (20.4 and 17.5%, respectively; $P = 0.03$). Interestingly, there was a difference in meat pH between the commercial Angus group and Simmental group ($P < 0.0001$). There was a tendency for steaks from Simmental steers to be more red (greater a* value) than their commercial Angus counterparts ($P = 0.053$). There was no difference in any other color measurement; there was also no difference in shear force values between breeds or treatments.

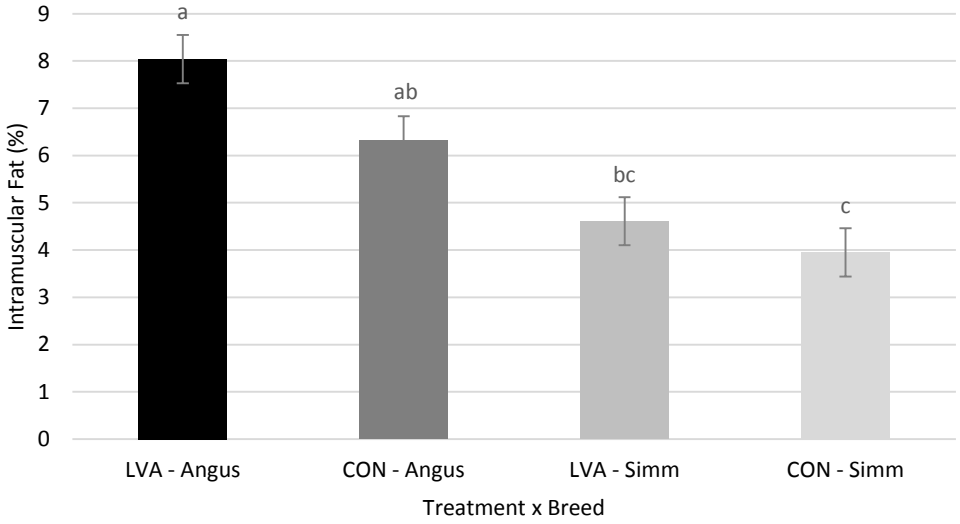

Figure 2.5. Intramuscular fat (B) in the longissimus of Angus-cross (Angus) or Simmental (Simm) steers fed a finishing diet with low vitamin A (LVA) or control (CON; supplemented with 2200 IU vitamin A/kg DM). Intramuscular fat was assessed by ether extract. [abc]Means not sharing a common superscript are different ($P < 0.05$).

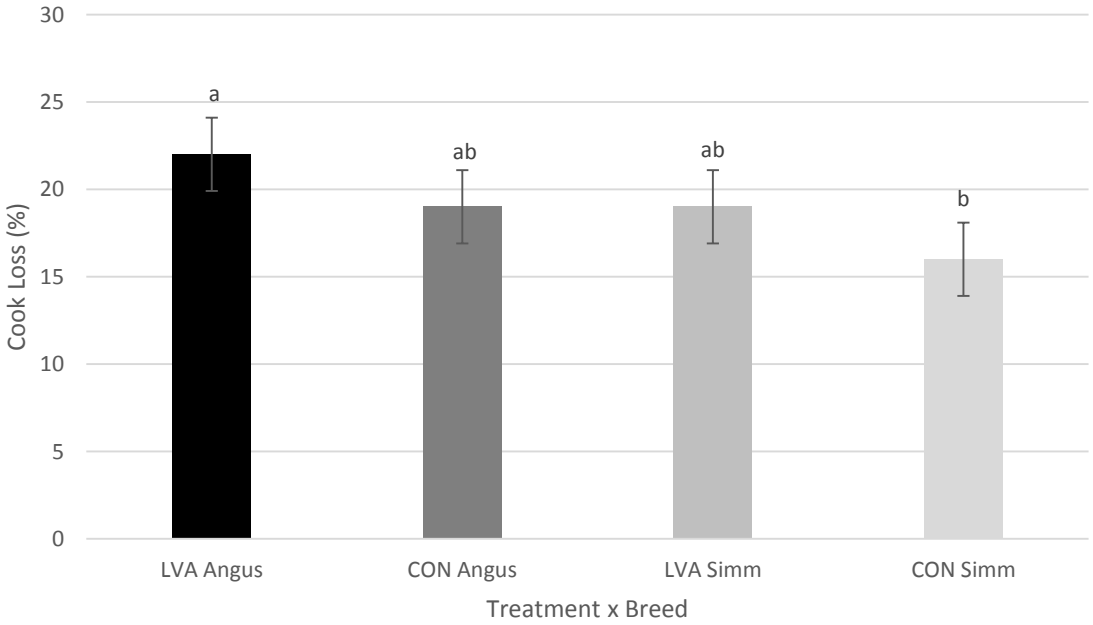

Figure 2.6. LVA Angus steaks exhibited significantly greater cook loss than CON Simmental steaks. [abc]Treatments not sharing a common superscript are different ($P < 0.05$)

Table 2.4. Effects of dietary vitamin A concentration and breed on carcass characteristics of Simmental and Commercial Angus steers

	Trt[1]		Breed[2]			*P*-value[3]		
Item	LVA	CON	Simm	Angus	SEM	Trt	Breed	Trt × Breed
HCW, kg	381	384	378	388	11	0.75	0.28	0.75
S. c. fat, cm	1.15	1.15	0.94	1.37	0.06	0.51	<0.001	0.80
Marbling	683	474	464	571	40	0.01	<0.001	0.008
Subj. Marbling	578	522	471	629	21	0.003	<0.001	0.52
IMF, %	6.29	5.21	4.32	7.17	0.06	0.03	<0.001	0.30
LM Area, cm^2	90.7	92.9	96.9	86.9	2.6	0.15	<0.001	0.22
Cook Loss, %	20.4	17.5	17.5	20.3	1.8	0.04	0.06	0.76
Shear Force, kg	2.26	2.48	2.46	2.28	0.16	0.11	0.23	0.74
pH	5.48	5.44	5.51	5.43	0.02	0.37	<0.001	0.16
KPH, %	1.85	1.77	1.69	1.93	0.05	0.04	<0.001	0.25
Yield Grade	2.85	2.74	2.27	3.32	0.16	0.23	<0.001	0.36
USDA QG								
Select, %	17	20	38	0	6	0.81	<0.001	0.81
Choice, %	69	80	62	87	11	0.30	0.02	0.15
Prime, %	14	0	0	13	9	0.02	0.02	0.02
Minolta Color								
L*	43.15	43.12	42.94	43.33	0.98	0.98	0.67	0.26
a*	25.51	26.02	26.18	25.35	0.46	0.21	0.05	0.28
b*	11.12	10.97	11.16	11.02	0.27	0.87	0.57	0.97

[1]Trt = treatment, LVA = basal diet containing 723 IU/kg DM vitamin A; CON = basal diet supplemented at 2,200 IU/kg DM.
[2]Simmental = pure bred Simmental steers, Angus = commercial Angus steers containing > 75% Angus.
[3]Trt = vitamin A level, Breed = Angus or Simmental.
[4]Sub. Marbling Score = scored at the NDSU Meats Laboratory by an experienced grader

Discussion

The data from this study support the hypothesis that vitamin A restriction during finishing increases marbling, however this was limited to cattle of high-marbling potential (i.e., commercial Angus steers). This is consistent with prior research on vitamin A restriction in Angus-based cattle (Ward et al., 2012; Gorocia-Buenfil et al., 2007; Siebert et al., 2006). This effect was not seen in Simmental cattle, which again is consistent with prior data showing that vitamin A restriction does not affect marbling score in cattle with a low propensity for marbling (Wang et al., 2007). This experiment confirms that the impact of vitamin A restriction on marbling is mitigated by breed propensity for intramuscular fat deposition. This mitigation may be explained by differences in GH that were detected between breed, as GH has been shown to limit fat accretion and favor a redistribution of energy to muscle growth (Etherton and Bauman, 1998). However, GH data may be influenced by measurement time, because GH is released in pulses. We also saw no difference in IGF-1, which should have been influenced by the difference in GH. In this experiment, only commercial Angus steers on the LVIT-A treatment produced prime-grade carcasses. Retinoic acid (a vitamin A metabolite) plays large roles in both preadipocyte commitment and terminal maturation of adipocytes. This is accomplished by the regulation of many adipogenic nuclear receptors that are responsible for the regulation of adipogenic genes. Retinoic acid is a major ligand for many of these nuclear receptors, and is allocated differently throughout adipocyte development (Wang et al., 2016). Restricting vitamin A may cause the reallocation of retinoic acid to different receptors that cause gene expression to be more favorable for adipose development. Genes that have been shown to have a crucial role in adipogenesis include PPARγ,δ, C/EBPα,β,δ, RXRα, LXR (reviewed by B. Féve, 2005).

