

EFFECT OF DIETARY VITAMIN A SUPPLEMENTATION ON SERUM AND LIVER
RETINOL CONTENT, GROWTH PERFORMANCE, CARCASS COMPOSITION, AND
MEAT QUALITY OF LAMBS AND CATTLE

by

AARON MATTHEW ARNETT

B.S., The Ohio State University, 2000

M.S., University of Kentucky, 2004

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Animal Sciences and Industry
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2007

Abstract

Two experiments were conducted to evaluate the effects of dietary vitamin A on growth, carcass characteristics, and meat quality of ruminants. In Experiment 1, 40 crossbred wethers (BW = 28.7 kg) were assigned to 1 of 4 treatments: backgrounding (BG) and finishing (FN) with no vitamin A (LL); BG with no vitamin A, FN with high vitamin A (6,600 IU·kg⁻¹ diet) (LH); BG with high vitamin A and FN with no vitamin A (HL); and BG and FN with high vitamin A (HH). During BG (d 1 to 56), intake was restricted to achieve 0.22 kg ADG. During FN (d 57 to 112), lambs consumed the same diet *ad libitum*. Lambs were humanely slaughtered after 112 d. There were no treatment differences ($P > 0.05$) in feed intake, ADG, or final BW. Carcasses from the HH group had higher ($P < .05$) marbling scores (514 vs. 459), and 25.8 % more extractable intramuscular lipid (IMF) than LL (3.88 vs. 3.08 % for HH and LL, respectively, $P < .05$); the LH and HL treatments were intermediate. There was a negative correlation ($r = -0.38$) between serum fatty acid content and %IMF. Experiment 1 data suggest that increased marbling may be achieved with high vitamin A for 112 d in lambs. In Experiment 2, Angus crossbred steers ($n = 48$), were either early-weaned (EW) at 137 ± 26 d of age or weaned at a traditional age (TW) 199 ± 26 d and allotted to either 42,180 IU vitamin A·hd⁻¹·d⁻¹ (HA) or no vitamin A (NA). Early- and TW steers consumed treatments for 235 ± 17 and 175 ± 18 d, respectively. Serum and liver retinol content diverged dramatically (both, $P < 0.01$) by the end of the experiment and TW steers tended ($P > 0.10$) to have higher ADG than EW steers (1.31 ± 0.2 and 1.48 ± 0.2 kg·hd⁻¹·d⁻¹, respectively). Steers were humanely slaughtered at 1.02 cm fat. Weights tended ($P = 0.08$) to be heavier and carcasses were fatter ($P < 0.05$) for HA than NA. Marbling score and % IMF were higher ($P < 0.05$) for EW-NA than other treatments. Percentage of USDA Choice and Prime carcasses doubled ($P < 0.05$) for NA than HA. Yield grades increased ($P < 0.05$) with EW-HA and were similar ($P > 0.10$) among other treatments. Feeding NA was effective for increasing marbling without increasing fat; EW enhanced these effects. Reasons for the contradictory results in these 2 experiments are unclear. Species differences in the ability to metabolize retinol are implicated.

EFFECTS OF DIETARY VITAMIN A SUPPLEMENTATION ON SERUM AND LIVER
RETINOL, GROWTH PERFORMANCE, AND CARCASS MARBLING
CHARACTERISTICS OF LAMBS AND CATTLE

by

AARON MATTHEW ARNETT

B.S., The Ohio State University, 2000
M.S., University of Kentucky, 2004

A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Animal Sciences and Industry
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2007

Approved by:

Major Professor
Michael E. Dikeman, Ph.D

Abstract

Two experiments were conducted to evaluate the effects of dietary vitamin A on growth, carcass characteristics, and meat quality of ruminants. In Experiment 1, 40 crossbred wethers (BW = 28.7 kg) were assigned to 1 of 4 treatments: backgrounding (BG) and finishing (FN) with no vitamin A (LL); BG with no vitamin A, FN with high vitamin A (6,600 IU·kg⁻¹ diet) (LH); BG with high vitamin A and FN with no vitamin A (HL); and BG and FN with high vitamin A (HH). During BG (d 1 to 56), intake was restricted to achieve 0.22 kg ADG. During FN (d 57 to 112), lambs consumed the same diet *ad libitum*. Lambs were humanely slaughtered after 112 d. There were no treatment differences ($P > 0.05$) in feed intake, ADG, or final BW. Carcasses from the HH group had higher ($P < .05$) marbling scores (514 vs. 459), and 25.8 % more extractable intramuscular lipid (IMF) than LL (3.88 vs. 3.08 % for HH and LL, respectively, $P < .05$); the LH and HL treatments were intermediate. There was a negative correlation ($r = -0.38$) between serum fatty acid content and %IMF. Lipid profiles were not affected by vitamin A treatments. Experiment 1 data suggest that increased marbling may be achieved with high vitamin A for 112 d in lambs. In Experiment 2, Angus crossbred steers ($n = 48$), were either early-weaned (EW) at 137 ± 26 d of age or weaned at a traditional age (TW) 199 ± 26 d and allotted to either 42,180 IU vitamin A·hd⁻¹·d⁻¹ (HA) or no vitamin A (NA). Early- and TW steers consumed treatments for 235 ± 17 and 175 ± 18 d, respectively. Serum and liver retinol content diverged dramatically (both, $P < 0.01$) by the end of the experiment and TW steers tended ($P > 0.10$) to have higher ADG than EW steers (1.31 ± 0.2 and 1.48 ± 0.2 kg·hd⁻¹·d⁻¹, respectively). Steers were humanely slaughtered at 1.02 cm fat. Weights tended ($P = 0.08$) to be heavier and carcasses were fatter ($P < 0.05$) for HA than NA. Marbling score and %IMF were higher ($P < 0.05$) for EW-NA than other treatments. Percentage of USDA Choice and Prime carcasses doubled ($P < 0.05$) for NA than HA. Yield grades increased ($P < 0.05$) with EW-HA and were similar ($P > 0.10$) among other treatments. Feeding NA is effective for increasing marbling without increasing fat; EW enhanced these effects. Reasons for the opposing results in these 2 experiments are unclear at this time. Species differences in the ability to metabolize retinol are implicated.

Table of Contents

List of Figures	ix
List of Tables	xi
Acknowledgements.....	xii
CHAPTER 1 - Review of Literature.....	1
Marbling.....	1
Methods to Increase Marbling in Cattle	1
Genetic Selection	1
Feeding Practices	2
Hormone Implants and β -adrenergic Agonists	2
Early Weaning	3
Health History and Disposition.....	4
Summary of Factors Involved in Marbling.....	4
Adipogenesis.....	5
Introduction to Adipogenesis	5
Maturation of the Adipocyte Phenotype	7
Transcription Factors Regulate Adipocyte Differentiation.....	7
The CAAT/EBP Family.....	8
The PPAR Family	8
Retinoid X Receptor (RXR) and Retinoic Acid Receptor (RAR)	9
Introduction to Vitamin A.....	10
Vitamin A History.....	10
Vitamin A Chemical Structure.....	10
Retinoids	11
Carotenoids	11
Quantifying Vitamin A	12
Vitamin A Solubilization and Digestion.....	13
Intestinal Enzymes	13
Pancreatic Enzymes	13

Cellular Uptake of Vitamin A.....	13
Intracellular and Intercellular Transport of Vitamin A.....	14
Incorporation of Vitamin A into Chylomicrons.....	14
Inhibition of Adipocyte Differentiation by Retinoic Acid.....	15
Vitamin A Biology in Cattle.....	22
Dietary Requirement.....	22
Vitamin A Supplementation in U.S. Feedlots.....	22
Tissue Storage of Vitamin A in Cattle.....	23
Seasonality of Quality Grade in the United States.....	23
Cattle Feeding in Asia.....	24
Vitamin A Studies in the U.S. Beef Industry.....	25
LITERATURE CITED.....	30
CHAPTER 2 - EFFECTS OF VITAMIN A SUPPLEMENTATION ON PERFORMANCE,	
SERUM LIPID, AND LONGISSIMUS MUSCLE LIPID COMPOSITION OF LAMBS ..	
ABSTRACT.....	37
INTRODUCTION	38
MATERIALS AND METHODS.....	38
Animals.....	39
Experimental Design and Treatments.....	39
Diets	39
Sample Collection.....	41
Vitamin A Determination	42
Fatty Acid Analysis.....	42
Statistical Analyses	43
RESULTS AND DISCUSSION.....	43
Vitamin A in Tissues	43
Growth Performance.....	45
Serum Retinol and Marbling.....	46
Serum Fatty Acid Concentration	47
Carcass Attributes	50
Backfat Thickness.....	50

Marbling Scores	51
Tissue Fatty Acid Concentration	52
Fatty Acid Concentration in the LT	52
Fatty Acid Composition of the LT	53
Fatty Acid Concentration in Liver	55
Fatty Acid Composition in the Liver	55
CONCLUSIONS	56
LITERATURE CITED	57
CHAPTER 3 - EFFECTS OF VITAMIN A SUPPLEMENTATION AND WEANING AGE ON SERUM AND LIVER RETINOL CONCENTRATIONS, CARCASS COMPOSITION, AND MEAT QUALITY IN MARKET BEEF CATTLE	59
ABSTRACT	59
INTRODUCTION	60
MATERIALS & METHODS	60
Animals	60
Diets	61
Endpoint Determination and Harvest	63
Fatty Acid Analysis	64
Retinol Analysis	65
Statistical Analysis	65
RESULTS AND DISCUSSION	66
Retinol Status	66
Growth Performance	68
Carcass Traits	69
Accounting for the cost of producing marbling	77
Fatty Acid Composition of the LM	83
CONCLUSIONS AND IMPLICATIONS	85
LITERATURE CITED	88
Appendix A - Regression Analyses and Prediction Equations	90
Regression of Carcass Marbling Score and % Intramuscular Fat on Liver Retinol Concentration	90

Appendix B - Effect of Supplementing either 1x or 7x NRC Levels of Dietary Vitamin A to

Early-weaned Steers	92
MATERIALS AND METHODS.....	92
Animals and experimental procedures	92
Statistical Analyses	92
Results and Discussion	93
Growth Performance	93
Carcass Traits	93
SUMMARY AND CONCLUSIONS	95

List of Figures

Figure 1.1. Adipocyte development from an undifferentiated, multipotent mesenchymal cell (adapted from Gerrard and Grant, 2003)	6
Figure 1.2. Photomicrographs of bovine preadipocytes cultured under different concentrations of retinol. (A) Without retinol, (B) 0.4 µg/100 ml retinol, (C) 4 µg/100 ml retinol, and (D) 40 µg/100 ml retinol (Ohyama et al., 1998).....	16
Figure 1.3 Effect of pharmacological doses of retinoic acid on glycerol-3-phosphate dehydrogenase activity of porcine preadipocytes in primary culture (Suryawan and Hu, 1997).	17
Figure 1.4 Effect of retinoic acid administration at different times on glycerol-3-phosphate dehydrogenase activity of porcine preadipocytes in primary culture (Suryawan and Hu, 1997).	18
Figure 1.5. Effect of exposure time of retinoic acid on glycerol-3-phosphate dehydrogenase activity of porcine preadipocytes in primary culture (Suryawan and Hu, 1997).....	19
Figure 1.6. The effect of increasing doses of retinoids on glycerol-3-phosphate dehydrogenase activity in primary cultures of differentiating porcine preadipocytes at d 10 (Brandebourg and Hu, 2005).....	20
Figure 1.7. Effect of retinoic acid receptor agonist, Ro61, on the ability of all-trans-retinoic acid and 4-(E-2-[5,6,7,8-tetrahydro-5,5,8,8,-tetramethyl-2-naphthalenyl]-1-propenyl) benzoic acid to inhibit glycerol-3-phosphate dehydrogenase activity in primary cultures of differentiating porcine preadipocytes at d 10 (Brandebouorg an Hu, 2005).	21
Figure 2.1. Serum retinol content during the backgrounding period.....	44
Figure 2.2. Serum retinol content during the last 28 d of the finishing period.....	45
Figure 2.3. Total serum fatty acid content during the backgrounding period.....	48
Figure 2.4. Total serum fatty acid content during the last 28 d of the finishing period.....	49
Figure 2.5. Total fatty acid content of the longissimus thoracis and liver at slaughter	52
Figure 3.1. Serum retinol concentrations on 3 sampling days	67
Figure 3.2. Liver retinol concentrations (log scale).....	68
Figure 3.3. Twelfth -rib fat thickness (cm) of carcasses.....	71

Figure 3.4. Ribeye area (cm ²) of carcasses	72
Figure 3.5. USDA Yield Grades of carcasses.....	73
Figure 3.6. Marbling scores for carcasses from the four treatment combinations. 400=Small ⁰⁰ , 410=Small ¹⁰ , ... 500= Modest ⁰⁰	74
Figure 3.7. Percentage of intramuscular fat in the LM of carcasses as determined by gas chromatography	76
Figure 3.8. Percent of carcasses with Modest ⁰⁰ or higher degrees of marbling.....	77
Figure 3.9. Marbling deposition (degrees) per day of age for steers	79
Figure 3.10. Marbling deposition (degrees) per day on the finishing diet.....	80
Figure 3.11. Marbling deposition (degrees) per USDA Yield Grade	81
Figure 3.12. Marbling deposition (degrees) per cm of twelfth-rib fat	82
Figure 3.13. Marbling deposition (degrees) per kg of hot carcass weight.....	83
Figure 3.14. Relative contribution of oleic acid (C18:1n-9 <i>cis</i>) to the total intramuscular fatty acids in the LM	84

List of Tables

Table 1-1. Conversions for various measures of vitamin A activity for cattle	12
Table 1-2. Summary of serum retinol and carcass traits compiled from experiments with 3 classes of cattle fed either 2,300 or 7,250 IU vitamin A per kg DM during the feedlot period (Pyatt et al., 2005).	27
Table 2-1. Composition of the treatment diets as fed	40
Table 2-2. Lamb weights, growth performance, backfat depth, marbling scores, liver fatty acids, and longissimus thoracis fatty acids	46
Table 2-3. Pearson correlations between final serum levels and content of fatty acids (FA) in several carcass depots	50
Table 2-4. Individual fatty acid concentrations in the longissimus thoracis ¹	54
Table 2-5. Concentration of individual fatty acids in the liver, caudal lobe ¹	56
Table 3-1. Composition (DM basis) of the preconditioning diet.....	62
Table 3-2. Average composition (DM basis) of the finishing diet	63
Table 3-3. Composition (DM basis) of the supplement for the finishing diet.....	63
Table 3-4. Effects of weaning age and dietary vitamin A level on steer growth performance attributes.....	69
Table 3-5. Effects of vitamin A level and weaning age on carcass attributes of steers. ^a For marbling score, 400= Small ⁰⁰ , 410= Small ¹⁰ , ...500=Modest ⁰⁰ , etc.....	70
Table 3-6. Selected fatty acids from the intramuscular fat of the <i>longissimus</i> muscle samples from the four treatment combinations.....	84

Acknowledgements

I would like to thank my major professor, Dr. Michael Dikeman, for the opportunity to study under him at Kansas State University. Many animal scientists understand the biology of living animals; many understand the biochemistry and value of meat in society; few can connect them. Dr. Dikeman is a legend in meat animal growth and carcass composition research. He and several others have made Kansas State University into an institution that is recognized globally for its excellence in research and teaching of animal and meat sciences. I am honored to have studied here.

I would also like to thank my parents, Kent Arnett and Patricia Ahr, and my grandparents, Don and Esther Arnett. No person is self-made. The love and discipline they gave to me as a child instilled the courage and work ethic necessary to attain a doctoral degree.

CHAPTER 1 - Review of Literature

Marbling

Marbling, or intramuscular fat, has been associated with increases in palatability and consumer satisfaction of eating beef. Although marbling appears to have only a low to moderate relationship to tenderness (Parrish, 1974; Tatum et al., 1980), marbling has been associated with juiciness and flavor intensity of beef when evaluated by trained sensory panels (May et al., 1992). Although there is a tendency in our society toward consumption of leaner meat products, it is apparent that many consumers are not willing to sacrifice the eating quality that is associated with well-marbled beef (Harrison et al., 1978; Savell et al., 1987). Furthermore, marbling score is the most important trait in determining USDA quality grades of carcasses from cattle younger than 42 mo of age at harvest (Aberle et al., 2001). In addition, many beef producers have shifted toward selling their cattle in a value-based marketing system where they are paid premiums for producing carcasses that meet USDA standards for marbling and receive severe discounts for carcasses that do not meet these standards. In 2006, approximately half of all fed cattle in the U.S. were marketed through value-based systems where the price was determined for individual carcasses based on USDA quality and yield grade (Anderson and Gleghorn, 2007). A decade of increased consumer demand for beef coupled with increased use of value-based price determinations substantiates the importance for researchers to continue finding methods of increasing marbling of beef without increasing waste fat production.

Methods to Increase Marbling in Cattle

Genetic Selection

The last 10 years have seen an unprecedented effort by multiple segments of the beef production chain to optimize the quality and consistency of beef. These production goals have been attained through application of research findings to the management decisions and genetic selections made on farms and ranches. Inherent differences among beef breeds to attain high degrees of marbling have been well-documented (Jeremiah et al., 1970; Albrecht et al., 2006). In Asian countries, the Japanese Black, Hanwoo, and Wagyu breeds are capable of producing the

highest marbling scores of any known cattle breeds (Oka et al., 1998). These breeds are not commonly used in U.S. beef production due to their inherent slow growth rate, inferior muscling, and high marbling that would generally be considered unhealthy by most American consumers. Among the beef breeds common to the U.S., British breeds of Angus, Red Angus, and Shorthorn are far more likely to produce carcasses with high marbling scores compared with Continental (European) and Brahman or Brahman-influenced breeds (Albrecht et al., 2006). Branded marketing programs like Certified Angus Beef® were founded upon the relatively high propensity of Angus cattle to produce carcasses with high marbling scores compared to most other breeds. In the mid-1990s, several beef cattle breed associations began using ultrasound images of the longissimus dorsi muscle (LM) in potential parent animals as a predictor of marbling potential of their progeny (Wilson, 1992; Baker et al., 2006). Further, genetic selection for increased marbling and higher USDA quality grades within these breeds has increased the genetic merit of the commercial beef cowherd for marbling.

Feeding Practices

Increasing marbling through management practices, such as feeding cattle high-energy grain diets, has been demonstrated extensively. Carcass marbling scores and USDA quality grades have been positively correlated to daily feed intake (Hicks et al., 1990), dietary energy density (Guenther et al., 1965; Prior et al., 1977; Burson et al., 1980) and time on feed (Moody et al., 1970; May et al., 1992; Duckett et al., 1993). Increasing the daily feed intake, energy-density, and days of feeding are practices that increase the caloric intake by cattle. Intuitively, storage of excess energy as marbling and other fat depots would be expected to increase. These feedlot management practices are common used standards in the U.S. cattle feeding industry to maximize genetic expression of marbling.

Hormone Implants and β -adrenergic Agonists

The use of growth-promoting metabolic modifiers, like steroid implants and β -adrenergic agonists, have been known to decrease marbling (Anderson and Gleghorn, 2007). By making informed decisions about the effects of the implant ingredients on marbling development, understanding the biological type of cattle and marketing goals for the animals being treated, and by following recommended implant protocols, suppression of carcass marbling can be mitigated (Dikeman, 2007). Under current market conditions, complete abandonment of growth-

promoting compounds is not practical. However, avoiding common application mistakes will certainly reduce the negative effects that many of these compounds have on marbling development.

Early Weaning

Conventionally, beef calves born in the spring are weaned from their mothers in the fall of the same year, averaging 200 to 220 d of age. Autumn is a logical time to wean calves for several reasons. Primarily, pasture lands usually become dry and feed resources are scarce by late summer or early autumn. In the fall months, spring-calving cows still nursing calves will be in their poorest body condition of the year and calves will have become largely dependent on forage rather than milk. When cattle are gathered from vast pastures to more concentrated winter feeding, it is a logical time to wean the calves. In Midwestern states, it is sometimes more convenient to wean calves in late fall, after crop harvest is completed and cows can graze crop residues during mid to late gestation.

Beginning in the mid-ninety's, research confirmed some long-held assumptions about the beneficial aspects of early weaning, particularly the increases in marbling scores and USDA quality grades (Berger and Faulkner, 2003). Early weaning is defined by most researchers and producers as the method of weaning calves between 75 and 150 d of age and placing them on a high-energy diet until slaughter (Wertz et al., 2001; Wertz et al., 2002; Berger and Faulkner, 2003). Compared to conventionally-weaned calves, early-weaned calves are equal to or heavier at 205 d of age, but have slightly lower average daily gain (ADG) in the feedlot, and slightly higher numeric USDA yield grades (Wertz et al., 2002). The percentage of carcasses grading at least premium Choice (i.e., Modest⁰ or higher marbling) is increased when calves are early-weaned (Pyatt et al., 2005). Pyatt et al. (2005) also reported that early-weaned calves harvested at 16.5 mo were 20% more efficient on feed at any marbling endpoint than conventionally-weaned calves harvested at 29 mo. The authors noted that total feed costs of early weaning will almost always be greater than for conventional weaning, but they are offset by reduced cow feed costs. It is important to consider that these data were published in 2005, before the sharp increase in grain prices associated with the demand for corn in ethanol production. Given that 2007 corn and soybean prices were almost double the prices of 2005, the economic advantages of early-weaning definitely diminished and may have become unfavorable.

Health History and Disposition

Greater communication among the production segments of the beef industry has enabled associations to be made among farm of origin, genetic makeup, age, vaccination status, sickness and treatment record, and carcass characteristics of cattle. Without question, this communication has been facilitated by recent improvements in animal identification and record-keeping.

Anderson and Gleghorn (2007) reported a strong negative correlation between death loss within a pen and USDA quality grades of surviving cattle from the same feedlot pen.

Calm disposition has long been considered an important convenience trait in beef cattle. Recently, disposition has been associated with carcass grade. Nkrumah et al. (2007) reported a direct correlation between docility scores of cattle in feedlot pens and USDA quality grades of the carcasses. Increased selection for quiet disposition of cattle is mutually beneficial to all segments of the beef industry.

Summary of Factors Involved in Marbling

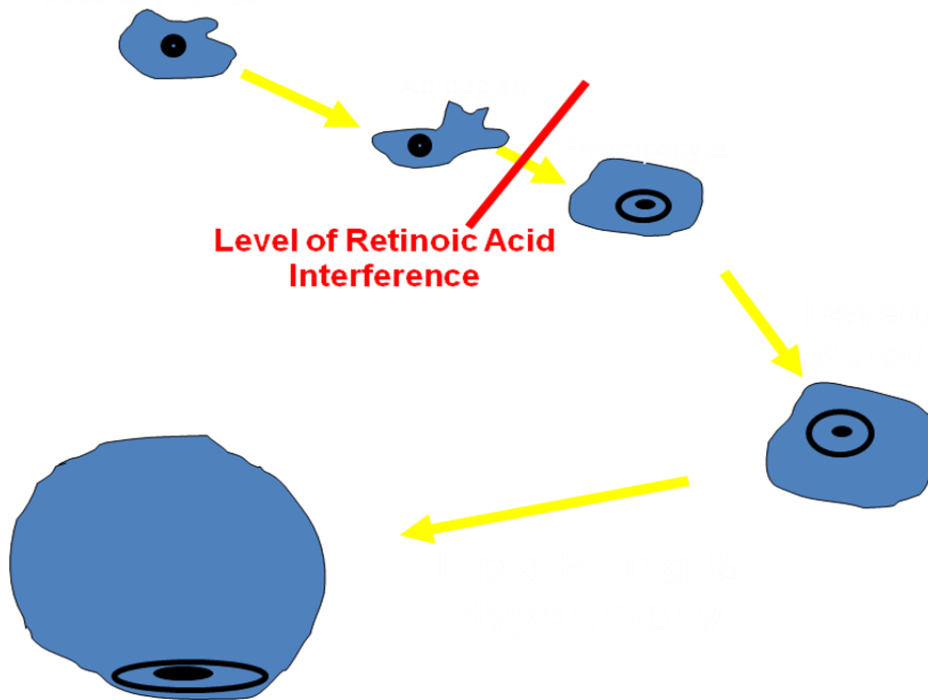
Feeding cattle with genetic potential for marbling is advantageous and can be accomplished using British breeds known for high marbling potential, and/or using lines of cattle that have been selected for increased marbling. Management and environmental choices such as early-weaning, feeding high-energy diets, disease prevention and selecting for docile animals have all been associated with increased marbling development. Proper use of hormone implants and/or β -adrenergic agonists can reduce the suppression in marbling development. Unfortunately, most of these management practices have at least one negative associative effect on beef production and profitability. For instance, long-term genetic *selection* for increased marbling may result in reduced carcass muscling and meat yield. Feeding high-energy diets and the use of early-weaning may be overshadowed by high costs of feed grains in response to the increased use of corn by the ethanol industry and will generally increase fat trim and decrease retail meat yields. Using less aggressive metabolic modifiers may decrease feed efficiency, carcass muscling and leanness. Ideal management solutions should cause little or now adverse side affects at the cost of improving marbling. Recent reports of increased marbling and carcass quality associated with reduction of vitamin A in cattle diets appears to be a “free lunch” opportunity to beef producers to increase carcass quality with no apparent side affects. This is the focus of my dissertation research.

Adipogenesis

Introduction to Adipogenesis

Fat deposited as marbling is metabolically unique from other fat storage depots. In livestock, marbling can be attained through management and genetics that are associated with manipulating of the metabolic processes of adipocyte proliferation, differentiation, and maturation. Adipogenesis begins in the developing embryo with organization of cells arising from a pool of undifferentiated multipotent stem cells of mesodermal origin (Gerrard and Grant, 2003). Muscle and cartilage tissues also originate from this pool of mesenchymal cells. The phenotypic fate of the mesenchymal cell depends largely upon the presence of signaling agents that are produced when either adipogenic or myogenic genes are expressed. For instance, in the presence of steroid compounds, mesenchymal cells predominantly give rise to fibroblasts, the precursors of muscle fibers (Cossu and Biressi, 2005). In the presence of insulin, cells typically follow the adipogenic pathway (Figure 1-1) (Dupont and LeRoith, 2001). Preadipocyte differentiation marks a point of no return and commitment of a cell to an adipogenic fate. Greater understanding of the mechanisms that signal and mediate cell proliferation and differentiation into unique phenotypes would have tremendous implications for the livestock and meat industries. This developmental “fork-in-the-road” is the focus of a great deal of research.

Figure 1.1. Adipocyte development from an undifferentiated, multipotent mesenchymal cell (adapted from Gerrard and Grant, 2003)



Because adipose tissue is a storage depot for energy, it is highly vascular and contains vast capillary networks. During early stages of adipose tissue development, there is an increase in connective tissue vascularization (Gerrard and Grant, 2003). Collections of adipoblasts form lobules that become loosely associated in a loosely-held sheath of collagen. Prenatal growth involves several proliferative divisions of adipoblasts. In cultures of stromal adipoblasts and preadipocytes, proliferation (hyperplasia) eventually ceases and the cells enter terminal differentiation. A similar process was presumed to occur *in vivo* such that in the peri-natal period, preadipocytes exited proliferative divisions and entered into early differentiation, resulting in fixed numbers of fat cells at birth. Under this theory, any postnatal increase in fatness was presumed to occur only through hypertrophy of existing adipocytes. This assumption generally was disproven by Allen et al. (1976) who demonstrated monomodal, bimodal, and ultimately trimodal distributions of adipocyte diameters that were contained in porcine backfat samples harvested periodically as pigs were fattened from 3.86 to 7.42 cm of backfat. The presence of cells with smaller diameters mixed with older cells having large

diameters indicated that both hyperplasia and hypertrophy were contributing to adiposity, particularly in fatter animals. Similarly, May et al. (1994) used subcutaneous and intramuscular adipose tissue cell cultures from Angus and Wagyu steers to demonstrate that proliferation and lipid filling (i.e., hypertrophy) can occur concurrently, even in mature cattle.

Maturation of the Adipocyte Phenotype

Adipoblasts are < 20 μm in diameter when lipid begins to accumulate in the center of cells (Scanes, 1995). Later, preadipocytes will contain several small intracellular lipid droplets known as multilocular lipid. As lipid droplets increase in size, they coalesce to form one large lipid particle, known as unilocular lipid. Interestingly, in cattle and sheep, most adipocytes have unilocular lipid at birth, whereas non-ruminants develop unilocular lipid during the first few postnatal weeks (Scanes, 1995). Adipocyte maturation is associated with continued lipid accumulation and hypertrophy. Ultimately, the lipid droplet may comprise > 95% of a cell's cytoplasmic volume (Gerrard and Grant, 2003). Lipid crowds the inside of the cell to the extent that the cell nucleus is forced to the outer boundaries of the cell against the cell membrane. Mature adipocytes are 6 to 10 times larger than adipoblasts, averaging 120 μm in diameter and attaining diameters of up to 300 μm in obese animals (Rule et al., 1995).

