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We have read this thesis
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FATTY ACID COMPOSITION OF BLOOD SERUM LIPIDS FROM LAMBS FED SUNFLOWER OIL IN A PROTECTIVE PROTEIN GEL SUPPLEMENT

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DEDICATION

This thesis is dedicated to my mother

SEBILE YILMAZ,

my father

HÜSMEN YILMAZ

for their support, understanding and love throughout this endeavor.

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ABSTRACT

This study investigated the effect of dietary supplemental α-tocopheryl acetate (vitamin E) and sunflower oil and α-tocopheryl acetate in a protective gel supplement (sunflower oil-gel) on the fatty acid composition of lamb serum lipids. Twenty-three crossbred lambs were assigned on the basis of fleece color and gender to a control (n=8), vitamin E (n=8) or sunflower oil-gel (n=7) diet treatment and were fed the assigned diet for twelve weeks. Blood samples were obtained from all lambs at the beginning and every two weeks of the feeding trial, and the fatty acid composition of blood serum lipids was determined. Lambs on the control diet received 38 g lipid per head daily; lambs on the vitamin E diet received 38.5 g lipid and 250 I.U. of α-tocopherol per head daily while lambs on the sunflower oil-gel diet received 103 g lipid (71% linoleic acid) and 233 I.U. of α-tocopherol per head daily.

Lambs fed the sunflower oil-gel diet had ($P<0.05$) lower levels of palmitic (C16:0) and oleic acid (C18:1) in blood serum lipids than lambs fed the control or vitamin E diets, and a higher level of linoleic (C18:2) acid (33.47 versus 23.04 and 24.93%, respectively). No differences were found ($P>0.05$) in levels of C16:0, C18:1 or C18:2 in serum lipids from lambs fed the control and those fed the vitamin E diet.

In lambs fed the sunflower oil-gel diet, blood serum levels of α-tocopherol correlated ($P<0.05$) with concentrations of C16:0 ($r=-0.74$), C18:1 ($r=-0.71$) and C18:2 ($r=0.63$) fatty acids of blood serum lipids. For these same lambs, blood serum levels of C18:1 correlated ($P<0.05$) with C18:1 levels ($r=0.88$) in the Longissimus, a white muscle,

and serum levels of C18:2 correlated ($P<0.05$) with Longissimus muscle levels of C18:2 ($r=0.83$). However, no significant correlations were found ($P>0.05$) between serum levels of C18:1 and C18:2 and levels of like fatty acids in the Psoas major, a red muscle.

Feeding lambs the protective gel containing sunflower oil and α-tocopheryl acetate increased C18:2 content of blood serum lipids by a factor of 1.5 and provided increased levels of vitamin E as an antioxidant. Also, levels of major fatty acids in blood serum lipids may be indicators of those acids in muscles of animals fed the supplement. This supplement made with a generally recognized as safe agent may have a significant impact at increasing polyunsaturated fat intake from animal products in the U.S.

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CHAPTER I

INTRODUCTION

Sheep as a bovidae family of ruminantia have 'true 4-chambered ruminant stomachs' that consist of rumen, reticulum, omasum and abomasum (Harfoot, 1981). Two major reactions that occur in rumen are lipolysis of the dietary lipids and hydrogenation of their unsaturated fatty acids (Noble, 1981). The fermentation process in the rumen results in formation of short chain (from C2 to C5) fatty acids. Their concentration depends on the diet given to the ruminants. These fatty acids can be used as a supply of energy in the ruminant animal. A concentration of 5-20 mM of volatile fatty acids enters the small intestine from the obamasum (Harfoot, 1981). The concentration of volatile fatty acids leaving the jejenum, approximately one third of the small intestines, is 2-4 mM, while it is 100-150 mM in the rumen (Harfoot, 1981). Volatile fatty acids can also be found in the large intestines and caecum, a part between the small intestine and the large intestine, at lower concentrations than in the rumen.

Myers et al. (1967) reported that volatile fatty acids produced in the ruminant caecum could be quickly transported by a simple diffusion mechanism across the caecal wall into the bloodstream. Increasing the pH from 6.2 to 7.5 and the osmolarity within the caecum decreased the absorption rate of individual VFA. However, the rate was not affected by the presence of other VFA. High concentration differences increased the absorption rate. Myers et al. (1967) showed that the absorption of VFA from the caecum does not occur by active transport, instead simple diffusion is the primary mechanism for the absorption of VFA from the caecum. Harfoot (1981) reported that 30% of the total

fatty acids that enter the bloodstream are from the hind gut and are caecal volatile fatty acids. The rest are largely from ruminal origin.

Plasma fatty acid composition can be altered by diet. Feeding the ruminants with different diets can greatly change the fatty acid composition of plasma and other tissues of the ruminants, but only if the fatty acids are protected from ruminal biohydrogenation. Palmquist et al. (1977) reported differences in palmitic acid and linoleic acid concentrations of plasma between the ruminants fed with 'protected saturated fat' (80% corn silage, 7% alfalfa pellets and 13% commercial protected tallow) and 'protected polyunsaturated fat' (81% corn silage and 19% commercial protected polyunsaturated oil) supplemented diets. The protected polyunsaturated diet decreased the palmitic acid proportion of ewe plasma triacylglycerols from 23.5% to 18.3%, compared to the control diet containing 78% corn silage and 22% alfalfa pellets while increasing linoleic acid from 10.4% to 31.4%. The differences in the fatty acid proportions of plasma triacylglycerols between control and 'protected saturated fat' diets were not significant.

Microorganisms in the rumen of animals convert most of the polyunsaturated fatty acids in ruminant diet into saturated and monounsaturated fatty acids via bio-hydrogenation. Therefore, increased desaturation of tissue fat through the ruminant diet containing unsaturated fat is very difficult to obtain unless feed that can escape from rumen is used. Solomon et al. (1992) reported that feeding Suffolk x Hampshire lambs with a diet containing 10.6% palm oil significantly increased plasma concentrations of triacylglycerols, total cholesterol and high-density lipoprotein (HDL) cholesterol after 60 days of feeding. They also reported that gender did not affect the concentrations of these three components of plasma lipids.

Lough et al. (1992) investigated the effect of canola seed and soy lecithin in lamb diet on the fatty acid composition of carcass tissues. They reported that addition of 6% canola seed to the basal diet (a high forage diet) reduced the total polyunsaturated fatty acids of Longissiumus muscle from 7.03% to 5.79% but did not affect the levels of the total saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA). However, addition of 4.8% deoiled soy lecithin to the basal diet of ram lambs significantly increased the total polyunsaturated fatty acids (PUFA) level of lean tissue to 8.44%. The canola seed diet decreased the level of SFA tissue from 45.21% to 40.45%, the level of MUFA from 38.43% to 33.94% and the level of PUFA from 4.72% to 3.39% in adipose. On the other hand, the deoiled soy lecithin diet did not have any effect on total SFA and MUFA levels of adipose tissue but increased the level of total PUFA concentration from 4.74% to 5.42% in adipose tissue of ram lambs compared with the basal diet.

Components of ruminant tissue fatty acids are different from components of non-ruminant ones (Christie, 1981a). Ruminant tissues contain odd-chain and/or branched chain fatty acids and positional and configurational isomers of mono- and di-unsaturated fatty acids at high concentration. However, polyunsaturated fatty acid content in ruminant tissues is comparatively low.

The most successful attempts to increase levels of unsaturated fatty acids in ruminant tissues by inclusion of oils in the diet have been achieved by protecting the oil with a formaldehyde-protein complex (Palmquist et al., 1977). However, formaldehyde is a carcinogen and cannot be fed to ruminants in the United States (Dje, 1994). Therefore, safe chemical agents that also can form a protective protein-oil complex against ruminal attack but allow absorption of nutrients after the rumen are needed. Two such chemicals,

acetaldehyde and 2,3-butanedione, were used successfully by Dje (1994) and Lee (1996) to increase levels of linoleic acid, respectively in ewe blood and milk and in lamb tissues. These latter two researchers fed the sheep a limited amount of ground whole soybean supplement treated with these chemicals to achieve a modest increase in the linoleic acid content. Inclusion of greater amounts of a polyunsaturated oil in the supplement and feeding a larger amount of it could further increase the level of polyunsaturated fatty acid in sheep blood serum and tissues than was found by Dje (1994) and Lee (1996). Therefore, although the first objective of this research was to test the efficacy that the oil-protein supplement could increase tissue deposition of a fat soluble component such as α-tocopheryl acetate; the second objective was to determine the differences in blood serum lipid fatty acid composition in lambs fed such a supplement. The second objective of the experiment was the primary objective for this thesis. In addition, levels of selected fatty acids were correlated with α-tocopherol content in the blood serum of the lambs and to concentrations of like fatty acids in the Psoas major and Longissimus of the lambs. Such correlations can show relationships between absorption of the vitamin and the oil into the blood stream from the diets and between fatty acids of blood serum and muscle tissues.

CHAPTER II

REVIEW OF THE LITERATURE

A. Composition of Plasma Lipids in Ruminant Animals

Sheep plasma and blood volumes are affected by several factors such as diet, age, sex, lactation, disease, water intake, environmental temperature and breed (Macfarlane, 1975). Concentrations of sheep plasma carbohydrates, polyols and organic acids other than lipid fatty acids are shown in Table 1. Christie and Moore (1971) isolated triacylglycerols from different parts of sheep including plasma, and reported that variation existed in the concentration of sheep plasma triacylglycerols in the literature because the response of animal plasma lipids to changes in diet is very rapid. Plasma lipid concentration of sheep (200 mg/100 mL) is usually lower than that of other ruminants. Table 2 shows plasma lipid compositions of mature, newborn, lactating and non-lactating sheep. Two major components of plasma lipids in sheep are cholesteryl esters (65-76 mg/100 mL) and phospholipids (46-75 mg/100 mL), and they account for about 70-80% of the lipids in sheep plasma (Lindsay and Leat, 1975). Fatty acid compositions of plasma cholesteryl esters, triacylglycerols and phospholipids are given in Table 3.

Plasma triacylglycerol concentrations of newborn cow, goat and sheep are lower than adults, and lactation slightly increases plasma triacylglycerols of these ruminants (Leat, 1967). Leat (1967) reported that the concentration of plasma triacylglycerols was maximal when sheep were 10 days old. After ten days, the concentration remained constant. However, the concentration of plasma phospholipids increased until 20 days. It decreased after 20 days post partum.

Table 1. Sheep plasma carbohydrate, polyols and organic acid composition (adapted from Lindsay and Leat, 1975).

Component	Concentration
	mg/L
Glucose	550 - 790
Ascorbic Acid	13 ± 0.7
Inositol	4 - 15
Glycerol	3.4 ± 0.04
Sorbitol	Trace
	µmol/L
Lactate	1240 - 1518
Pyruvate	60 - 120
α-oxo-glutarate	10 - 20
Succinate	30 - 70
Citrate	300 - 500
Formate	28 - 120
Acetate	400 - 1500
Propionate	10 - 20
Isobutyrate	2 - 12

Table 2. Plasma lipid compositions of mature, newborn, lactating and non-lactating sheep (wt % of the total).

Lipid	Mature [a]	Newborn[b]	Lactating[b]	Non-lactating[b]
Triglycerides	11.0	23.0	12.6	17.0
Cholesteryl esters	46.6	22.2	32.6	34.5
Cholesterol	8.0	16.4	10.6	8.5
Free Fatty Acids	4.3	-	-	-
Hydrocarbons	1.8	-	-	-
Phospholipids	28.2	38.4*	44.2*	39.9*

[a] Garton & Duncan (1964); [b] Leat (1967); * as lecithin

Table 3. Fatty acid composition of sheep plasma lipids and total fatty acids (wt % of the total).

Fatty	Triglycerides		Cholesteryl Esters		Phospholipids		Total F. Acids	
Acid	Mature [a]	Newborn [b]	Mature [c]	Newborn [b]	Mature [d]	Newborn [e]	Mature [b]	Newborn [b]
C14:0	1.4	-	-	0.9	-	-	1.2	1.8
C15:0	1.4	-	0.7	-	-	-	-	-
C16:0	27.8	37.0	10.3	32.8	20.2	30.0	16.7	22.5
C16:1	2.1	7.5	2.3	13.8	1.2	6.0	3.3	8.4
C17:0	1.5		2.6		25.5	-	-	-
C18:0	22.2	9.5	1.6	11.0	20.3	13.6	12.2	9.3
C18:1	31.5	45.0	27.4	35.7	15.8	25.8	28.3	50.0
C18:2	4.7	0.5	35.2	1.5	2.5	1.1	26.1	0.9
C18:3	0.9	-	6.5	<0.5	-	Tr	4.6	<0.5
C20:3	0.3	-	-	-	7.5	5.2	1.0	2.8
C20:4	1.1	-	-	-	} 4.7	5.4	2.1	1.2
C22:5	-	-	-	-		2.9	-	-
C22:6	-	-	-	-	-	5.2	-	-

[a] Christie and Moore (1971); [b] Leat (1966); [c] Nelson (1969); [d] Moore et al. (1968); [e] Noble et al. (1971)

Mature cattle, sheep and goats have saturated fatty acids such as palmitic acid (C16:0) and stearic acid (C18:0) in their triacylglycerols at high amounts and polyunsaturated fatty acids at small proportions (Christie, 1981a). However, newborn calves, lambs and goats contain higher amounts of monounsaturated fatty acids such as palmitoleic (C16:0) and oleic (C18:1) acids in their triacylglycerols. Newborn ruminants have higher amounts of palmitic acid (C16:0) but less stearic acid in triacylglycerol structures (Christie, 1981a).

Plasma lipids are transported in the ruminant body in lipoprotein form for metabolism (Christie, 1981a). Plasma lipid composition is dependent on lipid metabolism, which can be easily affected by changes in ruminant diet (Marchello, 1972; Palmquist et al., 1977; Noble et al., 1978; Christie, 1981b; Mandell et al., 1997; Smith and Isopenko, 1997; Speake et al., 1997).

B. Factors Affecting Composition of Plasma Lipids and Other Fat Depots in Ruminant Animals

Fasting, dietary change, pregnancy, lactation and other factors such as age, sex, stress, hormones, seasonal changes (Lindsay and Leat, 1975) and breed may affect plasma composition of ruminant animals. Starvation decreases plasma glucose levels of sheep. On the other hand free fatty acid concentration and plasma glycerol concentration increase after fasting for 3-5 days. Although pregnancy has little effect on the plasma carbohydrate or lipid concentration in sheep, it may interact with nutrition (Lindsay and Leat, 1975). Plasma lipid composition of ruminant animals can also be affected by similar factors such as fasting (Speake et al., 1997), changes in ruminant diet (Horgan and Masters, 1963; Miller and Rice, 1967; Cook et al., 1970; Marchello et al., 1972;

Solomon et al., 1992), age (Leat, 1966; Leat. 1967; Noble et al., 1978), lactation (Leat, 1967; Palmquist et al., 1977), pregnancy (Leat, 1966; Leat, 1967; Palmquist et al., 1977) and seasonal changes (Noble et al., 1976).

Plasma lipid composition of ruminants also depends on differences in the rumen microflora in addition to factors such as diet, time of feeding, age, breed, pregnancy, etc. (Christie, 1981a). Rumen contains a wide range of microorganisms that can be responsible for fermentation of feed in ruminant diets, such as fungi (Orpin and Joblin, 1988) and protozoa (Williams and Coleman, 1988) as well as a number of bacteria (Stewart and Bryant, 1988). Dietary lipids are hydrolyzed by rumen microorganisms. For example, *B. fibrisolvens* can ferment various kinds of substrates, and is considered to play an important role in the hydrolysis of plant cell wall, hydrogenation of dietary lipids, and proteolysis (Stewart and Bryant, 1988). Absorption of saturated fatty acids is slower that that of polyunsaturated fatty acids (Van Soest, 1982). Ruminants can easily hydrolyze and absorb triacylglycerols that by-pass the rumen fermentation. Plasma contains enzymes that metabolize lipids, e.g. lecithin-cholesterol acyl transferase. Lipid composition of an organ in the body may vary because of 'arterio-venous' differences (Christie, 1981a). Excess amount of unsaturated fatty acids in ruminant diet can suppress methane bacteria in rumen, and can cause serious changes in ruminal fermentation balance (Van Soest, 1982).

A recent study showed that low portions of a range of C20-C22 polyunsaturated fatty acids were detected in plasma; however, those fatty acids were undetectable in the adipose tissue (Speake et al., 1997). Speake et al. (1997) studied the effects of fatness and fasting on fatty acid composition of plasma and adipose triacylglycerols in Texel-Oxford

and Scottish Blackface sheep (Table 4 and 5). They found higher amounts of stearic (C18:0), linoleic (C18:2n-6) and linolenic (C18:3n-3) acids in plasma samples than in adipose tissues. In both adipose tissues and plasma samples, the existence of oleic (C18:1n-9) acid was dominant. However, its proportion in adipose tissues was much higher than in plasma. Speake et al. (1997) found higher proportions of the C20-C22 polyunsaturates in the plasma of fat lambs. In fat lambs, plasma lipids had a lower proportion of oleic (C18:1n-9) acid and a higher proportion of linoleic (C18:2n-6) acid. The differences in fat lambs were more noticeable in the Scottish Blackface breed than for the Texel-Oxfords. A period (48 h) of fasting doubled the plasma concentrations of total free fatty acids (FFA). Fasting reduced the C18:2n-6 fatty acid proportion of plasma FFA in fat and lean lambs. But, in the Scottish Blackface breed, a higher level of C18:2n-6 in fat lambs than in lean lambs was found in the fasted state.

C. Relationship between Dietary Fatty Acids and Composition of Plasma Lipids in Monogastric Animals

Rassias et al. (1991) showed that a polyunsaturated fat (linoleic acid) rich diet, which provided 23% of energy intake from polyunsaturated fatty acids, 11% from saturated and 11% from monounsaturated fatty acids, significantly reduced low-density lipoprotein (LDL) cholesterol level of human plasma, compared to a high saturated fat diet providing 30.5% energy intake from saturated fatty acids, 6.1% from polyunsaturated and 9.2% from monounsaturated fatty acids. However, the linoleic acid rich diet caused a significant increase in HDL cholesterol level compared with the high saturated fat diet. They found that plasma triacylglycerol level for the high polyunsaturated fatty acid diets

Table 4. Fatty acid composition of plasma total lipids for Texel-Oxford lambs. Lambs fasted for 48 h and lambs fed ad libitum within the previous 4 h. (adapted from Speake at al., 1997).

	Fed		Fasted	
Fatty acid	Lean	Fat	Lean	Fat
C16:0	19.61	19.78	18.18	18.35
C18:0	23.27	24.77	24.28	26.67
C18:1n-9	33.92	29.25	36.35	32.36
C18:1n-7	1.20	1.35	0.91	0.91
C18:2n-6	13.10	14.53	10.03	10.77
C18:3n-3	2.07	1.76	1.88	1.67
C20:4n-6	1.64	2.33	1.87	2.69
C20:5n-3	0.44	0.60	0.52	0.65
C22:5n-3	1.28	1.56	0.97	1.18
C22:6n-3	1.25	1.78	0.94	1.10

Table 5. Fatty acid composition of plasma total lipids for Scottish Blackface lambs. Lambs fasted for 48 h and lambs fed ad libitum within the previous 4 h. (adapted from Speake at al., 1997).

	Fed		Fasted	
Fatty acid	Lean	Fat	Lean	Fat
C16:0	21.33	22.03	19.31	18.60
C18:0	26.46	23.94	24.88	24.65
C18:1n-9	36.36	29.61	37.91	35.25
C18:1n-7	0.35	0.57	0.51	0.68
C18:2n-6	12.12	16.79	8.38	10.76
C18:3n-3	1.48	1.80	1.71	1.82
C20:4n-6	0.89	2.05	1.11	1.88
C20:5n-3	0.31	0.41	0.36	0.47
C22:5n-3	0.28	0.95	0.35	0.66
C22:6n-3	0.36	0.60	0.29	0.50

(1.23±0.52 mmol/L) was significantly higher than for the high-saturated fatty acid diets (1.14±0.33 mmol/L).

Katan et al. (1995) reported that replacement of 1% of dietary energy in carbohydrate form with saturated, monounsaturated or n-6 polyunsaturated fatty acids in human diet decreased blood serum triacylglycerol concentration while HDL level of blood serum increased. However, LDL levels of serum decreased for monounsaturated and n-6 polyunsaturated fatty acid diets. The diet high in saturated fatty acid increased LDL concentration, which is directly related to the coronary heart disease risk.

Bonanome and Grundy (1988) studied the effect of diets rich in palmitic, stearic and oleic acids on the cholesterol levels (HDL and LDL) and total plasma triglyceride level in the human body. They observed that diet changes did not have any significant effect on the plasma total triacylglycerols. On the other hand, a high oleic acid diet significantly reduced the palmitic acid level of the plasma triacylglycerols. The percentages of the palmitic acid in the plasma were 35.7, 28.5 and 23.2 for the diets rich in palmitic acid, stearic acid and oleic acid, respectively. In the same study, it was reported that the palmitic acid rich diet resulted in high palmitic acid ratio in the plasma while it caused a low fraction of oleic acid. Bonanome and Grundy (1988) also suggested that there was a rapid conversion of stearic acid into oleic acid in the human body when a high stearic acid diet was fed, i.e., the absorption rate of the oleic acid in the human body was higher compared to palmitic and stearic acids.

Perez-Jimenez et al. (1995) investigated the effect of oleic acid rich diets on the human plasma lipid composition. They used olive oil and oleic acid rich sunflower oil to compare the plasma lipid differences due to the diet effect. No significant differences in

plasma triacylglycerol concentrations were reported between olive oil that contained less total sterols and tocopherols, and sunflower oil diets.

Bright et al. (1994) reported that the dietary supplementation of n-3 fatty acids in diets of cats for 8 weeks significantly decreased linoleic acid (C18:2n-6), eicosa-11,14-dienoic acid (C20:2n-6), and eicosa-8,11,14-trienoic acid (C20:3n-6) levels in cat plasma. However, the supplementation significantly increased the plasma levels of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

D. Dietary Lipid Metabolism in Ruminant Animals

1. Composition of Ruminant Dietary Lipids

The ruminant diet usually contains low amounts of lipid because lipid content of most feed is low (1-4%) (Van Soest, 1982). A wide range of ruminal microorganisms start fermentation of feeds in rumen, which results in acid formation. Acid can then be removed by absorption across the rumen wall, and neutralized by salivary buffers (Van Soest, 1982). High amounts of unsaturated fatty acid in diet can stop the activity of methane bacteria and can cause large changes in fermentation balance in ruminants (Van Soest, 1982).

Although there is a great change in fatty acid composition of ruminant dietary lipids, most forage plants contain relatively high amounts of palmitic (C16:0), oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) acids (Table 6) with linolenic being the most predominant fatty acid.