Intramuscular fat was highly correlated with marbling score, which is consistent with other studies (Dow et al., 2010). The greater cook loss of steaks from the LVIT-A commercial Angus treatment was likely due to their greater fat content (as measured by marbling score and intramuscular fat percentage), as fat has a lower melting temperature than the cooking temperature and can thereby liquefy and be lost during cooking.

Liver is the primary retinol storage location. Liver vitamin A was not assessed in this study because serum retinol concentrations are highly correlated to liver retinol concentrations (Ward, et al., 2012). However, assessing the liver retinol stores may have given a more complete depiction of the retinol status of the cattle. Prior studies have shown that serum retinol concentrations are negatively correlated with marbling scores (Ward et al, 2012; Oka et al. 1992; Adachi et al., 1999). Low serum retinol concentration is likely evident of low liver stores, as the liver will release vitamin A stores to maintain serum retinol. In the current study, steers in each treatment had similar serum retinol levels at the beginning of the growing phase. There was no difference in start of growing and end of growing vitamin A concentration within each treatment group. However, at the end of growing, even though both treatment groups received the same diet and treatment up to this point and were randomly assigned to treatment groups (stratified by weight), steers in the CON group had greater serum retinol concentrations than the LVA group. Similarly, Ward et al. (2012) also reported differences in liver vitamin A concentrations at the start of test between steers randomly allocated to dietary treatment groups.

In contrast to prior studies, which have reported no difference or a reduction in growth with vitamin A restriction (Gorocia-Buenfil et al., 2007 a,b, 2008; Bryant et al., 2004, 1999); we report an increase in ADG in steers fed a low vitamin A diet. This is similar to what was found by Pyatt et al., though their low vitamin A treatment diet concentration contained 2,300 IU/kg

and high vitamin A treatment diet contained 7250 IU/kg with ADGs of 1.32 and 1.27 kg/d, respectively (Pyatt et al., 2005). In the current study, dietary vitamin A concentrations were lower: the LVA diet contained 723 IU/kg and CON diet contained 2,923 and were associated with differences in ADG of 1.52 and 1.44 kg/day respectively. Although ADG was higher in the LVA treatment, there was no difference in final body weight. Leptin has been shown to have a negative correlation with ADG in beef cattle, and is likely because fat steers gain weight slower than lean steers, and fat cattle produce more leptin (Foote, 2015; Nkrumah, 2006). In this experiment, we found that the LVA treatment had greater concentrations of serum leptin than that of the CON group. Interestingly, the LVA treatment animals began with higher levels of leptin at the beginning of the study. At the end of the study, there was no difference between treatment groups but a difference in breeds had developed, with Angus cross cattle having greater levels. Generally, leptin is known to decrease hunger, and therefore decrease DMI and ADG (Foote, 2015). In our study the opposite occurred, animals with high leptin levels had higher ADG. It is unclear why there would be any differences in leptin at the beginning of finishing, as these animals were in the same environment and on the same diet up to this point. Because adiposity is relatively low at this time, small differences can result in statistical significance; this difference in leptin could be a statistical artifact. Animals on the LVA treatment also had lower serum retinol levels. Retinoic acid suppresses leptin expression, which may explain the higher leptin levels in the LVA treatment (Kumar et al., 1999). The LVA treatment gained more intramuscular fat through this period, so an increase in leptin is expected as fatness and leptin are positively correlated (Nkrumah, 2006). There was no effect of treatment on BW, HCW, DMI or G:F. With these factors taken into account, it may be a reasonable assumption that the difference in ADG may be a statistical artifact. Further research with a

greater number of animals would be useful to further investigate if a low vitamin A diet can indeed cause increased ADG and as well as affect other production characteristics.

Conclusions

Increasing marbling has the potential to add significant value to a beef carcass. According to the results of this research, feeding a low vitamin A diet to cattle that have a genetic high propensity for marbling would function to increase the profits of cattle producers and packers without increasing cost of production. The increase in marbling without increasing other fat depots increases quality grade without a negative impact on yield grade. In cattle that have a lower propensity to marble there appears to be no effect on marbling, but vitamin A restriction increased ADG. No adverse effects on production characteristics or carcass characteristics were apparent when vitamin A was not supplemented in the diet of either breed of cattle. Therefore, it may be beneficial to reduce or remove vitamin A supplementation from cattle finishing diets, though care must be taken to avoid vitamin A deficiency.

Literature Cited

Adachi, K., H. Kawano, K. Tsuno, Y. Nomura, N. Yamamota, A. Arikawa, A. Tsuji, M. Adachi, T. Onimaru, and K. Ohwada. 1999. Relationship between serum biochemical Values and marbling scores in Japanese black cattle. J. Vet. Med. Sci. 61:961-964.

AOCA. 2010. Official Methods of Analysis, 18th ed. Rev. 3. Assoc. Off. Anal. Chem., Arlington, VA.

Bryant, T. C., J. D. Rivera, M. L. Galyean, G. C. Duff, D. M. Hallford, and T. H. Montgomery. 1999. Effects of dietary level of ruminally protected choline on performance and carcass characteristics of finishing beef steers and on growth and serum metabolites in lambs. J. Anim. Sci. 77:2893-2903. doi:10.2527/1999.77112893x

Bryant, T. C., J. J. Wagner, T. E., Engle, K. L. Dorton, P. D. Bruns, and M. L. Galyean. 2004. Effects of dietary supplemental vitamin A concentration on growth, intake, and marbling in yearling feedlot cattle. J. Anim. Sci. 82 (Suppl. 1):270.

Doughaday, W. H., I. K. Mariz, and S. L. Blethen. 1980. Inhibition of Access of Bound Somatomedin to Membrane Receptor and Immunobinding Sites: A Comparison of Radioreceptor and Radioimmunoassay of Somatomedin in Native and Acid-Ethanol-Extracted Serum. J. Clin. Endocrinol. Metab. 51:781-788. doi:10.1210/jcem-51-4-781

DiCostanzo, A and C. R. Dahlen. 2000. Grid pricing as a fed cattle marketing strategy. Minnesota Cattle Feeder Report B-470. University of Minnesota, St. Paul, MN, USA.

Dow, D. L., B. R. Wiegand, M. R. Ellersieck and C. L. Lorenzen. 2011. Prediction of fat percentage within marbling score on beef longissimus muscle using 3 different fat determination methods. J. Anim. Sci. 89:1173-1179. doi:10.2527/jas.2010-3382

Etherton, T. D. and D. E. Bowman. 1998. Biology of somatotropin in growth and lactation of domestic animals. Physiol. Rev. 78:745-761.

Feve, B. 2005. Adipogenesis: cellular and molecular aspects. Best Prac. Res. Clin. Endocrinol. Metab. 19:483-499.

Foote, A. P., K. E. Hales, L. A. Kuehn, D. H. Keisler, D. A. King, S. D. Shackelford, T. L. Wheeler, and H. C. Freetly. 2015. Relationship of leptin concentrations with feed intake, growth and efficiency in finishing beef steers. J. Anim. Sci. 93:4401-4407. doi:10.2527/jas2015-9339

Gorocica-Buenfil, M. A., F. L. Fluharty, T. Bohn, S. J. Schwartz, and S. C. Loerch. 2007a. Effect of low vitamin A diets with high-moisture or dry corn on marbling and adipose tissue

fatty acid composition of beef steers. J. Anim. Sci. 85:3355–3366. doi:10.2527/jas.2007-0172

Gorocica-Buenfil, M. A., F. L. Fluharty, and S. C. Loerch. 2008. Effect of vitamin A restriction on carcass characteristics and immune status of beef steers. J. Anim. Sci. 86:1609–1616. doi:10.2527/jas.2007-0241

Gorocica-Buenfil, M. A., F. L. Fluharty, C. K. Reynolds, and S. C. Loerch. 2007b. Effect of dietary vitamin A concentration and roasted soybean inclusion on marbling, adipose cellularity, and fatty acid composition of beef. J. Anim. Sci. 85:2230–2242. doi:10.2527/jas.2006-780

Klindt, J., J. J. Ford, J. G. Berardinelli, and L. L. Anderson. 1983. Growth hormone secretion after hypophysial stalk transection in pigs. Proc. Soc. Exp. Biol. Med. 172:508–513.