Transcription Factors Regulate Adipocyte Differentiation

Most of what is known about early development and differentiation of adipose tissue has come from studies of secondary cell lines, such as 3T3-L1 preadipocytes (Cornelius et al., 1994). Generally, preadipocytes are plated in 10 % fetal bovine serum and incubated until they reach confluence. Differentiation inducing factors can be added to the culture media causing commitment of cells to specific and predictable gene expression and phenotype development (Hwang et al., 1997). One of the pitfalls of cultured lines of preadipocytes is the absence of paracrine factors that are present *in vivo* which might influence either the rate or extent of cell growth in a manner that might go undetected in cell culture. Nonetheless, preadipocyte cell lines have enabled the recent discovery and continued understanding of the series of transcriptional events that control adipocyte development. These transcriptional events are involved in coordinating the expression of genes responsible for creating and maintaining the adipocyte phenotype (Tontonoz et al., 1994). Research has indicated that members of the CAAT/enhancer binding protein (C/EBP) and the peroxisome proliferator-activated receptor (PPAR) families are

highly expressed in adipose and act cooperatively to commit cells to an adipogenic program (Hwang et al., 1997).

The CAAT/EBP Family

The CAAT/EBP family of transcription factors plays a critical role in the induction and regulation of adipocyte differentiation. These are proteins that belong to the “basic region/leucine zipper transcription factor” family of nuclear transcription factors (Gerrard and Grant, 2003). These transcription factors mediate dimerization of other transcription factors and their binding at the promoter regions of DNA on adipose-relevant genes (Hurst, 1994). Expression of two isomers, C/EBP β and C/EBP δ , is induced at the inception of differentiation and their expression decreases in the terminal phase of differentiation (Yeh et al., 1995). These isomers are also known inducers of PPAR γ and C/EBP α that are markers of intermediate and late stages of adipocyte differentiation. Cells that lack the C/EBP β and C/EBP δ isomers do not develop into adipocytes (Gerrard and Grant, 2003), clearly demonstrating the importance of C/EBP β and C/EBP δ for affecting early differentiation of adipocytes.

The C/EBP α isomer is an antimitotic factor that is expressed during the terminal phase of differentiation and immediately prior to the expression of many adipose-specific genes (Umek et al., 1991; Hwang et al., 1997). It has been speculated that this isomer may cause the cessation of growth that ends the clonal expansion phase of differentiation (Freytag and Geddes, 1992).

The PPAR Family

Peroxisome proliferator-activated receptors (PPAR) are hormone receptors that were named based on their initial discovery as inducers of peroxisome proliferation. Peroxisomes are membrane-bound organelles found in plants and animals, which contain enzymes that are used to break down fatty acids (Campbell, 1996). Recently, their ubiquity and functional diversity in the cell has been recognized. Expression of the PPAR family has caused adipose differentiation and lipid accumulation in preadipocytes (Chawla et al, 1994), myoblasts (Teboul et al., 1995), multipotent C3H10T1/2 cells, and has induced adipocyte differentiation in NIH-3T3 fibroblasts (Forman et al., 1995).

PPARs become active transcription factors by forming heterodimers with nuclear ‘retinoid X receptors’ (RXR). Once dimerized, these transcription factors attach to peroxisome proliferator response elements in the promoter regions of DNA. The response elements are

direct repeats of the GGTC A base sequence separated by one random base (GGTCA-R-GGCTA) (Mangelsdorf and Evans, 1995). These sequences are found in the promoter regions of most genes that have been implicated in adipogenesis, including genes for lipoprotein lipase, fatty acid-binding protein, and steroyl-CoA desaturase (Hwang et al., 1997).

The gamma isomer is the most abundantly expressed isoform in adipose tissue (Zhu et al., 1995) and is described as the “master regulator of adipogenesis” by many writers (Pyatt and Berger, 2005). The gamma isoform is expressed early in the differentiation phase of adipocyte development and is either coincident with or slightly preceding C/EBP α expression (Tontonoz et al., 1994). Currently, the mechanism behind the interaction among C/EBP β , C/EBP α and PPAR γ is poorly characterized and more research is needed to understand the regulatory roles of these proteins. Brandebourg and Hu (2005) presented evidence that these transcription factors may not be interdependent for adipogenesis by demonstrating differences in the timing and sequence of transcriptional events between clonal cell lines and primary cultures of porcine preadipocytes. These discrepancies could also be caused by differences in the developmental stages of the two cell types and/or because a more homogeneous population of cells would likely be used in cultured cell lines.

Retinoid X Receptor (RXR) and Retinoic Acid Receptor (RAR)

Biological processes are regulated by intricate systems involving binding of a hormone or transcription factors to their receptors. Activated receptors initiate gene transcription through a series of cell signals, such as a second messenger (i.e., protein hormones), or bind to the promoter region of target genes to initiate transcription. These processes are exquisitely regulated by the binding kinetics of ligands to receptors. When two or more substances capable of binding to the same receptor are present, selective or preferential binding of substrates become important considerations for understanding the extent of the transcription factor’s ability to regulate gene expression.

Nuclear receptors have been characterized into a phylogenic tree that consists of several subfamilies of receptors. These include thyroid hormone receptor-like, retinoid X receptor-like (RXR), estrogen receptor-like, and a few others. The RXR is capable of binding with several members of the nuclear hormone receptor superfamily, including PPAR γ , retinoic acid receptor (RAR), thyroid hormone receptor, vitamin D receptor, liver X receptor, and fatty acid-activated

receptor (Kawada et al., 1996). When these ligands form dimers with RXR, they are capable of binding to *cis*-acting DNA elements in the promoter regions of DNA sequences, which is the first step in controlling gene expression. Retinoic acid acts as a hormone by activating intracellular RAR. For instance, the interactions of retinoic acid with genes that control adipocyte development are important for my dissertation research.

Introduction to Vitamin A

Vitamin A History

Reports as early as 1906 indicated that factors other than carbohydrates, proteins, and fats were necessary to keep cattle healthy. These were the first investigations that led to characterization of a class of nutrients known as vitamins. Vitamin A was first reported in 1913 by biochemists working independently; Elmer McCollum at the University of Wisconsin-Madison and Lafayette Mendel at Yale University were researching fat-soluble nutrients contained in butterfat and cod liver oil (cited by Semba, 1999). The Yale scientists chose to name this compound “fat-soluble factor A,” since “water-soluble factor B” (vitamin B) had recently been named (Wolf, 2001). Vitamin A was first synthesized in laboratory conditions in 1947 by Dutch chemists, David Adriaan van Dorp and Jozef Ferdinand Arens (Wolf, 2001). In 1967, George Wald won the Nobel Prize in Medicine for demonstrating the role of vitamin A in retina pigments, demonstrating the importance of vitamin A in vision.

Pre-formed vitamin A is available from animal-derived sources, such as liver and eggs, and from a variety of pigmented vegetables in the form of carotenes, which must be converted to vitamin A by intestinal enzymes. Although vitamin A deficiency is rarely implicated in sickness of humans and animals from developed countries, approximately 500,000 malnourished children go blind annually due to vitamin A deficiency (WHO, 2007). In livestock, dietary vitamin A status has been correlated to the immune response, fertility, embryo survival, vision, growth performance, and overall health since the 1930’s (NRC, 2001) and recently has been associated with carcass marbling (Oka, 1998; Arnett et al., 2007).

Vitamin A Chemical Structure

Vitamin A is a highly lipophilic, nonpolar, and polyunsaturated hydrocarbon that is important for many biological functions. In animals, vitamin A activity is found predominately

in the form of retinol and its esters, retinal, and to a lesser extent, retinoic acid (Gregory, 1996). For a compound to have vitamin A activity, it must have certain structural properties that make it similar to retinol (Figure 5). These include having at least one non-oxygenated β -ionone ring and having an isoprenoid (2-methyl-1,3-butadiene) side chain terminating in an alcohol, aldehyde, or carboxyl functional group (Gregory, 1996). Nutritionally active sources of vitamin A are generally classified as derivatives of either retinoids or carotenoids. Although there are many nutritionally active isomers of retinoids and carotenoids, the all-*trans* isomers of both compounds exhibit the greatest vitamin A activity and are the predominant naturally occurring forms (Gregory, 1996).

Retinoids

The term retinoid refers to a class of compounds, including retinol and its derivatives having four isoprenoid subunits. Many different isomers of retinol, retinal, and retinoic acid exist as a result of either *cis* or *trans* configurations of the four double bonds in the polyene chain (Semba, 1999). In addition, synthetic all-*trans* retinyl acetate and all-*trans* retinyl palmitate are widely used to fortify human and animal diets.

Animal-derived vitamin A is usually ingested in precursor form as retinyl esters. Milk fat (butter), liver, and eggs are excellent sources of animal-derived vitamin A. Hydrolysis of these esters in the small intestine yields the biologically active retinol. Some animal-derived retinyl esters are hydrolyzed to retinal, which can be reversibly reduced to retinol or it can be irreversibly oxidized to produce retinoic acid (Gregory, 1996). The 11-*cis*-retinal isomer is important because it is the chromophore of rhodopsin, the vertebrate photosensor molecule. The process of vision relies on the light-induced isomerisation of the chromophore from 11-*cis* to all-*trans* retinal, which results in a conformational change and activation of the photoreceptor (Semba, 1999).

Carotenoids

Carotenoids, sometimes known as provitamin A, contribute significantly to the vitamin A activity of both plant and animal-derived foods. Of the approximately 600 known carotenoids, approximately 50 have some provitamin A activity in vivo (Gregory, 1996). Because carotenoids are plant pigments that reflect a variety of colors, many fruits and vegetables with concentrated or dark colors are excellent sources of provitamin A. Sweet potatoes, carrots,

tomatoes, apricots, and plums are a few examples. Structurally, carotenes resemble 2 retinoid molecules joined as mirror images.

Carotenes do not have vitamin A activity until they undergo cleavage of the C15 – C15' bond in the intestinal mucosa via two oxidative enzymatic steps (Gregory, 1996). This cleavage potentially yields two molecules of retinol. Conversion of β -carotene to active retinol is an inefficient process, but among the carotenoids, β -carotene has the greatest vitamin A activity. Although two molecules of active vitamin A are potentially produced from each molecule of β -carotene consumed, the hydrolysis generally produces only one active vitamin A molecule, making the conversion of β -carotene only 50% efficient when compared to the activity of retinol by mass (Gregory, 1996).

Quantifying Vitamin A

Measures of vitamin A are expressed in different ways and these measures must be carefully interpreted because the bioavailability depends upon the dietary source. For livestock, vitamin A requirements are traditionally referred to in international units (IU), which is a measure of the biological activity of a compound (NRC, 1996). This system allows for accurate comparison among the various forms of retinoids and carotenoids that may be ingested through grazing and/or supplemented in a diet. In 1974, the United States adopted the “retinol equivalent” to quantify vitamin A activity relative to retinol, the most biologically active form of vitamin A. With this system, 1 RE = 1 μg of all-*trans* retinol (Table 1-1).

Table 1-1. Conversions for various measures of vitamin A activity for cattle

International Units (IU)	Retinol Equivalents (RE) mass of all- <i>trans</i> retinol (μg)	β -carotene for cattle (μg)
3.33	1	8.33
400	120	1000
1	0.3	2.5

Consequently, regular use of mass equivalents (either mg or μg) of retinol became acceptable and allowed for easier measurement of vitamin supplements when mixing rations. For cattle, one IU of vitamin A corresponds to 0.3 μg (RE) of all-*trans* retinol (0.344 μg of all-*trans* retinyl acetate or 0.550 μg of all-*trans* retinyl palmitate), and to 2.5 μg of β -carotene (NRC,

2001). Most requirements for livestock continue to be reported in either IU or mass equivalents of retinol.

Vitamin A Solubilization and Digestion

Intestinal Enzymes

Retinyl esters, which were the source of vitamin A used in my dissertation research, must be hydrolyzed prior to intestinal absorption. Hydrolysis of these esters is catalyzed by lipases, located in the membranes of intestinal enterocytes. Retinyl ester hydrolase has been characterized extensively and is considered the primary enzyme endogenous to the small intestine that has retinyl ester cleaving properties (Rigtrup et al., 1994).

Pancreatic Enzymes

Recent data from studies of rats and mice suggest that pancreatic triglyceride lipase (PTL) is the enzyme primarily responsible for retinyl ester hydrolysis in the intestine. Rigtrup et al. (1994) ligated the common pancreatic ducts of rats for 48 h to prevent secretion of pancreatic enzymes into the intestinal lumen before obtaining brush border membranes from enterocytes comprising the villi of the small intestine.

In the absence of pancreatic enzymes, there was a marked decrease in the hydrolysis of short-chain retinyl esters and a 30% decrease in hydrolysis of long-chain retinyl esters. The authors concluded that hydrolysis of short-chain retinyl esters was likely carried out by the pancreatic lipases and that the majority (70%) of long-chain hydrolysis must be inherent to the brush border membranes of the enterocytes (i.e., retinyl ester hydrolase).

Cellular Uptake of Vitamin A

Defining the exact mechanisms of uptake of retinol by intestinal cells is difficult and complex because several mechanisms may be involved in a single cell. Harrison (2005) suggested that the general perception that retinol is efficiently absorbed and quantitatively transported may need reevaluation. Recovery of ingested retinol into lymph varies between 20 and 60 % in humans (Goodman et al., 1966) and rats (Huang and Goodman, 1965). Hollander (1980) demonstrated that approximately 60 and 30 % of absorbed retinol is secreted into lymph and portal circulation, respectively. Much of the ingested retinol is secreted into lymph in esterified form, but considerable amounts of free retinol are likely secreted into the portal

circulation. Studies of intestinal absorption of retinol by intestinal cells of cattle are not available, and published work in this specific area has been completed with the human intestinal cell line.

Intracellular and Intercellular Transport of Vitamin A

Two cellular retinol-binding proteins (CRBP) have been purified and extensively characterized (Harrison, 2005). After absorption by enterocytes, free retinol is likely sequestered by CRBP(II), which is one of the most abundant proteins expressed in the absorptive cells of the small intestine (Harrison, 2005). Harrison (2005) reported that CRBP(II) accounts for approximately 1 % of the total soluble proteins in the jejuna mucosa. Cell culture studies have demonstrated that CRBP(II) is important for shuttling retinoids to various intracellular enzymes to modulate their metabolism. Harrison (2005) speculated that CRBP(II) can bind to specific transporters on the brush border membrane and permit facilitated diffusion. Interestingly, levels of CRBP(II) mRNA are increased in the small intestine of vitamin A-deficient rats (Rajan et al., 1992). Treatment of human-derived Caco-2 cells with retinoic acid results in a two-to-three-fold increase in CRBP(II) mRNA expression as well as increased absorption and intracellular radiolabeled retinol (Lissoos, et al., 1995).

Incorporation of Vitamin A into Chylomicrons

Studies in humans and rats have consistently indicated that once retinol is incorporated by enterocytes, it is largely reesterified with long-chain fatty acids, incorporated into chylomicrons, and secreted into the lymph system with other dietary lipids (Harrison, 2005). Levin (1993) demonstrated that Caco-2 cells derived from the human intestine secreted only free retinol when cell were not supplemented with fatty acids. Retinol is primarily secreted into the lymph as retinyl palmitate that is present in small chylomicrons and is found in lesser amounts in large chylomicrons and very-low-density lipoproteins (Lemieux et al., 1998). Unlike many other lipids, retinyl esters are not associated with either high- or low-density lipoproteins, suggesting they have chemical attributes somewhat different from other neutral lipids. Secretion of retinyl esters with chylomicrons is independent of the rate of retinol uptake, and independent of the intracellular levels of free and esterified retinol (Harrison, 2005). Rather, secretion of retinyl esters is dependent on the formation and secretion of chylomicrons through the villi of the small intestine.

Inhibition of Adipocyte Differentiation by Retinoic Acid

Kawada et al. (1996) reported that RXR can be activated by binding with PPAR γ , RAR, thyroid hormone receptor, vitamin D receptor, liver X receptor, and fatty acid-activated receptor. Each of these ligands possesses unique affinity for RXR. Intuitively, when two or more RXR ligands are in the vicinity of RXR, the ligand with the highest binding affinity forms the dimer with RXR. It has been suggested that RAR in the presence of RXR binds to RXR with high affinity, and inhibits PPAR γ binding to the extent that adipocyte development is suppressed.

Ohyama et al. (1998) cultured stromal vascular cells collected from the peri-renal tissue of 21-mo old Japanese Black steers. The cell culture differentiation media contained 0, 0.4, 4.0, or 40 $\mu\text{g} \cdot 100 \text{ ml}^{-1}$ retinol, which was presumed to convert to retinoic acid, and thiazolidinedione, a specific ligand that promotes adipogenesis by stimulating PPAR γ . Glycerol-3-phosphate dehydrogenase (GPDH) activity was measured in this and several other studies as a marker of adipocyte differentiation because GPDH is expressed in terminally-differentiated adipocytes, but is not detected in undifferentiated stromal-vascular cells.

Addition of the lowest concentration of retinol reduced GPDH activity to levels as low as the controls, and decreased the number of lipid-laden cells in a dose-dependant manner. This suggests that low concentrations of retinol virtually inhibit the ability of thiazolidinedione to stimulate adipogenic gene expression in cultured cells.

Figure 1.2. Photomicrographs of bovine preadipocytes cultured under different concentrations of retinol. (A) Without retinol, (B) 0.4 $\mu\text{g}/100\text{ ml}$ retinol, (C) 4 $\mu\text{g}/100\text{ ml}$ retinol, and (D) 40 $\mu\text{g}/100\text{ ml}$ retinol (Ohyama et al., 1998).

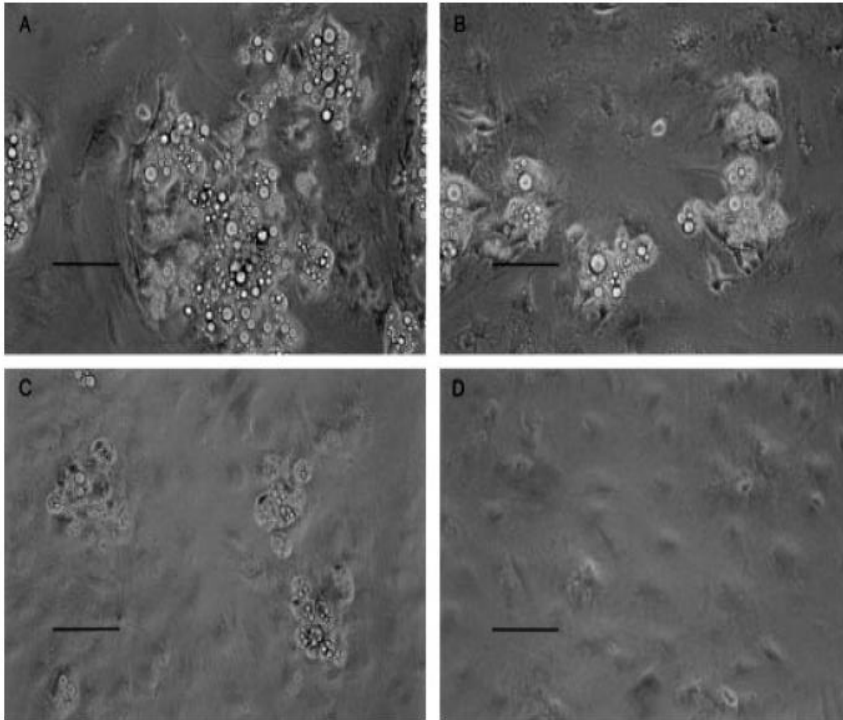
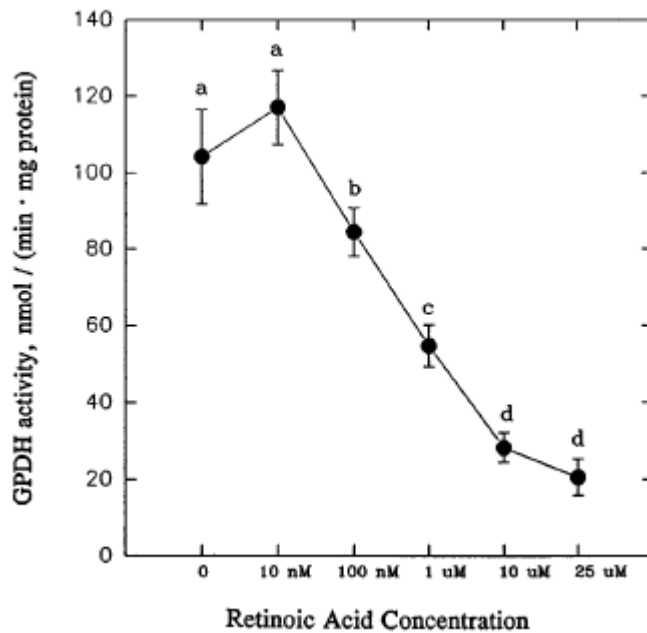


Figure 1-2 illustrates the dose-dependant response of bovine preadipocytes when cultured in different retinol concentrations.

Suryawan and Hu (1997) examined the effects of retinoic acid on preadipocyte differentiation in primary culture of stromal-vascular cells removed from crossbred newborn pigs. An inhibitory effect on GPDH activity was observed as concentrations of retinoic acid were increased in cultures. This suppressive effect of retinoic acid was also confirmed by oil red O staining that showed a reduction in the number of cells accumulating lipid.

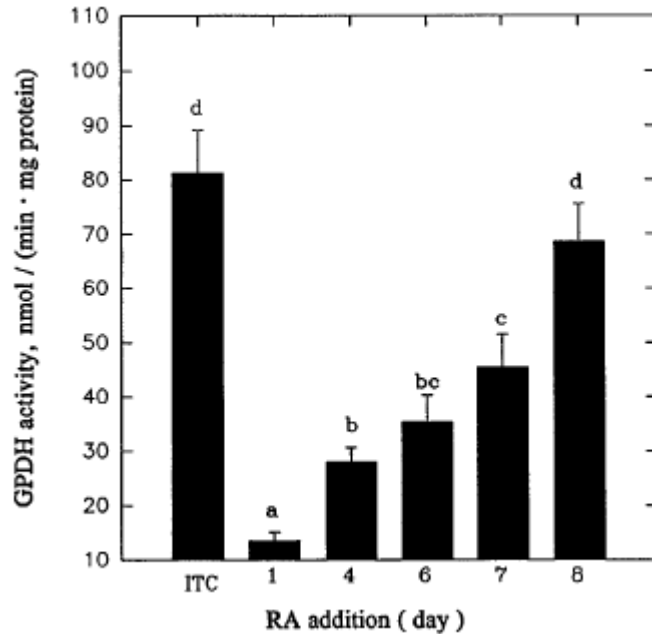
Because of the evidence presented by Safonava et al. (1994) that retinoic acid acted as an adipogenic promoter at physiological concentrations (i.e., 1 to 10 nM), Suryawan and Hu (1997) also examined the effect of physiological concentrations of retinoic acid on cultured porcine preadipocytes. They reported no concentration-dependant differences in preadipocyte differentiation and proposed that the results may indicate species differences in sensitivity to retinoids.

Figure 1.3 Effect of pharmacological doses of retinoic acid on glycerol-3-phosphate dehydrogenase activity of porcine preadipocytes in primary culture (Suryawan and Hu, 1997).



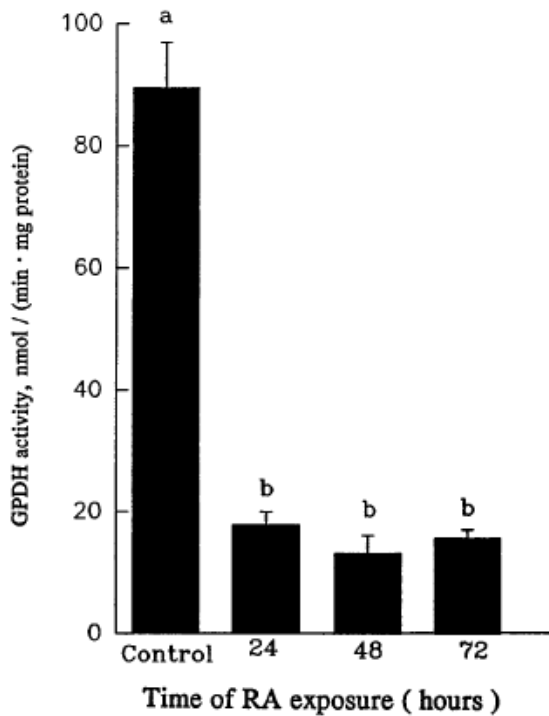
The inhibitory effect of retinoic acid on preadipocyte differentiation was time dependant. Exposure of cultures to retinoic acid early in the culture period was much more effective at inhibiting fat cell differentiation than when added several days into the culture period (Figure 1-3); withdraw of retinoic acid from the culture did not reverse the inhibitory effect on cell differentiation.

Figure 1.4 Effect of retinoic acid administration at different times on glycerol-3-phosphate dehydrogenase activity of porcine preadipocytes in primary culture (Suryawan and Hu, 1997).



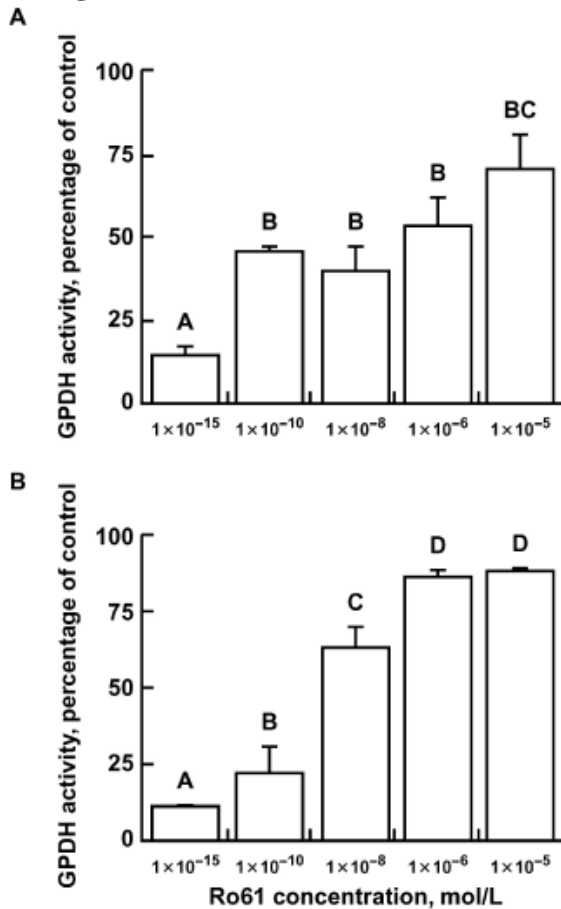
Furthermore, figure 1-4 shows that the maximum inhibitory effect of retinoic acid can be attained with only 24 h in culture and that exposure of preadipocytes to retinoic acid for either 48 or 72 h did not further suppress GPDH activity. This solidifies the hypothesis that retinoic acid interferes with fat cell growth during early stages of preadipocyte differentiation in culture.

Figure 1.5. Effect of exposure time of retinoic acid on glycerol-3-phosphate dehydrogenase activity of porcine preadipocytes in primary culture (Suryawan and Hu, 1997).



Working in the same lab, Brandebourg and Hu (2005) examined the effect of retinoids on the expression of known adipogenic transcription factors such as *C/EBP β* , *PPAR γ* , and *C/EBP α* . Porcine preadipocytes were harvested and cultured as described in Suryawan and Hu (1997). Cultures were continuously treated from d 0 to 10 with 0 to 10 μ M all-*trans*-retinoic acid (a nonspecific agonist for RAR and RXR), 10 nM to 10 μ M 9-*cis*-retinoic acid, 10 nM to 10 μ M methoprene acid (RXR-selective agonist), or 10 pM to 10 μ M 4-(E-2-[5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl]-1-propenyl) benzoic acid (TTNPB) (RAR-selective agonist). Treating the cultured stromal vascular cells with either all-*trans*-retinoic acid or 9-*cis*-retinoic acid decreased ($P < 0.01$) preadipocyte differentiation in an inverse concentration-dependant manner (Figure 1-14).

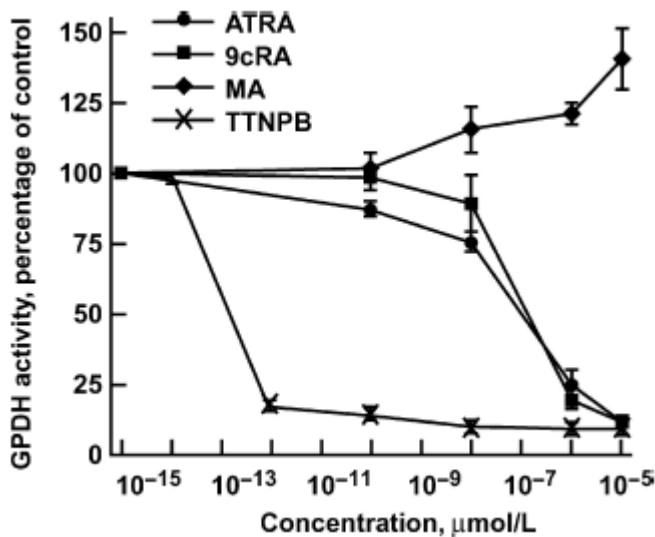
Figure 1.6. The effect of increasing doses of retinoids on glycerol-3-phosphate dehydrogenase activity in primary cultures of differentiating porcine preadipocytes at d 10 (Brandebourg and Hu, 2005).



