

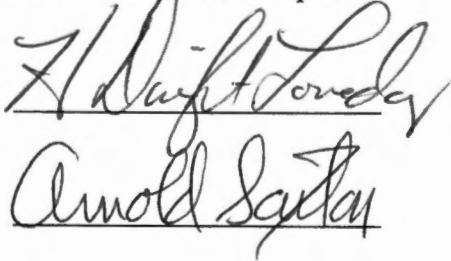
To the Graduate Council:

I am submitting herewith a thesis written by Jung Hoon Lee entitled "Levels of Fatty Acids, Tocopherols, and Flavor Volatiles in Meat from Lambs Fed Treated Ground Full-Fat Soybean Supplements." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science and Technology.



Sharon L. Melton, Major Professor

We have read this thesis and
recommended its acceptance



Accepted for the Council:



Associate Vice Chancellor and
Dean of The Graduate School

**LEVELS OF FATTY ACIDS, TOCOPHEROLS, AND FLAVOR
VOLATILES IN MEAT FROM LAMBS FED TREATED GROUND
FULL-FAT SOYBEAN SUPPLEMENTS**

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Jung Hoon Lee

May 1997

AD-VET-MED.

Thesis

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DEDICATION

This thesis is dedicated to my parents,

Mr. In Seon Lee

and

Mrs. Young Yun Kim

who have been giving me unlimited support and

invaluable educational opportunities,

my lovely wife, Chan Sook Park,

and my lovely daughter, Roo Ra Lee,

for their support, understanding and love throughout this study.

ACKNOWLEDGEMENT

My most sincere appreciation is extended to my major professor, Dr. Sharon L. Melton, for her great assistance, encouragement, knowledge, criticism, and guidance throughout this study. Also, I owe a great debt of thanks to Dr. Melton for completion of the writing of this these. Appreciation is extended also to my committee members, Dr. H. Dwight Loveday and Dr. Arnold M. Saxton, for their helpful suggestion and assistance in preparation of this dissertation.

Gratitude is also extended to staff in the Cherokee Farm of The University of Tennessee Agricultural Experimental Station. Special thanks to Dr. Loveday, Bobby Grindstaff, and meat science teaching assistants for help in preparing the meat samples.

I would like to thank Dr. Y. Dje for his assistance and knowledge in the beginning of this study. Also, special thanks to Kay Trigiano for her assistance in the flavor part of this study.

Special appreciation goes to my parents for their love and unlimited support. Also, special appreciation is extended to my lovely wife, Chan Sook, and my lovely daughter, Roo Ra, for their love, inspiration and support during my graduate study.

ABSTRACT

Supplements containing ground full-fat soybeans mixed with casein and either untreated (control) or treated with acetaldehyde or diacetyl to form gels were prepared. Each of the three supplements (approximately 200 g dry matter or DM), 1 kg of a ground corn basal diet and 0.33-kg hay were fed daily to each of six lambs from weaning to slaughter (90 days). Samples of the longissimus muscle (LD), intermuscular fat, back fat and kidney fat were obtained from each lamb carcass and analyzed for total lipid content. Fatty acid composition of LD nonpolar and polar lipids, intermuscular fat, back fat and kidney fat, and the α - and γ -tocopherol contents of the LD and back fat were determined. Flavor volatiles of a broiled LD from each lamb also were analyzed.

On a DM basis, the control (CO), acetaldehyde (AC), and diacetyl (DA) supplements contained, respectively, 49.7, 50.0 and 49.1% protein and 17.2, 15.5 and 17.4% fat. On a wet basis, the LD of lambs fed CO, AC and DA contained 3.7, 4.6, and 2.6% fat, respectively. Compared to lambs fed CO, lambs fed either AC or DA had ($P < 0.05$) a higher level of linoleic acid (C18:2) and linolenic acid (C18:3) in the LD nonpolar lipids, a higher level of C18:2 but lower level of oleic (C18:1) acid in the LD polar lipids, and a higher level of C18:2 in the back fat and had higher levels of C18:2, C18:3 and stearic (C18:0) acid but a lower concentration of C18:1 in kidney fat. In the LD nonpolar lipids, lambs fed CO had 4.8% C18:2 while those fed AC and DA had 6.4 and 6.8%, respectively. In kidney fat, lambs fed CO, AC and DA had, respectively, 24.9, 27.2 and 26.9% C18:0; 39.1, 35.4 and 36.3% C18:1; 4.5, 7.7 and 7.1% C18:2; and 0.5, 0.9 and