Kumar, M. V., G. D. Sunvold, P. J. Scarpace. 1999. Dietary vitamin A supplementation in rats: suppression of leptin and induction of UCP1 mRNA. J. Lipid Res. 40:824-829.

Nkrumah, J. D., D. H. Keisler, D. H. Crews, J. A. Basarab, Z. Wang, C. Li, M. A. Price, E. K. Okine, and S. S. Moore. 2007. Genetic and phenotypic relationships of serum leptin concentration with performance, efficiency of gain, and carcass merit of feedlot cattle. J. Anim. Sci. 85:2147-2155. doi:10.2527/jas.2006-764

Oka, A., Y. Maruo, T. Miki, M. Yamasaki, and T. Saito. 1998. Influence of vitamin A on the quality of beef from the Tajima strain of Japanese Black cattle. Meat Sci. 48:159-167. doi: 10.1016/S0309-1740(97)00086-7

Pearson, A. M. and R. B. Young. 1989. Postmortem changes during conversion of muscle to meat. In: A. M. Pearson, editor, Muscle and Meat Biochemistry. Academic Press, San Diego, CA. p. 391-419.

Pethick, D.W., G. S. Harper, and V. H. Oddy. 2004. Growth, development and nutritional manipulation of marbling in cattle: a review. Aust. J. Exp. Agr. 44:705-715. doi: 10.1071/EA02165

Rhoades, R. D., J. E. Sawyer, K. Y. Chung, M. L. Schell, D. K. Lunt, and S. B. Smith. 2007. Effect of dietary energy source on in vitro substrate utilization and insulin sensitivity of muscle and adipose tissues of Angus and Wagyu steers. J. Anim. Sci. 85:1719–1726. doi:10.2527/jas.2006-498

Pyatt, N. A., L. L. Berger, and T. G. Nash. 2005. Effects of vitamin A and restricted intake on performance, carcass characteristics, and serum retinol status in Angus x Simmental feedlot cattle. The Professional Animal Scientist. 21:318-331. doi:10.15232/S1080-7446(15)31223-7

Siebert, B. D., Z. A. Kruk, J. Davis, W. S. Pitchford, G. S. Harper, and C. D. K. Bottema. 2006. Effect of low vitamin A status on fat deposition and fatty acid desaturation in beef cattle. Lipids. 41:365-370. doi:10.1007/s11745-006-5107-5

Smith, S. B. and D.K. Lunt. 2007. Marbling: Management of cattle to maximize the deposition of intramuscular adipose tissue. In: Proceedings of the Plains Nutrition Council Spring Conference, San Antonio, TX. p. 26-40.

Wang, B., Q. Yang, C. L. Harris, M. L. Nelson, J. R. Busboom, M. J. Zhu, and M. Du. 2016. Nutrigenomic regulation of adipose tissue development – role of retinoic acid: A review. Meat Sci. 120:100-106. doi:10.1016/j.meatsci.2016.04.003

Wang, W. J., S. P. Wang, Y. S. Gong, J. Q. Wang, and Z. L. Tan. 2007. Effects of vitamin A supplementation on growth performance, carcass characteristics and meat quality in

Limosin x Luxi crossbreed steers fed a wheat straw based diet. Meat Sci. 77:450-458. doi:10.1016/j.meatsci.2007.04.019

Wang, Y. H., N. I. Bower, A. Reverter, S. H. Tan, N. De Jager, R. Wang, S. M. McWilliam, L. M. Café, P. L. Greenwood and S. A. Lehnert. 2009. Gene expression patterns during intramuscular fat development in cattle. J. Anim. Sci. 87:119-130. doi:10.2527/jas.2008-1082

Ward A. K., J. J. McKinnon, S. Hendrick and F. C. Buchanan. 2012. The impact of vitamin A restriction on the ADH1C genotype on marbling in feedlot steers. J. Anim. Sci. 90:1-8. doi:10.2527/jas.2011-4404

CHAPTER 3: EFFECT OF VITAMIN A RESTRICTION ON ADIPOGENIC GENE EXPRESSION AND INTRAMUSCULAR FAT DEVELOPMENT IN BEEF CATTLE[1]

Abstract

Commercial Angus steers consisting of a minimum of 75% Angus genetics and purebred Simmental steers were used to evaluate the effect of a low vitamin A diet on intramuscular fat development in cattle in a 2x2 factorial arrangement of treatments. After 3 months on a low vitamin A grower diet (1,017 IU vitamin A/kg of DM), 64 steers (32 commercial Angus, 32 Simmental) were allocated to one of two dietary treatments for finishing. The low vitamin A (LVA) diet contained 723 IU of vitamin A/kg of diet DM; the control diet (CON) consisted of the LVA diet supplemented with 2,200 IU vitamin A/kg of DM. Muscle biopsies were collected from the *longissimus* at the beginning, middle, and end of finishing. Immunohistology was used to visualize fatty acid binding protein 4 (FABP4). Gene expression was measured by qPCR. Target genes include *FABP4*, *PREF-1*, *PPARγ*, *ADH1C*, *RALDH1a1*, *C/EBPα*, and *RXR-α*, *β*, and *γ*. After 156 and 184 d of finishing steers were slaughtered at a commercial abattoir and marbling was assessed. No difference ($P = 0.24$) was found in FABP4 protein expression. Commercial Angus steers had greater ($P = 0.001$) gene expression of *FABP4*, as well as a tendency ($P < 0.10$) for greater expression of *RXR-α*, β, and γ. The CON group had greater ($P = 0.04$) expression of *FABP4* and a tendency ($P < 0.10$) for greater expression of *RXR-α*, *C/EBPα, PREF-1* and *ADH1C*.

---

[1]The material in this chapter was co-authored by E. E. Knutson, A. B. P. Fontoura, J. Gaspers, P. P. Borowicz, M. Bauer, K. C. Swanson, A. K. Ward. It has been submitted for publication to the Journal of Animal Science. E. E. Knutson and A. K. Ward had the responsibilities of collecting samples. E. E. Knutson is the author that developed the conclusions, did the original and corrections drafting, and developed the statistical modelling. A. K. Ward served as proofreader and checked the statistical analysis conducted by E. E. Knutson.

There was no differences ($P \geq 0.37$) in *PPARγ* or *RALDH1a1* expression. Therefore, we conclude that in steers of moderate to high marbling potential (such as commercial Angus), vitamin A restriction is associated with increased marbling and differential expression of adipogenic genes within the vitamin A pathway.

Key words: marbling, vitamin A, Angus, Simmental, gene expression, adipogenesis

Introduction

Intramuscular fat is the slowest developing and most complex fat depot affected by genetic propensity to marble, plane of nutrition throughout life, and environmental factors (Pethick et al., 2004). Recent studies have shown that serum vitamin A concentration is negatively correlated with marbling (Knutson et al., 2017; Ward et al., 2012; Adachi et al., 1999). Adipogenesis is under the control of many nutrients and metabolites; retinoic acid has a strong effect on adipogenesis. Retinoic acid achieves its effects largely through binding to nuclear receptors, which activate response elements to affect expression of target genes (Repa et al., 1993). *Peroxisome proliferator activated receptor γ* (*PPARγ), CCAAT-enhancer binding protein* (*C/EBPα, β,* and *δ*), *retinoid x receptor* (*RXRα, β,* and *γ*), *pre-adipocyte factor 1* (*PREF-1*), and *fatty acid binding protein 4* (*FABP4*) are key genes during adipogeneisis and most are effected by retinoic acid levels (Tan et al., 2002; Hudak and Sul, 2013; Rosen et al., 2002; Nielsen et al., 2008). Retinoic acid stimulates adipogenesis, whereas Retinaldehyde has an inhibitory effect (Ziouzenkova et al., 2007). During vitamin A restriction, the activity of RALDH1 (retinaldehyde dehydrogenase 1), which converts retinaldehyde to retinoic acid, is increased 3-fold; maintaining retinoic acid levels while decreasing retinaldehyde levels (Napoli et al., 1995). There is conflicting research on the effect of vitamin A restriction on marbling in cattle. The purpose of this study is to confirm that vitamin A restriction enhances marbling in

beef cattle and begin to understand the mechanisms that control intramuscular fat development. We hypothesize that expression of pro-adipogenic factors such as *PPARγ*, *C/EBPα*, and *FABP4* will be upregulated, and anti-adipogenic factors such as *PREF-1* downregulated in cattle with high marbling potential. We also hypothesize that several pro-adipogenic factors will be upregulated when dietary vitamin A is restricted.