Addition of only 100 pM TTNPB to cell cultures effectively decreased ($P < 0.001$) GPDH activity, whereas methoprene acid increased GPDH activity (Figure 1-6). These data suggest that RXR may not be involved in regulating adipogenesis because the RXR agonist, methoprene *acid* increased GPDH activity. Further evidence of the involvement of the RAR system in the suppression of adipogenesis was presented when the addition of Ro-61, a potent RAR-specific antagonist, prevented all-trans-retinoic acid and TTNPB from inhibiting adipocyte differentiation in a dose-dependant manner (Figure 1-7). This demonstrated that when RAR receptors were down-regulated and unable to form heterodimers with the retinoid agonists, preadipocyte cell differentiation was allowed to proceed without inhibition. The research

conducted by Brandebourg and Hu (2005) demonstrated the specific involvement of RAR in suppressing adipocyte development. While the use of TTNPB was highly effective in generating the hypothesized response, it should be questioned whether this is an accurate representation of the *in vivo* response. It is more likely that all-*trans*-retinoic acid and 9-*cis*-retinoic acid (i.e., the endogenous ligands) bind to receptors in a competitive environment with many paracrine factors affecting the affinity of retinoids to RAR and RXR.

Figure 1.7. Effect of retinoic acid receptor agonist, Ro61, on the ability of all-*trans*-retinoic acid and 4-(E-2-[5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl]-1-propenyl) benzoic acid to inhibit glycerol-3-phosphate dehydrogenase activity in primary cultures of differentiating porcine preadipocytes at d 10 (Brandebouorg an Hu, 2005).



These effects have been reported in other species. Decreased GPDH expression and suppressed adipogenesis has been associated with addition of retinol to ovine preadipocytes (Torii et al., 1996). Studies involving murine 3T3-L1 cell lines have demonstrated that all-*trans*-retinoic acid inhibits adipogenesis by down-regulating the genes expressing PPAR γ and C/EBP α . Xue et al. (1996) reported a decrease in PPAR γ mRNA and protein when mouse 3T3-L1 cells were treated with retinoic acid.

The suppressive effects of retinoids on adipocyte differentiation of cultured cells from multiple species provides compelling evidence that livestock diets containing high levels of vitamin A activity may be inhibiting adipose tissue development. The consequences of this could be beneficial for reducing excess fat and increasing retail yields of leaner beef, pork, and lamb. Intramuscular fat increases juiciness and flavor (Dolezal et al., 1982) and, in the United

States, Australia, and Asia, the amount of intramuscular fat is an important criterion used to determine beef quality grade and prices paid by processors to producers.

Vitamin A Biology in Cattle

Dietary Requirement

Dietary requirements for livestock are reported in the National Research Council's Handbook of Dietary Requirements (1996). They are species specific and are usually indicated for specific growth or production stages of the animals being supplemented. For beef feedlot cattle, the vitamin A requirement is 2,200 IU·kg⁻¹ of dry feed (NRC, 1996). This is the same value reported in the 1984 NRC and these values were determined from research published between 1935 and 1972. No recent research has been conducted to re-evaluate or update the vitamin A requirements for cattle.

Vitamin A Supplementation in U.S. Feedlots

Feeding little or no supplemental vitamin A in attempt to increase marbling is in distinct contrast to common practice in U.S. beef production. In a survey of feedlot nutritionists, most recommended levels of vitamin A supplementation far exceed NRC requirements. The average recommendation was 8,053 IU/kg DM for receiving diets and 4,554 IU/kg DM for finishing diets (Galyean and Gleghorn, 2002), with ranges from 3,520 to 15,400 IU/kg DM for receiving diets and 3,300 to 7,260 IU/kg for finishing diets. Because vitamin A fortification of cattle diets is an inexpensive and easy method for improving immune response, particularly in receiving cattle, it is likely that few have considered the potential negative consequences of such levels on marbling and quality grade.

In a review of the effects of vitamins A and D on marbling deposition, Pyatt and Berger (2005) noted the relationship between the seasonality of carcasses that grade USDA Choice and the intake of vitamin A. Cattle placed in feedlots in late spring and early summer are typically harvested in late summer or early fall, when quality grades are the lowest (Anderson and Gleghorn, 2007). Pyatt and Berger (2005) pointed out that these cattle are commonly backgrounded on lush pastures, including wheat pastures, that contain 100,000 to 300,000 IU/kg of vitamin A activity derived from the high levels of carotene (NRC, 1996). Because vitamin A is stored primarily in liver, it may take several months for circulating levels of vitamin A to be

depleted, even if vitamin A intake is quite low. This is supported by Oka et al. (1998), who reported a strong relationship ($r = .77$) between serum and liver vitamin A concentrations in cattle. In support of this theory, more heifers fed through the winter months graded USDA Prime and had higher marbling scores than heifers fed during the summer months (Kreikemeier and Mader, 2004). Similarly, Pusillo et al. (1991) observed lower quality grades in yearling steers fed from May to October than yearling steers placed on feed for 140 to 180 d in November, January, March, or September. While heat stress and reduced feed intake have been shown to reduce quality grade in summer-fed cattle, the suppressive effects of high vitamin A on marbling, either before or during the finishing period, is a real possibility for suppressing marbling deposition.

Tissue Storage of Vitamin A in Cattle

In cattle, 70 to 90 % of total vitamin A is stored in the liver (Sewell, 1993). Carotenes that escape conversion to retinyl esters are also stored in the liver. The remainder of carotenoids and retinoids are stored in fat and other fat-containing organs. Bodily storage of vitamin A is low at birth and in young animals. Riggs (1940) found a negative correlation between age and the number of days required to cause blindness when calves ranging from 3 to 16 mo of age were fed vitamin A deficient diets. Sewell (1993) reported that no appreciable storage of vitamin A takes place in the liver until the dietary intake of vitamin A becomes 3 to 5 times (5 to 10 times for carotene) higher than the basal requirement. Although the liver can prevent vitamin A deficiency for several months in older cattle, liver stores are highly variable and cannot be accurately assessed in live animals without biopsy samples (NRC, 1996).

Seasonality of Quality Grade in the United States

Seasonal variation in the percentage of beef carcasses grading USDA Choice or higher has been documented for decades. Reduction in feed intake during hot summer months has been implicated in the reduction of marbling that follows when cattle are harvested in late summer and early fall (Pusillo et al., 1991; Kreikemeier et al., 1998; Anderson et al., 2007). Pyatt and Berger (2005) suggested that cattle fed through the summer months and harvested in late summer and early fall represent a population of cattle that likely consumed extremely high levels of dietary vitamin A (via plant β -carotene). Most cattle harvested during the months of lowest USDA quality grades coincide to time spent in lush pastures consuming high levels of β -carotene during

the months prior to entering the feedlot. Pyatt and Berger (2005) pointed out that cattle fed through the summer and harvested in autumn months are commonly backgrounded on lush pastures, including wheat pastures, that contain 100,000 to 300,000 IU/kg of vitamin A activity derived from the high levels of carotene (NRC, 1989). Because vitamin A is stored primarily in liver, it may take several months for circulating levels of vitamin A to be depleted, even if vitamin A intake is quite low. This is supported by Oka et al. (1998), who reported a strong relationship ($r = .77$) between serum and liver vitamin A concentrations in cattle. Conversely, cattle harvested during intense grazing months likely consumed much lower levels of vitamin A during their growing phase. Kreikemeier and Mader (2004) reported a greater percentage of carcasses grading USDA Prime in winter-fed heifers compared to summer-fed heifers. Because depletion of vitamin A requires at least 100 days in cattle, pre-feedyard consumption of lush forage combined with heat stress presents a worst-case scenario for marbling development during the summer months. The effects of dietary vitamin A level on carcass traits are reviewed extensively below.

Cattle Feeding in Asia

In Asian countries, such as Korea and Japan, beef with a high degree of marbling is desirable because of its palatability attributes. Production of these highly-marbled carcasses involves the use of breeds with high genetic potential for marbling, such as Wagyu, Hanwoo, Angus, and crosses of these, harvested between 30 and 34 mo of age (Oka et al., 1998). Because of the climate, infrastructure, and other historical differences, these cattle are fed diets that are different from typical US diets for feedlot cattle. One of the most important considerations for beef producers in these countries to enhance marbling is to assure a low intake of vitamin A. Common feed ingredients include rice straw, Italian ryegrass hay, and timothy hay, which may contribute up to 20 % of the diet (Adachi et al., 1999). Barley, flaked corn, wheat bran, rice bran, soybean meal, and soybean hulls are the typical grain products that are fed. Interestingly, all of these ingredients are extremely low in carotene and vitamin A content (NRC, 1996).

Oka et al. (1998) fed 15 mo old Waygu steers ($n=57$) diets low in carotene and vitamin A and created a divergence in vitamin A status by injecting half of the steers with 303 mg of retinol i.m. every 60 d until slaughter at 30 months. Serum retinol concentrations at slaughter ranged from 3.8 to 39.7 $\mu\text{g dl}^{-1}$ and marbling scores ranged from 3 to 11 (Japanese scale of 1 to 12).

From this study, Oka et al. (1998) reported a significant negative correlation ($r = -0.38$, $P < 0.05$) between serum retinol concentrations and carcass marbling scores. They published a regression equation ($Y = 29.9 - 1.7X$; where Y is serum retinol concentration and X is marbling score) that continues to be frequently cited by animal scientists who are interested in methods of increasing marbling. By contrast to the reports in cattle, a positive correlation between marbling and circulating vitamin A was established in market lambs in our lab at Kansas State University (Arnett et al., 2007).

Adachi et al. (1999) obtained 13 Japanese Black steers from 6 different farms and finished them by Japanese standards until harvest at approximately 28 mo of age. Blood was sampled at 12, 17, 22, and 28 mo of age. Sixteen different blood parameters, including retinol, were analyzed to detect any correlations between carcass marbling scores and blood values. After marbling was evaluated by the Japanese Meat Grading Service, carcasses were classified as having either high (ranged 8 to 11) or low (ranged 4 to 5) marbling scores. Although not statistically significant, steers with high marbling scores had lower serum retinol immediately before harvest than steers with low marbling scores (31 ± 13 vs. 54 ± 19 IU/dL).

In an Australian study, Kruk et al. (2004) fed Angus steers ($n = 20$) with high Estimated Breeding Values for marbling, a standard feedlot ration for 10 mo. The basal diet consisted of 75 % grain and was determined to contain only trace amounts of vitamin A and β -carotene. Half of the steers were supplemented with 60,000 IU retinyl palmitate/100 kg/d while half received no supplemental vitamin A. After 100 d on the feedlot rations, serum retinol levels diverged to significance and remained different for the balance of the feeding period. Although no marbling scores were significantly different ($P > 0.05$) using either the Meat Standards Australia or USDA marbling standards, ether extractable lipid was 35% higher ($P < 0.05$) in the LM from cattle fed no supplemental vitamin A (13.0 vs. 9.6 % IMF for no and high supplemental vitamin A diets, respectively). No treatment differences ($P > 0.05$) in marbling and % IMF were observed for the *M. semitendinosus* in this study.

Vitamin A Studies in the U.S. Beef Industry

The effects of dietary vitamin A level on early-weaned, feedlot backgrounded heifers ($n = 48$) and steers ($n = 144$), and *traditionally-weaned*, pasture backgrounded yearling steers ($n = 42$)

were studied by Pyatt et al. (2005). Cattle were fed either 2,300 (1X NRC) or 7,250 (3.3X NRC) IU vitamin A / kg DM during the feedlot period. Heifers were early-weaned at 51 d of age, steers were early-weaned at 70 d of age, and traditional-weaned steers were weaned at 191 d of age. Early-weaned cattle were harvested when 12th rib fat thickness averaged 1.06 cm as determined by ultrasound. This was attained with 163 and 140 d of finishing for heifers and steers, respectively. Yearling steers were harvested after 105 d of finishing, regardless of backfat thickness. For all 3 experiments, carcass marbling scores and USDA Quality Grades were numerically in favor of cattle fed low levels of vitamin A, although no statistical differences were observed (Table 1-2). Interestingly, serum retinol became different between the low and high vitamin A groups by the end of the finishing period in both early-weaned experiments, but not in the yearling steer trial. Three factors likely contributed to this discrepancy. Obviously, the yearling steers were given fewer days in the feedlot to consume either the high or low vitamin A diets. However, the yearling cattle had grazed carotene-containing forages for several months prior to entering the feedlot. Consequently, they likely had much higher tissue stores of vitamin A than the early-weaned cattle at the initiation of the finishing period. Thirdly, because the yearling steers were older than the early-weaned cattle at the initiation of the feedlot finishing phase, they likely had accumulated more vitamin A stored in the liver simply as a function of age and maturity. For instance, vitamin A deficiency is most common in cattle less than 1 yr of age because younger animals are growing rapidly and utilizing more nutrients for growth compared to a metabolism of nutrient storage by older cattle that are physiologically more mature.

Table 1-2. Summary of serum retinol and carcass traits compiled from experiments with 3 classes of cattle fed either 2,300 or 7,250 IU vitamin A per kg DM during the feedlot period (Pyatt et al., 2005).

Carcass Trait	Treatment	Low Vitamin A	High Vitamin A	SEN	P
Final Serum retinol, ng/ml	Early-weaned	189.5	277.7	16.0	<0.01
	Early-weaned Steer	267.5	322.2	7.5	<0.001
	Yearling Steer	346.9	355.4	13.2	0.65
Marbling Score	Early-weaned	555	526	21.4	0.18
	Early-weaned Steer	449	449	7.8	0.96
	Yearling Steer	460	453	14.1	0.97
≥ Avg. Choice, %	Early-weaned Heifer	70.8	52.2	NA	0.16
	Early-weaned Steer	23.8	38.1	NA	0.32
	Yearling Steer	23.8	38.1	NA	0.32
Yield Grade	Early-weaned Heifer	2.6	2.5	0.1	0.87
	Early-weaned Steer	2.1	2.3	0.1	0.03
	Yearling Steer	2.7	2.7	0.1	0.72

Gorocica-Buenfil et al. (2007a) investigated the effects of dietary vitamin A level in the feedlot diets of Angus cross steers (n = 168) on beef carcass characteristics. Steers were backgrounded in the feedlot and fed to gain 1.1 kg / d for 84 d, then switched to a finishing program of an additional 84 d. The vitamin A treatments were either no supplemental vitamin A or 2,700 IU vitamin A/kg DM; the basal diet contained < 1,300 IU vitamin A / kg DM. Serum retinol at harvest was 44 % lower ($P < 0.01$) from steers fed no supplemental vitamin A (23.0 vs. 41.1 µg/dL). Quality grade tended to be higher ($P = 0.07$) in carcasses from steers fed no supplemental vitamin A. Marbling score and percentage of carcasses grading Choice or higher were 10% higher in the carcasses from steers receiving no supplemental vitamin A, although not significant ($P = 0.11$ and 0.13, respectively). Twelfth rib fat thickness and USDA yield grades were not different between treatments ($P > 0.20$). Although feeding no supplemental vitamin A in this study tended to increase marbling scores and USDA quality grades, the magnitude of the

effect was minimal and agrees with the report by Pyatt et al. (2005). There are many similarities between the designs, materials, and methods of these two studies. Interestingly, Gorocica-Buenfil et al. (2007a) used a treatment with no supplemental vitamin A, whereas Pyatt et al. (2005) used 2,300 IU vitamin A /kg DM as the “low” vitamin A treatment. Based upon the results of these two studies, supplementing with either no vitamin A or the NRC recommended level (1X) of vitamin A does not significantly increase carcass marbling scores and quality grades in Angus cross cattle fed in Midwestern feedlots. However, the trend for increased marbling and percentage of carcasses grading \geq USDA Choice⁺ suggests some efficacy to this feeding strategy. Longer periods of vitamin A depletion may be needed in order to realize a more drastic effect on marbling deposition.

Gorocica-Buenfil et al. (2007b) investigated the effects of the duration of vitamin A restriction on marbling development in Holstein steers ($n = 60$). All steers received an adaption diet containing 2,700 IU vitamin A/kg DM for the first 45 d in the feedlot. Thereafter, steers were randomly allotted to one of three treatments: 2,200 IU vitamin A/kg DM for 243 d (C); 2,200 IU vitamin A/kg DM for 112 d, then no supplemental vitamin A for an additional 131 d (short restricted); or no supplemental vitamin A / kg DM for 243 d of feeding (long restricted). The basal diet was calculated to contain 950 IU vitamin A/kg DM. Marbling scores were not affected by vitamin A treatment ($P = 0.36$), although a numerical advantage was observed for the percentage of carcasses grading USDA Choice⁰ or higher (28% in control to 50% in the long restricted). The authors offered no explanation for why the carcasses from short restricted steers had the lowest percentage of premium Choice and Prime quality grades. Percentage of ether extractable lipid from the LM was 33 % higher ($P < 0.05$) from long restricted steers than short restricted and controls (5.6 vs. 3.9 and 4.2 % ether extract, respectively). The authors concluded that restricting vitamin A intake for 131 d or less was not sufficient to improve marbling development in Holstein steers. However, restricting vitamin A intake for up to 243 d of feeding increased intramuscular fat deposition in the LM. Therefore, feeding low vitamin A diets may be an economically feasible strategy for affecting fat deposition in a site-specific manner.

In summary, the positive effects of depleting market cattle of vitamin A may be most apparent in populations of cattle with high genetic merit for marbling that can be depleted of vitamin A stores for extended periods (i.e., >150 d). Feed efficiency, average daily gain, general health and vision, and carcass cutability were not affected by feeding diets containing either no

or low levels of vitamin A. Plus, these reports suggest that the requirement for vitamin A in feedlot cattle may need to be re-evaluated since the NRC recommendation cites research published over 3 decades ago (Perry et al., 1965; Eaton et al., 1972) when modern techniques to analyze serum and liver vitamin A content (i.e., HPLC) were not available.

LITERATURE CITED

- Aberle, E.D., J.C. Forrest, D.E. Gerrard, and E.W. Mills. 2001. Principles of Meat Science. Fourth Ed. Kendall/Hunt Publishing Co., Dubuque, IA.
- Adachi, K., H. Kawano, K. Tsuno, Y. Nomura, N. Yamamoto, 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(8): 961-964.
- Albrecht, E., Teuscher, F., Ender, K., and J. Wenger. 2006. Growth- and breed-related changes of marbling characteristics in cattle. *J. Anim. Sci.* 84:1067-1075.
- Allen, C.E., D.C. Beitz, D.A. Cramer and R.G. Kauffman. 1976. Biology of fat in meat animals. North Central Regional Research Publication No. 234. Research Division, College of Agriculture and Life Sciences, University of Wisconsin-Madison.
- Anderson, P. and J. Gleghorn. 2007. Non-genetic factors that affect quality grade of fed cattle. Proc. of the 39th Beef Improvement Federation, Ft. Collins, CO, Speidel *Ed.* pp.31-43.
- Arnett, A.M., M.E. Dikeman, B.J. Johnson, C.W. Spaeth, and B. Hildabrand. 2007. Effects of vitamin A supplementation in young lambs on performance, serum lipid, and longissimus muscle lipid composition. *J. Anim. Sci.*
- Baker, M.J., Tedeschi, L.O., D.G. Fox, W.R. Henning, and D.J. Ketchen. 2006. Using ultrasound measurements to predict body composition of yearling bulls. *J. Anim. Sci.* 2666-2672.
- Batten, M.L., Y. Imanishi, T. Maeda, D.C. Tu, A.R. Moise et al. 2004. Lecithin-retinol acyltransferase is essential for accumulation of all-trans-retinyl esters in the eye and in the liver. *J. Biol. Chem.* 279:10422-10432.
- Berger, L.L., and D.B. Faulkner. 2003. Lifetime nutritional effects on feedlot performance and carcass merit. Proc. 2003 Plains Nutrition Council Spring Conference., San Antonio. Publication No. AREC0.-13 Texas A&M Research and Extension Center, Amarillo. pp. 63-78.
- Brandebourg, T.D. and C.Y. Hu. 2005. Regulation of differentiating pig preadipocytes by retinoic acid. *J. Anim. Sci.* 83:98-107.
- Burson, D.E., M.C. Hunt, D.M. Allen, C.L. Kastner, and D.H. Kropf. 1980. Diet energy density and time on feed effects on beef longissimus muscle palatability. *J. Anim. Sci.* 51:875-881.

- Campbell, N.A. 1996. Biology, 4th Rev. Edition. The Benjamin / Cummings Publishing Company, Manlo Park, CA p. 306 Figure 16.8.
- Chawla, A., E.J. Schwarz, E.E. Dimaculangan, and M.A. Lazar. 1994. Peroxisome proliferator-activated receptor (PPAR) γ : adipose-predominant expression and induction early in adipocyte differentiation. *Endocrinol.* 135:798-800.
- Cornelius, P., O.A. MacDonald, and M.D.lane. 1994. Regulation of adipocyte development. *Ann. Rev. Nutr.* 14:99-129.
- Cossu, G. and S. Biressi. 2005. Satellite cells, myoblasts and other occasional myogenic progenitors: possible origin, phenotypic features and role in muscle regeneration. *Seminars in Cell & Developmental Biology.* 16:623-631.
- Dolezal, H.G., G.C. Smith, J.W. Savellm and G.L. Carpenter. 1982. Effect of time on feed on the palatability of rib steaks from steers and heifers. *J. Food Sci.* 47:368-374.
- Duckett, S.K., D.G. Wagner, L.D. Yates, H.G. Dolezal, and S.G. May. 1993. Effects of time on feed on beef nutrient composition. *J. Anim. Sci.* 71:2079-2088.
- DuPont, J. and D. LeRoith. 2001. Insulin and insulin-like growth factor 1 receptors: similarities and differences in signal transduction. *Horm. Res.* 55(Suppl 2):22-26.
- Erlanson, C. and B. Borgström. 1968. The identity of vitamin A esterase activity of rat pancreatic juice. *Biochem. Biophys. Acta.* 167:629-631.
- Forman, B.M., P. Tontonoz, J. Chen, R.P. Brun, B.M. Spiegelman, and R.M. Evans. 1995. 15-Deoxy-delta 12, 14-prostaglandin J2 is a ligand for the adipocyte determination factor PPAR gamma. *Cell* 83:803-812.
- Freytag, S.O. and T.J. Geddes. 1992. Reciprocal regulation of adipogenesis by myc and C/EBP α . *Science* 256:379-382.
- Gaylean, M.L. and J.F. Gleghorn. 2002. Summary of the 2000 Texas Tech University consulting nutritionist survey. In: *Proc. Plain Nutr. Council Conf.* San Antonio, TX Texas A&M Research & Extension Center, Amarillo.
- Gerrard, D.E. and A.L. Grant. 2003. *Principles of Animal Growth and Development.* Kendall/Hunt Publishing Company, Dubuque, IA, pp. 119-124.
- Goodman, D.S., R. Blomstrand, B. Werner, H.S. Huang, and T. Shiratori. 1966. The intestinal absorption and metabolism of vitamin A in man. *J. Clin. Invest.* 45:1615-1623.
- Gorocica-Buenfil, M.A., F.L. Fluharty, C.K. Reynolds and S.C. Loerch. 2007a. Effect of dietary vitamin A concentration and roasted soybean meal inclusion on marbling, adipose cellularity, and fatty acid composition of beef. *J. Anim. Sci.* 85:2230-2242.

- Gorocica-Buenfil, M.A., F.L. Fluharty, C.K. Reynolds and S.C. Loerch. 2007b. Effect of dietary vitamin A restriction on marbling and conjugated linoleic acid content in Holstein steers. *J. Anim. Sci.* 85:1235-1238.
- Gregory, J.F. 1996. Vitamins. In: *Food Chemistry*, 3rd Ed. Fennema, Ed. Published by Marcel Dekker, Inc., New York, NY pp. 531-552.
- Guenther, J.J., D.H. Bushman, L.S. Pope and R.D. Morrison. 1965. Growth and development of the major carcass tissues in beef calves from weaning to slaughter weight, with reference to the effect of plane of nutrition. *J. Anim. Sci.* 24:1184-1189.
- Harrison, A.R., M.E. Smith, D.M. Allen, M.C. Hunt, C.L. Kastner, and D.H. Kropf. 1978. Nutritional regimen effects on quality and yield characteristics of beef. *J. Anim. Sci.* 47:383-388.
- Harrison, E.H. 2005. Mechanisms of digestion and absorption of dietary vitamin A. *Annu. Rev. Nutr.* 25:87-103.
- Helgerud, P., L.B. Peterson, and K.R. Norum. 1982. Acyl CoA:retinol acyltransferase in rat small intestine: its activity and some properties of the enzymatic reaction. *J. Lipid Res.* 23:609-618.
- Hicks, R.B., F. N. Owens, D. R. Gill, J. J. Martin, and C. A. Strasia. 1990. Effects of controlled feed intake on performance and carcass characteristics of feedlot steers and heifers. *J. Anim. Sci.* 68:233-244.
- Hollander, D. 1980. Retinol lymphatic and portal transport: influence of pH, bile, and fatty acids. *Am. J. Physiol.* 239:G210-214.
- Huang, H.S. and D.S. Goodman. 1965. Vitamin A and carotenoids. Intestinal absorption and metabolism of ¹⁴C-labeled vitamin A alcohol and β -carotene in the rat. *J Biol. Chem.* 240:2839-2844.
- Hurst, H. 1994. Transcription factors 1:bZIP proteins. *Protein Profile* 1:123.
- Hwang, Cheng-Shine, T.M. Loftus, S. Mandrup and M. D. Lane. 1997. Adipocyte differentiation and leptin expression. *Annu. Rev. Cell Dev. Biol.* 13:231-259.
- Jeremiah, L.E., G.C. Smith, and J.K. Hillers. 1970. Utilization of breed and traits determined from the live beef steer for prediction of carcass traits. *J. Anim. Sci.* 31:1089-1095.
- Kawada, T., Y. Mamei, and E. Sugimoto. 1996. The possibility of active form of vitamins A and D as suppressors on adipocyte development via ligand-dependant transcriptional regulators. *Intl. J. of Obesity* 20(Suppl 3):52-57.

- Kreikemeier, K.K., J.A. Unruh, and T.P. Eck. 1998. Factors affecting the occurrence of dark-cutting beef and selected carcass traits in finished beef cattle. *J. Anim. Sci.* 76:388-395.
- Kreikemeier, W.M. and T.L. Mader. 2004. Effects of growth-promoting agents and season on yearling feedlot heifer performance. *J. Anim. Sci.* 82:2481-2488.
- Kruk, Z.A., B.D. Siebert, W.S. Pitchford, J. Davis, G.S. Harper, and C.D.K. Bottema. 2004. The effect of vitamin A on fatness in lot-fed cattle: (I) Fat Quantity. Proc. 50th International Congress of Meat Sci. and Technol., University of Helsinki, Helsinki, Finland.
- Levin, M.S. 1993. Cellular retinol-binding proteins are determinants of retinol uptake and metabolism in stably transfected Caco-2 cells. *J. Biol. Chem.* 268:8267-8276.
- Lemieux, S., R. Fontani, K.D. Uffelman, G.F. Lewis, G. Steiner. 1998. Apolipoprotein B-48 and retinyl palmitate are not equivalent markers of postprandial intestinal lipoproteins. *J. Lipid Res.* 39:1964-1971.
- Lissoos, T.W., A.E. Davis, and M.S. Levin. 1995. Vitamin A trafficking in Caco-2 cells stably transfected with cellular retinol binding proteins. *Am. J. Physiol.* 268:G224-231.
- Mangelsdorf, D.L. and R.M. Evans. 1995. The RXR heterodimers and orphan receptors. *Cell* 83:841-850.
- May, S.G., H.G. Dolezal, D.R. Gill, F.K. Ray, and D.S. Buchanan. 1992. Effects of days fed, carcass grade traits, and subcutaneous fat removal on postmortem muscle characteristics and beef palatability. *J. Anim. Sci.* 70:444-453.
- May, S.G., J. W. Savell, D. K. Lunt, J. J. Wilson, J. C. Laurenz and S. B. Smith. 1994. Evidence for preadipocyte proliferation during culture of subcutaneous and intramuscular adipose tissues from Angus and Wagyu crossbred steers. *J. Anim. Sci.* 72:3110-3117.
- MacDonald, P.N. and D.E. Ong. 1988. Evidence for a lecithin-retinol acyltransferase activity in the rat small intestine. *J. Biol. Chem.* 263:12478-12482.
- Moody, W.G., J.E. Little, Jr., F.A. Thrift, L.V. Cundiff, and J.D. Kemp. 1970. Influence of length of feeding a high roughage ration on qualitative and quantitative characteristics of beef. *J. Anim. Sci.* 31:866-871.
- Nkrumah, J.D., D. H. Crews, Jr, J. A. Basarab, M. A. Price, E. K. Okine, Z. Wang, C. Li, and S. S. Moore. 2007. Genetic and phenotypic relationships of feeding behavior and temperament with performance, feed efficiency, ultrasound, and carcass merit of beef cattle. *J. Anim. Sci.* 85:2382-2390.
- NRC. 1996. Nutrient Requirements of Beef Cattle. 7th rev. ed. National Academy Press. Washington, DC.

