

Effects of Vitamin E Supplementation in Late Gestation Cattle and Evaluation of Vitamin E, Cholesterol, and Phospholipid Relationships in Bovine Serum and Serum Lipoproteins

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(ABSTRACT)

The effects of vitamin E supplementation during late gestation were evaluated in dairy or beef cattle in three experiments. Dairy cows were injected with vitamin E and selenium (Se) in one study; beef cows were offered oral vitamin E supplementation via a free-choice vitamin-mineral mix in two studies. Breed-related effects were also evaluated. Jerseys had higher blood Se and lower serum vitamin E concentrations than Holsteins at dry-off and higher blood Se concentrations than Holsteins 3-4 weeks pre-calving and at calving. Selenium supplementation increased blood Se concentrations at calving. Treatment did not affect serum vitamin E concentrations at calving or post-calving, nor blood Se concentrations post-calving. Beef cattle consuming supplemental vitamin E (treatment) had greater responses to treatment when calving in late winter than when calving in late summer. Treated multiparous cows calving in winter had increased serum and colostral vitamin E concentrations but treatment did not affect colostral or serum immunoglobulin G (IgG) concentrations of their calves. Calves from treated, multiparous cows calving in winter had increased 205-day adjusted weaning weights (AWWs). Treatment did not affect colostral vitamin E or IgG concentrations of nulliparous heifers calving in winter, nor serum vitamin E or IgG concentrations, or AWWs of their calves. Six hundred IU supplemental vitamin E/head/day did not affect serum vitamin E concentrations of dams, colostral vitamin E or IgG concentrations, serum vitamin E or IgG concentrations, or growth of calves in the summer-calving herd. Breed-related differences in vitamin E concentrations and AWW occurred in both herds. Consumption of 600-1000 IU vitamin E/cow/day (treatment) during late gestation via a free-choice vitamin-mineral mix increased vitamin E concentrations in serum and the lipoprotein fraction containing no apolipoprotein B (non-ApoB) from 1-2 weeks pre-calving to calving. Treatment group calves had higher vitamin E concentrations in serum and non-ApoB than control group calves. Treatment did not affect cholesterol or phospholipid

concentrations in serum or the lipoprotein fractions of either cows or calves. Treatment increased vitamin E cholesterol (VEC) and vitamin E phospholipid (VEPL) ratios in the serum and non-ApoB of cows and calves. Various breed-related differences also occurred.

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Table of Contents

Abstract	i
Grant Information	ii
Acknowledgments	iii
List of Tables	vii
List of Figures	x
List of Appendix Tables	xi

<u>Chapter</u>	<u>Page</u>
Chapter 1. Introduction	1
Chapter 2. Review of the Vitamin E Literature	3
Introduction.....	3
Historical Information.....	5
Absorption, Transport, and Tissue Uptake	6
Serum Lipoproteins of Cattle.....	8
General Function and Activity.....	9
Dietary Requirements of Production Animals.....	11
Factors Influencing Dietary Requirements	13
Research in Selected Production Species.....	14
Horses.....	14
Swine.....	15
Small Ruminants.....	15
Research in Cattle	16
Chapter 3. Effects of Dry Period Supplementation with Parenteral Vitamin E and Selenium in Jersey and Holstein Cows	19
Abstract.....	19
Introduction.....	19
Materials and Methods.....	20
Results.....	23
Discussion.....	23
Data Tables.....	26
Chapter 4. Effects of Oral Vitamin E Supplementation During Late Gestation in Beef Cattle Calving in Late Winter	27
Abstract.....	27
Introduction.....	28
Materials and Methods.....	29
Results.....	31
Discussion.....	33
Data Tables.....	38

Chapter 5. Effects of Oral Vitamin E Supplementation During Late Gestation in Beef Cattle Calving in Late Summer	41
Abstract	41
Introduction	41
Materials and Methods	43
Results	45
Discussion	46
Data Tables	49
Chapter 6. Effects of Oral Vitamin E Supplementation on Concentrations of Vitamin E, Phospholipid, and Cholesterol in Serum and Serum Lipoprotein Fractions of Beef Cattle	51
Abstract	51
Introduction	52
Materials and Methods	55
Results	56
Discussion	58
Data Tables	63
Figures	67
Chapter 7. Summary and Conclusions	68
Literature Cited	72
Appendices	92
A. Composition of free choice vitamin-mineral mix	92
B. Formula for calculating 205-day adjusted weaning weight	93
C. Analysis of vitamin E concentrations in serum and lipoprotein fractions	94
D. Analysis of colostrum vitamin E concentration	96
E. Analysis of serum IgG concentration	98
F. Analysis of colostrum IgG concentration	100
G. Elution of lipoprotein fractions containing no apolipoprotein B (non-ApoB) and containing apolipoprotein B (ApoB)	102
H. Analysis of phospholipid concentration	104
I. Analysis of cholesterol concentration	106
J. Linear regressions of vitamin E, cholesterol, and phospholipid in serum and two lipoprotein fractions of cows 1-2 weeks pre-calving, cows at calving, 24-48 hour-old calves, and analyte concentration changes pre-calving to calving	108
K. General Linear Model Tables – Chapter 3	112
L. General Linear Model Tables – Chapter 4	116
M. General Linear Model Tables – Chapter 5	123
N. General Linear Model Tables – Chapter 6	128
O. Additional Dairy Cow Data – Chapter 3	143
P. Additional Beef Cow Data – Chapter 4	144

Q. Additional Beef Cow Data – Chapter 5	147
Vita	149

List of Tables

<u>Table</u>	<u>Page</u>
Chapter 2.	
2-1. Alpha-tocopherol content of various feedstuffs on an as-fed basis	4
2-2. Clinical syndromes resulting from vitamin E and/or selenium deficiencies.....	12
Chapter 3.	
3-1. Effect of breed on serum vitamin E and blood selenium concentrations in periparturient Jersey and Holstein cows.	26
3-2. Effect of parenteral vitamin E or selenium (Se) supplementation during late gestation on serum vitamin E or blood Se concentrations of periparturient Jersey and Holstein cows.....	26
Chapter 4.	
4-1. Effect of oral vitamin E supplementation (avg. 1000 IU/head/day) during late gestation on serum vitamin E concentrations in a herd of periparturient Angus and Hereford cows (Trial 1) and a herd of nulliparous Angus and Hereford heifers (Trial 2) calving in late winter.....	38
4-2. Effect of oral vitamin E supplementation (avg. 1000 IU/head/day) during late gestation on concentrations of colostral vitamin E and immunoglobulin G (IgG) in a herd of periparturient Angus and Hereford cows (Trial 1) and a herd of nulliparous Angus and Hereford heifers (Trial 2) calving in late winter	38
4-3. Effect of oral vitamin E supplementation (avg. 1000 IU/head/day) during late gestation in a herd of periparturient Angus and Hereford cows (Trial 1) and a herd of nulliparous Angus and Hereford heifers (Trial 2) calving in late winter on serum vitamin E and immunoglobulin G (IgG) concentrations of 24-48 hour-old calves and 205-day adjusted weaning weights (AWWs).....	39

4-4. Effect of breed and oral vitamin E supplementation (avg. 1000 IU/head/day) during late gestation on serum vitamin E concentrations of 24-48 hour-old Angus and Hereford calves born in late winter to multiparous cows	39
4-5. Effect of breed on serum vitamin E concentrations during the periparturient period in a herd of multiparous Angus and Hereford cows (Trial 1) and a herd of nulliparous Angus and Hereford heifers (Trial 2) calving in late winter	40

Chapter 5.

5-1. Effect of oral vitamin E supplementation (avg. 600 IU/head/day) during late gestation on serum vitamin E concentrations in periparturient Angus and Hereford dams calving in late summer.....	49
5-2. Effect of oral vitamin E supplementation (avg. 600 IU/head/day) during late gestation on the IgG and vitamin E concentration of colostrum from Angus and Hereford dams calving in late summer	49
5-3. Effect of supplementing Angus and Hereford dams calving in late summer with oral vitamin E (avg. 600 IU/head/day) during late gestation on serum vitamin E and immunoglobulin G concentrations of their 24-48 hour-old calves and on 205-day adjusted weaning weights of their calves.....	50
5-4. Effect of breed on serum vitamin E and colostral IgG concentrations in Angus and Hereford dams calving in late summer and on serum vitamin E and IgG concentrations and 205-day adjusted weaning weights of their calves.....	50

Chapter 6.

6-1. Effects of time, breed, and consumption of 600-1000 IU/head/day supplemental vitamin E (treatment) during late gestation on concentrations of vitamin E, cholesterol, and phospholipid in serum and two lipoprotein fractions of beef cows between 1-2 weeks pre-calving and calving	63
6-2. Effects of time, breed, and consumption of 600-1000 IU/head/day supplemental vitamin E (treatment) during late gestation on vitamin E cholesterol (VEC) and vitamin E phospholipid (VEPL) ratios in serum and two lipoprotein fractions of beef cows between 1-2 weeks pre-calving and calving	64

6-3. Effects of maternal consumption of 600-1000 IU/head/day supplemental vitamin E (treatment) during late gestation and breed on concentrations of vitamin E, cholesterol, and phospholipid in serum and two lipoprotein fractions of beef calves at 24-48 hours of age 65

6-4. Effects of maternal consumption of 600-1000 IU/head/day supplemental vitamin E (treatment) during late gestation and breed on vitamin E cholesterol (VEC) and vitamin E phospholipid (VEPL) ratios in serum and two lipoprotein fractions of beef calves at 24-48 hours of age 65

6-5. Effect of treatment by time interaction on LDL+VLDL cholesterol concentrations, serum vitamin E cholesterol (VEC) ratios, and serum and HDL vitamin E phospholipid (VEPL) ratios of beef cows from 1-2 weeks pre-calving until calving 66

6-6. Effect of breed by time interaction on serum vitamin E cholesterol (VEC) and vitamin E phospholipid (VEPL) ratios and on HDL VEC ratios in beef cows between 1-2 weeks pre-calving and calving 66

List of Figures

<u>Figure</u>	<u>Page</u>
Chapter 2.	
2-1. Structure of alpha-tocopherol and alpha-tocopheryl acetate	4
2-2. Function and activity of vitamin E at the cellular level.....	10
2-3. Antioxidant system locations within the cell.....	11
Chapter 6.	
6-1. Relationships between vitamin E and cholesterol, and vitamin E and phospholipid in the serum of beef cows 1-2 weeks pre-calving, cows at calving, and 24-48 hour-old calves	67

List of Appendix Tables

<u>Table</u>	<u>Page</u>
Appendix K: General Linear Model Tables- Chapter 3.....	110
1-4. Serum vitamin E concentrations.....	110
5-8. Blood Se concentrations	112
Appendix L: General Linear Model Tables- Chapter 4.....	114
1-8. Serum vitamin E concentrations.....	114
9-10. Colostral vitamin E concentrations.....	117
11-12. Calf serum IgG concentrations	118
13-14. Colostral IgG concentrations	119
15-16. Calf 205-day adjusted weaning weights	120
Appendix M: General Linear Model Tables- Chapter 5.....	121
1-5. Serum vitamin E concentrations.....	121
6. Colostral vitamin E concentrations.....	123
7. Calf serum IgG concentrations	124
8. Colostral IgG concentrations	124
9. Calf 205-day adjusted weaning weights	125
Appendix N: General Linear Model Tables- Chapter 6.....	126
1-6. Vitamin E concentrations	126
7-12. Cholesterol concentrations.....	129
13-18. Phospholipid concentrations.....	132
19-24. Vitamin E cholesterol (VEC) ratios.....	135
25-30. Vitamin E phospholipid (VEPL) ratios	138
Appendix O: Additional Dairy Cow Data (Chapter 3).....	141
1. Selenium concentrations of pastures used in dry cow study.....	141
2. Body condition scores of periparturient cows by breed.....	141
3. Body condition scores of periparturient cows by treatment group	141

Appendix P: Additional Data from the Winter-calving Herd (Chapter 4)..... 142

1. Fat, lactose, and protein percentage of colostrum in treated and untreated Angus and Hereford cattle calving in late winter..... 142
2. Fat, lactose, and protein percentage and vitamin E concentration of colostrum from Angus, Hereford, and Continental breed cattle calving in late winter 142
3. Body condition scores and weights in treated and untreated Angus and Hereford cattle calving in late winter..... 143
4. Body condition scores and weights of Angus, Hereford, and Continental breed cattle calving in late winter..... 143
5. Birth weights and gender distributions of calves from treated and untreated Angus and Hereford cattle calving in late winter..... 144
6. Birth weights and gender distributions of calves from Angus, Hereford, and Continental breed cattle calving in late winter 144

Appendix Q: Additional Data from the Summer-calving Herd (Chapter 5)..... 145

1. Fat, lactose, and protein percentage of colostrum in treated and untreated Angus and Hereford cattle calving in late summer 145
2. Fat, lactose, and protein percentage and vitamin E concentration of colostrum from Angus, Hereford, and Continental breed cattle calving in late summer..... 145
3. Body condition scores and weights in treated and untreated Angus and Hereford cattle calving in late summer..... 146
4. Body condition scores and weights of Angus, Hereford, and Continental breed cattle calving in late summer 146
5. Birth weights and gender distributions of calves from treated and untreated Angus and Hereford cattle calving in late summer 147
6. Birth weights and gender distributions of calves from Angus, Hereford, and Continental breed cattle calving in late summer 147

Chapter 1

Introduction

Sound nutritional programs are cornerstones of successful ventures in animal agriculture. Other considerations, such as genetics and management, are also important. However, even the most genotypically superior animal will perform poorly without proper nutrition. Finishing steers would not gain five pounds a day without exceptional feed bunk management. Broilers could not attain market-ready weights in five to six weeks without adequate nutrient intake to support such a rapid growth rate. Individual dairy cows could not produce 50,000 pounds of milk in a year, nor could dairy herds average greater than 30,000 pounds of milk production over the same time period, without consuming properly formulated rations.

Diets must deliver adequate amounts of nutrients in order for animals to produce at optimal levels. Furthermore, the supplementation of individual essential nutrients can at times enhance animal performance or alleviate a recognized deficiency. Such scenarios, particularly those of enhanced performance through single nutrient supplementation, continue to be identified on a regular basis. Examples include providing supplemental protein to cattle on western range lands, “flushing” ewes with increased dietary energy pre-breeding to enhance ovulation rate/fertility, and giving supplemental calcium to recently fresh dairy cows to decrease metabolic disorders and periparturient disease. Supplementing Se to ruminants in Se-deficient geographic regions and increasing the dietary copper and zinc content for young, growing horses to reduce the incidence of developmental orthopedic disease are two more examples of this practice.

Vitamin E, a dietary essential, fat-soluble vitamin, can enhance animal performance when provided in amounts above minimal requirements. Claims attributed to supranutritional provision of vitamin E vary from preventing cancer and cataracts in humans, to enhancing fertility in rats, improving immunity in swine, and preventing mastitis in dairy cows. Research on vitamin E supplementation in beef cattle has revealed several promising findings. However, uncertainties remain regarding such issues as the effects of vitamin E supplementation on colostral vitamin E and immunoglobulin concentration, passive immunity in calves, and calf growth.

Experiments included in this dissertation were therefore conducted to evaluate the effects of vitamin E supplementation during late gestation in both dairy and beef cattle. Effects of parenteral vitamin E and selenium supplementation during the dry period were determined in Holstein and Jersey cows. Effects of oral vitamin E supplementation during late gestation were examined in two herds of beef cattle—one calving in late winter and the other calving in late summer. Potential breed differences in vitamin E metabolism were also investigated in both the beef and dairy studies. Lipoprotein cholesterol and phospholipid concentrations were examined concurrently with vitamin E concentrations to attempt explanation of treatment and breed-related differences in the beef studies. Lastly, a novel means of indicating vitamin E status per lipoprotein particle, the vitamin E phospholipid (VEPL) ratio, was investigated.

Chapter 2

Review of the Vitamin E Literature

Introduction

Vitamin E is one of four fat-soluble vitamins required by all mammals. Natural forms of vitamin E are synthesized in plants and are comprised of a group of related compounds, the tocopherols and tocotrienols, which demonstrate various degrees of biological activity. Structurally, both families of compounds contain a hydroquinone nucleus and isoprenoid side chain but differ with respect to side chain saturation and methyl group placement on the chroman ring.¹ The Greek letters α , β , γ , and δ structurally designate each tocopherol (tocol) and tocotrienol. Structural differences control the vitamin E activity for the given compounds. Alpha tocopherol is the most prevalent and exhibits the highest degree of biological activity of the naturally occurring forms of vitamin E.^{2,3} Further evidence of its importance is provided by a 1982 study wherein pigs and cattle were fed diets containing a variety of tocols, but only absorbed and utilized α -tocopherol.⁴ Although α -tocopherol is of primary importance, β -tocopherol, γ -tocopherol, and α -tocotrienol demonstrate 15-40% of α -tocopherol's biological activity.⁵

Vitamin E is abundant in vegetable oils, the germ fraction of most grains, egg yolk, liver, and green, leafy plants. Vitamin E concentrations vary in common livestock feeds (Table 2-1). Commercially available sources are synthesized from deodorizer distillates, a byproduct of vegetable oil manufacture and refinement.² These distillates contain a mixture of α -, β -, γ -, and δ -tocopherols. Alpha-tocopherol is extracted, then methylated following purification by ultra-vacuum molecular distillation.² The resulting α -tocopherol concentrate is often acetylated to produce α -tocopheryl acetate (Figure 2-1), a compound more resistant to oxidation and therefore more stable during feed processing and storage. Oxidative destruction of vitamin E is enhanced by exposure to oxygen, heat, moisture,² and UV light,⁶ as well as contact with polyunsaturated fatty acids⁷ and certain trace minerals.²

Table 2-1. Alpha-tocopherol concentrations of various feedstuffs on an as-fed basis.⁸

<u>Feedstuff</u>	<u>Mean Concentration (mg/kg)</u>	<u>Range</u>
Barley	7.4	4-11
Oats	7.8	4-11
Corn	8.3	0-21
Wheat	11.6	5-30
Corn gluten feed	15.5	2-42
Wheat bran	16.5	10-25
Wheat middlings	20.1	2-41
Soybean meal	2.3	0-8
Whole soybeans	18.1	11-33
Grass	8.1	5-15
Grass silage	23.4	10-92
Grass hay	12.7	1-21
Corn silage	3.1	0-9
Fish meal	5.0	0-18
Meat and bone meal	1.6	0-5

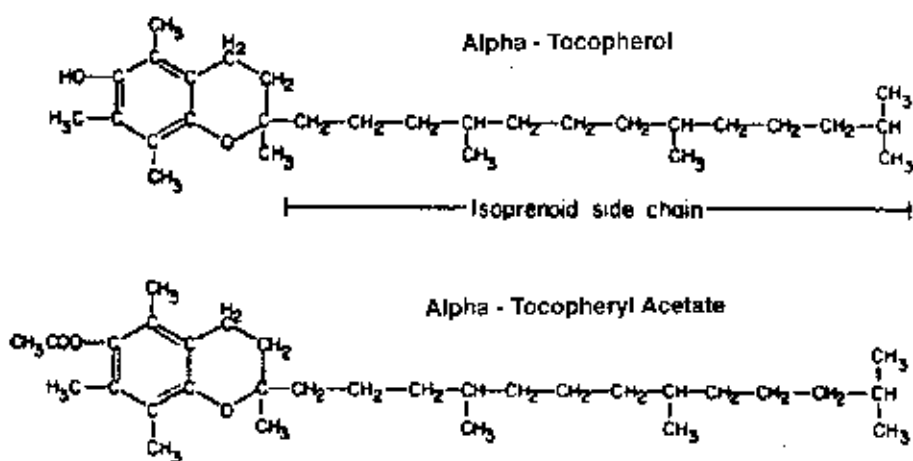


Figure 2-1. Structure of alpha-tocopherol and alpha-tocopheryl acetate.¹

Vitamin E is absorbed with fat and digestion is facilitated by the activity of bile and pancreatic lipase.⁹ Absorption occurs in the small intestine and is impaired by malabsorptive disorders.¹⁰ The alcohol form is absorbed directly by the intestinal wall, whereas the ester form must be hydrolyzed to the alcohol form within the gut lumen prior to absorption.² Once absorbed, vitamin E is incorporated into chylomicrons, enters the intestinal lacteals, and is delivered to the general circulation as a component of lymph.

The primary role of vitamin E in biological systems is that of an antioxidant. The vitamin neutralizes free radicals and prevents oxidative damage of intramembranous and intracellular lipids. Although vitamin E content varies throughout the body, the highest concentrations occur within tissues of high lipid content (e. g. adipose and liver).¹¹ Vitamin E primarily resides and exerts its biological activity within cell membranes and cellular organelles that possess high oxidation-reduction capabilities, specifically microsomes and mitochondria.¹²

Vitamin E also functions in a number of other biochemical and physiological roles. Supplementation of vitamin E at levels above those minimally required to prevent overt signs of deficiency enhances both cell mediated and humoral immunity.¹³ Neutrophil activity,^{14, 15} T-cell function,¹⁶ macrophage activity,¹⁷ and antibody production^{18, 19, 20} have all responded favorably to vitamin E supplementation. Enhanced monocyte function¹⁴ and lymphocyte stimulation^{18, 21} have also been reported in association with supplemental administration. Vitamin E may also function as a cofactor in cellular respiration, as it restricts the activity of cytochrome C reductase.⁷ It inhibits platelet aggregation by suppressing arachidonic acid peroxidation,²² an essential step in the formation of the prostaglandins involved with normal clotting function.^{7, 23} Lastly, roles in vitamin C, coenzyme Q, and DNA synthesis have also been suggested for vitamin E.⁷

Historical Information

Vitamin E was discovered in 1922 by Evans and Bishop at U.C.- Berkeley. They identified a previously unknown compound in wheat germ, lettuce, and alfalfa meal; this organic compound was determined essential for normal reproduction in female rats.²⁴ Evans et al. isolated the compound from wheat germ oil in 1936, definitively demonstrated all of its

previously attributed properties, and identified it as α -tocopherol.²⁵ The designation vitamin “E” was proposed by Sure,²⁶ as “E” was the next sequential letter to be arbitrarily assigned to an organic nutrient required in small amounts in the diet. “Tocopherol” is derived from the Greek “tokos” (childbirth or offspring), the Greek “pherein” (to bring forth), and “ol”, which structurally designates it as an alcohol.²⁷

The earliest studies involving vitamin E and farm animals occurred in the 1920’s and 30’s and focused on various aspects of reproductive performance. Hickman and Harris first suggested in 1946 that some biochemical functions of vitamin E could result from some type of antioxidant activity.²⁸ In 1957, Schwarz and Foltz proved the relationship between vitamin E and the previously unidentified (from a functional standpoint) element, selenium (Se).²⁹ They learned that liver necrosis in the rat and exudative diathesis in the chick could be prevented by the dietary inclusion of some types of brewer’s yeast²⁹ (which contained no vitamin E). The substance found functioning in place of vitamin E was determined to be Se. Further support and clarification of this functional relationship was achieved in 1973 when Rotruck et al. proved that Se was an essential component of glutathione peroxidase (GSH-Px).³⁰

Absorption, Transport, and Tissue Uptake

Ingested vitamin E associates with lipids within the gastrointestinal tract because of its hydrophobic nature. Vitamin E initially enters the gut lumen in association with triglycerides, but its absorption does not occur until these compounds are broken down via lipase-mediated hydrolysis into non-esterified fatty acids and β -monoglycerides,¹¹ structures small enough to traverse the brush border membrane of the small intestine. Non-esterified fatty acids and β -monoglycerides interact with bile salts to form mixed micelles. Because of their extremely small size, mixed micelles are uniformly dispersed within the aqueous phase of the gut lumen.¹¹ Except for bile salts, all components of mixed micelles are absorbed by enterocytes upon contact with the brush border. Specifically, vitamin E is absorbed in the alcohol form by simple diffusion.³¹ Only 20-30% of the vitamin E ingested is typically absorbed.^{32, 33} However, absorptive efficiency is inversely related to the amount present in the diet³⁴ and may be somewhat influenced by concentrations within the body.

Once inside the mucosal epithelial cells, absorbed vitamin E either enters cell organelles or their membranes, or remains within the cytosol. That fraction present in the cytosol is taken up by chylomicrons, the predominant lipoprotein secreted by the intestine.³⁵ Chylomicrons facilitate transport of absorbed dietary lipids to the general circulation.³⁵ Formation of chylomicrons begins in the smooth endoplasmic reticulum with the process of de novo triglyceride synthesis.¹¹ Once the developing triglyceride mass reaches a certain size, the Golgi apparatus adds an apoprotein moiety to the particle and packages it within a secretory vesicle.¹¹ The chylomicron (now relatively water soluble due to its apoprotein component) is then released via exocytosis and deposited in the lymphatic circulation. Thereafter, vitamin E (as a chylomicron component) enters the bloodstream as lymph empties into the right atrium via the thoracic duct.

Like other fat-soluble vitamins, vitamin E is transported through the bloodstream attached to a carrier molecule. However, unlike vitamin A, which utilizes a specific carrier protein, vitamin E is transported through circulation as a component of serum lipoproteins.³⁶⁻³⁹ Across species, vitamin E distribution among lipoprotein classes corresponds to the relative proportions of each lipoprotein class present (i. e. total lipid distribution). Cattle, for example, maintain the majority of their serum vitamin E in the high density lipoprotein (HDL) fraction,⁴⁰ which is the predominant lipoprotein class present in the bovine.⁴¹⁻⁴⁵ Conversely, humans contain the majority of their circulating lipids and vitamin E in the low density lipoprotein (LDL) fraction.^{39, 46}

Lipoproteins are molecular complexes comprised of varying amounts of triglyceride, free cholesterol, cholesteryl ester, phospholipid, and apolipoprotein.⁴⁷ Lipoproteins have a bi-layered globular structure. They consist of a hydrophobic core comprised of triglyceride and esterified cholesterol wrapped in a relatively more hydrophilic layer of phospholipid, unesterified cholesterol, and protein.⁴⁸ They are generally categorized into four density-dependent classes: high, low, and very low density lipoproteins (termed HDL, LDL, and VLDL, respectively), plus chylomicrons. These molecular complexes function to stabilize and transport circulating lipids in a water soluble form as well as facilitate the exchange of lipids between tissues.^{49, 50} Essentially all lipoproteins originate from either the liver or intestine.³⁵

Vitamin E moves between lipoprotein classes within the bloodstream independent of an exchange protein.¹¹ Transfer between lipoproteins is thermodynamically controlled by the relative lipid content of the lipoprotein particles involved in the exchange.⁵¹ The specific mechanism(s) of α -tocopherol uptake by cells are not fully known. However, evidence suggests the process involves lipoprotein lipase activity⁵² as well as receptor-mediated recognition and uptake of lipoprotein particles.¹¹ For example, human fibroblast cultures with a hereditary LDL-receptor deficiency,⁵³ or otherwise manipulated to inhibit LDL uptake,⁵⁴ exhibited significantly lower α -tocopherol uptake versus normal cells. Additional studies have substantiated this relationship by demonstrating a positive correlation between activity of tissue-specific LDL receptors and tissue vitamin E concentration.⁵⁵

Other methods of cellular vitamin E uptake may exist. Recent studies have identified the presence of a α -tocopherol binding protein in the cytosol of rat liver and heart,⁵⁶ rabbit heart,⁵⁶ and bovine heart.⁵⁷ Perhaps a similar protein functioning in cellular uptake also exists. Passive diffusion is another possibility, as is some specific form of HDL receptor-mediated transport.¹¹

Serum Lipoproteins of Cattle

Four classes of density-based lipoproteins are found in cattle: HDL, LDL, VLDL, and chylomicrons. High density lipoproteins predominate in adult cattle, comprising approximately 80% of the circulating lipoproteins.⁴⁴ The remaining 20% of lipoproteins in bovine serum exist as chylomicrons, VLDL, and LDL.^{40, 42, 44, 58} High density lipoproteins contain 68-90% of the lipids (weight-basis) present in bovine serum,^{42, 44, 59} with the remainder existing in the other lipoprotein classes and as volatile fatty acids (VFA) and non-esterified fatty acids (NEFA). Chylomicrons contain up to 2.5%,^{43, 58} VLDL less than 5%,⁴² and LDL approximately 4-7%⁴² of the total serum lipids (weight-basis) in cattle. Cholesteryl esters and phospholipids comprise approximately 90% (weight-basis) of the circulating lipids in cattle.⁴² These lipid structures exist in all lipoprotein fractions⁴⁸ but are found in highest amounts in the HDL fraction of cattle.^{40, 42, 60, 61}

The lipoprotein classes have different roles in lipid and energy metabolism. Chylomicrons transport triglycerides from the intestine to peripheral tissues.⁶² Very low density lipoproteins transport triglycerides from the liver to extrahepatic tissues such as the mammary

gland and adipose tissue.⁶³ Very low density lipoproteins ultimately evolve into LDL after transferring varying amounts of cholesterol and the majority of their triglyceride⁶² to peripheral tissues, plus a portion of their apolipoprotein C to HDL.⁶³ Low density lipoprotein is believed to deliver cholesterol to extrahepatic tissues for membrane or steroid synthesis.^{64, 65} High density lipoproteins transport phospholipid and cholesterol from extrahepatic tissues to the liver.^{48, 66}

Lipoproteins can be separated by ultracentrifugation,^{40, 42, 44} electrophoresis,^{43, 67-69} precipitation with sulfated polysaccharides,^{70, 71} affinity chromatography,^{46, 72, 73} or some combination^{60, 74} of these procedures. Lipoprotein classification is based upon density,^{40, 42, 44} electrophoretic mobility within agarose gel,⁶⁷⁻⁶⁹ or apolipoprotein content⁷²⁻⁷⁴ and is dependent upon the method(s) of separation used. One method of separation by apolipoprotein composition results in fractions designated as α - (no apolipoprotein B present) and β - (apolipoprotein B present).^{72, 73} Enzyme-linked immunosorbent assays^{66, 75} (ELISA) and single radial immunodiffusion assays⁷⁶ (SRID) have been developed to determine concentrations of apolipoproteins in bovine serum. Apolipoprotein A-1 is the primary protein moiety in the HDL of cattle^{62, 66} and other mammals.^{48, 62} The apolipoproteins B (B-48 or B-100) are the major protein moieties of mammalian LDL, VLDL, and chylomicrons.^{48, 62, 75}

General Function and Activity

Vitamin E functions primarily as an antioxidant. Vitamin E prevents formation of peroxides and free radicals from cellular lipids by interrupting the initial stages of free radical production and undergoing oxidation itself, thereby preserving cell membrane integrity. Membrane lipids are very susceptible to peroxidation due to the high degree of unsaturation required to facilitate fluidity.⁷ Lipid peroxidation can lead to membrane protein oxidation, further damaging cellular integrity and possibly leading to complete cellular destruction. Vitamin E also inhibits the conversion of existing lipid peroxides to the much more reactive and destructive peroxide radicals, thereby reducing further damage and helping maintain cell membrane integrity (Figure2-2).⁷⁷

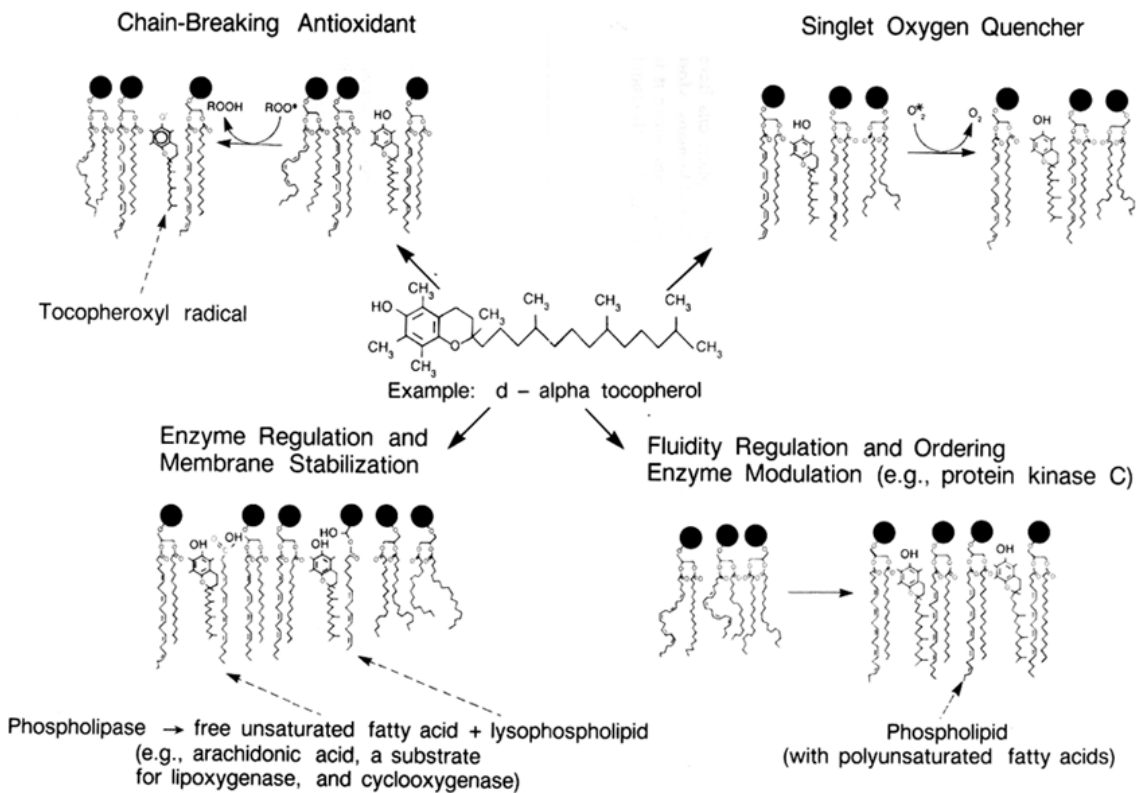


Figure 2-2. Function and activity of vitamin E at the cellular level.⁷⁷

Vitamin E functions in concert with Se, a primary antioxidant. Selenium is an essential component of glutathione peroxidase and phospholipid hydroperoxide glutathione peroxidase,⁷⁸ enzymes that function in hydrophilic environments (e. g. cell cytosol, interstitial fluid) and cellular membranes, respectively. The glutathione-containing enzymes are referred to as primary or preventive antioxidants, which function to suppress free radical formation through precursor removal and catalyst inactivation.⁷⁹ Vitamin E exerts its activity in hydrophobic environments such as cell and cellular organelle membranes (Figure 2-3). It acts as a secondary or chain-breaking antioxidant, inhibiting peroxidation by minimizing the chain length of the propagation reaction.⁷⁹ Vitamin E achieves this through direct action with peroxy radicals.