Materials and Methods

Animal care protocols described herein (protocol number A16004) were approved by the North Dakota State University Animal Care and Use Committee.

Experimental Design and Treatments

Cattle for this experiment were obtained from the North Dakota State University resident herd. A total of 32 commercial Angus (minimum of 75% Angus genetics) and 32 purebred Simmental steer calves were used in this study. After a 2-week acclimation period, all steers were fed a low vitamin A (1017 IU/kg DM) growing diet at for 95 days. This diet was designed to deplete the liver vitamin A stores, which can sustain serum retinol for 2 to 4 months (NRC, 1996). Following this period, steers were allocated to treatment.

Calves were stratified by weight and assigned to one of two nutritional treatments which were implemented from the end of the growing period until slaughter. The low vitamin A (LVA) treatment was a diet that contained 723 IU vitamin of A/kg of DM (Table 1). The control (CON) treatment consisted of the same diet as LVA plus 2,200 IU/kg of DM supplemental vitamin A, which is the NRC (2000) recommend level of vitamin A for finishing cattle. Steers were housed in 2 pens, which contained equal numbers of breed and treatment. Individual feed intake and feeding behaviors were monitored using the RIC feeding system (Insentec, Markness, The Netherlands). Steers were weighed 2 d consecutively at the start and end of finishing for an

average and weighed every 28 d throughout the finishing period. Weight gain and feed intake as well as visual pen checking were used to monitor for signs of vitamin A deficiency and illness throughout finishing. Steers were slaughtered at a commercial abattoir in two groups, following 154 (n = 24) or 184 d (n = 37) of finishing.

Table 3.1. Ingredient and nutrient composition (DM basis) of TMR growing and finishing diets fed

Item	Growing, %	Finishing, %
Ingredient		
Brome hay	15.0	---
Wheat straw	30.0	10.0
Barley	10.0	---
Corn	20.0	60.0
CSB[2]	---	5.0
DDGS[3]	20.0	20.0
Supplement[1]	5.0	5.0
Nutrient Composition		
CP	15.0	14.7
NDF	48.2	25.5
ADF	26.0	10.3
Ca	0.69	1.01
P	0.32	0.53

[1]Supplement contained ground corn, limestone, urea, salt, monensin, tylosin, (Elanco, Greenfield, IN), vitamin D (3,000 IU/kg), and a trace mineral premix.
[2]CSB = concentrated separator by-product (partially de-sugared beet molasses).
[3]DDGS = dried distillers grains with solutes.
Taken with permission from Knutson et al., 2017

Serum Retinol Analysis

Serum retinol concentrations are presented in Knutson et al. (2017) and repeated here for completeness. Blood was collected at the beginning of the growing period (d 0), beginning of finishing (d 96), and end of finishing (d 268). Jugular blood was collected using 10-mL untreated (serum) Vacuum blood collection tubes. Collected blood was stored on ice and protected from light. Samples were then centrifuged at 2,000 × *g* for 10 min and serum was collected. Serum was stored at -80^{0}C until retinol extraction was performed. Extraction and HPLC preparation was performed under dim red light to limit retinol degradation. Retinol extraction followed a procedure outlined previously (Gorocia-Buenfil et al., 2007b).

Muscle Biopsies

Muscle biopsies were performed on a subset of 36 steers, with an equal distribution of breeds and treatments. Biopsies were performed at d 29, 86, and 142 of finishing. Steers were selected randomly within the selection parameters of three steers per breed per treatment, for 12 steers per biopsy period. In preparation of the biopsy procedure, flunixin meglumine (Banamine,50 mg/mL, Merck & Co. Inc., Madison, NJ) was administered intravenously at 1.1 mg/kg of BW as an analgesic and anti-inflammatory. The surgical area was clipped of hair and scrubbed with 7.5% povidone-iodine scrub and 70% isopropanol, and sprayed with 10% povidone-iodone solution. Local anesthesia (10 ml lidocaine HCl and epinephrine 1:100,000, Hospira, Lake Forest, IL) was injected subcutaneously surrounding the incision site. Penicillin G procaine suspension (300,000 units/ml, VetOne, Boise, ID) was also administered at the time of biopsy (66 units/kg of BW intramuscularly).

The first biopsy was performed as an open surgical biopsy. An 8-cm incision was made horizontally along the skin above *longissimus* muscle near the 12th rib, approximately 30 cm

ventrally from the dorsum. A scalpel was used to dissect approximately a 1-cm^3 muscle biopsy from the *longissimus*. The incision was rinsed with sterile glycerol saline, sutured, and coated with sodium propionate, gentian violet, and acriflavine wound spray (Blu-Kote, Naylor Co. Inc., Morris, NY).

The second and third biopsies were performed using a 10-mm muscle bergstrom biopsy cannula (Millennium Surgical Corp., Narberth, PA) instead of an open surgical biopsy to reduce inflammation observed when using the open-surgical method. A 2-cm incision was made using a scalpel in the same location as described above. The biopsy cannula was inserted approximately 5 cm into the *longissimus*. Suction was applied to the cannula using a 50 mL glass syringe. A total of three cuts were made using the cannula plunger, at three different rotational positions. A portion of the biopsy sample was preserved in formalin to be used for histological mounting, the remainder was preserved in RNAlater (Thermo Fisher Scientific Inc., Waltham, MA) to preserve RNA.

Marbling Score

Carcass characteristics and production traits are reported in Knutson et al. (2017); marbling score is presented here for completeness. Steers were slaughtered in two slaughter groups on d 156 and 184. Steers were selected for slaughter date by subcutaneous fat thickness and BW, with 1.3 cm and 600 kg considered adequate, respectively. Steers were transported to a commercial abattoir, where they were slaughtered (Tyson Fresh Meats., Dakota City, NE). Marbling was assessed by computer image analysis.

Gene Expression

Muscle samples preserved in RNA*later* were stored at -80^{0}C until RNA extraction. The muscle RNA extraction was based upon the procedure presented in the Qiagen RNeasy Lipid

Tissue Mini Kit Handbook (Qiagen, Hilden, Germany). In short, 100 mg tissue was lysed in 1 ml QIAzol Lysis reagent (Qiagen), and extracted with 200 µl chloroform (VWR, West Chester, PA). The aqueous phase was combined with 500 µl isopropanol (Merck, Darmstadt, Germany) and followed the kit protocol thereon. The RNA quality and concentration was assessed using absorbance at 260 and 280 nm (Synergy H1 microplate, BioTek Instruments Inc., Winooski, VT). Extracted RNA was then reverse transcribed to cDNA using the QuantiTect Reverse Transcription Kit (Quiagen, Hilden, Germany). The 20 µL qPCR cocktail included 10 µL of iTaq Universal SYBR Greed Supermix (Bio-Rad Laboratories Inc., Hercules, California), 1 µL each of 10 µM forward and reverse primers (Integrated DNA Technologies, Coralville, IA; Table 2), 6 µL RNAase-free water, and 2 µl (1 ng/µl) cDNA. Primers were designed using Primer-BLAST (National Center for Biotechnology Information, Bethseda, MD), and were selected to span exon to exon junctions. Expression of genes of interest was quantified in triplicate using an Applied Biosystems 7500 Fast qPCR machine (Thermo Fisher Scientific Inc., Waltham, MA). Relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method (LiVak and Schmittgen, 2001). Values were normalized to *GAPDH*, which was chosen as a reference gene because of its consistent expression between treatments compared to four other candidate reference genes (*ACTB, HPRT1*, *YWHAZ*, *UBC*).

Table 3.2. Primer sequences used for real-time quantitative PCR analysis.