Table 6. Fatty acid composition (% of total) of various ruminant forages.

Forage	C12:0	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	Other
Mixed grasses[a]	2.9	3.3	9.4	3.0	1.5	13-19	20-26	30-39	1.4
Mixed grasses[b]	-	1.1	16.9	2.5	2.0	3.4	13.2	61.3	0.5
Clover pasture[c]	-	-	8.9	7.9	2.8	9.5	8.1	58.9	3.0

[a]Garton (1959); [b]Garton (1960); [c]Shorland et al. (1955).

2. Biohydrogenation of Dietary Unsaturated Fatty Acids

In monogastric animals dietary lipid digestion and absorption take place across the small intestine. In the small intestine, there are only minor changes in lipids. However, in ruminant animals, dietary lipids are subject to hydrolysis by lipases of rumen microorganisms. Unsaturated fatty acids are hydrogenated by rumen bacteria, and this biohydrogenation results in formation of stearic acid (C18:0) as the end product (Barnett and Reid, 1961a; Harfoot and Hazlewood, 1988). Absorption of free fatty acids in the rumen wall is faster than that of anions. Absorption of saturated fatty acids in the ruminant is slower than that of unsaturated fatty acids; also, absorption decreases with increasing chain length (Van Soest, 1982). Harfoot and Hazlewood (1988) reported the mechanisms of the biohydrogenation of α-linolenic acid (C18:3) (Figure 1) and linoleic acid (C18:2) (Figure 2) by rumen microorganisms. Both dietary fatty acids are finally hydrogenated to stearic acid (C18:0) by two classes of bacteria that are capable of biohydrogenation of dietary lipids (group A and group B). Bacteria classified under group A are mainly some strains of *Butyrivibrio fibrisolvens*, *Eubacterium*, *Ruminococcus albus* F2/6, and *Micrococcus* sp. Group B bacteria include *Fusocillus babrahamensis* P2/2, *Fusocillus* T344, and R8/5 Gram-negative rod. Biohydrogenation end products of dietary linoleic and linolenic acids depend on the two groups of bacteria.

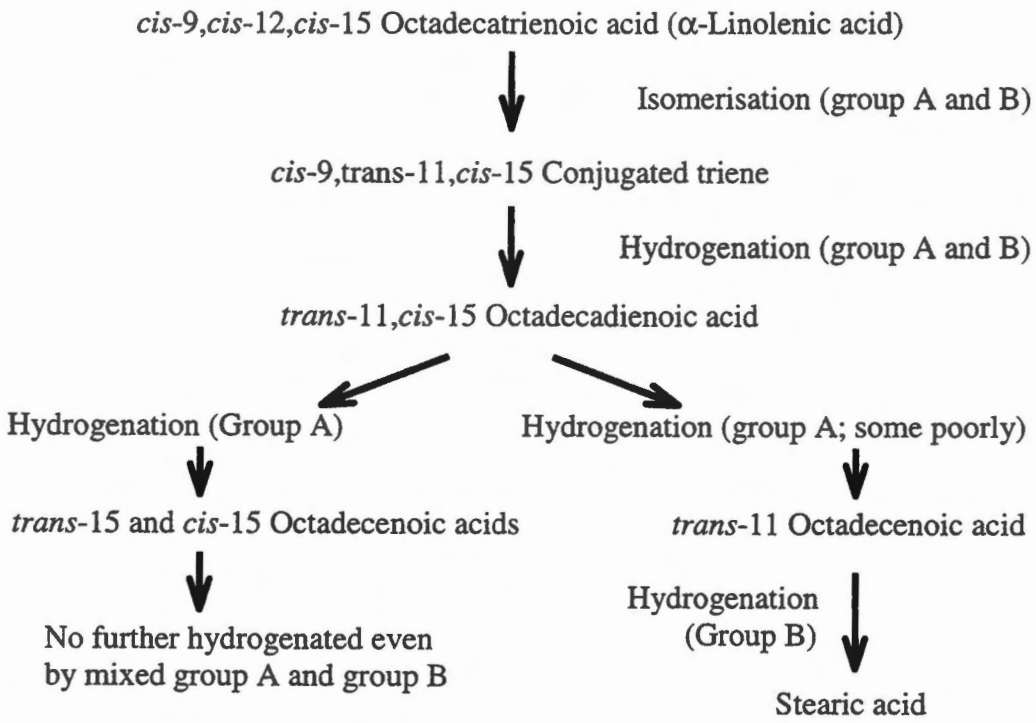


Figure 1. Mechanism for the biohydrogenation of α-linolenic acid by rumen bacteria (Harfoot and Hazlewood, 1988)

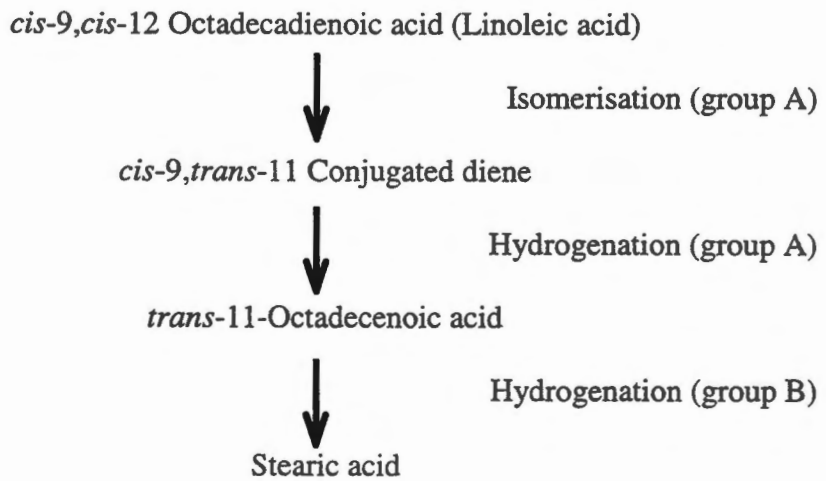


Figure 2. Mechanism for the biohydrogenation of linoleic acid by rumen bacteria (Harfoot and Hazlewood, 1988)

For example, biohydrogenation of *trans*-11,*cis*-15-Octadecadienoic acid by group B bacteria may results in formation of both *trans*-15- and *cis*-15-Octadecenoic acids (Harfoot and Hazlewood, 1988).

Excessive presence of polyunsaturated fatty acids in the intestine causes a 'swamped' absorption process for these fatty acids. Christie (1981b) reported a mechanism for the absorption process of fatty acids in the intestine. Polyunsaturated fatty acids that are not hydrogenated by the rumen bacteria are used by specific intestinal acyl transferases for the phospholipid synthesis in intestinal wall membranes when sheep are fed a normal low fat forage based diet. However, high amounts of saturated and monounsaturated fatty acids reaching the intestines are rapidly used for the triacylglycerol synthesis. Triacylglycerols are then absorbed and distributed quickly in the animal. "When large amounts of polyunsaturated fatty acids reach the intestines, the mechanism for segregating these components is not able to cope and the excess is utilized for triglyceride biosynthesis" (Christie, 1981b).

Hughes et al. (1982) purified the cis-9,trans-11-octadecadienoate reductase enzyme that catalyzes the biohydrogenation pathway of linoleic acid by *Butyrivibrio fibrisolvens*, an anaerobic rumen bacterium. Hughes and Tove (1980a) suggested that deoxy-α-tocopherolquinol and α-tocopherolquinone might serve as an electron donor for biohydrogenation of unsaturated fatty acids by that bacterium in the rumen. The α-tocopherolquinol was also identified as an electron donor for the biohydrogenation of cis-9, trans-11-octadecadienoate by *B. fibrisolvens* (Hughes and Tove, 1980b).

Barnett and Reid (1961a) summarized the formation of volatile fatty acids such as formic, acetic, butyric, valeric, caproic, acrylic, propionic and other acids from pyruvate

and lactate degradation in the ruminant metabolism. Fatty acids in the ruminant body may form from different sources other than glucose degradation products mentioned above. Proteins are partially broken down by the rumen microflora. Some goes to the abomasum without any change (Barnett and Reid, 1961a). Ammonia forms through the deamination of amino acids, and this ammonia may be converted to microbial protein or directly absorbed by the rumen wall. This deamination step may cause the formation of hydroxy, keto and fatty acids.

Hydrogenation of dietary linoleic acid acid (C18:2) in the rumen results in stearic, vaccenic acid (11t-C18:1) and isomers and a conjugated dienoic acid (9c,11t-18:2) and re-esterification of these fatty acids takes place in the intestines, and they are transferred in the animal body. "Some unchanged linoleic acid may then be converted to arachidonic acid and other long chain fatty acids, while the stearic acid may be desaturated to oleic acid." Thus, dietary supplementation of ruminant feed with linoleic acid may not elevate the concentration of linoleic acid in tissues but may increase the proportions of C18:0 or C18:1 fatty acids (Christie, 1981b).

Fatty acid composition of ruminant lipids can be altered by modification of rumen fermentation and the end product of this fermentation. High absorption rate of fats in the diet may alter serum fatty acid characteristics, which in turn may change the lipid metabolism of the ruminant (Marchello et al., 1972).

E. Relationship between Dietary Fatty Acids and Lipid Composition in Plasma and Other Fat Depots of Ruminants

In several studies, unprotected dietary lipids were used to investigate the effect of dietary lipids on fatty acid composition of animal tissues (Miller and Rice, 1967;

Marchello et al., 1972; Lough et al., 1992; Solomon et al., 1992; Mandell et al., 1997). However, biohydrogenation of dietary unsaturated fatty acids by ruminal bacteria in ruminant bodies has caused researchers to investigate possible techniques to protect fatty acids against the rumen biohydrogenation process (Palmquist et al., 1977; Noble et al, 1978; Dje, 1994; Smith and Isopenko, 1997).

1. Unprotected Dietary Fatty Acids

Marchello et al. (1972) studied the effect of animal fat (6%) and safflower oil (6%) additions to the control diet on the fatty acid compositions of serum phospholipids, cholesterol esters, free fatty acids and triacylglycerols in steers. To manipulate lipid composition of diets, treatments of control, animal fat and safflower diets were supplemented with 60, 75 and 90% concentrate, which was composed of equal parts of alfalfa roughage and cottonseed hull. They collected blood samples over five periods of time (period 1, just before the treatment diets; period 2, after feeding with 60% concentrate diets; period 3, after feeding with 75% concentrate diets; period 4, after feeding with 90% concentrate diets and period 5, at time of slaughter). Marchello et al. (1972) reported that fatty acid levels of the serum glyceride fraction from steers fed with the animal fat diet were similar to the fatty acid profiles of the 90% concentrate diet (period 4) and at slaughter time period (period 5) (Table 7).

Major fatty acid fractions of the major components of steer serum lipids are shown in Table 7. For period 3, which had 75% concentrate diets, 6% animal fat addition significantly increased palmitic (C16:0) acid contents of the glyceride fraction of steer

Table 7. LSMEANS of palmitic, stearic, oleic and linoleic acid percents of steer serum lipids for phospholipids, cholesterol esters and glyceride fractions by diets [a] within periods [b] (adapted from Marchello et al., 1972).

Fatty Acid	Period 1			Period 2			Period 3			Period 4			Period 5		
	control	6% AF	6% SO	control	6% AF	6% SO	control	6% AF	6% SO	control	6% AF	6% SO	control	6% AF	6% SO
Phospholipids															
C16:0	19.4	19.4	20.2	18.8	19.6	17.7	18.2	19.3	17.1	17.6	18.1	18.0	16.9 ^{cd}	19.5 ^{c}	14.7 ^{d}
C18:0	27.6	25.7	26.3	30.2	26.3	27.0	26.7	24.6	27.2	27.0	25.7	29.0	27.7 ^{c}	21.7 ^{cd}	21.5 ^{d}
C18:1	25.3	22.9	23.9	19.3 ^{c}	26.7 ^{d}	25.2 ^{d}	13.2 ^{c}	25.8 ^{d}	19.9 ^{e}	15.9 ^{cd}	19.8 ^{c}	14.2 ^{d}	14.1 ^{c}	24.6 ^{d}	19.3 ^{e}
C18:2	10.7	12.1	10.7	19.0	13.2	18.6	25.4 ^{c}	13.9 ^{d}	22.7 ^{c}	22.8	20.2	22.4	24.2 ^{c}	15.3 ^{d}	26.1 ^{c}
Cholesteryl esters															
C16:0	7.1	6.8	7.9	6.8	6.6	6.2	5.0	6.2	6.6	5.1	5.7	5.2	4.5	7.1	6.4
C18:0	0.8	0.9	0.9	0.9	0.8	0.5	0.9	0.8	1.5	1.0	0.8	0.7	0.7	0.8	1.4
C18:1	11.4	10.5	12.7	8.3	10.8	7.7	4.7 ^{c}	11.2 ^{d}	6.8 ^{c}	8.1	8.0	4.6	6.1	11.1	8.1
C18:2	41.5	42.8	43.3	62.4 ^{cd}	53.3 ^{c}	69.7 ^{d}	70.3 ^{c}	56.2 ^{d}	70.9 ^{c}	70.3	65.5	76.9	71.4	55.7	67.3
Glycerides															
C16:0	32.1	30.6	30.9	23.3 ^{c}	28.2 ^{d}	21.4 ^{c}	22.2 ^{c}	27.4 ^{d}	15.8 ^{e}	21.4 ^{c}	24.6 ^{d}	17.9 ^{e}	21.5 ^{c}	26.4 ^{d}	15.3 ^{e}
C18:0	32.2	30.4	29.9	26.4	20.9	19.9	25.6	21.8	22.0	23.8	20.7	27.4	21.5	26.0	16.5
C18:1	18.1	18.4	19.4	25.0 ^{c}	28.6 ^{c}	41.1 ^{d}	25.1 ^{c}	27.5 ^{c}	39.4 ^{d}	32.1	30.2	29.5	35.5 ^{c}	28.4 ^{c}	45.7 ^{d}
C18:2	1.7	2.3	2.3	6.6	3.7	4.3	6.1 ^{c}	4.4 ^{c}	9.5 ^{d}	7.1 ^{c}	6.5 ^{c}	10.8 ^{d}	5.4 ^{cd}	4.0 ^{c}	8.1 ^{d}

[a] AF= added animal fat; SO= added safflower oil; [b] Period 1, start of alfalfa hay ratio; Period 2, 60% concentrate diets; Period 3, 75% concentrate diets; Period 4, 90% concentrate diets; and Period 5, at the time of slaughter; [cde] Values within the same row with unlike superscripts significantly differ ($P<0.05$)

serum lipids. However, safflower addition (6%) decreased the palmitic (C16:0) acid level increased n-3 fatty of that fraction in serum lipids. Oleic (C18:1) acid levels of the serum glyceride fraction were increased significantly by safflower supplementation of the steer diet for the same period. In the cholesteryl ester fractions of serum lipids for period 3, 6% animal fat addition to the diet significantly decreased linoleic (C18:2) acid levels, while increasing the oleic (C18:1) acid proportion.

The highest linoleic (C18:2) acid content of the glyceride fraction of serum lipids was reported for the steers on the highly unsaturated 6% safflower oil diet at the time of slaughter. The oleic (C18:1) acid content in the serum lipid glyceride fraction for period 5 was significantly higher compared to that for other periods. The saturated animal fat addition increased palmitic (C16:0) acid levels in the glyceride fraction of steer serum during periods 4 and 5. Oleic (C18:1) and linoleic (C18:2) acids of serum lipid fractions within each of the diets by period varied inversely over time i.e. while C18:1 acid levels decreased, C18:2 acid levels increased (Marchello et al., 1972).

Marchello et al. (1972) concluded that dietary lipid treatment changes the concentration of certain fatty acids of a lipid fraction. They stated that "increased levels of unsaturated fatty acids in the serum of steers fed the 6% added safflower oil and not the 6% animal fat diet suggested that increased absorption of the fats themselves may have altered the characteristics of the serum fatty acids and thus the lipid metabolism of the animal."

Mandell et al. (1997) investigated the effects of fishmeal (5% and 10%) containing 5.87 g/kg EPA (eicosapentaenoic acid) and 9.84 g/kg DHA (docosahexaenoic acid) on some properties of beef cattle including fatty acid composition of longissimus

muscle after 56, 112 and 168 days of feeding. They reported that muscle lipid concentrations of n-3 fatty acids, including EPA and DHA, were increased by a 5% fishmeal diet from 0.8% (% total fatty acids) to 1.1, 1.5 and 1.7% after feeding 56, 112 and 168 days, respectively. Moreover, 10% fishmeal supplementation acid concentration in the muscle lipids from 0.8% to 1.5, 1.6 and 1.8 after feeding 56, 112 and 168 days, respectively. However, the arachidonic acid level significantly decreased from 0.7% to 0.5% when animals were fed with 5% fishmeal supplementation, and to 0.3% when animals were fed with 10% fishmeal supplementation after 168 days of feeding. Mandell et al. (1997) demonstrated that n-3 fatty acid concentrations in beef could be increased with a proper dietary strategy such as fishmeal supplementation of steer diet.

In sheep, small amounts of fatty acids from dietary lipids such as linoleic acid (C18:2) and linolenic acid (C18:3) acids may escape from hydrogenation in the rumen, but the amounts account for only 0.3% of the total energy intake (Lindsay and Leat, 1975). The concentrations of C18:2 and C18:3 are low in sheep plasma triacylglycerols and free fatty acids, while stearic acid concentrations are high. The reason is the hydrogenation of unsaturated fatty acids in the rumen.

"Despite the absorption of such small amounts of polyunsaturated fatty acids, high concentrations of C18:2 acids are found in the plasma cholesteryl esters and phospholipids" (Lindsay and Leat, 1975) (Tables 3, 4, 5 and 7).

High concentrations of dietary free fatty acids may influence the absorption of the products in the rumen, and this may change the lipid metabolism of ruminants. As a result of this change, fatty acid components of other animal tissues may be modified

(Christie, 1981b). The effects of fats in ruminant diets may be avoided by the protection of fats and oils against rumen hydrolysis and biohydrogenation of fats and oils.

2. Protected Dietary Fatty Acids

Fatty acids of plasma may vary from diet to diet given to ruminants. Plasma triacylglycerol changes due to dietary fat have been reported by several researchers (Palmquist et al., 1977). Protection of dietary fat in the diet and the type of protected fat in the diet may influence the plasma fatty acid composition. Protection of polyunsaturated fats in the diet may reduce the saturated fatty acid fraction of plasma lipids while increasing the polyunsaturated fatty acid fraction of plasma lipids (Palmquist et al., 1977).

Noble et al. (1978) reported that the linoleic acid level of plasma significantly increased in ewes fed with a supplemental diet containing the polyunsaturated fatty acid protected by 'a formaldehyde-treated protein coat' when they were closer to parturition.

Smith and Isopenko (1997) reported supplementation of sheep diet with 'protected polyunsaturated fatty acids' prepared from linseed oil and casein increased the plasma selenium concentration, but the increase was not significant. On the other hand, the linolenic acid concentration of plasma was significantly increased by the diet with the protected polyunsaturated fatty acids between 49 days and 63 days of feeding. The use of protected polyunsaturated fatty acids in the diet increased the linolenic acid (C18:3) level (% total fatty acids) from 9.1 to 15.7%.

Palmquist et al. (1977) studied the effect of 'protected saturated' and 'protected polyunsaturated fat' feeding of lambs on milk composition, plasma fatty acids and

growth. They reported changes in ewe plasma fatty acid proportions depending on the diet. 'Protected polyunsaturated' oil supplements in the diet significantly decreased the palmitic acid percentage of plasma, while it caused a significant increase in the linoleic acid (C18:2n-6) proportion. However, feeding the ewes with the diet containing 'protected saturated fat supplement' significantly increased the palmitic acid proportion of plasma compared to the diet with 'protected polyunsaturated fat' supplement. However, the difference in C16:0 and C18:2n-6 fatty acid proportions between control and the 'protected saturated fatty acid' supplemented diet was not significant.

Dje (1994) used acetaldehyde and diacetyl as alternative protective agents to formaldehyde, which is carcinogenic to animals, to increase unsaturated fatty acid contents of ewes' milk. Gelled emulsions of defatted soy flour-casein-sunflower oil, ground full-fat soybean seeds-casein and ground full-fat sunflower seeds-casein were fed to ewes. Dje (1994) reported that feeding with the gelled emulsions containing ground full-fat soybeans treated with 2.2% formaldehyde, acetaldehyde, diacetyl significantly increased the serum linoleic acid levels of lactating ewes, compared to those fed with the control diet (non-treated emulsion). Addition of formaldehyde, acetaldehyde and diacetyl treated supplements into the diet increased linoleic acid levels by 42, 41 and 25% respectively, over that in serum of ewes fed the control diet only. Enhanced growth in lambs fed with protected supplements was reported; however, protection did not influence ewe weights. Moreover, linoleic acid levels of ewe milk increased by 114, 52 and 43% when lactating ewes were fed with formaldehyde, acetaldehyde and diacetyl treated supplements, respectively.

Atwal et al. (1991) reported that feeding cows a diet containing 4% Protec®, which is a mixture of formalin-treated-canola seed and canola meal, increased both saturated and unsaturated C18 fatty acids in milk fat.

Cook et al. (1970) reported that formaldehyde-treated safflower oil supplementation of animal feed increased the proportion of linoleic acid in depot fats by 3 to 5 fold after 6 weeks. They suggested that in lambs there was a rapid exchange between the fatty acids of plasma triacylglycerols and depot fats.

F. Relationship between Fatty Acids of Plasma and Other Components in Ruminant Animals

The most marked difference between plasma lipids and lipids of other tissues of many animals is that plasma lipids contain a wide range of fatty acids from medium chain fatty acids such as myristic and palmitic to long chain fatty acids such as C22:5n-6 and C22:6n-6. Sheep plasma lipids usually contain high proportions of palmitic, stearic, oleic and linoleic acids (Moore et al, 1968).

Speake et al. (1997) reported a negative relationship between the proportions of C18:1n-9 in the fractions of plasma free fatty acids and adipose tissues triacylglycerols and backfat depth of fed Texel-Oxford and Blackface sheep. However, concentrations of C18:2n-6 in both fractions and backfat depth had a positive relationship. In fed state, the proportions of C20:4n-6, C22:5n-3 and C22:6n-3 plasma FFA also were positively related to backfat depth. In the plasma of fasting lambs, a negative relationship between C18:1n-9 concentration and fatness was shown, while the relationship between C18:2n-6 and backfat depth was not significant. On the other hand, C18:0 concentration in plasma showed a positive relationship with backfat depth.