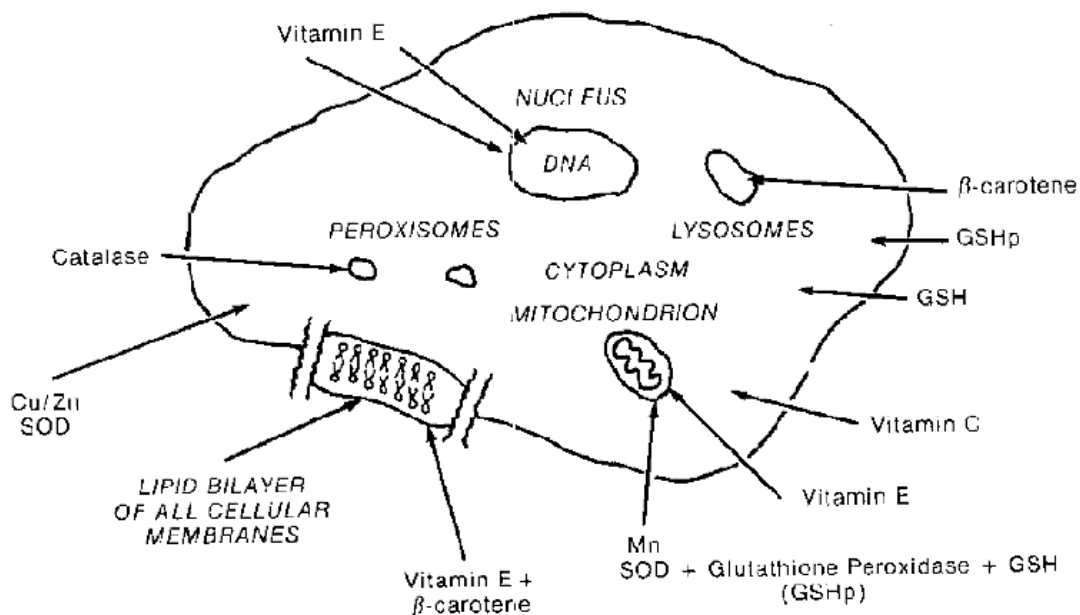


Figure 2-3. Antioxidant system locations within the cell.⁸⁰

Based upon in vitro studies, α -tocopherol has been determined to have near optimal structural and behavioral properties for neutralizing peroxy radicals.⁸¹⁻⁸³ The tocopheroxyl radical formed in the reaction between α -tocopherol and a peroxy radical is relatively stable. Alpha-tocopherol effectively “traps” the peroxy radical and prevents further oxidative damage.⁷⁹ Each α -tocopherol molecule is capable of trapping two peroxy radicals. Once oxidized (in the tocopheroxyl radical form), the molecule can potentially undergo regeneration to the metabolically active form (tocopherol) via reduction by vitamin C.⁷⁹ If not regenerated, the tocopheroxyl radical is irreversibly converted to tocopheryl quinone, a metabolically inactive compound excreted in bile.²⁷

Dietary Requirements of Production Animals

The National Research Council’s (NRC) published nutrient requirements may not exceed the minimum dietary concentrations known or believed required for prevention of clinical signs of deficiency. These requirements may not address the fact that vitamin E appears to demonstrate specific functions when provided at levels greater than those which prevent deficiency.⁸⁴ Nutrient levels required to optimize/maximize immunity, reproduction,

performance, etc. are often 2-20 fold higher than NRC maintenance requirements, thus providing a basis for additional dietary provision.

White muscle disease is the best known and most commonly reported manifestation of vitamin E and/or Se deficiency in livestock.⁸⁵⁻⁸⁹ However, vitamin E deficiency, alone or in conjunction with selenium deficiency, has been linked to several other diverse clinical syndromes that are variably manifested by the common production species (Table 2-2). The scope of its involvement in various body systems is perhaps best described by Blaxter and Brown, who stated that "...deficiency of no other vitamin results in such a wide diversity of clinical signs and pathological changes."⁹⁰

Table 2-2. Clinical syndromes associated with vitamin E and/or selenium deficiencies.⁹¹

<u>Species</u>	<u>Clinical Syndromes</u>
Poultry	Encephalomalacia, Exudative Diathesis, Muscular Dystrophy
Horses	White Muscle Disease, Steatitis, Neonatal Weakness, Myodegeneration, Degenerative Myeloencephalopathy
Pigs	White Muscle Disease, Mulberry Heart Disease, Hepatosis Dietetica, Mastitis/Metritis/Agalactia
Cattle and/or Sheep	White Muscle Disease, Retained Placenta, Mastitis, Metritis, Abortions and Stillbirths, Neonatal Weakness, Myodegeneration, Ill Thrift and Poor Weight Gain, Male and Female Infertility, Impaired Immune Function

(Adapted from Blodgett, D. J., 1998)

Dietary vitamin E requirements of horses are largely extrapolated from research in other species.^{92, 93} Roneus et al. reported no adverse effects in maintenance horses consuming only 15 IU vitamin E/kg ration dry matter but also stated that 100 IU/kg were required to maximize liver and muscle vitamin E concentrations.⁹⁴ The NRC recommends 50 IU vitamin E/kg ration dry matter for maintenance horses and 80-100 IU/kg ration dry matter for growing horses, pregnant and lactating mares, and horses in work.⁹⁵

Lactating sows and recently weaned pigs have the greatest metabolic demand for vitamin E in swine. Growing swine rations should contain from 11 total IU⁹⁶ up to 30 IU of supplemental⁹⁷ vitamin E per kg of ration dry matter. Recommendations of 20-30 IU vitamin

E/kg of ration dry matter for gestating and lactating sows, and 22 IU/kg for breeding boars have also been proposed.⁹⁸

Vitamin E requirements for beef cattle have not been definitively established, however several recommendations exist. The NRC recommends young calves consume 15-60 IU vitamin E/kg dietary dry matter,⁹⁹ whereas optimum weight gain in finishing steers was facilitated by supplementing the basal ration with 50-100 IU vitamin E/animal/day.¹⁰⁰ BASF Corporation recommends the following levels of dietary vitamin E provision: 100-200 IU/head/day for mature beef cows, 800-1600 IU/head/day for recently received and growing cattle, and 600-1000 IU/head/day for finishing cattle.¹⁰¹

Definitive requirements for dietary provision of vitamin E have also not been established in dairy cattle. NRC dietary recommendations for vitamin E intake are 15-60 IU/kg dietary dry matter for young calves, 121 IU/head/day for 6-12 month old calves, 242 IU/head/day for 12-24 month old heifers, 154 IU/head/day for dry cows, and 308 IU/head/day for lactating cows.¹⁰² BASF Corporation recommends the daily provision of 300 IU vitamin E for nursing calves, 200-400 IU for recently weaned calves, 100-200 IU for replacement heifers, and 800-1600 IU for mature cows.¹⁰¹ Similarly, Erdman suggests providing calves, heifers, dry cows, and lactating cows with 100-150, 200-300, 300-1000, and 300-500 IU vitamin E/head/day, respectively.¹⁰³

Factors Influencing Dietary Requirements

Specific requirements for vitamin E are difficult to establish, as numerous variables influence the dietary concentrations required. For example, species of concern and stage of production^{95, 99, 103, 101} will affect estimated requirements. Metabolic oxidative and energy demand¹⁰⁴ and the presence of other dietary components, such as high dietary levels of gossypol,^{105, 106} Fe,¹⁰⁷ Cu,¹⁰⁸ vitamin A,⁷ carotenoids,¹⁰⁹ PUFAs,⁷ or nitrates,¹¹⁰ may increase dietary requirements of vitamin E. The presence of Se, sulfur-containing amino acids, vitamin C, and/or certain fat-soluble antioxidants (e. g. ethoxyquin,⁷ BHT⁷⁹) will decrease requirements.^{7, 111, 112}

Dietary Se content may have the greatest influence on recommendations for provision of vitamin E in the diet, due to the similar biological functions of these two nutrients. Selenium nutriture may be influenced by dietary vitamin E and sulfur content⁷ as well as the form of Se

consumed.¹¹³ This micromineral should typically be present in the ration dry matter at the recommended¹¹⁴ or legally allowed limit for supplementation¹¹⁵ of 0.1-0.3 mg/kg, depending upon the production specie of concern. Selenium supplementation is important to animals grazing in geographic regions known to be Se deficient,¹¹⁶ or consuming forages and grains known to be marginal to deficient in Se content.^{117, 118}

Research in Selected Production Species

Horses

Supranutritional supplementation of vitamin E in horses results in several positive effects, manifested by multiple body systems. Unfit horses experiencing moderate to heavy work may benefit from high dietary concentrations of vitamin E. Horses and zebras fed 100 IU vitamin E/kg dietary dry matter experienced no muscle soreness or lameness when captured and restrained, but developed both when fed only 50 IU vitamin E/kg dry matter.⁹⁵ Although vitamin E and/or Se deficiencies have been incriminated in the pathogenesis of exertional rhabdomyolysis,¹¹⁹ other reports refute any causal relationship.¹²⁰ Valentine et al. report no significant association between a horse's vitamin E or Se status and its response to dietary treatment of polysaccharide storage myopathy (also known as exertional rhabdomyolysis).¹²¹

Vitamin E deficiency has been established as a risk factor for equine motor neuron disease (EMND).¹²² Similarly, the development of equine degenerative myeloencephalopathy (EDM) is associated with low concentrations of vitamin E in plasma,¹²³ and a vitamin E deficiency is suspected as significant predisposing factor.^{124, 125} Furthermore, oral vitamin E supplementation at 2000 IU/day decreased the incidence of EDM, while long term treatment with 6000 IU/day improved the clinical signs of some affected horses, especially when treated early in the course of the disease.^{125, 126}

Baalsrud and Overnes reported enhanced humoral immune response to novel antigens in horses supplemented orally with 600 IU vitamin E and/or 5 mg Se.¹²⁷ Reproductive capabilities in horses given supplemental vitamin E are not enhanced,¹²⁸ contrary to other species.

Swine

Studies from as early as 1977 have documented the immunoenhancing effects of vitamin E supplementation in swine.¹²⁹ Improvements in both humoral^{130, 131} and cellular^{132, 133} immunity have been reported. Another benefit to porcine immune function was demonstrated when 900 IU of intramuscular α -tocopherol reduced the detrimental effects of endotoxin by suppressing the post-exposure production of interleukin-6 and cortisol in young pigs.¹³⁴

Feeding sows 50 IU supplemental vitamin E/kg ration dry matter increases milk and colostrum vitamin E content,¹³⁵ possibly conferring enhanced immunity in neonatal pigs. As is the case in cattle¹³⁶ and other ruminants, very little vitamin E crosses the porcine placenta.^{135, 137} Therefore, colostrum is a vital source of vitamin E to newborn pigs.¹³⁵ Serum vitamin E concentrations decrease in the periparturient sow,¹³⁸ similar to what has been reported in dairy cows.^{139, 140}

Vitamin E supplementation benefits other body systems in swine. Spermatazoa concentration was increased in boars fed 1000 IU vitamin E/day for seven weeks.¹⁴¹ Piatkowski et al. reported that 22 IU supplemental vitamin E/kg ration dry matter maintained tissue vitamin E concentrations in gilts through the duration of pregnancy.¹⁴² Supplementing the grower-finisher ration with 100 IU vitamin E/kg of feed reduced lipid oxidation and increased palatability of the resulting retail cuts.¹⁴³ In-vitro research has shown that dietary vitamin E supplementation may decrease the extent and severity of the intramuscular peroxidation reactions that occur in pigs suffering from malignant hyperthermia.¹⁴⁴

Small Ruminants

Most research on vitamin E supplementation in small ruminants has involved sheep. Many of the supplementation benefits reported in other species have also been observed in sheep.

Supplementing lambs with 250 IU vitamin E and 0.3 mg Se/kg feed increased body mass gain and altered several measurements of rumen function, including increased volatile fatty acid production and total protozoa numbers, and decreased rumen pH.¹⁴⁵ To the best of the author's knowledge, these favorable alterations in rumen function have not been reported in association with vitamin E or selenium supplementation in other ruminant species.

Like swine and cattle, pregnant ewes transfer minimal amounts of vitamin E across the placenta to the developing fetus(es), and colostrum is the primary vitamin E source for the newborn lamb.^{146, 147} Supplementing ewe lambs with 15, 30, and 60 mg of *dl*- α -tocopheryl acetate per day during the last 28 days of pregnancy resulted in a dose-dependent increase in α -tocopherol concentrations in colostrum and serum of 3 day-old lambs.¹⁴⁶ Kott et al. reported additional benefits of vitamin E supplementation in ewes during late gestation. Ewes supplemented with 330 IU vitamin E/day for the last three weeks of pregnancy showed no difference in weight, body condition score, prolificacy, or fertility versus unsupplemented controls.¹⁴⁸ However, mortality rates in the early portion of the lambing season were lower in lambs from supplemented ewes.¹⁴⁸ A significant increase in pounds of lamb weaned per ewe was associated with the mortality difference.¹⁴⁸

Vitamin E supplementation enhances both cellular¹⁴⁹ and humoral¹⁵⁰ immunity in sheep. It also lengthens product shelf-life, where retail cuts from lambs supplemented with oral vitamin E underwent less lipid oxidation than retail cuts derived from unsupplemented controls.¹⁵¹

The effects of vitamin E supplementation on ovine fertility vary. The previously discussed work by Kott et al. found no effect of oral vitamin E supplementation on ewe fertility.¹⁴⁸ Conversely, Segerson and Ganapathy found a trend towards improved ova fertility in ewes given multiple intramuscular injections of 136 IU vitamin E and 10 mg Se.¹⁵² Segerson et al. found an increase in uterine contractions moving towards the oviducts of ewes given 136 IU vitamin E and 10 mg Se via intramuscular injection.¹⁵³

Research in Cattle

Vitamin E supplementation in cattle has been investigated for more than 60 years. Vogt-Moller and Bay, in 1931 reported that wheat germ oil injections cured sterility in some cows identified as repeat breeders.¹⁵⁴ Salisbury determined in 1944 that supplemental vitamin E did not affect the semen quality, sperm characteristics, or fertility of dairy bulls used for artificial insemination.¹⁵⁵

Vitamin E supplementation has reduced the occurrence of mastitis,^{139, 156} cystic ovaries,¹⁵⁷ retained placentas,¹⁵⁸⁻¹⁶⁰ and metritis^{159, 160} in dairy cows. In addition, vitamin E supplementation has been associated with a shorter anestrous period post-calving,^{157, 161}

decreased services per conception,¹⁶² and a reduction in days open.¹⁶¹ Hurley and Doane indicated that cows supplemented with vitamin E and Se demonstrated enhanced sperm transport and an increase in uterine contractions which moved toward the oviducts.¹⁶² Reduced udder edema was reported for dairy heifers fed 1000 IU/head/day vitamin E and at least 0.15 ppm Se for the last 40 days of gestation.¹⁶³ Lacetera et al. demonstrated that parenteral supplementation of 0.25 IU *dl*- α -tocopheryl acetate and 0.05 mg Se per kg of body weight given 3 and 1.5 weeks before calving increased colostrum and early lactation milk production in Holsteins by 22 and 10%, respectively.¹⁶⁴ Lacetera's results contrast a recent review of the bovine vitamin E literature by Corah, et al. The authors of this review concluded that a production response to supplemental vitamin E occurred more frequently when the vitamin E was provided orally rather than parenterally.¹⁶⁵

Beneficial effects of vitamin E supplementation on bovine immune function include stimulation of serum antibody synthesis (particularly IgG)¹⁶⁶ and reduced glucocorticoid production.^{167, 168} Supplementation of vitamin E also prevents periparturient inhibition of neutrophil chemotaxis in dairy cows.¹⁵ Cipriano et al. reported that vitamin E and selenium supplementation elevated antibody titers and enhanced phagocytic activity in dairy calves.¹⁶⁸ Enhanced calf growth rate¹⁶⁹ and a trend towards improved passive immune status¹⁶⁶ in calves have also occurred in conjunction with vitamin E supplementation. Jackson et al. evaluated the effects of vitamin E supplementation on the signs associated with fescue toxicosis. Neither 1000 nor 2000 IU oral vitamin E per day affected feed intake, milk yield, rectal temperature, or body weight changes in Holstein cows consuming endophyte-infected fescue hay.¹⁷⁰ With respect to dairy product quality, adequate dietary vitamin E provision increases milk shelf life, through reduction of the rate and extent of milk fat autoxidation.^{104, 171}

Newly received feedlot cattle supplemented with vitamin E have demonstrated increased average daily gains and improved feed-to-gain ratios,^{172, 173} as well as reductions in morbidity versus unsupplemented controls.¹⁷² Benefits of supplementation on weight gain may not be restricted to the feedlot, however. Wright et al. assessed the effects of pre-weaning vitamin E supplementation on early post-weaning weight gain in 1996.¹⁷⁴ In this study, beef calves supplemented with 500 IU vitamin E per day for 48 days before weaning gained an average of 0.11 kg per day more than their control counterparts for the first 4 weeks after weaning.

Similar to its effects on milk quality, vitamin E supplemented during the finishing period increases beef product shelf-life. This finding is economically significant to the beef industry, as reports estimate annual savings of \$175 million¹⁷⁵ to \$1 billion¹⁷⁶ by extending retail product shelf-life by 1-2 days. Faustman et al. showed that feeding 370 IU vitamin E/head/day increased the stability of sirloin steak color from Holstein steers.¹⁷⁷ Steers in Faustman's study were supplemented with vitamin E beginning at 110 kg of body weight and continuing until a slaughter weight of 545 kg was reached. In a study by Garber et al., beef steers fed 500 IU vitamin E/head/day for 119-133 days produced meat with an extended shelf-life because of decreased metmyoglobin formation and lipid oxidation.¹⁷⁸ Several other studies also report improved retail product quality resulting from decreased lipid oxidation in association with vitamin E supplementation.¹⁷⁹⁻¹⁸¹

Nockels et al. reported that parenteral supplementation of vitamin E to brood cows approximately one month before calving enhanced the passive immune status of their calves.¹⁸² Similarly, increases in IgG concentrations of colostrum and calf serum have been obtained with prepartum Se supplementation in cows grazing Se-deficient pastures.¹⁸³ However, other studies involving maternal vitamin E supplementation in both beef^{184, 185} and dairy cows¹⁶⁴ have found no difference in passive immune status between treatment and control group calves. Reduced incidence of neonatal diarrhea has also been reported in association with maternal supplementation of 1000 IU/head/day vitamin E during late gestation.¹⁸⁵ Vitamin E content of the colostrum from treated cows in this study increased, as did the plasma vitamin E concentration of their calves at two days of age.¹⁸⁵

Results of studies examining the effects of vitamin E supplementation in beef cows are variable and limited, but have shown enough benefits to merit additional investigation.¹⁸⁶ Research undertaken in this dissertation is intended to enhance the evolving database of knowledge on vitamin E supplementation in late gestation cattle and the relationship between vitamin E and serum lipoproteins in cattle.

Chapter 3

Effects of Supplementation with Parenteral Vitamin E and Selenium during the Dry Period in Jersey and Holstein Cows

Abstract

The effects of supplementation with parenteral vitamin E and selenium (Se) during late gestation on parturient and post-parturient blood concentrations of these micronutrients were evaluated in 16 Jersey and 36 Holstein cows. Cows were allotted to blocks by breed and expected calving date. Cows within blocks were randomly assigned vitamin E or Se treatment in a 2X2 factorial design. Treatments were administered as subcutaneous injections approximately 3-4 weeks prior to anticipated calving. Dosages were empirically based on body weights of the two breeds. Holsteins were given 3000 IU and 32.5 mg of vitamin E and Se, respectively, while Jerseys were given 2400 IU of vitamin E and 22.5 mg of Se. All groups were similarly housed, managed and fed through the study period. Blood samples were taken at dry-off, 3-4 weeks prior to predicted calving date (the time of treatment), within 24 hours after calving, and 2-3 weeks post-calving for assessment of serum vitamin E and blood Se concentrations. Jerseys had higher blood Se ($P=0.0001$) and lower serum vitamin E ($P=0.005$) concentrations than Holsteins at dry-off. The breed effect for Se persisted, with Jerseys having higher blood Se concentrations than Holsteins at the pre-calving ($P=0.0001$) and calving ($P=0.0002$) samplings. Selenium supplementation resulted in higher blood Se concentrations at calving ($P=0.04$). Treatment did not affect serum vitamin E concentrations at calving or post-calving, nor blood Se concentrations post-calving. A periparturient decline in serum vitamin E concentrations was observed in both breeds and all treatment groups.

INTRODUCTION

Vitamin E and Se supplementation is frequently associated with a decreased incidence of retained fetal membranes,¹⁵⁸⁻¹⁶⁰ metritis,^{159, 160, 187} cystic ovaries,^{157, 187} and periparturient mastitis^{139, 156, 188} in dairy cows. Other studies, however, report no benefit to such

supplementation.¹⁸⁹ These nutrients are often supplemented in lactating cow rations. Vitamin E and Se supplementation is commonly recommended for dry cows,^{157, 187} but does not occur on some dairies. Vitamin E and Se are sometimes administered parenterally during the dry period.¹⁹⁰

Dietary concentrations of Se and vitamin E in dry cow rations vary between dairies because of the different concentrates, forage types, and forage qualities fed during the dry period. Weiss et al. reported lower dietary Se concentrations for dry period rations than for early lactation rations in nine Ohio dairies.¹³⁹ Some dairy producers feed a dry cow ration consisting primarily, if not exclusively, of hay. This and other stored forages typically have reduced vitamin E content as compared to vegetative, good quality pasture.^{8, 191, 192}

Minimum requirements for vitamin E and Se are well established for lactating dairy cows.¹⁰² However, data are limited on provision of supplemental vitamin E and Se to dry cows. Dietary vitamin E recommendations for dry cows vary, ranging from 200-1000 IU/head/day.^{102, 103, 193-195} Furthermore, only two reports of potential breed-related differences in serum concentrations of vitamin E exists in cattle,^{178, 196} and none are known to exist for Se. Lastly, studies utilizing parenteral supplementation of these nutrients during the dry period have yielded varied results. Therefore, objectives of this study were to determine: 1) if parenteral supplementation of vitamin E and Se during lactation maintains adequate circulating concentrations of these nutrients through the dry period; 2) if differences exist in circulating concentrations of vitamin E and Se between Holstein and Jersey breeds during the dry period; and 3) if parenteral treatment with vitamin E and Se affects circulating concentrations of these nutrients through the dry period and into early lactation.

MATERIALS AND METHODS

Cows and Experimental Design

Sixteen multiparous Jersey and thirty-six multiparous Holstein cows bred to calve between July and September of 1995 were included in the study. Cows were blocked by breed and expected calving date, then randomly assigned within blocks to vitamin E or Se treatment in a 2X2 factorial design. All cows were maintained, treatments were administered, and samples

were collected in accordance with the *Guidelines for the Care and Use of Agricultural Animals in Agricultural Research and Teaching*.

Vitamin E and Se were administered parenterally 3-4 weeks prior to anticipated calving date. The dosage employed was empirically based on estimated average body weights for the two breeds and followed product label guidelines. Holsteins and Jerseys were estimated to weigh an average of 591 and 409 kg, respectively. Holsteins were given 3000 IU of vitamin E in the form of *d*- α tocopherol (VITAL E™ - 300, 300 IU/ml *d*- α tocopherol, Schering-Plough Animal Health, Kenilworth, NJ) and Jerseys were given 2400 IU. Each dose was administered by a single subcutaneous injection behind the right shoulder. Selenium (MU-SE®, 5 mg/ml Se and 50 mg/ml *d*- α tocopheryl acetate, Schering-Plough Animal Health, Kenilworth, NJ) was administered at the dose of 32.5 mg for Holsteins and 22.5 mg for Jerseys via a single subcutaneous injection on the right side of the neck. Vitamin E was administered in combination with Se because no pure Se product was available. Therefore, Holsteins and Jerseys given Se also respectively received 442 and 306 IU vitamin E as *d*- α tocopheryl acetate.

All cows were similarly housed, managed and fed for the duration of the study, which began in May and ran through the end of October. During the dry period, cows had free-choice access to pasture consisting primarily of tall fescue, orchardgrass, and some white clover. Pasture Se concentrations ranged from 30-60 μ g Se/kg pasture DM. Pasture analysis for vitamin E content was not performed, but concentrations were estimated to average 50 mg α -tocopherol/kg pasture DM.⁸ Cows were fed a transition ration, which contained 0.3 mg total Se/kg ration DM, beginning approximately 1 week prior to calving. Early lactation rations were similar in feed composition and nutrient content, and also contained 0.3 mg total Se/kg ration DM. Transition and early lactation rations were not analyzed for vitamin E content but were estimated to contain approximately 40.5 and 32 mg α -tocopherol/kg ration DM, respectively.

Sample Collection and Analysis

Blood samples were collected via coccygeal venipuncture into Vacutainers® (Beckton-Dickinson, Franklin Lakes, NJ) four times from each cow: dry-off, 3-4 weeks prior to anticipated calving date, within 24 hours post-calving, and 2-3 weeks after parturition. Ten ml of blood was collected in EDTA tubes for determination of Se concentration and ten ml of blood was collected

in tubes with no additives for determination of vitamin E concentration. All samples taken for assessing vitamin E concentrations were protected from light and heat during handling, storage, and analysis. Serum was harvested by allowing the collected blood samples to clot for 30-60 minutes, then spinning at 3433 X g for 5 minutes. Separated serum was pipetted off the packed red cell clot and stored frozen at -20°C in polypropylene tubes until the time of analysis for vitamin E content. Blood was stored refrigerated in tubes containing EDTA until analyzed for Se concentration. Serum samples were analyzed for vitamin E content by high-pressure liquid chromatography (HPLC), and blood Se concentrations were determined by atomic absorption spectrophotometry using a Varian SpectrAA•20 (Varian, Sugar Land, TX).¹⁹⁷

Vitamin E analysis was modified from a previously reported technique.¹⁹⁸ Vitamin E extraction was performed by adding to a 1 ml serum sample: 100 µl of 1000 µg/ml α-tocopheryl acetate (internal standard), followed by 2 ml of 95% ethanol, and 3 ml of HPLC-grade cyclohexane. Between additions, the solution was vortexed for 10, 20, and 45 seconds, respectively. After centrifugation at 3064 X g for 10 minutes, the top (cyclohexane) layer of the centrifugate was pipetted into a 5 ml conical vial and fully evaporated under nitrogen on a heating block at 60°C. One hundred µl of 95% ethanol was then added to the vial, which was then vortexed intermittently for 1 minute to reconstitute the vitamin E. In order to minimize photodegradation, the entire vitamin E extraction procedure was carried out in subdued light.

Samples underwent HPLC analysis on a Varian Star Chromatograph (Varian, Sugar Land, TX) employing a Supelco C18 reverse-phase, 5 µm, 4.6 x 150 mm column, guard column, and a 10 µl injection loop. Pure methanol (100%) was used as the mobile phase at a flow rate of 2 ml/min. Ultraviolet detection was carried out at 294 nm. The concentration of α-tocopherol in each sample was determined by comparing its peak area with that of known standards, and using peak area of the α-tocopheryl acetate internal standard to correct for percent recovery.

Statistical Analysis

Data were analyzed by the GLM procedure of SAS.¹⁹⁹ The model included the main effects of vitamin E treatment, Se treatment, breed, and all two- and three-way interactions.

RESULTS

Holstein cows had lower blood Se ($P=0.0001$) and higher serum vitamin E ($P=0.005$) concentrations than Jerseys at dry-off (Table 3-1). The breed-related difference in blood Se concentrations persisted through the time at which the immediate post-calving sample was taken. Jersey cows had higher Se concentrations than Holstein cows at the mid-dry period ($P=0.0001$) and calving ($P=0.0002$) samplings (Table 3-1). There were no other differences in serum vitamin E concentrations between breeds through the study period (Table 3-1).