Gene[1]	Accession No.	Forward	Reverse
GAPDH	NM_001034034	AGGTCGGAGTGAACGGATTC	ATGGCGACGATGTCCACTTT
FABP4	NM_174314.2	TGGGGGTGTGGTCACCATTA	ACGATGCTCTTGACTTTCCTGT
PREF-1	XM_005222109.3	GACCTAGACATCCGGGCTTG	CACTCGTACTGGCCGTCATC
PPARγ	NM_181024.2	CCGGGTCTGTGGGGATAAAG	TCCTCCGGAAGAAACCCTTG
PPARβ	NM_001083636.1	CATGTCGCACAATGCCATCC	GACTCCCCTCATTGGCTGTC
ADH1C	NM_001206387.1	ATGGTGGCCACAGGAATCTG	CAATGCTCTCCACAATGCCG
RALDH1α1	NM_174239.2	CCGTGTGGGTGAACTGCTAT	CCCAGTTCTCGACCATTTCCA
C/EBPα	NM_176784.2	GCCTTCAACGACGAGTTCCT	GTAGTCAAAGTCGTTGCCGC
RXRα	NM_001304343.1	ATGGTGTCCTCAAAGTGCCG	CTTGGCGAACTCCACAGTCT
RXRβ	NM_001083640.1	GGGAGCCATCTTCGATAGGGT	CCCTTGGCATCTGGATTGAACA
RXRγ	NM_001075408.1	TCTGACTGAGCTGGTCTCCA	GAGAGACCCTTGGCATCTGG

[1]GAPDH – glyceraldehyde 3-phosphate dehydrogenase; FABP4 – Fatty acid binding protein 4; PREF-1 – pre-adipocyte factor 1 (also referred to as DLK1); PPARγ – Peroxisome proliferator actiVated receptor gamma; ADH1C – Alcohol dehydrogenase class 1 member C; RALDH1α1 – Retinaldehyde dehydrogenase; C/EBPα – CCAAT/ enhancer-binding protein alpha; RXRα,β,γ – Retinoid X receptors alpha, beta, and gamma.

Histology

Samples preserved for histology were placed in formalin (Richard Allan Scientific Co., San Diego, CA) for 24 h, then processed using a series of alcohol and xylenes to prepare samples for paraffin embedding. Samples were embedded in paraffin (Leica Microsystems, Wetzlar, Germany), and stored at room temperature until slide mounting. Slides were cut using a microtome (Leica RM2255, Leica Microsystems, Wetzlar, Germany) set to the cutting depth of 5 μm, laid in a water bath, and picked up using ProbeOn Plus microscope slides (Thermo Fisher Scientific Inc., Waltham, MA). Slides were stained using rabbit FABP4 primary antibody (Abcam, Cambridge, UK), and a goat anti-rabbit secondary antibody with a CF555 dye (Biotium, Fremont, California). Slides were also stained with DAPI to visualize cells and nuclei. Images were taken using a Zeiss AXIO Imager 2, with 20X zoom, using Axiovision 4.8 software (Carl Zeiss Ag., Oberkochen, Germany). Images were analyzed for intensity and area of luminescence using Image Pro – Premier 3D 9.1 software (Media Cybernetics, Inc., Rockville, MD).

Statistical Analysis

Serum Vitamin A. Data were analyzed using the mixed procedure of SAS (v. 9.4; SAS Inst., Cary, NC). Fixed effects included breed, treatment, date, and the interaction between each and between all three. If the interaction P-Value was > 0.05, it was removed from the model. Date was analyzed as a repeated measure, with animal as the subject. Means were separated using the LSMEANS procedure, and *P*-Values ≤ 0.05 were considered significant.

Marbling. The experiment was a randomized complete block (slaughter date) design with a 2 x 2 factorial treatment arrangement, comparing breed (commercial Angus vs. Simmental) with vitamin A treatment (LVA vs. CON). Data were analyzed using the mixed procedure of

SAS (v. 9.4; SAS Inst., Cary, NC). Breed, treatment, and their interaction were used as fixed effects of interest; slaughter date was used as a fixed effect to remove Variation. Steer served as the experimental unit. Means were separated using the LSMEANS procedure, and P-Values ≤ 0.05 were considered significant.

Gene Expression. The experiment was a completely randomized block (slaughter date) design with a 2 x 2 factorial treatment arrangement, comparing breed (commercial Angus vs. Simmental) with vitamin A treatment (LVA vs. CON). Data were analyzed using the mixed procedure of SAS (v. 9.4; SAS Inst., Cary, NC). Fixed effects of interest included breed, treatment, biopsy date, and the interaction between each and between the three. If the interaction was not significant, it was removed from the model. Date was used as a repeated measure, with animal as the subject. Means were separated using the LSMEANS procedure, and *P*-Values ≤ 0.05 were considered significant.

Results and Discussion

Serum Retinol

This data is presented in Knutson et al. (2017), but is repeated here for completeness. There was an interaction ($P = 0.02$) between vitamin A supplementation and time of sampling. Serum retinol concentration was similar between treatments at the beginning of the growing phase ($P = 0.23$) (Figure 1). Serum retinol levels at this the beginning of the growing phase were below the normal reference range for serum retinol levels for cattle, which range from 25 to 60 μg/dl (Frye et al., 1991). At the end of the 95-day growing phase, during which steers were fed the same vitamin A poor diet, steers that would be assigned to the CON treatment had greater ($P = 0.01$) serum retinol than steers that would be assigned to the LVA treatment, 29.21 and 22.45 μg/dl respectively. This appears to be a statistical anomaly, as vitamin A level was not a factor in

assigning steers to treatment. At the beginning of finishing time point, the CON treatment steers had moved above the threshold into normal serum retinol levels, whereas the LVA treatment steers remained below the threshold for normal vitamin A levels (Frye et al., 1991). At the conclusion of finishing, steers in the CON treatment had greater serum retinol concentration ($P < 0.01$) than the LVA steers, 45.60 and 32.74 respectively. This is a similar pattern for serum retinol levels that was found in similar vitamin A restriction studies (Ward et al., 2012; Pickworth et al., 2012).

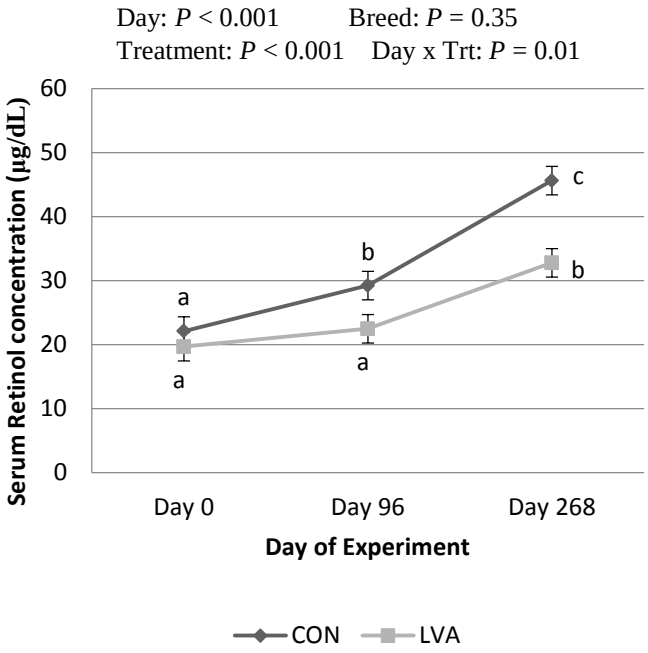

Figure 3.1. Serum retinol concentration increased more over time in the CON treatment than in the LVA treatment. Day 0 corresponds to the beginning of backgrounding, d 96 corresponds to the beginning of finishing, and d 268 corresponds to the end of finishing. LVA = basal diet containing 1,017 IU vitamin A/kg DM, CON = basal diet supplemented at 2,200 IU vitamin A/kg DM

Marbling

This data is presented in Knutson et al. (2017), but is repeated here for completeness. Marbling score resulted in an interaction ($P = 0.008$) between treatment and breed. Low vitamin A commercial Angus steers had greater marbling scores than any other group ($P < 0.01$)(Figure 2). This data support the hypothesis that a low vitamin A diet during finishing increases marbling score, but this effect was limited to cattle of high marbling potential (i.e. Angus-cross steers). This effect has been shown in several previous studies in Angus-based cattle (Ward et al., 2012; Gorocia-Buenfil et al., 2007; Siebert et al., 2006). Our results are supported by Wang et al. (2007) who reported that vitamin A restriction did not affect marbling score in Limosin × Luxi crossbreed steers (cattle that presumably have a low propensity to marble). The difference in marbling score led to quality grade differences, with the LVA commercial Angus group achieving more prime carcasses than any other group (Knutson et al., 2017). Prime carcasses award a premium price over base; this adds significant Value to a carcass, resulting in increased profits for producers.

Gene Expression

RXRα, β, and γ. There was a three way interaction ($P= 0.04$) between biopsy time point, treatment, and breed for expression of the *RXRγ* gene (Table 3, Figure 3). Within the commercial Angus cattle, the LVA group maintained relatively consistent expression; in contrast, the CON treatment began high and diminished throughout the finishing phase. Steady expression of RXRγ may enhance intramuscular fat development or lipid filling.