0.8% C18:3. Lambs fed CO had lower levels of α -tocopherol in the LD ($P < 0.10$) and back fat ($P < 0.05$) than did lambs fed AC and DA. Compared with lambs fed AC, lambs fed DA had higher levels of γ -tocopherol in the LD muscle ($P < 0.05$) and back fat ($P < 0.10$). In broiled LD muscle, twenty-one volatiles were identified and included seven alkanals, seven 2-alkenals, two 2,4-alkadienals, and five other compounds, but most differences existing in the volatile concentrations among lambs fed the different supplements did not correspond to like concentration differences in their precursor fatty acids. Results imply that compared with the CO supplement, the AC and DA supplements protected C18:2, C18:3 and vitamin E in the oil of the soybeans from degradation in the rumen of the lambs and increased their deposition in the lamb tissues.

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CHAPTER I

INTRODUCTION

At the present time, heart disease is the major cause of death in the United States and many other economically well-developed countries. By far the most common form of heart disease is coronary heart disease. High blood cholesterol level (>250 mg/dL) is one of the heart disease risk factors (Gallagher and Allred, 1992a; Kuller, 1983; Mattson and Grundy, 1985). The cholesterol that is in the blood serum comes from one of two sources: diet or synthesis. The consumption of saturated fatty acids (SFA) has been shown to increase plasma low density lipoprotein (LDL)-cholesterol in human (Mattson and Grundy, 1985); however, people on the polyunsaturated fatty acids (PUFA) rich diet tend to have lower blood serum cholesterol levels (Ginsberg et al., 1990; Grundy and Vega, 1988; Keys et al., 1965), and the PUFA provide essential fatty acids for growth and development (Holman et al., 1982; Simopoulos, 1994). Currently, health professionals are recommending diets low in calorie and limited in SFA and cholesterol to reduce the risk of heart disease and many other health problems; moreover, one-third of the recommended total fat intake calories should be from the PUFA (NRC, 1988; Simopoulos, 1994). Based on the scientific evidence and health professionals' recommendations, the diet and health conscious population keeps growing in the United States; therefore, the consumption of meat and dairy products, which contain 40 to 50% of SFA, has been decreasing. Conversely, the consumption of fish and vegetable oils is

growing because these products have relatively less SFA and more PUFA than ruminant animal products.

The meat and dairy industries have responded to this consumer demand by reducing the fat in meat (leaner) and dairy products (low and non-fat). In addition, the researchers have investigated substituting the levels of SFA with PUFA in ruminant animals' body fat; unlike monogastric animals, the body fat of ruminants cannot be changed through the consumption of high levels of PUFA in the diet because the rumen microorganisms are able to hydrogenate PUFA during the digestion. The efforts to increase the levels of PUFA in the ruminants' meat and milk products have had very limited success by several researchers (Church, 1983; Garton, 1960; Hartfoot and Hazlewood, 1988; Hungate, 1966; Shorland et al., 1955; Van Soest, 1984). Australian researchers (Cook et al., 1970; Scott et al., 1971; 1972) however, produced promising supplements that could increase the levels of PUFA greatly through preventing the ruminal biohydrogenation of unsaturated dietary fat during the digestion. These supplements were made by particles of oil (safflower) encased in spray-dried protein (casein) treated with formaldehyde (37%). Unfortunately, formaldehyde is a known carcinogen that is not allowed in animal feed additives in the United States. In the efforts to find an alternative to formaldehyde, researchers (Dje, 1994; Newberry, 1990) have produced soybean oil-protein supplements treated with acetaldehyde or diacetyl. Feeding these supplements, which contain ground, whole soybeans, to lactating ewes increased the polyunsaturation in blood serum and milk fat. There is a high probability that feeding such supplements to ruminant animals would increase polyunsaturation in their meat fat. However, additional

research is need to determine if this can be accomplished. Therefore, the objectives of this research were (1) to produce a gel containing ground whole soybeans, the oil of which contains high levels of linoleic acid (C18:2), in combination with casein treated with acetaldehyde or diacetyl, (2) to determine the extent of PUFA deposition in the body and muscle fats of lambs fed the two treated (acetaldehyde and diacetyl) soybean gel supplements, (3) to determine the effect of feeding the two treated soybean gel supplements on the accumulation of vitamin E in the lamb fat, and (4) to evaluate the effect of feeding the supplements on the flavor volatile compounds in cooked longissimus muscle.