Treatment with vitamin E did not affect serum vitamin E concentrations at calving ($P=0.15$) or post-calving, even though the treatment group tended ($P=0.07$) towards higher serum vitamin E concentrations immediately prior to vitamin E administration (Table 3-2). Treatment with Se increased blood Se concentrations at calving ($P=0.04$). Selenium treatment did not affect post-calving blood Se concentrations (Table 3-2). No significant two- or three-way interactions occurred.

DISCUSSION

A periparturient decline in serum vitamin E concentrations was observed in both breeds irrespective of treatment group. This finding has been reported in several other studies^{140, 141, 200} and is correlated with a decrease in circulating lipoprotein concentrations.²⁰¹ Lipoproteins transport vitamin E in the bloodstream, and therefore affect the concentration of vitamin E in circulation.^{36, 40, 201, 202}

Plasma or serum vitamin E concentrations are commonly used as a practical means of evaluating vitamin E status in cattle.^{187, 190, 201} Serum vitamin E concentrations of $> 4.0 \mu\text{g/ml}$ are considered adequate,⁹ between 2.0 and $4.0 \mu\text{g/ml}$ marginal, and $< 2.0 \mu\text{g/ml}$ deficient by the VMRCVM Toxicology Laboratory. Regardless of breed, all groups had adequate mean concentrations of vitamin E in serum at all samplings except calving. Serum vitamin E concentrations at calving averaged in the marginal range (Tables 3-1 and 3-3). The periparturient decline in serum vitamin E concentrations can be at least partially attributed to a concurrent decrease in serum lipoprotein concentrations.^{40, 42, 43, 201} Other contributing factors

may include a periparturient decrease in vitamin E intake (as related to a periparturient decline in DM intake),¹⁰² and removal of vitamin E from the bloodstream as it contributes to colostrum synthesis.^{140, 203}

Blood Se concentrations are often used as a measure of Se status.^{190, 204} Blood Se concentrations > 100 ng/ml are generally considered adequate, between 50 and 100 ng/ml marginal, and < 50 ng/ml deficient in this region of the United States.^{204, 205} Blood Se concentrations were adequate at each sample period irrespective of breed or treatment group.

Blood Se concentrations differed between Jerseys and Holsteins at dry-off. This difference persisted through the mid-dry period sampling and was still evident at calving despite supplementation of all cows with 0.3 mg total Se/kg ration DM for approximately the last week of gestation. Comparable Se concentrations between breeds at the post-calving sampling can be attributed to longer-term provision (3-4 weeks) of dietary Se^{197, 203} prior to sampling.

The breed-related difference in blood Se concentrations observed in this study has not been previously reported. This finding suggests a breed-related difference between Holsteins and Jerseys in Se metabolism as has previously been reported for Cu, Fe, and Zn.²⁰⁶ Breed-related differences in hepatic concentrations of certain trace minerals have also been reported in beef cattle.²⁰⁷

Serum vitamin E concentrations were lower at dry-off for Jerseys than for Holsteins, even though all cows were consuming the same late lactation ration (data not shown). As was anticipated, Jersey cows demonstrated an early dry period increase in serum vitamin E concentrations. This increase was expected for all cows, because the ad-libitum provision of pasture throughout the dry period should have raised serum vitamin E concentrations as a result of its presumed high vitamin E content.^{191, 192, 208} As was evident for Se, the breed-related differences in serum vitamin E concentrations at dry-off and changes through the dry period might suggest possible breed-related differences in serum lipoprotein concentrations.^{40, 201} Limited evidence exists for cattle that suggests breed-related differences in vitamin E metabolism. Garber et al. reported differences in serum vitamin E concentration, response to supplementation, and metabolism of this nutrient between finishing beef and dairy steers.¹⁷⁸ Charmley et al. have suggested a breed-related difference in serum vitamin E concentrations between nonlactating dairy cows and pregnant, crossbred beef heifers.¹⁹⁶

In this study, all treatment groups maintained mean blood Se concentrations within the adequate range for the duration of the dry period. This occurred even though forages consumed were Se-deficient and no supplemental Se was provided (except by experimental treatment) until the transition ration was fed beginning approximately 1 week prior to calving. Maintenance of adequate blood Se concentrations during the dry period depends in part upon blood Se concentrations attained during late lactation,¹³⁹ and also reflects Se intake²⁰⁹ during the dry period.^{139, 203}

Mean concentrations of serum vitamin E remained adequate through the majority of the dry period but dropped into the marginal range at parturition. Just as dietary Se content influences blood Se concentrations,^{197, 203} the adequate concentrations of vitamin E in serum observed in this study are at least in part attributable to the presumed high concentration of vitamin E present in the pasture.^{8, 190, 191} Cows turned dry during periods of low pasture availability or otherwise managed such that fresh forage intake is limited would very likely demonstrate lower concentrations of serum vitamin E at parturition as well as for the majority of the dry period.^{190, 210} Cows so managed might not maintain adequate serum vitamin E concentrations in the periparturient period, possibly resulting in sub-optimal immune function¹⁵⁶ and an increased incidence of disease.^{158, 159, 187, 211}

In summary, mid-dry period supplementation of parenteral Se administered according to product label directions and at the time relative to calving employed in this study elevated blood Se concentrations in dairy cows at parturition. Mid-dry period supplementation of parenteral vitamin E at the time administered in this study and at recommended label dosages did not affect serum vitamin E concentrations in dairy cows at parturition.

Further research is needed to substantiate the apparent breed-related differences in Se metabolism suggested by the differences in blood Se concentrations observed between Jerseys and Holsteins in this study. Additional research is also needed to investigate the possibility of differences in vitamin E metabolism suggested by the observed differences in serum vitamin E concentrations between Jersey and Holstein cows at dry-off.

Table 3-1. Effect of breed on serum vitamin E and blood selenium concentrations in periparturient Jersey and Holstein cows.[‡]

Sample Period	Serum Vitamin E (µg/ml)			Blood Selenium (ng/ml)		
	Jersey	Holstein	<i>P</i>	Jersey	Holstein	<i>P</i>
Dry Off	3.6 ± 0.2	5.5 ± 0.4	0.005	177 ± 5	141 ± 4	0.0001
3-4 weeks pre-calving	4.8 ± 0.3	4.7 ± 0.2	0.76	145 ± 5	108 ± 4	0.0001
Within 24 hr post-calving	2.6 ± 0.2	2.9 ± 0.1	0.16	155 ± 4	124 ± 5	0.0002
2-3 weeks post-calving	4.1 ± 0.3	4.1 ± 0.2	0.98	167 ± 4	157 ± 7	0.36

[‡]Values are least squares means ± standard error of 16 and 36 observations for Jerseys and Holsteins, respectively.

Table 3-2. Effect of parenteral vitamin E or selenium (Se) supplementation during late gestation on serum vitamin E or blood Se concentrations of periparturient Jersey and Holstein cows. Jerseys and Holsteins were given 2400 and 3000 IU vitamin E, or 22.5 and 32.5 mg Se respectively, as subcutaneous injections immediately after taking ‘3-4 weeks pre-calving’ sample.[‡]

Sample Period	Serum Vitamin E (µg/ml)			Blood Selenium (ng/ml)		
	Control	Vit. E treatment	<i>P</i>	Control	Se treatment	<i>P</i>
3-4 weeks pre-calving	4.4 ± 0.2	5.1 ± 0.3	0.07	127 ± 6	126 ± 6	0.84
Within 24 hr post-calving	2.5 ± 0.2	2.9 ± 0.2	0.15	131 ± 7	148 ± 5	0.04
2-3 weeks post-calving	4.3 ± 0.3	4.0 ± 0.2	0.49	159 ± 7	165 ± 7	0.61

[‡]Values are least squares means ± standard error and are based on 26 observations for the control and treatment groups, respectively.

Chapter 4

Effects of Oral Vitamin E Supplementation During Late Gestation in Beef Cattle Calving in Late Winter

Abstract

The effects of oral vitamin E supplementation during late gestation were examined in two beef cattle herds calving in late winter. One herd consisted of multiparous cows (trial 1) and the other of nulliparous heifers (trial 2). All cattle consumed an estimated average of 200 and 319 IU vitamin E/head/day in their basal late gestation and periparturient rations, respectively. Cattle in the treatment group of each trial consumed an average of an additional 1000 IU/head/day of supplemental vitamin E in a free-choice vitamin-mineral mix beginning 30 days prior to the onset of a 65-day calving season and continuing until they calved. In trial 1, treatment increased serum vitamin E concentrations of cows pre-calving ($P=0.04$), calving ($P=0.009$), and post-calving ($P=0.01$) and also increased colostral vitamin E concentrations ($P=0.07$). Treatment did not affect calf serum or colostral IgG concentrations. Calves from treated cows had heavier 205-day adjusted weaning weights (AWWs) than calves from untreated cows ($P=0.02$). A treatment by breed interaction occurred whereby calves from treated Angus cows had higher serum vitamin E concentrations at 24-48 hours of age than calves from untreated Angus cows ($P=0.002$). Angus cows had higher serum vitamin E concentrations than Hereford cows pre-calving ($P = 0.01$), and tended toward higher serum vitamin E concentrations at calving ($P=0.09$) and post-calving ($P=0.09$). Angus cows had higher colostral vitamin E concentrations than Hereford cows ($P=0.06$). In trial 2, treatment increased serum vitamin E concentrations of heifers at calving ($P=0.01$), but did not affect pre-calving ($P=0.13$) or post-calving concentrations of serum vitamin E. Treatment tended to increase 205-day AWWs of calves in trial 2 ($P=0.08$). Treatment did not affect vitamin E or IgG concentrations in colostrum or the serum of 24-48 hour-old calves in trial 2. Angus heifers in trial 2 had higher serum vitamin E concentrations at calving ($P=0.02$) and post-calving ($P=0.06$) than Hereford heifers.

INTRODUCTION

Calves are born with physiologically low stores of vitamin E, a fat-soluble vitamin that crosses the bovine placenta in limited amounts.^{137, 212} Adequate vitamin E status is necessary for proper musculoskeletal development^{111, 168} and optimal immune system function.^{169, 213} Colostrum is the primary source of vitamin E for neonatal calves.^{137, 212} Producers sometimes supplement calves with vitamin E during the first few days of life in an attempt to improve vitamin E status. Supplemental vitamin E can be given parenterally to neonatal calves. Less commonly, calves are given oral vitamin E or cows are supplemented during late gestation via oral or parenteral means to increase colostrum vitamin E content.

Enhancement of the vitamin E status of neonatal calves may be most important when dams are consuming low levels of vitamin E during late gestation. Harvested forages and dormant winter pastures typically contain less vitamin E than growing, vegetative forages^{2, 214, 215} and usually comprise the majority of the late gestation rations of beef cows calving in late winter and early spring. Increased provision of vitamin E to cows during late gestation should increase vitamin E provision to the newborn calf, as vitamin E is incorporated in colostrum during synthesis in late gestation¹³⁶ and vitamin E concentration of colostrum is related to maternal intake.^{216, 217}

Studies on vitamin E supplementation in late gestation dairy cattle have focused on enhancing cow immunity and performance, whereas such studies in beef cattle have focused on the benefits to the calf. The effects of vitamin E supplementation in pregnant beef cows have varied across experiments. Parenteral administration of 3000 IU of vitamin E to crossbred beef cows approximately one month prior to parturition increased plasma vitamin E concentrations in calves and enhanced their passive immune status.¹⁸² However, Hayek et al. reported no effect of injecting beef cows with 1000 IU vitamin E approximately two weeks prepartum on either the passive immune status or serum vitamin E concentration of their calves.¹⁸⁴ Weiss et al. increased the colostrum vitamin E content of Holstein cows by feeding 70 IU vitamin E/kg diet dry matter during the dry period,²⁰³ indicating the potential for oral vitamin E supplementation of the dam to increase vitamin E provision to the newborn calf. This experiment seeks to enhance the presently disparate and limited knowledge base on the effects of vitamin E supplementation

during late gestation in beef cows and to determine if breed-related differences in vitamin E nutriture may exist.

Experimental objectives were to assess the effects of brood cow exposure to a free-choice vitamin-mineral mix formulated to provide 1000 IU vitamin E/head/day during late gestation. Response variables examined were serum vitamin E concentrations in cows and calves, colostral vitamin E and immunoglobulin G (IgG) concentrations, serum IgG concentrations of calves, and calf growth. A secondary objective of the study was to evaluate the data for any potential breed differences in vitamin E status among beef cattle.

MATERIALS AND METHODS

Experimental Design

In trial one, thirty-eight Angus and sixteen Hereford cows were allotted to groups homogenous with respect to breed, age, and serum vitamin E concentration. All cows were multiparous and between three and nine years of age. In trial two, twenty Angus and five Hereford nulliparous heifers were allotted to groups homogenous with respect to breed and yearling weight ratio. Members of homogenous groups within each trial were then randomly assigned to treatment and control groups approximately 1 month prior to the beginning of a 65-day calving season that extended from mid-January through March. One Angus cow in the trial 1 treatment group was originally misidentified as a Hereford by virtue of her ID number configuration. This oversight created an equal number of Angus in the treatment and control groups of trial 1, but also resulted in a disparate number of Herefords in the treatment and control groups. Because of a sorting error at establishment of the treatment and control groups for trial 2, one Angus heifer initially designated as a control group member was included with the treatment group and remained there for the duration of the study. The sorting error and uneven number of Hereford heifers involved in the study resulted in numerical differences between Angus and Herefords in the treatment and control groups of trial 2.

For the purposes of this study, ‘supplemental vitamin E’ refers to *dl*- α -tocopheryl acetate originating from a commercial premix (Rovamix E™ 125, Roche Vitamins and Fine Chemicals, Nutley, NJ) and added to a free-choice vitamin-mineral mix (Appendix A). Cattle in the

treatment group of each trial were exposed to supplemental vitamin E beginning one month prior to the start of the calving season and ending at parturition.

Vitamin-mineral mix provision and intake were monitored 2-3 times per week. The concentration of vitamin E in the treatment groups' vitamin-mineral mixes was adjusted weekly using the average per head per day consumption rate of the previous week. The average consumption rate of the previous week served as expected level of average consumption for the forthcoming week. Samples of the vitamin-mineral mix with added vitamin E were analyzed by a commercial laboratory to ensure agreement between calculated and actual vitamin E concentrations. Exposure of the treatment groups to supplemental vitamin E ceased at parturition, however all cows still had free-choice access to the vitamin-mineral mix.

Stored forages comprised the majority of the brood cow rations for the duration of supplemental vitamin E provision. Forages fed during the study were analyzed for vitamin E content by high-pressure liquid chromatography (HPLC) at a commercial laboratory and were stored frozen at -20°C until analysis. In an attempt to minimize any potential pasture effects, treatment and control groups within each trial were alternated between two pastures on a biweekly basis so that each group spent an equal amount of time on each of two respective pastures utilized during the supplementation phase of the experiment. The vitamin-mineral mix offered free-choice to all cattle contained 30 mg Se/kg (as-fed basis).

Cattle were moved to small pastures 1-2 weeks prior to their anticipated due dates so they could be more closely observed in the periparturient period. Cattle and their calves were placed in maternity stalls shortly after parturition and typically remained there for 2-3 days after calving in order to monitor and ensure calf well-being. Calves were allowed to voluntarily nurse their dams and colostrum intake was not monitored. Upon leaving the maternity stalls, all dams and calves from both trials were returned to pasture, managed, and fed as a single herd.

Sample Collection and Analysis

Blood samples were collected via coccygeal venipuncture three times from each dam after vitamin E supplementation began: approximately 1-2 weeks prior to calving, within 6 hours post-calving, and again 2-3 days post-calving. A single blood sample was collected from each calf at 24-48 hours of age via jugular venipuncture. All blood samples were collected into

Vacutainers[®] (Beckton-Dickinson, Franklin Lakes, NJ) without additives. Blood samples were allowed to clot for 30-60 minutes, centrifuged, and the serum from each sample was then harvested and frozen at -20°C until analysis.

Colostrum samples were obtained from each dam within 6 hours of calving for determination of vitamin E and IgG concentrations. For each dam, a teat (or teats) was wiped clean, stripped 3-4 times, and 20-30 ml of colostrum was collected in a Whirl-Pak[®]. Pre-suckle colostrum samples were desired and were successfully obtained from fifty-five cattle (thirty-five in trial 1 and twenty in trial 2). All colostrum and serum samples were stored frozen at -20°C until the time of analysis. Samples were protected from light and heat during collection, handling, and analysis to reduce vitamin E degradation.

Calves were weighed at birth and again at weaning (late August) to determine 205-day adjusted weaning weights (AWWs) (Appendix B). Calves averaged 204 days of age at weaning. All calf weights were obtained using platform scales.

Serum vitamin E concentrations were determined by HPLC (Appendix C). Colostrum vitamin E concentrations were also determined by HPLC, but sample composition necessitated modification of the vitamin E extraction method used (Appendix D). Serum and colostrum IgG concentrations were determined via a single radial immunodiffusion (SRID) technique using a commercial kit (VMRD, Pullman, WA) (Appendices E and F).

Statistical Analysis

Data from both trials were analyzed using the GLM procedure of SAS (version 6.12).²¹⁸ Factors in the model included treatment, breed, and their two-way interaction. Significant two-way interactions were evaluated using the SLICE option to compare means of one factor within each level of the other factor.²¹⁸

RESULTS

Trials 1 and 2

Hay fed during mid- and late gestation contained an average of 6.7 mg/kg (100% DM basis) vitamin E. The hay and corn silage fed during the last 10-16 days of gestation and first 2-3 days after calving (the periparturient period) averaged 13.7 and 5.9 mg/kg (100% DM basis)

vitamin E, respectively. Based upon the concentrations of vitamin E in forage, all cattle consumed an estimated average of 200 and 322 IU vitamin E/head/day in the basal late gestation and periparturient rations, respectively. This estimation includes vitamin E (approximately 15-25 IU/head/day) provided by the vitamin-mineral mix that was offered free-choice to all cattle. Cattle in the treatment groups of each trial therefore consumed an average total of 1200 and 1322 IU vitamin E/head/day in their late gestation and periparturient rations, respectively. All cattle involved in trials 1 and 2 consumed an average of 2.3 mg Se/head/day for the duration of supplemental vitamin E provision.

Trial 1

One calf was born dead. This occurrence reduced the sample size used to evaluate calf serum vitamin E and IgG concentrations by one observation. The sample size for analysis of 205-day AWW was further reduced because of the death of one calf at three days of age and the sale of five cow-calf pairs prior to weaning. The mean concentration of serum vitamin E for the treatment (vitamin E-exposed) and control groups did not differ approximately 1 month prior to the beginning of supplemental vitamin E provision (Table 4-1).

Cows exposed to supplemental vitamin E provided in a free-choice vitamin-mineral mix (treatment) had increased concentrations of serum vitamin E at pre-calving ($P=0.04$), calving ($P=0.009$), and post-calving ($P=0.01$) (Table 4-1). Treated cows had higher concentrations of colostral vitamin E ($P=0.07$) but colostral IgG concentrations did not differ between treatment and control groups (Table 4-2). Treatment did not affect serum IgG concentrations of 24-48 hour-old calves (Table 4-3). A treatment by breed interaction occurred whereby calves from Angus cows exposed to the supplemental vitamin E had higher concentrations of serum vitamin E at 24-48 hours of age than calves from unexposed Angus cows ($P=0.002$) (Table 4-4). Calves from treated cows had heavier 205-day AWWs than calves from control cows ($P=0.02$) (Table 4-3).

Angus cows had higher concentrations of serum vitamin E than Hereford cows at the initial sampling ($P=0.007$) and pre-calving ($P = 0.01$), and tended towards higher concentrations of serum vitamin E at calving ($P=0.09$) and post-calving ($P=0.09$) (Table 4-5). Angus cows had higher ($P=0.06$) concentrations of vitamin E in colostrum than Hereford cows (16.1 ± 2.2 and

8.7±2.6 µg/ml, respectively). Angus calves tended toward heavier 205-day AWWs than Hereford calves (239.0±4.4 and 224.9±7.5 kg, respectively) (P=0.09).

Trial 2

One heifer experienced a dystocia and delivered a dead calf, reducing the sample size for calf serum vitamin E and IgG concentrations by one observation. One cow-calf pair involved in this trial was sold prior to weaning, reducing the sample size for 205-day AWW by one additional observation.

Exposure of nulliparous heifers to supplemental vitamin E during late gestation (treatment) increased their serum vitamin E concentrations at parturition (P=0.01), but did not affect their pre-calving or post-calving serum vitamin E concentrations (Table 4-1). Treatment did not affect vitamin E or IgG concentrations of colostrum (Table 4-2), or vitamin E or IgG concentrations of serum from their 24-48 hour-old calves (Table 4-3). Calves from treated heifers tended toward heavier 205-day AWWs than calves from control heifers (P=0.08) (Table 4-3).

Angus heifers had higher concentrations of serum vitamin E at calving (P=0.02) and post-calving (P=0.06) than Hereford heifers (Table 4-5). Angus calves had heavier 205-day AWWs than Hereford calves (248.8±4.4 and 226.6±8.3 kg, respectively) (P=0.03).

DISCUSSION

In trial 1, beef cows exposed to supplemental vitamin E in a free-choice vitamin-mineral mix during late gestation had higher serum vitamin E concentrations at all sample periods and also had higher colostrum vitamin E concentrations than control cows. These results are comparable to those of Zobell et al. who recently reported the effects of late gestation vitamin E supplementation in 134 crossbred beef cows.¹⁸⁵ Supplemented cows in Zobell's study were fed 1000 IU vitamin E/head/day for the last 60-100 days of gestation. Supplemented cows had higher serum vitamin E concentrations pre-calving and at calving, higher colostrum vitamin E concentrations, and their calves had higher serum vitamin E concentrations at 24-48 hours of age. Similar results were obtained in the present trial, except for a treatment by breed interaction involving vitamin E concentrations in serum of 24-48 hour-old calves.

In trial 2, treatment increased serum vitamin E concentrations of nulliparous heifers only at parturition. Treatment did not affect serum vitamin E concentrations of nulliparous heifers pre-calving or post-calving. In this respect, the heifers in trial 2 responded somewhat differently than the cows in trial 1. Growth increases tissue demand for vitamin E.¹⁹⁶ It is therefore possible the different responses to treatment observed in the two trials are age-related, with heifers having a greater tissue demand for vitamin E resulting from growth. Relative to cows, increased tissue demand by heifers could reduce the concentration difference in serum vitamin E afforded by supplementation, as surplus serum vitamin E leaves circulation in an attempt to satisfy tissue requirements. Serum vitamin E concentrations can differ because of differences in serum lipoprotein concentration.^{38, 40} Therefore, serum lipoprotein concentrations may differ between cows and heifers, resulting in an age-related difference in serum vitamin E concentrations.

In trial 1, only calves from treated Angus cows had higher serum vitamin E concentrations. It is unknown why calves from treated Hereford cows did not respond similarly. Based on the difference in serum vitamin E concentrations between calves from treated and control Angus cows, the higher serum vitamin E concentrations of calves from treated Angus cows may have resulted from increased maternal consumption of vitamin E. Other factors that may have caused or contributed to the treatment by breed interaction include differences in vitamin E absorption,² serum lipoprotein concentration,^{38, 40} and vitamin E concentration within lipoprotein particles.⁴⁰

The higher concentrations of serum vitamin E for Angus versus Hereford cows at the initial sampling and pre-calving indicate that a breed-related difference in serum vitamin E concentrations existed in trial 1. Further evidence of this difference is provided by the trend towards higher concentrations of serum vitamin E for Angus versus Hereford cows at parturition and again post-calving. Increased concentrations of serum lipoproteins^{38, 40} and higher degrees of vitamin E saturation per lipoprotein particle⁴⁰ increase circulating concentrations of vitamin E. Angus cattle of all ages may have higher serum lipoprotein concentrations than comparably aged Herefords, or Angus cattle may attain a higher degree of vitamin E saturation per lipoprotein particle. Either possibility would create the potential for a physiologic state that facilitates the higher concentrations of serum vitamin E observed in Angus cows and calves in trial 1.

Therefore, it is likely such a physiologic state existed in all Angus calves, but the provision of additional vitamin E in colostrum heightened its manifestation in those calves from treated Angus cows.

In trial 1, calves from treated cows had heavier 205-day AWWs than calves from control cows. In trial 2, treatment group calves had 205-day AWWs that tended to be heavier than those of control group calves. Oral vitamin E supplementation has previously facilitated increased weight gain in both post-weaned dairy¹⁶⁹ and beef calves.^{173, 174} However, this is the first study reporting a growth response in pre-weaned calves as a result of maternal vitamin E supplementation during late gestation.

Weaning weight and maternal milk production are highly correlated.²¹⁹⁻²²² The heavier 205-day AWWs of treatment group calves in trial 1 and the trend towards heavier 205-day AWWs of treatment group calves in trial 2 may have therefore resulted from increased milk production. Lacetera et al. reported that administering 5 mg sodium selenite and 25 IU *dl*- α -tocopherol acetate/100 kg of body weight to Holstein cows 3 and 1.5 weeks prior to calving increased colostrum production by 22 percent and milk production during the first 12 weeks of lactation by 10 percent.¹⁶⁴ Since vitamin E and Se exhibit similar biological functions,^{2, 27} the supranutritional level of vitamin E supplementation may have increased milk production by the treated cows in trial 1 (and to a lesser extent, the treated heifers in trial 2) as Se and vitamin E supplementation did in Lacetera's study. Additional research is needed to assess the repeatability of the 205-day AWW response to treatment, and to elucidate reasons for it.

Colostrum IgG concentrations were not affected by oral vitamin E supplementation in either trial of the present study. This finding agrees with those of Arthington, who fed yearling heifers either 400 or 800 IU vitamin E/head/day beginning 36 days prior to calving.²²³ Neither level of supplemental vitamin E provision affected colostrum IgG concentrations relative to those of their respective control group in Arthington's study.²²³ The author is unaware of any data that contradict the findings of Arthington or those reported herein concerning the effects of vitamin E supplementation on colostrum IgG concentrations.

Oral vitamin E supplementation of dams did not affect serum IgG concentrations of calves in either trial, a finding in agreement with Arthington,²²³ and Zobell et al.¹⁸⁵ Conversely, Nockels et al. reported a 46.7% increase (statistically insignificant) in serum IgG concentrations

of 36-40 hour-old calves from cows treated with 3000 IU of parenteral vitamin E approximately 1 month before calving.¹⁸² The response difference seen by Nockels et al. could possibly be attributed to the method of vitamin E supplementation, but Hayek et al. also supplemented vitamin E (1000 IU/cow 2 weeks prior to parturition) parenterally and found no difference in serum IgG concentrations of calves from treated and control dams.¹⁸⁴ The difference in response to injected vitamin E could have been dose-dependent, or related to the vaccine given concurrently with treatment to the cows in Nockels' study. Considering vaccination as a potential confounder was not reported by Nockels et al. as a possible explanation for the numerical difference in passive immune status attributed solely to treatment.

Vitamin E has been reported to function as an adjuvant in sheep.^{224, 225} Perhaps when administered concurrently with a vaccine, vitamin E has "remote adjuvant" capabilities as well. Differences in the immunoenhancing capabilities of vitamin E based on route of administration and the presence or absence of a concurrent immune challenge have been suggested.²²⁶

Breed-related differences in serum vitamin E concentrations were discovered. Angus cows in trial 1 tended toward higher concentrations of serum vitamin E than Hereford cows at calving and post-calving, and had higher concentrations of serum vitamin E than Hereford cows at the initial sampling and pre-calving. Similarly, Angus heifers in trial 2 had higher concentrations of serum vitamin E than Hereford heifers at calving and post-calving. Breed-related differences in tissue concentrations of trace minerals have been reported for cattle.^{206, 207} Garber et al. have reported differences in serum vitamin E concentrations between vitamin E-supplemented beef and dairy steers,¹⁷⁸ and Charmley et al. have suggested a breed-related difference between nonlactating dairy cows and pregnant, crossbred beef heifers.¹⁹⁶ However, differences in serum vitamin E concentrations among British beef breeds have not been previously reported to the author's knowledge.

Other breed-related differences also occurred. In trial 1, Angus cows produced colostrum with a higher concentration of vitamin E than did Hereford cows. This finding has not been previously reported to the author's knowledge. Calves from Angus cows tended to have heavier 205-day AWWs than did calves from Hereford cows. Angus heifers produced calves with higher 205-day AWWs than did Hereford heifers in trial 2. Similar findings on Angus and

Hereford weaning weights have been reported previously,²²⁷ and probably result from differences in average milk yield for the Angus and Hereford breeds.²²⁸

In summary, supplementing vitamin E in a free-choice vitamin-mineral mix (average consumption of 1000 IU/head/day) during late gestation resulted in more differences in the multiparous cow herd (trial 1) than the nulliparous heifer herd (trial 2). Increased concentrations of serum vitamin E in calves born to treated Angus cows may have resulted in improved calf health or performance as indicated by variables not evaluated in the present study.^{174, 185, 223} The heavier 205-day AWWs attained by calves from treated cows and the tendency towards the same in calves from treated heifers show tangible benefits may result from maternal vitamin E supplementation during late gestation in certain production scenarios. However, additional research is needed to assess the repeatability of the 205-day AWW response to treatment, and to elucidate reasons for it.

The total cost of supplemental vitamin E per dam averaged approximately \$2.50, given that the average dam calved 30 days into the calving season. The differences in weaning weight between treatment and control group calves across the two trials resulted in greater than a 5-to-1 return on investment (excluding any added labor costs), given a price of \$65 per hundred weight for weaned calves. Therefore, supplementing beef cattle in late gestation with oral vitamin E at the rate of 1000 IU/head/day appears economically justified when the cattle are consuming stored forages and otherwise similarly managed to the herd utilized in this experiment.

Table 4-1. Effect of oral vitamin E supplementation (avg. 1000 IU/head/day) during late gestation on serum vitamin E concentrations in a herd of periparturient Angus and Hereford cows (Trial 1) and a herd of nulliparous Angus and Hereford heifers (Trial 2) calving in late winter.[‡]

Sample Period	Serum Vitamin E ($\mu\text{g/ml}$)					
	Cows			Heifers		
	Control	Treatment	<i>P</i>	Control	Treatment	<i>P</i>
Initial set-up [§]	7.3 \pm 0.3	7.5 \pm 0.3	0.76	----	----	----
1-2 weeks pre-calving	4.2 \pm 0.2	5.1 \pm 0.4	0.04	3.5 \pm 0.2	4.2 \pm 0.3	0.13
Parturition	3.3 \pm 0.2	4.3 \pm 0.3	0.01	3.1 \pm 0.2	4.3 \pm 0.3	0.01
2-3 days post-calving	3.0 \pm 0.1	3.6 \pm 0.2	0.01	3.1 \pm 0.2	3.6 \pm 0.2	0.27

[‡]Values are least squares means \pm standard error of 28 and 26 observations for the control and treatment groups in the cow herd, and 11 and 14 observations for the control and treatment groups in the heifer herd, respectively.