There was no three way interaction for either *RXRα* or *RXRβ*, though *RXRα* did exhibit a tendency for three way interaction ($P= 0.08$, Table 3). There was no treatment, breed, or time effect ($P \geq 0.22$) on *RXRα* expression. *RXRβ* exhibited a breed effect ($P = 0.02$), with

Simmentals having greater expression. There was also a tendency for a treatment effect (P = 0.08), with the LVA tending to have higher expression. The RXR proteins act as polygamous heterodimers to many other nuclear receptors that have an effect on adipogenesis. These include peroxisome proliferator actiVated receptor (PPAR), liver X receptor (LXR), vitamin D receptor (VDR), and retinoic acid receptor (RAR). Members of the RXR family are one of the most abundant receptors in the body, with at least one isoform being expressed in every cell of the body (Mangelsdorf, 1992). The primary ligand for the RXR receptor is 9-*cis* retinoic acid. It has been proposed that as adipocytes are differentiating there is a shift in heterodimerization from RAR-RXR to PPARγ-RXR, which can actiVate response elements, altering translation from repressed to actiVated states for adipogenic genes (Fajas et al., 1998). The roles of each RXR family member have not been well established, particularly in muscle and intramuscular fat of cattle. It has been shown in mice that RXRα is necessary for adipocyte hypertrophy, with the remoVal of RXRα resulting in increased number of small adipocytes; indicating that it is likely involved in differentiation (Metzger et al., 2005). In this study, Metzger et al. (2005) proposed that the PPARγ-RXRα heterodimer is responsible for lipogenesis, and the PPARγ-RXRγ or the PPARγ-RXRα heterodimers maintain adipocyte surviVal; the RXRα isoform is more abundant in adipocytes. In the current study, *RXRα* expression was numerically greater in both the LVA treatment and the Angus cross breed. Expression of *RXRγ* was more Variable than *RXRα* or *β*, but with a larger sample size, the differences in *RXRα* may become more apparent.

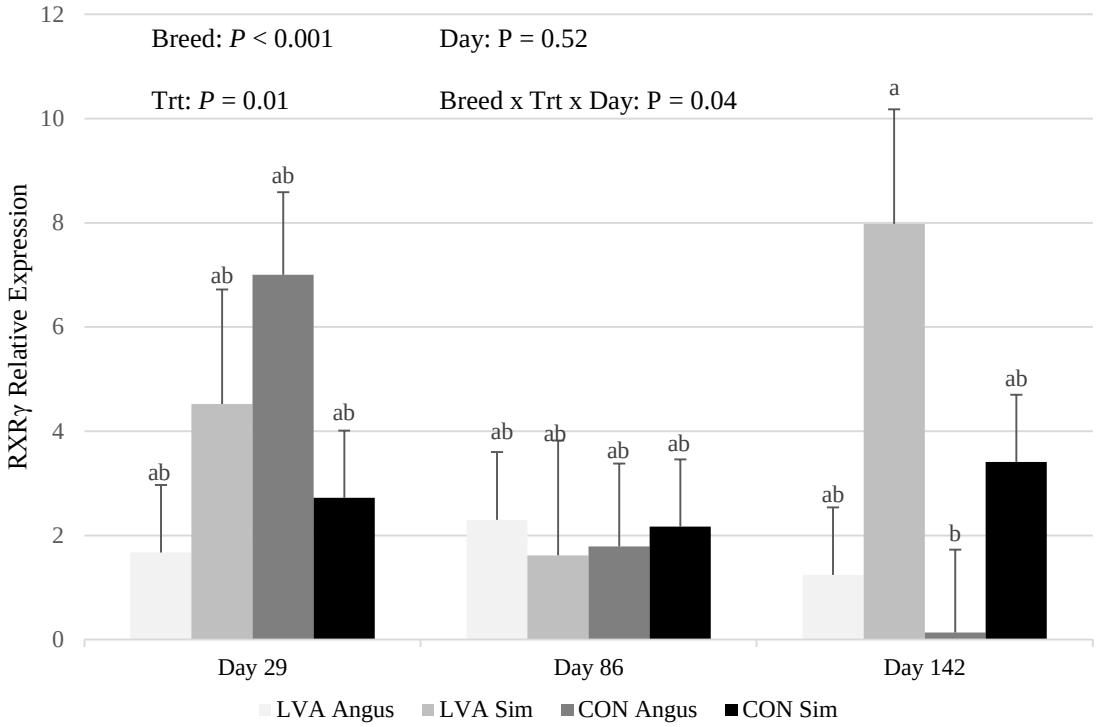

Figure 3.2. Relative expression of RXRγ, calculated using the $2^{\Delta\Delta Ct}$ method. Days 29, 86, and 142 are days of finishing. LVA = basal diet containing 1,017 IU/kg DM vitamin A, CON = basal diet supplemented at 2,200IU/kg DM. Simm = Pure bred Simmental steers, Angus = Commercial Angus Angus steers containing > 75% Angus. [abc]Treatments not sharing a common superscript are different ($P < 0.05$).

Table 3.3. The effect of vitamin A restriction, breed, and time on gene expression in the *longissimus* muscle of finishing cattle.

Item	Vitamin A[1]		Breed[2]		Day			SEM	P-Value		
	LVA	CON	Simm	Angus	29	86	142		VA	Breed	Day
C/EPBα	272.65	381.52	287.95	366.23	188.53	487.26	305.48	287.87	0.5060	0.6328	0.2556
PPARγ	52.99	39.84	45.73	47.10	48.06	46.91	44.27	12.24	0.3698	0.9253	0.9744
FABP4	9.28	10.56	4.12	15.72	6.20	9.89	13.67	4.44	0.7987	0.0282	0.4500
ADH1C	88.32	119.10	38.66	168.76	40.79	140.21	130.13	107.60	0.6970	0.1094	0.4706
RALDH1α1	4.83	4.52	4.02	5.33	4.05	5.48	4.49	2.17	0.7461	0.1784	0.3984
PREF-1	82.53	119.61	92.73	109.40	54.34	162.26	86.60	96.42	0.5266	0.7759	0.2385
RXRα	1.14	1.03	1.28	0.89	1.07	0.86	1.33	0.25	0.7155	0.2199	0.4204
RXRβ	3.46	1.97	3.73	1.70	2.73	1.81	3.61	0.69	0.0835	0.0217	0.1713
RXRγ	3.29	2.19	3.78	1.70	2.84	2.06	3.32	0.80	0.2783	0.0416	0.5211

[1]LVA = basal diet containing 1,017 IU of vitamin A/kg of DM , CON = basal diet supplemented with 2,200 IU vitamin A/kg of DM

[2]Simm = Purebred Simmental steers, Angus = Crossbred Angus steers containing > 75% Angus

PPARγ. There was no treatment or breed effect ($P \geq 0.37$) for *PPARγ* expression (Table 3.3). This gene is regarded as the master coordinator of adipogenic differentiation (Fajas, 1998). Loss of function studies have been performed that show that PPARγ is conditionally necessary for adipogenesis both *in vivo* and *in vitro* (Barak et al. 1999). It is a positive regulator of adipogenesis, meaning that while it is present on the peroxisome proliferator response element (PPRE) it increases adipocyte development. In order to activate PPRE, PPARγ must dimerize with RXR (Tontonoz et al., 1994). In contrast to prior research where *PPARγ* expression increased in finishing cattle and was associated with increased marbling (Wang et al., 2009), we found no difference in *PPARγ* over time with breed and treatment. There may have been no difference because all groups of cattle were in the adipocyte lipid filling stage, meaning they had already differentiated and matured, which is when the large changes in PPARγ are observed. Once mature, PPARγ levels are maintained to keep adipocytes in a mature state, but no difference in expression may be detected. Measuring this gene expression during earlier stages in adipocyte development may yield larger differences between treatments and breeds.