G. Relationship between Dietary Vitamin E Supplementation and Tissue Properties in Ruminant Animals

1. Tocopherol

Tocopherol was derived from the Greek tocos (childbirth or offspring) and pherein (to bring forth) (Anonymous, 1987; McDowell, 1989; Drevon, 1993). Tocopherols are insoluble in water, but highly soluble in fats, oils and some organic solvents such as alcohol, benzene, and chloroform (McDowell, 1989; Kijima, 1993; Perry, 1995). They are heat resistant but oxidized very easily. The acetate form of vitamin E (Figure 3) when absorbed by the body is converted to the alcohol form (McDowell, 1989). Vitamin E is a biological antioxidant that can protect blood and other parts of an aerobic organism against free radical attack (McDowell, 1989; Niki, 1993).

Eight forms of vitamin E found in nature are alpha-, beta-, gamma-, and delta-tocopherols and alpha-, beta-, gamma-, and delta-tocotrienols. Vitamin E is stored mainly in the liver, but may be found in all body tissues. Consumption of vitamin E by polyunsaturated fatty acids in the tissues is very rapid, and the rate is proportional to polyunsaturated fatty acid (PUFA) intake (McDowell, 1989). Deficiency of vitamin E in ruminants can cause 'white muscle disease' which is known as nutritional muscular dystrophy (McDowell, 1989; Perry, 1995).

Estimated dietary vitamin E requirements for most animals are usually from 5 to 50 μg/kg of diet. High levels of vitamin E are found in cereal grains (Diplock, 1983; Perry, 1995), as well as in most vegetable oil grains (Diplock, 1983). Vitamin E supplements generally consist of α-tocopheryl acetate (Figure 3), usually in the fully

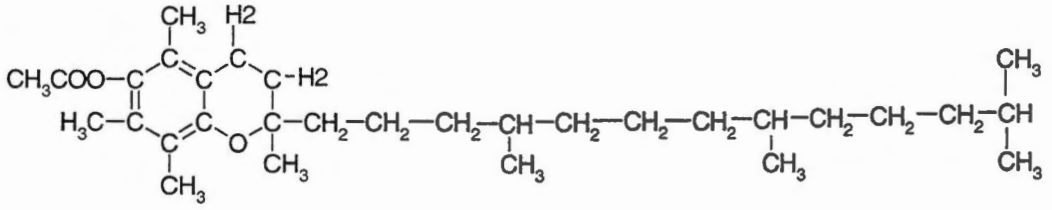


Figure 3. Structure of alpha-tocopheryl acetate.

racemic form, all-rac-α-tocopheryl acetate. Most animal species hydrolyze this form to the free alcohol form, tocopherol, in the small intestine, where it becomes free alcohol after micellar solubilization (Diplock, 1983).

The α-tocopherol has the major source of the vitamin E activity in plasma and other animal tissues (Coelho, 1991). Vitamin E requirement in the diet is related to the proportion of the PUFA intake such as soybean and cottonseed oil in the feed. Since vitamin E is a fat-soluble vitamin, its absorption is poor from low fat diets (Combs, 1991).

2. Absorption of Vitamin E in Ruminant Body

Impairment of pancreatic function or bile production will result in decreased absorption of vitamin E. Also, the type of fat in the diet affects vitamin E absorption. Medium-chain triacylglycerols increase its absorption while high levels of linoleic acid decrease absorption of vitamin E (Diplock, 1983).

The vitamin E, upon absorption, is transported via the blood stream to the cell walls, where it is an integral part of the structure along with highly polyunsaturated phospholipids. Vitamin E acts as a biological antioxidant in the cell wall protecting the

highly polyunsaturated phospholipids in the cellular membranes from peroxidative deterioration (Diplock, 1983). Tocopherol acts as an antioxidant by transferring the hydrogen on the alcohol group to reduce the free radicals within the cell. Donation of the H atom on the sixth carbon by α-tocopherol results in the formation of 8-α-hydroxy-tocoperone or 8-α-alkoxy-tocoperone, both of which are irreversibly converted to tocopheryl quinones (Diplock, 1983). Thus, vitamin E is an antioxidant which prevents or delays deterioration of substances when exposed to oxygen (Ramay, 1996).

"Factors such as vitamin bioavailability, stability during storage and physiological condition of animals may influence the level of vitamins and other nutrients in the diet" (Anonymous, 1987).

In mammals, the absorption of vitamin E takes place in the small intestine, and vitamin E is carried to the plasma and general circulation via the lymph (McDowell, 1989; Burton et al., 1983).

3. The Effect of Dietary Vitamin E on Meat Quality

Most animal species have a vitamin E plasma concentration of 1-5 μg/mL α-tocopherol except when they are fed supra nutritional levels of vitamin E in their diet. Atwal et al. (1991) reported a marked increase in α-tocopherol level in plasma when cow diets were supplemented with 400 IU vitamin E/kg dry matter daily. Monahan et al. (1992) fed pigs a diet supplemented with 3% beef tallow or 3% soya oil with and without 200 mg/kg α-tocopherol acetate daily. Feeding the vitamin E supplement with beef tallow increased the plasma α-tocopherol level from 2.23±0.27 to 7.27±0.54 μg/mL plasma. Feeding the vitamin E supplement with soya oil increased α-tocopherol levels in

pig plasma from 2.03±0.25 to 6.85±0.35 mg/mL. The α-tocopherol supplementation also increased the oxidative stability of the pork muscles and compared with the beef tallow, the soya oil increased the C18:2/C18:1 ratio in pork muscles and adipose tissues.

Other investigators have investigated the effect of dietary vitamin E supplementation on tissue concentration of α-tocopherol and oxidative stability of different muscles in several different animals. Leskanich et al. (1997) found that increasing all-rac-α-tocopheryl acetate concentration in the diet of pigs from 100 mg to 250 mg/kg of feed significantly increased the vitamin E content of longissimus muscle. Bartov et al. (1983) studied the influence of dietary vitamin E on the stability and sensory quality of turkey meat and reported that stability of breast and drumstick was increased by the supplementation of vitamin E from 5 to 45 mg/kg in the diet.

The α-tocopheryl acetate supplementation of broiler diet (100 mg/kg feed) significantly increased the oxidative stability of both dark and white meat (Lin et al., 1989). Besides dietary α-tocopherol supplementation increasing tissue levels in nonruminant animals, it also increases the tissue levels of α-tocopherol in ruminant animals and oxidative stability of those tissues. Faustman et al. (1989) reported that dietary supplementation with alpha-tocopheryl acetate (370 I.U./head/day) increased the stability of pigments and lipids in beef from Holstein steers. They concluded that vitamin E supplementation of steer diets could be used to increase the shelf-life of beef. Arnold et al. (1993) found that tissue vitamin E concentration of Holstein and beef breed steers was increased by feeding the animals with a-tocopheryl acetate (0, 360 and 1290 I.U./head/day) supplemented diets for 252 days. Vitamin E supplementation also decreased the lipid oxidation while it extented the shelf life of beef based on its red color.

Wulf et al. (1995) investigated the effect of dietary vitamin E supplementation (500 or 1000 I.U./head/day) on the storage and caselife properties of lamb retail cuts. Vitamin E supplementation increased the caselife of lamb cuts around 4 days with the effect of 1000 I.U. vitamin E on the storage and caselife properties adding little additional effect compared to 500 I.U. supplementation.

In this study, the acetate form of vitamin E was used because of high concentration of linoleic acid in the gelled emulsion diet of lambs. Thus, mono and polyunsaturated fatty acids both in diets and ruminant tissues were protected against oxidation.

CHAPTER III

MATERIALS AND METHODS

A. ANIMALS

Twenty-four crossbred lambs were used to monitor the effect of finishing diets on the blood serum fatty acid composition. Eight lambs were assigned to a treatment with four lambs assigned to each of two pens, based on gender and color of coat. Each pen had two male and two female lambs, and as much as possible, three lambs with brown to black wool and one with white wool. The twenty-four newly weaned lambs were in pens that provided 0.46 m^2 of space for each animal. The lambs were on the dietary treatments from July 28 through October 20, 1997 and had access to an unlimited supply of fresh, clean water daily. Lambs were weighed individually on July 28 and every fourteen days during the feeding trial except for the last weigh date which was on October 17, 11 days from the next-to-last weigh day, to avoid undue stress of the lambs prior to harvest. The average daily weight gain for each lamb during the feeding trial was then determined.

Blood samples were collected from each lamb on July 28 and every 14 days during the trial and on October 20, prior to the death of each lamb. The blood was collected via jugular vein puncture into 20-mL vacuum tubes and immediately placed on ice and transported to the University of Tennessee Department of Animal Science where they were stored no longer then 24 h at 5-6°C. The serum was separated from the blood sample by centrifugation at 2000 g for 20 min in a Sorvall Superspeed Model %RC2-B Automatic Refrigerated Centrifuge (Ivan Sorvall Inc., Newton, CT) and sealed in 10-mL

vials under nitrogen. The blood sera samples were stored at -18°C until extracted and analyzed for fatty acid composition.

B. TREATMENTS

Three different diets were used to investigate fatty acid composition of blood serum lipids from lambs fed supplemental α-tocopheryl acetate with and without additional sunflower oil. Treatment 1 (control) lambs were fed 5.121 kg of basal diet per pen of four lambs daily. The composition of the control diet is shown in Table 8. Treatment 2 lambs were fed the same level of control diet plus 1000 I.U. of d-α-tocopheryl acetate per pen of four lambs daily. Treatment 3 animals were fed 2 kg of a protective supplement (gel) containing 1000 I.U. vitamin E from d-α-tocopheryl acetate mixed with 3.121 kg of basal diet per pen of four lambs daily. The gel contained water, defatted soybean flour, casein, sunflower oil, lecithin, sodium hydroxide and gelling agent. All pens were allowed mixed grass alfalfa hay ad libitum.

Table 8. Basal corn diet.

Component	Percentage
Coarse ground corn	63.8
Alfalfa Pellets	19.9
Soybean Meal (48 % crude protein)	10.0
Rolled oats	5.0
Trace mineral mix	0.5
Limestone	0.3
Antibiotic	0.2
Ammonium chloride	0.3

C. Chemical and Chromatographic Analyses

1. Lipid Extraction From Serum Samples

Prior to lipid extraction by a method described by Dje (1994), serum samples were thawed in a water bath at 25°C. A 1.5-mL sample of serum was transferred into a stainless steel, 60-mL container with a sterile pipette, and 8 mL of 0.05% butylated hydroxytoluene (BHT)-chloroform solution and 18 mL of methanol were added to the serum. An aliquot (1.0 mL) of an internal standard solution containing 1.0 $mg(mL)^{-1}$ of tridecanoic (C13:0) and heneicosanoic (C21:0) fatty acids was added to the mixture. The mixture was then homogenized at a low speed for 2 min with a Virtis Model 23 homogenizer (The Virtis Company, Inc., Gardiner, NY). After this step, 9 mL of BHT-chloroform solution was added to the mixture and homogenized for 30 sec at the same speed. After the addition of 9 mL of aqueous zinc acetate (4.6 g/L) and homogenization for 30 more seconds, the extract was poured into a separatory funnel and kept one hour at 4-6°C to allow separation between the aqueous methanol and chloroform layers. The lower chloroform layer was transferred into a 125-mL Erlenmeyer flask, and solvent was evaporated with a rotary evaporator at 55°C under vacuum. Fatty acid methyl esters were prepared from the dried lipids for gas chromatographic analysis of the serum lipid fatty acids.

2. Preparation of Fatty Acid Methyl Esters

Fatty acid methyl esters (FAME) of blood serum lipids were prepared according to the AOCS (1975) method. For each serum sample, an aliquot (4 mL) of 0.5 N sodium

hydroxide in methanol and a few glass boiling beads were added to the dried lipids in the 125-mL Erlenmeyer flask. The flasks were attached to water-cooled condensers and the flask contents boiled under reflux for 10 min. Then, 5 mL of boron trifluoride (14%) in methanol (Supelco, Inc. Bellafonte, PA) were transferred to the flask and allowed to react for 2 min. After the contents of the flask were cooled, 7 mL of pentane was added and the flask contents were boiled for an additional minute under reflux. The flask was then removed from the condenser. Saturated sodium chloride solution was added to bring the pentane layer containing the FAME into the neck of the flask, and the pentane layer was transferred to a 10-mL vial. A small amount of anhydrous sodium sulfate was added to the vial to absorb any residual water. The vial was flushed with nitrogen, sealed, and stored at –18°C until analysis by gas chromatography.

3. GC Analyses of Fatty Acid Methyl Esters

Blood serum fatty acid methyl esters were analyzed on a 30 m by 0.25 mm i.d. fused silica SP2330 column (Supelco, Inc., Bellefonte, PA) that was attached to a Shimadzu Model GC-9A gas chromatograph (Shimadzu Scientific Instruments, Inc., Columbia, MD) equipped with a flame ionization detector. An automatic injector, Model AOC-9 from the same company was used to inject 1.5 μL of FAME samples into the GC. Data were collected and processed with a Shimadzu Model CR501 Chromatopac data processor (Shimadzu Scientific Instruments, Inc., Columbia, MD). Data from the data processor was transferred to a personal computer by the software Chromatopac Data Archive Utility Version 3.1 (Shimadzu Scientific Instruments, Inc., Columbia MD). GC conditions for FAME analysis were as follows: injector and detector temperatures,

250°C, and column temperature program, 130°C initial temperature, raised at 2°C/min to 220°C final temperature with a 20 min hold at 220°C. Helium was the carrier gas with a 2.0 mL/min flow rate and was used as the make-up gas at a flow rate of 50 mL/min. The flow rates for the FID gases were: air flow rate of 400 mL/min and the hydrogen flow rate of 40 mL/min.

Standards of the methyl esters of fatty acids (FAME) listed in Table 9 were purchased in solutions of known concentrations, as well as single FAME, from Supelco™ 37 (Supelco, Inc, Bellefonte, PA) and Sigma® Oil Reference Standard, AOCS No.6 (Sigma Chemical Co., St. Lois, MO). A solution of FAME was prepared that was similar in qualitative and quantitative composition to the blood serum lipid fatty acids. This standard with FAME of known weight percentages was analyzed under exactly the same GC conditions as the samples. Individual fatty acids in the blood serum samples were identified by matching their respective retention times with those of individual standard FAME. The correction factors (CF) for specific fatty acids were calculated as given in the AOCS (1975) method from multiple analyses of the standard FAME solution. Two different sets of CF (Table 9) were obtained because part of the serum FAME samples and the standard FAME were analyzed before the GC column was heated without carrier gas flow for a period of time. The other blood serum samples were analyzed after this column exposure which changed the retention times and resolution of some of the FAME and altered some of the FAME CF. Therefore, the standard FAME solution was analyzed again and the CF recalculated as shown by Correction Factor 2 in Table 9. The weight percentage of each fatty acid identified in each blood serum sample from each lamb and sampling time was calculated using the AOCS (1975) method.

Table 9. Correction factors for the fatty acid methyl esters for determining fatty acid composition of blood serum lipids (AOCS (1975) method was used to calculate the correction factors).

Fatty Acid	Name	Correction Factor 1	Correction Factor 2
C14:0	Myristic acid	1.0545	1.1432
C14:1	Myristoleic acid	1.0074	1.0523
C16:0	Palmitic acid	1.0000	1.0000
C16:1	Palmitoleic acid, cis	1.0420	1.1999
C16:1*trans*	Palmitoleic acid , trans	1.0420	1.1999
C17:0	Heptadecanoic acid	1.1117	1.2523
C17:1	*cis*-10-Heptadecenoic acid	1.1095	1.0889
C18:0	Stearic acid	0.9256	0.8626
C18:1	Oleic acid	1.0142	1.0330
C18:2	Linoleic acid	0.8006	1.8515
C18:2*trans*	Linoelaidic acid	0.9854	1.1213
C18:3n-3	Linolenic acid	2.4699	2.8968
C18:3n-6	γ-Linolenic acid	0.7641	1.4183
C20:0	Arachidic acid	2.2829	2.8968
C20:1	*cis*-11-Eicosaenoic acid	1.1740	1.8120
C20:2n-6	*cis*-11,14-Eicosadienoic acid	1.0000	1.0000
C20:3n-6	*cis*-8,11,14-Eicosatrienoic acid	0.7602	1.2406
C20:4n-6	Arachidonic acid	1.0000	1.2406
C20:5n-3	*cis*-5,8,11,14,17-Eicosapentaenoic a.	1.0000	1.0000
C22:5n-3	*cis*-4,7,10,13,16-Docosapentaenoic a.	1.0000	1.0000
C22:6n-3	*cis*-4,7,10,13,16,19-Docosahexaenoic a.	0.5098	0.5008
C24:0	Lignoceric acid	0.9947	0.5028
C24:1	Nervonic acid	1.0300	1.0040

D. Statistical Analyses

A split plot design with the whole plot being the pen with repeated measures was used as a statistical design. The following statistical model was used;

$Y = \mu +$ Fixed effects + Random effects + E

Fixed Effects = D + S + B + T + D*S + D*B + D*T + S*T + B*T + D*S*T + D*B*T

Random Effects = P(T) + S*B*P(T)

μ = Mean

Y = Fatty acid measurement (wt %)

D =Diet effect (control, vit. E and sunflower oil-gel diets)

S =Sex effect (male vs. female)

B =Breed effect (black-brown vs. white)

T =Time (7 different testing times)

P = Pen effect

E =Error term

The rest of the terms in the model show the interactions of the factors mentioned above. D, S and B were whole plot factors, while T was the subplot (repeated measure) factor.

Proc Freq (SAS Inc, Cary, NC) was used to obtain percentages of each fatty acid in every sample. Unknown peak areas were not taken into consideration. For the fatty acids that could not be identified in some samples, their percentage was assumed to be zero. Data from one of the lambs on sunflower oil-gel diet were not included in the analyses because of no weight gain during the experiment. Moreover, nine observations were deleted because they were outliers. The percentages of each fatty acid were corrected using their related correction factors (Table 9) and then adjusted to sum to 100 percent. Corrected percentages of each fatty acid were used in the analysis of variance with Proc Mixed (SAS Inc, Cary, NC). The 'pdmix612.sas' program (Saxton, 1988) was used to generate letter groups of the least significant difference mean separation for treatments and interactions. Rank transformation was used to eliminate normality

problems for the following fatty acids; C14:0, C14:1, C17:1, C18:2*trans*, C20:0, C20:2n-6, C20:3n-6, and C22:5n-3.

Proc Corr (SAS, Inc, Cary, NC) was used to calculate simple correlations between the vitamin E content (α-tocopherol) of the serum and each fatty acid identified in the blood serum of the lambs. Moreover, it was used to determine the correlation between the fatty acid levels in serum lipids and the fatty acid levels of Longissimus or Psoas major muscles.

CHAPTER IV

RESULTS AND DISCUSSION

Each lamb on the control diet, vitamin E diet and sunflower oil plus gel diet ingested an average of 1061 g, 1072 g and 897 g of dry matter per day, respectively. On the average, the gel contained 52.77±0.87% moisture, 15.18±0.60% lipid, 15.1±0.1% protein and 465±67 I.U. tocopheryl acetate /kg. Major fatty acids of the lipids in gel supplements were linoleic (C18:2) and oleic (C18:1) acids (Table 10).

Table 10. Fatty acid composition (weight percentage fatty acids) of lipids in gel fed to lambs (Melton, 1998).

Fatty Acid	Percentages (wt %)
C16:0	7.0±0.4
C18:0	2.6±0.7
C18:1	17.2±1.1
C18:2n-6	71.4±0.7
C18:3n-3	0.6±0.1
C20:0	0.1±0.0
C20:1	0.2±0.0
C20:4n-6	0.6±0.2
C22:5n-3	0.3±0.5

At the beginning of the experiment lambs weighed 28.6 to 39.5 kg, and at the end of the experiment 40.0 to 54.0 kg. The average daily gain (ADG) of the lambs was 362.9 g/head, and it was not affected ($P>0.05$) by treatment, breed or gender.

A. Treatment Effect

Significant differences were found among the relative percentages of specific serum fatty acids (C16:0, C17:0, C17:1, C18:1, and C18:2) in lambs fed with three different diets averaged across testing times ($P<0.05$) (Table 11). However, there were no

Table 11. The effect of three different diets on the fatty acid composition of serum lipids in lambs. Fatty acid percentages were averaged across testing times, gender and breed.

Fatty Acid	Concentration (wt % of fatty acids)*		
	Control Diet	Vit. E Diet	Sunflower oil plus Gel Diet
C14:0	0.041	0.069	0.041
C14:1	0.067	0.080	0.024
C16:0	16.660[a]	16.400[a]	12.360[b]
C16:1*trans*	0.432	0.730	0.371
C16:1*cis*	0.802	1.008	0.581
C17:0	1.192[a]	1.330[a]	0.724[b]
C17:1	0.648[a]	0.777[a]	0.253[b]
C18:0	18.910	17.480	22.940
C18:1	23.910[a]	23.410[a]	16.980[b]
C18:2	23.04[a]	24.930[a]	33.470[b]
C18:2*trans*	0.246	0.196	0.256
C18:3n-3	0.764	0.368	0.394
C18:3n-6	0.610	0.726	0.394
C20:0	0.019	0.018	0.014
C20:1	0.340	0.341	0.302
C20:2n-6	0.110	0.125	0.052
C20:3n-6	0.361	0.391	0.479
C20:4n-6	4.270	4.300	4.590
C20:5n-3	0.727	0.583	0.393
C22:5n-3	1.407	1.090	0.738
C22:6n-3	4.540	4.220	3.830
C24:0	0.576	0.641	0.537
C24:1	0.432	0.523	0.390

*Least square means (LSMEANS) in the same row with different superscript letters differ ($P<0.05$)

significant differences in levels of these fatty acids in serum lipids between the lambs fed the control and those fed vitamin E supplemented diet. The diet with gel containing sunflower oil decreased the serum lipid proportion of palmitic acid (C16:0) averaged across feeding time ($P<0.05$) to around 12.36% compared to the control (16.66%) or vitamin E supplemented diets (16.40%). The relative percentage of oleic acid (C18:1) averaged across testing times was significantly lower in the serum of lambs fed with sunflower oil plus gel diet (16.98%) compared with control (23.91%) and vitamin E (23.41%) diets. However, sunflower oil plus gel diet increased the serum level of linoleic acid (C18:2) from around 24% to 33.5% ($P<0.05$). Feeding lambs with sunflower oil plus gel diet lowered major saturated (specifically C16:0) and monounsaturated (specifically C18:1) fatty acid levels of blood serum lipids in lambs, while increasing the diunsaturated fatty acid level, especially C18:2 fatty acid.

The diets investigated in this study also changed the levels of minor fatty acids in lamb blood serum. For example, levels of minor fatty acids, heptadecanoic acid (C17:0) and *cis*-10-heptadecenoic acid (C17:1) in lamb serum were reduced ($P<0.05$) by the diet with sunflower and gel (Table 10). In addition to palmitic acid, the major fatty acids of the lamb serum are oleic and linoleic acids.