[§]Samples taken approximately 1 month prior to the beginning of supplemental vitamin E provision.

Table 4-2. Effect of oral vitamin E supplementation (avg. 1000 IU/head/day) during late gestation on concentrations of colostrum vitamin E and immunoglobulin G (IgG) in a herd of periparturient Angus and Hereford cows (Trial 1) and a herd of nulliparous Angus and Hereford heifers (Trial 2) calving in late winter.[‡]

Colostrum Analyte	Cows			Heifers		
	Control	Treatment	<i>P</i>	Control	Treatment	<i>P</i>
Vitamin E ($\mu\text{g/ml}$)	8.8 \pm 2.0	15.9 \pm 2.9	0.07	12.3 \pm 3.4	15.2 \pm 3.4	0.65
IgG (g/L)	67.6 \pm 4.9	65.1 \pm 3.1	0.70	91.7 \pm 8.4	93.5 \pm 8.0	0.90

[‡]Values are least squares means \pm standard error of 28 and 26 observations for the control and treatment groups in the cow herd, and 11 and 14 observations for the control and treatment groups in the heifer herd, respectively.

Table 4-3. Effect of oral vitamin E supplementation (avg. 1000 IU/head/day) during late gestation in a herd of multiparous Angus and Hereford cows (Trial 1) and a herd of nulliparous Angus and Hereford heifers (Trial 2) calving in late winter on serum vitamin E and immunoglobulin G (IgG) concentrations of 24-48 hour-old calves and 205-day adjusted weaning weights (AWWs).[‡]

Calf Variable	Cows			Heifers		
	Control	Treatment	<i>P</i>	Control	Treatment	<i>P</i>
Serum vitamin E (µg/ml)	1.6 ± 0.1 (27)	1.9 ± 0.2 (26)	0.08	1.4 ± 0.2 (10)	1.7 ± 0.2 (14)	0.32
Serum IgG (g/L)	20.4 ± 2.1 (27)	22.4 ± 1.9 (26)	0.53	17.7 ± 2.2 (10)	24.3 ± 3.1 (14)	0.21
205-day AWW (kg)	222.1 ± 5.5 (23)	241.7 ± 5.2 (24)	0.02	229.1 ± 7.2 (9)	246.3 ± 5.0 (14)	0.08

[‡]Values are least squares means ± standard error of (n) observations.

Table 4-4. Effect of breed and oral vitamin E supplementation (avg. 1000 IU/head/day) during late gestation on serum vitamin E concentrations of 24-48 hour-old Angus and Hereford calves born in late winter to multiparous cows.[‡]

Breed	Serum Vitamin E (µg/ml)		<i>P</i> [§]
	Control	Treatment	
Angus	1.7 ± 0.1 (18)	2.3 ± 0.2 (19)	0.002
Hereford	1.4 ± 0.1 (9)	1.4 ± 0.2 (7)	0.96

[‡]Values are least squares means of (n) observations ± standard error.

[§]*P* values were derived using the SLICE option of SAS.

Table 4-5. Effect of breed on serum vitamin E concentrations during the periparturient period in a herd of multiparous Angus and Hereford cows (Trial 1) and a herd of nulliparous Angus and Hereford heifers (Trial 2) calving in late winter.[‡]

Sample Period	Serum Vitamin E (µg/ml)					
	Cows			Heifers		
	Angus	Hereford	<i>P</i>	Angus	Hereford	<i>P</i>
Initial set-up [§]	8.0 ± 0.2	6.8 ± 0.4	0.007	----	----	----
1-2 weeks pre-calving	5.2 ± 0.3	4.1 ± 0.2	0.01	4.0 ± 0.2	3.6 ± 0.4	0.40
Parturition	4.1 ± 0.2	3.5 ± 0.2	0.09	4.2 ± 0.3	3.1 ± 0.4	0.02
2-3 days post-calving	3.5 ± 0.2	3.1 ± 0.2	0.09	3.7 ± 0.2	3.0 ± 0.3	0.06

[‡]Values are least squares means ± standard error of 38 and 16 observations for the Angus and Hereford breeds in the cow herd and 20 and 5 observations for the Angus and Hereford breeds in the heifer herd, respectively.

[§]Samples taken approximately 1 month prior to the beginning of supplemental vitamin E provision.

Chapter 5

Effects of Oral Vitamin E Supplementation During Late Gestation in Beef Cows Calving in Late Summer

Abstract

The effects of oral vitamin E supplementation were examined in a beef cattle herd calving in late summer. Thirty-seven Angus and Hereford cattle were allotted to groups homogenous with respect to breed, age, and serum vitamin E concentration. Cattle were then randomly assigned to treatment and control groups approximately 1 month prior to the beginning of a 65-day calving season. Free-choice pasture served as the basis of all late gestation and periparturient rations. Cattle in the treatment group consumed an average of 600 IU/head/day of supplemental vitamin (treatment) E in a free-choice vitamin-mineral mix beginning 30 days prior to the start of a 65-day calving season and continuing until they calved. Treatment did not affect maternal concentrations of serum vitamin E at pre-calving, calving, or post-calving. Treatment did not affect colostrum vitamin E or IgG concentrations, nor did it affect serum vitamin E or IgG concentrations of 24-48 hour-old calves. 205-day adjusted weaning weights (AWWs) did not differ between treatment and control group calves. Several breed-related differences occurred. Angus calves had higher concentrations of vitamin E ($P=0.007$) and IgG ($P=0.006$) in serum at 24-48 hours of age than Hereford calves. Angus calves also had heavier 205-day AWWs than Hereford calves ($P=0.0002$). Angus dams had higher post-calving concentrations of vitamin E in serum ($P=0.07$) and colostrum ($P=0.03$) than Hereford dams.

INTRODUCTION

Calves are born with physiologically low stores of vitamin E, a fat-soluble vitamin that crosses the bovine placenta in limited amounts.^{136, 212} Adequate vitamin E status is necessary for proper musculoskeletal development^{91, 148} and optimal immune system function.^{169, 213} Colostrum is the primary source of vitamin E for neonatal calves.^{136, 212} Because calves are born with a low vitamin E status, attempts are often made to increase their vitamin E nutriture within the first few days of life. Supplemental vitamin E can be given to neonatal calves in an injectable form. Less commonly, calves are given oral vitamin E or cows are supplemented

during late gestation via oral or parenteral means in an attempt to increase colostrum vitamin E content.

Studies of vitamin E supplementation during late gestation in dairy cattle have primarily focused on enhancing cow immunity and performance, whereas such studies in beef cattle have centered on the benefits to the calf. The effects of vitamin E supplementation in pregnant beef cows have varied across experiments. Parenteral administration of 3000 IU of vitamin E to crossbred beef cows approximately one month prior to parturition increased plasma vitamin E concentrations in calves and enhanced their passive immune status.¹⁸² However, Hayek et al. reported no effect of injecting beef cows with 1000 IU vitamin E approximately two weeks prepartum on either the passive immune status or serum vitamin E concentration of their calves.¹⁸⁴ Weiss et al. increased the colostrum vitamin E content of Holstein cows by feeding 70 IU vitamin E/kg diet dry matter during the dry period,²⁰³ indicating the potential for oral vitamin E supplementation of the dam to increase vitamin E provision to the newborn calf.

Enhancement of the vitamin E status of neonatal calves may be justified when dams are consuming low concentrations of vitamin E during late gestation, but the potential for benefit is unknown when pregnant cows are on vitamin E-replete diets. Fresh, green forages such as good quality pasture generally contain a minimum of 50 mg α -tocopherol/kg dry matter,⁸ approximately 5-6 times the average α -tocopherol content of hay.²¹⁵ These amounts exceed the 15 mg α -tocopherol/kg ration dry matter recommended for mature beef cattle by the NRC. This study sought to determine whether supranutritional vitamin E supplementation of late gestation beef cattle consuming a presumably vitamin E-replete diet results in any production-related benefits.

Experimental objectives were to assess the effects of exposing late gestation cows on an ad-libitum fresh forage diet to a free-choice vitamin-mineral mix formulated to provide 600 IU supplemental vitamin E/head/day. Response variables examined were vitamin E concentrations in dam and calf serum, vitamin E and immunoglobulin G (IgG) concentrations of colostrum, calf passive immune status, and calf growth. A secondary objective of the study was to evaluate the data for any potential breed-related differences in vitamin E status among beef cattle.

MATERIALS AND METHODS

Experimental Design

Thirty-seven beef cattle calving in late summer were divided into groups homogenous with respect to vitamin E concentrations in serum, breed, and age. Cattle were then randomly assigned to treatment and control groups approximately 1 month prior to the beginning of a 65-day calving season that extended from late August through October. The study population was comprised of fifteen Angus and twenty-two Hereford cattle. Thirteen cattle involved in the study were nulliparous heifers. All cattle involved in the study were between two and six years of age, with the exception of one Angus cow that was eleven.

For the purposes of this study, 'supplemental vitamin E' refers to *dl*- α -tocopheryl acetate originating from a commercial premix (Rovamix E 125, Roche Vitamins and Fine Chemicals, Nutley, NJ) and added to a free-choice vitamin-mineral mix (Appendix A). Cattle in the treatment group were exposed to supplemental vitamin E beginning one month prior to the start of the calving season and ending at parturition. The free-choice vitamin-mineral mix that was available to both the treatment and control groups contained a basal concentration of 125 IU vitamin E/lb (as-fed basis).

Vitamin-mineral mix provision and intake were monitored 2-3 times per week. The concentration of supplemental vitamin E in the treatment group's vitamin-mineral mix was adjusted weekly using the previous week's average per head per day consumption rate. The average consumption rate of the previous week served as the basis of supplemental vitamin E provision for the forthcoming week. Samples of the vitamin-mineral mix with added vitamin E were analyzed by a commercial laboratory to ensure agreement between calculated and actual vitamin E concentrations. Exposure of the treatment group to supplemental vitamin E ceased at parturition, however all cows still had free-choice access to the vitamin-mineral mix.

Free-choice pasture comprised the majority of the brood cow rations for the duration of vitamin E supplementation. Pasture samples were analyzed for vitamin E content by high-pressure liquid chromatography (HPLC) at a commercial laboratory and were stored frozen at -20°C until analysis. In an attempt to minimize any potential pasture effects, treatment and control groups were alternated between pastures on a biweekly basis so that each group spent an equal amount of time on each of the pastures utilized during the supplementation phase of the

experiment. The vitamin-mineral mix offered free-choice to all cattle contained 30 mg Se/kg (as-fed basis).

Cattle were moved to small pastures 1-2 weeks prior to their anticipated due dates so they could be more closely observed in the periparturient period. Cows and their calves were placed in maternity stalls shortly after parturition and typically remained there for 2-3 days after calving in order to monitor and ensure calf well-being. Upon leaving the maternity stalls, all cows and calves were returned to pasture, managed, and fed as a single herd.

Sample Collection and Analysis

Blood samples were collected from cattle approximately 5 weeks prior to the beginning of vitamin E supplementation in order to use vitamin E concentrations in serum as a criterion for establishment of treatment and control groups. Blood samples were collected via coccygeal venipuncture three times from each cow after vitamin E supplementation began: approximately 1-2 weeks prior to calving, within 6 hours post-calving, and again 2-3 days post-calving. A single blood sample was collected from each calf at 24-48 hours of age via jugular venipuncture. All blood samples were collected into Vacutainers[®] (Beckton-Dickinson, Franklin Lakes, NJ) without additives. Blood samples were allowed to clot for 30-60 minutes, centrifuged, and the serum from each sample was then harvested and frozen at -20°C until analysis.

Colostrum samples were obtained from each dam within 6 hours of calving for determination of vitamin E and IgG concentrations. For each dam, a teat (or teats) was wiped clean, stripped 3-4 times, and 20-30 ml of colostrum was collected in a Whirl-Pak[®]. Pre-suckle colostrum samples were desired and were successfully obtained from twenty-eight cattle. Colostrum and serum samples were stored frozen at -20°C until the time of analysis. All samples were protected from light and heat during collection, handling, and analysis to reduce vitamin E degradation.

Calves were weighed at birth and again at weaning (late March) to determine 205-day adjusted weaning weights (AWWs) (Appendix B). All calf weights were obtained using platform scales. Calves averaged 204 days of age at weaning.

Serum vitamin E concentrations were determined by high-pressure liquid chromatography (HPLC) (Appendix C). Colostrum vitamin E concentrations were also determined by HPLC, but sample composition necessitated modification of the vitamin E

extraction method used (Appendix D). Serum and colostral IgG concentrations were determined via a single radial immunodiffusion (SRID) technique using a commercial kit (VMRD, Pullman, WA) (Appendices E and F).

Statistical Analysis

Data were analyzed using the general linear model of SAS (version 6.12).²¹⁸ The model included treatment, breed, and age, plus all two- and three-way interactions. Age was divided into two categories: nulliparous dams were categorized as heifers and multiparous dams as cows. Significant two-way interactions were evaluated using the SLICE option to compare means of one factor within each level of the other factor.²¹⁸

RESULTS

One Angus cow did not calve. Three Hereford dams had twins that were not sampled. These occurrences reduced the number of calves available for serum vitamin E and IgG analysis. One calf died at 7 days of age due to septicemia resulting from omphalitis. In addition, eight cow-calf pairs were sold prior to weaning. The combination of calf mortality and sold cow-calf pairs further reduced the number of calves available to evaluate 205-day AWWs.

Pastures contained a reported average of 1.7 mg/kg (100% DM basis) α -tocopherol. This is a very low α -tocopherol concentration for vegetative forage and is believed to be an erroneous value resulting from laboratory error or α -tocopherol degradation during storage of the samples before analysis.

Mean concentrations of serum vitamin E did not differ for the treatment and control groups approximately five weeks prior to the beginning of supplemental vitamin E provision (Table 5-1). Cattle in the treatment group consumed an average of 600 IU/head/day supplemental vitamin E for the duration of supplemental vitamin E provision. Cattle in both the treatment and control groups consumed an average of 3.0 mg of Se per day during late gestation.

Exposure of cattle to supplemental vitamin E provided in a free-choice vitamin-mineral mix (treatment) had no effect on serum vitamin E concentrations of dams at pre-calving, calving, or post-calving (Table 5-1). Treatment did not affect concentrations of vitamin E or IgG (P=0.14) in colostrum (Table 5-2), nor did it affect concentrations of vitamin E (P=0.18) or IgG

in serum of 24-48 hour-old calves from treated dams (Table 5-3). 205-day AWWs did not differ between treatment and control group calves (Table 5-3).

Several breed-related differences occurred. At 24-48 hours of age, Angus calves had higher concentrations of vitamin E ($P=0.007$) and IgG ($P=0.006$) in serum than Hereford calves (Table 5-4). Angus calves also had higher 205-day AWWs than Hereford calves ($P=0.0002$) (Table 5-4). Angus dams had higher post-calving serum vitamin E ($P=0.07$) and colostral vitamin E ($P=0.03$) concentrations than Hereford dams (Table 5-4).

DISCUSSION

Mean concentrations of vitamin E in the serum of cows were consistently higher in the present study than for the winter-calving herd (Chapter 4) at any given sample period. Mean serum vitamin E concentrations of prepartum cows in the present study are similar to those reported for nonlactating dairy cattle fed pasture during the dry period.¹⁹¹ Mean concentrations of serum vitamin E in 24-48 hour-old calves in the present study are similar to those in the winter-calving herd (Chapter 4) and those reported by Zobell et al.¹⁸⁵ Mean concentrations of serum IgG in 24-48 hour-old calves in the present study averaged 16% higher than in the winter-calving herd (Chapter 4) but 56% lower than those reported by Zobell et al.¹⁸⁵ Colostral vitamin E concentrations in the present study are similar to those of supplemented cows and all heifers in the winter-calving herd, but approximately 80% higher than those of unsupplemented cows in the winter-calving herd (Chapter 4).

Zobell et al.¹⁸⁵ and Bass (Chapter 4) both supplemented late gestation beef cows with oral vitamin E at the rate of 1000 IU/head/day. Zobell's study was conducted during the fall and winter in Alberta, Canada; and it is therefore probable that the cows consumed only harvested forage during the supplementation phase of the study. Cattle participating in Bass' study (Chapter 4) also consumed only harvested forage during the period of supplemental vitamin E provision. Cattle in the present study were grazing pasture and are presumed to have a greater total intake of vitamin E.^{8, 190, 191} This assumption provides one possible explanation for the lack of a treatment effect on the response variables measured.

Vitamin E has no true tissue reservoir, unlike the liver which can function as a storage depot for vitamin A.² Attainment and maintenance of optimal vitamin E concentrations within

the body are therefore dependent upon consistent and adequate vitamin E intake,¹⁹⁶ such as that afforded to cattle by ad-libitum consumption of vegetative pasture.^{191, 215} Once vitamin E intake, tissue demand, and body concentrations attain homeostasis, supplemental vitamin E may not affect concentrations of vitamin E in serum or colostrum unless it is consumed in amounts well above those provided in the basal diet. Charmley et al. fed pregnant, crossbred beef heifers 0, 1000, 2000, and 4000 IU of supplemental vitamin E/day for 21 days (basal dietary concentrations of vitamin E were not reported).¹⁹⁶ Heifers supplemented with vitamin E had higher concentrations of serum vitamin E than unsupplemented heifers, but the response to supplementation appeared to plateau with the 1000 IU/head/day treatment.¹⁹⁶

Other researchers have reported that cattle respond in a dose-dependent manner to the total amount of vitamin E consumed. Quigley and Bernard reported a dose-dependent increase in serum vitamin E concentrations of neonatal dairy calves fed averages of 11, 111, and 1011 IU vitamin E.²²⁹ Weiss et al. reported higher concentrations of vitamin E in serum for periparturient dairy cows consuming 4905 IU vitamin E/head/day than for those consuming 940 and 1900 IU vitamin E/head/day.²¹¹ Weiss et al. reported in another study that dairy cows consuming an average of 1474 IU vitamin E/head/day during the dry period had higher concentrations of serum vitamin E at calving than those consuming an average of 574 IU vitamin E/head/day.²⁰³ Garber et al. reported a linear, dose-dependent increase in serum vitamin E concentrations of crossbred, yearling beef steers fed 166, 465, 972, and 1753 IU vitamin E/head/day for 112 days.¹⁷⁸

It appears as though approximately a two-fold increase in the amount of vitamin E consumed is necessary to increase concentrations of serum vitamin E. The dairy studies^{203, 211, 229} mentioned above report dose-dependent increases in serum vitamin E concentrations when the total amount of vitamin E fed was at least 2.5-fold higher than the next level of vitamin E provision. Garber's study involving beef steers reports dose-dependent increases in serum vitamin E concentration for levels of vitamin E provision as low as 1.8-fold higher than the next level of vitamin E fed.¹⁷⁸ Bass reported higher concentrations of serum vitamin E for treated cattle (Chapter 4) that were associated with at least a four-fold increase in the total amount of vitamin E consumed. A similar difference in total vitamin E intake probably occurred in the study by Zobell et al.,¹⁸⁵ but the vitamin E content of the basal ration was not reported. Since treatment did not affect serum vitamin E concentrations in the present study, it is likely that the amount of supplemental vitamin E consumed did not result in a sufficient difference in

proportional intake of vitamin E between the treatment and control groups. Therefore supplemental vitamin E, relative to the amount in the basal diet, did not affect concentrations of vitamin E in serum or colostrum. In fact, consumption of an average of 600 IU/head/day supplemental vitamin E did not affect any response variable measured in this study.

Several breed-related differences in vitamin E status were found. Angus calves had higher concentrations of vitamin E in serum than Hereford calves at 24-48 hours of age (Table 5-4). Angus dams had higher concentrations of vitamin E in serum 2-3 days post-calving than Hereford dams and also higher concentrations of colostrum vitamin E (Table 5-4). Many of these results agree with those reported by Bass (Chapter 4), wherein Angus cows had higher serum and colostrum vitamin E concentrations than Hereford cows. In that same study, nulliparous Angus heifers had higher concentrations of vitamin E in serum at calving and 2-3 days post-calving than nulliparous Hereford heifers. The repeatability of these findings between differently designed studies provides additional evidence for the author's belief that the Angus and Hereford breeds differ with respect to vitamin E metabolism or nutriture.

In summary, supplementing vitamin E in a free-choice vitamin-mineral mix (average consumption of 600 IU/head/day) during late gestation did not benefit the cattle in this study. The absence of any response to treatment is attributed to the presumably high content of vitamin E in the basal diet^{8, 190, 191} that negated any response to the amount of supplemental vitamin E provided. Based upon these findings, oral vitamin E supplementation at the rate of 600 IU/head/day beginning one month prior to the onset of the calving season does not appear justified in pregnant beef cattle consuming fresh, vegetative forages.

Table 5-1. Effect of oral vitamin E supplementation (avg. 600 IU/head/day) during late gestation on serum vitamin E concentrations in periparturient Angus and Hereford dams calving in late summer.[‡]

Sample Period	Serum Vitamin E (µg/ml)		<i>P</i>
	Control	Treatment	
Initial set-up [§]	6.4 ± 0.2 (18)	6.2 ± 0.3 (19)	0.62
1-2 weeks pre-calving	6.3 ± 0.3 (18)	5.9 ± 0.4 (19)	0.42
Parturition	4.3 ± 0.2 (17)	4.8 ± 0.3 (18)	0.26
2-3 days post-calving	3.4 ± 0.2 (17)	3.8 ± 0.3 (17)	0.36

[‡]Values are least squares means of (n) observations ± standard error.

[§]Samples taken approximately 5 weeks prior to the beginning of supplemental vitamin E provision.

Table 5-2. Effect of oral vitamin E supplementation (avg. 600 IU/head/day) during late gestation on the immunoglobulin G (IgG) and vitamin E concentrations of colostrum from Angus and Hereford dams calving in late summer.[‡]

	Control	Treatment	<i>P</i>
Colostrum IgG (g/L)	117.4 ± 12.7 (17)	89.9 ± 9.6 (18)	0.14
Colostrum vitamin E (µg/ml)	16.8 ± 2.8 (16)	15.4 ± 2.7 (18)	0.73

[‡]Values are least squares means of (n) observations ± standard error.

Table 5-3. Effect of supplementing Angus and Hereford dams calving in late summer with oral vitamin E (avg. 600 IU/head/day) during late gestation on serum vitamin E and immunoglobulin G (IgG) concentrations of their 24-48 hour-old calves and on 205-day adjusted weaning weights (AWWs) of their calves.[‡]

	Control	Treatment	<i>P</i>
Serum vitamin E (µg/ml)	1.6 ± 0.2 (15)	2.1 ± 0.2 (18)	0.18
Serum IgG (g/L)	22.6 ± 3.1 (15)	26.4 ± 2.6 (18)	0.40
205-day AWW (kg)	227.4 ± 12.9 (9)	214.7 ± 11.2 (15)	0.36

[‡]Values are the least squares means of (n) observations ± standard error.

Table 5-4. Effect of breed on serum vitamin E and colostral IgG concentrations in Angus and Hereford dams calving in late summer and on serum vitamin E and immunoglobulin G (IgG) concentrations and 205-day adjusted weaning weights (AWWs) of their calves.[‡]

Variable	Breed		<i>P</i>
	Angus	Hereford	
Dam serum vit. E (µg/ml) 2-3 days post-calving	4.0 ± 0.4 (14)	3.2 ± 0.2 (20)	0.07
Colostral vit. E (µg/ml)	20.6 ± 3.6 (13)	11.6 ± 1.9 (21)	0.03
Serum vit. E (µg/ml) in 24-48 hr.-old calves	2.4 ± 0.3 (14)	1.3 ± 0.1 (19)	0.007
Serum IgG (g/L) in 24-48 hr.-old calves	31.4 ± 2.6 (14)	17.6 ± 2.5 (19)	0.006
Calf 205-day AWW (kg)	254.8 ± 6.3 (11)	187.3 ± 9.1 (13)	0.0002

[‡]Values are least squares means of (n) observations ± standard error.

Chapter 6

Effects of Oral Vitamin E Supplementation on Concentrations of Vitamin E, Phospholipid, and Cholesterol in Serum and Serum Lipoprotein Fractions of Beef Cattle

Abstract

The effects of oral vitamin E supplementation were evaluated on vitamin E, cholesterol, and phospholipid concentrations in periparturient beef cows and their 24-48 hour-old calves. Serum and two lipoprotein fractions, one containing no apolipoprotein B (non-ApoB) and another containing apolipoprotein B (ApoB), were assayed in all animals. Consumption of 600-1000 IU vitamin E/cow/day during late gestation (treatment) via a free-choice vitamin-mineral mix increased vitamin E concentrations in serum ($P=0.07$) and non-ApoB ($P=0.06$) from 1-2 weeks pre-calving to calving. Calves from treated cows had higher vitamin E concentrations in serum ($P=0.07$) and non-ApoB ($P=0.06$) at 24-48 hours of age than calves from untreated cows. Treatment did not affect cholesterol or phospholipid concentrations in serum or either lipoprotein fraction in either cows or calves. Vitamin E, cholesterol, and phospholipid concentrations decreased in serum and both lipoprotein fractions ($P\leq 0.0001$) of cows from 1-2 weeks pre-calving to calving. Effects of treatment on vitamin E cholesterol (VEC) and vitamin E phospholipid (VEPL) ratios in serum, non-ApoB, and ApoB were evaluated in both cows and calves. Treatment increased VEC ($P=0.004$) and VEPL ($P=0.003$) ratios in serum of cows; it also increased VEC ($P=0.003$) and VEPL ($P=0.003$) ratios in non-ApoB of cows. Calves from treated cows had higher VEC ($P=0.01$) and VEPL ($P=0.01$) ratios in serum, as well as higher VEC ($P=0.06$) and VEPL ($P=0.04$) ratios in non-ApoB than calves from untreated cows. All VEC and VEPL ratio values in serum and both lipoprotein fractions of cows decreased from pre-calving to calving ($P\leq 0.0001$), or were affected by a time by treatment interaction ($P\leq 0.05$), with the exception of the ApoB VEPL ratio. Various breed-related differences occurred between Angus and Hereford cows and calves in vitamin E, cholesterol, and/or phospholipid concentrations, and/or VEC and VEPL ratios.

INTRODUCTION

Vitamin E nutriture of cattle is commonly assessed by measuring concentrations of the vitamin in plasma or serum.^{14, 140, 146, 200, 230, 231} The resulting concentration is often used as the sole factor to decide whether an animal is deficient, marginal, or adequate with respect to vitamin E status. Like other fat-soluble vitamins, vitamin E is transported through the bloodstream attached to a carrier molecule. However, vitamin E uses serum lipoproteins rather than a specific carrier protein for transport through circulation.³⁶⁻³⁹ Vitamin E distributes proportionately among serum lipoprotein fractions in a concentration-dependent manner that follows the distribution of lipoprotein classes for a given species.⁴⁰ The predominant lipoprotein class present in the bovine is HDL,^{40-45, 231} which does not contain apolipoprotein B.^{48, 62}

Herdt and Smith advocated measuring vitamin E in conjunction with lipoproteins as a more accurate means of assessing vitamin E nutriture, given that serum vitamin E concentrations are related to and partially controlled by serum lipoprotein concentrations.⁴⁰ However, serum lipoprotein concentrations are controlled by lipid metabolism, a dynamic process that occurs independent of variations in vitamin E intake and metabolism. Thus, serum vitamin E concentrations are in part dependent upon an animal's lipoprotein status, which may vary considerably. A means of assessing circulating vitamin E concentrations that accounts for serum lipoprotein status was therefore sought.

Reporting vitamin E concentrations relative to a lipid component is not a new idea. As early as 1947, the tocopherol concentration of bovine colostrum was expressed per unit of fat.²¹⁷ Horwitt et al. suggested that plasma vitamin E concentrations be expressed per gram of total plasma lipid in 1972.²³³ Weiss et al. reported that plasma vitamin E concentrations declined by 49 and 35% from dry-off to calving in dairy cows receiving no supplemental vitamin E or 1000 IU/head/day through the dry period, respectively.²³⁴ However, when expressed per unit of plasma cholesterol, vitamin E concentrations at calving for these same two groups of cows were 92 and 116% of their respective dry-off values, thus demonstrating a relationship between concentrations of plasma vitamin E and cholesterol in dairy cows. This relationship served as the basis of Herdt and Smith's evaluation of the vitamin E cholesterol (VEC) ratio as a means to correct for the variability in serum vitamin E concentrations of dairy cattle resulting from changes in lipoprotein concentrations.⁴⁰

The relative concentrations of vitamin E and cholesterol do not differ between HDL, LDL and very low density lipoprotein (VLDL) fractions of dairy cattle.⁴⁰ Distributions of vitamin E and cholesterol across bovine lipoprotein fractions results in consistent VEC ratios in HDL, LDL and VLDL fractions.⁴⁰ Use of the VEC ratio associates changes in serum vitamin E concentration with changes in serum lipoprotein concentration and thereby increases accuracy in determination of a cow's vitamin E status.⁴⁰ This relationship holds true for Holstein cattle across various stages of lactation, with the possible exception of early lactation (0-20 days in milk).⁴⁰

Herd and Smith acknowledge that their study does not address any of several factors that influence circulating cholesterol and lipoprotein concentrations. Increased parity, milk yield, and stage of lactation increase serum cholesterol concentrations.²³⁵ Serum cholesterol concentrations also vary through the estrous cycle.²³⁶ Cholesterol is a primary precursor for the synthesis of bovine steroid hormones²³⁷ and the ovary exhibits one of the highest rates of cholesterol uptake per gram of organ tissue,²³⁸ however, the relative impact of these two factors on circulating cholesterol concentrations is minimal.

Serum cholesterol concentrations are determined by serum lipoprotein concentrations, which may be modified by external factors. Supplemental fat in the ration increases²³⁹⁻²⁴¹ and high ambient temperatures decrease²⁴² serum lipoprotein and total cholesterol²⁴³ concentrations in dairy cattle. Puppione reported that the onset of lactation increases HDL concentrations in bovine plasma.⁶²

Because extrinsic and intrinsic factors modify serum cholesterol concentrations, a different means of assessing vitamin E status relative to lipoprotein concentration is proposed. Phospholipids, like cholesterol, are an integral lipoprotein constituent; and serum cholesterol and phospholipid concentrations are positively correlated.^{42, 45, 244, 245} However, phospholipid concentrations may vary less than cholesterol concentrations, as phospholipids exist in lipoproteins solely as a structural component of the more hydrophilic particle exterior.⁴⁸ Additionally, vitamin E is associated with phospholipids in membranes,^{246, 247} suggesting a better structural relationship with phospholipid than cholesterol. Lastly, serum phospholipid concentrations are equally, if not more consistent than cholesterol concentrations across both changes in lipoprotein density and variations in animal age and production stage.^{42, 45, 244, 245} These considerations offer reasonable evidence of the potential for a consistent relationship

between vitamin E and phospholipid in bovine serum and across bovine lipoprotein fractions. A vitamin E phospholipid (VEPL) ratio may therefore be a more accurate indicator of bovine vitamin E concentration per lipoprotein particle than is the VEC ratio.