FABP4. There was a breed difference for *FABP4* gene expression, with commercial Angus cattle having greater levels (Table 3.3, $P = 0.03$). Histological analysis of FABP4 protein expression showed no difference in treatment, breed, or biopsy period. It has been shown that FABP4 genotype has a significant effect on marbling deposition (Michal et al., 2006). The FABP4 protein binds fatty acids (FA) and transports them to adipocytes for lipid filling. In addition, it can transport them to the nucleus where the FABP4-FA complex activates PPARγ, which further increases the production of FABP4 and other pro-adipogenic genes (Tan et al., 2002). In porcine adipocytes, FABP4 was increased 46-fold during adipocyte proliferation, and has long been portrayed as the main fatty acid transporter in white adipose tissue (Samulin et al.,

2008). The higher levels of FABP4 found in commercial Angus cattle could be indicative why this group of cattle has higher levels of marbling than their Simmental counterparts.

C/EBPα. There was no differences ($P > 0.23$) between treatments, breeds, or biopsy periods for *C/EBPα* expression. This gene is of interest because it acts alongside *PPARγ*, inducing each other's expression as a positive feedback loop; together these transcription factors promote and maintain the differentiated state of adipocytes. Expression of *C/EBPα* and *PPARγ* generally follows the upregulated expression of *C/EBPβ* and *C/EBPδ* (Rosen et al., 2002). The C/EBPα protein is also pivotal in insulin sensitivity; it mediates sensitivity partially by direct transcriptional induction of the insulin receptor and has been show to play a part in post receptor regulation as well (Wu et al., 1999). Insulin sensitivity is particularly important for intramuscular fat development, as this fat depot is relatively sensitive to insulin in comparison to other fat depots.

ADH1C. No differences ($P \geq 0.11$) were found in the mRNA expression of *ADH1C*. This may be due to sufficient retinaldehyde being produced in both treatments and breeds to meet physiological demands. Both treatments were within the threshold of normal serum retinol levels at the end of finishing, therefore, *ADH1C* upregulation may not have been needed to maintain retinaldehyde or retinoic acid levels. The enzyme ADH1C is responsible for the oxidation of retinol to retinaldehyde (Molotkov et al., 2002). The genotype of cattle at the loci for the ADH1C enzyme has been shown to have an effect on the intramuscular fat deposition when vitamin A is limited in the diet (Ward et al., 2015). Retinaldehyde is a potent inhibitor of adipogensesis (Ziouzenkova et al., 2007); therefore, an increase in ADH1C without an increase in RALDH1α1 would likely substantially inhibit adipogensis.

RALDH1α1. Retinaldehyde dehydrogenase 1 alpha 1 (*RALDH1α1*) showed a three-way interaction between treatment, breed, and biopsy date (Figure 3.3, $P = 0.002$). At d 86, the second biopsy, LVA commercial Angus cattle had greater expression of *RALDH1α1* than every other group ($P < 0.05$). It is possible that cattle with higher marbling scores, generally need higher retinoic acid levels during the middle portion of finishing to stimulate intramuscular lipid development. RALDH1α1 is responsible for the oxidation of retinaldehyde to retinoic acid. This oxidation can occur to both the *9-cis* conformation as well as the *all-trans* conformation of retinaldehyde, and the conformation is conserved in the product, which can alter the function of retinoic acid by determining whether it will preferentially bind to RAR or RXR (Patterson et al., 2013). It has been shown that in times of vitamin A deficiency RALDH expression is increased 3-fold (Napoli et al., 1995). It has been proposed that when retinol is limiting, retinoic acid levels are maintained at the cost of retinaldehyde (Ward et al., 2012). As our results show, the commercial Angus LVA steers had the greatest expression of *RALDH1α1* during the second biopsy period, which is consistent with the upregulation of RALDH1α1 reported by Napoli et al. (1995). Though retinoic acid was not measured, it is possible that its levels were higher in this group of cattle and help promote intramuscular fat development. As this group had the greatest marbling, it is possible that a high level of RALDH1α1 at this time point is beneficial to the marbling of cattle.

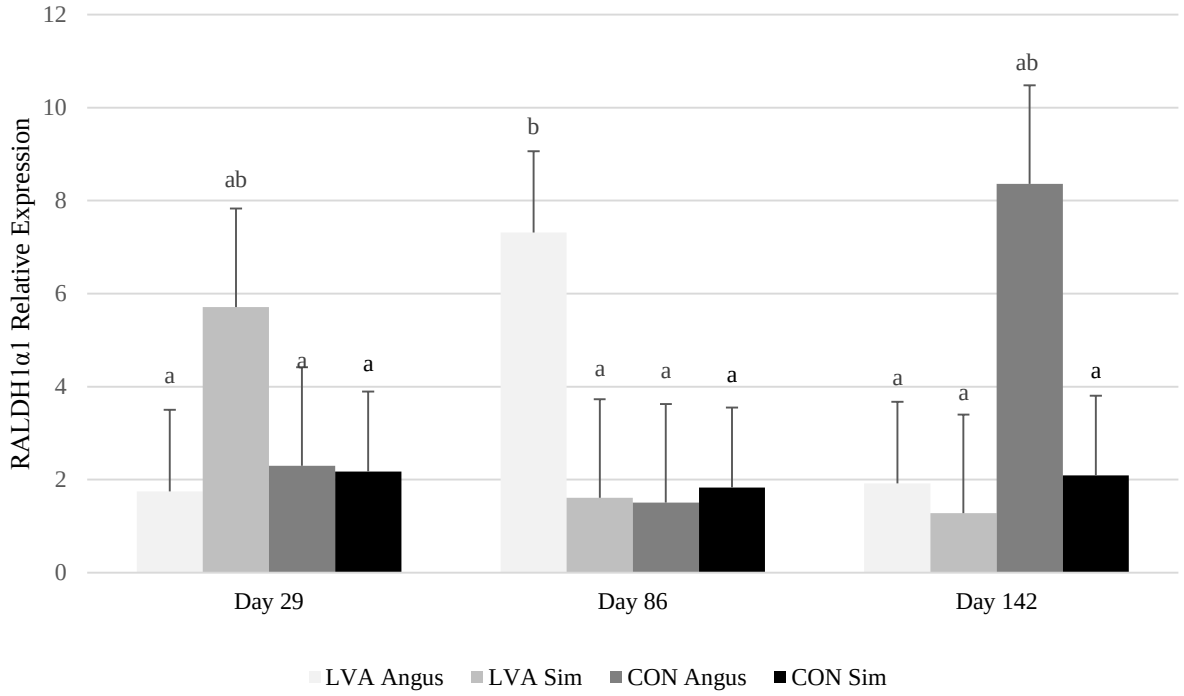

Figure 3.3. Relative expression of RALDH1α1, presented using the $2^{\Delta\Delta Ct}$ transformation. Days 29, 86, and 142 are days of finishing, respectively. LVA = basal diet containing 1,017 IU/kg DM vitamin A, CON = basal diet supplemented at 2,200IU/kg DM. Simm = Pure bred Simmental steers, Angus = Commercial Angus Angus steers containing > 75% Angus. [abc]Treatments not sharing a common superscript are different ($P < 0.05$).

PREF-1. Pre-adipocyte factor 1 (PREF-1) has been shown to be a potent inhibitor of adipogeneis. There was no effect of treatment, breed, or biopsy period on *PREF-1* expression (Table 3.3). PREF-1 has been shown to inhibit adipocyte differentiation by induction of Sox9, which in turns limits the translation of C/EBPβ and C/EBPδ by binding their promotor region. C/EBPβ and C/EBPδ are known to begin a cascade of transcriptional changes that promote adipocyte differentiation, thus PREF-1 functions to maintain cell in a preadipocyte state (Hudak and Sul, 2013).

Conclusions

This study confirms that a restricted vitamin A diet during finishing results in increased marbling scores in commercial Angus steers; this result is not observed in Simmental steers. Certain genes in the adipogenic pathway were expressed differentially throughout and at different points during finishing. Markedly, *FABP4* was higher in commercial Angus steers, and *RALDH1α1* was higher in the LVA commercial Angus group at 86 days of finishing. A similar study with a larger sample size and a broader array of adipogenic genes would be useful to extend the findings of this study. The specific cascade of hormones, transcription factors, and genes necessary to promote intramuscular fat development is not well understood. Nonetheless, vitamin A restriction has been shown to be useful tool to increase marbling in commercial Angus cattle.

Literature Cited

Adachi, K., H. Kawano, K. Tsuno, Y. Nomura, N. Yamamota, A. Arikawa, A. Tsuji, M. Adachi, T. Onimaru, and K. Ohwada. 1999. Relationship between serum biochemical Values and marbling scores in Japanese black cattle. J. Vet. Med. Sci. 61:961-964.