Lindsay and Leat (1975) reported plasma lipids of sheep are more resistant to dietary changes than those of non-ruminants because of biohydrogenation of lipids by rumen microflora. We found that a vitamin E supplemented diet did not create any change in the composition of lamb serum lipid fatty acid levels different from the control diet when fatty acid percentages were averaged across testing times. It was reasonable to expect different results, particularly with the polyunsaturated fatty acid (PUFA) levels in

the blood serum lipids of lambs fed the vitamin E supplemented diet. This latter diet increased ($P<0.05$) the α-tocopherol level in blood serum from 0.32 (control diet) to 1.41 μg/mL (Melton, 1998), and since α-tocopherol is a powerful antioxidant, it could have protected against oxidation of the PUFA and increased their levels in the blood serum of lambs fed the vitamin E supplemented diet.

On the other hand, protection of the polyunsaturated fatty acids in the sunflower oil-gel supplement against rumen biohydrogenation changed the ratio of major fatty acids such as palmitic, oleic and linoleic acids in blood serum lipids of lambs fed that supplement (Table 11). Therefore, dietary effect of polyunsaturated oils on the serum lipid fatty acids in ruminants is enhanced when dietary lipids are protected, i.e., "when the rumen is by-passed and oils are introduced into the duodenum or abomasum" (Lindsay and Leat, 1975). The reason for the major change in the ratio was because linoleic acid in the protective sunflower oil-gel (Table 10) by passed the rumen of the lamb and was absorbed into the lamb blood, increasing the relative percentage of linoleic acid and decreasing those of palmitic and oleic acids. In addition, some additional protection against oxidation of PUFA may have been obtained from an increased level ($P<0.05$) of α-tocopherol in the blood serum (1.97 μg/mL) of lambs fed the protective supplement over that of lambs fed the control or vitamin E supplemented diet (Melton, 1998).

Moore et al. (1969) reported that intra-abomasal infusions of linseed oil (21.3% C18:2 and 71.1% C18:3), maize oil (22.9% C18:1 and 64.4% C18:2) and linoleic acid (15.2% C16:0, 10.9% C18:1 and 71.1% C18:2) changed the concentrations of linoleic and linolenic acids in plasma lipid components. The response of plasma triacylglycerols

to the infusion of emulsions was faster than that of plasma phospholipids or cholesteryl esters. Linoleic or linolenic acid levels of plasma triacylglycerols increased 1.5 h after infusion of the emulsions while there was a decrease in the concentrations of palmitic and stearic acids. Moore et al. (1969) reported that "the concentrations of linolenic or linoleic acid in the plasma phospholipids and cholesteryl esters did not begin to increase until 8-9 h and 24-25 h, respectively, after the infusions of the emulsions had begun". Moore et al. (1969) reported that linoleic and linolenic acids absorbed from the small intestine are transported to the liver in triacylglycerol form. Complete or partial hydrolysis of triacylglycerols occurs in the liver. These two fatty acids then are used for the synthesis of phospholipids and cholesteryl esters.

Dje (1994) used acetaldehyde and diacetyl as alternative chemical agents to formaldehyde for forming a supplement protective of polyunsaturated fatty acids in the rumen. This researcher fed lactating ewes a ground full-fat soybean (16% oil containing 53-54% linoleic acid) supplement treated with acetaldehyde and increased ($P<0.05$) the serum linoleic acid levels (33.64%) of lactating ewes compared to that (23.85%) of ewes fed the control diet (non-treated supplement containing the ground full-fat soybean. Treatment of the supplement containing the full-fat soybeans with diacetyl and feeding that supplement to the ewes, however, increased the serum linoleic acid level (29.90%) less than the acetaldehyde treatment, but the level was still greater ($P<0.093$) than in ewes fed the control diet. The alternative agents in the diets fed by Dje (1994) did not change the proportion of other fatty acids in serum lipids of lactating ewes. However, in our study, we found that the gel-protected treatment containing sunflower oil increased the

level of linoleic acid while decreasing the levels of palmitic and oleic acids in the serum lipids of lambs.

In looking at the effect of dietary lipids on the fatty acid composition of the blood serum of lambs, the level of dietary lipids fed per lamb in each treatment also should be considered. In the control treatment, lambs received an average of 38.0 g of dietary lipid (mainly from corn) per lamb daily during the 12-week feeding trial. In the treatment containing supplemental vitamin E, lambs received an average of 38.5 g of dietary lipid per head daily, and in the treatment containing the sunflower oil-gel supplement, lambs received an average of 103.0 g of dietary lipid per head daily, 2.7 times more lipid than the lambs in the other treatments. The level of dietary lipid alone fed in the treatment containing the sunflower oil-gel supplement would have been great enough to affect the metabolism of the lambs profoundly and would have caused diarrhea unless the lipid had been protected from ruminal digestion (Waller, 1998). The fact that the lambs fed this much lipid had the same average daily weight gain as, and otherwise, were as healthy as the lambs fed the other diets indicates also that the lipid was protected against digestive attack.

These facts, the results of Dje (1994) showing that a diacetyl-treated full-fat soybean supplement also protected polyunsaturated oil from ruminal biohydrogenation, and our results of increased levels of linoleic acid in the lamb blood serum lipids from feeding the sunflower oil-gel supplement, suggest that the polyunsaturated sunflower oil in the supplemental gel was at least partially protected from biohydrogenation in the rumen of the lamb. This lack of saturation of the dietary linoleic acid resulted in

increased absorption of linoleic acid and significantly increased levels of linoleic acid in lamb blood serum lipids.

B. Time Effect

When averaged across diets, two-week increments had significant differences ($P<0.05$) in the levels of each fatty acid in lamb serum lipids except for C16:1*trans*, C16:1*cis*, C17:0 and C17:1 fatty acids (Table 12). The relative percentages of most fatty acids in serum lipids averaged across diets did not have a specific pattern, meaning that their levels in serum lipids neither continuously increased nor decreased over testing time. While palmitic acid (C16:0) concentration of serum averaged across treatment diets decreased from 17.69% to 13.70%, the oleic acid (C18:1) proportion of lamb serum lipids decreased from 23.79% to 19.70% during the first four two-week periods, then increased to 23.06% on October 20, 1997. In comparison, linoleic acid levels increased from 18.02% to 30.35% during the first four weeks of the feeding trial and did not change levels significantly until the twelfth week of the trial when percentage of C18:2 decreased to 21.72% (Table 12).

C. Interaction of Treatment with Testing Time

The diet and testing time interaction significantly affected ($P<0.05$) the relative percentages of C16:0, C16:1*cis*, C18:1, C18:2 and C18:2*trans* fatty acids in lamb serum lipids when averaged across sex and breed during this feeding trial. Within the testing time, the sunflower oil plus gel diet decreased the level of palmitic acid (C16:0) in serum lipids compared to the control diet with the exception of the first testing time (July 28)

Table 12. Fatty acid composition of serum lipids averaged across diets, gender and breed in lambs.

Fatty Acid	Testing Times						
	28-Jul-97	11-Aug-97	25-Aug-97	08-Sep-97	22-Sep-97	06-Oct-97	20-Oct-97
	Concentration (wt % of fatty acids)*						
C14:0	0.245^{a}	0.042^{bc}	0.065^{ab}	0.000^{c}	0.000^{c}	0.000^{c}	0.000^{c}
C14:1	0.142^{a}	0.114^{a}	0.112^{a}	0.003^{b}	0.031^{b}	0.000^{b}	0.001^{b}
C16:0	17.694^{a}	15.039^{bc}	14.530^{cd}	15.844^{b}	14.621^{bcd}	14.541^{bcd}	13.696^{d}
C16:1*trans*	1.178	1.000	0.915	0.449	0.468	0.779	0.792
C16:1*cis*	0.588	0.511	0.476	0.334	0.377	0.563	0.730
C17:0	1.291	1.263	1.195	0.983	0.915	0.989	0.937
C17:1	0.726	0.510	0.524	0.409	0.469	0.599	0.681
C18:0	19.954^{abc}	21.463^{a}	18.015^{c}	18.719^{c}	18.650^{bc}	20.691^{ab}	20.942^{ab}
C18:1	23.786^{a}	21.655^{ab}	20.153^{b}	19.703^{b}	19.754^{b}	21.930^{ab}	23.060^{a}
C18:2	18.020^{d}	25.817^{b}	30.349^{a}	32.406^{a}	31.950^{a}	29.768^{a}	21.717^{c}
C18:2*trans*	0.138^{bc}	0.326^{a}	0.322^{a}	0.320^{ab}	0.058^{c}	0.132^{c}	0.330^{bc}
C18:3n-3	0.654^{ab}	0.303^{c}	0.265^{c}	0.632^{b}	0.725^{ab}	0.848^{a}	0.773^{ab}
C18:3n-6	0.445^{b}	0.895^{a}	1.010^{a}	0.386^{b}	0.324^{b}	0.398^{b}	0.576^{b}
C20:0	0.034^{b}	0.048^{a}	0.026^{b}	0.000^{c}	0.012^{c}	0.002^{c}	0.000^{c}
C20:1	0.608^{a}	0.433^{b}	0.478^{b}	0.106^{c}	0.134^{c}	0.165^{c}	0.369^{b}
C20:2n-6	0.230^{a}	0.226^{a}	0.213^{a}	0.001^{b}	0.000^{b}	0.000^{b}	0.000^{b}
C20:3n-6	0.487^{a}	0.587^{a}	0.595^{a}	0.241^{c}	0.187^{bc}	0.416^{ab}	0.359^{c}
C20:4n-6	4.564^{b}	3.698^{cd}	4.298^{bc}	3.489^{d}	4.678^{b}	3.619^{cd}	6.358^{a}
C20:5n-3	1.213^{a}	0.568^{b}	0.495^{bc}	0.355^{c}	0.427^{bc}	0.338^{c}	0.575^{b}
C22:5n-3	1.975^{a}	1.267^{ab}	1.188^{abc}	0.676^{e}	0.933^{bcd}	0.699^{de}	0.808^{cde}
C22:6n-3	5.329^{a}	3.666^{bc}	4.076^{b}	3.821^{bc}	4.260^{b}	2.773^{c}	5.459^{a}
C24:0	0.366^{c}	0.179^{c}	0.172^{c}	0.993^{ab}	0.740^{b}	0.420^{c}	1.220^{a}
C24:1	0.341^{bc}	0.386^{bc}	0.453^{bc}	0.484^{b}	0.429^{bc}	0.330^{c}	0.713^{a}

* LSMEANS in the same row with all unlike superscript letters differ ($P<0.05$)

(Figure 4). A similar trend was also found for the relative concentration of oleic acid (C18:1) (Figure 5). Although the sunflower oil plus gel diet decreased the serum lipid levels of palmitic and oleic acids, it increased the level of linoleic acid in lamb serum (Figure 6). Besides these three major fatty acids of serum lipids, the diet and testing time interaction was significant ($P<0.05$) for minor fatty acids such as C16:1*cis* and C18:2*trans*. In general, the level of C16:1*cis* fatty acid of serum lipids in the lambs fed the sunflower oil plus gel diet was lower than that in the lambs fed the control or vitamin E diets (Table 13).

D. Breed and Sex Effects

Breed or sex differences did not have any main affect ($P>0.05$) on the relative percentages of fatty acids in serum lipids when averaged across testing times and treatments. However, interaction of breed (Appendix B-4) or gender (Appendix B-5) with testing times was significant ($P<0.05$) for the levels of several fatty acids in blood serum lipids of lambs. The interaction of breed (black and white) with testing time was significant for the levels of palmitoleic acids (both C16:1*trans* and C16:1*cis*), *cis*-10-heptadecenoic acid (C17:1*cis*), stearic acid (C18:0) (Figure 7), arachidic acid (C20:0) and *cis*-11-eicosenoic acid (C20:1) in plasma lipids of the lambs. The interaction of breed with testing time did not follow a definite pattern, for any of the fatty acids, which made the interpretation more difficult to present. However, for the most predominant of these fatty acids, stearic, the interaction of breed with testing time can be seen in Figure 7. On the testing date of August 11, lambs with a brown-black coat had higher levels of stearic acid (C18:0) than those with a white coat in their blood serum lipids. However, on July

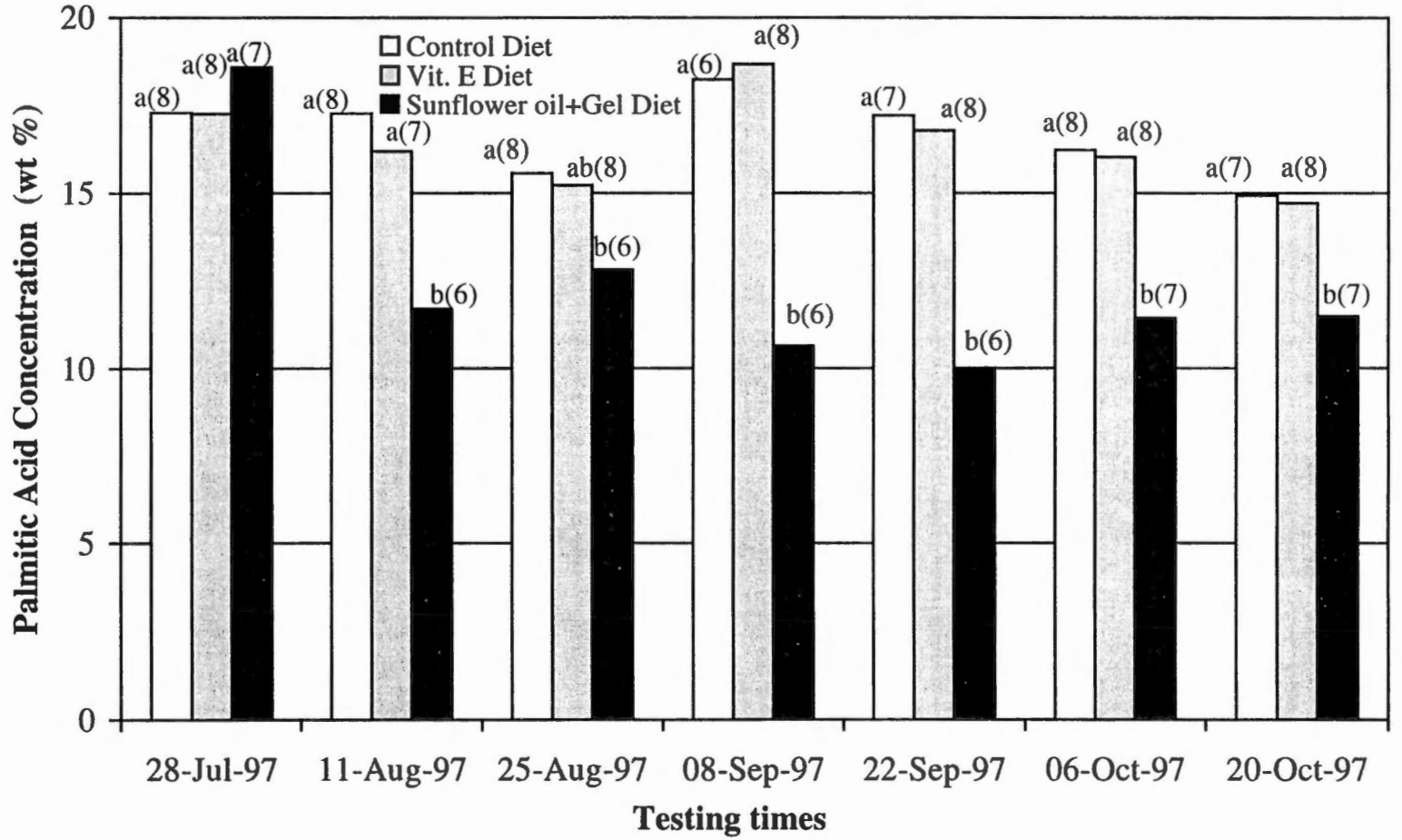


Figure 4. Relative concentration of palmitic acid (C16:0) in blood serum lipids of lambs affected by diets over testing times. Unlike letters within a testing time differ significantly ($P<0.05$). Numbers in parenthesis following the letters represent the number of the observations per testing time (n).

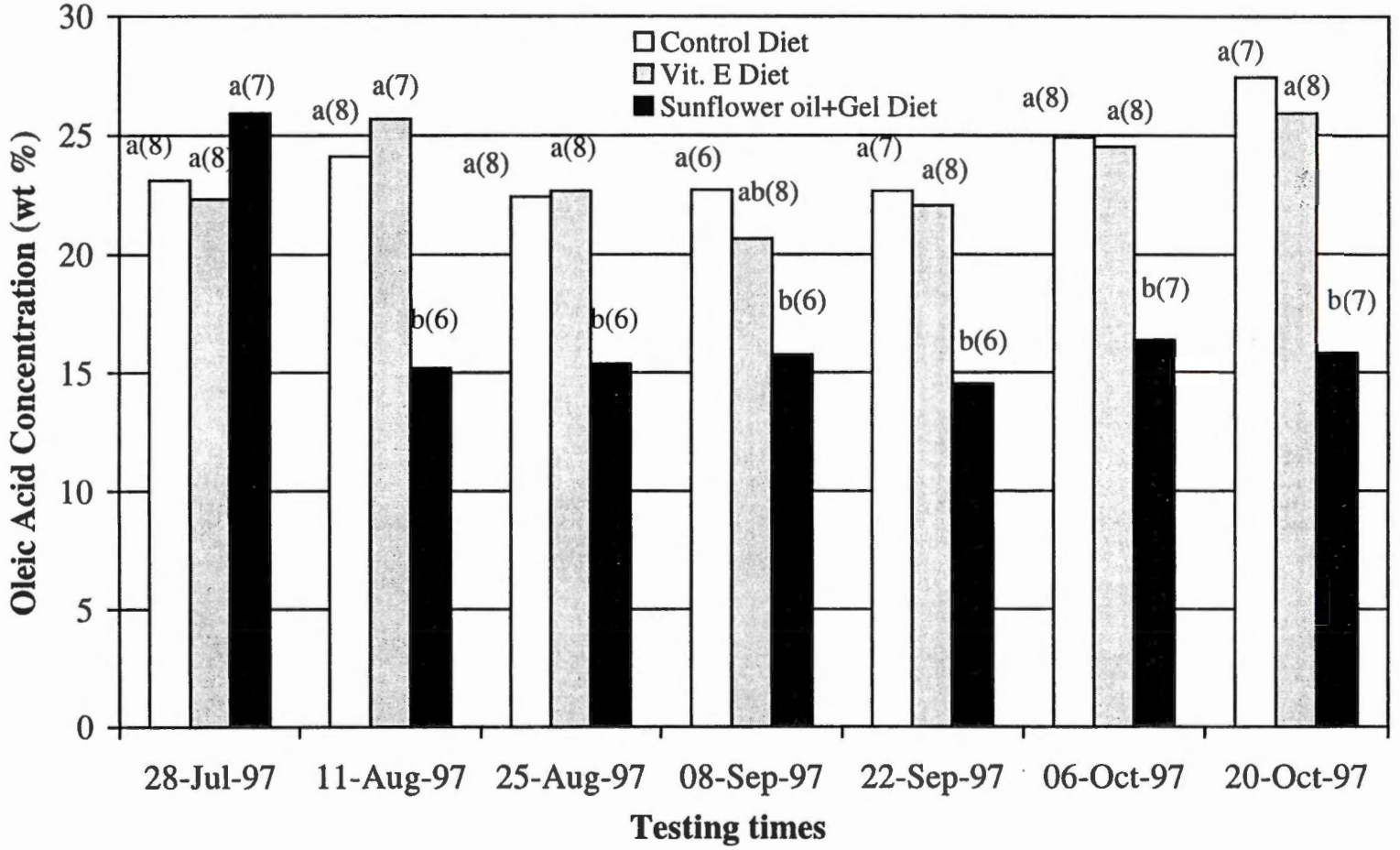


Figure 5. Relative concentration of oleic acid (C18:1) in blood serum lipids of lambs affected by diets over testing times. Unlike letters within a testing time differ significantly ($P<0.05$). Numbers in parenthesis following the letters represent the number of the observations per testing time (n).

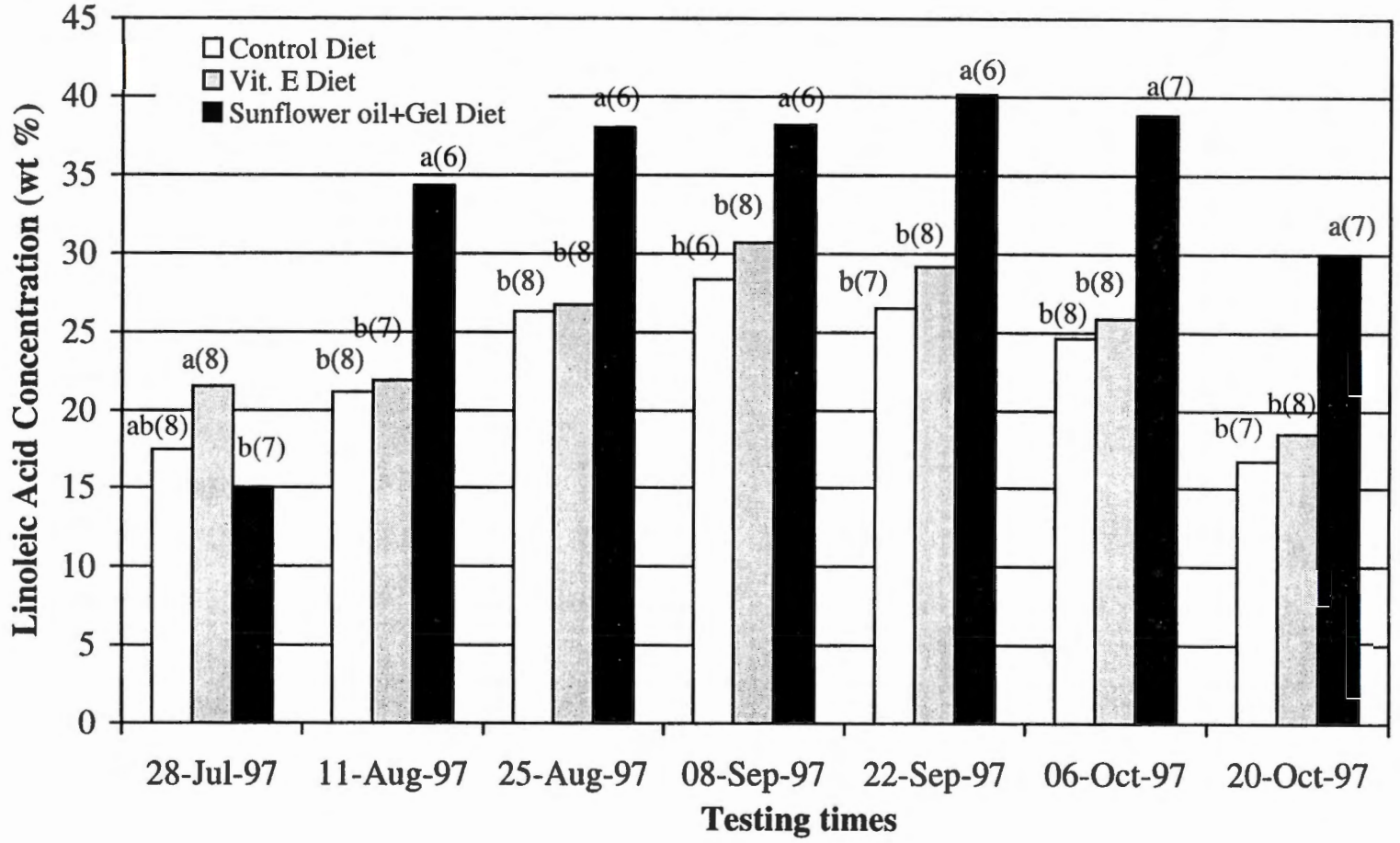


Figure 6. Relative concentration of linoleic acid (C18:2) in blood serum lipids of lambs affected by diets over testing times. Unlike letters within a testing time differ significantly ($P<0.05$). Numbers in parenthesis following the letters represent the number of the observations per testing time (n).