- NRC. 2001. Nutrient requirements of Dairy Cattle. 7th rev. ed. National Academy Press. Washington, DC.
- Ohyama, M., K. Matsuda, S. Torii., T. Matsui, H. Yano, T. Kawada, and T. Ishihara. 1998. The uninteraction between vitamin A and thiazolidinedione on bovine adipocyte differentiation in primary culture. *J. Anim. Sci.* 76:61-65.
- Oka, A., Y. Maruo, T. Miki, T. 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.
- Parrish, F.C. 1974. Relationship of marbling to meat tenderness. *Proc. Meat Ind. Res. Conf.* p. 117.
- Pitt, G.A. 1965. Chemical structure and vitamin A activity. *J. Nutr.* 24:153-159.
- Prior, R.L., R.H. Kolhmeier, L.V. Cundiff, M.E. Dikeman, and J.D. Crouse. 1977. Influence of dietary energy and protein on growth and carcass composition in different biological types of cattle. *J. Anim. Sci.* 45:132-138.
- Pusillo, G.M., M.P. Hoffman, and H.L. Self. 1991. Effects of placing cattle on feed at two-month intervals and housing on feedlot performance and carcass grades. *J. Anim. Sci.* 69:443-450.
- Pyatt, N.A. and L.L. Berger. 2005. Review: Potential effects of vitamins A and D on marbling deposition in beef cattle. *Prof. Anim. Sci.* 21:174-181.
- 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.
- Quick, T.C. and D.E. Ong. 1990. Vitamin A metabolism in the human Caco-2 intestinal cell line. *Biochemistry* 29:11116-11123.
- Rajan, N., G.L. Kidd, D.A. Talmage, W.S. Blaner, A. Suhara, and D.S. Goodman. 1991. Cellular retinoic acid-binding protein messenger RNA: levels in rat tissues and localization in rat testis. *J. Lipid Res.* 32:1195-1204.
- Rhoades, R. and Pflanzner R., Eds. 1992. The Gastrointestinal System. *Human Physiology*, 2nd Ed., pp. 777-822
- Riggs, J.K. 1940. The length of time required for depletion of vitamin A reserves in range cattle. *J. Nutr.* 20:491-500.
- Rigtrup, K.M., L.R. McEwen, H.M. Said, and D.E. Ong. 1994. Retinyl ester hydrolytic activity associated with human intestinal brush border membranes. *Am. J. Clin. Nutr.* 60:111-116.

- Rule, D.C., S.B. Smith, and J.R. Romans. 1995. Fatty acid composition of muscle and adipose tissue of meat animals. In: *Biology of Fat in Meat Animals*. Smith and Smith, *Eds.* American Society of Animal Science, Champaign, IL pp. 144-165.
- Safonova, I., E. Amri, and G. Ailhaud. 1994. Retinoids are positive effectors of adipose differentiation. *Mol. Cell. Endocrinol.* 104:201-211.
- Savell, J.W., R.E. Branson, H.R. Cross, D.M. Stiffler, J.W. Wise, D.B. Griffin, and G.C. Smith. 1987. National consumer beef retail study: palatability evaluations of beef loin steaks that differed in marbling. *J. Food Sci.* 52:517.
- Scanes, C. 1995. Adipose tissue and its hormonal control in poultry. In: *Biology of Fat in Meat Animals*. Smith and Smith, *Eds.* American Society of Animal Science, Champaign, IL pp. 121-124.
- Semba, R.D. 1999. Vitamin A as “anti-infective” therapy, 1920-1940. *J. Nutr.* 129:783-791.
- Sewell, H.B. 1993. Vitamins for beef cattle. Available at <http://muextension.missouri.edu/explore/agguides/ansci/g02058.htm> Retrieved May 16th, 2007.
- Suryawan, A. and C.Y. Hu. 1997. Effect of retinoic acid on differentiation of cultured pig preadipocytes. *J. Anim. Sci.* 75:112-117.
- Tatum, J.D., G.C. Smith, B.W. Berry, C.E. Murphey, F.L. Williams, and Z.L. Carpenter. 1980. Carcass characteristics, time on feed and cooked beef palatability attributes. *J. Anim. Sci.* 50:833-840.
- Teboul, L. D. Gaillard, L. Staccini, H. Inadera, E-Z. Amri, and P.A. Grimaldi. 1995. Thiazolidine-diones and fatty acids convert myogenic cells into adipose-like cells. *J. Biol. Chem.* 270:28183-28187.
- Tontonoz, P., E. Hu and B.M. Spiegelman. 1994. Stimulation of adipogenesis in fibroblasts by PPAR γ , a lipid-activated transcription factor. *Cell* 79:1147-1156.
- Torii, S., T. Matsui, and H. Yano. 1996. Development of intramuscular fat in Wagyu beef cattle depends on adipogenic or antiadipogenic substances present in serum. *Anim. Sci.* 63:73-78.
- Umek, R.M., A.D. Friedman, and S.L. McKnight. 1991. CCAAT-enhancer binding protein: a component of a different switch. *Science* 251:288-292.
- University of Wisconsin-Madison, School of Medicine, Dept. of Anatomy. 1992. Available at: www.rocq.inria.fr/.../Fig/EnterocytBio.jpg Accessed 9-14-2007.

- Wertz, A.E., L.L. Berger, P.M. Walker, D.B. Faulkner, F.K. McKeith, and S. Rodriguez-Zas. 2001. Early weaning and post weaning nutritional management affect feedlot performance of Angus X Simmental heifers and the relationship of 12th rib fat to marbling score and feed efficiency. *J. Anim. Sci.* 79:1660-1669.
- Wertz, A.E., L.L. Berger, P.M. Walker, D.B. Faulkner, F.K. McKeith, and S. Rodriguez-Zas. 2002. Early weaning and post weaning nutritional management affect feedlot performance, carcass merit, and the relationship of 12th rib fat, marbling score, and feed efficiency among Angus and Wagyu heifers. *J. Anim. Sci.* 80:28-37.
- Wilson, D.E. 1992. Application of ultrasound for genetic improvement. *J. Anim. Sci.* 70:973-983.
- Wolf, G. 2001. Discovery of vitamin A. In: *Encyclopedia of Life Sciences*. Published by John Wiley & Sons, Ltd., Chichester, UK. <http://www.els.net> [doi:10.1038/npg.els.0003419] Retrieved 7-21-2007.
- World Health Organization. 2007. Statistics in vitamin A deficiency. <http://www.who.int/research/vita/> Retrieved on 7-12-2007.
- 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 PPAR γ . *Mol. Cell. Biol.* 16:1567-1575.
- Yeh, W.C., Z. Cao, M. Classon, and S.L. McKnight. 1995. Cascade regulation of terminal adipocyte differentiation by three members of the C/EBP family of leucine zipper proteins. *Genes Dev.* 9(2):168-181.
- Zhu, Y. C. Qi, J.R. Korenberg, X.N. Chen, and D. Noya. 1995. Structural organization of mouse peroxisome proliferator-activated receptor gamma (mPPAR gamma) gene: alternative promoter use and different splicing yield two mPPAR gamma isoforms. *Proc. Natl. Acad. Sci. USA* 92:7921-7925.

CHAPTER 2 - EFFECTS OF VITAMIN A SUPPLEMENTATION ON PERFORMANCE, SERUM LIPID, AND LONGISSIMUS MUSCLE LIPID COMPOSITION OF LAMBS

ABSTRACT

Forty crossbred wethers (BW = 28.7 kg) were used to evaluate the effects of diets containing high and low levels of vitamin A on LM lipid composition. Four treatments arranged as a 2 X 2 factorial with a completely random design were investigated: backgrounding (BG) and finishing (FN) with no supplemental vitamin A (LL); BG with no supplemental vitamin A and FN with high vitamin A (6,600 IU·kg⁻¹ diet) supplementation (LH); BG with high vitamin A supplementation and FN with no vitamin A supplementation (HL); and BG and FN with high vitamin A (HH) supplementation. Diets included cracked corn (62.4%), soybean meal (16.0%), cottonseed hull pellets (14.8%), and supplement (7%) and contained <100 IU vitamin A kg⁻¹ from carotenes before vitamin A was added. During the BG period (d 1 to 56), feed intake was restricted to achieve 0.22 kg ADG. During the FN period (d 57 to 112), lambs consumed the same diet *ad libitum*. Lambs were weighed every 14 d and blood sampled every 28 d to map changes in serum fatty acids (FA) and vitamin A levels. Lambs were humanely slaughtered after 112 d. Lipid composition was determined for liver and longissimus tissues. There were no treatment differences ($P > 0.05$) in feed intake, ADG, or final BW. Carcass weights were not affected by vitamin A treatment ($P > 0.20$), although backfat thickness tended to be different between HL and LL lambs (0.80 vs. 0.64 cm, respectively; $P = 0.08$). Carcasses from the HH group had higher ($P < .05$) marbling scores (514 vs. 459), and 25.8 % more extractable intramuscular lipid than LL (3.88 vs. 3.08 % for HH and LL, respectively, $P < .05$); the LH and HL treatments were intermediate. Interestingly, the LL group had the greatest increase in serum FA throughout the experimental period (change of 127 vs. 41 $\mu\text{g}\cdot\text{g}^{-1}$ for LL and HH, respectively; $P < .01$). Degree of saturation of fatty acids was not affected by treatment ($P = .18$) in the serum but was affected in longissimus thoracis fat. Oleic acid increased and linoleic acid decreased in the longissimus thoracis of HH-treated lambs ($P < 0.02$). These data suggest that

increases in total intramuscular lipid may be achieved with high levels of vitamin A supplementation for 112 d in young lambs.

INTRODUCTION

Intramuscular fat (marbling) is a major indicator of consumer satisfaction associated with beef consumption in many developed countries, especially the United States and Japan. Marbling is also the major factor used to determine USDA Quality Grade and prices paid by processors to beef producers. Currently, many producers sell their cattle based on carcass grade, where considerable premiums can be paid for carcasses with increased marbling (Schroeder et al., 2002).

Recently, the relationship between vitamin A intake and marbling development has been investigated. Increased marbling scores and chemically extractable intramuscular lipid (IMF) have been demonstrated in cattle (Oka et al., 1998; Nade et al., 2003; Kruk et al., 2004) and swine (D'Souza et al., 2003) fed either no or very low supplemental levels of vitamin A. Furthermore, marbling scores have been negatively correlated with concentration of vitamin A (retinol) in cattle blood (Oka et al., 1998; Adachi et al., 1999) and liver (Oka et al., 1998; Chae et al., 2003). Preadipocyte differentiation has been either suppressed or completely inhibited in the presence of retinoids in cultured porcine (Brandebourg and Hu, 2005), bovine (Ohyama et al., 1998), and ovine (Torii et al., 1995) stromal-vascular cells. Several authors have theorized that vitamin A and its metabolites inhibit preadipocyte differentiation by activating retinoic acid receptors (RAR) and down-regulating the expression of peroxisome proliferator-activated receptor gamma (PPAR γ), a marker of preadipocyte differentiation (Xue et al., 1996; Brandebourg and Hu, 2005).

To minimize economic risks and to conduct a study with shorter duration, we used lambs as a 'model species' to evaluate the effects of high and low vitamin A diets on growth performance, carcass traits, and lipid composition of market lambs using a combination of backgrounding (BG) and finishing (FN) periods. Specifically, it sought to determine the relationships between vitamin A status and the content and composition of fatty acids (FA) in serum, liver, and carcass fat depots.

MATERIALS AND METHODS

Animals

Forty crossbred wethers (Rambouillet X Finn ewes mated to Suffolk X Hampshire rams) were purchased at approximately 90 d of age (BW = from a single source, and weaned the day of purchase. Lambs had *ad libitum* access to creep feed from birth to weaning, which was on the day of purchase. They were vaccinated and treated for internal parasites prior to weaning. Upon arrival at Kansas State University, lambs were managed in the care of trained university personnel using methods described in the experimental protocol that was approved by the Institutional Animal Care and Use Committee (IACUC) at Kansas State University.

Experimental Design and Treatments

A completely random design with a 2 X 2 factorial treatment structure was used for this research. There were 4 pens (2 per treatment) each with either 10 or 11 lambs during the 56 d BG period. During the 56 d FN period, 8 pens (2 per treatment) each contained either 5 or 6 lambs. This was accomplished by constructing a fence through the middle of each pen on the last d of BG, thus doubling the number of pens. Consequently, pen size and number of animals per pen were halved for the FN period. However, this method allowed the stocking rate and the amount of pen space per animal to remain the same throughout the experiment. Lambs were weighed prior to assignment of treatments and initial pen weights were balanced by stratifying weights onto treatment. Lambs were randomly assigned to one of 2 BG treatments: high vitamin A (H); low vitamin A (L). After 56 d on the BG diets, a similar method of stratifying animal weight onto treatment created 4 treatment combinations for the combined feeding periods: high vitamin A during the BG and FN periods (HH); high vitamin A during the BG period, then low vitamin A during the FN period (HL); low vitamin A during the BG period and high vitamin A during the FN period (LH); or low vitamin A during the BG and FN periods (LL).

Diets

To examine the effect of level of vitamin A intake during the growing phase in lambs, a 56-d BG period was included in this study immediately preceding the 56-d FN period. Diets were mixed at the Kansas State University feed mill (Table 2-1) and the lambs were adjusted to a basal diet for 14 d prior to initiation of the trial. The same diet formulation was used for the BG and FN periods. Intake was restricted to produce an average daily gain of 0.22 kg/hd per d during the BG period; roughly half of the estimated *ad libitum* growth rate potential. During the

FN period which was initiated on d 57, the lambs had *ad libitum* access to feed. Lambs were adjusted up to *ad libitum* access by increasing the amount of feed offered each day, in increments determined at the discretion of trained personnel, to the level that, at the end of a feeding event, some feed was left in the bunk. This stepping up process was complete after approximately 4 d; thereafter, management was used for determining the appropriate amount of feed to be offered in each bunk daily, such that all of the feed was consumed each day. A free-choice mineral containing no vitamin A (Vita-Ferm[®] Custom Sheep Mineral, Biozyme Inc., St. Joseph, MO) was provided throughout the experiment.

Table 2-1. Composition of the treatment diets as fed

Ingredient	Amount
	———— % ————
Cracked corn	62.4
Soybean meal	16.0
Cottonseed hulls (pelleted)	14.8
Molasses product	5.2
CaCO ₃	1.6
Total	100.0
	———— IU/kg ————
Vitamin D	136
Vitamin E	6.8
High vitamin A	6,600
Low vitamin A	0

To create a large differential in circulating vitamin A levels, high vitamin A diets were supplemented with 6,600 IU retinol/kg of feed in wheat middling carrier and low vitamin A diets contained no supplemental vitamin A. Although forages may be a considerable source of vitamin A in ruminant diets, lambs in the present study were allowed only dry prairie hay that was harvested during the previous year and were housed in a drylot with no access to growing forages. Therefore, the lambs were considered to be provided no vitamin A from carotenes. Additionally, the grain diet contained either low or no detectable carotene and levels were confirmed by two private laboratories (Medallion Labs, Minneapolis, MN; NPAL, St. Louis, MO). Lambs, especially those receiving diets low in vitamin A, were observed daily for signs of

blindness and other physical illness. No negative effects on vision or general health condition were observed. The BG and FN diets were analyzed for retinoid and carotene activity by two private laboratories, both using HPLC, to verify the vitamin A levels.

Sample Collection

Lambs were weighed every 14 d and blood was collected every 28 d to document the onset and extent of the differences among treatments in vitamin A concentrations in circulation. Blood samples were also used to evaluate changes in circulating FA during the experiment. Blood was collected via jugular venipuncture into 10 ml red-topped, non-heparinized tubes (Kendall, Monoject 16 X 100 mm; Tyco Healthcare Group LP, Mansfield, MA). Blood sampling was conducted in a dimly lighted room and care was taken to avoid exposing the tubes to light. Filled tubes were immediately placed on ice in a cooler at the sheep unit. When sampling was completed, blood tubes were returned to the laboratory on campus and allowed to cool in a dark refrigerator at 4⁰ C for 24 hr. Tubes were then centrifuged (Beckman Coulter, Fullerton, CA) for 25 min at 2,200 x g and 4⁰ C. Serum was pipetted into two 5ml plastic tubes under UV-filtered light conditions and frozen at -27⁰ C for no longer than 90 d before vitamin A analyses were conducted.

Lambs were weighed off test on d 112 and were harvested at the Kansas State University abattoir over 2 consecutive d using humane procedures. Liver samples were collected from the caudate lobe, quick frozen in liquid nitrogen, and then stored at -27⁰ C for no more than 90 d until chemical analyses were conducted. Carcasses were allowed to chill for 24 h before ribbing between the 12th and 13th ribs. Marbling scores were determined by three experienced evaluators using the marbling score system that is utilized for USDA Beef Quality Grading where there are marbling picture standards. The USDA Lamb Quality Grade standards use “flank fat streaking”, which is a very subjective system with no reference points. Backfat thickness was measured midway over the longissimus dorsi muscle and then adjusted subjectively for body wall thickness. Sections (2.54 cm thick) of the longissimus thoracis (**LT**) muscle and overlying subcutaneous fat were removed and stored at -27⁰ C before chemical analyses.

Vitamin A Determination

Blood serum and liver were analyzed for vitamin A content by HPLC using the methods described by Barua and Olson (1998). Retinyl acetate was obtained from the Department of Human Nutrition at Kansas State University and used as the internal standard. Because retinol comprised nearly 85% of the detected retinoids in our samples, vitamin A content was interpreted as the total of retinol esters present in each sample. Likewise, retinol is metabolized to a number of metabolites, namely, retinoic acid (Barua and Olson, 1998). Analyses were conducted under yellow light to minimize deterioration of retinol. The mobile phase contained methanol with the flow rate set at 1.0 ml/min. The reverse phase was measured using a 25 cm, C-18 column. Vitamin A data were interpreted with chromatography software (Gold Chromatography Data System Version 1.6, licensed to Beckman Coulter, Fullerton, CA) using a 320 nm spectrum with a 4 nm band. All analyses were conducted in duplicates and the mean was reported as the value for each sample.

Fatty Acid Analysis

Blood serum, liver from the caudate lobe, and a section of the longissimus dorsi muscle obtained at the 12th/13th rib juncture, were analyzed for lipid content and FA profiles using a Shimadzu GC-17A (Shimadzu, Kyoto, Japan) gas chromatograph (GC). After 500 µl samples were freeze dried overnight, 1 ml of benzene, containing the internal standard (1000 µg/ml, methyl-13:0) was added and the tubes were vortexed to break up the pellet. Then, 4ml of boron trifluoride:methanol reagent (Supelco B1252, Supelco Inc., Bellefonte, PA) was added and the tubes were mixed gently. The tubes were incubated at 60⁰ C for 60 min. Tubes were cooled at room temperature, and 4ml of ddH₂O and 1 ml of hexane were added and mixed vigorously. The tubes were centrifuged at 1,000 X g for 5 min and the upper layer (1 to 2 ml) from each was transferred to a GC vial. Samples were injected at 260⁰ C through a Supelco SP-2560 capillary column and detected at 260⁰ C. The detector temperature was 260⁰ C and the final oven temperature was 240⁰ C, which was held for 15 min. Column flow rate was set at 1.1 ml/min with a split ratio of 48:1. Supelco 37 FA methyl ester mix was used as the external standard. All GC analyses were run in duplicate. Individual FA were expressed as proportion of sample weight for liver and muscle, and as a proportion of the total fatty acid content for serum.

Statistical Analyses

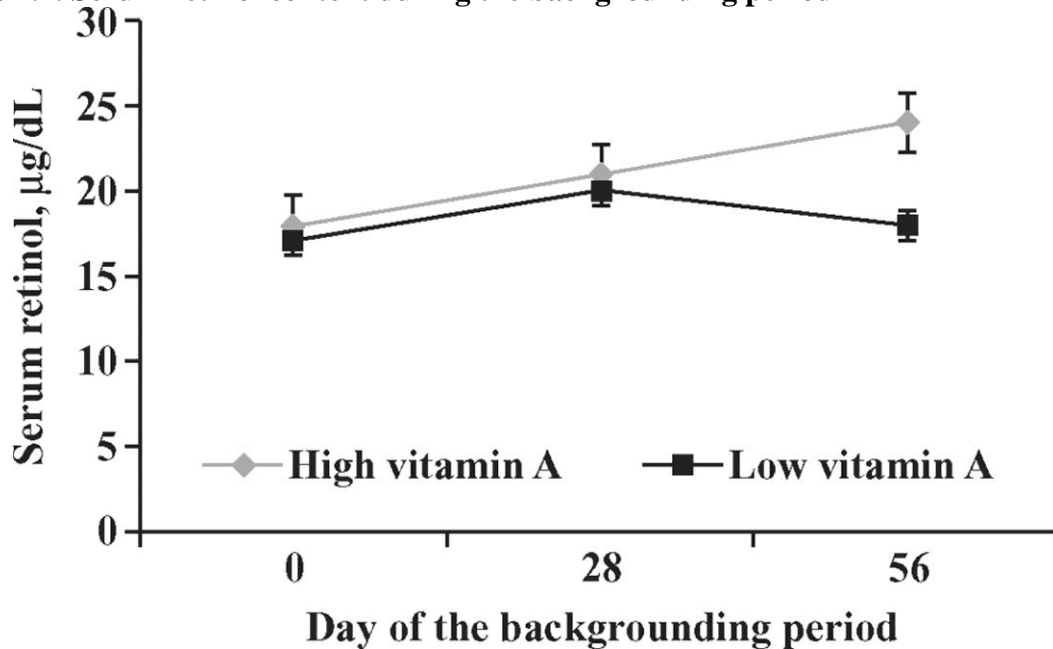
A completely random design with a 2 X 2 factorial arrangement of treatments was used. There were 2 pens per treatment, with either 10 or 11 lambs and either 5 or 6 lambs per pen for the BG and FN periods, respectively. Differences in means were detected using the PROC MIXED procedure of SAS[®], Cary, NC. Differences in serum retinoids and FA measured over time were analyzed with the PROC MIXED procedure, using a repeated-measures model with an unstructured covariance, which allowed the data to determine the best correlation model. The model contained vitamin A status as the main effects of the BG and FN periods and their interaction. Additionally, Pearson correlation coefficients (PROC CORR of SAS) were determined for serum retinol, marbling score, % IMF in the LT, backfat thickness, liver FA concentration, and serum FA content. Paired t-tests were used to compare individual fatty acid components in serum, muscle, backfat, and liver.

RESULTS AND DISCUSSION

Vitamin A in Tissues

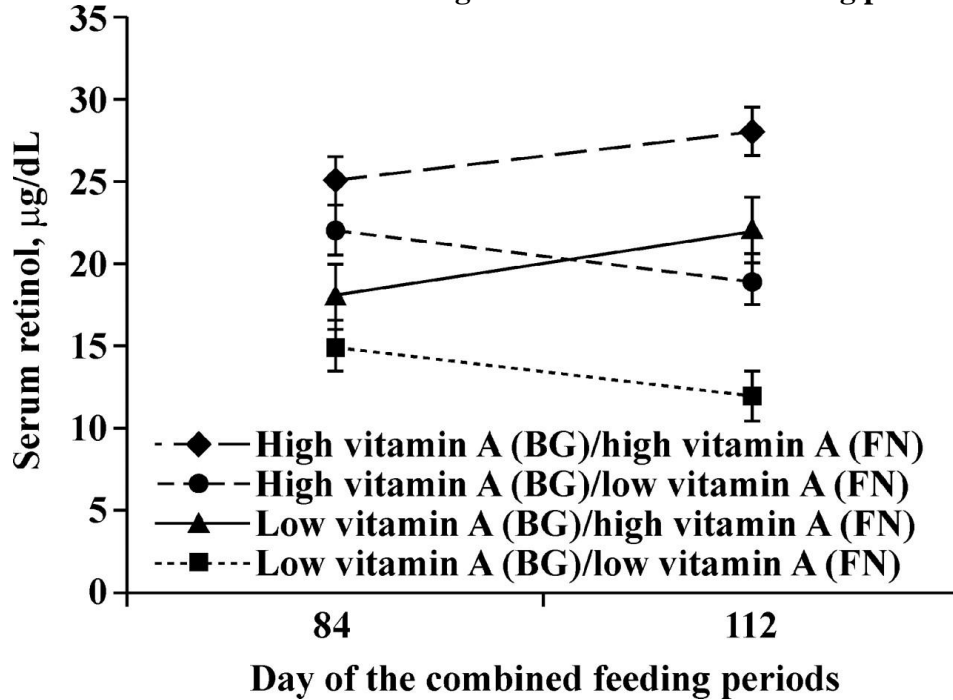
Liver is the primary storage site for vitamin A, and its depletion from the liver is critical in establishing divergent treatment effects (Goodman, 1984). Serum levels of retinol were used as an indicator of vitamin A status in the liver because a significant logarithmic relationship between serum and liver concentrations of vitamin A has been demonstrated in lambs (May et al. 1987) and in steers ($r = 0.77$, $P < 0.01$; Oka et al. 1998). Serum retinol was not different ($P > 0.10$) between lambs from high and low vitamin A BG treatments on any sampling day during the BG period (18, 21, and 24 vs. 17, 20, and 18 $\mu\text{g}\cdot\text{dl}^{-1}$ serum on d 0, 28, and 56 for H and L vitamin A treatments, respectively; Figure 2-1).

Figure 2.1. Serum retinol content during the backgrounding period



Serum levels differed ($P < 0.10$) by d 84 between the HH and LL lambs (25 vs. 15 $\mu\text{g}\cdot\text{dl}^{-1}$, respectively; Figure 2-2). This difference remained throughout the remainder of the experiment. Divergence in serum levels of retinol is often delayed and levels in blood sometimes rise when dietary vitamin A sources are removed and hepatic stores are mobilized (McDowell, 1989). Thus, disappearance of vitamin A from the blood tends to take longer in animals with greater stores of hepatic vitamin A, and hepatic stores are generally higher in animals with high dietary intake of vitamin A and/or older animals that may have accumulated significant reserves (Riggs, 1940). There were no differences in serum retinol ($P > 0.10$) between HL and LH lambs throughout the FN period. Because liver is the primary storage site, vitamin A was not measured in fat depots.

Figure 2.2. Serum retinol content during the last 28 d of the finishing period



Growth Performance

Average initial BW was 28.8 kg for all lambs (Table 2-2) and were not different ($P > 0.10$) among treatments. Although a divergence in circulating vitamin A was achieved by d 56, no negative effects on growth performance among treatments were detected ($P > 0.10$) for the BG, FN, or combined feeding periods. Average final BW for all treatments was 60.9 kg (Table 2-2). Average daily gain for all treatments was 0.28 kg/hd over the entire experimental period and were not different among treatments ($P > 0.10$) for the BG, FN, or combined feeding periods. These findings are consistent with those of May et al. (1987), who reported no significant linear or quadratic effects of vitamin A levels for live weight gain, feed intake, or feed efficiency when lambs received vitamin A supplementation ranging from 2 to 64 $\mu\text{g}/\text{kg}$ of BW per d for 16 wk.

Table 2-2. Lamb weights, growth performance, backfat depth, marbling scores, liver fatty acids, and longissimus thoracis fatty acids

Item	Treatment ¹				SEM
	HH	HL	LH	LL	
Initial weight, kg (d 0)	28	29	29	29	
Final weight, kg (d 112)	61	60	62	61	
ADG, kg/d	0.28	0.27	0.29	0.28	0.05
12th-rib fat, cm	0.66 ^a	0.81 ^b	0.74 ^b	0.64 ^a	0.06
Marbling score	514 ^c	445 ^d	519 ^c	459 ^d	28.7
Liver fatty acids, % of sample	3.72 ^e	3.65 ^e	3.65 ^e	3.37 ^f	0.14
Longissimus thoracis fatty acids, % of sample	3.88 ^c	3.45 ^{cd}	3.4 ^{cd}	3.09 ^d	0.24

^{a,b}Values within a row containing different superscripts tended to differ ($P = 0.08$).

^{c,d}Values within a row containing different superscripts differed ($P < 0.05$).

^{e,f}Values within a row containing different superscripts differed ($P < 0.01$).

¹HH = high vitamin A during the backgrounding (BG) and finishing (FN) periods; HL = high vitamin A during the BG period, then low vitamin A during the FN period; LH = low vitamin A during the BG period and high vitamin A during the FN period; LL = low vitamin A during the BG and FN periods.

Bruns and Webb (1990) reported similar growth rates between vitamin A-deficient and vitamin A-sufficient lambs during initial feeding, but decreased growth performance in vitamin A-deficient lambs during later stages of growth. A similar pattern of growth performance was reported by Oka et al. (1998) in cattle fed high or low vitamin A diets. Also, feeding less than half the NRC recommended level of vitamin A to Angus cross steers for 168 d did not reduce DMI, ADG, or G/F (Gorocica-Buenfil et al., 2005). However, retinoic acid, a form of vitamin A, regulates growth hormone gene expression (Bedo et al., 1989) and has increased growth rates of cattle (Perry et al., 1968). Several factors, including the extent and duration of depletion, chronological age, phase of growth, specie, and other environmental conditions likely contribute to the discrepancies in growth performance reported in these studies. Research is warranted to describe how vitamin A depletion over time affects growth performance of ruminants in US livestock production systems.