Herd and Smith did not evaluate vitamin E or cholesterol concentrations of the same animal at different physiological states. Examination of the changes in lipoprotein vitamin E, cholesterol, and phospholipid concentrations in the same animals across time may yield further evidence of the interrelationship and dynamics of the aforementioned lipoprotein components. Such data might support the superiority of one ratio (VEPL or VEC) over the other.

This study sought to evaluate the relationships of vitamin E, cholesterol, and phospholipid in serum and two lipoprotein fractions of beef cattle. Serum lipoproteins are commonly separated and classified based upon density^{40, 42, 44} or apolipoprotein content.⁷²⁻⁷⁴ Ultracentrifugation is used to separate lipoproteins into density-dependent classes designated as HDL, LDL, and VLDL.^{40, 42, 44} Lipoprotein fractions evaluated in the present study were separated based upon apolipoprotein content. These lipoprotein fractions either did not contain apolipoprotein B (non-ApoB) or did contain apolipoprotein B (ApoB) and equate with HDL and LDL+VLDL, respectively.

Specific objectives included determination of vitamin E, cholesterol, and phospholipid concentrations and relationships in serum, non-ApoB, and ApoB of beef cows between pre-calving and calving. Vitamin E, cholesterol, and phospholipid concentrations and relationships were also evaluated in serum, non-ApoB, and ApoB of 24-48 hour-old beef calves. The effects of breed and provision of supplemental vitamin E (treatment) were examined on concentrations of vitamin E, cholesterol, and phospholipid in the serum and lipoprotein fractions of cows and calves. Vitamin E phospholipid and VEC ratios were calculated for serum, non-ApoB, and ApoB of cows at pre-calving and calving, and of their 24-48 hour-old calves. The effects of breed and provision of supplemental vitamin E in a free-choice vitamin-mineral mix on the VEPL and VEC ratio values were also examined.

MATERIALS AND METHODS

Experimental Design

Blood samples were obtained from beef cattle involved in two studies evaluating the effects of oral vitamin E supplementation during late gestation (refer to Chapters 4 and 5). Fifty-two Angus and thirty-eight Hereford cows and heifers from two herds calving in either late winter or late summer were chosen at random for inclusion in the study. Dystocia, neonatal calf death, the birth of three sets of twins, and one cow not calving reduced calf numbers to forty-nine and thirty-five for the Angus and Hereford breeds, respectively.

Sample Collection and Analysis

Blood samples were collected via coccygeal venipuncture twice from each cow after the start of vitamin E supplementation: approximately 1-2 weeks prior to calving and within 6 hours post-calving. A single blood sample was collected from each calf at 24-48 hours of age via jugular venipuncture. All blood samples were collected into Vacutainers[®] (Beckton-Dickinson, Franklin Lakes, NJ) without additives. Blood samples were allowed to clot, centrifuged, and the serum from each sample was then harvested and frozen at -20°C until analysis. All samples were protected from light and heat during collection, handling, preparation, and analysis to reduce vitamin E degradation.

Two lipoprotein fractions, non-ApoB and ApoB, were derived from serum and separated via heparin affinity chromatography (Appendix G) using a commercial kit (LDL Direct Plus, Isolab Inc., Akron, OH). Vitamin E concentrations in serum, non-ApoB, and ApoB were determined by high-pressure liquid chromatography (HPLC) (Appendix C). Phospholipid and cholesterol concentrations in serum, non-ApoB, and ApoB were determined by enzymatic colorimetric reactions using commercial kits (Appendices H and I, respectively). Colostrum was analyzed for fat content at a DHIA laboratory via near-infrared reflectance spectroscopy (NIRS).

Data Evaluation and Statistical Analysis

Data were analyzed using the GLM procedure of SAS (version 6.12).²¹⁸ Vitamin E, cholesterol, and phospholipid concentrations of cows were evaluated within serum, non-ApoB, and ApoB using a split-plot in time repeated measures analysis of variance. The model included

breed and treatment as whole-plot factors with time as the sub-plot factor; it also included all their interactions. Cow nested within treatment and breed was used as the whole-plot error term. Vitamin E phospholipid and VEC ratios were calculated on an equivalent mass basis for serum and each lipoprotein fraction, then examined with the same repeated measures analysis of variance model described above. Significant two-way interactions were evaluated using the SLICE option to compare means of one factor within a fixed level of the other factor.²¹⁸

Concentrations of vitamin E, cholesterol, and phospholipid in serum, non-ApoB, and ApoB of calves were evaluated by analysis of variance. The model included treatment, breed, and treatment by breed interaction. This model was also used to evaluate VEPL and VEC ratios in serum, non-ApoB, and ApoB of calves. Vitamin E phospholipid and VEC ratios were not statistically compared, as they were not independent of one another.

Linear regressions were performed for vitamin E versus phospholipid and vitamin E versus cholesterol in serum and each lipoprotein fraction of cows pre-calving, cows at calving, and 24-48 hour-old calves. The main effect of breed on colostral vitamin E and lipid content was tested ad hoc by analysis of variance in an attempt to explain breed-related differences in concentrations of vitamin E, phospholipid, and cholesterol in serum and non-ApoB of 24-48 hour-old calves.

RESULTS

Treated cows had higher vitamin E concentrations in serum ($P=0.07$) and non-ApoB ($P=0.06$) from 1-2 weeks pre-calving to calving (Table 6-1). Serum VEC and VEPL ratios were higher for treated versus untreated cows ($P=0.004$ and $P=0.003$, respectively) (Table 6-2). Treatment did not affect VEC or VEPL ratios in ApoB of cows (Table 6-2). Cholesterol and phospholipid concentrations of serum and both lipoprotein fractions were not affected by treatment in cows from 1-2 weeks pre-calving to calving (Table 6-1). Treatment also did not affect vitamin E concentrations in ApoB of cows (Table 6-1).

Calves from treated cows had higher vitamin E concentrations in serum ($P=0.07$) and non-ApoB ($P=0.06$) at 24-48 hours of age than control group calves; mean concentrations of vitamin E in ApoB did not differ between the two groups of calves (Table 6-3). Treatment did not affect concentrations of cholesterol or phospholipid in serum or either lipoprotein fraction of

calves (Table 6-3). Calves from treated cows had higher VEC ratios in serum (P=0.014) and non-ApoB (P=0.009), as well as higher VEPL ratios in serum (P=0.06) and non-ApoB (P=0.04) than control group calves (Table 6-4). Treatment did not affect VEPL (P=0.14) but tended to affect VEC (P=0.09) ratios in ApoB of calves (Table 6-4).

All response variables of cows except the VEPL ratio of ApoB decreased from pre-calving to calving or were affected by a treatment-by-time or breed-by-time interaction (Tables 6-1 and 6-2). The VEPL ratio of ApoB averaged 6.18×10^{-3} across the pre-calving to calving time period (Table 6-2). Concentrations of vitamin E, cholesterol, and phospholipid in serum and non-ApoB, as well as concentrations of vitamin E and phospholipid in ApoB, all decreased between 1-2 weeks pre-calving and calving (P≤0.0001) (Table 6-1). The VEC ratio in ApoB of cows decreased from 1-2 weeks pre-calving to calving (P=0.06) (Table 6-2).

Cholesterol concentration in ApoB differed between treated and untreated cows from pre-calving to calving (P=0.04). Cholesterol concentrations in ApoB decreased over time in treated cows (P=0.0001), but decreased to a lesser extent in untreated cows (P<0.0001) (Table 6-5). There was an interaction between time and treatment for VEC ratios in non-ApoB of cows (P=0.05). Vitamin E cholesterol ratios of non-ApoB did not change from pre-calving to calving for treated cows but decreased for untreated cows (P=0.03) (Table 6-5). Similarly, there was an interaction between time and treatment for VEPL ratios in non-ApoB of cows (P=0.02). Vitamin E phospholipid ratios did not change in non-ApoB from pre-calving to calving in treated cows (P=0.16), but tended to decrease in untreated cows (P=0.07) (Table 6-5).

Breed and time interacted on VEC (P=0.05) and VEPL (P=0.04) ratios in the serum of cows. Serum VEC and VEPL ratios of Angus cows did not change between pre-calving and calving but serum VEC and VEPL ratios of Hereford cows decreased (P=0.003 and P=0.0007, respectively) during the same period (Table 6-6). The change in VEPL ratios of non-ApoB differed for Angus and Hereford cows between pre-calving and calving (P=0.04). Vitamin E phospholipid ratios in non-ApoB of Angus and Hereford cows numerically increased (P=0.19) and decreased (P=0.12), respectively, between pre-calving and calving (Table 6-6).

Angus cows had lower cholesterol concentrations in non-ApoB (P=0.048) and higher phospholipid concentrations in ApoB (P=0.014) than Hereford cows (Table 6-1). Angus cows had higher VEC ratios in non-ApoB than Hereford cows (P=0.049) (Table 6-2). At 24-48 hours of age, Angus calves had higher vitamin E concentrations in serum (P=0.009) and non-ApoB

($P=0.001$) than Hereford calves (Table 6-3). Angus calves had higher concentrations of cholesterol and phospholipid in serum ($P=0.0002$ and $P=0.0001$, respectively) and non-ApoB ($P=0.0001$ for both), than Hereford calves (Table 6-3). No breed-related effects occurred for calf VEC or VEPL ratios (Table 6-4).

Ad hoc analysis of colostrum composition revealed the Angus breed had higher colostrum vitamin E ($P=0.03$) and fat ($P=0.04$) content than Herefords. Mean colostrum vitamin E concentration for the Angus breed was 16.9 ± 1.7 $\mu\text{g/ml}$ versus 10.9 ± 2.0 $\mu\text{g/ml}$ for Herefords. Angus and Hereford colostrum contained an average of $7.0 \pm 0.6\%$ and $5.0 \pm 0.7\%$ fat, respectively.

Linear regressions of cholesterol, phospholipid, and vitamin E in serum, non-ApoB, and ApoB were performed for cows pre-calving, cows at calving, the concentration differences between the two physiologic states, and 24-48 hour old calves (Appendix J). All linear regressions of cholesterol versus vitamin E and phospholipid versus vitamin E were significant ($P \leq 0.005$) with the exception of phospholipid versus vitamin E in ApoB of calves ($P=0.52$). Relationships between cholesterol and vitamin E and phospholipid and vitamin E respectively explained 29% and 31% of the variation in serum vitamin E concentrations in cows 1-2 weeks before calving (Figure 6-1). In parturient cows, relationships between cholesterol and vitamin E and phospholipid and vitamin E explained 22% and 25% of the variation in serum vitamin E concentrations, respectively (Figure 6-1). Relationships between cholesterol and vitamin E and phospholipid and vitamin E respectively explained 32% and 26% of the variation in serum vitamin E concentrations for 24-48 hour-old calves (Figure 6-1).

DISCUSSION

Results of this study indicate oral vitamin E supplementation does not alter circulating concentrations of cholesterol or phospholipid in serum lipoproteins of cattle. This finding agrees with those of Weiss et al. who determined that feeding 890 IU/head/day supplemental vitamin E to dry dairy cows did not affect plasma cholesterol concentrations at any time from 2 weeks pre- to 2 weeks post-calving but increased plasma vitamin E concentrations at parturition.²⁰⁰

Serum cholesterol concentrations observed for beef cows in this study averaged 128 mg/dl 1-2 weeks pre-calving and 109 mg/dl at calving. Comparable concentrations of serum

cholesterol were reported by Herdt and Smith for late gestation and periparturient dairy cattle,⁴⁰ and by Marcos et al. for dairy cows with normal liver triglyceride content in early lactation.⁷⁶ Serum cholesterol concentrations of cows reported in the present study are also similar to those reported by Raphael et al. for Holsteins 0-15 weeks pre-calving⁴² and by Puppione et al. for lactating, non-pregnant dairy cows.²⁴⁴ However, cholesterol concentrations within lipoprotein fractions might differ for beef and dairy cows. Results of the current study indicate periparturient beef cows have higher non-ApoB and lower ApoB cholesterol concentrations than late gestation dairy cows with similar concentrations of serum cholesterol.

Serum phospholipid concentrations in the present study averaged 131 and 113 mg/dl for pre-calving and calving cows, respectively. These serum phospholipid concentrations are similar to those reported by Raphael et al. for Holsteins 0-15 weeks pre-calving.⁴² However, beef cows in this study distribute serum phospholipid differently between lipoprotein fractions than do dairy cattle. As noted above for cholesterol, a greater concentration of phospholipid exists in non-ApoB and a lower concentration in ApoB of periparturient beef versus late gestation dairy cattle. Differences in cholesterol and phospholipid concentrations in lipoprotein fractions between beef and dairy cows likely reflect overall differences in lipoprotein fraction distributions.⁴⁰ These differences could result from differences in energy balance,²³⁵ genetics, diet,²³⁹⁻²⁴¹ method of sample analysis, or some combination of the above.

Calves in this study had serum cholesterol and phospholipid concentrations of approximately 50 and 75 mg/dl, respectively. These serum cholesterol concentrations are 50% lower than those reported for two-month-old Angus calves⁶⁰ and 40% lower than those reported for five-week-old Holstein-Friesian male calves fed a conventional milk replacer diet.²⁴⁸ In contrast, Forte et al. reported serum cholesterol concentrations for newborn calves (1-9 days of age) that were 12% lower than those reported in the present study.⁴⁵ Serum phospholipid concentrations of neonatal beef calves have not been previously reported to the author's knowledge. Although one study reporting the lipoprotein composition of newborn calves exists, breed is not specified.⁴⁵ Serum phospholipid concentrations of calves in the present study were 58% higher than those reported by Forte et al.⁴⁵ In contrast, serum phospholipid concentrations of calves in the present study were 44 and 37% lower than those previously reported for sixteen-²³⁹ and five-week-old²⁴⁸ Holstein calves, respectively. The differences in serum cholesterol and

phospholipid concentrations between neonatal beef calves and pre-ruminant dairy calves result in part from age-related differences in overall serum lipoprotein concentrations.^{45, 60}

Vitamin E, cholesterol, and phospholipid concentrations in the serum of 24-48 hour-old calves were 35, 42, and 61%, respectively, of the corresponding values for mature cows in the present study. Day-old calves differ from cows in their lipoprotein lipid and vitamin E concentrations because of the rapid conversion from a fetal lipoprotein profile where LDL (ApoB) predominates to a mature, HDL (non-ApoB)-predominant profile.⁴⁵ The calves' high dietary fat intake (in the form of colostrum) also supports a higher proportion of ApoB particles in serum when compared to the forage-based rations of adult cows.²³⁹

Significant effects of time on vitamin E, cholesterol, and phospholipid concentrations of periparturient cows were expected, but have not been previously reported for beef cattle. The periparturient decline in serum and lipoprotein concentrations of vitamin E, cholesterol, and phospholipid for beef cows in the present study is similar to the decline reported in dairy cattle,^{140, 200, 234, 235, 249} and is attributed to corresponding decreases in lipoprotein concentrations.^{42, 43} Findings of this study suggest that, as for dairy cattle,⁴⁰ the majority of lipids and vitamin E in beef cattle serum are associated with the non-ApoB (HDL) fraction.

Concentrations of vitamin E, cholesterol, and phospholipid differed between the Angus and Hereford breeds. Angus cows had lower non-ApoB cholesterol and higher ApoB phospholipid concentrations across time than Hereford cows. Angus calves had higher concentrations of vitamin E, cholesterol, and phospholipid in serum and non-ApoB than Hereford calves. The higher vitamin E and lipid content of Angus colostrum could account for the higher vitamin E, cholesterol, and phospholipid concentrations of Angus calves, as increasing dietary fat content is associated with higher serum lipoprotein concentrations in cattle.²³⁹⁻²⁴¹ Greater lipid deposition in colostrum could explain the lower cholesterol concentrations in non-ApoB of Angus cows.

Serum VEC ratios for cows in the present study are higher than those previously reported for dairy cows.⁴⁰ This difference results from higher concentrations of serum vitamin E for beef cows. The decline in VEC and VEPL ratios from pre-calving to calving in the present study indicates that factors other than decreasing lipoprotein concentrations may be involved in the periparturient decline in serum vitamin E concentrations. Possible reasons for the disproportional concentration changes of vitamin E and serum lipoproteins during the

periparturient period include deposition of vitamin E in colostrum,^{140, 203} a change in the saturation properties of lipoprotein particles,^{38, 39, 201} and decreased vitamin E intake.¹⁰²

Although values were not compared statistically, VEC and VEPL ratios of calves were consistently lower than those of cows. Lower ratios in calves support the premise that neonates have a lower vitamin E status than adult cattle. Serum VEC and VEPL ratios for calves suggest they are 80% and 55% as vitamin E-replete, respectively, as adult cattle. A comparison of serum vitamin E concentrations between cows and calves suggests calves are 35% as vitamin E-replete as cows. The variation in results among these three comparisons provides no support for any particular method(s). The author does not know which comparison best reflects actual vitamin E nutriture in cows and calves, but suspects the difference is more accurately represented by serum VEPL ratios or vitamin E concentrations. This assumption is based on the higher incidence of white muscle disease in calves as compared to adult cattle.^{2, 88, 136}

Differences in vitamin E concentrations caused the differences in VEC and VEPL ratios that occurred in the present experiment. For example, VEC and VEPL ratios in serum and non-ApoB for both cows and calves were increased by treatment because vitamin E concentrations in cow and calf serum and non-ApoB were also increased by treatment. This consistency suggests the ratios are sensitive indicators of changes in vitamin E concentration in light of cholesterol or phospholipid concentration. However, the author is not certain that VEC or VEPL ratios are substantial improvements over serum vitamin E concentrations for assessing vitamin E nutriture, or if the accuracy of one ratio exceeds that of the other.

The relationship between concentrations of phospholipid and vitamin E in serum explained a greater percentage of the variation in serum vitamin E concentrations for cows than did the relationship between concentrations of cholesterol and vitamin E in serum; the opposite was true for calves. However, the coefficients of determination for serum vitamin E versus cholesterol and serum vitamin E versus phospholipid were similar to one another in all linear regression analyses performed. Furthermore, no regression relationship explained more than 33% of the variation in serum vitamin E concentration. Simple linear regression therefore did not provide evidence that supported one ratio over the other.

Based upon the comparisons discussed above, results of this study suggest either serum cholesterol or phospholipid may be used as a lipoprotein “marker” against which to compare serum vitamin E concentrations for beef cows and calves.

In summary, vitamin E, cholesterol, and phospholipid appear to exhibit similar concentrations within and distributions among the serum lipoprotein fractions for beef cows and neonatal beef calves. The vitamin E and cholesterol data reported herein generally agree with those previously reported for dairy cows. VEC ratios for beef cows tend to be higher but exhibit the same periparturient changes as for dairy cows. Vitamin E supplementation does not affect concentrations of the major lipid components of bovine lipoproteins. Supplemental vitamin E affects VEC and VEPL ratios only when vitamin E concentrations are concurrently affected.

The findings of this study suggest that using either the VEPL or VEC ratio will result in a relatively equal assessment of vitamin E concentration relative to lipoprotein concentration for periparturient cow and neonatal calf serum. These ratios more accurately depict vitamin E concentration per lipoprotein particle, but may not reflect overall vitamin E status better than serum vitamin E concentrations alone. To potentially validate one or both ratios, the author believes research that compares ratio values to defined levels of vitamin E nutriture and also establishes ratio reference intervals needs to be conducted.

Table 6-1. Effects of time, breed, and consumption of 600-1000 IU/head/day supplemental vitamin E (treatment) during late gestation on concentrations of vitamin E, cholesterol, and phospholipid in serum, a lipoprotein fraction containing no apolipoprotein B (non-ApoB), and a lipoprotein fraction containing apolipoprotein B (apoB) of beef cows between 1-2 weeks pre-calving and calving.[‡]

Analyte	Fraction	Treatment		Time		Breed		<i>P</i>				
		Control	Supplement	Pre-calving	Calving	Angus	Hereford	Treatment	Time	Breed	Trt x Time	Brd x Time
Vitamin E (µg/ml)	Serum	4.8±0.2	5.4±0.2	5.7±0.2	4.4±0.2	5.2±0.2	5.0±0.2	0.07	0.0001	0.63	0.19	0.08
	Non-ApoB	4.2±0.2	4.8±0.2	5.0±0.2	4.1±0.2	4.6±0.2	4.5±0.2	0.06	0.0001	0.66	0.10	0.06
	ApoB	0.5±0.03	0.6±0.04	0.8±0.03	0.4±0.02	0.6±0.03	0.5±0.03	0.57	0.0001	0.53	0.55	0.52
Cholesterol (mg/dl)	Serum	120±3	117±3	128±3	109±2	116±2	121±3	0.55	0.0001	0.25	0.46	0.95
	Non-ApoB	112±3	111±3	120±3	103±2	107±2	116±3	0.78	0.0001	0.05	0.41	0.19
	ApoB	13±0.5	13±0.7	17±0.5	9±0.3	13±0.6	13±0.6	0.37	0.0001	0.71	0.04	0.94
Phospholipid (mg/dl)	Serum	124±2	121±2	131±2	113±2	122±2	122±3	0.41	0.0001	0.98	0.53	0.74
	Non-ApoB	107±2	105±2	116±2	96±2	103±2	109±3	0.40	0.0001	0.13	0.92	0.50
	ApoB	10±0.5	9±0.5	13±0.5	6±0.2	10±0.5	9±0.5	0.54	0.0001	0.01	0.61	0.27

[‡]Values are least squares means ± standard error of 90 pre-calving and calving observations, 43 and 47 control and supplement group observations, and 52 and 38 Angus and Hereford breed observations, respectively.

Table 6-2. Effects of time, breed, and consumption of 600-1000 IU/head/day supplemental vitamin E (treatment) during late gestation on vitamin E cholesterol (VEC) and vitamin E phospholipid (VEPL) ratios in serum, a lipoprotein fraction containing no apolipoprotein B (non-ApoB), and a lipoprotein fraction containing apolipoprotein B (apoB) of beef cows between 1-2 weeks pre-calving and calving.[‡]

Ratio (Value x 10 ⁻³)	Fraction	Treatment		Time		Breed		<i>P</i>				
		Control	Supplement	Pre-calving	Calving	Angus	Hereford	Treatment	Time	Breed	Trt x Time	Brd x Time
VEC	Serum	3.95±0.12	4.61±0.15	4.47±0.14	4.08±0.14	4.42±0.14	4.14±0.13	0.004	0.01	0.21	0.09	0.05
	Non-ApoB	3.73±0.10	4.35±0.14	4.12±0.12	3.96±0.13	4.24±0.13	3.83±0.11	0.003	0.26	0.05	0.05	0.13
	ApoB	4.20±0.17	4.37±0.27	4.63±0.27	3.94±0.18	4.39±0.24	4.18±0.19	0.55	0.06	0.47	0.78	0.91
VEPL	Serum	3.81±0.12	4.42±0.13	4.34±0.13	3.89±0.12	4.17±0.13	4.07±0.12	0.003	0.002	0.63	0.10	0.04
	Non-ApoB	3.89±0.12	4.59±0.15	4.26±0.13	4.22±0.15	4.38±0.14	4.10±0.13	0.003	0.74	0.23	0.03	0.04
	ApoB	5.98±0.28	6.43±0.31	6.33±0.28	6.08±0.31	5.89±0.26	6.53±0.35	0.33	0.54	0.16	0.63	0.70

[‡]Values are least squares means ± standard error of 90 pre-calving and calving observations, 43 and 47 control and supplement group observations, and 52 and 38 Angus and Hereford breed observations, respectively.

Table 6-3. Effects of maternal consumption of 600-1000 IU/head/day supplemental vitamin E (treatment) during late gestation and breed on concentrations of vitamin E, cholesterol, and phospholipid in serum, a lipoprotein fraction containing no apolipoprotein B (non-ApoB), and a lipoprotein fraction containing apolipoprotein B (apoB) of beef calves at 24-48 hours of age.[‡]

Analyte	Fraction	Treatment		Breed		<i>P</i>	
		Control	Supplement	Angus	Hereford	Treatment	Breed
Vitamin E (µg/ml)	Serum	1.6±0.1	1.9±0.4	2.0±0.1	1.5±0.1	0.07	0.009
	Non-ApoB	1.3±0.1	1.5±0.1	1.6±0.1	1.1±0.1	0.06	0.001
	ApoB	0.4±0.03	0.4±0.03	0.4±0.03	0.4±0.03	0.23	0.94
Cholesterol (mg/dl)	Serum	51±2	49±1	54±2	46±1	0.37	0.0002
	Non-ApoB	38±2	37±1	42±1	33±1	0.57	0.0001
	ApoB	13±0.5	12±0.4	13±0.5	12±0.5	0.35	0.36
Phospholipid (mg/dl)	Serum	76±3	75±2	83±3	68±2	0.85	0.0001
	Non-ApoB	63±3	62±2	69±2	55±2	0.94	0.0001
	ApoB	12±0.6	11±0.4	12±0.5	11±0.5	0.47	0.25

[‡]Values are least squares means ± standard error of 39 and 45 control and supplement group observations, and 49 and 35 Angus and Hereford breed observations, respectively.

Table 6-4. Effects of maternal consumption of 600-1000 IU/head/day supplemental vitamin E (treatment) during late gestation and breed on vitamin E cholesterol (VEC) and vitamin E phospholipid (VEPL) ratios in serum, a lipoprotein fraction containing no apolipoprotein B (non-ApoB), and a lipoprotein fraction containing apolipoprotein B (apoB) of beef calves at 24-48 hours of age.[‡]

Ratio (Value x 10 ⁻³)	Fraction	Treatment		Breed		<i>P</i>	
		Control	Supplement	Angus	Hereford	Treatment	Breed
VEC	Serum	3.14±0.18	3.84±0.20	3.68±0.19	3.31±0.21	0.01	0.19
	Non-ApoB	3.24±0.18	4.01±0.21	3.86±0.20	3.39±0.22	0.01	0.11
	ApoB	2.76±0.22	3.31±0.23	2.90±0.19	3.17±0.29	0.09	0.41
VEPL	Serum	2.14±0.13	2.51±0.14	2.40±0.13	2.24±0.14	0.06	0.42
	Non-ApoB	2.00±0.12	2.38±0.13	2.34±0.13	2.04±0.12	0.04	0.10
	ApoB	3.27±0.29	3.90±0.30	3.34±0.25	3.83±0.37	0.14	0.25

[‡]Values are least squares means ± standard error of 39 and 45 control and supplement group observations, and 49 and 35 Angus and Hereford breed observations, respectively.

Table 6-5. Effect of a treatment by time interaction on cholesterol concentrations in a lipoprotein fraction containing apolipoprotein B (apoB), vitamin E cholesterol (VEC) ratios in a lipoprotein fraction containing no apolipoprotein B (non-apoB), and vitamin E phospholipid (VEPL) ratios in non-ApoB of beef cows from 1-2 weeks pre-calving until calving.[‡]

Analyte or Ratio	Fraction	Group	Time		P [§]
			Pre-calving	Calving	
Cholesterol (mg/dl)	ApoB	Treatment	18±1	9±0	0.0001
		Control	16±1	10±0	<0.0001
VEC ratio (x 10 ⁻³)	Non-ApoB	Treatment	4.29±0.19	4.40±0.21	0.55
		Control	3.94±0.15	3.52±0.13	0.03
VEPL ratio (x 10 ⁻³)	Non-ApoB	Treatment	4.45±0.19	4.73±0.23	0.16
		Control	4.08±0.17	3.70±0.16	0.07

[‡]Values are least squares means ± standard error of 47 and 44 pre-calving observations, and 47 and 43 calving observations for the control and treatment groups, respectively.

[§]P-values derived from the SLICE option of SAS (version 6.12).

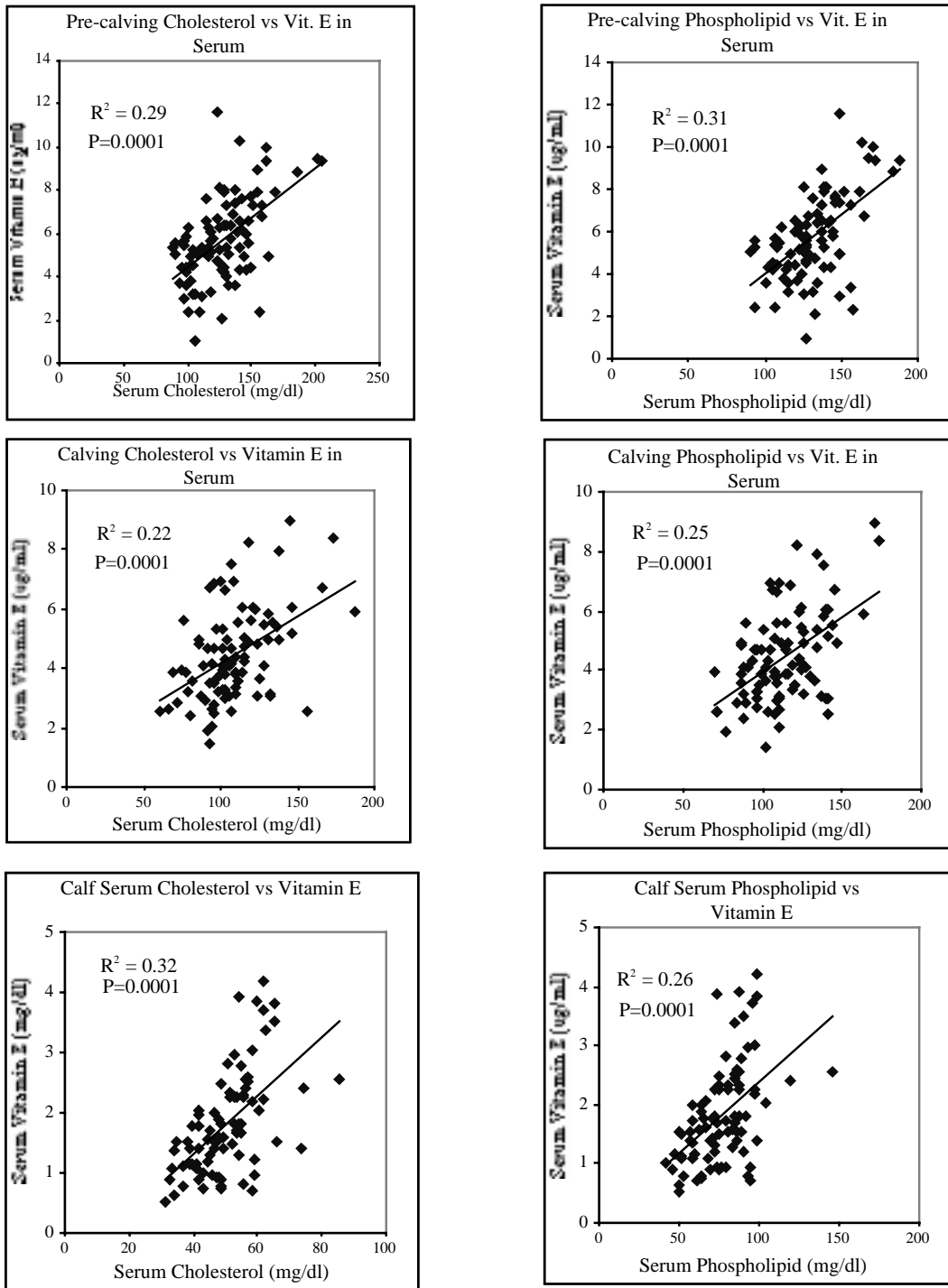
Table 6-6. Effect of a breed by time interaction on vitamin E cholesterol (VEC) and vitamin E phospholipid (VEPL) ratios in serum and on VEC ratios in a lipoprotein fraction containing no apolipoprotein B (non-ApoB) of beef cows between 1-2 weeks pre-calving and calving.[‡]

Ratio (x 10 ⁻³)	Fraction	Breed	Time		P [§]
			Pre-calving	Calving	
VEC	Serum	Angus	4.47±0.21	4.37±0.19	0.62
		Hereford	4.48±0.18	3.79±0.17	0.003
VEPL	Serum	Angus	4.24±0.19	4.09±0.17	0.38
		Hereford	4.43±0.16	3.70±0.17	0.0007
	Non-ApoB	Angus	4.26±0.18	4.50±0.22	0.19
		Hereford	4.27±0.18	3.93±0.18	0.12

[‡]Values are least squares means ± standard error of 54 and 38 pre-calving observations, and 53 and 38 calving observations for the Angus and Hereford breeds, respectively.