Table 13. Interaction of treatment diets with testing time averaged across breed and gender for palmitoleic (C16:1*cis*) and linoelaidic (C18:2*trans*) acids in blood serum lipids of lambs.

Fatty Acid	Treatment	Testing Times						
		28-Jul-97	11-Aug-97	25-Aug-97	08-Sep-97	22-Sep-97	06-Oct-97	20-Oct-97
		Concentration (wt % of fatty acids)*						
C16:1*cis*	Control	1.063^{a}	1.083^{a}	0.880^{ac}	0.228^{b}	0.544^{bc}	0.957^{ac}	0.862^{a}
	Vit. E	1.110^{ac}	1.354^{a}	1.333^{a}	0.628^{b}	0.714^{bc}	1.027^{abc}	0.890^{bc}
	Sunflower oil plus gel	1.360^{a}	0.562^{bc}	0.532^{bc}	0.492^{bc}	0.145^{b}	0.354^{bc}	0.622^{c}
C18:2*trans*	Control	0.117^{ab}	0.366^{a}	0.349^{a}	0.064^{b}	0.105^{ab}	0.301^{a}	0.416^{ab}
	Vit. E	0.173^{ab}	0.354^{a}	0.428^{a}	0.324^{a}	0.043^{b}	0.039^{bc}	0.007^{c}
	Sunflower oil plus gel	0.124^{ab}	0.257^{a}	0.189^{ab}	0.571^{a}	0.025^{b}	0.056^{b}	0.568^{a}

* For any single fatty acid, LSMEANS in the same row with all unlike superscript letters differ ($P<0.05$)

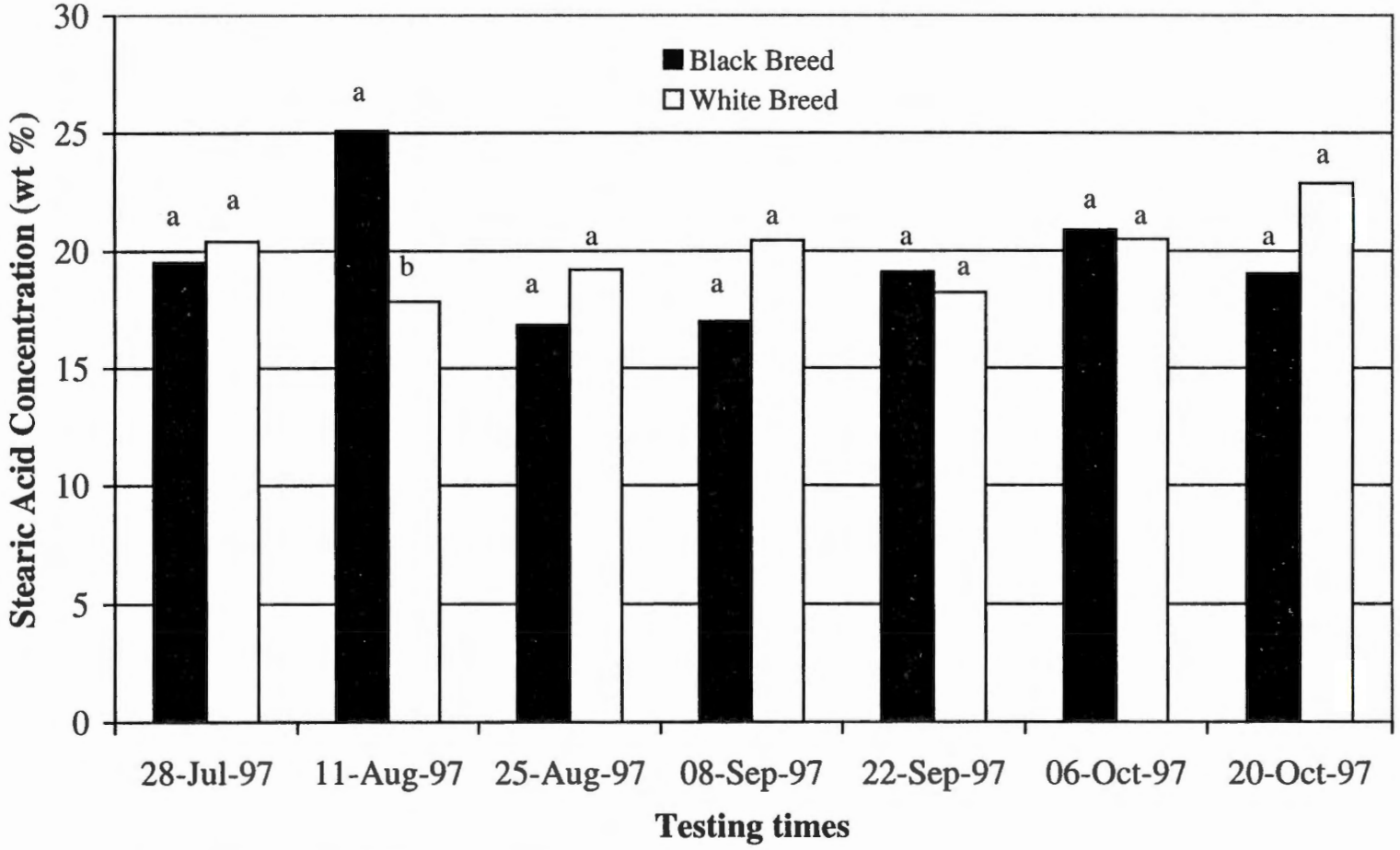


Figure 7. Changes in relative concentration of stearic acid (C18:0) in blood serum lipids of lambs averaged across diets and sex over testing times for black-brown and white breeds. Unlike letters within a testing time differ significantly ($P<0.05$).

28, August 25, September 8, September 22, October 6 and October 20, there were no differences in levels of this fatty acid of blood serum lipids between the two breeds ($P<0.05$). Generally, the interaction of breed with testing time as found in this study had little practical significance in feeding a protective supplement to change fatty acid composition of ruminant products as measured by percentage fatty acid composition.

E. Correlation between Fatty Acid Percentages of Serum Lipids and Serum Vitamin E Content in Lambs

The correlation coefficients between blood serum vitamin E content and fatty acid percentages of serum lipids in lambs averaged across testing times, sex and breed are given in Table 14. There was a positive correlation (0.63) between the vitamin E content and linoleic acid proportion of serum lipids in lambs fed with the sunflower oil-gel protected supplement ($P<0.05$). As linoleic acid levels of lamb serum increased, vitamin E levels of serum also increased. On the other hand, the proportions of major fatty acids such as palmitic acid and oleic acid, were negatively correlated to vitamin E content of serum ($P<0.05$) in lambs fed with the sunflower oil-gel diet. The significant negative correlations between lamb blood serum α-tocopherol content and the levels of palmitic acid (C16:0) and oleic acid (C18:1) in blood serum lipids of lamb fed the sunflower oil-gel supplement also are to be expected. Feeding the sunflower oil supplement, which also contained supplemental α-tocopheryl acetate, decreased the levels of palmitic and oleic acids in (Figures 4 and 5) and increased ($P<0.05$) levels of the α-tocopherol in the blood serum of these lambs (Melton, 1998).

Table 14. Correlation coefficients between the fatty acid proportions (wt %) of fatty acids and vitamin E content in serum of lambs fed with vitamin E diet or sunflower oil-gel diet (Vitamin E contents of serum were obtained from Melton (1998)).

Fatty acid	Correlation Coefficients [a]		
	Vitamin E Diet [b]		Sunflower Oil+Gel Diet [c]
C14:0	-0.42*	(23)	-0.22
C14:1	-0.27	(24)	-0.36
C16:0	0.14	(23)	-0.74*
C16:1*trans*	-0.33	(24)	-0.29
C16:1*cis*	-0.27	(23)	-0.57*
C17:0	0.25	(24)	-0.37
C17:1	0.32	(23)	-0.65*
C18:0	0.13	(24)	0.14
C18:1	-0.02	(23)	-0.71*
C18:2	0.40	(24)	0.63*
C18:2*trans*	0.10	(23)	0.05
C18:3n-3	0.14	(24)	-0.07
C18:3n-6	0.12	(23)	-0.03
C20:0	-0.26	(24)	-0.36
C20:1	-0.36	(23)	-0.75*
C20:2n-6	-0.28	(24)	-0.53*
C20:3n-6	-0.19	(23)	-0.13
C20:4n-6	-0.50*	(24)	0.06
C20:5n-3	-0.56*	(23)	-0.72*
C22:5n-3	-0.75*	(24)	-0.36
C22:6n-3	-0.37	(23)	-0.76*
C24:0	-0.33	(24)	-0.02
C24:1	-0.16	(23)	0.41

* Significantly correlated ($P<0.05$); [a] Interaction of vitamin E content with testing time was insignificant ($P>0.05$), correlation represents coefficients averaged across testing times; [b] Number of observations used in the analyses; [c] Number of observations is 19.

There was no significant correlation between vitamin E content and major fatty acid proportions of lipids in plasma such as palmitic, oleic, and linoleic acids for the vitamin E supplemented diet. Feeding α-tocopheryl acetate also increased ($P<0.05$) the level of α-tocopherol in lamb blood serum over that of lambs just fed the control diet (Melton, 1998) while not greatly affecting the concentrations of the major fatty acids in the lamb blood serum lipids (Figures 3, 4 and 5).

Combs (1991) reported that mechanical actions such as chewing and gastric churning cause emulsification of dietary fats or oils and vitamin E. Upon entering the duodenum, vitamin E becomes dissolved in the emulsion droplets. Large droplets are broken down in the small intestine, and this process depends on the release of lipase enzymes and bile from the gall bladder (Combs, 1991). The products of the hydrolysis of these large droplets form small particles called "mixed micelles". The hydrophobic part of these "mixed micelles" is a good place for non-polar lipids such as cholesterol and fat soluble vitamins like vitamin E. Mixed micelles serve as a carrier for vitamin E. Table 10 shows that the major fatty acid of the gel used to prepare the sunflower oil-gel diet is linoleic acid. Therefore, linoleic acid is a major carrier for vitamin E in the gel diet.

F. Relationship between Fatty Acids of Serum Lipids and Fatty Acids of Longissimus and Psoas Major Muscles in Lambs

Correlation coefficients between the fatty acid proportions of serum lipids on October 20 1997 and Longissimus and Psoas major muscles are shown in Table 15. The correlation between the fatty acids of blood serum lipids and the fatty acids of these

Table 15. Correlation coefficients between the fatty acids percentages (wt %) of serum lipids on October 20, 1997 and fatty acid percentages (wt %) of Longissimus muscle or Psoas major muscle in lambs fed with vitamin E supplemented diet or sunflower oil-gel supplemented diet (Fatty acid percentages of L. muscle lipids and Psoas major lipids were obtained from Melton (1998)).

	Correlation Coefficients			
	Longissimus Muscle		Psoas major Muscle[c]	
Fatty Acids	Vit. E Diet[a]	Sunflower Oil-Gel Diet[b]	Vit. E Diet	Sunflower Oil-Gel Diet
C16:0	0.33	0.59	0.53 (5)	0.65 (4)
C16:1*trans*	-0.29	-0.21	0.42 (4)	0.33 (4)
C16:1*cis*	-0.20	-0.21	0.04 (4)	-0.98 (3)
C17:0	-0.04	-0.41	0.98 (3)	-0.43 (3)
C17:1	0.19	0.54	0.61 (5)	-0.96* (4)
C18:0	0.26	0.23	-0.16 (3)	-0.39 (3)
C18:1	-0.02	0.88*	-0.37 (5)	0.77 (4)
C18:2	-0.00	0.83*	-0.20 (3)	0.94 (3)
C18:3n-3	0.49	-0.43	-0.49 (3)	0.57 (3)
C20:1	0.45	-0.08	-0.60 (4)	-1.00* (3)
C20:3n-6	0.29	-0.46	0.95* (5)	-
C20:4n-6	-0.28	0.60	0.93 (3)	-0.71 (3)
C20:5n-3	-	-0.27	-0.33 (5)	0.93 (4)
C22:5n-3	-0.60	-	0.91 (3)	-0.99* (3)
C24:0	-	0.36	0.37 (4)	-
C24:1	-	0.40	-	-

* Significantly correlated ($P<0.05$); [a] Number of observations is 8; [b] Number of observations is 7; [c] Numbers in parenthesis show the number of observations

two muscles was not significant (P>0.05) for the lambs fed with the vitamin E supplemented diet with the exception of C20:3n-6. The C20:3n-6 fatty acid in serum lipids was positively (0.95) correlated to that in P. major (P<0.05). Although we could not detect any significant correlation between the predominant fatty acid (C16:0, C18:0, C18:1 and C18:2) levels of serum lipids and the levels of the like fatty acids in the Longissimus muscle of the lambs fed with the vitamin E supplemented diet, oleic and linoleic acid proportions of serum lipids were positively correlated (P<0.05) to those respective fatty acids of Longissimus muscle in lambs fed with sunflower oil-gel supplemented diet. Correlation coefficients for oleic and linoleic acids were 0.88 (n=7) and 0.83 (n=7), respectively. For the same diet group, we found that C17:1, C20:1 and C22:5n-3 fatty acids of serum lipids were significantly correlated with the levels of the respective fatty acids in the Psoas major muscle (P<0.05) (Table 15). Dryden and Merchello (1973) reported that diets with sunflower oil increased the level of unsaturated fatty acids in Longissimus muscle tissues of steers, whereas animal fat supplementation of diet increased the saturation of intermuscular fatty acids. They also reported that changes in dietary lipids in steer diets were related to changes in the free fatty acid and glyceride proportions of the serum lipids. In our study we found a positive correlation between linoleic acid levels in serum lipids and Longissimus muscle for the lambs on sunflower oil-gel diet, meaning that increase in serum level of this fatty acid also increased the level of linoleic acid in the Longissimus muscle. Although the sunflower oil-gel diet decreased the level of oleic acid in blood serum lipids of lambs, there was a positive correlation between the oleic acid level in plasma lipid and the oleic acid level in Longissimus muscle lipids.

The Longissimus muscle is a white muscle, which has a more limited blood supply than the red muscle, Psoas major (Cassens, 1978). The Longissimus muscle, therefore, relies more on glycogen for energy than does the Psoas major muscle and tends to store more blood serum fatty acids than the red muscle, which uses the blood serum fatty acids for energy. Therefore, higher correlation coefficients between the levels of the major fatty acids in the blood serum lipids and levels of like muscle fatty acids would be more likely in the Longissimus muscle than the Psoas major muscle of lambs fed the sunflower oil-gel supplement. More research is needed to determine relationships between blood serum fatty acids and like fatty acids in lipids of different muscles of ruminants, particularly in studies where large differences in levels of major fatty acids among the animals exist.

CHAPTER V

SUMMARY

The primary objective of this study was to investigate the effect of different diets on the fatty acid composition of blood serum lipids in lambs. These diets included (1) a corn-based ration with no supplemental α-tocopheryl acetate or sunflower oil (control); (2) the same ration with supplemental α-tocopheryl acetate (250 IU/lamb daily) (Vitamin E diet) and (3) a diet with a protective gel supplement containing sunflower oil and α-tocopheryl acetate at the same level as the vitamin E diet (sunflower oil-gel diet). For this purpose, relative percentages of the fatty acids in blood serum lipids were monitored every two weeks in lambs fed the different diets, producing seven testing times. The response of serum lipids to the diets studied in this research was rapid, and gel protection of dietary lipids from biohydrogenation in rumen significantly changed the relative concentrations of palmitic acid (C16:0), oleic acid (C18:1) and linoleic acid (C18:2) in lamb serum lipids over testing times. In general, significant differences were found between the fatty acid concentrations of serum lipids in lambs fed the control or vitamin E supplemented diets and the serum lipid fatty acid concentrations in lambs fed the sunflower oil-gel supplemented diet after the first testing time. The sunflower oil-gel diet decreased the level of palmitic acid from 16.7% to 12.4% and oleic acid from 23.9% to 17.0% in serum lipids, while increasing the level of linoleic acid from 23.0% to 33.5% when averaged across the testing times. Feeding a protective gel supplement containing sunflower oil produced with a generally recognized as safe (Anonymous, 1998) chemical agent, 2,3-butanedione, increases the polyunsaturated fatty acids in lamb blood serum to

the same extent feeding a protective supplement treated with the carcinogen formaldehyde, to ewes increased the C18:2 content in ewe plasma (Palmquist et al., 1977). This result indicates it is possible to use such a supplement to increase polyunsaturation in the fat of ruminant animal products in the U.S. significantly with a "safe" diet.

A secondary objective of this study was to determine the relationship between the fatty acids of serum lipids and the vitamin E content of lamb serum. Correlation analyses between the fatty acid percentages of serum lipids and vitamin E contents of serum samples showed that the levels of C16:0, C16:1*cis*, C17:1, C18:1, C20:1, C20:2n-6, C20:5n-3 and C22:6n-3 fatty acids in serum lipids are negatively correlated to the blood serum vitamin E content in lambs fed the sunflower oil-gel supplemented diet. However, there was a positive correlation between the vitamin E content and linoleic acid (C18:2) level in serum lipids of the same lambs. The sunflower oil, which contained 71% linoleic acid in the gel served as a carrier for part of the α-tocopherol to the blood in lambs. The positive relationship ($P<0.05$) of vitamin E with C18:2 content in blood serum indicates that when a protective gel supplement containing additional vitamin E and sunflower oil was fed to lambs, higher levels of vitamin E was associated with increased levels of C18:2. These results indicate that feeding such a combination to ruminant animals not only increases polyunsaturated fatty acids in blood serum significantly but also provides protection of those fatty acids from oxidation.

Positive correlations were found also between the levels of oleic acid of blood serum lipids and Longissimus muscle lipids, and between the levels of linoleic acid of serum and Longissimus muscle lipids in lambs fed with gel protected sunflower oil diet.

However, these correlations were not found for the lambs on the vitamin E diet. The levels of C17:1, C20:1 and C22:5n-3 fatty acids in Psoas major muscle lipids were negatively correlated with the levels of those fatty acids in serum lipids for the lambs on sunflower oil-gel diet. On the other hand, in lambs on the vitamin E diet, the level of C20:3n-6 in Psoas major lipids was positively correlated to that in serum lipids. Except for linoleic acid levels in the Longissimus muscle, it is not clear that the fatty acid proportions of these two muscle lipids are linearly related to the levels of fatty acids in blood serum lipids. However, if levels of blood serum fatty acids were related to levels of like fatty acids in all muscle lipids, particularly for the polyunsaturated fatty acids, the information could be used to predict end-point levels of those fatty acids in muscle from ruminants fed the protective gel. Further research is needed to explain the relationship between the fatty acids of serum lipids and muscle lipids of ruminants.

The implications of this research are that a protective protein gel made with a safe chemical agent and containing polyunsaturated oil and vitamin E supplement, can change the fatty acid composition of blood serum lipids when fed to lambs and provide additional antioxidant activity to increased levels of polyunsaturated fatty acids in the serum. Also, significant relationships between major fatty acid levels in the blood serum and those acids in muscle lipids in lambs fed the protective supplement indicate blood serum fatty acid levels could predict muscle fatty acid levels. However, research in which the effect of feeding the protective supplement to cattle has on fatty acid composition of meat and milk is needed. The cost of the protective gel, which currently is approximately $1.00/kg, also must be reduced for the protective gel to be widely used in the U.S. and to

have a significant chance at increasing levels of polyunsaturated in the diet of American consumers.

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APPENDICES

APPENDIX A

APPENDIX A. Analyses of variances for the individual fatty acid levels in blood serum lipids from lambs fed supplemental α-tocopheryl acetate with and without additional sunflower oil.

A-1. Analysis of variance for myristic (C14:0) acid in serum lipids of lambs.

Tests of Fixed Effects

Source	NDF	DDF	Type III F	Pr > F
TRT	2	3	0.00	0.9971
SEX	1	5	0.06	0.8116
BREED	1	5	2.36	0.1852
TIME	6	81	4.19	0.0010
TRT*SEX	2	5	0.44	0.6696
TRT*BREED	2	5	0.39	0.6943
TRT*TIME	12	81	0.18	0.9989
SEX*TIME	6	81	0.36	0.8994
BREED*TIME	6	81	1.80	0.1084
TRT*SEX*TIME	12	81	0.81	0.6350
TRT*BREED*TIME	12	81	0.76	0.6870

A-2. Analysis of variance for myristoleic (C14:1) acid in serum lipids of lambs.

Tests of Fixed Effects

Source	NDF	DDF	Type III F	Pr > F
TRT	2	3	0.58	0.6140
SEX	1	5	0.40	0.5568
BREED	1	5	0.25	0.6351
TIME	6	81	18.05	0.0001
TRT*SEX	2	5	1.80	0.2578
TRT*BREED	2	5	0.56	0.6025
TRT*TIME	12	81	1.09	0.3813
SEX*TIME	6	81	1.43	0.2117
BREED*TIME	6	81	2.03	0.0711
TRT*SEX*TIME	12	81	1.03	0.4279
TRT*BREED*TIME	12	81	1.52	0.1347

A-3. Analysis of variance for palmitic (C16:0) acid in serum lipids of lambs.