Serum Retinol and Marbling

Several investigators in countries supplying Asian beef markets have associated serum retinol content with beef carcass marbling scores. Oka et al. (1998) reported a correlation between serum vitamin A concentration and beef marbling score (Japanese scale) of - 0.38 ($P <$

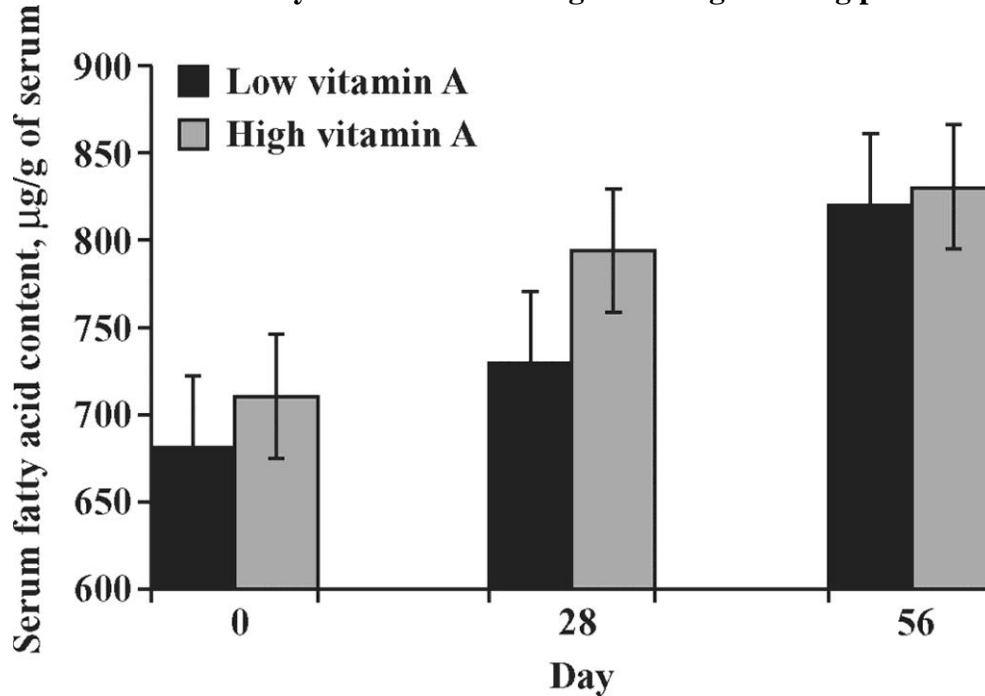
0.05). Similarly, Adachi et al. (1999) demonstrated a negative correlation between vitamin A level in cattle blood and marbling score. Currently, there is little information available about the relationship between serum retinol status and marbling development in US lamb and beef production. In our study however, serum retinol concentration was positively correlated to, and a moderate predictor of marbling score ($r = 0.30$; $P = 0.07$) and total extractable lipid ($r = 0.31$; $P = 0.06$) in lamb carcasses.

Results from the Japanese studies likely differed partially due to feeding cattle much longer and to older chronological ages than is deemed ideal in most US beef production systems. Depletion of dietary vitamin A for extended periods in the Japanese studies would have likely caused greater depletion of liver stores, thus increasing the correlation between serum vitamin A and marbling score in these studies. Our data oppose the findings of Oka et al (1998) and Adachi et al. (1999) and tend not to support those of Pyatt et al. (2005), who studied the effects of dietary vitamin A level on marbling development in a US beef production model. Surprised by our findings, we suggest that the relationship between serum levels of vitamin A and marbling deposition in lambs are different than those reported in cattle. The mechanism by which this relationship may be opposite in sheep is not clear, although differences in fat levels contained in the liver and IMF storage depots suggest a preferential effect related to blood retinol. These fat differences are reported herein.

Serum Fatty Acid Concentration

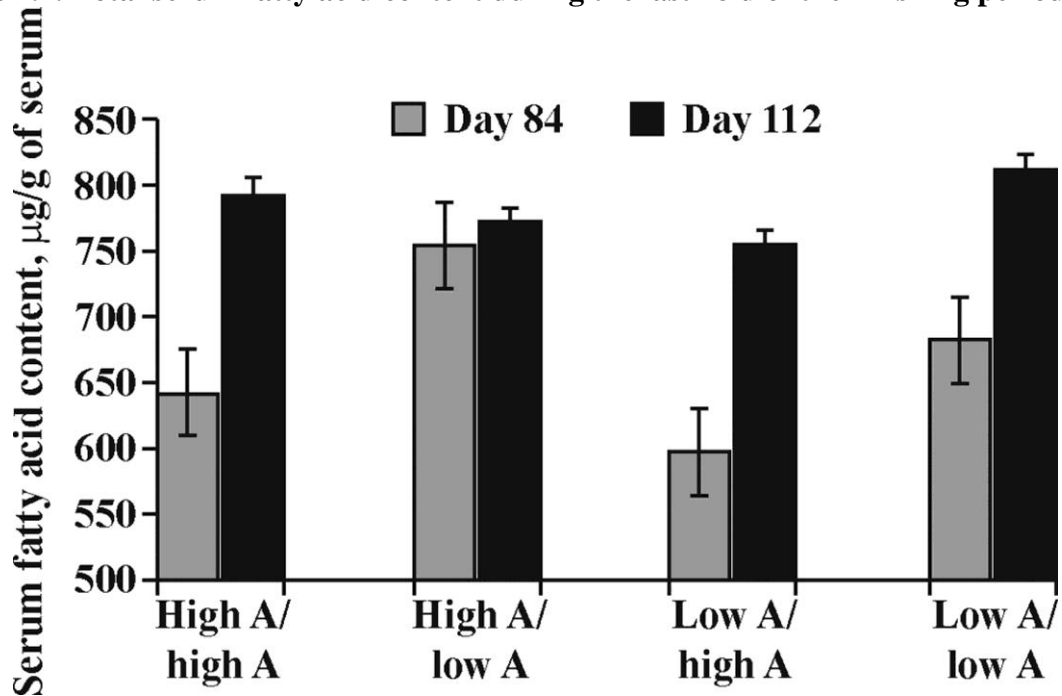
Concentration of FA in serum increased with time throughout the BG period in both treatments (Figure 2-3). On the last day of BG (d 56), serum FA had increased to 831 and 820 ug/g for high and low vitamin A treatments, respectively.

Figure 2.3. Total serum fatty acid content during the backgrounding period



Interestingly, serum FA concentration peaked on d 56 in all treatments, then decreased ($P < 0.01$) in all treatments 28 d into the FN period (d 84) (Figure 2-4). Following the decrease in serum FA midway through the FN period (d 84), serum FA concentration increased in all treatments for the remainder of the FN period. Serum FA concentrations were not different ($P > 0.10$) by treatment on the last d of the FN period. The magnitude of FA decline (d 56 to 84) was considerably higher ($P < 0.05$) in HL and LH treatments. The reason for the decrease in these two treatments is not clear; however, the stress of co-mingling lambs from different BG treatments to new FN treatments and the establishment of new social hierarchies early in the FN period may be partly responsible. Lambs that were switched from high to low, or low to high vitamin A treatments were also presented with more new pen mates than lambs remaining on the same treatment through the BG and FN periods. Additionally, all of the lambs endured extremely hot days during the FN period that may have affected FA circulation and storage. These data are supported by Oka et al. (1998), who reported no differences in serum FA concentrations of Japanese black steers fed diets containing high or low vitamin A, despite differences in the FA content of liver and muscle. Adachi et al. (1999) found no differences in non-esterified FA in serum of Japanese Black steers finished on diets containing either high or low levels of vitamin A.

Figure 2.4. Total serum fatty acid content during the last 28 d of the finishing period



Although serum FA level did not differ by vitamin A treatment, a moderate negative correlation was observed between final serum FA and carcass fat deposition (Table 2-3). The correlation of serum FA on the last day of the trial with 12th rib backfat depth was -0.36 ($P = 0.03$), while it was -0.33 ($P = 0.04$) with marbling score and -0.38 ($P = 0.01$) with %IMF in the LT. These data indicate a negative relationship between serum FA and fat deposition at two distinct anatomical locations, suggesting that serum FA content may be useful in predicting total FA content in the various tissues.

Table 2-3. Pearson correlations between final serum levels and content of fatty acids (FA) in several carcass depots

Item	Backfat depth	Marbling score	Longissimus thoracis FA	Liver FA	Serum FA	Serum retinol
Backfat depth	1.00					
Marbling score	0.20	1.00				
Longissimus thoracis FA	0.34*	0.68***	1.00			
Liver FA	0.08	0.09	0.09	1.00		
Serum FA	-0.36*	-0.36*	-0.38*	0.15	1.00	
Serum retinol	0.03	0.30†	0.31†	0.36*	-0.04	1.00

† $P < 0.10$, * $P < 0.05$, and *** $P < 0.001$.

Because of the moderate correlations reported here, more research would be useful in characterizing the relationship between serum and tissue FA concentrations as affected by vitamin A status. Currently, there are no published data describing the effects of vitamin A level on serum or tissue FA content in lambs.

Carcass Attributes

Backfat Thickness

Because genetic composition and live weights were similar, carcass weights and loin eye area were not considered in the analysis. Fat depth was measured midway over the LT at the 12th rib and then adjusted for differences in thickness of the body wall. Average fat thickness was 0.71 cm for all treatments (Table 2-2). Carcasses from HL and LH treatments tended to be fatter ($P = 0.08$) than the LL and HH carcasses. Fat thickness is an important consideration here because similar studies involving beef cattle reported that dietary vitamin A content may influence marbling deposition in the LM without increasing backfat thickness (Gorocica-Buenfil et al., 2005; Oka et al., 1998). Determination of feeding practices such as these which have the potential to increase marbling without increasing deposition of backfat, are desirable for livestock producers, processors, and consumers.

Marbling Scores

It is common practice in the U.S. to grow cattle, and sometimes lambs, on forage-based diets for several months (i.e., BG) before finishing on high-energy grain diets. When BG is done on lush spring pastures, it is likely that these animals are consuming 100,000 to 300,000 IU/kg forage of vitamin A (Pyatt and Berger, 2005). Large amounts of vitamin A may become stored in liver and fat tissues during this period. The effect of high vitamin A intake, from either BG on lush green pastures or from high supplemental intake during the FN period, may be detrimental to carcass marbling deposition. Consequently, to examine these effects in lambs, a BG period was included in our study immediately preceding the FN period. Average marbling score for all treatments was 484 degrees (i.e., Small⁸⁴). Lambs fed high vitamin A diets during the FN period tended to have higher ($P < 0.10$) marbling scores than those fed low vitamin A diets (Table 2-2). The HH and LH treatments produced carcasses containing Modest degrees of marbling (514 and 518 degrees, respectively); whereas carcasses from lambs fed HL and LL diets contained Small degrees of marbling (445 and 459 degrees, respectively). Marbling score was higher ($P < 0.05$) in HH and LH lambs than HL lambs and LL lambs (531 and 519 versus, 445, and 459 marbling degrees, respectively; Table 2-2). These data suggest that feeding high vitamin A diets to lambs for 56 d prior to harvest will increase marbling scores. Interestingly, when Japanese Black steers were fed either low or high vitamin A diets after 23 mo of age and slaughtered at 33 mo, there were no differences in marbling score using the Japanese scale. However, when steers were placed on high or low vitamin A diets beginning at 15 mo of age and harvested at 31 mo, marbling score was higher in cattle fed low vitamin A diets (Oka et al., 1998). When Angus steers (12 mo of age) were fed either high or low vitamin A diets for 10 mo, there were no differences in marbling scores when either the Australian or USDA grading system was used (Kruk et al., 2004). Similarly, Pyatt et al., (2005) found no difference ($P > 0.05$) in marbling score or percentage of carcasses grading low or premium Choice between Angus X Simmental steers and heifers fed either low or high vitamin A (3.3x NRC) diets. Gorocica-Buenfil et al. (2005) reported a trend for a 10% increase in marbling scores and percentage of carcasses graded USDA Choice from beef steers that were provided no supplemental vitamin A for 168 d versus those provided 2,700 IU vitamin A/kg diet DM ($P = 0.11$ for marbling score and $P = 0.13$ for percentage of Choice). Vitamin A effects on cattle marbling scores are not consistent and generally contradict our findings in lambs. Clearly, the effects of age and time on feed are

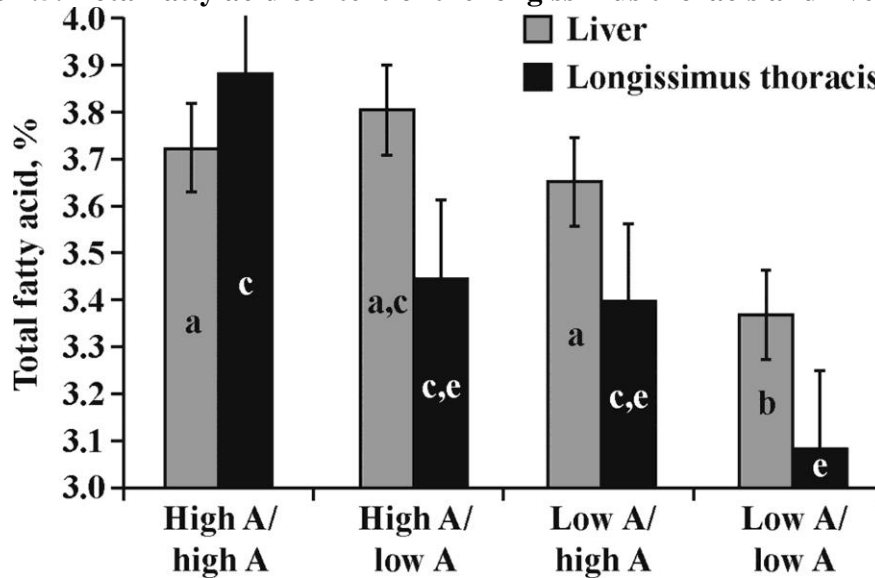
important considerations in depleting vitamin A and altering marbling deposition. These effects should be considered further in lambs to clarify the potential for high or low vitamin A diets to affect marbling deposition in different species of livestock.

Tissue Fatty Acid Concentration

Fatty Acid Concentration in the LT

Content of fatty acids in the LT provided an objective measure of IMF deposition to evaluate the effects of vitamin A treatment. This is arguably the most important determination made in this study because of being interested in extrapolating these differences to potential USDA Quality Grade differences in cattle that might be observed when cattle were treated similarly (i.e., no supplemental vitamin A and high vitamin A). The percentage of IMF in the LT was assessed by GC, and was intended to substantiate the marbling scores by using an objective method. Percent IMF is reported as total FA as a proportion of the LT sample weight (Table 2-2 and Figure 2-5).

Figure 2.5. Total fatty acid content of the longissimus thoracis and liver at slaughter



The results of our study in lambs contradict the findings from similar research conducted using cattle. In general, feeding lambs low levels of vitamin A decreased IMF, feeding high levels increased IMF, and feeding high and low levels in BG and F combinations resulted in intermediate IMF, regardless of the combination (HL or LH). Lambs from the LL treatment produced carcasses with the lowest % IMF and were different ($P < 0.05$) from the HH treatment (3.1 vs. 3.9 % IMF for LL and HH, respectively). The HL and LH treatments produced carcasses containing intermediate IMF (3.5 and 3.4 % IMF, respectively) compared to the LL or HH treatments and were not different compared to the LH and HL treatments ($P > 0.10$). Furthermore, this relationship suggests that a linear model may explain the relationship between vitamin A status and marbling deposition in lambs.

Interestingly, an almost opposite effect has been reported in cattle and swine. When Angus steers (12 mo old) were fed either high or no supplemental vitamin A for 10 mo, non-supplemented steers produced carcasses with 35% higher ($P < 0.05$) IMF in the LT than steers supplemented with high vitamin A (Kruk et al., 2004). When Large White X Landrace X Duroc finisher gilts were fed diets containing no supplemental vitamin A, they had higher ($P = 0.002$) IMF content in the longissimus muscle than supplemented gilts (D'Souza et al., 2003). Research reports describing the relationship between dietary vitamin A intake and IMF accumulation in the LT are inconsistent and further investigation is warranted to mitigate these discrepancies.

Fatty Acid Composition of the LT

Changes in unsaturated fatty acids were of particular interest because increased concentrations of these fatty acids in human diets have been associated with reduction of certain cancers and the risk of heart diseases, such as coronary artery disease (Simopoulos, 1991). There were no differences ($P > 0.10$) in the unsaturated FA content in s.c. fat deposited over the LT among the 4 treatments, which includes all monounsaturated and polyunsaturated FA that were detected by GC. Therefore, these data are not shown. Oleic acid (C18:1n-9 cis) was higher ($P < 0.02$) in the LT of carcasses from the HH lambs compared to LL lambs (Table 2-4). This increase accounts for more than half of the increase in total FA observed in the HH carcasses.

Table 2-4. Individual fatty acid concentrations in the longissimus thoracis¹

Item	Treatment				SEM
	HH	HL	LH	LL	
Fatty acids, ² % of total fatty acids					
16:0	23.7	23.8	22.5	22.8	0.52
18:0	12.6	12.5	13.4	12.7	0.28
18:1n-9 <i>trans</i>	3.1 ^a	3.2 ^a	2.9 ^a	3.6 ^b	0.17
18:1n-9 <i>cis</i>	41.2 ^a	39.1 ^{ab}	40.5 ^{ab}	38.3 ^b	0.87
18:2n-6 <i>cis</i>	6.4 ^a	7.5 ^b	7.1 ^b	7.8 ^b	0.23
20:4n-6	1.7 ^a	2.0 ^{ab}	2.0 ^{ab}	2.3 ^b	0.18
Total fatty acids, % of sample weight	3.9 ^a	3.5 ^{ab}	3.4 ^{ab}	3.1 ^b	0.21

^{a,b}Values within a row containing different superscripts differ, $P < 0.05$.

¹HH = high vitamin A during the backgrounding (BG) and finishing (FN) periods; HL = high vitamin A during the BG period, then low vitamin A during the FN period; LH = low vitamin A during the BG period and high vitamin A during the FN period; LL = low vitamin A during the BG and FN periods.

²Fatty acids not listed contributed less than 2% of total fatty acids.

Interestingly, this advantage in IMF deposition in HH lambs did not come in the form of linoleic acid, which has been shown to be of potential benefit to human health (Scollan et al., 2006). Rather, linoleic acid was the lowest in HH lambs and highest in concentration in IMF from LL lambs (6.4 vs. 7.8 % for HH and LL, respectively; $P < 0.05$). In retrospect, assays of desaturase enzyme activities would have strengthened our understanding of this relationship and may have explained how vitamin A affects the activity of these enzymes in vivo. Concentrations of oleic and linoleic acids from the LT of both crossover treatments (HL and LH) were intermediate, substantiating the likelihood of a linear relationship between dietary vitamin A status and content of unsaturated FA content of lamb loin meat. These data suggest that feeding diets high in vitamin A for at least 112 d will increase the concentration of monounsaturated fat, but will decrease the amount of polyunsaturated fat in the LT. This feeding protocol may help characterize lamb meat as a source of heart-healthy protein. This contradicts the findings of Daniel et al. (2004), who fed growing lambs vitamin A for 21 d and increased levels of palmitoleic and oleic acids in liver and adipose tissues, but concluded that manipulation of dietary vitamin A was not a suitable method of increasing unsaturated fat content in lamb meat. It is questionable whether feeding either high or low vitamin A for only 21 d in the study by Daniel et al. (2004) was enough time to create significant changes in tissue FA concentrations.

Fatty Acid Concentration in Liver

Although the relationships between vitamin A treatment and IMF in our study do not concur with similar studies in cattle, our results do concur with a similar trend in the fatty acid concentrations in lamb liver. Generally, feeding low vitamin A diets to lambs for 112 d (LL) reduced the concentration of FA in the liver, whereas feeding high levels of vitamin A (HH), particularly during the BG period (HL and HH), resulted in higher FA concentration in liver (Table 2-2 and Figure 2-5). Lambs from the LL treatment had lower ($P < 0.01$) concentration of liver FA than other treatments (3.4 vs. 3.7, 3.8, and 3.6 for LL, HH, HL, and LH treatments, respectively). Although total FA content from liver of HL lambs was numerically the highest, it was not different from the HH or LH treatments ($P > 0.10$). Nonetheless, this trend of higher FA in HH lambs and lower FA in liver of LL lambs is consistent with the FA profiles of the LT muscle. Coupled with the higher FA content of LL and lower FA content of HH serum of these lambs, vitamin A appears to be interacting with the mechanism of uptake of long-chain fatty acids from blood and subsequent storage.

Fatty Acid Composition in the Liver

Although lamb liver from the HH and HL treatments contained more total FA ($P < 0.02$) than liver from the LL treatment, concentration of monounsaturated FA was higher in the LL treatment. Both isomers of oleic acid were higher ($P < 0.05$) in liver from LL lambs than HH (Table 2-5). The *cis* isomer contributed more to the total FA content was higher in LH and LL than other treatments ($P < 0.02$). By contrast the *cis* isomer for IMF was highest in the HH treatment. High vitamin A is caused increased monounsaturation as fat became stored as marbling compared to those FA stored in liver.

Table 2-5. Concentration of individual fatty acids in the liver, caudal lobe¹

Item	Treatment				SEM
	HH	HL	LH	LL	
Fatty acids, ² % of total fatty acids					
16:0	14.4 ^a	13.1 ^{ab}	13.8 ^b	13.6 ^b	0.31
18:0	23.5	22.8	22.3	22.1	0.51
18:1n-9 <i>trans</i>	3.8 ^a	4.1 ^{ab}	4.3 ^{ab}	4.6 ^b	0.18
18:1n-9 <i>cis</i>	14.1 ^a	14.9 ^b	16.3 ^c	15.0 ^b	0.30
18:2n-6 <i>cis</i>	13.2 ^a	13.6 ^b	13.1 ^a	13.0 ^a	0.24
20:4n-6	10.9	10.5	10.7	10.8	0.21
Total fatty acids, % of sample weight	3.7 ^a	3.8 ^a	3.6 ^{ab}	3.4 ^b	0.14

^{a,b}Values within a row containing different superscripts differ, $P < 0.05$.

¹HH = high vitamin A during the backgrounding (BG) and finishing (FN) periods; HL = high vitamin A during the BG period, then low vitamin A during the FN period; LH = low vitamin A during the BG period and high vitamin A during the FN period; LL = low vitamin A during the BG and FN periods.

²Fatty acids not listed contributed less than 2% of total FA.

The mechanisms responsible for differences in FA saturation by storage depot are likely related to the desaturase enzymes, but the means by which they can be regulated at different depots in vivo remains unclear. Concentration of linoleic acid was not different in the liver tissue ($P > 0.20$). In our study, there is no clear relationship between the levels of linoleic acid in liver and IMF depots.

CONCLUSIONS

Feeding and management practices that effectively increase IMF accretion without increasing backfat and adversely affecting USDA Yield Grade are desirable for both lamb and beef production in the United States. Japanese and Australian reports have indicated a negative association between vitamin A content in the diet and/or serum with IMF (marbling) scores in cattle. This relationship in US lambs has not been demonstrated and only a weak or negligible relationship has been reported in US beef cattle. In our study, the effects of vitamin A tended to oppose the findings of Australian and Asian studies conducted in cattle, suggesting that higher dietary levels of vitamin A promote increased marbling in the LT of lambs. The effects of manipulating vitamin A levels are inconclusive in U.S. livestock production systems. More work is justified to clarify the effects of feeding high or low vitamin A diets on marbling deposition in sheep.

LITERATURE CITED

- Adachi, K., H. Kawano, K. Tsuno, Y. Nomura, N. Yamamoto, 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(8): 961-964.
- Barua, A.B., and J. A. Olson. 1998. Reversed-phase gradient high-performance liquid chromatographic procedure for simultaneous analysis of very polar to nonpolar retinoids, carotenoids and tocopherols in animal and plant samples. *J. of Chromatography.* 707: 69-79.
- Bedo, G., P. Santisteban, and A. Aranda. 1989. Retinoic acid regulates growth hormone gene expression. *Nature* 339: 231-234.
- Brandebourg, T.D. and C.Y. Hu. 2005. Regulation of differentiating pig preadipocytes by retinoic acid. *J. Anim. Sci.* 83: 98-107.
- Bruns, N.J., and K.E. Webb. 1990. Vitamin A deficiency: serum cortisol and humoral immunity in lambs. *J. Anim. Sci.* 68: 454-459.
- Chae, S.H., K.K. Jung, and C.B. Choi. 2003. Correlated relationships between vitamin A concentration in serum and liver and carcass characteristics of Hanwoo steers. *J. Anim. Sci. Tech.* 45: 585-592
- Daniel, Z.C.T.R., A.M. Satler, and P.J. Buttery. 2004. Vitamin A regulation of stearoyl-coA desaturase mRNA levels and fatty acid composition in sheep tissues. *Anim. Sci.* 82(1): 237-243.
- D'Souza, D.N., D.W. Pethick, F.R. Dunshea, J.R. Pluske, and B.P. Mullan. 2003. Nutritional manipulation increases intramuscular fat levels in the *Longissimus* muscle of female finisher pigs. *Austr. J. Ag. Res.* 54(8):745-749.
- Goodman, D.S. 1984. Vitamin A and retinoids in health and disease. *The New England J. of Med.* 310: 1023-1031.
- Gorocica-Buenfil, M.A., F.L. Fluharty, and S.C. Loerch. 2005. Effect of dietary vitamin A intake on marbling. *J. Anim. Sci.* 83 Suppl. 1: 370 (Abstr.)
- Kruk, Z.A., B.D. Siebert, W.S. Pitchford, J. Davis, G.S. Harper, and C.D.K. Bottema. 2004. The effect of vitamin A on fatness in lot-fed cattle: (I) Fat Quantity. *Proc. 50th International Congress of Meat Sci. and Technol., Helsinki, Finland.*
- May, B.J., M.C. Calhoun, and G.R. Engdahl. 1987. A re-evaluation of the minimum vitamin A requirement of growing-finishing lambs. *J. Anim. Sci.* 65: 1626-1632.
- 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: 353-358.

- Nakai, A., K. Kita, K. Hasegawa, and M. Hiramitsu. 1992. Vitamin A deficiency in Japanese black cattle. *J. Clin. Vet. Med.* 10:2143-2148.
- Ohyama, M., K. Matsuda, S. Torii, T. Matsui, H. Yano, T. Kawada, and T. Ishihara. 1998. The interaction between vitamin A and thiazolidinedione on bovine adipocyte differentiation in primary culture. *J. Anim. Sci.* 76: 61-65.
- Oka, A., Y. Maruo, T. Miki, T. 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.
- Perry, T.W., W.M. Beeson, W.H. Smith, R.B., Harrington, and M.T. Mohler. 1968. Interrelationships among vitamins A, E and K when added to the rations of fattening beef cattle. *J. Anim. Sci.* 27: 190-194.
- Pyatt, N.A., and L.L. Berger. 2005. Review: Potential effects of vitamins A and D on marbling deposition in beef cattle. *Prof. Anim. Sci.* 21:174-181.
- 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.
- SAS. 2002. SAS/STAT User's Guide (Version 9.1). SAS Inst., Inc., Cary, NC.
- Schroeder, T.C., J.D. Lawrence, C.E. Ward, and D.M. Fuez. 2002. Fed cattle marketing trends and concerns: Cattle feeder survey results. Kansas State Agric. Exp. Stn. MF-2561, Kansas State University, Manhattan, KS.
- Scollan, N., J.F. Hocquette, K. Nuernberg, D. Dannenberger, I. Richardson, and A. Moloney. 2006. Innovations in beef production systems that enhance the nutritional and health value of beef lipids and their relationship with meat quality. *Meat Sci.* 74:17-33.
- Simopoulos, A.P. 1991. Omega-3 fatty acids in health and disease and in growth and development. *Am. J. Clin. Nutr.* 54: 438-463.
- Torii, S., T. Matsui, and H. Yano. 1996. Development of intramuscular fat in Wagyu beef cattle depends on adipogenic or antiadipogenic substances present in serum. *Anim. Sci.* 63: 73-78.
- Torii, S., T. Matsumoto, T. Matsui, and H. Yano. 1995. Effect of vitamin A, C and D on glycerol-3-phosphate dehydrogenase activity of sheep preadipocytes in primary culture. *Anim. Sci. Technol. (Jpn.)* 66: 1039-1042.
- Xue, J., E.J. Schwarz, A. Chawla, and M.A. Lazar. 1996. Distinct stages in adipogenesis revealed by retinoid inhibition of differentiation after induction of PPAR γ . *Mol. Cell Biol.* 16: 1567-1575.

CHAPTER 3 - EFFECTS OF VITAMIN A SUPPLEMENTATION AND WEANING AGE ON SERUM AND LIVER RETINOL CONCENTRATIONS, CARCASS COMPOSITION, AND MEAT QUALITY IN MARKET BEEF CATTLE

ABSTRACT

Angus crossbred steers ($n = 48$) were either early-weaned (EW) at 137 ± 26 d of age or weaned at a traditional age (TW) of 199 ± 26 d to determine the effects weaning age and dietary vitamin A on growth performance and carcass traits. Steers from both weaning ages were allotted to receive either $42,180 \text{ IU vitamin A} \cdot \text{hd}^{-1} \cdot \text{d}^{-1}$ (HA) or no supplemental vitamin A (NA). Early-weaned and TW steers consumed treatment levels of vitamin A for 235 ± 17 and 175 ± 18 d, respectively. Serum and liver retinol contents diverged significantly (both, $P < 0.01$) by the end of the feeding period, and TW steers tended ($P > 0.10$) to have higher ADG than EW steers (1.31 ± 0.2 and $1.48 \pm 0.2 \text{ kg} \cdot \text{hd}^{-1} \cdot \text{d}^{-1}$, respectively). When ultrasound 12^{th} rib fat thickness averaged 1.02 cm, steers were humanely slaughtered and tissue samples and carcass data were collected following carcass chilling. Live and hot carcass weights tended ($P > 0.10$) to be heavier and carcasses were fatter ($P < 0.05$) for HA than NA steers. Marbling score and % intramuscular fat were higher ($P < 0.05$) for EW-NA steers than other treatments. Percentage of USDA Choice and Prime carcasses was double ($P < 0.05$) for NA than HA carcasses. Yield grade was numerically increased ($P < 0.05$) in EW-HA carcasses but was similar ($P > 0.10$) among other treatments. Ratios of marbling to age at slaughter, d on finishing diet, USDA yield grade, 12^{th} rib fat thickness, and carcass weight favored NA regardless, of weaning age, but were higher ($P < 0.05$) for EW-NA than other treatments. Proportion of individual fatty acids, including conjugated linoleic acid, of the IMF from the longissimus muscle were not affected ($P > 0.10$) by treatment. Supplementing with HA tended to increase ADG and decrease carcass marbling, especially in the EW cattle. Feeding NA to cattle was an effective method for

increasing carcass marbling deposition while tending to decrease waste fat; EW tended to enhance these effects.