Barak, Y., M. C. Nelson, E. S. Ong, Y. Z. Jones, P. Ruiz-Lozano, K. R. Chien, A. Koder, and R. M. EVans. 1999. PPAR gamma is required for placental, cardiac, and adipose tissue development. Mol. Cell. 4:585–595.

Fajas, L., J. C. Fruchart, and J. Auwerx. 1998. Transcriptional control of adipogenesis. Curr. Opin. Cell Biol. 10:165-173.

Frye, T. M., S. N. Williams, and T. W. Graham. 1991. Vitamin deficiencies in cattle. Vet. Clin. North Am. Food Anim. Pract. 7:217-275.

Gorocica-Buenfil, M. A., F. L. Fluharty, T. Bohn, S. J. Schwartz, and S. C. Loerch. 2007a. Effect of low vitamin A diets with high-moisture or dry corn on marbling and adipose tissue fatty acid composition of beef steers. J. Anim. Sci. 85:3355–3366. doi:10.2527/jas.2007-0172

Hudak, C. S. and H. S. Sul. 2013. Pref-1, a gatekeeper of adipogenesis. Front. Endocrinol. 4:1-6. doi: 10.3389/fendo.2013.00079

Knutson, E. E., X. Sun, A. B. P. Fontoura, J. Gaspers, J. H. Liu, K. R. Carlin, M. Bauer, K. C. Swanson, and A. K. Ward. 2017. Effect of a low vitamin A diet on carcass and production characteristics of Angus cross and Simmental steers. J. Anim. Sci. Submitted.

LiVak, K. J. and T. D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C}{}_T$ method. Methods 25:402-408. doi:10.1006/meth.2001.1262

Mangelsdorf DJ, Borgmeyer U, Heyman RA, Zhou JY, Ong ES, Oro AE, Kakizuka A, EVans RM. Genes Dev. 1992 Mar; 6(3):329-44.

Michal, J. J., Z. W Zhang, C. T. Gaskins, and Z. Jiang. 2006. The bovine *fatty acid binding protein 4* gene is significantly associated with marbling and subcutaneous fat depth in Wagyu x Limousin F_2 crosses. Anim. Genet. 37:400–402. doi:10.1111/j.1365-2052.2006.01464.x

Molotkov, A., X. Fan, and G. Duester. 2002. Excessive vitamin A toxicity in mice genetically deficient in ether alcohol dehydrogenase Adh1 or Adh3. Eur. J. Biochem. 269:2607–2612

Napoli, J. L. 1996. Retinoic acid biosynthesis and metabolism. FASEB J. 10:993−1001.

NRC. 2000. Nutrient requirements of beef cattle. 7th rev. ed. National Academy Press, Washington, D.C.

Pethick, D. W., G .S. Harper, and V. H. Oddy. 2004. Growth, development and nutritional manipulation of marbling in cattle: a review. Aust. J. Exp. Agric. 44:705-715.

Pickworth, C. L., S. C. Loerch, and F. L. Fluharty. 2012. Restriction of vitamin A and D in beef cattle finishing diets on feedlot performance and adipose accretion. J. Anim. Sci. 90:1866-1878. doi:10.2527/jas.2010-3590

Repa, J. J., K. K. Hanson, and M. Clagett-Dame. 1993. *All-trans* retinol is a ligand for the retinoic acid receptors. PNAS. 90:7293−7297.

Rosen, E. D., C. Hsu, X. Wang, S. Sakai, M. W. Freeman, F. J. Gonzalex, and B. M. Spiegelman. 2002. C/EBPα induces adipogenesis through PPARγ: a unified pathway. Genes Dev. 16: 22-26.

Siebert, B. D., Z. A. Kruk, J. Davis, W. S. Pitchford, G. S. Harper, and C. D. K. Bottema. 2006. Effect of low vitamin A status on fat deposition and fatty acid desaturation in beef cattle. Lipids. 41:365-370. doi:10.1007/s11745-006-5107-5

Tan N. S., N. S. Shaw, N. Vinckenbosch, P. Liu, R. Yasmin, and B. Desvergne. 2002. Selective cooperation between fatty acid binding proteins and peroxisome proliferator-actiVated receptors in regulating transcription. Mol. Cell. Biol. 22:5114–5127.

Tontonoz, P., E. Hu, R. A. Graves, A. I. BudaVari, and B. M. Spiegelman. 1994. mPPARy2: tissue-specific regulator of an adipocyte enhancer. Genes Dev. 8:1224-1234.

Wang, Y. H., N. I. Bower, A. Reverter, S. H. Tan, N. De Jager, R. Wang, S. M. McWilliam, L. M. Café, P. L. Greenwood, and S. A. Lehnert. 2009. Gene expression patterns during intramuscular fat development in cattle. J. Anim. Sci. 87:119-130. doi:10.2527/jas.2008-1082

Ward A. K., J. J. McKinnon, S. Hendrick and F. C. Buchanan. 2012. The impact of vitamin A restriction on the ADH1C genotype on marbling in feedlot steers. J. Anim. Sci. 90:1-8. doi:10.2527/jas.2011-4404

ZiouzenkoVa, O., G. Orasanu, M. Sharlach, T. E. Akiyama, J. P. Berger, J. Viereck, J. A. Hamilton, G. Tang, G. G. Dolnikowski, S. Vogel, G. Duester, and J. Plutzky. 2007. Retinaldehyde represses adipogenesis and diet-induced obesity. Nat. Med. 13:695−702.

CHAPTER 4: CONCLUSION

The results of this thesis and previous research have shown that VA restriction does indeed increase marbling, but this effect is only seen in cattle with high genetic marbling potential. The reason for this difference is unknown. In our study, Simmental cattle had greater GH levels, which may have had a negative effect on adipocyte development and lipid filling. There was also a tendency for the LVA treatment to have lower IGF-1 serum concentration; IGF-1 is generally regarded as an anabolic and lipolytic hormone, therefore lower levels may allow adipocyte development and lipid filling. Glucose contributes to a substantially larger percentage (50-75%) of the acetyl units in intramuscular fat than in subcutaneous fat (1-10%), whereas acetate contributes the majority of acetyl groups in subcutaneous fat. There was no difference in insulin between treatments or breeds in our study, the sensitivity of insulin receptors and the abundance of GLUT4 transporters was not measured. It may be possible that cattle with high propensity to marble maintain higher sensitivity to insulin and mobilize more glucose into the cell for use in fatty acids, increasing intramuscular fat accumulation. It would also be interesting to measure circulating glucose between breeds and treatments, as there could be a difference in ruminal production or passage of glucose between groups.

Retinoic acid exerts its greatest negative effects during the differentiation stage of adipocyte development; adipocytes in cattle with low propensity to marble may be differentiating at a time when VA was not restricted, therefore reducing preadipocytes differentiation. Future studies would be useful to determine if intramuscular fat could be increased in cattle with low propensity to marble by manipulating VA at differing time periods, or if VA restriction could further increase cattle with high propensity to marble.

A more descriptive picture of what is occurring in the adipogenic gene pathway could be discovered if multiple surgeries were performed on the same animal throughout development. This would allow us to use repeated measures statistical analysis, which would increase statistical power by removing Variation between subjects.

Another way to improve gene expression analysis is to find a way to specifically isolate and obtain intramuscular fat, instead of performing qPCR on intact muscle. If we could find a way to locate the mesenchymal stem cells and preadipocytes that the genes of interest are being altered in and develop into intramuscular fat, this would give us the most accurate representation of gene expression throughout adipose development. On the other side of this suggestion, we could have also included more genes that are found in muscle to have a lipolytic effect. This is logical because VA effected the intramuscular fat but not the subcutaneous fat quantity; this leads me to believe that it may be a difference in muscle lipolytic capacity/ oxidative capacity/energy absorptive capacity, which leads to the development of fat around the muscle. But this difference in effect of VA on intramuscular and subcutaneous fat development may also be dictated by the amount of blood flow and nutrient supply to these tissues/regions.

In conclusion, the contents of this thesis show that removing VA supplementation from the growing and finishing diets of Angus cross steers has a positive effect on marbling and quality grade. There was no apparent negative effects of removing VA, other positives were also apparent, such as increased ADG. Increasing marbling without increasing other fat depot's leads to increased premiums at market, which leads to increased profits for producers. Particularly recently, with the Chinese meat trade re-opened, there will be a large market for high quality, highly marbled beef. I believe that removing VA supplementation from the growing and

finishing rations of cattle with high propensities to marble, the U.S. beef industry could increase profits substantially at no extra cost to the producer.