Tests of Fixed Effects

Source	NDF	DDF	Type III F	Pr > F
TRT	2	3	29.69	0.0105
SEX	1	5	0.69	0.4439
BREED	1	5	0.31	0.6029
TIME	6	81	7.92	0.0001
TRT*SEX	2	5	0.82	0.4911
TRT*BREED	2	5	1.92	0.2410
TRT*TIME	12	81	4.66	0.0001
SEX*TIME	6	81	1.25	0.2889
BREED*TIME	6	81	0.37	0.8932
TRT*SEX*TIME	12	81	0.91	0.5436
TRT*BREED*TIME	12	81	1.46	0.1558

A-4. Analysis of variance for palmitoleic (C16:1trans) acid in serum lipids of lambs.

Tests of Fixed Effects

Source	NDF	DDF	Type III F	Pr > F
TRT	2	3	6.07	0.0882
SEX	1	5	0.09	0.7799
BREED	1	5	2.20	0.1984
TIME	6	81	2.19	0.0518
TRT*SEX	2	5	0.35	0.7198
TRT*BREED	2	5	3.49	0.1127
TRT*TIME	12	81	1.83	0.0569
SEX*TIME	6	81	0.79	0.5780
BREED*TIME	6	81	2.72	0.0187
TRT*SEX*TIME	12	81	0.55	0.8773
TRT*BREED*TIME	12	81	1.41	0.1776

A-5. Analysis of variance for palmitoleic (C16:1*cis*) acid in serum lipids of lambs.

Tests of Fixed Effects

Source	NDF	DDF	Type III F	Pr > F
TRT	2	3	3.69	0.1553
SEX	1	5	0.39	0.5602
BREED	1	5	0.51	0.5069
TIME	6	81	10.02	0.0001
TRT*SEX	2	5	0.16	0.8526
TRT*BREED	2	5	0.16	0.8588
TRT*TIME	12	81	2.25	0.0163
SEX*TIME	6	81	0.72	0.6379
BREED*TIME	6	81	2.25	0.0468
TRT*SEX*TIME	12	81	1.14	0.3382
TRT*BREED*TIME	12	81	0.74	0.7124

A-6. Analysis of variance for heptadecanoic (C17:0) acid in serum lipids of lambs.

Tests of Fixed Effects

Source	NDF	DDF	Type III F	Pr > F
TRT	2	3	15.14	0.0271
SEX	1	5	2.74	0.1588
BREED	1	5	0.13	0.7312
TIME	6	81	1.85	0.0993
TRT*SEX	2	5	0.89	0.4687
TRT*BREED	2	5	0.29	0.7617
TRT*TIME	12	81	1.10	0.3741
SEX*TIME	6	81	0.06	0.9990
BREED*TIME	6	81	0.68	0.6692
TRT*SEX*TIME	12	81	0.39	0.9619
TRT*BREED*TIME	12	81	0.44	0.9438

A-7. Analysis of variance for *cis*-10-heptadecenoic (C17:1) acid in serum lipids of lambs.

Tests of Fixed Effects

Source	NDF	DDF	Type III F	Pr > F
TRT	2	3	17.81	0.0216
SEX	1	5	0.00	0.9677
BREED	1	5	0.25	0.6371
TIME	6	81	2.12	0.0593
TRT*SEX	2	5	0.05	0.9525
TRT*BREED	2	5	2.89	0.1463
TRT*TIME	12	81	1.15	0.3323
SEX*TIME	6	81	0.45	0.8414
BREED*TIME	6	81	2.76	0.0170
TRT*SEX*TIME	12	81	0.98	0.4759
TRT*BREED*TIME	12	81	1.19	0.3035

A-8. Analysis of variance for stearic (C18:0) acid in serum lipids of lambs.

Tests of Fixed Effects

Source	NDF	DDF	Type III F	Pr > F
TRT	2	3	5.51	0.0989
SEX	1	5	0.00	0.9555
BREED	1	5	0.03	0.8647
TIME	6	81	3.41	0.0047
TRT*SEX	2	5	0.62	0.5766
TRT*BREED	2	5	0.91	0.4589
TRT*TIME	12	81	1.35	0.2066
SEX*TIME	6	81	0.86	0.5244
BREED*TIME	6	81	4.99	0.0002
TRT*SEX*TIME	12	81	3.19	0.0009
TRT*BREED*TIME	12	81	1.56	0.1206

A-9. Analysis of variance for oleic (C18:1) acid in serum lipids of lambs.

Tests of Fixed Effects

Source	NDF	DDF	Type III F	Pr > F
TRT	2	3	17.66	0.0219
SEX	1	5	0.25	0.6395
BREED	1	5	0.09	0.7748
TIME	6	81	2.57	0.0248
TRT*SEX	2	5	0.62	0.5732
TRT*BREED	2	5	1.30	0.3516
TRT*TIME	12	81	2.86	0.0025
SEX*TIME	6	81	2.12	0.0593
BREED*TIME	6	81	0.61	0.7206
TRT*SEX*TIME	12	81	0.91	0.5393
TRT*BREED*TIME	12	81	0.97	0.4796

A-10. Analysis of variance for linoleic (C18:2) acid in serum lipids of lambs.

Tests of Fixed Effects

Source	NDF	DDF	Type III F	Pr > F
TRT	2	3	34.20	0.0086
SEX	1	5	0.14	0.7257
BREED	1	5	0.03	0.8675
TIME	6	81	26.57	0.0001
TRT*SEX	2	5	0.08	0.9255
TRT*BREED	2	5	0.09	0.9133
TRT*TIME	12	81	5.24	0.0001
SEX*TIME	6	81	0.81	0.5631
BREED*TIME	6	81	2.02	0.0723
TRT*SEX*TIME	12	81	1.17	0.3194
TRT*BREED*TIME	12	81	0.53	0.8920

A-11. Analysis of variance for linoelaidic (C18:2*trans*) acid in serum lipids of lambs.

Tests of Fixed Effects

Source	NDF	DDF	Type III F	Pr > F
TRT	2	3	0.76	0.5407
SEX	1	5	1.72	0.2471
BREED	1	5	4.60	0.0848
TIME	6	81	5.22	0.0001
TRT*SEX	2	5	3.26	0.1240
TRT*BREED	2	5	2.18	0.2080
TRT*TIME	12	81	4.01	0.0001
SEX*TIME	6	81	2.36	0.0374
BREED*TIME	6	81	1.46	0.2033
TRT*SEX*TIME	12	81	1.58	0.1131
TRT*BREED*TIME	12	81	2.17	0.0209

A-12. Analysis of variance for linolenic (C18:3n-3) acid in serum lipids of lambs.

Tests of Fixed Effects

Source	NDF	DDF	Type III F	Pr > F
TRT	2	3	4.76	0.1172
SEX	1	5	0.04	0.8491
BREED	1	5	1.51	0.2732
TIME	6	81	7.41	0.0001
TRT*SEX	2	5	0.01	0.9931
TRT*BREED	2	5	0.75	0.5189
TRT*TIME	12	81	1.47	0.1533
SEX*TIME	6	81	0.10	0.9959
BREED*TIME	6	81	0.81	0.5663
TRT*SEX*TIME	12	81	0.83	0.6233
TRT*BREED*TIME	12	81	0.21	0.9976

A-13. Analysis of variance for γ-linolenic (C18:3n-6) acid in serum lipids of lambs.

Tests of Fixed Effects

Source	NDF	DDF	Type III F	Pr > F
TRT	2	3	3.77	0.1518
SEX	1	5	0.52	0.5045
BREED	1	5	0.19	0.6785
TIME	6	81	7.79	0.0001
TRT*SEX	2	5	0.34	0.7284
TRT*BREED	2	5	0.97	0.4404
TRT*TIME	12	81	1.67	0.0901
SEX*TIME	6	81	0.24	0.9614
BREED*TIME	6	81	0.49	0.8143
TRT*SEX*TIME	12	81	0.31	0.9860
TRT*BREED*TIME	12	81	0.45	0.9352

A-14. Analysis of variance for arachidic (C20:0) acid in serum lipids of lambs.

Tests of Fixed Effects

Source	NDF	DDF	Type III F	Pr > F
TRT	2	3	0.06	0.9438
SEX	1	5	0.49	0.5170
BREED	1	5	0.04	0.8508
TIME	6	81	10.33	0.0001
TRT*SEX	2	5	0.05	0.9492
TRT*BREED	2	5	1.47	0.3155
TRT*TIME	12	81	1.35	0.2063
SEX*TIME	6	81	1.08	0.3827
BREED*TIME	6	81	2.52	0.0277
TRT*SEX*TIME	12	81	0.52	0.8981
TRT*BREED*TIME	12	81	0.77	0.6804

A-15. Analysis of variance for *cis*-11-eicosaenoic (C20:1) acid in serum lipids of lambs.

Tests of Fixed Effects

Source	NDF	DDF	Type III F	Pr > F
TRT	2	3	0.42	0.6929
SEX	1	5	1.15	0.3321
BREED	1	5	0.07	0.8015
TIME	6	81	18.38	0.0001
TRT*SEX	2	5	0.70	0.5398
TRT*BREED	2	5	0.55	0.6107
TRT*TIME	12	81	1.28	0.2488
SEX*TIME	6	81	0.73	0.6307
BREED*TIME	6	81	3.22	0.0069
TRT*SEX*TIME	12	81	1.37	0.1991
TRT*BREED*TIME	12	81	1.36	0.2031

A-16. Analysis of variance for *cis*-11,14-eicosadienoic (C20:2n-6) acid in serum lipids of lambs.

Tests of Fixed Effects

Source	NDF	DDF	Type III F	Pr > F
TRT	2	3	2.45	0.2336
SEX	1	5	0.30	0.6078
BREED	1	5	0.04	0.8478
TIME	6	81	61.70	0.0001
TRT*SEX	2	5	0.37	0.7056
TRT*BREED	2	5	0.76	0.5136
TRT*TIME	12	81	9.28	0.0001
SEX*TIME	6	81	0.26	0.9541
BREED*TIME	6	81	1.92	0.0874
TRT*SEX*TIME	12	81	0.81	0.6442
TRT*BREED*TIME	12	81	0.75	0.6956

A-17. Analysis of variance for *cis*-8,11,14-eicosatrienoic (C20:3n-6) acid in serum lipids of lambs.

Tests of Fixed Effects

Source	NDF	DDF	Type III F	Pr > F
TRT	2	3	0.41	0.6971
SEX	1	5	0.18	0.6907
BREED	1	5	0.03	0.8641
TIME	6	81	5.31	0.0001
TRT*SEX	2	5	1.01	0.4279
TRT*BREED	2	5	0.19	0.8355
TRT*TIME	12	81	0.62	0.8228
SEX*TIME	6	81	0.41	0.8715
BREED*TIME	6	81	0.25	0.9560
TRT*SEX*TIME	12	81	0.35	0.9771
TRT*BREED*TIME	12	81	0.33	0.9815

A-18. Analysis of variance for arachidonic (C20:4n-6) acid in serum lipids of lambs.

Tests of Fixed Effects

Source	NDF	DDF	Type III F	Pr > F
TRT	2	3	0.72	0.5543
SEX	1	5	0.34	0.5831
BREED	1	5	0.00	0.9591
TIME	6	81	14.52	0.0001
TRT*SEX	2	5	4.25	0.0835
TRT*BREED	2	5	3.98	0.0926
TRT*TIME	12	81	1.16	0.3260
SEX*TIME	6	81	0.95	0.4635
BREED*TIME	6	81	2.16	0.0555
TRT*SEX*TIME	12	81	1.15	0.3350
TRT*BREED*TIME	12	81	1.45	0.1621

A-19. Analysis of variance for *cis*-5,8,11,14,17-eicosapentaenoic (C20:5n-3) acid in serum lipids of lambs.

Tests of Fixed Effects

Source	NDF	DDF	Type III F	Pr > F
TRT	2	3	7.00	0.0741
SEX	1	5	1.11	0.3399
BREED	1	5	2.16	0.2019
TIME	6	81	29.89	0.0001
TRT*SEX	2	5	0.15	0.8677
TRT*BREED	2	5	2.00	0.2307
TRT*TIME	12	81	1.64	0.0958
SEX*TIME	6	81	0.63	0.7060
BREED*TIME	6	81	0.42	0.8653
TRT*SEX*TIME	12	81	0.63	0.8141
TRT*BREED*TIME	12	81	0.42	0.9503

A-20. Analysis of variance for *cis*-4,7,10,13,16-docosapentaenoic (C22:5n-3) acid in serum lipids of lambs.

Tests of Fixed Effects

Source	NDF	DDF	Type III F	Pr > F
TRT	2	3	0.61	0.6010
SEX	1	5	0.00	0.9726
BREED	1	5	4.08	0.0995
TIME	6	81	4.71	0.0004
TRT*SEX	2	5	0.05	0.9544
TRT*BREED	2	5	0.08	0.9270
TRT*TIME	12	81	0.76	0.6845
SEX*TIME	6	81	0.55	0.7707
BREED*TIME	6	81	1.09	0.3757
TRT*SEX*TIME	12	81	0.44	0.9425
TRT*BREED*TIME	12	81	0.33	0.9804

A-21. Analysis of variance for *cis*-4,7,10,13,16,19-docosahexaenoic (C22:6n-3) acid in serum lipids of lambs.

Tests of Fixed Effects

Source	NDF	DDF	Type III F	Pr > F
TRT	2	3	2.12	0.2663
SEX	1	5	0.35	0.5805
BREED	1	5	4.94	0.0768
TIME	6	81	6.44	0.0001
TRT*SEX	2	5	0.65	0.5616
TRT*BREED	2	5	3.11	0.1325
TRT*TIME	12	81	0.93	0.5237
SEX*TIME	6	81	2.35	0.0386
BREED*TIME	6	81	1.03	0.4112
TRT*SEX*TIME	12	81	0.87	0.5770
TRT*BREED*TIME	12	81	1.24	0.2726

A-22. Analysis of variance for lignoceric (C24:0) acid in serum lipids of lambs.

Tests of Fixed Effects

Source	NDF	DDF	Type III F	Pr > F
TRT	2	3	0.60	0.6057
SEX	1	5	0.03	0.8638
BREED	1	5	1.21	0.3206
TIME	6	81	17.43	0.0001
TRT*SEX	2	5	0.24	0.7933
TRT*BREED	2	5	1.03	0.4217
TRT*TIME	12	81	0.53	0.8879
SEX*TIME	6	81	1.40	0.2254
BREED*TIME	6	81	1.66	0.1420
TRT*SEX*TIME	12	81	0.95	0.5012
TRT*BREED*TIME	12	81	1.97	0.0379

A-23. Analysis of variance for nervonic (C24:1) acid in serum lipids of lambs.

Tests of Fixed Effects

Source	NDF	DDF	Type III F	Pr > F
TRT	2	3	1.58	0.3396
SEX	1	5	0.50	0.5111
BREED	1	5	2.40	0.1821
TIME	6	81	6.92	0.0001
TRT*SEX	2	5	1.41	0.3265
TRT*BREED	2	5	0.20	0.8251
TRT*TIME	12	81	1.30	0.2361
SEX*TIME	6	81	0.35	0.9063
BREED*TIME	6	81	1.67	0.1381
TRT*SEX*TIME	12	81	1.58	0.1148
TRT*BREED*TIME	12	81	1.52	0.1334

APPENDIX B

APPENDIX B. Lsmeans of the fatty acid proportions of blood serum lipids from the lambs fed supplemental α-tocopheryl acetate with and without additional sunflower oil (significant differences among the lsmeans are shown only).

B-1. The effect of testing time* on percentage lsmeans of myristic (C14:0), myristoleic (C14:1), palmitic (C16:0), palmitoleic (C16:1*trans*), stearic (C18:0), oleic (C18:1), linoleic (C18:2), linoelaidic (C18:2*trans*), linolenic (C18:3n-3), γ-linolenic (C18:3n-6), arachidic (C20:0), *cis*-11-eicosaenoic (C20:1), *cis*-11,14-eicosadienoic (C20:2n-6), *cis*-8,11,14-eicosatrienoic (C20:3n-6), arachidonic (C20:4n-6), *cis*-5,8,11,14,17-eicosapentaenoic (C20:5n-3), *cis*-4,7,10,13,16-docosapentaenoic (C22:5n-3), *cis*-4,7,10,13,16,19-docosahexaenoic (C22:6n-3), lignoceric (C24:0) and nervonic (C24:1) acids averaged across treatment diets, breed and sex in blood serum lipids of lambs.

FA[a]	TIME	LSMEAN	Std Error	DF	t	Pr > |t|	LetGrp
140	1	0.24483254	0.04450833	81	5.50	0.0001	A
140	2	0.04248080	0.04524995	81	0.94	0.3506	BC
140	3	0.06537357	0.04524995	81	1.44	0.1524	AB
140	4	0.00000362	0.04949542	81	0.00	0.9999	C
140	5	-0.00045206	0.04600296	81	-0.01	0.9922	C
140	6	0.00000000	0.04450833	81	0.00	1.0000	C
140	7	-0.00021724	0.04527797	81	-0.00	0.9962	C

[a]Myristic acid (C14:0)

FA[b]	TIME	LSMEAN	Std Error	DF	t	Pr > |t|	LetGrp
141	1	0.14151878	0.02541965	81	5.57	0.0001	A
141	2	0.11408425	0.02575362	81	4.43	0.0001	A
141	3	0.11174732	0.02575362	81	4.34	0.0001	A
141	4	0.00277972	0.02776575	81	0.10	0.9205	B
141	5	0.03059707	0.02608476	81	1.17	0.2442	B
141	6	0.00000000	0.02541965	81	0.00	1.0000	B
141	7	0.00082606	0.02575577	81	0.03	0.9745	B

[b]Myristoleic acid (C14:1)

FA[c]	TIME	LSMEAN	Std Error	DF	t	Pr > |t|	LetGrp
160	1	17.69415102	0.48472100	81	36.50	0.0001	A
160	2	15.03909123	0.49250123	81	30.54	0.0001	BC
160	3	14.52980973	0.49250123	81	29.50	0.0001	CD
160	4	15.84393531	0.53701415	81	29.50	0.0001	B
160	5	14.62104831	0.50027901	81	29.23	0.0001	BCD
160	6	14.54105277	0.48472100	81	30.00	0.0001	BCD
160	7	13.69556275	0.49265307	81	27.80	0.0001	D

[c]Palmitic acid (C16:0)

FA[d]	TIME	LSMEAN	Std Error	DF	t	Pr > |t|	LetGrp
1612	1	1.17751672	0.10105111	81	11.65	0.0001	A
1612	2	0.99951111	0.10237873	81	9.76	0.0001	AB
1612	3	0.91510312	0.10237873	81	8.94	0.0001	B
1612	4	0.44915328	0.11058118	81	4.06	0.0001	C
1612	5	0.46763299	0.10369260	81	4.51	0.0001	C
1612	6	0.77933702	0.10105111	81	7.71	0.0001	B
1612	7	0.79166299	0.10238018	81	7.73	0.0001	B

[d]Palmitoleic acid (C16:1*trans*)

FA[e]	TIME	LSMEAN	Std Error	DF	t	Pr > |t|	LetGrp
180	1	19.95364225	0.97536112	81	20.46	0.0001	ABC
180	2	21.46333066	0.98437965	81	21.80	0.0001	A
180	3	18.01499933	0.98437965	81	18.30	0.0001	C
180	4	18.71895063	1.03766020	81	18.04	0.0001	BC
180	5	18.64958651	0.99444879	81	18.75	0.0001	C
180	6	20.69137898	0.97536112	81	21.21	0.0001	AB
180	7	20.94168549	0.98609690	81	21.24	0.0001	AB

[e]Stearic acid (C18:0)

FA[f]	TIME	LSMEAN	Std Error	DF	t	Pr > |t|	LetGrp
181	1	23.78586249	1.00982829	81	23.55	0.0001	A
181	2	21.65451390	1.02621291	81	21.10	0.0001	AB
181	3	20.15289692	1.02621291	81	19.64	0.0001	B
181	4	19.70296528	1.12050449	81	17.58	0.0001	B
181	5	19.75369443	1.04255844	81	18.95	0.0001	B
181	6	21.93034601	1.00982829	81	21.72	0.0001	AB
181	7	23.05967306	1.02649789	81	22.46	0.0001	A

[f]Oleic acid (C18:1)

FA[g]	TIME	LSMEAN	Std Error	DF	t	Pr > |t|	LetGrp
182	1	18.02046987	1.03555678	81	17.40	0.0001	D
182	2	25.81679165	1.05035568	81	24.58	0.0001	B
182	3	30.34862897	1.05035568	81	28.89	0.0001	A
182	4	32.40645273	1.13555511	81	28.54	0.0001	A
182	5	31.94984969	1.06625298	81	29.96	0.0001	A
182	6	29.76817690	1.03555678	81	28.75	0.0001	A
182	7	21.71724749	1.05208991	81	20.64	0.0001	C

[g]Linoleic acid (C18:2)

FA[h]	TIME	LSMEAN	Std Error	DF	t	Pr > |t|	LetGrp
1821	1	0.13830715	0.05484203	81	2.52	0.0136	BC
1821	2	0.32587763	0.05584818	81	5.84	0.0001	A
1821	3	0.32209370	0.05584818	81	5.77	0.0001	A
1821	4	0.31961424	0.06154061	81	5.19	0.0001	AB
1821	5	0.05788762	0.05683661	81	1.02	0.3115	C
1821	6	0.13205436	0.05484203	81	2.41	0.0183	BC
1821	7	0.33003646	0.05584828	81	5.91	0.0001	BC

[h]Linoelaidic acid (C18:2*trans*)

FA[i]	TIME	LSMEAN	Std Error	DF	t	Pr > |t|	LetGrp
1833	1	0.64536056	0.08203857	81	7.87	0.0001	AB
1833	2	0.30309983	0.08292923	81	3.65	0.0005	C
1833	3	0.26274266	0.08292923	81	3.17	0.0022	C
1833	4	0.63185143	0.08813966	81	7.17	0.0001	B
1833	5	0.72494601	0.08394955	81	8.64	0.0001	AB
1833	6	0.84754557	0.08203857	81	10.33	0.0001	A
1833	7	0.77325515	0.08314473	81	9.30	0.0001	AB

[i]Linolenic acid (C18:3n-3)

FA[j]	TIME	LSMEAN	Std Error	DF	t	Pr > |t|	LetGrp
1836	1	0.44518123	0.09670983	81	4.60	0.0001	B
1836	2	0.89530243	0.09817568	81	9.12	0.0001	A
1836	3	1.00969604	0.09817568	81	10.28	0.0001	A
1836	4	0.38596370	0.10658830	81	3.62	0.0005	B
1836	5	0.32407846	0.09971368	81	3.25	0.0017	B
1836	6	0.39829463	0.09670983	81	4.12	0.0001	B
1836	7	0.57571477	0.09829719	81	5.86	0.0001	B

[j]γ-Linolenic acid (C18:3n-6)