INTRODUCTION

In the US, Asia, and other developed nations, beef with high degrees of intramuscular fat (marbling) is associated with consumer satisfaction, quality grade, and price of beef. Increasingly, beef producers market finished cattle in the U.S. via a value-based system where price is significantly influenced by the amount of marbling deposited in the longissimus muscle. Despite the efforts of researchers, producers, and processors to meet the beef quality demands of American consumers, the percentage of carcasses graded USDA Choice and Prime has not increased significantly over the past 2 decades.

When carcasses with high degrees of marbling are produced, unsatisfactory yields of lean retail product due to excessive fat trim have been problematic. Improved methods of cattle feeding and management that encourage increased deposition of intramuscular fat without increasing subcutaneous waste fat are desperately needed to protect the long-term sustainability of the U.S. beef industry. In Asian countries, cattle diets comprised of rice straw, barley, and other commonly used feed ingredients retain virtually no β -carotene activity compared to grazed forages and Oka et al. (1998) was the first to associate the restricted levels of dietary vitamin A in cattle diets with high marbling scores of beef carcasses. Investigation of vitamin A restriction under US production systems has been primarily at three land grant institutions in the Midwest (Pyatt et al., 2005; Arnett et al., 2007; Gorocica-Buenfil et al., 2007a,b). These investigators have reported subtle increases in beef carcass marbling when dietary vitamin A was restricted.

MATERIALS & METHODS

Animals

Angus crossbred steers (n = 48), born March through May of 2006, from Kansas State University's commercial cowherd, were either early-weaned (EW) at an average of 137 ± 26 d of age or weaned at a traditional age (TW) of 199 ± 26 d. All calves were vaccinated with CattleMaster 4 + VL5 (Pfizer, Exton, PA) and Vision 7/Somnus (Bayer, Kansas City, MO) 14 d prior to and again on the d of maternal separation. On the d of maternal separation, calves were

treated for internal and external parasites (Ivomec; Merck, Whitehouse Station, NJ) and placed in a drylot near the KSU Manhattan campus for 14 d where they were familiarized with bunk feeding and grain provided through a preconditioning diet, and closely monitored and treated accordingly for sickness that is commonly associated with weaning. The EW calves were then transported approximately 260 km from Manhattan to the KSU Hays Experiment Station where they were weighed and randomly allotted to one of 2 pens (12 animals per pen) so that the mean initial pen weights were similar. This weaning-management and allotment protocol was repeated 60 d later for the TW calves. Weights of the EW and TW calves upon arrival to the Hays Experiment Station were 191 ± 31.7 and 234 ± 37.8 kg, respectively. Animals were continually managed under the care of trained university personnel according to the guidelines recommended in the *Guide for the Care and Use of Agriculture Animals in Agriculture Research and Teaching* (FASS, 1998), and all experimental procedures were approved by the Institutional Animal Care and Use Committee at Kansas State University.

Diets

The feeding period consisted of a preconditioning, growing and a finishing phase for the EW calves and a preconditioning and finishing phase for the TW calves. The composition of the preconditioning and grower diets was the same (Table 3-1). The preconditioning phase, as described previously by Olson et al. (2007), was designed to have EW calves consuming 2.0% of their BW (DM basis) of a 75 to 85% concentrate diet within 7 to 10 d of maternal separation. The preconditioning diet and the supplement (Table 3-1) were formulated by a collaborating ruminant nutritionist at Kansas State University. Briefly, the preconditioning diet was placed into the bunk first and the hay was layered loosely over the concentrate diet. This was done with the intent of attracting calves to a familiar feed, which allowed them to encounter the underlying concentrate as the hay was consumed. The preconditioning diet was consumed by calves for 14 d. The additional 61 d on the preconditioning (i.e., “grower” diet for the EW calves) was intended to increase skeletal growth and maximize lean tissue growth while preventing premature and/or excess fat accretion in the EW calves. Vitamin A treatments were initiated for all calves upon arrival at the Hays experiment station following the 14 d preconditioning period. For EW calves, this meant consuming either high or low vitamin A in the grower and finishing diets, whereas vitamin A treatments were initiated at the start of the finishing period for the TW calves. Thus, EW calves consumed either high or low vitamin A diets for an additional 61 d than

the TW calves. This decision was made to pattern what might commonly happen when EW calves are fed for extended periods in feedlots using varied levels of vitamin A supplementation. The low vitamin A diet (NA) contained no supplemental vitamin A because others who fed no supplemental vitamin A reported no detrimental effects on health or growth performance (Oka et al., 1998; Kruk et al., 2004). No animals exhibited symptoms of deficiency during our study. The high vitamin A diet (HA) was supplemented to provide 42,180 IU vitamin A \cdot hd⁻¹ \cdot d⁻¹; or 7 times the recommended level (NRC, 1996). The vitamin A treatment levels were continued for the EW steers and initiated for the TW steers with the finishing diets.

Table 3-1. Composition (DM basis) of the preconditioning diet

Ingredient	Percent (DM basis)
Ground Sorghum Grain	48.2
Corn Gluten Feed	24.2
Tallgrass Prairie Hay (chopped)	14.8
Whole Soybeans (raw)	9.6
Supplement ^a	3.2
Total	100.0

^aProvided NRC (1996) recommended levels of salt, trace minerals, and vitamin A. Bovatec 91 (Alpharma, Fort Lee, NJ) was included at 1.2% (DM) of the diet.

The finishing phase (d 0) was initiated immediately following the last d of the growing (EW) and preconditioning (TW) phases using a diet that consisted of sorghum silage, ground sorghum, and supplement (Tables 3-2 and 3-3). Feed intake was increased daily over a 3 wk period with the goal of averaging 2.8% of their BW (DM basis) for the entire finishing period. The finishing diet was placed into the bunk first and the supplement was top-dressed daily. A feedbunk scoring system with a scale from 0 to 5 was used daily to monitor intake and to determine how much to feed. A score of zero implied that the feedbunk was empty; score of 1 meant something less than or equal to 2.54 cm of feed was left in the bottom of the bunk; score of 2 meant that approximately 5.08 cm of feed was left; etc.

Table 3-2. Average composition (DM basis) of the finishing diet

Ingredient	Percent (DM basis)
Ground Sorghum Grain	80.2
Sorghum Silage	15.9
Supplement ^a	3.9
Total	100.0

^aComposition of the supplement, including vitamin A treatments are located in Table 3-3.

Table 3-3. Composition (DM basis) of the supplement for the finishing diet

Ingredient	Percent (DM basis)	
	Low vitamin A	High Vitamin A
Soybean Meal	53.6	53.2
Trace Mineral	0.645	0.645
Rumensin 80	0.074	0.074
Tylan 40	0.022	0.022
Calcium	24.7	24.7
Urea	14.8	14.8
Salt	6.2	6.2
Vitamin A (60,000 IU/g)	0.000	0.346
Total	100.0	100.0

Endpoint Determination and Harvest

Steers were individually weighed every 28 d to monitor growth performance. Finishing diets were continued until steers averaged 1.02 cm of 12th rib fat thickness as determined by periodic ultrasound with a 3.5 MHz probe (Aloka 500V, Aloka America, Inc., Wallingford, CT). Ultrasound data were analyzed with software to predict days to a desired fat thickness, quality and yield grade (Cattle Performance Enhancement Company, Oakley, KS). To minimize variation in fat thickness and body composition, the steers were harvested in 2 groups, 35 d apart. One-half of the steers from each of the 4 treatment combinations was harvested on each day. Approximately 15 h prior to slaughter, steers were loaded and transported approximately

350 km to Tyson Fresh Meats[®], Emporia, Kansas where they were held off feed and provided access to water until humane slaughter.

Liver samples were removed from the caudal lobe within 15 min of exsanguination, packed in dry ice, and returned to Kansas State University's meat chemistry laboratory with minimal exposure to direct light. Samples were cut into 2 pieces (approximately 100 g each), placed in a plastic bag, overwrapped with aluminum foil, and stored at -80°C prior to vitamin A and fatty acid analyses.

Carcasses were chilled for 24 hr at 1 to 3°C before being ribbed between the 12th and 13th ribs for USDA quality and yield grade determinations. All carcass measurements and estimates were performed by experienced university faculty and graduate students. Yield grade was calculated according to USDA standards. Marbling scores were evaluated by 3 experienced university employees. The mean of the 3 scores was used for the statistical analysis.

Vacuum-packaged, boneless-strip loins were obtained upon carcass fabrication at Tyson Fresh Meats[®] (Emporia, KS) approximately 72 hr postmortem. They were packed in coolers with dry ice, and returned to the abattoir at Kansas State University where they were aged at 4°C until 14 d postmortem. On d 15, samples of subcutaneous fat and of the LM were obtained when the strip loins were fabricated. Samples were placed in plastic bags and stored at -80°C until fatty acid analyses were conducted.

Fatty Acid Analysis

Blood was collected via jugular venipuncture at 60 d intervals. Filled 10 ml red-topped, non-heparinized tubes (Kendall, Monoject 16 X 100 mm; Tyco Healthcare Group LP, Mansfield, MA) were immediately placed on ice in an insulated and covered container. Samples were returned to the laboratory at the Manhattan campus and stored in dark refrigeration at 4°C for 24 hr. Tubes were then centrifuged for 25 min. at 2,600 rpm and 4°C using a JA-10 rotor (Beckman Coulter, Fullerton, CA). Serum was pipetted into two 5ml plastic tubes under dim lighting and stored at -80°C for no longer than 60 d before fatty acid analyses were conducted. In addition, liver from the caudate lobe, and a section of the LM obtained at the 12th/13th rib juncture, were analyzed for lipid content and FA profiles using a Shimadzu GC-17A (Kyoto, Japan) gas chromatograph (GC). After 500 µl samples were freeze dried overnight, 1 ml of benzene, containing the internal standard (1000 µg·ml⁻¹ methyl-C13:0) was added and the tubes were vortexed to break up the pellet. Then, 4ml of boron trifluoride:methanol reagent (Supelco

B1252, Supelco Inc., Bellefonte, PA) was added and the tubes were mixed gently. The tubes were incubated at 60 °C for 60 min. Tubes were cooled at room temperature and then 4ml of ddH₂O and 1 ml of hexane were added and mixed vigorously. Tubes were centrifuged at 1000 X g for 5 min and the upper layer (1 to 2 ml) from each was transferred to a GC vial. Samples were injected at 260 °C through a Supelco SP-2560 capillary column and detected at 260 °C. The detector temperature was 260 °C and the final oven temperature was 240 °C held for 15 min. Column flow rate was set at 1.1 ml·min⁻¹ with a split ratio of 48:1. Supelco 37 FA methyl ester mix was used as the external standard. All GC analyses were run in duplicates. Individual FA were expressed as proportions of sample weight for liver and muscle, and as a proportion of the total fatty acid content in serum.

Retinol Analysis

Blood and liver samples were collected as described above and retinol content was determined by HPLC using the methods described by Barua and Olson (1998), with slight modifications. Retinyl acetate was obtained from the Department of Human Nutrition at Kansas State University and used as the internal standard. Analyses were conducted under yellow light to minimize deterioration of retinol. The samples were extracted with hexane and dried under N₂ gas at 37°C. Samples were reconstituted with ethanol, and injected into a HPLC equipped with a 25 cm, C-18 reverse-phase column. The mobile phase contained methanol, with the flow rate set at 1.0 ml·min⁻¹. Chromatographs were interpreted by calculating the area under the curve with specialized chromatography software (Gold Chromatography Data System Version 1.6, licensed to Beckman-Coulter, Fullerton, CA) using a 320 nm spectrum with a 4 nm band. All analyses were conducted in duplicates and the mean was used as the value for each sample. Because retinol comprised nearly 85% of the detected retinoids in our samples, vitamin A content was interpreted as the total of the retinol esters present in the sample. Half of the liver samples (n = 24) were analyzed by an independent laboratory to substantiate our results (Nestlé-Purina Analytical Laboratories, St. Louis, MO).

Statistical Analysis

A completely random design with a 2 X 2 factorial arrangement of treatments was used. There was 1 pen per treatment containing 12 steers per pen. Differences in means were detected using the PROC MIXED procedure of SAS[®], Cary, NC. Differences in serum retinoids and fatty

acids measured over time were analyzed with the PROC MIXED procedure, using a repeated-measures model with an unstructured covariance, which allowed the data to determine the best correlation model. The model was tested using dietary vitamin A level and weaning method main effects as well as the vitamin A level X weaning method interaction. Additionally, Pearson correlation coefficients (PROC CORR procedure of SAS[®]) were determined for liver and serum retinol and fatty acid concentrations, marbling score, % IMF, and 12th rib fat thickness. Animal was the experimental unit.

RESULTS AND DISCUSSION

Retinol Status

Concentrations of serum retinol on three sampling days are presented in figure 3-1. The initial sampling d was August 30th and October 30th for the EW and TW steers, respectively. Although these samples were collected on different days, they represent a baseline concentration of retinol on the d vitamin A treatments were initiated. The other sampling days represented the middle and end of the finishing period. As expected, serum retinol levels were similar among the four treatment groups on the first d of vitamin A treatments; levels were especially similar within vitamin A treatments. Although not statistically different, initial serum retinol levels were higher for the TW calves; most likely because they consumed pasture-derived carotenes for approximately 2 mo longer than EW calves. On the December 15th sampling, steers had been consuming treatment levels of vitamin A for either 107 (EW) or 46 (TW) d. Figure 3-1 illustrates the divergence in serum retinol levels by treatment. Although we did not collect antemortem liver samples, the serum data suggest that within 45 d of dietary vitamin A depletion (i.e., TW-NA), liver stores were depleted to an extent that circulating levels could not be maintained according to Blaner and Olson (1994) who reported that serum levels remain relatively constant unless hepatic stores are depleted. Given the age classification of these calves, this result seems reasonable because younger animals generally have minimal stores of hepatic vitamin A (Blaner and Olson, 1994). On the last sampling d, steers had consumed either very high or no supplemental vitamin A for either 213 (EW) or 153 (TW) d and serum retinol levels had diverged dramatically ($P < 0.01$) between high and no supplemental vitamin A treatments. No effects of weaning age were detected for serum retinol on the last sampling d (P

> 0.10), and serum means for the steers that consumed high vitamin A were almost identical for both weaning ages on March 29th. A maximum physiological level for blood may have been reached by feeding 7 times NRC levels for an extended period and large amounts of vitamin A must have been stored in the liver and other fat depots. This theory is supported by the retinol content of the livers (Figure 3-2).

Figure 3.1. Serum retinol concentrations on 3 sampling days

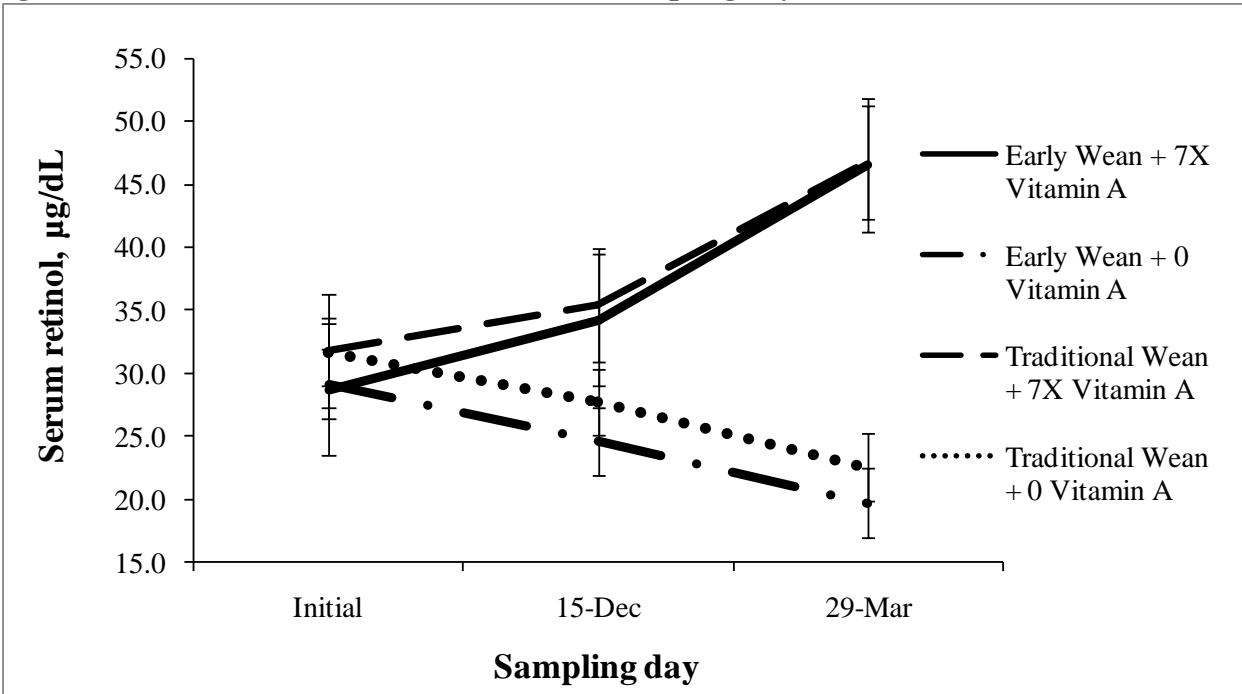
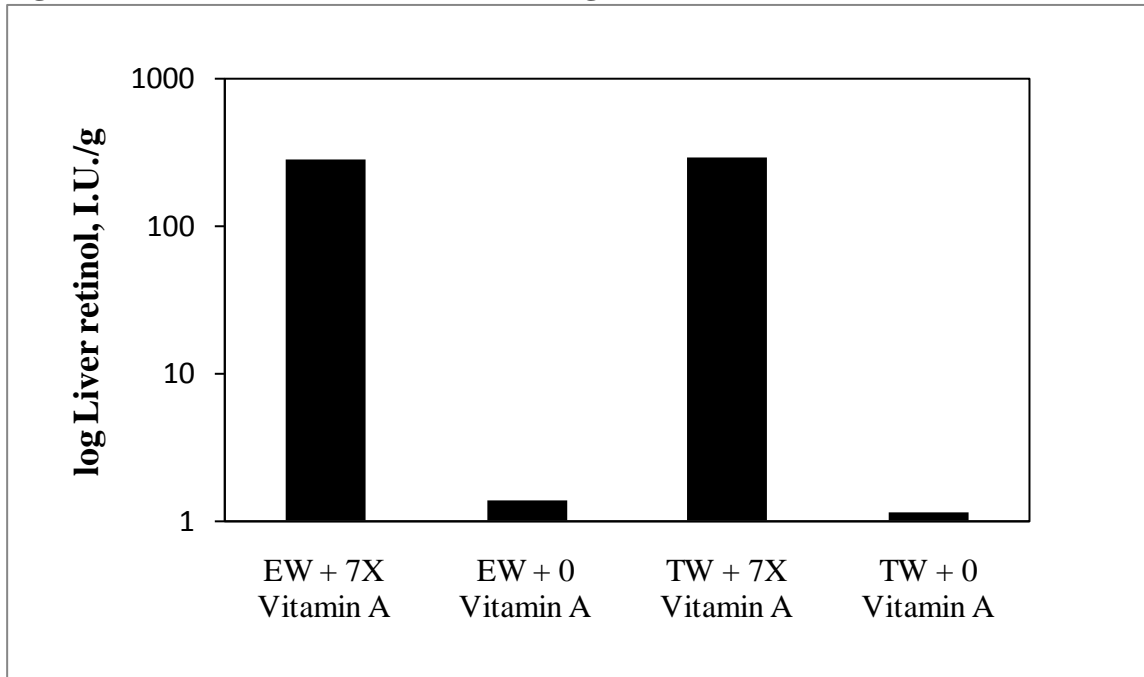


Figure 3.2. Liver retinol concentrations (log scale)



Liver retinol content was vastly different between vitamin A treatments (294 ± 85 and 1.3 ± 0.63 IU/g for high and low vitamin A, respectively; Figure 3-2). Although the vitamin A treatments were designed to represent extremes in supplementation scenarios, we were surprised that hepatic retinol from the high vitamin A steers was 226 times greater than the low vitamin A treatment. Because liver is the primary storage tissue for vitamin A, these results provided a biological indicator that the dietary treatment levels were effective.

Growth Performance

Growth performance characteristics are reported in table 3-4. Although the diet compositions were similar, we sometimes have referred to the preconditioning diet as the “grower diet” in the context of the EW steers for reasons explained in the Materials and Methods section. Early-weaned steers weighed 191 ± 31.7 kg at the initiation of the grower phase and gained $1.15 \text{ kg} \cdot \text{hd}^{-1} \cdot \text{d}^{-1}$ for 61 d before the finishing period was initiated. Early-weaned steers that were fed NA had slightly higher ADG and were 4 kg heavier at the end of the grower phase than steers fed high supplemental vitamin A (both; $P > 0.10$).

Early-weaned steers were heavier ($P < 0.01$) than TW steers on d 0 of the finishing period as was expected because of increased BW gain achieved during the grower phase while

TW siblings remained on pastures with their dams during this time (Table 3-4). Body weights were similar for both vitamin A treatment levels within weaning age at the beginning of the finishing period ($P > 0.10$). The TW steers tended ($P = 0.11$) to have higher ADG than EW steers. Compensatory gain is a common phenomenon in TW steers and was likely responsible for the advantage in ADG in our study. Ideally, all of the steers should have been heavier at slaughter to have been more acceptable on a carcass pay weight basis.

Table 3-4. Effects of weaning age and dietary vitamin A level on steer growth performance attributes

Item	High Vitamin A		No Vitamin A		SEM	P value			
	Weaning age	Early	Traditional	Early		Traditional	Vit. A	Wean	Vit. A X Wean
Initial BW, grower, kg		193.2	NA	188.2	NA				
Final BW, grower, kg		257.5	NA	260.7	NA				
BW gain, grower, kg/d		1.10	NA	1.19	NA				
Initial BW, finishing, kg (d0)		257.5	237.0	260.7	230.2	32.0	0.96	0.43	0.87
Final BW, finishing, kg		488.9	488.2	482.2	479.6	36.2	0.84	0.96	0.97
BW gain, finishing, kg/d		1.35	1.48	1.26	1.47	0.1	0.64	0.11	0.67

Carcass Traits

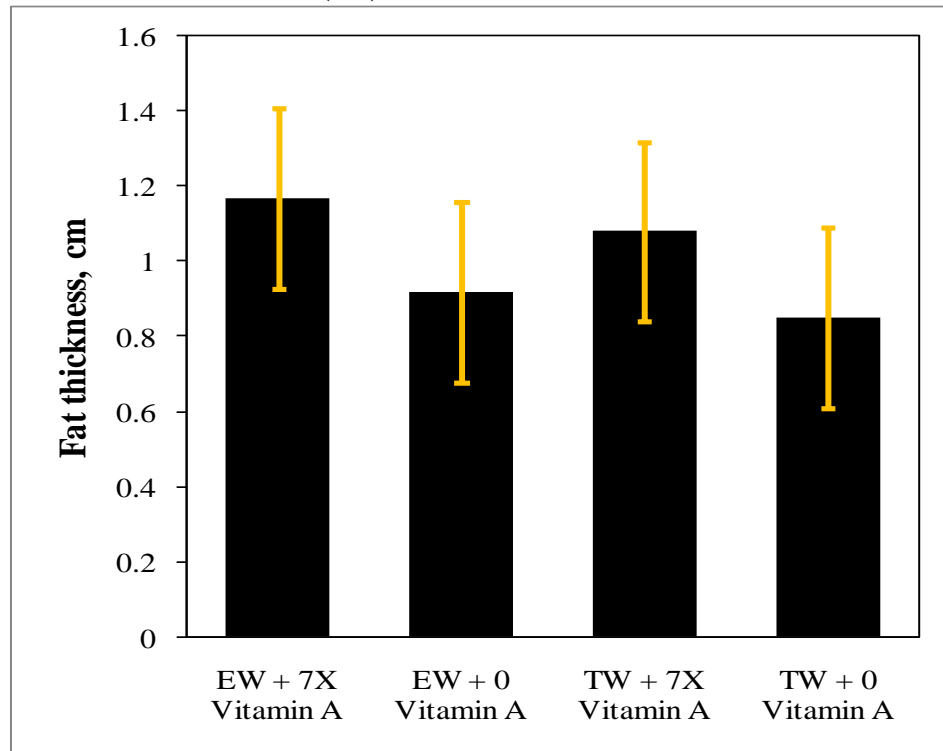
Treatment means for carcass traits are presented in table 3-5. Because final live weights tended to be heavier in HA steers, hot carcass weights also tended to be heavier ($P = 0.08$). There were no differences in hot carcass weights due to weaning age ($P > 0.10$). Average dressing percent was 62.5 ± 1.28 for all steers and there were no treatment differences (data not presented).

Table 3-5. Effects of vitamin A level and weaning age on carcass attributes of steers. ^aFor marbling score, 400= Small⁰⁰, 410= Small¹⁰, ...500=Modest⁰⁰, etc.

Item	High vitamin A		No vitamin A		SEM	P value			
	Weaning age	Early	Traditional	Early		Traditional	Vit. A	Wean	Vit. A X Wean
Hot carcass weight, kg		318	318	314	312	33.2	0.84	0.96	0.97
Ribeye area, sq. cm		72.7	78.1	76.8	75.5	5.0	0.88	0.69	0.50
12th rib fat, cm		1.17	1.08	0.92	0.85	0.2	0.33	0.74	0.98
KPH, %		2.3	2.2	2.2	2.2	0.2	0.79	0.79	0.62
USDA Yield Grade		3.2	2.8	2.7	2.7	0.4	0.40	0.57	0.64
Marbling score ^a		430	440	480	450	64.9	0.63	0.88	0.77
Intramuscular fat, %		4.8	4.8	6.2	5.3	1.2	0.44	0.72	0.73
Premium Choice and Prime, %		17	18	36	36				

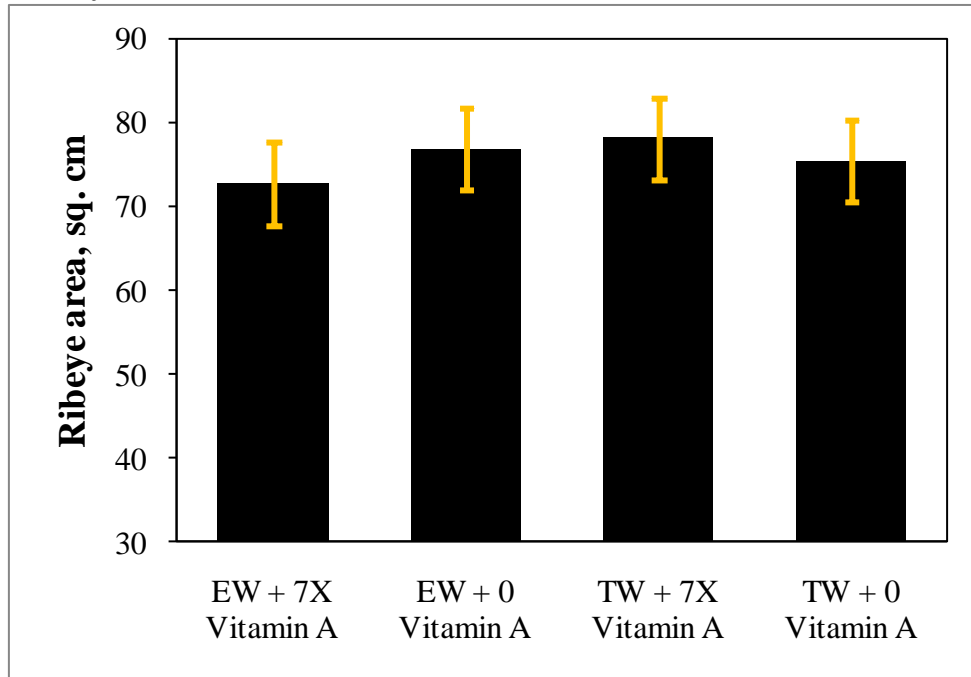
Twelfth-rib fat thickness of 1.02 cm was deemed as the target endpoint and market readiness for the steers, and was determined by periodic ultrasound evaluations. The HA steers tended to be getting fatter than the NA steers, so the decision was made to harvest approximately half of the cattle of each treatment in two harvest groups, with the fattest half harvested in one group and the remainder in another group when they averaged 1.02 cm backfat thickness. This meant that some steers from NA treatments were harvested at slightly less than desired fat thicknesses because we reasoned that it was more desirable to slaughter approximately equal numbers of steers from each treatment on each slaughter d, rather than waiting for all the pens to average 1.02 cm of backfat. Because of this discrepancy in the live animals, carcasses from HA steers tended to be heavier and fatter than carcasses from NA steers. Although mean fat thicknesses were within acceptable limits for all treatments, the EW-HA steers produced carcasses with the most ($P < 0.05$) 12th rib fat. These results suggest that vitamin A may affect adipogenesis in a depot-specific manner because HA steers were fatter and than NA steers (Figure 3-3) and this effect was more pronounced when the vitamin A treatments were fed for a longer period (i.e., EW).