[§]P-values derived from the SLICE option of SAS (version 6.12).

Figure 6-1. Relationships between vitamin E and cholesterol, and vitamin E and phospholipid in the serum of beef cows 1-2 weeks pre-calving, cows at calving, and 24-48 hour-old beef calves.



Chapter 7

Summary and Conclusions

This dissertation focused on evaluating the effects of vitamin E supplementation in beef and dairy cattle during late gestation. A second objective was investigation of the relationship between vitamin E and lipoproteins in the serum of beef cattle. A synopsis of results and conclusions follows.

The study involving dairy cows (Chapter 3) revealed two previously unreported differences in circulating concentrations of micronutrients in dairy cattle. Jersey and Holstein cows had different blood Se concentrations through the dry period. Results also indicated the potential for breed-related differences in vitamin E metabolism, given the different serum vitamin E concentrations of Jerseys and Holsteins at dry-off. Previous reports of the duration of efficacy for supplemental vitamin E administered by parenteral means were confirmed. Injecting dairy cows with vitamin E at the recommended label dosages did not affect serum vitamin E concentrations 3-4 weeks post-administration. Therefore, this form of supplemental vitamin E provision was deemed impractical for beef cattle in most production scenarios due to labor considerations. Oral vitamin E supplementation was thus chosen in the beef cattle studies that followed. The author believes the oral route is the best means of providing supplemental vitamin E to cattle.

Beef cattle in both the winter- and summer-calving studies (Chapters 4 and 5, respectively) were provided with supplemental vitamin E in a free-choice vitamin-mineral mix. This means of vitamin E provision seems particularly appropriate for cow-calf herds, the majority of which subsist on 100% forage rations for most if not all of the year. Other methods of oral vitamin E provision require the daily feeding of some form of concentrate-- an impractical consideration for cattle on good quality pasture and a significant labor increase for herds consuming only pasture or hay. Providing supplemental vitamin E in the mineral mix would result in a minimal increase in labor.

Oral vitamin E supplementation enhanced production responses of cattle that consumed stored forages during late gestation. Conversely, cattle consuming vegetative forage did not respond to oral vitamin E supplementation during late gestation. In the presence of an abundance of dietary vitamin E,^{8, 191, 215} supplemental vitamin E may yield no responses unless

provided at levels approximating at least twice that of the basal diet.^{178, 203, 211} The author believes continuous, adequate vitamin E intake is essential for maintaining optimal beef cattle productivity. Results of the present experiments suggest that if beef cows are to attain optimal productivity, they may require higher dietary levels of vitamin E than those recommended by the National Research Council⁹⁹ and BASF Corporation.¹⁴⁵ Based on these results, the author believes beef cattle should consume a minimum of 1200 IU vitamin E/head/day during late gestation.

Most producers will adopt a new management practice if they anticipate tangible and economically justifiable results. Cows and calves in the treatment group of the winter-calving study may have derived unnoticed benefits (e.g. enhanced immune function^{15, 167, 168}) from the supplemental vitamin E, but this possibility was not evaluated. However, calves from supplemented cows in the winter-calving herd had increased 205-day adjusted weaning weights (AWWs) and calves from supplemented heifers in the winter-calving herd tended towards the same. This result economically justifies the cost of vitamin E supplementation.

Supplemental vitamin E cost approximately \$2.50 per head, given that the average cow in the winter-calving herd calved 30 days into the calving season. This yields an overall cost of approximately \$200 for vitamin E supplementation in the winter-calving herd. The twenty-four calves from treated cows in trial 1 and the fourteen calves from treated heifers in trial 2 had 205-day AWWs that averaged 19.6 and 17.2 kilograms heavier, respectively, than calves in the control groups. Attributing all differences in weight gain to the effects of supplementation would result in 711 additional kilograms of weaned calf from these two study populations. Even with an average price of \$65.00 per hundred-weight for weaned calves, greater than a 5-to-1 return on investment (excluding any added labor costs) was achieved for all cattle involved in the winter study. Thus, the heavier 205-day AWWs of calves in the treatment groups paid for the cost of supplementation more than five times over. Although the author would not recommend supplemental vitamin E under all circumstances, it appears cost-effective in cow-calf herds that are consuming stored forages during late gestation, are similarly managed, and of similar breed composition to that involved in the winter study. Further research should be done to test the repeatability of these findings and thereby provide additional evidence for or against the results on which these potential recommendations are based.

Vitamin E concentrations in serum and colostrum differed between Angus and Hereford cattle in several instances. Angus cows in the winter-calving herd had higher colostrum vitamin E concentrations and often had higher serum vitamin E concentrations than Herefords. Angus calves in the winter-calving herd had higher serum vitamin E concentrations at 24-48 hours of age than Hereford calves. Increased concentrations of serum vitamin E in Angus calves appear to be primarily of colostrum origin. Angus colostrum contains higher concentrations of both lipid and vitamin E than Hereford colostrum. These differences afford Angus calves a physiologic basis for higher concentrations of serum lipoproteins and also provide additional vitamin E that facilitates increased concentrations of this nutrient in serum.

Angus cows may not utilize the same mechanism as their calves for breed-related differences in serum vitamin E concentrations. Angus cows had statistically and numerically higher pre-calving concentrations of serum vitamin E than Hereford cows in the winter- and summer-calving herds, respectively. Although one might suspect Angus cows of having higher lipoprotein concentrations at this time, results indicate otherwise. Angus cows had lower concentrations of cholesterol in the lipoprotein fraction containing no apolipoprotein B (non-ApoB) and higher concentrations of phospholipid in the lipoprotein fraction containing apolipoprotein B (ApoB) than Hereford cows between pre-calving and calving. No breed-related differences in serum cholesterol and phospholipid concentrations occurred and the potential differences in lipoprotein fraction concentrations effectively cancelled each other out. Therefore, one might logically conclude that Angus cows have higher concentrations of serum vitamin E than Hereford cows with comparable concentrations of serum lipoproteins. Breed-related differences in lipoprotein saturation capabilities^{200, 226, 231} might explain this result, and the difference in VEC ratios in non-ApoB between Angus and Hereford cows provides some support for this theory. However, no breed-related differences in VEC or VEPL ratios occurred in serum or ApoB of cows. The variability of these results suggests additional research is required before more definitive conclusions are drawn.

Beef and dairy cattle exhibit similar vitamin E and lipoprotein relationships. Serum lipoprotein concentrations appear to be the primary determinant of serum vitamin E concentrations in beef cattle consuming diets that are not deficient in vitamin E. High-density lipoprotein (equated with non-ApoB in this dissertation), the predominant lipoprotein fraction in cattle, accounts for the majority of changes in serum vitamin E and lipoprotein concentrations

that occur over time.⁴⁰ Low-density lipoprotein and VLDL concentrations (equated with ApoB in this dissertation) influence serum vitamin E concentrations in a similar manner, but to a lesser extent than HDL.⁴⁰

Serum vitamin E can be reported relative to lipoproteins or some lipid component of lipoproteins.^{40, 230} Advocates believe such methods enhance the accuracy of assessing vitamin E nutriture by concurrently evaluating circulating vitamin E and lipid concentrations.⁴⁰ The VEC ratio proposed by Herdt and Smith⁴⁰ was evaluated against a novel method of assessing vitamin E concentration per lipoprotein particle proposed by the author, the VEPL ratio. Experimental results did not support greater accuracy of one ratio over the other and suggested no practical difference between the two. Consideration of analytical methods yields further impartiality to ratio selection, as the phospholipid and cholesterol assays are similar in cost (as utilized in this dissertation research), use the same equipment, and require the same amount of time and technical proficiency to run.

The working knowledge of vitamin E metabolism and homeostasis in ruminants presently contains as many assumptions, theories, and extrapolations as it does facts. Future research may reveal one of these ratios as more accurate or of greater practical application than the other. Vitamin E cholesterol and VEPL ratios do account for circulating lipid concentrations, but valid reference intervals have not yet been established for either ratio. Until this work is done, the added time and expense of performing the phospholipid or cholesterol analysis required for ratio determination is not justifiable from a diagnostic standpoint. The author therefore believes serum vitamin E concentrations presently remain the best means of assessing and predicting an animal's vitamin E nutriture.

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Appendix A

Composition of Free-choice Vitamin-mineral Mix (as-fed basis):

<u>Nutrient</u>	<u>Concentration</u>
Calcium	(min.) 12.50%(max.) 15.00%
Phosphorus	(min.) 7.00%
Salt (NaCl)	(min.) 11.00%(max.) 13.00%
Magnesium	(min.) 12.00%
Sulfur	(min.) 1.00%
Potassium	(min.) 1.00%
Zinc	(min.) 0.11%
Manganese	(min.) 0.10%
Copper	(min.) 0.028%
Iodine	(min.) 0.005%
Cobalt	(min.) 0.0011%
Selenium	(min.) 0.003%
Vitamin A	200,000 IU/lb.
Vitamin D	50,000 IU/lb.
Vitamin E	250 IU/lb.

Appendix B

Formula for Calculating 205-day Adjusted Weaning Weight (BIF Standard Adjustment Factors)

$$\begin{aligned} &(((\text{Actual Calf Weight} - \text{Birth Weight}) / \text{Days of Age}) * 205) + \text{Birth Weight} \\ &+ \text{Age of Dam and Calf Gender Adjustment} \end{aligned}$$

This equation adjusts calves to the same age, thus removing this variable from consideration. Age of dam and calf gender are also adjusted to remove two additional sources of variation. Calves from cows age 5-10 years receive no adjustment (adjustment factor=0). The following adjustment factors were used in calculating 205-day adjusted weaning weights for calves in chapters 4 and 5.

Age of Dam (years)	Weaning Weight Adjustment (kg)	
	Male	Female
2	27.27	24.55
3	18.18	13.36
4	9.09	8.18
5-10	0	0
11 and older	9.09	8.18

Appendix C

Analysis of Vitamin E Concentrations in Serum and Lipoprotein Fractions

A. Sample Collection and Handling

1. Blood samples were collected from each cow at the outset of the experiment, 1-2 weeks pre-calving, within 6 hours post-calving, and 2-3 days post-calving via coccygeal venipuncture. Blood samples were taken from each calf at 24-48 hours of age via jugular venipuncture. Vacutainers[®] (Beckton-Dickinson, Franklin Lakes, NJ) without additives were used to collect all blood samples.
2. Blood samples were allowed to clot for 30-60 minutes at room temperature, centrifuged at 3433 x g for 6 minutes, and the serum from each sample was then harvested.
3. Serum samples were stored frozen at -20°C until the time of analysis.
4. Samples were protected from light and heat to the greatest extent possible during collection, handling, and analysis.

B. Sample Preparation

1. Serum samples were removed from freezer and allowed to thaw and warm to room temperature.
2. After reaching room temperature, each serum sample was vortexed (10-15 seconds) to eliminate any settling or separation that may have occurred.
3. Serum samples were subsequently analyzed for vitamin E concentration.
4. Lipoprotein fractions were generated as indicated in Appendix G and were handled and analyzed by the same procedure as used for serum.

C. Vitamin E Extraction Procedure

1. Place sample (serum- 0.5 or 1.0 ml, HDL or LDL+VLDL eluate- 2.5 ml) in 12 X 80 mm glass test tube. Lipoprotein fraction eluates (2.5 ml each) used for vitamin E analysis were derived from 400 µl of serum.
2. Add 100 µl of 1000 µg/ml vitamin E acetate in ethanol (internal standard) to sample. Vortex test tube twice in succession for 7-8 seconds each time.
3. Add 2 ml HPLC-grade ethanol to sample and vortex twice for 5-6 seconds, then thrice more in succession for 9-10 seconds each time (precipitates serum proteins and deproteinates sample).
4. Add 3 ml HPLC-grade cyclohexane to sample. Vortex tube 3 times for 4-6 seconds each time, then thrice more in succession for 10-13 seconds each time (separates lipid-soluble components of serum into the added cyclohexane).
5. Centrifuge sample for 10 minutes at 3064 x g.
- When finished, centrifuged sample will exist in 3 phases: 1) top layer- cyclohexane layer (containing vitamin E), 2) bottom layer- ethanol layer, and 3) proteinaceous centrifugate.
6. Using a transfer pipette, remove **entire** cyclohexane (top) layer and place in 1 dram screw-cap vial.

7. Uncap vial and affix vial to a rotary evaporator (Büchi Rotavapor R-114, Brinkmann Instruments, Inc., Westbury, NY, 11590). Evaporate under vacuum at near-maximum rotary speed (160-180 rpm), with sample immersed in 38-39°C water bath until solvent is completely evaporated (approximately 3-4 minutes).

D. Sample Reconstitution

1. Reconstitute sample with 100 µl of 20% cyclohexane in ethanol, being certain to completely “rinse” vial with solvent in order to return all vitamin E to solution. Vortex thoroughly to enhance rinsing process. Serum samples of 0.5 and 1.0 ml have been concentrated 5- and 10-fold, respectively. Lipoprotein fraction samples have been concentrated 4-fold.
2. Pipette the entire 100 µl sample into a 150 µl HPLC sample insert within a brown HPLC vial in preparation for HPLC analysis. Cap tightly with top containing intact silicone septum.
3. Ensure that no air bubbles exist at the bottom of the sample insert vial.
4. Place prepared samples on HPLC machine for analysis.

E. HPLC Analysis for Vitamin E Concentration

1. Samples underwent HPLC analysis on a Varian Star Chromatograph (Varian, Sugar Land, TX). Serum and lipoprotein fractions were respectively analyzed with Supelco and Phenomenex[®] C18 reverse phase, 5 µm, 4.6 x 150 mm columns and guard column.
2. Pure methanol (100%) was employed as the mobile phase at a flow rate of 2 ml/min (isocratic elution).
3. Ultraviolet detection was carried out at 294 nm using a 10 µl injection loop for all serum samples and for cow lipoprotein fraction samples; injection volumes of 30 µl were used to ensure proper filling of sample loop. A 20 µl injection loop was used for all calf lipoprotein fraction samples; injection volumes of 60 µl were used on calf lipoprotein fraction samples to ensure proper filling of sample loop.
4. Alpha-tocopherol standards were analyzed with each batch of samples. Peak areas of six standard α -tocopherol solutions (5, 10, 25, 37.5, 50, 75, and 100 µg/ml) were used to generate a standard curve and associated regression equation.
5. The concentration of α -tocopherol in each sample was determined by comparing its peak area with those of standard solutions.
6. The peak area of each sample was plugged into the standard curve equation and the vitamin E (α -tocopherol) concentration was calculated. Percent recovery was corrected for using peak area of the α -tocopheryl acetate internal standard; sample concentration factors were also accounted for.

Appendix D

Analysis of Colostral Vitamin E Concentration

A. Sample Collection and Handling

1. Colostrum samples were obtained from each dam within 6 hours of calving for determination of IgG concentrations. For each dam, a teat (or teats) was wiped clean, stripped 3-4 times, and 20-30 ml of colostrum was collected in a Whirl-Pak[®].
2. Colostrum samples were stored frozen at -20°C until the time of analysis.
3. Samples were protected from light and heat to the greatest extent possible during collection, handling, and analysis.

B. Sample Preparation

1. Remove colostrum samples from freezer (-20°C), thaw, and allow them to warm to room temperature.
2. Vortex each colostrum sample thoroughly (30-40 seconds). Remove test tube cap and ring tube with wooden applicator stick to remove any lipid component that has not returned to suspension. Replace cap and vortex thoroughly again (additional 30-40 seconds).

C. Vitamin E Extraction Procedure

1. Place 500 mg colostrum sample in 12 X 80 mm glass test tube.
2. Add 100 μl of 1000 $\mu\text{g/ml}$ vitamin E acetate in ethanol (internal standard) to sample. Vortex test tube twice in succession for 10-12 seconds each time.
3. Add 3 ml HPLC-grade ethanol to sample and vortex twice for 5-6 seconds, then thrice more in succession for 9-10 seconds each time (precipitates serum proteins and deproteinates sample).
4. Add 3 ml HPLC-grade cyclohexane to sample. Vortex tube twice for 4-6 seconds, then thrice more in succession for 10-13 seconds each time (separates lipid-soluble components of serum into the added cyclohexane).
5. Centrifuge sample for 10 minutes at 3064 x g.
- When finished, centrifuged sample will exist in 3 phases: 1) top layer- cyclohexane layer (containing vitamin E), 2) bottom layer- ethanol layer, and 3) proteinaceous centrifugate.
6. Using a transfer pipette, remove **entire** cyclohexane (top) layer and place in 3 dram screw-cap vial.
7. Repeat steps 4-6 twice more, adding the resulting cyclohexane layers to the same aforementioned 3 dram vial.
8. Uncap vial and affix vial to a rotary evaporator (Büchi Rotavapor R-114, Brinkmann Instruments, Inc., Westbury, NY, 11590). Evaporate under vacuum at near-maximum rotary speed (160-180 rpm), with sample immersed in 38-39 $^{\circ}\text{C}$ water bath until solvent is completely evaporated (approximately 5-7 minutes).

D. Sample Reconstitution

1. Reconstitute sample with 100 μ l of 20% cyclohexane in ethanol, being certain to completely “rinse” vial with solvent in order to return all vitamin E to solution. Vortex thoroughly to enhance rinsing process. Colostrum samples have been concentrated 5-fold.
2. Pipette the entire 100 μ l sample into a 150 μ l HPLC sample insert within a brown HPLC vial in preparation for HPLC analysis. Cap tightly with top containing intact silicone septum.
3. Ensure that no air bubbles exist at the bottom of the sample insert vial.
4. Place prepared samples on HPLC machine for analysis.

E. HPLC Analysis for Vitamin E Concentration

1. Samples underwent HPLC analysis on a Varian Star Chromatograph (Varian, Sugar Land, TX). Serum and lipoprotein fractions were respectively analyzed with a Supelco C18 reverse phase, 5 μ m, 4.6 x 150 mm column and guard column.
2. Pure methanol (100%) was employed as the mobile phase at a flow rate of 2 ml/min (isocratic elution).
3. Ultraviolet detection was carried out at 294 nm using a 10 μ l injection loop. Injection volumes of 30 μ l were used to ensure proper filling of sample loop.
4. Alpha-tocopherol standards were analyzed with each batch of samples. Peak areas of six standard α -tocopherol solutions (5, 10, 25, 37.5, 50, 75, and 100 μ g/ml) were used to generate a standard curve and associated regression equation.
5. The concentration of α -tocopherol in each sample was determined by comparing its peak area with those of standard solutions.
6. The peak area of each sample was plugged into the standard curve equation and the vitamin E (α -tocopherol) concentration was calculated. Percent recovery was corrected for using peak area of the α -tocopheryl acetate internal standard; sample concentration factors were also accounted for.

Appendix E

Analysis of Serum IgG Concentration

A. Sample Collection and Handling

1. Blood samples were collected from each calf at 24-48 hours of age via jugular venipuncture. Vacutainers[®] (Beckton-Dickinson, Franklin Lakes, NJ) without additives were used to collect all blood samples.
2. Blood samples were allowed to clot for 30-60 minutes at room temperature, centrifuged at 3433 x g for 6 minutes, and the serum from each sample was then harvested.
3. Serum samples were stored frozen at -20°C until the time of analysis.

B. Sample Preparation

1. Serum samples were removed from freezer and allowed to thaw and warm to room temperature. IgG determination kits were stored refrigerated with agar plates inverted as directed by the manufacturer until used.
2. After reaching room temperature, each serum sample was vortexed (10-15 seconds) to eliminate any settling or separation that may have occurred.
3. Serum samples were subsequently analyzed for IgG content. Commercially available kits (VMRD, Inc., Bovine IgG SRID Kit) were utilized. Kit directions were followed as indicated below.

C. Single Radial Immunodiffusion Procedure

1. Add 3 µl of each bovine IgG reference standard (3300, 1650, 825, and 412 mg IgG/dl) to each of the first 4 wells in an anti-bovine IgG-containing agarose gel plate.
2. Remove 3 µl serum sample with disposable VMRD micropipette, and add to unfilled well on the agarose gel plate. Use a new pipette for each subsequent sample.
3. When all wells on plate are full, place cover firmly on plate.
4. Allow plate to incubate undisturbed, right-side up, and at room temperature for 18-22 hours prior to reading ring diameters.

D. Determination of Serum IgG Concentrations

1. Ring diameters of standards and samples were measured with an electronic RID measuring device (Electronic RID Plate Reader, Alta Diagnostic Machines, Ltd., Birmingham, England) that measured to the nearest 0.01 mm. For the purposes of calculation, each ring diameter was measured twice, with the resulting average value used as the basis for calculating IgG concentration.
2. Standards were plotted in semi-log form (ring diameter (mm) vs. log(IgG concentration)), with a computer generated linear regression fit to the resulting scatter plot using CricketGraph[®] computer software.

3. The computer-generated linear equation for each standard curve was used to calculate IgG concentrations on each serum sample tested on a given plate. Ring diameter was plugged into the regression equation as the x value with the resulting y value used to calculate the IgG concentration of each sample.
4. Standard solutions were assayed on each agar plate used. Therefore, a new standard curve was generated and served as the basis of concentration calculation on each agar plate used for determination of serum IgG concentration.

Appendix F

Analysis of Colostral IgG Concentration

A. Sample Collection and Handling

1. Colostrum samples were obtained from each dam within 6 hours of calving for determination of IgG concentrations. For each dam, a teat (or teats) was wiped clean, stripped 3-4 times, and 20-30 ml of colostrum was collected in a Whirl-Pak[®].
2. Colostrum samples were stored frozen at -20°C until the time of analysis.

B. Sample Preparation

1. Remove colostrum samples from freezer (-20°C), thaw, and allow them to warm to room temperature.
2. Vortex each colostrum sample thoroughly (30-40 seconds). Remove test tube cap and ring tube with wooden applicator stick to remove any lipid component that has not returned to suspension. Replace cap and vortex thoroughly again (additional 30-40 seconds).
3. Add 500 μl of colostrum to 1500 μl of 0.9% sterile saline, using Eppendorf[®] micropipette. This dilution results in a final volume of 2 ml. Vortex solution thoroughly (10-15 seconds).

C. Single Radial Immunodiffusion Procedure

1. Add 3 μl of each bovine IgG reference standard (3300, 1650, 825, and 412 mg IgG/dl) to each of the first 4 wells in an anti bovine IgG-containing agarose gel plate.
2. Remove 3 μl diluted colostrum sample with disposable VMRD micropipette, and add to unfilled well on the agarose gel plate. Use a new pipette for each subsequent sample.
3. When all wells on plate are full, place cover firmly on plate.
4. Allow plate to stand undisturbed, right-side up, and at room temperature for 18-22 hours prior to reading ring diameters.

D. Determination of Colostral IgG Concentrations

1. Ring diameters of standards and samples were measured with an electronic RID measuring device (Electronic RID Plate Reader, Alta Diagnostic Machines, Ltd., Birmingham, England) that measured to the nearest 0.01 mm. For the purposes of calculation, each ring diameter was measured twice, with the resulting average value used as the basis for calculating IgG concentration.
2. Using ring diameters from standard solutions (3300, 1650, 825, and 412 mg IgG/dl), ring diameter (mm) versus $\log(\text{IgG concentration})$ was plotted in CricketGraph[®] and served a standard curve for the samples on the respective agarose gel plate.
3. Using the formulated regression curve equation, colostral IgG concentrations were determined by plugging sample ring diameters into the equation as the x value with the resulting y value used to calculate concentration. Account for 1:3 dilution of colostrum with sterile saline by multiplying equation-generated concentration by four.

4. Standard solutions were assayed on each agar plate used. Therefore, a new standard curve was generated and served as the basis of concentration calculation on each agarose gel plate used for determination of colostral IgG concentration.

Appendix G

Elution of Lipoprotein Fractions Containing no Apolipoprotein B (non-ApoB) and Containing Apolipoprotein B (ApoB)

A. Sample Collection and Handling

1. Blood samples were collected from each cow at the outset of the experiment, 1-2 weeks pre-calving, and within 6 hours post-calving via coccygeal venipuncture. Blood samples were taken from each calf at 24-48 hours of age via jugular venipuncture. Vacutainers[®] (Beckton-Dickinson, Franklin Lakes, NJ) without additives were used to collect all blood samples.
2. Blood samples were allowed to clot for 30-60 minutes at room temperature, centrifuged at 3433 x g for 6 minutes, and the serum from each sample was then harvested.
3. Serum samples were stored frozen at -20°C until the time of analysis.
4. Samples were protected from light and heat to the greatest extent possible during collection, handling, and analysis.

B. Sample Preparation

1. Serum samples were removed from freezer and allowed to thaw and warm to room temperature.
2. After reaching room temperature, each serum sample was vortexed (10-15 seconds) to eliminate any settling or separation that may have occurred.

C. Derivation of non-ApoB Fraction

1. Drain columns (Isolab LDL-Direct Plus[™], Isolab Inc., Akron, OH) by removing top cap, then snapping off tip. Allow columns to fully drain.
2. Equilibrate columns by adding 1.25 ml of non-ApoB (α) fraction elution agent. Allow equilibration volume to fully drain.
3. Place receptacle tube (glass, 12 X 80 mm) under column. Then place 440 microliters of thawed, vortexed serum on column. Allow serum volume to fully enter top disc and column. Collect all drained eluate.
4. Place 110 microliters of non-ApoB (α) elution agent on top disc. Wait 5 minutes. Collect all drained eluate into the same tube as in step 3 above.
5. After the 5 minutes have passed, place 2.2 ml of non-ApoB (α) elution agent on column. Allow column to fully drain into the same tube used to collect eluate from steps 3 and 4.
6. Total non-ApoB eluate volume should equal approximately 2.75 ml. Determine exact total volume eluted by subtracting dry, empty tube weight from full tube weight, assuming 1ml eluate weighs 1g.

D. Derivation of ApoB Fraction

1. Place a new tube under the column once drainage of the non-ApoB (α) fraction elution is complete.

2. Add 2.75 ml ApoB (β) fraction elution agent to column. Allow column to fully drain, collecting all eluate.
3. Total ApoB eluate volume should equal approximately 2.75 ml. Determine exact total volume eluted by subtracting dry, empty tube weight from full tube weight, assuming 1ml eluate weighs 1g.

E. Subsequent Analysis of non-ApoB and ApoB Lipoprotein Fractions

1. Total volume (weight) eluted for each fraction is then determined.
2. Eluted fractions can then be assayed for vitamin E, phospholipid, and/or cholesterol content.
3. For determining fraction concentrations of phospholipid and cholesterol, 250 microliters of eluate is removed from each tube (using 100-1000 μ l Eppendorf[®] Reference Pipette). These samples are combined from each duplicate sample, with the resulting aggregate eluate thoroughly mixed, then used for phospholipid and cholesterol concentration determination (Appendices H and I).

Appendix H

Analysis of Phospholipid Concentration

A. Sample Collection and Handling

1. Blood samples were collected from each cow via coccygeal venipuncture two times for phospholipid concentration analysis: 1-2 weeks pre-calving and no greater than 6 hours post-calving.
2. Blood samples were collected from each calf at 24-48 hours of age via jugular venipuncture for phospholipid analysis.
3. Vacutainers[®] (Beckton-Dickinson, Franklin Lakes, NJ) without additives were used to collect all blood samples. All blood samples were allowed to clot for 30-60 minutes at room temperature, centrifuged at 3433 x g for 6 minutes, and the serum from each sample was then harvested.
4. Serum samples were stored frozen at -20°C until the time of analysis.

B. Sample Preparation

1. Serum samples were removed from freezer and allowed to thaw and warm to room temperature.
2. After reaching room temperature, each serum sample was vortexed (10-15 seconds) to eliminate any settling or separation that may have occurred.
3. Serum samples were then ready for phospholipid concentration analysis or lipoprotein fractionation. Lipoprotein fractions were generated as described in Appendix G.

C. Microplate Assay for Phospholipid Content

Serum

1. Dilute 90-100 µl of serum 1:1 with distilled water or sterile saline. Vortex the diluted serum thoroughly (8-10 seconds).
2. Add 10 µl of diluted serum to microplate well(s). Add 290 µl of phospholipid color reagent (Phospholipids B Test Kit, Wako Chemicals USA, Inc.) to well(s).
3. Place filled microwell plate (including standards) in microplate reader (SPECTRAmax[™] 250, Molecular Devices Corp., Sunnyvale, CA 94089) and mix for 5 seconds. Incubate plate for 12 min. at 37°C.
4. Read absorbance of well(s) at 505 nm and compare to regression curve equation generated (SOFTmax[®] PRO, Version 1.1, Molecular Devices Corp., Sunnyvale, CA, 94089) from standard solution absorbances to determine serum phospholipid concentration(s).
5. Phospholipid standard solution provided by Wako Chemicals as a component of the Phospholipids B test kit. Standard concentrations of 300, 150, 75, 37.5, 18.75, and 9.375 mg/dl used to generate a standard curve (see section D).

Non-ApoB and ApoB Lipoprotein Fractions

1. Add 70 μl of undiluted lipoprotein fraction eluate to plate well(s).
2. Add 230 μl of phospholipid color reagent to well(s) containing a lipoprotein fraction eluate sample.
3. Place filled microwell plate in microplate reader (SPECTRAMax™ 250, Molecular Devices Corp., Sunnyvale, CA 94089) and mix for 5 seconds. Incubate plate for 12 min. at 37°C.
4. Convert standard solutions from serum concentration-basis to fraction concentration-basis by dividing each standard by 14 (70 μl fraction versus 5 μl serum). Conversions: 300 mg/dl = 21.4 $\mu\text{g}/100 \mu\text{l}$, 150 mg/dl = 10.71 $\mu\text{g}/100 \mu\text{l}$, 75 mg/dl = 5.35 $\mu\text{g}/100 \mu\text{l}$, 37.5 mg/dl = 2.68 $\mu\text{g}/100 \mu\text{l}$, 18.75 mg/dl = 1.34 $\mu\text{g}/100 \mu\text{l}$, and 9.375 mg/dl = 0.67 $\mu\text{g}/100 \mu\text{l}$. Micrograms per 100 μl equals mg/dl.
5. Following 12 minute incubation, read absorbance of well(s) at 505 nm and compare to regression curve equation generated (SOFTmax® PRO, Version 1.1, Molecular Devices Corp., Sunnyvale, CA, 94089) from “lipoprotein fraction” standard solution absorbances to determine phospholipid concentrations of lipoprotein fractions.
6. The total eluate volume generated during lipoprotein fractionation was predetermined (Appendix G). Divide this value by 4.4 (total number of 100 μl units of serum from which the lipoprotein fractions were generated). This yields a conversion factor for each sample.
7. Multiply sample absorbance (from step 5 above) by the appropriate sample conversion factor (from step 6 above). The result is the concentration (mg/dl) of phospholipid in each lipoprotein fraction.