FA[k]	TIME	LSMEAN	Std Error	DF	t	Pr > |t|	LetGrp
200	1	0.03374949	0.01086775	81	3.11	0.0026	B
200	2	0.04759005	0.01104207	81	4.31	0.0001	A
200	3	0.02617654	0.01104207	81	2.37	0.0201	B
200	4	-0.00006724	0.01204283	81	-0.01	0.9956	C
200	5	0.01184759	0.01121330	81	1.06	0.2939	C
200	6	0.00160484	0.01086775	81	0.15	0.8830	C
200	7	-0.00047263	0.01104175	81	-0.04	0.9660	C

[k]Arachidic acid (C20:0)

FA[l]	TIME	LSMEAN	Std Error	DF	t	Pr > |t|	LetGrp
201	1	0.60841857	0.04217484	81	14.43	0.0001	A
201	2	0.43341128	0.04289442	81	10.10	0.0001	B
201	3	0.47787458	0.04289442	81	11.14	0.0001	B
201	4	0.10606597	0.04698737	81	2.26	0.0267	C
201	5	0.13369463	0.04362172	81	3.06	0.0030	C
201	6	0.16506436	0.04217484	81	3.91	0.0002	C
201	7	0.36899942	0.04291810	81	8.60	0.0001	B

[l]*cis*-11-Eicosaenoic acid (C20:1)

FA[m]	TIME	LSMEAN	Std Error	DF	t	Pr > |t|	LetGrp
2026	1	0.23048607	0.02199589	81	10.48	0.0001	A
2026	2	0.22550090	0.02228366	81	10.12	0.0001	A
2026	3	0.21337795	0.02228366	81	9.58	0.0001	A
2026	4	0.00113245	0.02394973	81	0.05	0.9624	B
2026	5	-0.00028542	0.02259908	81	-0.01	0.9900	B
2026	6	-0.00000000	0.02199589	81	-0.00	1.0000	B
2026	7	-0.00001530	0.02232735	81	-0.00	0.9995	B

[m]*cis*-11,14-Eicosadienoic acid (C20:2n-6)

FA[n]	TIME	LSMEAN	Std Error	DF	t	Pr > |t|	LetGrp
2036	1	0.48737753	0.09339446	81	5.22	0.0001	A
2036	2	0.58737473	0.09461105	81	6.21	0.0001	A
2036	3	0.59503324	0.09461105	81	6.29	0.0001	A
2036	4	0.24066190	0.10169782	81	2.37	0.0203	C
2036	5	0.18691671	0.09580456	81	1.95	0.0545	BC
2036	6	0.41578094	0.09339446	81	4.45	0.0001	AB
2036	7	0.35896186	0.09460289	81	3.79	0.0003	C

[n]*cis*-8,11,14-Eicosatrienoic acid (C20:3n-6)

FA[o]	TIME	LSMEAN	Std Error	DF	t	Pr > |t|	LetGrp
2046	1	4.56423963	0.25518646	81	17.89	0.0001	B
2046	2	3.69817447	0.25933763	81	14.26	0.0001	CD
2046	3	4.29756759	0.25933763	81	16.57	0.0001	BC
2046	4	3.48856108	0.28416346	81	12.28	0.0001	D
2046	5	4.67754855	0.26349453	81	17.75	0.0001	B
2046	6	3.61850961	0.25518646	81	14.18	0.0001	CD
2046	7	6.35800316	0.25941307	81	24.51	0.0001	A

[o]Arachidonic acid (C20:4n-6)

FA[p]	TIME	LSMEAN	Std Error	DF	t	Pr > |t|	LetGrp
2053	1	1.21331045	0.06407789	81	18.93	0.0001	A
2053	2	0.56766735	0.06498600	81	8.74	0.0001	B
2053	3	0.49548738	0.06498600	81	7.62	0.0001	BC
2053	4	0.35469162	0.07064530	81	5.02	0.0001	C
2053	5	0.42732907	0.06589481	81	6.49	0.0001	BC
2053	6	0.33805597	0.06407789	81	5.28	0.0001	C
2053	7	0.57538867	0.06499899	81	8.85	0.0001	B

[p]*cis*-5,8,11,14,17-Eicosapentaenoic acid (C20:5n-3)

FA[r]	TIME	LSMEAN	Std Error	DF	t	Pr > |t|	LetGrp
2253	1	1.97456518	0.09523990	81	20.73	0.0001	A
2253	2	1.26688305	0.09694085	81	13.07	0.0001	AB
2253	3	1.18838461	0.09694085	81	12.26	0.0001	ABC
2253	4	0.67597461	0.10662141	81	6.34	0.0001	E
2253	5	0.93290222	0.09862056	81	9.46	0.0001	BCD
2253	6	0.69925122	0.09523990	81	7.34	0.0001	DE
2253	7	0.80814578	0.09695102	81	8.34	0.0001	CDE

[r]*cis*-4,7,10,13,16-Docosapentaenoic acid (C22:5n-3)

FA[s]	TIME	LSMEAN	Std Error	DF	t	Pr > |t|	LetGrp
2263	1	5.32891579	0.36346292	81	14.66	0.0001	A
2263	2	3.66603124	0.37011996	81	9.90	0.0001	BC
2263	3	4.07644271	0.37011996	81	11.01	0.0001	B
2263	4	3.82150360	0.40778772	81	9.37	0.0001	BC
2263	5	4.25977489	0.37666444	81	11.31	0.0001	B
2263	6	2.77285303	0.36346292	81	7.63	0.0001	C
2263	7	5.45932622	0.37012587	81	14.75	0.0001	A

[s]*cis*-4,7,10,13,16,19-Docosahexaenoic acid (C22:6n-3)

FA[t]	TIME	LSMEAN	Std Error	DF	t	Pr > |t|	LetGrp
240	1	0.36558723	0.09517494	81	3.84	0.0002	C
240	2	0.17928537	0.09690049	81	1.85	0.0679	C
240	3	0.17240073	0.09690049	81	1.78	0.0790	C
240	4	0.99301506	0.10667440	81	9.31	0.0001	AB
240	5	0.74027810	0.09860176	81	7.51	0.0001	B
240	6	0.42002588	0.09517494	81	4.41	0.0001	C
240	7	1.22037216	0.09690748	81	12.59	0.0001	A

[t]Lignoceric acid (C24:0)

FA[u]	TIME	LSMEAN	Std Error	DF	t	Pr > |t|	LetGrp
241	1	0.34128975	0.05443237	81	6.27	0.0001	BC
241	2	0.38594083	0.05524427	81	6.99	0.0001	BC
241	3	0.45273113	0.05524427	81	8.20	0.0001	BC
241	4	0.48447717	0.06012556	81	8.06	0.0001	B
241	5	0.42947367	0.05604448	81	7.66	0.0001	BC
241	6	0.32986045	0.05443237	81	6.06	0.0001	C
241	7	0.71316140	0.05524365	81	12.91	0.0001	A

[u]Nervonic acid (C24:1)

*Time=1 is July 28, 1997; Time=2 is August 11, 1997: Time=3 is August 25, 1997; Time=4 is September 8, 1997; Time=5 is September 22, 1997; Time=6 is October 6, 1997 and Time=7 is October 20, 1997.

B-2. The effect of treatment diets* on percentage lsmeans of palmitic (C16:0), heptadecanoic (C17:0), cis-10-heptadecenoic (C17:1), oleic (C18:1), linoleic (C18:2), acids averaged across testing times, breed and gender in blood serum lipids of lambs.

FA[a]	TRT	LSMEAN	Std Error	DF	t	Pr > |t|	LetGrp
160	1	16.66049258	0.43764017	3	38.07	0.0001	A
160	2	16.39679306	0.43216869	3	37.94	0.0001	A
160	3	12.35613627	0.44639029	3	27.68	0.0001	B

[a]Palmitic acid (C16:0)

FA[b]	TRT	LSMEAN	Std Error	DF	t	Pr > |t|	LetGrp
170	1	1.19166155	0.08017659	3	14.86	0.0007	A
170	2	1.32952386	0.07852444	3	16.93	0.0004	A
170	3	0.72446417	0.08302795	3	8.73	0.0032	B

[b]Heptadecanoic acid (C17:0)

FA[c]	TRT	LSMEAN	Std Error	DF	t	Pr > |t|	LetGrp
171	1	0.64823595	0.06420055	3	10.10	0.0021	A
171	2	0.77743732	0.06320152	3	12.30	0.0012	A
171	3	0.25317663	0.06552420	3	3.86	0.0307	B

[c]*cis*-10-Heptadecenoic acid (C17:1)

FA[d]	TRT	LSMEAN	Std Error	DF	t	Pr > |t|	LetGrp
181	1	23.91247073	0.90801100	3	26.34	0.0001	A
181	2	23.40557391	0.89643083	3	26.11	0.0001	A
181	3	16.98479197	0.92655482	3	18.33	0.0004	B

[d]Stearic acid (C18:0)

FA[e]	TRT	LSMEAN	Std Error	DF	t	Pr > |t|	LetGrp
182	1	23.03750758	0.94188152	3	24.46	0.0001	B
182	2	24.93484080	0.92968538	3	26.82	0.0001	B
182	3	33.46805903	0.95586739	3	35.01	0.0001	A

[e]Linoleic acid (C18:2)

*Trt=1 is the control diet; Trt=2 is the vitamin E supplemented diet; Trt=3 is the vitamin E plus gel supplemented diet.

B-3. The interaction lsmeans of treatment diets* with testing times** on the percentages of palmitic (C16:0), palmitoleic (C16:1*cis*), oleic (C18:1), linoleic (C18:2), linoelaidic (C18:2*trans*), *cis*-11,14-eicosadienoic (C20:2n-6) acids averaged across breeds and sexes in blood serum lipids of lambs.

FA[a]	TRT	TIME	LSMEAN	Std Error	DF	t	Pr > |t|	LetGrp
160	1	1	17.26955511	0.83956140	81	20.57	0.0001	ABC
160	1	2	17.24856136	0.83956140	81	20.54	0.0001	ABC
160	1	3	15.56086947	0.83956140	81	18.53	0.0001	CDE
160	1	4	18.24496304	0.88009721	81	20.73	0.0001	AB
160	1	5	17.18632023	0.88009721	81	19.53	0.0001	ABCD
160	1	6	16.19709406	0.83956140	81	19.29	0.0001	BCDE
160	1	7	14.91608477	0.88013451	81	16.95	0.0001	DEF
160	2	1	17.23523957	0.83956140	81	20.53	0.0001	ABCD
160	2	2	16.17801878	0.83956140	81	19.27	0.0001	BCDE
160	2	3	15.21767281	0.83956140	81	18.13	0.0001	CDEF
160	2	4	18.67109454	0.83956140	81	22.24	0.0001	A
160	2	5	16.76377843	0.83956140	81	19.97	0.0001	ABCDE
160	2	6	16.01040154	0.83956140	81	19.07	0.0001	BCDE
160	2	7	14.70134575	0.83956140	81	17.51	0.0001	EF
160	3	1	18.57765837	0.83956140	81	22.13	0.0001	A
160	3	2	11.69069355	0.87936936	81	13.29	0.0001	GH
160	3	3	12.81088689	0.87936936	81	14.57	0.0001	FG
160	3	4	10.61574836	1.05642006	81	10.05	0.0001	GH
160	3	5	9.91304627	0.87924820	81	11.27	0.0001	H
160	3	6	11.41566271	0.83956140	81	13.60	0.0001	GH
160	3	7	11.46925773	0.83956140	81	13.66	0.0001	GH

[a]Palmitic acid (C16:0)

FA[b]	TRT	TIME	LSMEAN	Std Error	DF	t	Pr > |t|	LetGrp
1612	1	1	1.06313617	0.17502566	81	6.07	0.0001	ABCDE
1612	1	2	1.08257083	0.17502566	81	6.19	0.0001	ABCD
1612	1	3	0.87987344	0.17502566	81	5.03	0.0001	ABCDEF
1612	1	4	0.22767406	0.18184007	81	1.25	0.2142	GH
1612	1	5	0.54436643	0.18184007	81	2.99	0.0037	FGH
1612	1	6	0.95732990	0.17502566	81	5.47	0.0001	ABCDEF
1612	1	7	0.86201363	0.18184432	81	4.74	0.0001	ABCDEF
1612	2	1	1.11037648	0.17502566	81	6.34	0.0001	ABC
1612	2	2	1.35369023	0.17502566	81	7.73	0.0001	A
1612	2	3	1.33333743	0.17502566	81	7.62	0.0001	A
1612	2	4	0.62819468	0.17502566	81	3.59	0.0006	DEFGH
1612	2	5	0.71393034	0.17502566	81	4.08	0.0001	CDEFG
1612	2	6	1.02682919	0.17502566	81	5.87	0.0001	ABCDEF
1612	2	7	0.89052428	0.17502566	81	5.09	0.0001	BCDEF
1612	3	1	1.35903751	0.17502566	81	7.76	0.0001	AB
1612	3	2	0.56227227	0.18183695	81	3.09	0.0027	EFGH
1612	3	3	0.53209848	0.18183695	81	2.93	0.0045	FGH
1612	3	4	0.49159109	0.21529977	81	2.28	0.0250	FGH
1612	3	5	0.14460221	0.18185052	81	0.80	0.4288	H
1612	3	6	0.35385196	0.17502566	81	2.02	0.0465	GH
1612	3	7	0.62245104	0.17502566	81	3.56	0.0006	CDEFG

[b]Palmitoleic acid (C16:1*cis*)

FA[c]	TRT	TIME	LSMEAN	Std Error	DF	t	Pr > |t|	LetGrp
181	1	1	23.11671790	1.74907391	81	13.22	0.0001	ABC
181	1	2	24.10321331	1.74907391	81	13.78	0.0001	ABC
181	1	3	22.41560314	1.74907391	81	12.82	0.0001	BC
181	1	4	22.72076670	1.83425282	81	12.39	0.0001	ABC
181	1	5	22.67109235	1.83425282	81	12.36	0.0001	ABC
181	1	6	24.91604546	1.74907391	81	14.25	0.0001	ABC
181	1	7	27.44385623	1.83432879	81	14.96	0.0001	A
181	2	1	22.32243497	1.74907391	81	12.76	0.0001	BC
181	2	2	25.68431424	1.74907391	81	14.68	0.0001	AB
181	2	3	22.67289124	1.74907391	81	12.96	0.0001	ABC
181	2	4	20.65112144	1.74907391	81	11.81	0.0001	CD
181	2	5	22.06361813	1.74907391	81	12.61	0.0001	BC
181	2	6	24.51888862	1.74907391	81	14.02	0.0001	ABC
181	2	7	25.92574873	1.74907391	81	14.82	0.0001	AB
181	3	1	25.91843460	1.74907391	81	14.82	0.0001	AB
181	3	2	15.17601415	1.83289317	81	8.28	0.0001	E
181	3	3	15.37019637	1.83289317	81	8.39	0.0001	E
181	3	4	15.73700770	2.20817344	81	7.13	0.0001	DE
181	3	5	14.52637283	1.83265108	81	7.93	0.0001	E
181	3	6	16.35610394	1.74907391	81	9.35	0.0001	DE
181	3	7	15.80941422	1.74907391	81	9.04	0.0001	DE

[c]Oleic acid (C18:1)

FA[d]	TRT	TIME	LSMEAN	Std Error	DF	t	Pr > |t|	LetGrp
182	1	1	17.48641276	1.79363695	81	9.75	0.0001	IJ
182	1	2	21.19289862	1.79363695	81	11.82	0.0001	HI
182	1	3	26.30014448	1.79363695	81	14.66	0.0001	DEFG
182	1	4	28.34639587	1.87700020	81	15.10	0.0001	DEF
182	1	5	26.52301467	1.87700020	81	14.13	0.0001	DEFG
182	1	6	24.63097550	1.79363695	81	13.73	0.0001	FGH
182	1	7	16.78271117	1.87823630	81	8.94	0.0001	IJ
182	2	1	21.56825510	1.79363695	81	12.02	0.0001	GHI
182	2	2	21.92997626	1.79363695	81	12.23	0.0001	GHI
182	2	3	26.73899681	1.79363695	81	14.91	0.0001	DEF
182	2	4	30.70245141	1.79363695	81	17.12	0.0001	CD
182	2	5	29.17239860	1.79363695	81	16.26	0.0001	DEF
182	2	6	25.85851011	1.79363695	81	14.42	0.0001	EFGH
182	2	7	18.57329733	1.79363695	81	10.36	0.0001	IJ
182	3	1	15.00674176	1.79363695	81	8.37	0.0001	J
182	3	2	34.32750007	1.86948028	81	18.36	0.0001	BC
182	3	3	38.00674562	1.86948028	81	20.33	0.0001	AB
182	3	4	38.17051091	2.20569837	81	17.31	0.0001	AB
182	3	5	40.15413579	1.86863466	81	21.49	0.0001	A
182	3	6	38.81504508	1.79363695	81	21.64	0.0001	AB
182	3	7	29.79573397	1.79363695	81	16.61	0.0001	CDE

[d]Linoleic acid (C18:2)

FA[e]	TRT	TIME	LSMEAN	Std Error	DF	t	Pr > |t|	LetGrp
1821	1	1	0.11727804	0.09498918	81	1.23	0.2205	BCDEF
1821	1	2	0.36638678	0.09498918	81	3.86	0.0002	ABD
1821	1	3	0.34893620	0.09498918	81	3.67	0.0004	AB
1821	1	4	0.06429726	0.10012683	81	0.64	0.5226	EFG
1821	1	5	0.10485211	0.10012683	81	1.05	0.2981	ABDEF

1821	1	6	0.30103863	0.09498918	81	3.17	0.0022	ABD
1821	1	7	0.41557198	0.10012683	81	4.15	0.0001	ABDEF
1821	2	1	0.17334125	0.09498918	81	1.82	0.0717	BDEF
1821	2	2	0.35433708	0.09498918	81	3.73	0.0004	AC
1821	2	3	0.42798653	0.09498918	81	4.51	0.0001	A
1821	2	4	0.32373934	0.09498918	81	3.41	0.0010	ABD
1821	2	5	0.04341165	0.09498918	81	0.46	0.6489	EFG
1821	2	6	0.03929731	0.09498918	81	0.41	0.6802	FG
1821	2	7	0.00666355	0.09498918	81	0.07	0.9442	G
1821	3	1	0.12430218	0.09498918	81	1.31	0.1944	ABDE
1821	3	2	0.25690904	0.10012633	81	2.57	0.0121	AB
1821	3	3	0.18935837	0.10012633	81	1.89	0.0622	ABDEF
1821	3	4	0.57080611	0.12262502	81	4.65	0.0001	AB
1821	3	5	0.02539911	0.10012627	81	0.25	0.8004	EFG
1821	3	6	0.05582713	0.09498918	81	0.59	0.5584	DEF
1821	3	7	0.56787386	0.09498918	81	5.98	0.0001	AB

[e]Linoelaidic acid (C18:2*trans*)

FA[f]	TRT	TIME	LSMEAN	Std Error	DF	t	Pr > |t|	LetGrp
2026	1	1	0.15120829	0.03809799	81	3.97	0.0002	C
2026	1	2	0.31879745	0.03809799	81	8.37	0.0001	AB
2026	1	3	0.30359335	0.03809799	81	7.97	0.0001	AB
2026	1	4	-0.00219167	0.03975495	81	-0.06	0.9562	DE
2026	1	5	-0.00078998	0.03975495	81	-0.02	0.9842	DE
2026	1	6	-0.00000000	0.03809799	81	-0.00	1.0000	DE
2026	1	7	-0.00004591	0.03979551	81	-0.00	0.9991	DE
2026	2	1	0.21736095	0.03809799	81	5.71	0.0001	BC
2026	2	2	0.35102739	0.03809799	81	9.21	0.0001	A
2026	2	3	0.30699455	0.03809799	81	8.06	0.0001	ABC
2026	2	4	0.00000000	0.03809799	81	0.00	1.0000	DE
2026	2	5	-0.00000000	0.03809799	81	-0.00	1.0000	DE
2026	2	6	-0.00000000	0.03809799	81	-0.00	1.0000	DE
2026	2	7	-0.00000000	0.03809799	81	-0.00	1.0000	DE
2026	3	1	0.32288898	0.03809799	81	8.48	0.0001	ABC
2026	3	2	0.00667787	0.03957449	81	0.17	0.8664	DE
2026	3	3	0.02954595	0.03957449	81	0.75	0.4575	D
2026	3	4	0.00558902	0.04615617	81	0.12	0.9039	DE
2026	3	5	-0.00006627	0.03955442	81	-0.00	0.9987	E
2026	3	6	0.00000000	0.03809799	81	0.00	1.0000	E
2026	3	7	0.00000000	0.03809799	81	0.00	1.0000	E

[f]*cis*-11,14-Eicosadienoic acid (C20:2n-6)

*Trt=1 is the control diet; Trt=2 is the vitamin E supplemented diet; Trt=3 is the vitamin E plus gel supplemented diet.

**Time=1 is July 28, 1997; Time=2 is August 11, 1997: Time=3 is August 25, 1997; Time=4 is September 8, 1997; Time=5 is September 22, 1997; Time=6 is October 6, 1997 and Time=7 is October 20, 1997.

B-4. The interaction lsmeans of breed with testing times* on the percentages of palmitoleic (C16:1*trans* and C16:1*cis*), *cis*-10-heptadecenoic (C17:1), stearic (C18:0), arachidic (C20:0) and *cis*-11-eicosaenoic (C20:1) acids averaged across treatment diets and sexes in blood serum lipids of lambs.