Figure 3.3. Twelfth -rib fat thickness (cm) of carcasses



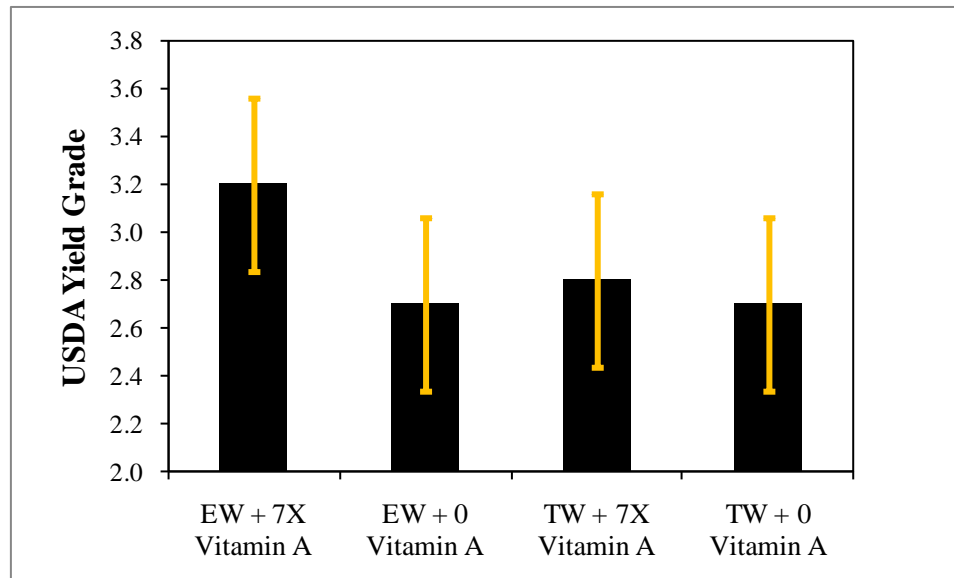
Given the relatively light live and carcass weights of these steers, ribeye areas were generally acceptable among all treatments. Ribeye area is presented in figure 3-4. Supplementing diets with HA may affect the extent of muscle accretion and/or the onset of signaling processes that cause nutrients to be partitioned to fat rather than muscle because carcasses from EW-HA steers tended to be fatter than EW-NA steers. Likewise, within the TW treatment, carcasses from HA steers were fatter ($P < 0.05$) than carcasses from NA steers.

Figure 3.4. Ribeye area (cm²) of carcasses



Percentage of kidney, pelvic, and heart fat (KPH) was similar ($P > 0.20$) among treatments (Table 3-5). Yield grades of the carcasses from all cattle were very desirable, ranging from 1.6 to 3.7. Furthermore, the yield grades should be considered exceptional relative to the carcass marbling (Figures 3-9 and 3-11). Yield grades were similar among TW-HA, TW-NA, and EW-NA treatments ($P > 0.20$). The combination of smaller ribeyes in the fattest carcasses caused USDA yield grades to be numerically higher (i.e., lower cutability) in carcasses from EW-HA steers than other treatments ($P < 0.05$).

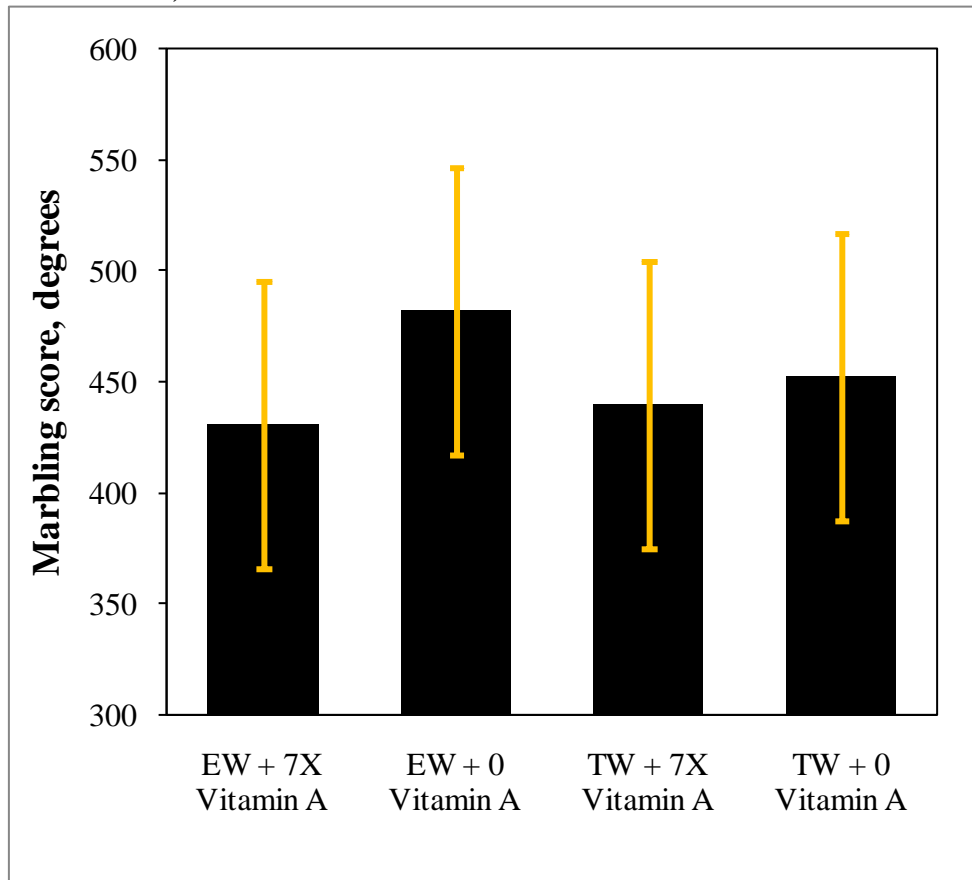
Figure 3.5. USDA Yield Grades of carcasses



Marbling scores for all treatments were very acceptable and this research confirms that when steers with relatively high genetic potential for marbling are managed on a high plane of nutrition with little sickness, high degrees of marbling can be attained by 12 to 13 mo of age without sacrificing cutability. Feeding NA increased ($P < 0.05$) marbling scores compared with feeding HA (Table 3-5; 435 for HA steers and 465 for NA steers), suggesting that feeding NA for at least 150 d increases marbling scores by 0.30 degree, regardless of weaning age. However, the response was more pronounced with EW-NA steers (Figure 3-6). Compared to HA steers at both weaning ages, EW-NA steers produced carcasses that averaged 0.45 degree higher ($P < 0.05$) marbling scores (480 vs. 430 and 440 for EW-NA, EW-HA, and TW-HA steers, respectively). Within the steers fed NA, EW steers produced carcasses with 0.30 degree higher ($P < 0.05$) marbling score than TW steers (480 vs. 450, respectively). These data suggest that either weaning age and/or longer periods of vitamin A depletion are beneficial for enhancing carcass marbling scores. In a related study, Gorocica-Buenfil et al. (2007a) fed either NA or 2,700 IU/kg DM to Angus-cross steers for 168 d but evaluated only TW. They reported a trend ($P = 0.11$) for increased marbling scores in steers fed NA; a result similar to ours. In another study using Holstein steers, Gorocica-Buenfil et al. (2007b) reported numerical increases in marbling scores ($P = 0.36$) and significant increases ($P < 0.05$) in % ether extract in the LM with 243 d of vitamin A depletion. The two studies by Gorocica-Buenfil et al. (2007a,b) did not evaluate EW as a factor. Pyatt et al. (2005) evaluated the effects of vitamin A supplementation levels in the diets of EW steers but they used a “low” vitamin A treatment designed to meet NRC

recommended levels. Thus, my study was the first to feed NA to EW cattle and, taken in summary with these other reports, suggests that EW is favorable for enhancing the effect of feeding NA on carcass marbling scores.

Figure 3.6. Marbling scores for carcasses from the four treatment combinations. 400=Small⁰⁰, 410=Small¹⁰, ... 500= Modest⁰⁰.



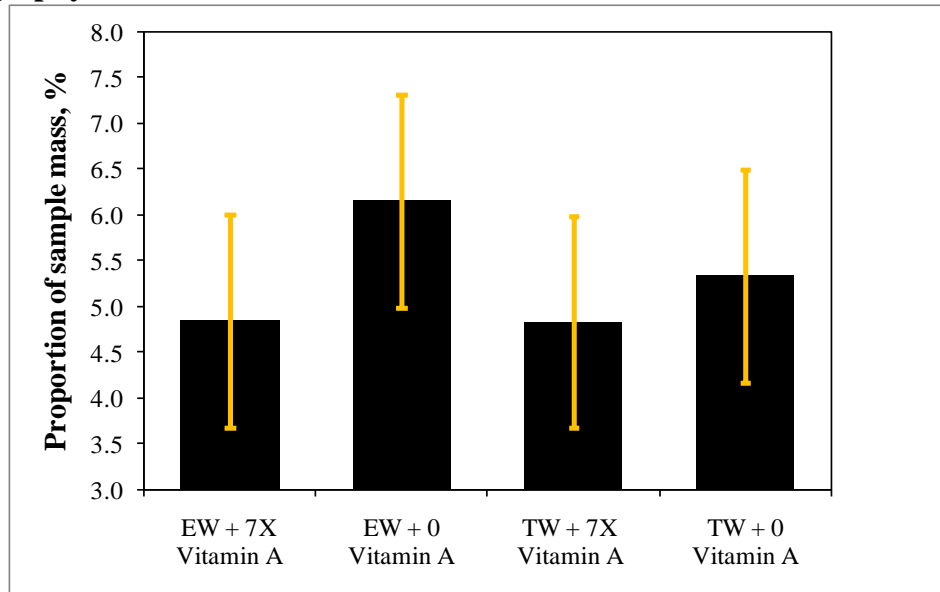
In my study, weaning age could be considered confounded with length of vitamin A treatment because treatment levels of dietary vitamin A were initiated with the growing period of EW steers and did not commence in TW steers until the beginning of the finishing period. Oka et al. (1998) suggested that intramuscular adipose tissue is an immature depot during early stages of fattening and that adipocytes might still be capable of differentiation and proliferation in younger animals. Pyatt et al. (2005) supplemented vitamin A to yearling Angus X Simmental steers to either meet or be 3.3 times greater than NRC recommended levels for 105 d and found no significant advantages to feeding low vitamin A on carcass marbling scores. However, this

study differed from mine in that their steers were fed treatment levels of vitamin A for fewer days and had similar serum retinol levels at harvest. Plus, the yearling steers used in that study had been pastured for 8 mo prior to initiation of the vitamin A treatments. Additional research would be needed to determine if older cattle weaned at similar ages (i.e., yearlings coming off of grass into the feedlot) would respond to the same vitamin A treatments that were used in my research. One obvious challenge would be that yearling cattle would be expected to complete the feedlot finishing period in much fewer days than the scenarios used in my study.

Oka et al. (1998) reported increased carcass marbling scores when 15 mo old Tajima steers were either fed NA or injected i.m. with approximately 1 million IU of vitamin A every 60 d for a 450 d feeding period. In the same publication, Oka et al. (1998) conducted two other experiments and found no difference in carcass marbling scores when 23 and 25 mo old steers received the previous vitamin A treatments and were harvested after 300 and 180 d feeding periods, respectively. It is not possible to determine from their research whether animal age, extent of the vitamin A depletion, or a combination of these factors caused the differentiation in marbling scores in the first experiment, although serum retinol concentrations were similar in the NA steers at harvest in all 3 experiments.

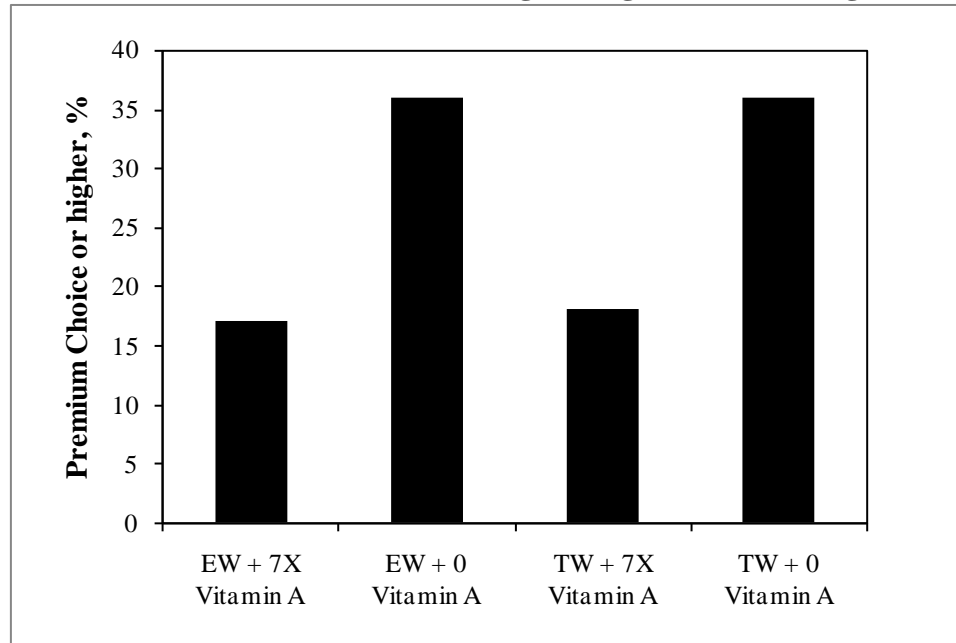
A more objective measure of carcass quality, which is %IMF determined by gas chromatography, supported the marbling scores for the four treatment combinations. Steers fed NA produced carcasses with more ($P < 0.05$) IMF than steers fed HA, regardless of weaning age (Table 3-5 and Figure 3-7). This effect was most pronounced in EW-NA steers, which contained 30% more ($P < 0.05$) lipid than the steers fed HA from both weaning ages. Additionally, the %IMF in the LM of EW-NA steers tended to be higher ($P > 0.10$) than in TW-NA steers, and contained 17% more lipid. These results substantiate the importance of EW in enhancing the effect of feeding NA. My findings agree with those of Kruk et al. (2004) who reported increased IMF in the LM of Angus steers from 9.6 to 13.0% by feeding NA for 300 d, and Gorocica-Buenfil et al. (2007b) who increased the % IMF in the LM of Holstein steers with 243 d of vitamin A depletion. By contrast, Gorocica-Buenfil et al. (2007a) reported IMF values from LM samples that were almost identical between vitamin A treatments, but admitted that reducing the sample size to 2 animals per pen may have compromised their ability to detect differences.

Figure 3.7. Percentage of intramuscular fat in the LM of carcasses as determined by gas chromatography



The percentage of carcasses that qualified for “premium Choice” brands (i.e., black hided cattle with marbling scores of Modest⁰⁰ or higher, such as Certified Angus Beef[®] and others) was doubled ($P < 0.01$) in carcasses from steers fed NA (Figure 3-8). Based on current market premiums for these carcasses, this increase is clearly meaningful to producers and processors. Gorocica-Buenfil (2007a) pointed out that, although the proportion of carcasses that qualified for premium Choice brands was not statistically different due to feeding NA (52.4 vs. 45.7%, $P = 0.32$), the implications to the beef industry were still important. In the marketing system used in their study, carcasses that qualified for premium Choice brands were valued at \$8.00/ 45.4 kg more on a carcass basis. The differences in percent premium Choice and Prime in my study are even more dramatic than in the report by Gorocica-Buenfil et al. (2007a) and underscore that feeding NA is advantageous to carcass value, irrespective of the weaning age.

Figure 3.8. Percent of carcasses with Modest⁰⁰ or higher degrees of marbling



Accounting for the cost of producing marbling

Cattle enthusiasts at all levels seem to have forgotten, or perhaps have never understood, the basic biology of fattening. Marbling is one of several depots used to store excess energy as fat. Relative to protein (i.e., muscle), fat accretion requires approximately 2.25 times more energy per unit, making it a very inefficient process. This explains the reduced gains and feed efficiencies that are usually associated with the fattening phase of any growth model. Due to the increased demand for corn-derived ethanol, increases in the cost of feed ingredients for livestock have intensified the high cost of fattening cattle.

Fortunately, the process of fat accretion (i.e., marbling) continues to be consumer driven and for the first time in history, cattlemen are being rewarded with considerable premiums paid by processors for carcasses with high degrees of marbling. The beef industry should be praised for acting on these market signals and end-user requests (Smith et al., 2006). For instance, expert researchers have developed, and progressive cattle breeders have adopted, objective comparisons of potential parent animals through expected progeny differences (EPD). While these selection tools have propelled cattle breeding to far-reaching expectations, a considerable segment of the purebred and commercial beef industry has used EPD for single trait selection of marbling. A hallmark in animal breeding is that intense selection for one trait will always come at the expense of other traits. We live in a world with exponential population growth that needs

affordable, high-quality animal protein that is produced using water, fossil fuels, and other energy resources that are increasing in demand and decreasing in supply. Frankly, the beef industry desperately needs to recognize the economic and biological costs of producing marbling. Unchecked expenditure for marbling production is short-sighted and does nothing to protect the long-term sustainability of the U.S. beef industry.

These concerns do not spell demise for the beef industry. Every carcass that is evaluated for marbling by USDA graders is conveniently accompanied by a predictor of carcass cutability. The USDA Yield Grade system needs to become more highly regarded by all who are interested in the viability of beef production. Yield grades should be used as a yardstick of the biologic and economic expenditures in the relentless quest for marbling.

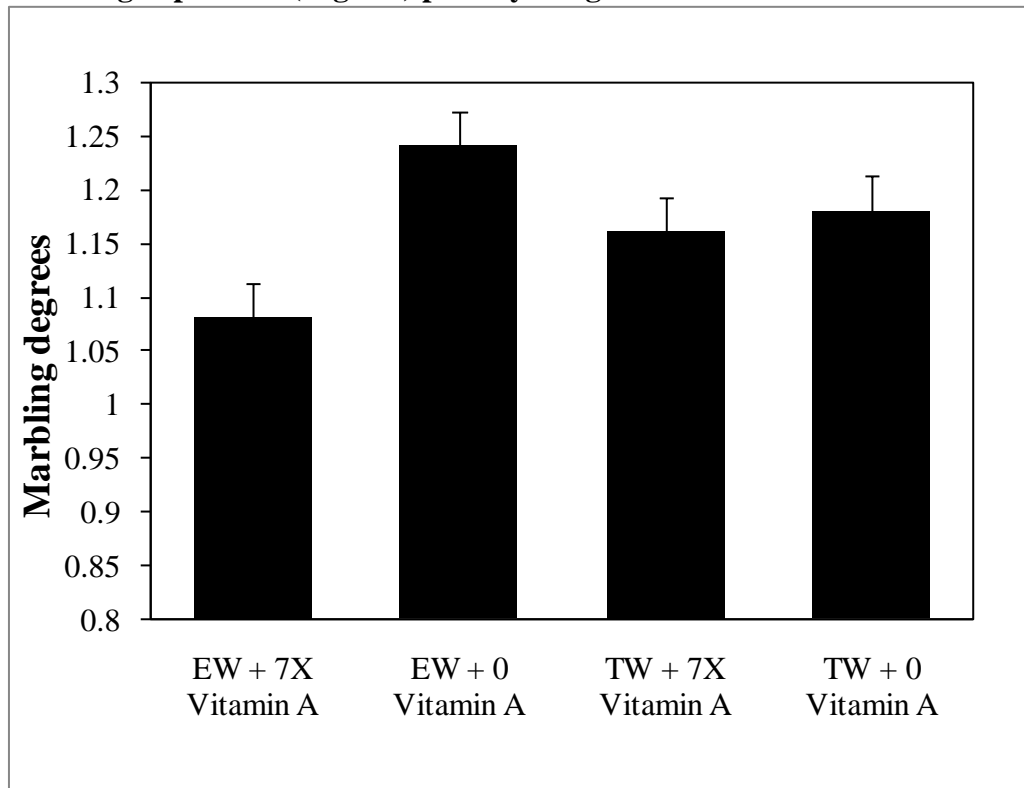
The next several figures illustrate the relationships between both marbling score or % IMF and important measures of production, such as cutability or efficiency characteristics. These ratios are not commonly reported by researchers or understood by producers. Yet, the general inverse relationship between marbling production and percent retail product from a carcass suggests that increases in marbling generally have a price or sacrifice that is paid through decreases in cutability (i.e., increased numeric yield grade). Thus, production strategies that increase marbling without sacrificing carcass cutability are desirable and should be quantified. Feeding NA in the diets of finishing market steers is an example of a feeding practice that has been demonstrated to improve marbling deposition with no obvious sacrifice in USDA yield grade.

Figures 3-9 through 3-13 illustrate that when marbling is expressed in ratios relative to animal age, days on the finishing diet, hot carcass weight, fat thickness, and yield grade, the resultant values support the aforementioned findings, but with an added element of accountability. These ratios are very favorable for NA treatments and this is especially true for EW calves. It is unlikely that these relationships are linear at the extremes of physiological possibilities; but within practical limits, measures such as marbling per yield grade and marbling per fat thickness fit a linear model and could be used as a more practical and effective measure of marbling production.

Although there were no large differences in age at harvest in our study, the EW-NA steers produced the most ($P < 0.05$) marbling per d of age (Figure 3-9). By contrast, EW-HA steers were the least ($P < 0.05$) desirable in marbling per d of age. This is another measure that

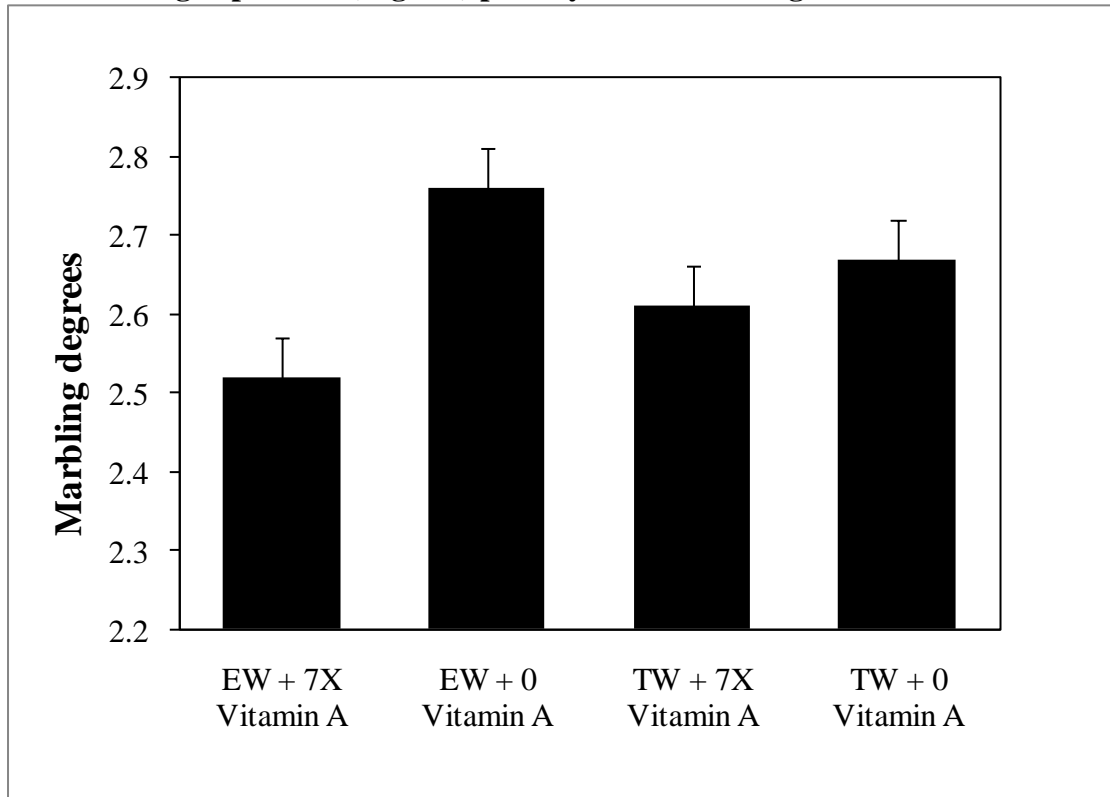
substantiates the magnitude of the differential marbling response when either HA or NA is fed to EW steers. The response was intermediate for TW steers at both levels of vitamin A. This ratio may be particularly useful when EW or calf-fed steers are being fed. In addition, tenderness of beef would benefit from selection pressure that promotes harvesting younger animals.

Figure 3.9. Marbling deposition (degrees) per day of age for steers



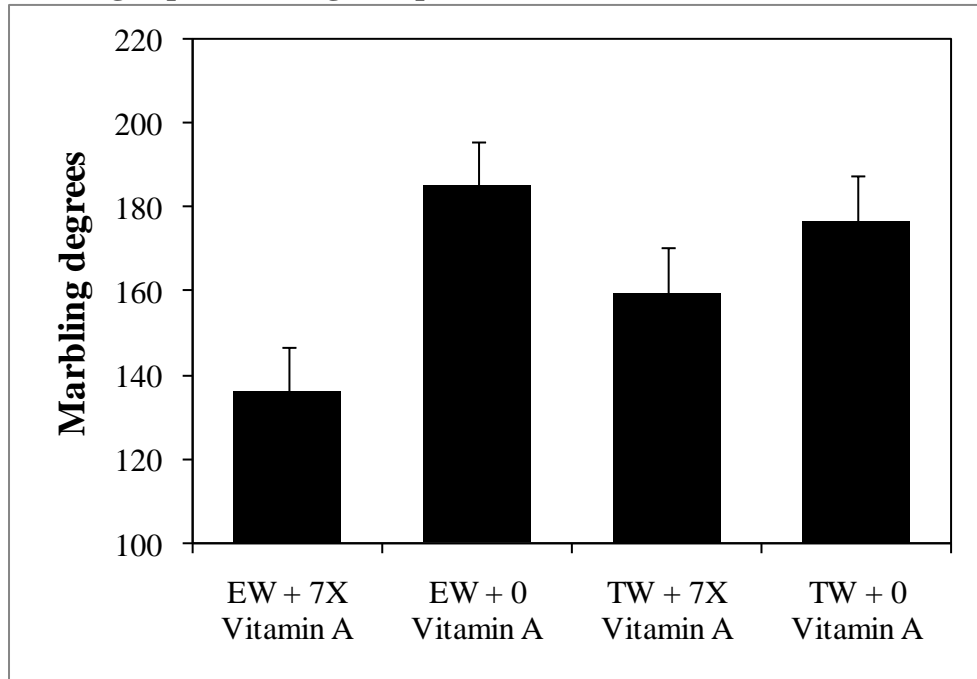
An almost identical response pattern resulted for marbling deposition per d on the finishing diet (Figure 3-10). However, when compared to marbling per d of age, the daily marbling deposition was more concentrated in the comparison of marbling per d on the finishing diet. Intuitively, the high energy finishing diet produced marbling ratios more than double the marbling per d of age but the graphical response was similar for both ratios. The EW-NA steers produced a ratio of over 0.0275 degrees of marbling per d while consuming the finishing diet whereas EW-HA steers produced a ratio slightly more than 0.025 degrees of marbling per d during the finishing period. As with marbling per d of age, TW steers were intermediate in marbling production per d on the finishing diet at both levels of vitamin A. Currently, the high cost of gains make this ratio particularly useful and important.

Figure 3.10. Marbling deposition (degrees) per day on the finishing diet



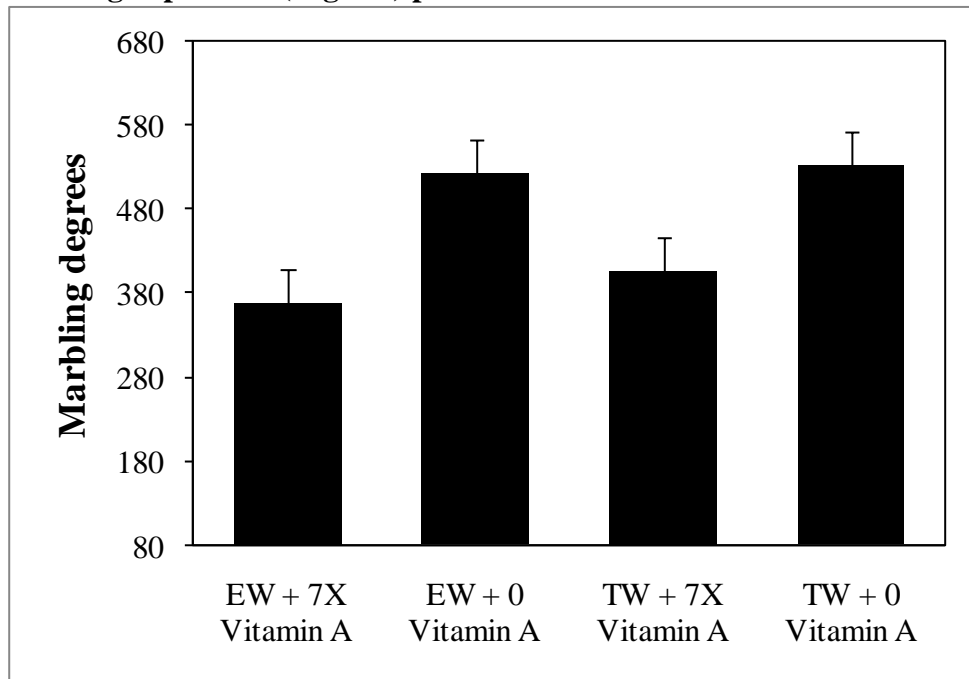
Perhaps the most useful measure of overall efficiency of marbling deposition is the ratio of marbling deposition per USDA Yield Grade (Figure 3-11). Astonishingly, marbling was produced most efficiently (i.e., lowest sacrifice of USDA yield grade) in EW-NA steers. Based on traditional logic, the EW-NA steers might have been expected to produce fatter, lower yielding carcasses because they deposited higher amounts of marbling per d. However, the opposite was true. Not only did EW-NA steers produce more marbling per d of age and per d of finishing than other treatments, they also produced leaner, higher yielding carcasses. Similar to the previous ratios, TW steers at both levels of vitamin A were intermediate in marbling per USDA Yield Grade. Cumulatively, these 3 ratios reveal a synergy that is infrequent in the beef industry and solidifies the merit of EW-NA management of market steers.