D. Making Standard Solutions for Microplate Phospholipid Analysis

Concentrations:

300 mg/dl Standard: 300 mg/dl **stock solution** (Phospholipids B Test Kit, Wako Chemicals USA, Inc.) diluted 1:1 with double-distilled water (ddH₂O) or sterile saline.

150 mg/dl Standard: 300 mg/dl solution diluted 1:1 with ddH₂O or sterile saline.

75 mg/dl Standard: 300 mg/dl solution diluted 1:3 with ddH₂O or sterile saline.

37.5 mg/dl Standard: 300 mg/dl solution diluted 1:7 with ddH₂O or sterile saline.

18.75 mg/dl Standard: 300 mg/dl solution diluted 1:15 with ddH₂O or sterile saline.

9.38 mg/dl Standard: 300 mg/dl solution diluted 1:31 with ddH₂O or sterile saline.

- All dilutions are on a volume:volume basis.
- To minimize degradation of standards they were individually frozen at -70°C in volumes required for the phospholipid assay. The required standards were then thawed as needed.

Appendix I

Analysis of Cholesterol Concentration

A. Sample Collection and Handling

1. Blood samples were collected from each cow via coccygeal venipuncture two times for cholesterol concentration analysis: 1-2 weeks pre-calving and no greater than 6 hours post-calving.
2. Blood samples were collected from each calf at 24-48 hours of age via jugular venipuncture for cholesterol analysis.
3. Vacutainers[®] (Beckton-Dickinson, Franklin Lakes, NJ) without additives were used to collect all blood samples. All blood samples were allowed to clot for 30-60 minutes at room temperature, centrifuged at 3433 x g for 6 minutes, and the serum from each sample was then harvested.
4. Serum samples were stored frozen at -20°C until the time of analysis.

B. Sample Preparation

1. Serum samples were removed from freezer and allowed to thaw and warm to room temperature.
2. After reaching room temperature, each serum sample was vortexed (10-15 seconds) to eliminate any settling or separation that may have occurred.
3. Serum samples were then ready for cholesterol concentration analysis or lipoprotein fractionation. Lipoprotein fractions were generated as described in Appendix G.

C. Microplate Assay for Cholesterol Content

Serum

1. Dilute 20 µl of serum with 230 µl sterile saline. Vortex thoroughly (8-10 seconds).
2. Add 60 µl of diluted serum to microplate well(s). Add 240 µl of cholesterol color reagent (Isolab LDL-Direct Plus[™], Isolab, Inc. Akron, OH) to well(s).
3. Place filled microwell plate (including standards) in microplate reader (SPECTRAmax[™] 250, Molecular Devices Corp., Sunnyvale, CA 94089) and mix for 5 seconds. Incubate plate for 12 min. at 37°C.
4. Read absorbance of well(s) at 505 nm and compare to regression curve equation generated (SOFTmax[®] PRO, Version 1.1, Molecular Devices Corp., Sunnyvale, CA, 94089) from standard solution absorbances to determine serum cholesterol concentration(s).
5. Cholesterol standards obtained from Stanbio and Sigma. Standard concentrations of 400, 200, 100, 50, 25, and 12.5 mg/dl used to generate a standard curve (see section D).

Non-ApoB and ApoB Lipoprotein Fractions

1. Add 60 µl of undiluted lipoprotein fraction to plate well(s).
2. Add 240 µl of cholesterol color reagent to well(s) containing a lipoprotein fraction eluate sample.

3. Place filled microwell plate in microplate reader (SPECTRAmax™ 250, Molecular Devices Corp., Sunnyvale, CA 94089) and mix for 5 seconds. Incubate plate for 12 min. at 37°C.
4. Convert standard solutions from serum concentration-basis to fraction concentration-basis by dividing each standard by 12.5 (60 µl fraction versus 4.8 µl serum).
Conversions: 400 mg/dl = 32 µg/100 µl, 200 mg/dl = 16 µg/100 µl, 100 mg/dl = 8 µg/100 µl, 50 mg/dl = 4 µg/100 µl, 25 mg/dl = 2 µg/100 µl, and 12.5 mg/dl = 1 µg/100 µl. Micrograms per 100 µl equals mg/dl.
5. Following 12 minute incubation, read absorbance of well(s) at 505 nm and compare to regression curve equation generated (SOFTmax® PRO, Version 1.1, Molecular Devices Corp., Sunnyvale, CA, 94089) from “lipoprotein fraction” standard solution absorbances to determine cholesterol concentration(s) of lipoprotein fractions.
6. The total eluate volume generated during lipoprotein fractionation was predetermined (Appendix G). Divide this value by 4.4 (total number of 100 µl units of serum from which the lipoprotein fractions were generated). This yields a conversion factor for each sample.
7. Multiply sample absorbance (from step 5 above) by the appropriate sample conversion factor (from step 6 above). The result is the concentration (mg/dl) of cholesterol in each lipoprotein fraction.

D. Making Standard Solutions for Microplate Cholesterol Analysis

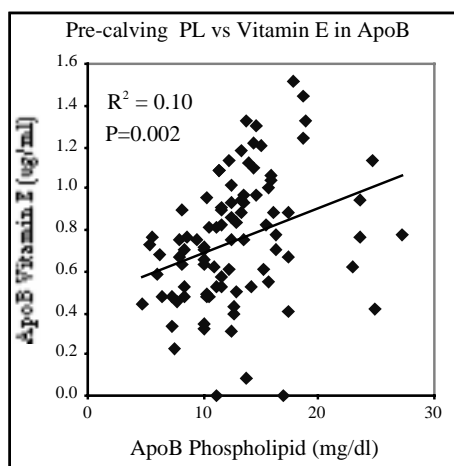
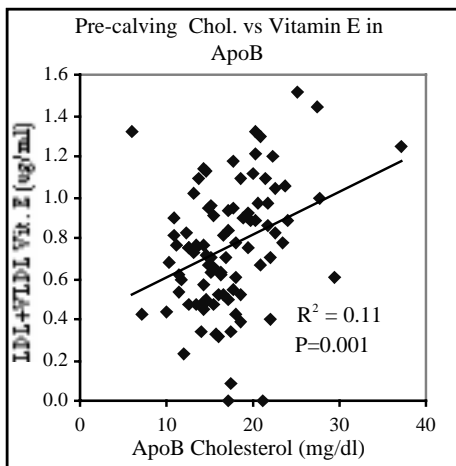
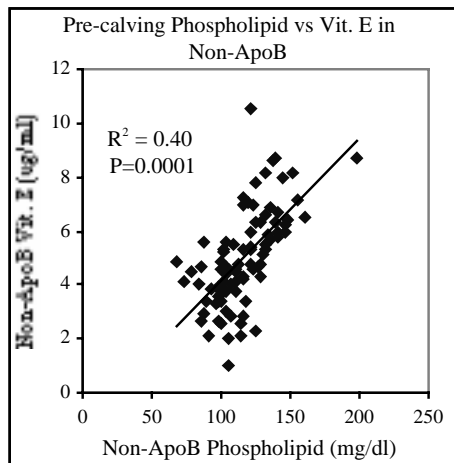
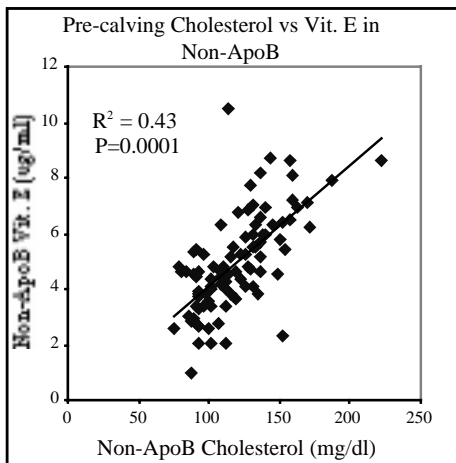
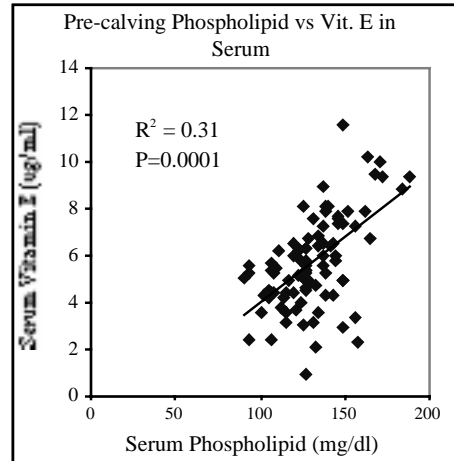
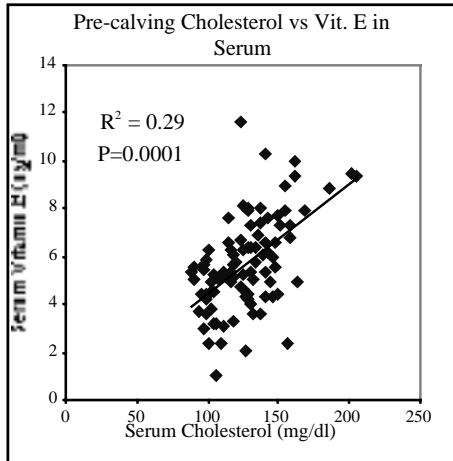
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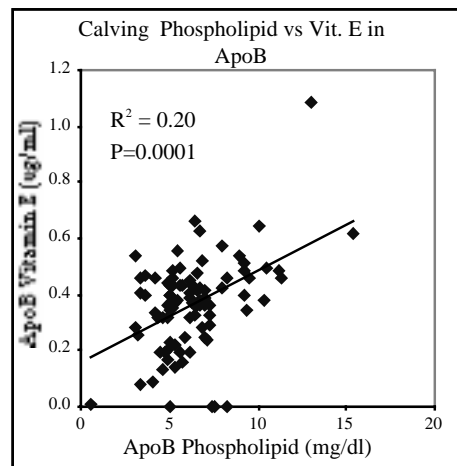
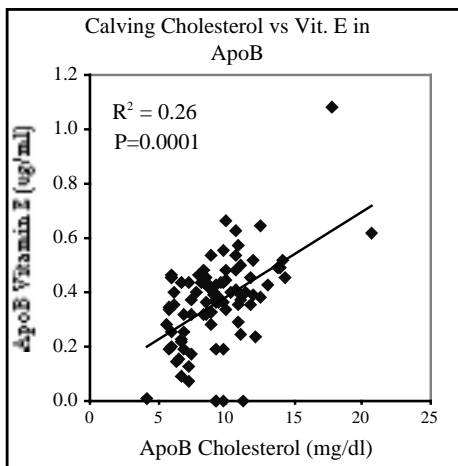
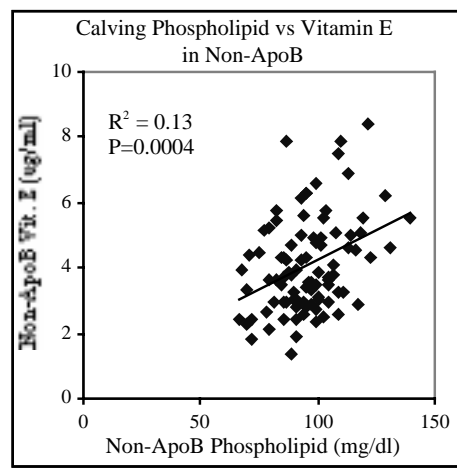
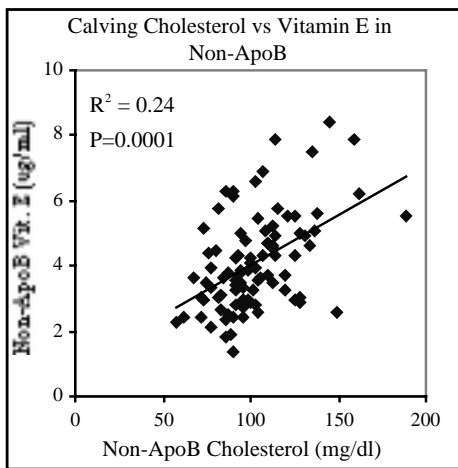
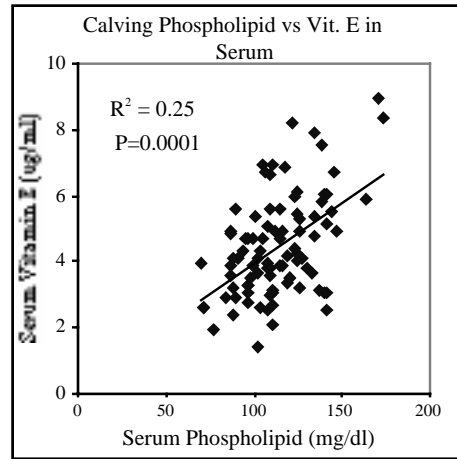
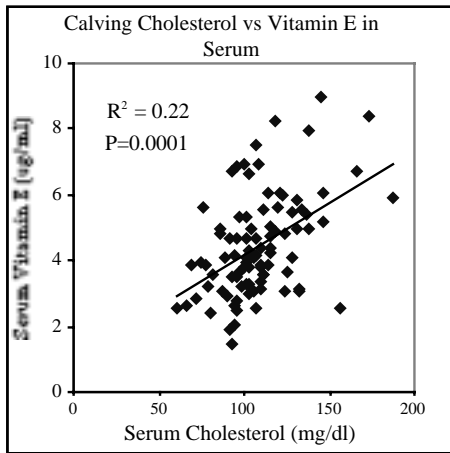
- 400 mg/dl:** 220 µl of 200 mg/dl **stock solution** diluted with 1155 µl sterile saline.
- 200 mg/dl:** 200 µl 200 mg/dl **stock solution** diluted with 2300 µl ml of sterile saline.
- 100 mg/dl:** 200 mg/dl solution diluted 1:1 with sterile saline.
- 50 mg/dl:** 200 mg/dl solution diluted 1:3 with sterile saline.
- 25 mg/dl:** 200 mg/dl solution diluted 1:7 with sterile saline.
- 12.5 mg/dl:** 200 mg/dl solution diluted 1:15 with sterile saline.
- 6.25 mg/dl:** 200 mg/dl solution diluted 1:31 with sterile saline.

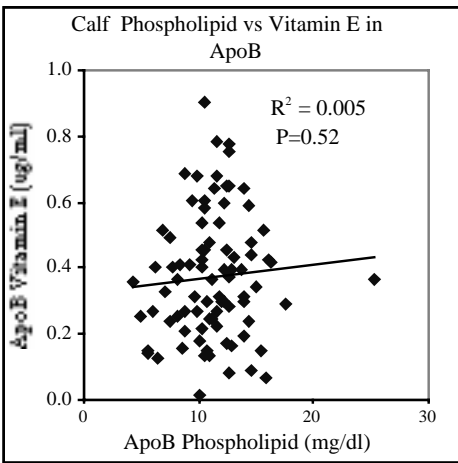
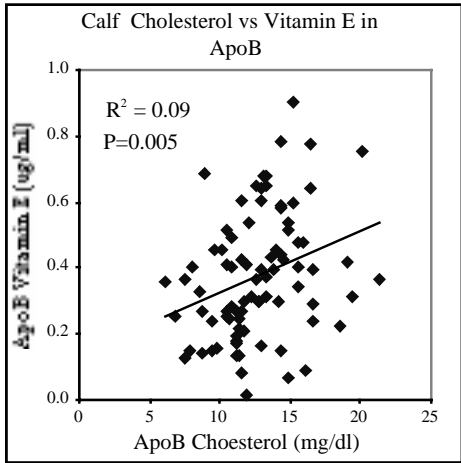
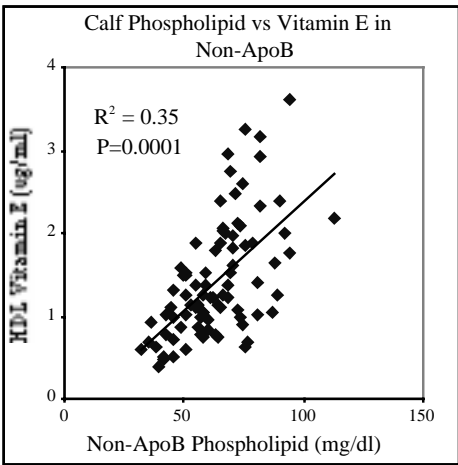
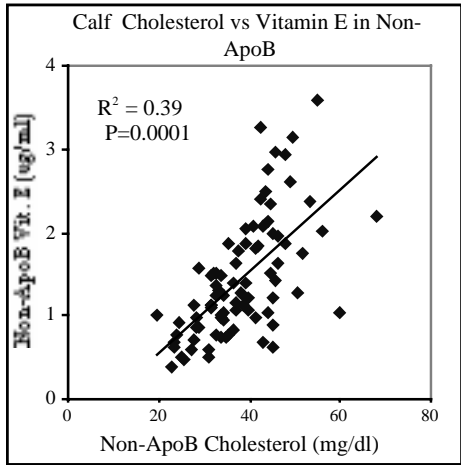
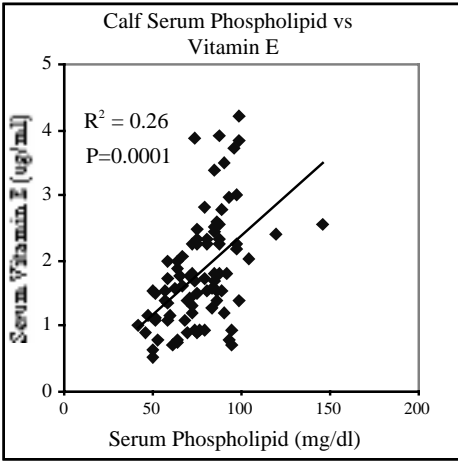
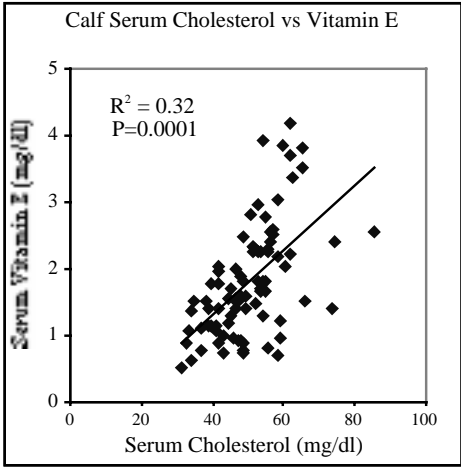
- All dilutions are on a volume:volume basis.
- To minimize degradation of standards they were individually frozen at -70°C in volumes required for the cholesterol assay. The required standards were then thawed as needed.

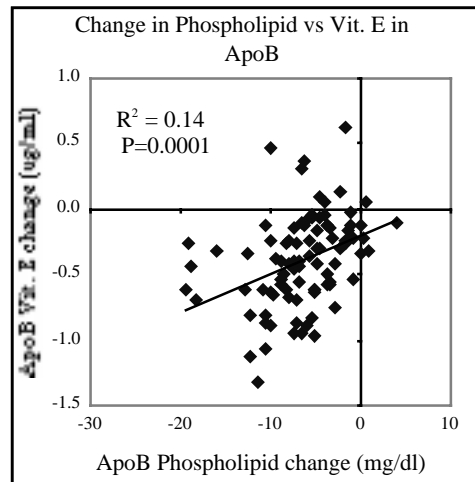
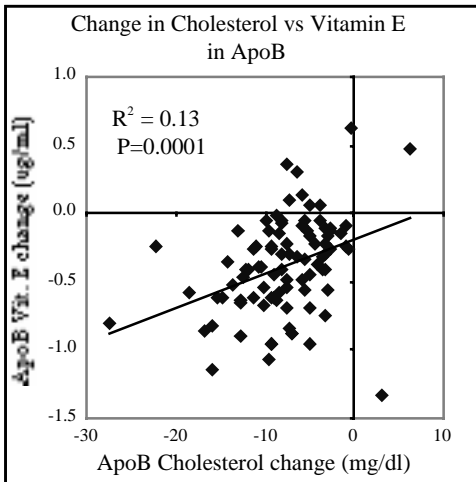
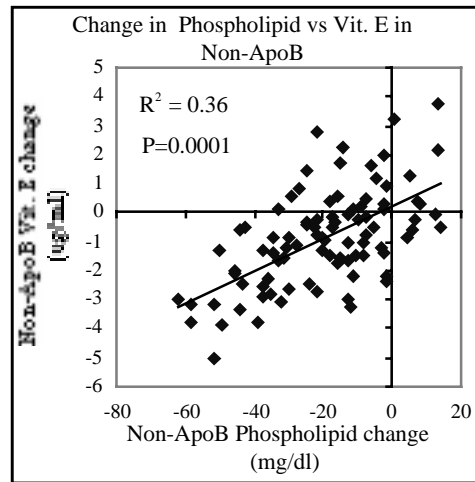
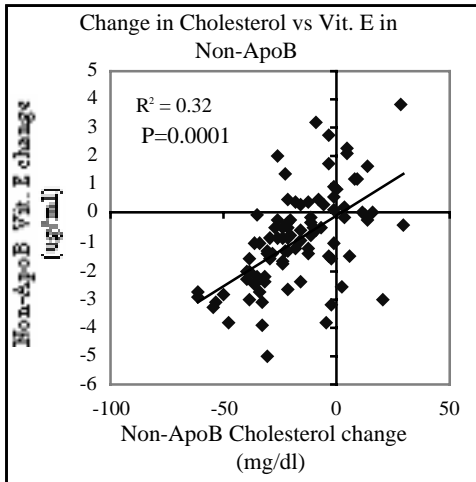
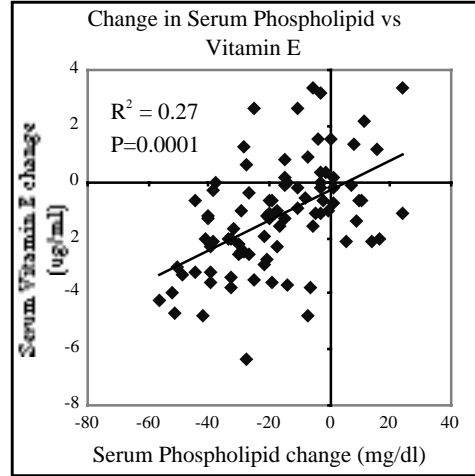
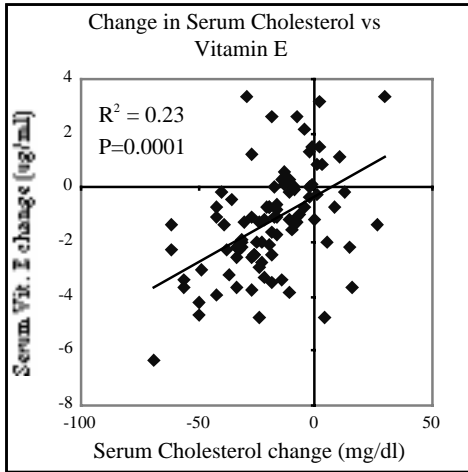
Appendix J

Linear Regressions of Vitamin E, Cholesterol, and Phospholipid in Serum and two Lipoprotein Fractions of Cows 1-2 Weeks Pre-calving, Cows at Calving, 24-48 Hour-Old Calves, and Analyte Concentration Changes Pre-calving to Calving









Appendix K

General Linear Model Tables- Chapter 3

Serum Vitamin E Concentrations

Table 1. Dry-off.

Source	DF	Type III SS	F Value	Pr > F
Breed (brd)	1	40.075	8.57	0.0054
Vitamin E (E)	1	2.060	0.44	0.5103
Se	1	0.544	0.12	0.7348
E*Se	1	0.179	0.04	0.8458
brd*E	1	2.434	0.52	0.4744
brd*Se	1	2.087	0.45	0.5076
brd*E*Se	1	0.040	0.01	0.9263
error	44	205.750		

Table 2. Mid-dry period.

Source	DF	Type III SS	F Value	Pr > F
Breed (brd)	1	0.143	0.09	0.7622
Vitamin E (E)	1	5.363	3.46	0.0396
Se	1	0.600	0.38	0.5385
E*Se	1	1.772	1.14	0.2908
brd*E	1	0.356	0.23	0.6344
brd*Se	1	0.0005	0.00	0.9853
brd*E*Se	1	0.027	0.02	0.8943
error	44	68.209		

Table 3. Calving.

Source	DF	Type III SS	F Value	Pr > F
Breed (brd)	1	1.604	2.09	0.1552
Vitamin E (E)	1	1.663	2.17	0.1479
Se	1	0.960	1.25	0.2692
E*Se	1	0.185	0.24	0.6259
brd*E	1	0.209	0.27	0.6039
brd*Se	1	0.034	0.04	0.8338
brd*E*Se	1	0.669	0.87	0.3552
error	44	33.743		

Table 4. Post-calving.

Source	DF	Type III SS	F Value	Pr > F
Breed (brd)	1	0.0013	0.00	0.9755
Vitamin E (E)	1	0.655	0.48	0.4903
Se	1	0.026	0.02	0.8900
E*Se	1	0.626	0.46	0.4999
brd*E	1	0.073	0.05	0.8177
brd*Se	1	0.073	0.05	0.8177
brd*E*Se	1	0.874	0.65	0.4258
error	43	58.138		

Blood Selenium Concentrations

Table 5. Dry-off.

Source	DF	Type III SS	F Value	Pr > F
Breed (brd)	1	14008.889	27.08	0.0001
Vitamin E (E)	1	149.488	0.29	0.5936
Se	1	22.013	0.04	0.8375
E*Se	1	481.090	0.93	0.3401
brd*E	1	71.949	0.14	0.7110
brd*Se	1	403.398	0.78	0.3820
brd*E*Se	1	1100.013	2.13	0.1519
error	44	22761.750		

Table 6. Mid-dry period.

Source	DF	Type III SS	F Value	Pr > F
Breed (brd)	1	15427.284	28.07	0.0001
Vitamin E (E)	1	0.547	0.00	0.9750
Se	1	23.558	0.04	0.8369
E*Se	1	133.547	0.24	0.6245
brd*E	1	1522.085	2.77	0.1032
brd*Se	1	5.558	0.01	0.9204
brd*E*Se	1	1214.778	2.21	0.1443
error	44	24185.833		

Table 7. Calving.

Source	DF	Type III SS	F Value	Pr > F
Breed (brd)	1	10468.815	16.69	0.0002
Vitamin E (E)	1	324.503	0.52	0.4762
Se	1	2887.523	4.60	0.0380
E*Se	1	103.128	0.16	0.6873
brd*E	1	796.378	1.27	0.2666
brd*Se	1	46.065	0.07	0.7878
brd*E*Se	1	354.586	0.57	0.4565
error	40	25091.469		

Table 8. Post-calving.

Source	DF	Type III SS	F Value	Pr > F
Breed (brd)	1	1126.196	0.88	0.3551
Vitamin E (E)	1	62.134	0.05	0.8272
Se	1	348.143	0.27	0.6058
E*Se	1	866.399	0.67	0.4167
brd*E	1	77.683	0.06	0.8072
brd*Se	1	1.355	0.00	0.9743
brd*E*Se	1	144.160	0.11	0.7396
error	40	51466.042		

Appendix L

General Linear Model Tables– Chapter 4

Serum Vitamin E Concentrations

Table 1. Cows 1-2 weeks pre-calving.

Source	DF	Type III SS	F Value	Pr > F
Treatment (trt)	1	8.264	4.37	0.0416
Breed (brd)	1	12.899	6.83	0.0118
Trt*brd	1	2.652	1.40	0.2418
error	50	94.487		

Table 2. Cows at calving.

Source	DF	Type III SS	F Value	Pr > F
Treatment (trt)	1	11.149	7.32	0.0093
Breed (brd)	1	4.452	2.92	0.0935
Trt*brd	1	1.066	0.70	0.4069
error	50	76.157		

Table 3. Cows 2-3 days post-calving.

Source	DF	Type III SS	F Value	Pr > F
Treatment (trt)	1	4.742	7.00	0.0109
Breed (brd)	1	2.070	3.05	0.0866
Trt*brd	1	0.088	0.13	0.7199
error	50	33.883		

Table 4. 24-48 hour-old calves from cows.

Source	DF	Type III SS	F Value	Pr > F
Treatment (trt)	1	1.091	3.11	0.0839
Breed (brd)	1	3.472	9.90	0.0028
Trt*brd	1	1.191	3.40	0.0713
error	49	17.180		

Table 5. Nulliparous heifers 1-2 weeks pre-calving.

Source	DF	Type III SS	F Value	Pr > F
Treatment (trt)	1	1.958	2.52	0.1271
Breed (brd)	1	0.563	0.73	0.4038
Trt*brd	1	0.572	0.74	0.4001
error	21	16.289		

Table 6. Nulliparous heifers at calving.

Source	DF	Type III SS	F Value	Pr > F
Treatment (trt)	1	6.158	8.00	0.0101
Breed (brd)	1	4.782	6.21	0.0211
Trt*brd	1	0.104	0.14	0.7167
error	21	16.166		

Table 7. Nulliparous heifers 2-3 days post-calving.

Source	DF	Type III SS	F Value	Pr > F
Treatment (trt)	1	0.702	1.26	0.2742
Breed (brd)	1	2.151	3.87	0.0627
Trt*brd	1	0.335	0.60	0.4464
error	21	11.685		

Table 8. 24-48 hour-old calves from nulliparous heifers.

Source	DF	Type III SS	F Value	Pr > F
Treatment (trt)	1	0.342	1.04	0.3195
Breed (brd)	1	0.213	0.65	0.4295
Trt*brd	1	1.775	5.41	0.0306
error	20	6.557		

Colostrals Vitamin E Concentrations

Table 9. Cows.

Source	DF	Type III SS	F Value	Pr > F
Treatment (trt)	1	565.072	3.56	0.0649
Breed (brd)	1	611.991	3.86	0.0551
Trt*brd	1	0.469	0.00	0.9569
error	50	7931.603		

Table 10. Nulliparous heifers.

Source	DF	Type III SS	F Value	Pr > F
Treatment (trt)	1	32.422	0.21	0.6480
Breed (brd)	1	2.079	0.01	0.9078
Trt*brd	1	163.302	1.08	0.3104
error	21	3174.036		

Calf Serum IgG Concentrations

Table 11. Calves from cows.

Source	DF	Type III SS	F Value	Pr > F
Treatment (trt)	1	439421.9	0.41	0.5265
Breed (brd)	1	2464130.9	2.28	0.1373
Trt*brd	1	883198.2	0.82	0.3702
error	49	52907661.2		

Table 12. Calves from nulliparous heifers.

Source	DF	Type III SS	F Value	Pr > F
Treatment (trt)	1	1704689.6	1.67	0.2108
Breed (brd)	1	1124351.1	1.10	0.3063
Trt*brd	1	171017.6	0.17	0.6865
error	20	20399258.6		

Colostrals IgG Concentrations

Table 13. Cows.