FA[a]	BREED	TIME	LSMEAN	Std Error	DF	t	Pr > |t|	LetGrp
1611	black	1	0.44079221	0.09982423	81	4.42	0.0001	CD
1611	black	2	0.64413261	0.10926387	81	5.90	0.0001	ABC
1611	black	3	0.33083171	0.10106559	81	3.27	0.0016	DE
1611	black	4	0.40528892	0.10903283	81	3.72	0.0004	CDE
1611	black	5	0.13466273	0.10269464	81	1.31	0.1935	E
1611	black	6	0.29326831	0.09982423	81	2.94	0.0043	DE
1611	black	7	0.89128208	0.10119950	81	8.81	0.0001	A
1611	white	1	0.73460932	0.17201184	81	4.27	0.0001	ABC
1611	white	2	0.37800214	0.17750006	81	2.13	0.0362	BCDE
1611	white	3	0.62103727	0.17289583	81	3.59	0.0006	ABCD
1611	white	4	0.26241705	0.19518692	81	1.34	0.1826	CDE
1611	white	5	0.61899541	0.17353367	81	3.57	0.0006	ABCD
1611	white	6	0.83222667	0.17201184	81	4.84	0.0001	AB
1611	white	7	0.56799238	0.17281358	81	3.29	0.0015	ABCD

[a]Palmitoleic acid (C16:1*trans*)

FAb	BREED	TIME	LSMEAN	Std Error	DF	t	Pr > |t|	LetGrp
1612	black	1	1.10163676	0.11042738	81	9.98	0.0001	A
1612	black	2	1.08682915	0.11925450	81	9.11	0.0001	A
1612	black	3	0.64954632	0.11158860	81	5.82	0.0001	BDE
1612	black	4	0.47136311	0.11918392	81	3.95	0.0002	DE
1612	black	5	0.41131049	0.11290550	81	3.64	0.0005	E
1612	black	6	0.55308393	0.11042738	81	5.01	0.0001	BDE
1612	black	7	0.99025019	0.11164489	81	8.87	0.0001	AC
1612	white	1	1.25339668	0.18761914	81	6.68	0.0001	A
1612	white	2	0.91219306	0.19288296	81	4.73	0.0001	ABD
1612	white	3	1.18065991	0.18837007	81	6.27	0.0001	A
1612	white	4	0.42694344	0.20996654	81	2.03	0.0453	DE
1612	white	5	0.52395550	0.18902157	81	2.77	0.0069	BDE
1612	white	6	1.00559011	0.18761914	81	5.36	0.0001	AB
1612	white	7	0.59307578	0.18833830	81	3.15	0.0023	BCDE

[b]Palmitoleic acid (C16:1*cis*)

FAc	BREED	TIME	LSMEAN	Std Error	DF	t	Pr > |t|	LetGrp
171	black	1	0.58451969	0.08410014	81	6.95	0.0001	ABCD
171	black	2	0.53649065	0.09144831	81	5.87	0.0001	ABCDE
171	black	3	0.37121004	0.08519549	81	4.36	0.0001	CDE
171	black	4	0.55720543	0.09151153	81	6.09	0.0001	ABCDE
171	black	5	0.71786729	0.08633612	81	8.31	0.0001	AB
171	black	6	0.56711153	0.08410014	81	6.74	0.0001	ABCDE
171	black	7	0.74535607	0.08526953	81	8.74	0.0001	AB
171	white	1	0.86767033	0.15001490	81	5.78	0.0001	A
171	white	2	0.48423213	0.15425443	81	3.14	0.0024	BCDE
171	white	3	0.67682268	0.15063170	81	4.49	0.0001	ABC
171	white	4	0.25987835	0.16800024	81	1.55	0.1258	DE
171	white	5	0.21954279	0.15127975	81	1.45	0.1506	E
171	white	6	0.63074925	0.15001490	81	4.20	0.0001	ABCD

171	white	7	0.61597664	0.15067359	81	4.09	0.0001	ABCDE

c*cis*-10-Heptadecenoic acid (C17:1)

FA[d]	BREED	TIME	LSMEAN	Std Error	DF	t	Pr > |t|	LetGrp
180	black	1	19.50177189	1.07132873	81	18.20	0.0001	BCD
180	black	2	25.09718215	1.12721228	81	22.26	0.0001	A
180	black	3	16.83483093	1.07954584	81	15.59	0.0001	EF
180	black	4	16.99217297	1.12834153	81	15.06	0.0001	DF
180	black	5	19.09530871	1.08873519	81	17.54	0.0001	BCE
180	black	6	20.88707570	1.07132873	81	19.50	0.0001	BC
180	black	7	19.01575581	1.08111193	81	17.59	0.0001	BCDE
180	white	1	20.40551261	1.87209411	81	10.90	0.0001	BCDE
180	white	2	17.82947917	1.90462559	81	9.36	0.0001	CDE
180	white	3	19.19516773	1.87680855	81	10.23	0.0001	BCDE
180	white	4	20.44572830	2.01268451	81	10.16	0.0001	ABCDE
180	white	5	18.20386431	1.88210930	81	9.67	0.0001	CDE
180	white	6	20.49568225	1.87209411	81	10.95	0.0001	BCDE
180	white	7	22.86761516	1.87770981	81	12.18	0.0001	AB

[d]Stearic acid (C18:0)

FA[e]	BREED	TIME	LSMEAN	Std Error	DF	t	Pr > |t|	LetGrp
200	black	1	0.00985011	0.01188749	81	0.83	0.4098	C
200	black	2	0.05712772	0.01298258	81	4.40	0.0001	A
200	black	3	0.07890763	0.01202927	81	6.56	0.0001	A
200	black	4	0.00197071	0.01295333	81	0.15	0.8795	C
200	black	5	0.01211642	0.01222159	81	0.99	0.3244	C
200	black	6	0.00026883	0.01188749	81	0.02	0.9820	C
200	black	7	-0.00020380	0.01204677	81	-0.02	0.9865	C
200	white	1	0.05764887	0.02029070	81	2.84	0.0057	A
200	white	2	0.03805238	0.02093062	81	1.82	0.0728	AB
200	white	3	-0.02655455	0.02039511	81	-1.30	0.1966	BC
200	white	4	-0.00210519	0.02299499	81	-0.09	0.9273	C
200	white	5	0.01157875	0.02046749	81	0.57	0.5732	C
200	white	6	0.00294086	0.02029070	81	0.14	0.8851	C
200	white	7	-0.00074147	0.02038443	81	-0.04	0.9711	C

[e]Arachidic acid (C20:0)

FA[f]	BREED	TIME	LSMEAN	Std Error	DF	t	Pr > |t|	LetGrp
201	black	1	0.79774966	0.04661615	81	17.11	0.0001	A
201	black	2	0.36076315	0.05099465	81	7.07	0.0001	BCD
201	black	3	0.45260082	0.04726563	81	9.58	0.0001	B
201	black	4	0.09684506	0.05100968	81	1.90	0.0612	E
201	black	5	0.05782460	0.04793149	81	1.21	0.2312	E
201	black	6	0.14333287	0.04661615	81	3.07	0.0029	E
201	black	7	0.34005715	0.04728966	81	7.19	0.0001	BCD
201	white	1	0.41908749	0.08305906	81	5.05	0.0001	BCD
201	white	2	0.50605940	0.08559040	81	5.91	0.0001	B
201	white	3	0.50314835	0.08342819	81	6.03	0.0001	B
201	white	4	0.11528689	0.09375750	81	1.23	0.2224	E
201	white	5	0.20956466	0.08380167	81	2.50	0.0144	CDE
201	white	6	0.18679584	0.08305906	81	2.25	0.0272	DE
201	white	7	0.39794169	0.08343893	81	4.77	0.0001	BC

[f]*cis*-11-Eicosaenoic acid (C20:1)

*Time=1 is July 28; Time=2 is August 11; Time=3 is August 25; Time=4 is September 8; Time=5 is September 22; Time=6 is October 6 and Time=7 is October 20, 1997.

B-5. The interaction lsmeans of sex with testing times* on the percentages of linoelaidic (C18:2*trans*) and *cis*-4,7,10,13,16,19-docosahexaenoic (C22:6n-3) acids averaged across treatment diets and breeds in blood serum lipids of lambs.

FA[a]	SEX	TIME	LSMEAN	Std Error	DF	t	Pr > |t|	LetGrp
1821	female	1	0.12760593	0.07755834	81	1.65	0.1038	BCD
1821	female	2	0.39876271	0.08175276	81	4.88	0.0001	A
1821	female	3	0.31716231	0.07755834	81	4.09	0.0001	AB
1821	female	4	0.34683769	0.08574223	81	4.05	0.0001	ABC
1821	female	5	0.04316797	0.07755834	81	0.56	0.5793	DE
1821	female	6	0.09700754	0.07755834	81	1.25	0.2146	DE
1821	female	7	0.05198913	0.07755834	81	0.67	0.5046	E
1821	male	1	0.14900838	0.08574398	81	1.74	0.0860	BCD
1821	male	2	0.25299255	0.09200940	81	2.75	0.0074	ABC
1821	male	3	0.32702509	0.08830353	81	3.70	0.0004	AB
1821	male	4	0.29239078	0.09557191	81	3.06	0.0030	ABCD
1821	male	5	0.07260728	0.09079116	81	0.80	0.4262	CDE
1821	male	6	0.16710118	0.08574398	81	1.95	0.0548	BCD
1821	male	7	0.60808380	0.08830378	81	6.89	0.0001	AB

[a]Linoelaidic acid (C18:2*trans*)

FA[b]	SEX	TIME	LSMEAN	Std Error	DF	t	Pr > |t|	LetGrp
2263	female	1	6.31806587	0.51401419	81	12.29	0.0001	A
2263	female	2	3.43667497	0.54176403	81	6.34	0.0001	CDE
2263	female	3	3.86279714	0.51401419	81	7.51	0.0001	CDE
2263	female	4	2.87006319	0.56816012	81	5.05	0.0001	DE
2263	female	5	3.66827124	0.51401419	81	7.14	0.0001	CDE
2263	female	6	3.14687356	0.51401419	81	6.12	0.0001	DE
2263	female	7	5.44651322	0.51401419	81	10.60	0.0001	AB
2263	male	1	4.33976572	0.56826407	81	7.64	0.0001	BCD
2263	male	2	3.89538750	0.60971771	81	6.39	0.0001	BCDE
2263	male	3	4.29008827	0.58519913	81	7.33	0.0001	BCD
2263	male	4	4.77294402	0.63330152	81	7.54	0.0001	ABC
2263	male	5	4.85127855	0.60167039	81	8.06	0.0001	ABC
2263	male	6	2.39883250	0.56826407	81	4.22	0.0001	E
2263	male	7	5.47213922	0.58521407	81	9.35	0.0001	AB

[b]*cis*-4,7,10,13,16,19-Docosahexaenoic acid (C22:6n3)

*Time=1 is July 28; Time=2 is August 11; Time=3 is August 25; Time=4 is September 8; Time=5 is September 22; Time=6 is October 6 and Time=7 is October 20, 1997.

B-6. The interaction lsmeans of treatment diets*, gender and testing times** on the percentages of stearic (C18:0) acid averaged across breeds in blood serum lipids of lambs.

FA	TRT	SEX	TIME	LSMEAN	Std Error	DF	t	Pr > |t|	LetGrp
180	1	female	1	21.95786843	1.88028898	81	11.68	0.0001	BCDEFK
180	1	female	2	23.76199112	1.88028898	81	12.64	0.0001	ABD

180	1	female	3	17.51390088	1.88028898	81	9.31	0.0001	GHJLM
180	1	female	4	23.27093551	2.11711630	81	10.99	0.0001	ABCDEI
180	1	female	5	16.89793451	1.88028898	81	8.99	0.0001	HJLM
180	1	female	6	18.09510850	1.88028898	81	9.62	0.0001	FGHJLM
180	1	female	7	18.80047978	1.88028898	81	10.00	0.0001	CEFGHJLM
180	1	male	1	18.34466926	2.80721663	81	6.53	0.0001	BCDEFGHJLM
180	1	male	2	16.88860783	2.80721663	81	6.02	0.0001	EFGHJLM
180	1	male	3	16.26591083	2.80721663	81	5.79	0.0001	FGHJLM
180	1	male	4	14.38385907	3.09113545	81	4.65	0.0001	JLM
180	1	male	5	19.78123781	2.93401152	81	6.74	0.0001	BCDEFGHJLM
180	1	male	6	19.00956838	2.80721663	81	6.77	0.0001	BCDEFGHJLM
180	1	male	7	19.72237666	2.93914016	81	6.71	0.0001	BCDEFGHJLM
180	2	female	1	17.96255128	2.80721663	81	6.40	0.0001	BCDEFGHJLM
180	2	female	2	16.47854796	2.97105368	81	5.55	0.0001	FGHIJLM
180	2	female	3	19.20872460	2.80721663	81	6.84	0.0001	BCDEFGHJM
180	2	female	4	17.72667944	2.80721663	81	6.31	0.0001	BCDEFGHJLM
180	2	female	5	12.93000449	2.80721663	81	4.61	0.0001	LN
180	2	female	6	17.86588597	2.80721663	81	6.36	0.0001	BCDEFGHJLM
180	2	female	7	19.93752727	2.80721663	81	7.10	0.0001	BCDEFGHJM
180	2	male	1	17.86670702	1.88028898	81	9.50	0.0001	FGHIJLM
180	2	male	2	19.13254655	2.11711630	81	9.04	0.0001	BCDEFGHJL
180	2	male	3	15.74224536	1.88028898	81	8.37	0.0001	JLM
180	2	male	4	13.63079341	1.88028898	81	7.25	0.0001	MN
180	2	male	5	17.71921257	1.88028898	81	9.42	0.0001	FGHIJL
180	2	male	6	19.68813278	1.88028898	81	10.47	0.0001	BCDEFGHJ
180	2	male	7	18.85299817	1.88028898	81	10.03	0.0001	BCDEFGHJL
180	3	female	1	19.94549086	2.30287429	81	8.66	0.0001	BCDEFGHJL
180	3	female	2	28.54872756	2.30287429	81	12.40	0.0001	A
180	3	female	3	16.72216156	2.30287429	81	7.26	0.0001	HJKLM
180	3	female	4	17.09860917	2.49998495	81	6.84	0.0001	FGHIJLM
180	3	female	5	24.62493348	2.30287429	81	10.69	0.0001	ABCD
180	3	female	6	24.05628496	2.30287429	81	10.45	0.0001	ABCDE
180	3	female	7	22.83196658	2.30287429	81	9.91	0.0001	BCDEFG
180	3	male	1	23.64456666	3.10615655	81	7.61	0.0001	ABCDEFGH
180	3	male	2	23.96956297	3.20694119	81	7.47	0.0001	ABCDEFGH
180	3	male	3	22.63705275	3.20694119	81	7.06	0.0001	ABCDEFGHJ
180	3	male	4	26.20282719	3.25498130	81	8.05	0.0001	ABC
180	3	male	5	19.94419622	3.20527949	81	6.22	0.0001	DEFGHJLM
180	3	male	6	25.43329328	3.10615655	81	8.19	0.0001	ABCD
180	3	male	7	25.50476445	3.10615655	81	8.21	0.0001	ABCD

*Trt=1 is the control diet; Trt=2 is the vitamin E supplemented diet; Trt=3 is the vitamin E plus gel supplemented diet.
**Time=1 is July 28; Time=2 is August 11; Time=3 is August 25; Time=4 is September 8; Time=5 is September 22; Time=6 is October 6 and Time=7 is October 20, 1997.

B-7. The interaction lsmeans of treatment diets*, breeds and testing times** on the percentages of linoelaidic (C18:2*trans*) and lignoceric (C24:0) acids averaged across gender in blood serum lipids of lambs.

FA[a]	TRT	BREED	TIME	LSMEAN	Std Error	DF	t	Pr > |t|	LetGrp
1821	1	black	1	0.10799633	0.09498918	81	1.14	0.2589	EFGHJ
1821	1	black	2	0.44241259	0.09498918	81	4.66	0.0001	ABCDI

1821	1	black	3	0.47895798	0.09498918	81	5.04	0.0001	ABC
1821	1	black	4	0.24899910	0.12664649	81	1.97	0.0527	ABCDEFGHJ
1821	1	black	5	0.07541970	0.10012683	81	0.75	0.4535	HJK
1821	1	black	6	0.21297733	0.09498918	81	2.24	0.0277	ABCDEFG
1821	1	black	7	0.40222639	0.10012683	81	4.02	0.0001	FGHJ
1821	1	white	1	0.12655974	0.18189044	81	0.70	0.4885	ABCDEFGHJK
1821	1	white	2	0.29036096	0.18189044	81	1.60	0.1143	ABCDEFGHJ
1821	1	white	3	0.21891443	0.18189044	81	1.20	0.2323	ABCDEFGHJ
1821	1	white	4	-0.12040458	0.20025114	81	-0.60	0.5493	KLM
1821	1	white	5	0.13428452	0.18462548	81	0.73	0.4691	ABCDEFGHJ
1821	1	white	6	0.38909993	0.18189044	81	2.14	0.0354	ABCDEFGHJ
1821	1	white	7	0.42891757	0.18462548	81	2.32	0.0227	ABCDEFGHJ
1821	2	black	1	0.09828274	0.09498918	81	1.03	0.3039	GHJK
1821	2	black	2	0.40963607	0.12262502	81	3.34	0.0013	ABCDE
1821	2	black	3	0.50381879	0.09498918	81	5.30	0.0001	ABC
1821	2	black	4	0.45535735	0.09498918	81	4.79	0.0001	ABCDEF
1821	2	black	5	0.06483391	0.09498918	81	0.68	0.4968	GHJK
1821	2	black	6	0.23045897	0.09498918	81	2.43	0.0175	BCDEFGHJ
1821	2	black	7	0.59423978	0.09498918	81	6.26	0.0001	BCDEFGHJ
1821	2	white	1	0.24839975	0.18189044	81	1.37	0.1758	ABCDEFGHJ
1821	2	white	2	0.29903809	0.19773235	81	1.51	0.1343	ABCDEFGHJ
1821	2	white	3	0.35215426	0.18189044	81	1.94	0.0563	ABCDEFGH
1821	2	white	4	0.19212134	0.18189044	81	1.06	0.2940	ABCDEFGHJ
1821	2	white	5	0.02198940	0.18189044	81	0.12	0.9041	JKL
1821	2	white	6	-0.15186434	0.18189044	81	-0.83	0.4062	LM
1821	2	white	7	-0.58091269	0.18189044	81	-3.19	0.0020	M
1821	3	black	1	0.08824217	0.12263051	81	0.72	0.4739	DEFGHJ
1821	3	black	2	0.34339492	0.12665141	81	2.71	0.0082	ABCDE
1821	3	black	3	0.16134065	0.12665141	81	1.27	0.2063	ABCDEFGHJ
1821	3	black	4	0.12456413	0.12263051	81	1.02	0.3128	FGHIJK
1821	3	black	5	0.03080815	0.12665136	81	0.24	0.8084	GHJK
1821	3	black	6	0.05477034	0.12263051	81	0.45	0.6563	FGHIJK
1821	3	black	7	0.78510351	0.12263051	81	6.40	0.0001	A
1821	3	white	1	0.16036218	0.19773576	81	0.81	0.4197	ABCDEFGHJK
1821	3	white	2	0.17042316	0.20025426	81	0.85	0.3973	ABCDEFGHJ
1821	3	white	3	0.21737610	0.20025426	81	1.09	0.2809	CDEFGHJKL
1821	3	white	4	1.01704809	0.25130705	81	4.05	0.0001	AB
1821	3	white	5	0.01999007	0.20025422	81	0.10	0.9207	FGHIJKL
1821	3	white	6	0.05688391	0.19773576	81	0.29	0.7743	CDEFGHJKL
1821	3	white	7	0.35064422	0.19773576	81	1.77	0.0799	ABCDEFGHJK

[a]Linoelaidic acid (18:2*trans*)

FA[b]	TRT	BREED	TIME	LSMEAN	Std Error	DF	t	Pr > |t|	LetGrp
240	1	black	1	0.53303395	0.16484783	81	3.23	0.0018	EFGHIKL
240	1	black	2	0.13902179	0.16484783	81	0.84	0.4015	KL
240	1	black	3	0.12844390	0.16484783	81	0.78	0.4382	L
240	1	black	4	0.67976253	0.21920120	81	3.10	0.0027	DEFGHIK
240	1	black	5	0.71665231	0.17369465	81	4.13	0.0001	DEFGH
240	1	black	6	0.36660419	0.16484783	81	2.22	0.0289	GHIKL
240	1	black	7	1.44111640	0.17369490	81	8.30	0.0001	ABC
240	1	white	1	0.24374222	0.31514109	81	0.77	0.4415	GHIJKL
240	1	white	2	0.07412990	0.31514109	81	0.24	0.8146	HIKL
240	1	white	3	0.07576044	0.31514109	81	0.24	0.8106	HIKL
240	1	white	4	1.20666363	0.34672746	81	3.48	0.0008	ABCDEF
240	1	white	5	0.53692646	0.31985767	81	1.68	0.0971	DEFGHIKL

240	1	white	6	0.72320214	0.31514109	81	2.29	0.0243	CDEFGHIKL
240	1	white	7	1.19271198	0.31985781	81	3.73	0.0004	ABCDEF
240	2	black	1	0.31700159	0.16484783	81	1.92	0.0580	GHIKL
240	2	black	2	0.15723773	0.21229677	81	0.74	0.4610	IKL
240	2	black	3	0.29596888	0.16484783	81	1.80	0.0763	GHIKL
240	2	black	4	1.88596629	0.16484783	81	11.44	0.0001	A
240	2	black	5	0.99085934	0.16484783	81	6.01	0.0001	CDE
240	2	black	6	0.35355698	0.16484783	81	2.14	0.0350	GHIKL
240	2	black	7	1.51182971	0.16484783	81	9.17	0.0001	AB
240	2	white	1	0.61498018	0.31514109	81	1.95	0.0545	DEFGHIKL
240	2	white	2	0.19174282	0.34235803	81	0.56	0.5770	GHIJKL
240	2	white	3	0.08169900	0.31514109	81	0.26	0.7961	HIKL
240	2	white	4	-0.00191271	0.31514109	81	-0.01	0.9952	KL
240	2	white	5	0.74584200	0.31514109	81	2.37	0.0203	CDEFGHIKL
240	2	white	6	0.74237055	0.31514109	81	2.36	0.0209	CDEFGHIKL
240	2	white	7	1.08786228	0.31514109	81	3.45	0.0009	BCDEFJ
240	3	black	1	0.12454929	0.21269558	81	0.59	0.5598	KL
240	3	black	2	0.15963732	0.21963594	81	0.73	0.4694	HIKL
240	3	black	3	0.23047501	0.21955570	81	1.05	0.2970	GHIKL
240	3	black	4	1.09917757	0.21269558	81	5.17	0.0001	BCD
240	3	black	5	0.43631574	0.21963142	81	1.99	0.0504	FGHIKL
240	3	black	6	0.21139244	0.21269558	81	0.99	0.3232	GHIKL
240	3	black	7	1.62167046	0.21269558	81	7.62	0.0001	AB
240	3	white	1	0.36021615	0.34260547	81	1.05	0.2962	DEFGHIKL
240	3	white	2	0.35394264	0.34690605	81	1.02	0.3106	DEFGHIKL
240	3	white	3	0.22205715	0.34695684	81	0.64	0.5240	GHIJKL
240	3	white	4	1.08843302	0.43457648	81	2.50	0.0143	ABCDEFGHI
240	3	white	5	1.01507275	0.34690432	81	2.93	0.0045	BCDEFG
240	3	white	6	0.12302900	0.34260547	81	0.36	0.7205	GHIKL
240	3	white	7	0.46704215	0.34260547	81	1.36	0.1766	DEFGHIKL

[b]Lignoceric acid (C24:0)

*Trt=1 is the control diet; Trt=2 is the vitamin E supplemented diet; Trt=3 is the vitamin E plus gel supplemented diet.

**Time=1 is July 28; Time=2 is August 11; Time=3 is August 25; Time=4 is September 8; Time=5 is September 22; Time=6 is October 6 and Time=7 is October 20, 1997.

VITA

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