Figure 3.11. Marbling deposition (degrees) per USDA Yield Grade



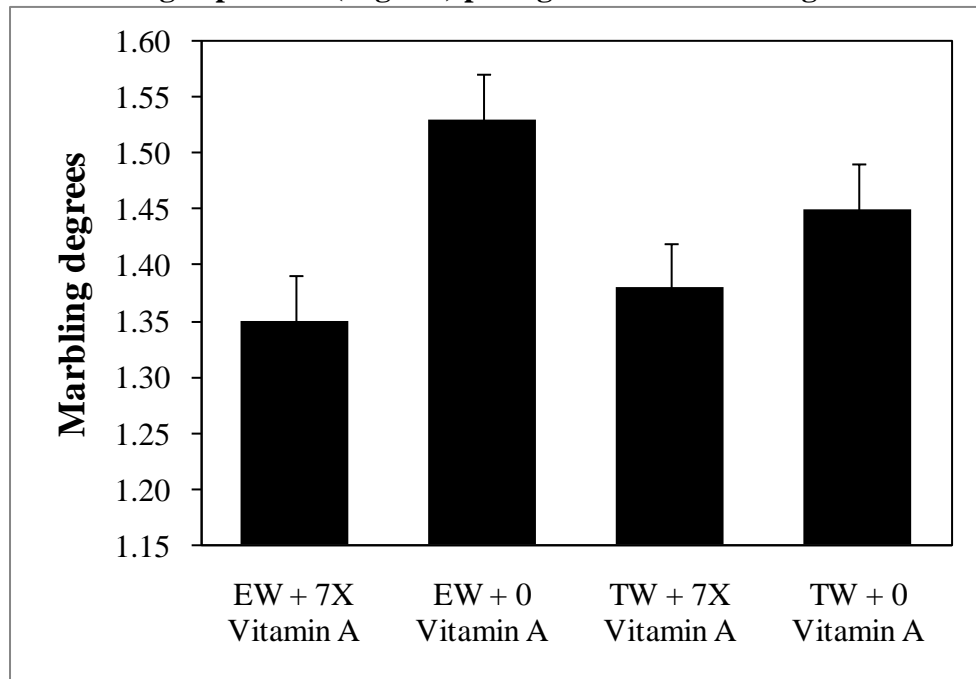
Marbling deposition with respect to USDA yield grade can be further evaluated in detail using ratios of marbling production to yield grade components, such as fat thickness (Figure 3-12) and hot carcass weight (Figure 3-13). Steers fed NA produced carcasses with more desirable combinations of marbling and leanness. Consequently, marbling production per cm of fat was superior in both NA treatments. This ratio suggests that high levels of vitamin A suppress the rate of marbling production and may result in more subcutaneous fat. In contrast, feeding NA apparently allows intramuscular adipocytes to develop and fill with lipid at an accelerated rate that causes marbling to accrue faster than subcutaneous fat. The manner by which dietary vitamin A, or the absence of vitamin A, directs the depots of fat deposition is poorly understood. Future research that measures the 12th rib fat required by HA steers to produce marbling scores comparable to the NA steers in our study would expand our understanding of the relationship between marbling and fat thickness in beef cattle. Regression analyses would likely be the most useful tool to determine if the relationship between marbling and fat thickness is affected by the level supplemental vitamin A and to determine the shape of the response curve as fat and marbling increase.

Figure 3.12. Marbling deposition (degrees) per cm of twelfth-rib fat



Live and carcass weights are the almost exclusive methods for the sale of beef in the U.S. Not surprisingly, EW-NA steers produced the most ($P < 0.05$) marbling in relation to carcass weight (Figure 3-13). Similar to the previous ratios, EW-HA steers produced the least marbling per hot carcass weight. Because profitability is largely determined by weight, this ratio has obvious implications for maximizing marbling production and saleable carcass weight. This ratio may also be useful for measuring the efficiency of marbling production relative to live weight gains and feedlot efficiencies because the initial weights of steers in this study were similar.

Figure 3.13. Marbling deposition (degrees) per kg of hot carcass weight



Fatty Acid Composition of the LM

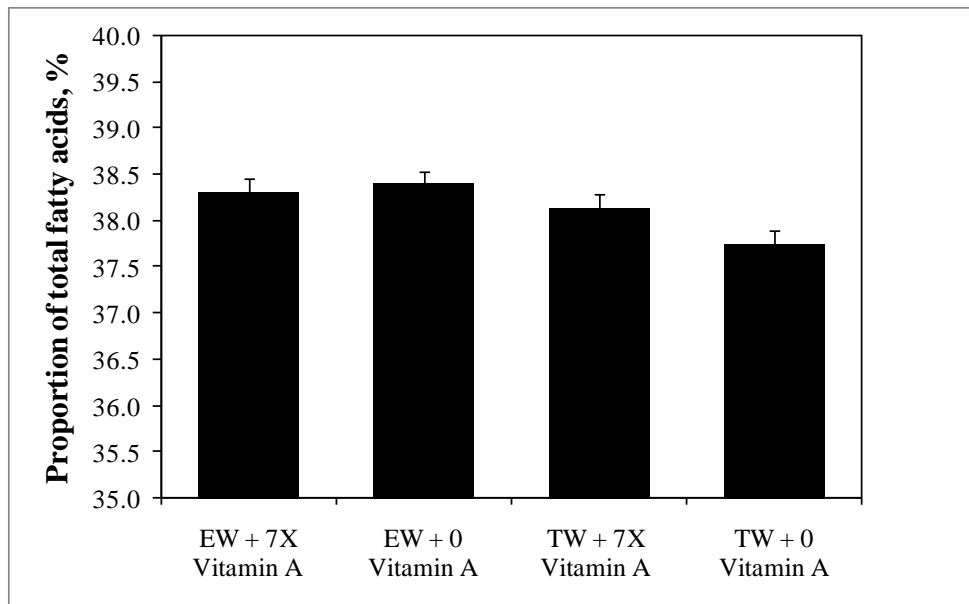
Data on the effect of dietary vitamin A level of cattle on the fatty acid composition and conjugated linoleic acid (CLA) content of beef are very limited. Means for selected fatty acids from the IMF of the LM are presented in table 3-6. There were no important differences among treatments for any of the saturated or unsaturated fatty acids ($P > 0.10$).

Table 3-6. Selected fatty acids from the intramuscular fat of the *longissimus* muscle samples from the four treatment combinations.

Item	High vitamin A		No vitamin A		SEM	P value		
	Weaning age		Weaning age			Vit. A	Wean	Vit. A X Wean
	Early	Traditional	Early	Traditional				
Fatty acid	% of total fatty acids							
14:0	3.4	3.3	3.4	3.2	0.09	0.68	0.87	0.53
16:0	26.9	26.1	26.3	27.0	0.38	0.57	0.31	0.91
16:1	4.5	4.4	4.2	4.1	0.11	0.19	0.36	0.99
17:0	1.6	1.6	1.6	1.6	0.02	0.15	0.22	0.48
18:0	13.3	13.3	14.0	14.0	0.26	0.26	0.54	0.61
18:1n-9 <i>trans</i>	1.9	2.5	2.4	2.2	0.06	0.89	0.65	0.81
18:1n-9 <i>cis</i>	38.3	38.2	38.4	37.8	0.35	0.29	0.72	0.37
18:2n-6 <i>cis</i>	2.7	2.9	2.7	2.8	0.08	0.77	0.84	0.12
18:2n-9 <i>cis</i> ,11 <i>trans</i> CLA	0.32	0.44	0.35	0.39	0.03	0.44	0.16	0.79

Monounsaturated oleic acid is the most significant fatty acid found in beef fat (Table 3-6). Human diets rich in monounsaturated fats have been shown to be as effective as diets with high levels of polyunsaturated fats at reducing serum cholesterol levels (Mensick and Katan, 1989). Treatment means for oleic acid are presented graphically in Figure 3-14. Although beef is an excellent source of monounsaturated fat, there were no differences in oleic acid content of the IMF due to vitamin A or weaning age in my study ($P > 0.10$).

Figure 3.14. Relative contribution of oleic acid (C18:1n-9 *cis*) to the total intramuscular fatty acids in the LM



Gorocica-Buenfil et al. (2007,b) noted that because stearoyl co-A desaturase is required for the synthesis of CLA in ruminants, retinol might cause a reduction in CLA content of ruminant fat because Alam and Alam (1985) presented evidence that retinol reduces the enzymatic activity of stearoyl co-A desaturase. Scollan et al. (2006) reported that the amount of CLA in beef is very small relative to the recommended daily intake for human health benefits. The CLA differences in our study are not likely to be of any practical significance and it is unlikely that feeding NA diets to market steers will alter the CLA content of IMF dramatically. With these data, it would be misleading to suggest that the vitamin A level of cattle diets results in a human health benefit from eating beef from these animals.

CONCLUSIONS AND IMPLICATIONS

There were no apparent negative consequences to health or growth performance from feeding NA to beef steers for up to 235 d (i.e., the duration that my EW steers were fed). Furthermore, there were no apparent toxic effects that resulted from feeding 7X NRC levels of vitamin A. Because of the advantages of feeding NA on carcass marbling and carcass composition, the requirement for feeding vitamin A to cattle may need to be re-evaluated to account for differences in animal age and background. For example, the research by Oka et al. (1998) indicates that older animals may require even longer depletion times to affect marbling deposition.

Feeding no supplemental vitamin A is an effective method for optimizing carcass quality and cutability for either EW or TW management. My results suggest that longer periods of vitamin A depletion (i.e., 235 vs. 175 d) tend to enhance these beneficial carcass outcomes; although the percentage of premium Choice carcasses was increased by feeding NA regardless of the length of vitamin A depletion (i.e., increased marbling for NA at both weaning ages).

Similarly, supplementing high levels of vitamin A to cattle for extended periods may suppress marbling, even as cattle deposit more backfat. On average, EW cattle were fed 60 d longer than TW cattle and supplementing vitamin A at 7 times the NRC-recommendation for 235 d reduced carcass marbling scores and retail yields compared to feeding for 175 d.

Because of the experimental design, it is impossible to know for sure if the duration of vitamin A depletion/supplementation or the younger age when EW calves were started on diets

with treatment levels of vitamin A was responsible for the effects on carcass marbling deposition. It is likely that both factors are important. Vitamin A level has been demonstrated by other researchers to affect carcass marbling, especially when treatment levels were maintained for long periods (Oka et al., 1998). Additionally, younger cattle (i.e., EW) usually have lower hepatic reserves of vitamin A, so creating a treatment-induced divergence in vitamin A status was more expedient and sustainable in EW calves, although this theory is not necessarily supported by the serum retinol data. More research is needed to clarify the effects of vitamin A depletion when initiated at various ages.

Like most management decisions in agriculture, EW of calves is a practice that has advantages and disadvantages. In practice, the decision to EW calves must be weighed against the increased cost of gain in the feedlot and usually a decrease in endpoint live and carcass weights. The recent increase in feed ingredient costs caused by growth of demand for corn-derived ethanol and other novel uses for corn, soybeans, and their byproducts makes EW less appealing economically than ever before. Even with the increase in carcass quality and yield that is associated with EW-NA, the practice of EW should most likely be used only to mitigate production constraints such as drought or other unforeseen shortages of forage for cows and calves.

The use of marbling ratios in this dissertation was particularly useful to demonstrate marbling efficiencies compared to other important production costs. The ratios of marbling versus animal age, d in the feedlot, carcass yield, fat thickness, and weight have fortified the value of practicing EW-NA management of market cattle. If the beef industry must continue to reach for maximum marbling production, producers must be willing to recognize the metabolic, and therefore, economic costs that are associated. Use of ratios give a measure of accountability to marbling that is produced with resources that tend to be increasing in cost and diminishing in supply.

Although beef is an excellent source of monounsaturated fats in human diets, there is little contribution of CLA from beef. The effect of vitamin A supplementation in cattle diets on fatty acid composition of beef is poorly understood at present and there were no significant modifications in proportion of saturated or unsaturated fatty acids due to vitamin A level in this study.

Preliminary research investigated the effect of dietary vitamin A level on marbling deposition in lambs and generally contradicts the results when cattle were used as the ruminant model. Increases in marbling and %IMF in the LM of wether lambs were associated with high levels of dietary vitamin A supplementation. The biological mechanism(s) responsible for these contrasting results in ruminants are not well-understood. There seemed to be some disconnect between the higher circulating levels of fatty acids in lambs fed high vitamin A and the decreased deposition of IMF in the LM muscle. I do not have an explanation for this. Seibert et al. (2000) suggested that cattle might not metabolize retinoids and carotenoids in the same manner as sheep. My study is the only report on the effect of vitamin A supplementation on carcass quality of lambs in the U.S. In general, the effect of vitamin A on carcass traits of lambs remain poorly characterized and further investigation is needed to explain the differences that were found between cattle and lambs in our experiments.

LITERATURE CITED

- Alam, S.Q., and B.S. Alam. 1985. Microsomal fatty acid desaturase activities in vitamin A-deficient rat liver. *Biochem. Biophys. Acta.* 833:175-177.
- Arnett, A.M., M.E. Dikeman, B.J. Johnson, C.W. Spaeth, and B. Hildabrand. 2007. Effects of vitamin A supplementation in young lambs on performance, serum lipid, and longissimus muscle lipid composition. *J. Anim. Sci.* 85:3062-3071.
- Barua, A.B., and J. A. Olson. 1998. Reversed-phase gradient high-performance liquid chromatographic procedure for simultaneous analysis of very polar to nonpolar retinoids, carotenoids and tocopherols in animal and plant samples. *J. of Chromatography.* 707: 69-79.
- Blaner, W.S., and J.A. Olson. 1994. Retinol and retinoic acid metabolism. In *The Retinoids: Biology, Chemistry, and Medicine*. 2nd ed. M.B. Sporn, A.B. Roberts, and D.S. Goodman, *eds.* Raven Press, Ltd, New York, NY
- Gorocica-Buenfil, M.A., F.L. Fluharty, C.K. Reynolds and S.C. Loerch. 2007a. Effect of dietary vitamin A concentration and roasted soybean meal inclusion on marbling, adipose cellularity, and fatty acid composition of beef. *J. Anim. Sci.* 85:2230-2242.
- Gorocica-Buenfil, M.A., F.L. Fluharty, C.K. Reynolds and S.C. Loerch. 2007b. Effect of dietary vitamin A restriction on marbling and conjugated linoleic acid content in Holstein steers. *J. Anim. Sci.* 85:1235-1238.
- Kruk, Z.A., B.D. Siebert, W.S. Pitchford, J. Davis, G.S. Harper, and C.D.K. Bottema. 2004. The effect of vitamin A on fatness in lot-fed cattle: (I) Fat Quantity. *Proc. 50th International Congress of Meat Sci. and Technol., University of Helsinki, Helsinki, Finland.*
- Mensick, R.P., and M.B. Katan. 1989. Effect of diet enriched with monounsaturated or polyunsaturated fatty acids on levels of low-density and high-density lipoprotein cholesterol in women and men. *N. Engl. J. Med.* 321:436-452.
- NRC. 1996. *Nutrient Requirements of Beef Cattle*. 7th rev. ed. National Academy Press. Washington, DC.
- NRC. 2001. *Nutrient requirements of Dairy Cattle*. 7th rev. ed. National Academy Press. Washington, DC.
- Oka, A., Y. Maruo, T. Miki, T. 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.

- Olson, K.C., R.J. Rasby, T.B. Schmidt, and J.R. Jaeger. 2007. Early weaning as a drought mitigation strategy for commercial cattle ranches. Proc. Western Nutrition Conference 28(1):349-364. Published by Animal Nutrition Association of Canada, Saskatoon, SK.
- 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.
- SAS. 2002. SAS/STAT User's Guide (Version 9.1). SAS Inst., Inc., Cary, NC.
- Scollan, N., J.F. Hocquette, K. Nuernberg, D. Dannenberger, I. Richardson, and A. Moloney. 2006. Innovations in beef production systems that enhance the nutritional and health value of beef lipids and their relationship with meat quality. Meat Sci. 74:17-33.
- Smith, G.C., J.W. Savell, J.B. Morgan, and T.E. Lawrence. 2006. Report of the June-September, 2005 National beef quality audit: A new benchmark for the US beef industry. Proc. 38th Beef Improvement Federation, Choctaw, MS, Published by Mississippi State University, MS State, MS pp. 6-11.

Appendix A - Regression Analyses and Prediction Equations

Regression of Carcass Marbling Score and % Intramuscular Fat on Liver Retinol Concentration

This dissertation research has practical implications for adding value to beef and increasing profitability to producers. Regression analyses provide unique opportunities for understanding the relationships between factors that were investigated in the experiment. Because the liver is the master depository and reservoir for retinol storage, the relationship between retinol status of the liver and carcass marbling scores provides an important contribution to scientific knowledge. In addition, these regressions may have predictive value for marbling and can serve as models for future research. Oka et al. (1998) was the first to regress marbling on serum retinol.

Figure A- 1. Regression of carcass marbling score on liver retinol concentration

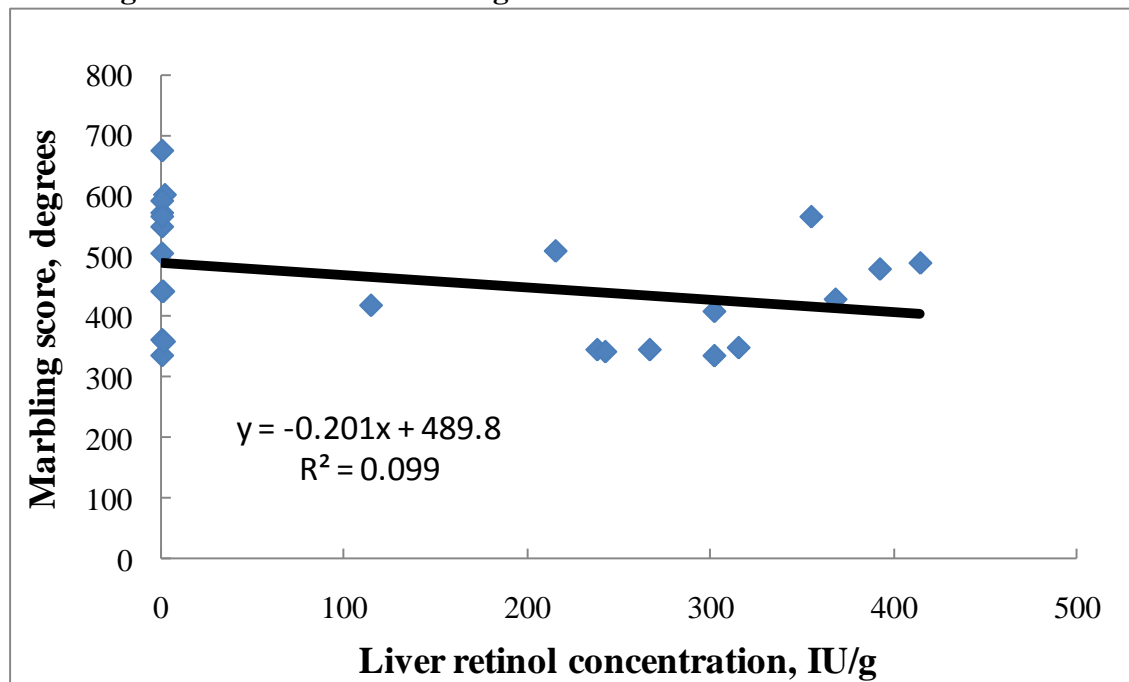
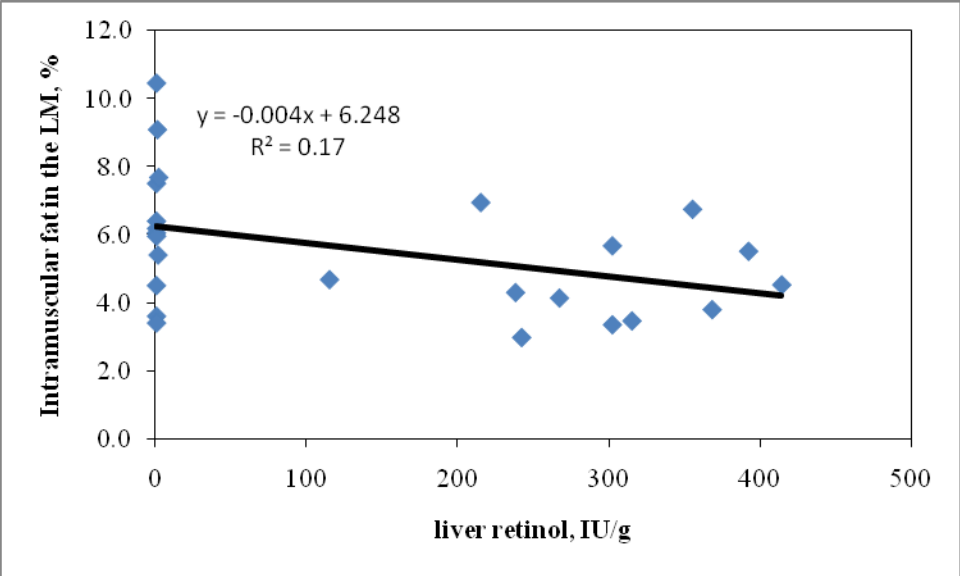


Figure A- 2. Regression of % intramuscular fat in the longissimus muscle on liver retinol concentration



Appendix B - Effect of Supplementing either 1x or 7x NRC Levels of Dietary Vitamin A to Early-weaned Steers

MATERIALS AND METHODS

Animals and experimental procedures

Early-weaned steers (n = 191) were used to evaluate the effects of dietary vitamin A level on carcass marbling development. Calves were genetically similar to and were managed using the same procedures and locations described for experiment 1. Upon arrival to the Hays experiment station, calves were randomly allotted to one of 10 pens (5 replications per treatment combination) to receive either 6,025 (1X) or 42,180 IU hd·d⁻¹ (7X the recommended level) of supplemental vitamin A in the preconditioning and finishing diets (NRC, 1996). Calves in experiment 2 were managed and harvested according to the protocol described for experiment 1. The cattle in experiment 2 were harvested in 3 groups to minimize variation in fat thickness, although the first and second harvest days were the same day as for experiment 1. While complete carcass data were collected as described for experiment 1, muscle and fat samples were not collected in experiment 2. Cattle in experiment 2 were covered under the same protocol approved by Kansas State University's IACUC.

Statistical Analyses

A completely random design with a single treatment structure was used. There were 5 pens per treatment containing either 19 or 20 animals per pen. Differences in means were detected using the PROC MIXED procedure of SAS[®], Cary, NC.

Results and Discussion

Growth Performance

Initial average BW on d 0 of the finishing period was (258±29.5 kg) and weights were not different ($P > 0.10$) between treatments. Treatment levels of dietary vitamin A were initiated on October 20, 2006 and continued until harvest on April 3rd, May 8th, or June 12th, 2007. Steers gained 1.4±0.3 kg·d⁻¹ during the finishing period when the experimental levels of vitamin A were fed.

Table B- 1. Growth performance and carcass traits of early-weaned steers fed either 1X or 7X recommended levels of vitamin A. ^aFor marbling score, 400= Small⁰⁰, 410= Small¹⁰,...500=Modest⁰⁰.

Item	7X vitamin A	1X vitamin A	SEM	P value
Finishing period initial weight, kg	260	255		
Finishing period final weight, kg	492	495		
Body weight gain, kg/d	1.4	1.4		
12th rib fat, cm	1.00	1.10		
USDA Yield Grade	3.1	3.1		
Marbling score ^a	483	475		
Premium Choice and Prime, %	34.0	33.7		

Carcass Traits

In general, carcass yield and quality grades were very acceptable, and were not affected by the level of supplemental vitamin A in the diet. Mean twelfth rib fat thickness was similar and USDA yield grades were identical for the two levels of vitamin A.

Marbling scores tended to be higher ($P = 0.13$) in the carcasses from the HA steers, although this difference of only 0.08 degree is of little practical significance and would not affect carcass value. The proportion of carcasses that qualified for premium Choice brands (i.e., Modest⁰⁰ and higher marbling) were nearly identical and extremely favorable compared to industry averages. When marbling deposition was measured in relation to yield grade and

external fatness of the carcasses, feeding 7X appears to be favorable to 1X vitamin A (Figures B-1 and B-2).

Figure B- 1. Marbling deposition per USDA Yield Grade for carcasses from steers in Experiment 2

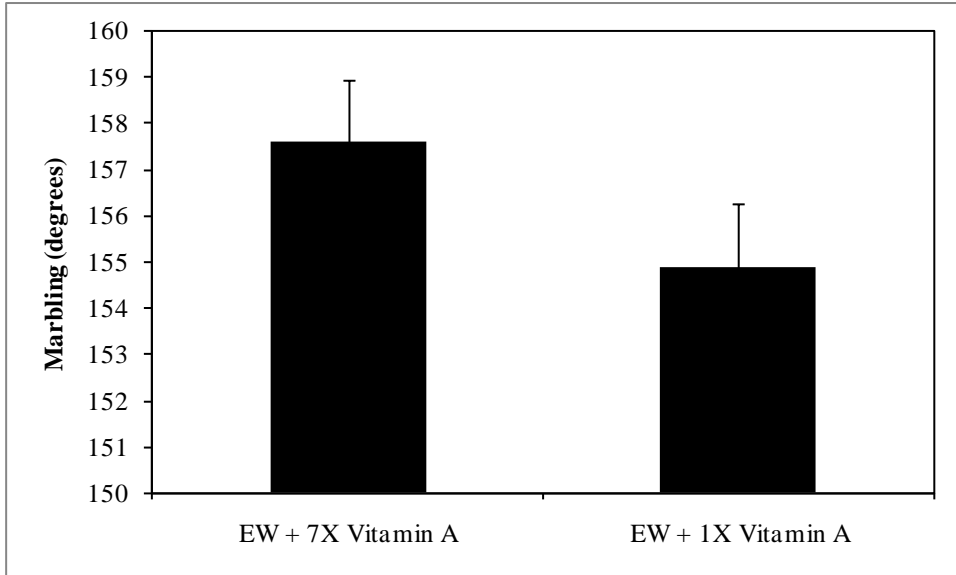
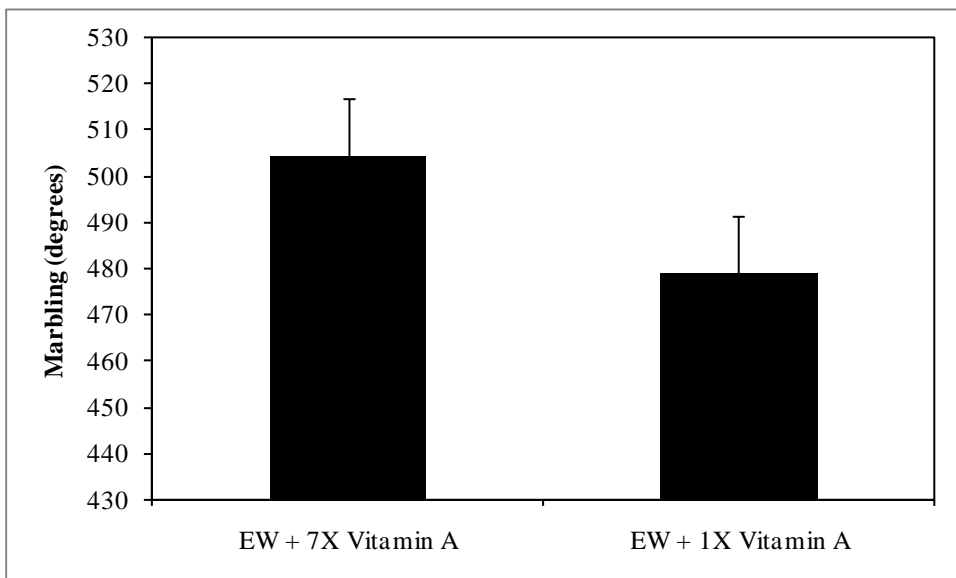


Figure B- 2. Marbling production per cm of 12th rib fat for carcasses from steers in Experiment 2



SUMMARY AND CONCLUSIONS

The importance of feeding NA vs. feeding at the published requirement is apparent in our data and is confirmed by the findings of others. Because of the potential for reduced growth performance and possibly more serious health consequences, the decision was made to feed the larger group of EW cattle the published requirement of vitamin A as the “low” vitamin A treatment for experiment 2, rather than feeding NA as in experiment 1. The HA diets were the same in experiments 1 and 2. Similarly, Pyatt et al. (2005) used the published requirement for vitamin A as the “low” treatment level compared to feeding 3.3 times the published requirement fed to EW steers and heifers. These authors found no difference in carcass marbling scores and our data support their findings. Considering the more favorable outcomes of feeding NA as in experiment 1, Gorocica-Buenfil et al. (2007), and others, it appears that feeding only basal levels of supplemental vitamin A may be detrimental to carcass marbling production in cattle.