Source	DF	Type III SS	F Value	Pr > F
Treatment (trt)	1	682458.9	0.15	0.7039
Breed (brd)	1	2073020.3	0.44	0.5084
Trt*brd	1	6287835.5	1.35	0.2515
error	50	233604061.2		

Table 14. Nulliparous heifers.

Source	DF	Type III SS	F Value	Pr > F
Treatment (trt)	1	129653.4	0.02	0.9022
Breed (brd)	1	11939032.5	1.42	0.2459
Trt*brd	1	6696219.9	0.80	0.3815
error	21	175982111.1		

Calf 205-day Adjusted Weaning Weights

Table 15. Calves from cows.

Source	DF	Type III SS	F Value	Pr > F
Treatment (trt)	1	17974.268	5.93	0.0191
Breed (brd)	1	9307.001	3.07	0.0869
Trt*brd	1	6923.692	2.28	0.1381
error	43	130376.32		

Table 16. Calves from nulliparous heifers.

Source	DF	Type III SS	F Value	Pr > F
Treatment (trt)	1	5322.996	3.49	0.0773
Breed (brd)	1	8967.804	5.88	0.0255
Trt*brd	1	3975.564	2.61	0.1230
error	19	28989.287		

Appendix M

Chapter 5- General Linear Model Tables

Serum Vitamin E Concentrations

Table 1. Dams at establishment of treatment and control groups.

Source	DF	Type III SS	F Value	Pr > F
Treatment (trt)	1	0.232	0.25	0.6192
Breed (brd)	1	0.948	1.03	0.3182
Trt*brd	1	0.043	0.05	0.8308
Age	1	8.598	9.36	0.0047
Trt*age	1	3.321	3.62	0.0672
Brd*age	1	1.030	1.12	0.2984
Trt*brd*age	1	0.014	0.02	0.9027
error	29	26.639		

Table 2. Dams 1-2 weeks calving.

Source	DF	Type III SS	F Value	Pr > F
Treatment (trt)	1	1.268	0.66	0.4244
Breed (brd)	1	2.352	1.22	0.2788
Trt*brd	1	1.198	0.62	0.4373
Age	1	4.135	2.14	0.1541
Trt*age	1	0.019	0.01	0.9214
Brd*age	1	0.137	0.07	0.7921
Trt*brd*age	1	4.038	2.09	0.1589
error	29	55.990		

Table 3. Dams at calving.

Source	DF	Type III SS	F Value	Pr > F
Treatment (trt)	1	1.758	1.33	0.2593
Breed (brd)	1	2.593	1.96	0.1730
Trt*brd	1	1.868	1.41	0.2452
Age	1	0.0002	0.00	0.9915
Trt*age	1	2.917	2.20	0.1493
Brd*age	1	0.453	0.34	0.5636
Trt*brd*age	1	0.243	0.18	0.6715
error	27	35.747		

Table 4. Dams 2-3 days post-calving.

Source	DF	Type III SS	F Value	Pr > F
Treatment (trt)	1	0.935	0.86	0.3632
Breed (brd)	1	3.894	3.57	0.0701
Trt*brd	1	0.105	0.10	0.7593
Age	1	1.440	1.32	0.2610
Trt*age	1	2.189	2.01	0.1685
Brd*age	1	0.216	0.20	0.6601
Trt*brd*age	1	0.132	0.12	0.7303
error	26	28.370		

Table 5. 24-48 hour-old calves.

Source	DF	Type III SS	F Value	Pr > F
Treatment (trt)	1	1.360	1.89	0.1816
Breed (brd)	1	6.339	8.80	0.0065
Trt*brd	1	0.0053	0.01	0.9322
Age	1	0.048	0.07	0.7981
Trt*age	1	0.0003	0.00	0.9841
Brd*age	1	0.120	0.17	0.6863
Trt*brd*age	1	0.081	0.11	0.7405
error	25	18.003		

Colostrals Vitamin E Concentrations

Table 6. Dams.

Source	DF	Type III SS	F Value	Pr > F
Treatment (trt)	1	12.633	0.12	0.7327
Breed (brd)	1	526.013	4.96	0.0348
Trt*brd	1	2.851	0.03	0.8710
Age	1	124.537	1.18	0.2883
Trt*age	1	17.016	0.16	0.6919
Brd*age	1	288.991	2.73	0.1107
Trt*brd*age	1	301.156	2.84	0.1038
error	26	2755.594		

Serum IgG Concentrations

Table 7. 24-48 hour-old calves.

Source	DF	Type III SS	F Value	Pr > F
Treatment (trt)	1	812393.9	0.72	0.4030
Breed (brd)	1	10329261.0	9.20	0.0056
Trt*brd	1	1728.929	0.00	0.9690
Age	1	1057105.7	0.94	0.3411
Trt*age	1	561117.5	0.50	0.4861
Brd*age	1	4273438.0	3.81	0.0623
Trt*brd*age	1	1358532.3	1.21	0.2818
error	25	28062832.2		

Colostrals IgG Concentrations

Table 8. Dams.

Source	DF	Type III SS	F Value	Pr > F
Treatment (trt)	1	49094227.7	2.33	0.1388
Breed (brd)	1	19114972.1	0.91	0.3496
Trt*brd	1	2734849.5	0.13	0.7216
Age	1	47130727.6	2.23	0.1466
Trt*age	1	2655498.6	0.13	0.7255
Brd*age	1	19558711.1	0.93	0.3442
Trt*brd*age	1	1758615.5	0.08	0.7750
error	27	569630002.8		

205-day Adjusted Weaning Weights

Table 9. Calves at weaning.

Source	DF	Type III SS	F Value	Pr > F
Treatment (trt)	1	622.307	0.15	0.6992
Breed (brd)	1	93826.976	23.34	0.0002
Trt*brd	1	1417.283	0.35	0.5610
Age	1	1057.697	0.26	0.6150
Trt*age	1	573.607	0.14	0.7106
Brd*age	1	9724.684	2.42	0.1394
Trt*brd*age	1	98.603	0.02	0.8775
error	16	64322.736		

Appendix N

Chapter 6- General Linear Model Tables

Vitamin E Concentrations

Table 1. Cow serum.

Source	DF	Type III SS	F Value	Pr > F
Time	1	76.564	42.01	0.0001
Trt*time	1	3.255	1.79	0.1849
Brd*time	1	5.648	3.10	0.0819
Trt*brd*time	1	0.025	0.01	0.9066
Cow(trt*brd)	87	369.024		

Tests of hypotheses using the Type III MS for Cow(trt*brd) as the error term

Treatment (trt)	1	14.667	3.46	0.0663
Breed (brd)	1	1.008	0.24	0.6272
Trt*brd	1	9.687	2.28	0.1344

Table 2. Cow non-ApoB fraction.

Source	DF	Type III SS	F Value	Pr > F
Time	1	38.076	28.13	0.0001
Trt*time	1	3.818	2.82	0.0967
Brd*time	1	4.902	3.62	0.0604
Trt*brd*time	1	0.011	0.01	0.9291
Cow(trt*brd)	87	315.102		

Tests of hypotheses using the Type III MS for Cow(trt*brd) as the error term

Treatment (trt)	1	13.596	3.75	0.0559
Breed (brd)	1	0.716	0.20	0.6577
Trt*brd	1	7.740	2.14	0.1474

Table 3. Cow ApoB fraction.

Source	DF	Type III SS	F Value	Pr > F
Time	1	6.654	104.67	0.0001
Trt*time	1	0.022	0.35	0.5539
Brd*time	1	0.026	0.42	0.5210
Trt*brd*time	1	0.003	0.05	0.8276
Cow(trt*brd)	87	5.410		

Tests of hypotheses using the Type III MS for Cow(trt*brd) as the error term

Treatment (trt)	1	0.020	0.33	0.5691
Breed (brd)	1	0.025	0.40	0.5294
Trt*brd	1	0.109	1.75	0.1888

Table 4. Calf serum.

Source	DF	Type III SS	F Value	Pr > F
Treatment (trt)	1	2.239	3.43	0.0679
Breed (brd)	1	4.729	7.24	0.0087
Trt*brd	1	0.032	0.05	0.8242
error	80	52.282		

Table 5. Calf non-ApoB fraction.

Source	DF	Type III SS	F Value	Pr > F
Treatment (trt)	1	1.596	3.67	0.0591
Breed (brd)	1	4.797	11.02	0.0014
Trt*brd	1	0.007	0.02	0.9009
error	80	34.827		

Table 6. Calf ApoB fraction.

Source	DF	Type III SS	F Value	Pr > F
Treatment (trt)	1	0.0543	1.48	0.2277
Breed (brd)	1	0.0002	0.01	0.9359
Trt*brd	1	0.0096	0.26	0.6112
error	80	2.938		

Cholesterol Concentrations

Table 7. Cow serum.

Source	DF	Type III SS	F Value	Pr > F
Time	1	15826.686	83.17	0.0001
Trt*time	1	105.569	0.55	0.4584
Brd*time	1	0.676	0.00	0.9526
Trt*brd*time	1	54.563	0.29	0.5937
Cow(trt*brd)	86	78127.788		

Tests of hypotheses using the Type III MS for Cow(trt*brd) as the error term

Treatment (trt)	1	325.935	0.36	0.5508
Breed (brd)	1	1214.378	1.34	0.2508
Trt*brd	1	648.618	0.71	0.4005

Table 8. Cow non-ApoB fraction.

Source	DF	Type III SS	F Value	Pr > F
Time	1	13276.016	70.28	0.0001
Trt*time	1	127.281	0.67	0.4140
Brd*time	1	327.345	1.73	0.1915
Trt*brd*time	1	5.029	0.03	0.8708
Cow(trt*brd)	86	89517.182		

Tests of hypotheses using the Type III MS for Cow(trt*brd) as the error term

Treatment (trt)	1	78.480	0.08	0.7843
Breed (brd)	1	4182.838	4.02	0.0481
Trt*brd	1	1064.043	1.02	0.3148

Table 9. Cow ApoB fraction.

Source	DF	Type III SS	F Value	Pr > F
Time	1	2639.271	209.40	0.0001
Trt*time	1	57.514	4.56	0.0355
Brd*time	1	0.068	0.01	0.9415
Trt*brd*time	1	0.727	0.06	0.8107
Cow(trt*brd)	86	1591.728		

Tests of hypotheses using the Type III MS for Cow(trt*brd) as the error term

Treatment (trt)	1	15.013	0.81	0.3703
Breed (brd)	1	2.576	0.14	0.7100
Trt*brd	1	5.243	0.28	0.5959

Table 10. Calf serum.

Source	DF	Type III SS	F Value	Pr > F
Treatment (trt)	1	68.041	0.80	0.3741
Breed (brd)	1	1284.549	15.08	0.0002
Trt*brd	1	95.243	1.12	0.2935
error	80	6814.423		

Table 11. Calf non-ApoB fraction.

Source	DF	Type III SS	F Value	Pr > F
Treatment (trt)	1	22.068	0.33	0.5673
Breed (brd)	1	1372.181	20.51	0.0001
Trt*brd	1	67.865	1.01	0.3168
error	80	5351.121		

Table 12. Calf ApoB fraction.

Source	DF	Type III SS	F Value	Pr > F
Treatment (trt)	1	8.329	0.89	0.3477
Breed (brd)	1	7.968	0.85	0.3583
Trt*brd	1	0.401	0.04	0.8363
error	80	746.732		

Phospholipid Concentrations

Table 13. Cow serum.

Source	DF	Type III SS	F Value	Pr > F
Time	1	13713.704	75.69	0.0001
Trt*time	1	70.598	0.39	0.5341
Brd*time	1	20.754	0.11	0.7359
Trt*brd*time	1	14.867	0.08	0.7752
Cow(trt*brd)	86	58981.735		

Tests of hypotheses using the Type III MS for Cow(trt*brd) as the error term

Treatment (trt)	1	461.871	0.67	0.4141
Breed (brd)	1	0.459	0.00	0.9794
Trt*brd	1	1150.041	1.68	0.1988

Table 14. Cow non-ApoB fraction.

Source	DF	Type III SS	F Value	Pr > F
Time	1	17239.062	103.14	0.0001
Trt*time	1	1.525	0.01	0.9241
Brd*time	1	76.501	0.46	0.5005
Trt*brd*time	1	170.397	1.02	0.3155
Cow(trt*brd)	86	42708.393		

Tests of hypotheses using the Type III MS for Cow(trt*brd) as the error term

Treatment (trt)	1	352.842	0.71	0.4016
Breed (brd)	1	1173.951	2.36	0.1278
Trt*brd	1	611.971	1.23	0.2701

Table 15. Cow ApoB fraction.

Source	DF	Type III SS	F Value	Pr > F
Time	1	1797.343	175.17	0.0001
Trt*time	1	2.722	0.27	0.6079
Brd*time	1	12.641	1.23	0.2701
Trt*brd*time	1	4.135	0.40	0.5272
Cow(trt*brd)	86	1303.398		

Tests of hypotheses using the Type III MS for Cow(trt*brd) as the error term

Treatment (trt)	1	5.870	0.39	0.5354
Breed (brd)	1	95.098	6.27	0.0141
Trt*brd	1	0.001	0.00	0.9927

Table 16. Calf serum.

Source	DF	Type III SS	F Value	Pr > F
Treatment (trt)	1	9.403	0.04	0.8479
Breed (brd)	1	4617.635	18.17	0.0001
Trt*brd	1	805.580	3.17	0.0788
error	80	20328.710		

Table 17. Calf non-ApoB fraction.

Source	DF	Type III SS	F Value	Pr > F
Treatment (trt)	1	1.271	0.01	0.9369
Breed (brd)	1	3922.140	19.45	0.0001
Trt*brd	1	547.758	2.72	0.1033
error	80	16133.549		

Table 18. Calf ApoB fraction.

Source	DF	Type III SS	F Value	Pr > F
Treatment (trt)	1	5.393	0.52	0.4711
Breed (brd)	1	13.747	1.34	0.2511
Trt*brd	1	13.224	1.29	0.2602
error	80	822.751		

Vitamin E Cholesterol (VEC) Ratios

Table 19. Cow serum.

Source	DF	Type III SS	F Value	Pr > F
Time	1	0.00000672	7.00	0.0097
Trt*time	1	0.00000275	2.87	0.0941
Brd*time	1	0.00000383	4.00	0.0487
Trt*brd*time	1	0.00000011	0.12	0.7324
Cow(trt*brd)	86	0.00018528		

Tests of hypotheses using the Type III MS for Cow(trt*brd) as the error term

Treatment (trt)	1	0.00001903	8.83	0.0038
Breed (brd)	1	0.00000348	1.61	0.2073
Trt*brd	1	0.00000500	2.32	0.1312

Table 20. Cow non-ApoB fraction.

Source	DF	Type III SS	F Value	Pr > F
Time	1	0.00000106	1.30	0.2569
Trt*time	1	0.00000320	3.94	0.0503
Brd*time	1	0.00000195	2.39	0.1255
Trt*brd*time	1	0.00000001	0.01	0.9189
Cow(trt*brd)	86	0.00015679		

Tests of hypotheses using the Type III MS for Cow(trt*brd) as the error term

Treatment (trt)	1	0.00001671	9.17	0.0033
Breed (brd)	1	0.00000730	4.00	0.0486
Trt*brd	1	0.00000361	1.98	0.1631

Table 21. Cow ApoB fraction.

Source	DF	Type III SS	F Value	Pr > F
Time	1	0.00002033	3.78	0.0551
Trt*time	1	0.00000043	0.08	0.7773
Brd*time	1	0.00000008	0.01	0.9061
Trt*brd*time	1	0.00000051	0.09	0.7591
Cow(trt*brd)	86	0.00033597		

Tests of hypotheses using the Type III MS for Cow(trt*brd) as the error term

Treatment (trt)	1	0.00000140	0.36	0.5505
Breed (brd)	1	0.00000202	0.52	0.4742
Trt*brd	1	0.00000949	2.43	0.1228

Table 22. Calf serum.

Source	DF	Type III SS	F Value	Pr > F
Treatment (trt)	1	0.00001001	6.25	0.0144
Breed (brd)	1	0.00000282	1.76	0.1879
Trt*brd	1	0.00000001	0.00	0.9643
error	80	0.00012800		

Table 23. Calf non-ApoB fraction.

Source	DF	Type III SS	F Value	Pr > F
Treatment (trt)	1	0.00001191	7.09	0.0094
Breed (brd)	1	0.00000442	2.63	0.1089
Trt*brd	1	0.00000001	0.00	0.9503
error	80	0.00013451		

Table 24. Calf ApoB fraction.

Source	DF	Type III SS	F Value	Pr > F
Treatment (trt)	1	0.00000624	2.88	0.0933
Breed (brd)	1	0.00000151	0.70	0.4059
Trt*brd	1	0.00000105	0.49	0.4878
error	80	0.00017296		

Vitamin E Phospholipid (VEPL) Ratios

Table 25. Cow serum.

Source	DF	Type III SS	F Value	Pr > F
Time	1	0.00000867	10.54	0.0017
Trt*time	1	0.00000233	2.83	0.0961
Brd*time	1	0.00000365	4.43	0.0382
Trt*brd*time	1	0.00000002	0.02	0.8866
Cow(trt*brd)	86	0.00015737		

Tests of hypotheses using the Type III MS for Cow(trt*brd) as the error term

Treatment (trt)	1	0.00001661	9.08	0.0034
Breed (brd)	1	0.00000043	0.24	0.6279
Trt*brd	1	0.00000275	1.50	0.2235

Table 26. Cow non-ApoB fraction.

Source	DF	Type III SS	F Value	Pr > F
Time	1	0.00000010	0.11	0.7428
Trt*time	1	0.00000475	5.23	0.0247
Brd*time	1	0.00000378	4.16	0.0444
Trt*brd*time	1	0.00000030	0.33	0.5687
Cow(trt*brd)	86	0.00019757		

Tests of hypotheses using the Type III MS for Cow(trt*brd) as the error term

Treatment (trt)	1	0.00002146	9.34	0.0030
Breed (brd)	1	0.00000342	1.49	0.2257
Trt*brd	1	0.00000427	1.86	0.1762

Table 27. Cow ApoB fraction.

Source	DF	Type III SS	F Value	Pr > F
Time	1	0.00000274	0.39	0.5345
Trt*time	1	0.00000169	0.24	0.6258
Brd*time	1	0.00000108	0.15	0.6961
Trt*brd*time	1	0.00000192	0.27	0.6033
Cow(trt*brd)	86	0.00077559		

Tests of hypotheses using the Type III MS for Cow(trt*brd) as the error term

Treatment (trt)	1	0.00000084	0.98	0.3250
Breed (brd)	1	0.00001800	2.00	0.1613
Trt*brd	1	0.00000393	0.44	0.5108

Table 28. Calf serum.

Source	DF	Type III SS	F Value	Pr > F
Treatment (trt)	1	0.00000283	3.64	0.0602
Breed (brd)	1	0.00000050	0.65	0.4242
Trt*brd	1	0.00000012	0.15	0.6951
error	80	0.00006234		

Table 29. Calf non-ApoB fraction.

Source	DF	Type III SS	F Value	Pr > F
Treatment (trt)	1	0.00000302	4.62	0.0346
Breed (brd)	1	0.00000178	2.73	0.1027
Trt*brd	1	0.00000020	0.30	0.5827
error	80	0.00005219		

Table 30. Calf ApoB fraction.

Source	DF	Type III SS	F Value	Pr > F
Treatment (trt)	1	0.00000812	2.18	0.1440
Breed (brd)	1	0.00000495	1.33	0.2528
Trt*brd	1	0.00000031	0.08	0.7728
error	80	0.00029857		

Appendix O
Additional Dairy Cow Data (Chapter 3)

Table O-1. Selenium concentrations (100% dry matter basis) of pastures used in dry cow study.

Pasture Name	Selenium content ($\mu\text{g}/\text{kg}$)
Airport	50
Meatpacker	60
Research Center	30

Table O-2. Body condition scores of periparturient dairy cows by breed.[‡]

Sample Period	Body Condition Score	
	Jersey	Holstein
3-4 weeks pre-calving	3.1 \pm 0.1 (16)	3.1 \pm 0.1 (36)
Within 24 hours post-calving	2.9 \pm 0.1 (16)	2.8 \pm 0.1 (35)
2-3 weeks post-calving	3.0 \pm 0.1 (15)	2.6 \pm 0.1 (36)

[‡] Values are means \pm standard error of (n) observations.

Table O-3. Body condition scores of periparturient dairy cows by treatment group.[‡]

Sample Period	Treatment Group			
	Vitamin E	Se	Vitamin E+Se	Control
3-4 weeks pre-calving	3.1 \pm 0.1 (13)	3.1 \pm 0.1 (13)	3.0 \pm 0.1 (13)	3.1 \pm 0.1 (13)
Within 24 hours post-calving	2.7 \pm 0.2 (12)	2.8 \pm 0.1 (13)	2.8 \pm 0.1 (13)	2.9 \pm 0.1 (13)
2-3 weeks post-calving	2.9 \pm 0.1 (13)	2.8 \pm 0.1 (13)	2.6 \pm 0.1 (13)	2.6 \pm 0.2 (12)

[‡] Values are means \pm standard error of (n) observations.

Appendix P

Additional Data from the Winter-calving Herd (Chapter 4)

Table P-1. Fat, lactose, and protein percentage of colostrum in treated (average consumption of 1000 IU/head/day of supplemental vitamin E during late gestation) and untreated Angus and Hereford cattle calving in late winter.[‡]

Colostrum analyte (%)	Group			
	Control		Treatment	
	cow (28)	heifer (10)	cow (26)	heifer (13)
Fat	4.9 ± 0.7	8.2 ± 1.6	6.4 ± 0.9	8.5 ± 1.0
Lactose	3.0 ± 0.1	2.3 ± 0.2	2.9 ± 0.1	2.5 ± 0.1
Protein	14.4 ± 0.7	17.4 ± 1.2	14.9 ± 0.6	17.5 ± 1.2

[‡]Values are means ± standard error of (n) observations.

Eight Continental breed (Gelbvieh and Limousin) dams were in the winter-calving herd and participated in the study. Including data from these cattle in Chapter 4 would have resulted in empty cells in the experimental design and the potential for reporting misrepresentative breed-related data (because of small sample size). The data are reported in this appendix for the purposes of completeness.

Table P-2. Fat, lactose, and protein percentage and vitamin E concentration of colostrum from Angus, Hereford, and Continental breed cattle calving in late winter.[‡]

Colostrum analyte	Breed					
	Angus		Hereford		Continental	
	cow (38)	heifer (18)	cow (16)	heifer (5)	cow (6)	heifer (2)
Fat (%)	6.1 ± 0.7	8.5 ± 1.1	4.6 ± 1.1	7.7 ± 1.1	5.7 ± 1.2	7.3 ± 2.9
Lactose (%)	3.0 ± 0.1	2.4 ± 0.1	2.9 ± 0.1	2.6 ± 0.3	3.0 ± 0.2	2.7 ± 0.3
Protein (%)	14.8 ± 0.5	17.2 ± 0.9	14.4 ± 0.9	18.4 ± 2.3	12.5 ± 1.6	18.2 ± 1.7
Vitamin E (µg/ml)	16.1 ± 2.2	13.5 ± 2.6	8.3 ± 2.6	13.2 ± 5.9	13.3 ± 4.6	7.0 ± 3.7

[‡]Values are means ± standard error of (n) observations.

Table P-3. Body condition scores (1-9 scale) and weights in treated (average consumption of 1000 IU/head/day of supplemental vitamin E during late gestation) and untreated Angus and Hereford cattle calving in late winter.[‡]

Variable	Group			
	Control		Treatment	
	cow (28)	heifer (11)	cow (26)	heifer (14)
<u>Body condition score</u>				
2 months pre-calving	6.0 ± 0.2	5.4 ± 0.2	6.0 ± 0.2	5.6 ± 0.1
At calving	5.7 ± 0.2	5.3 ± 0.1	5.5 ± 0.2	5.1 ± 0.2
<u>Body Weight (kg)</u>				
2 months pre-calving	645 ± 14	544 ± 11	662 ± 15	536 ± 11
Within 6 hr post-calv	591 ± 13	502 ± 12	596 ± 14	482 ± 12

[‡]Values are means ± standard error of (n) observations.

Table P-4. Body condition scores (1-9 scale) and weights of Angus, Hereford, and Continental breed cattle calving in late winter.[‡]

Variable	Breed					
	Angus		Hereford		Continental	
	cow (38)	heifer (20)	cow (16)	heifer (5)	cow (6)	heifer (2)
<u>Body condition score</u>						
2 months pre-calving	5.7 ± 0.1	5.5 ± 0.1	6.7 ± 0.3	5.6 ± 0.2	5.8 ± 0.3	6.0 ± 0.0
At calving	5.3 ± 0.1	5.2 ± 0.2	6.3 ± 0.2	5.4 ± 0.2	5.7 ± 0.2	5.5 ± 0.5
<u>Body Weight (kg)</u>						
2 months pre-calving	642 ± 12	539 ± 7	679 ± 18	543 ± 27	619 ± 27	544 ± 2
Within 6 hr post-calv	577 ± 11	489 ± 8	630 ± 15	495 ± 26	552 ± 19	482 ± 2

[‡]Values are means ± standard error of (n) observations.

Table P-5. Birth weights and gender distribution of calves from treated (average consumption of 1000 IU/head/day of supplemental vitamin E during late gestation) and untreated Angus and Hereford cattle calving in late winter.[‡]

Variable	Group			
	Control		Treatment	
	cow (27)	heifer (11)	cow (25)	heifer (14)
Calf birth weight (kg)	38.3 ± 0.7	36.9 ± 1.3	41.6 ± 1.0	35.9 ± 1.2
Male calves (%)	56	55	60	64

[‡]Values are means ± standard error or percentages of (n) observations.

Table P-6. Birth weights and gender distribution of calves from Angus, Hereford, and Continental breed cattle calving in late winter.[‡]

Variable	Breed					
	Angus		Hereford		Continental	
	cow (36)	heifer (20)	cow (16)	heifer (5)	cow (6)	heifer (2)
Calf birth weight (kg)	39.4 ± 0.7	35.7 ± 1.0	41.0 ± 1.4	38.8 ± 1.4	43.3 ± 3.0	35.2 ± 2.5
Male calves (%)	56	60	63	60	83	50

[‡]Values are means ± standard error or percentages of (n) observations.

Appendix Q

Additional Data from the Summer-calving Herd (Chapter 5)

Table Q-1. Fat, lactose, and protein percentage of colostrum in treated (average consumption of 600 IU/head/day of supplemental vitamin E during late gestation) and untreated Angus and Hereford cattle calving in late summer.[‡]

Colostrum Analyte (%)	Group	
	Control (n=16)	Treatment (n=19)
Fat	5.8 ± 0.9	5.8 ± 1.0
Lactose	1.5 ± 0.2	2.2 ± 0.2
Protein	21.7 ± 1.4	17.7 ± 1.4

[‡]Values are means ± standard error of (n) observations.

Five Continental breed (Gelbvieh) dams were in the summer-calving herd and participated in the study. Including data from these cattle in Chapter 5 would have resulted in empty cells in the experimental design and the potential for reporting misrepresentative breed-related data (because of small sample size). The data are reported in this appendix for the purposes of completeness.

Table Q-2. Fat, lactose, and protein percentage and vitamin E concentration of colostrum from Angus, Hereford, and Continental breed cattle calving in late summer.[‡]

Colostrum Analyte	Breed		
	Angus (n=13)	Hereford (n=22)	Continental (n=5)
Fat (%)	6.4 ± 1.1	5.4 ± 0.8	8.1 ± 2.7
Lactose (%)	2.2 ± 0.3	1.7 ± 0.2	2.2 ± 0.4
Protein (%)	17.4 ± 1.6	20.8 ± 1.3	19.2 ± 2.1
Vitamin E (µg/ml)	19.8 ± 3.6	12.4 ± 1.9	32.0 ± 13.0

[‡]Values are means ± standard error of (n) observations.

Table Q-3. Body condition scores (1-9 scale) and weights in treated (average consumption of 600 IU/head/day of supplemental vitamin E during late gestation) and untreated Angus and Hereford cattle calving in late summer.[‡]

Variable	Group	
	Control (n=17)	Treatment (n=19)
<u>Body condition score</u>		
2 months pre-calving	6.4 ± 0.2	6.4 ± 0.2
At calving	6.1 ± 0.1	6.1 ± 0.2
<u>Body Weight (kg)</u>		
2 months pre-calving	632 ± 15	610 ± 19
Within 6 hr post-calv	613 ± 17	592 ± 20

[‡]Values are means ± standard error of (n) observations.

Table Q-4. Body condition scores (1-9 scale) and weights of Angus, Hereford, and Continental breed cattle calving in late summer.[‡]

Variable	Breed		
	Angus (n=14)	Hereford (n=22)	Continental (n=5)
<u>Body condition score</u>			
2 months pre-calving	6.1 ± 0.2	6.6 ± 0.2	5.6 ± 0.2
At calving	5.7 ± 0.2	6.4 ± 0.2	5.0 ± 0.0
<u>Body Weight (kg)</u>			
2 months pre-calving	619 ± 23	622 ± 14	566 ± 15
Within 6 hr post-calv	586 ± 24	612 ± 15	558 ± 19

[‡]Values are means ± standard error of (n) observations.

Table Q-5. Birth weights and gender distribution of calves from treated (average consumption of 600 IU/head/day of supplemental vitamin E during late gestation) and untreated Angus and Hereford cattle calving in late summer.[‡]

Variable	Group	
	Control (n=14)	Treatment (n=19)
Calf birth weight (kg)	39.6 ± 1.1	41.1 ± 1.1
Male calves (%)	86	68

[‡]Values are means ± standard error or percentages of (n) observations.

Table Q-6. Birth weights and gender distribution of calves from Angus, Hereford, and Continental breed cattle calving in late summer.[‡]

Variable	Breed		
	Angus (n=14)	Hereford (n=19)	Continental (n=5)
Calf birth weight (kg)	40.0 ± 1.0	40.8 ± 1.2	41.3 ± 2.1
Male calves (%)	71	68	40

[‡]Values are means ± standard error or percentages of (n) observations.

Vita

Roger Thompson Bass, II, son of Mr. Roger T. and Mrs. Phyllis K. Bass, was born on July 12, 1968 in Richmond, Virginia. He grew up in Chesterfield County and graduated from Lloyd C. Bird High School in June 1985. His grandfather, a VPI and SU alumnus, immersed him in a sea of Virginia Tech propaganda from an early age. This immersion had the desired effect, and Tom departed for Blacksburg in the fall of the same year. He received Bachelor of Science degrees in Animal Science and Biology from Virginia Polytechnic Institute and State University in May 1990 and a Doctor of Veterinary Medicine degree from the Virginia-Maryland Regional College of Veterinary Medicine in May 1994.

Tom began his Ph.D./Residency program in Large Animal Clinical Nutrition at the Virginia-Maryland Regional College of Veterinary medicine in August 1994. He received his Certificate of Residency in June 1998. He is a member of the American Veterinary Medical Association, American Association of Bovine Practitioners, and American Association of Veterinary Nutrition.