CHAPTER II

LITERATURE REVIEW

STRUCTURAL AND FUNCTIONAL PROPERTIES OF LIPIDS

Lipids are generally hydrophobic and immiscible with water, and they exist in adipose tissues in all living cells. Based on chemical structures, lipids can be classified into hydrocarbons, aliphatic alcohols, aliphatic fatty acids, wax, glycerol-containing lipids, sphingosine-containing lipids, sterols, and miscellaneous lipids (Wan, 1991). From physical form, lipids can be divided into fats and oils; fats are solid and oils are liquid at room temperature. Almost all of the lipids (99%) are glycerol esters of fatty acids in both plant and animal origins (Nawar, 1985). The fatty acids (FA) in the natural sources of fats and oils exist predominately as even carbon number straight chain molecules with a carboxylic acid ($-\text{COOH}$) group on one end and a methyl ($-\text{CH}_3$) group on the other end (Nawar, 1985; Wan, 1991). Based on the number of double bonds ($\text{C}=\text{C}$) present in the carbon chain containing from 2 to more than 20 carbons, FA can be classified into saturated fatty acids or SFA (no double bond) and unsaturated fatty acids or USFA (at least one double bond). The USFA can be divided into monounsaturated fatty acids or MUFA (one double bond) and polyunsaturated fatty acids or PUFA (at least two double bonds). The most common and important FA contain from 12 to 22 carbons (Wan, 1991). Animal fats contain mainly palmitic or $\text{C}_{16:0}$ (21%), stearic or $\text{C}_{18:0}$ (28%), and

oleic or C18:1 (37%) acids, especially in mutton tallow, but vegetable oils (soybean, corn, peanut, sunflower, and safflower) have a lower amount of SFA than animal fats (Rossell, 1992; Weiss, 1983).

Lipids impart unique properties to foods such as high energy, good flavor, and tender texture (Nawar, 1985; Wan, 1991). From a nutritional viewpoint, lipids provide more calories (9 kcal/g) than proteins or carbohydrates (4 kcal/g) and essential fatty acids; moreover, they carry fat soluble vitamins such as vitamin A, D, E, and K (Nawar, 1985; Wan, 1991). Plastic fats (shortenings) provide functionality in bakery and confectionary products by allowing aerating of whipped batters and lubricating proteins and carbohydrates during baking (Wan, 1991). In addition, fats also contribute flavor by reacting with other food ingredients (Nawar, 1985; Wan, 1991).

METABOLISM OF FATTY ACIDS AND HUMAN HEALTH

Liver and adipose tissue are the most important sites where FA biosynthesis occurs in mammals, and the end products are usually C16:0 and C18:0 (Simopoulos, 1994). In order to function, cell membranes need USFA to maintain fluidity (Simopoulos, 1994). In SFA (C16:0 or C18:0), one double bond is introduced to form MUFA (C16:1 or C18:1) by the enzyme delta (δ)-9 desaturase (Brenner, 1989). Oleic (C18:1 ω 9) acid is the major MUFA in the diet (Brenner, 1989). Diets rich in cholesterol increase δ -9 desaturase activity, resulting in an increased ratio of MUFA to SFA, such as palmitoleic/palmitic (C16:1/C16:0) or oleic/stearic (C18:1/C18:0), and lead to an increase in membrane

fluidity, thus offsetting the hardening effects of their enhanced cholesterol content (Holman, 1960). When MUFA are not available in diets, δ -9 desaturase enters into action by increasing MUFA from SFA via the introduction of a single double bond (Holman, 1960). The C18:1 ω 9 is metabolized through a series of enzymatic steps involving desaturases and elongases to C18:2 ω 9, C20:2 ω 9, and C20:3 ω 9 (Holman, 1960). This action increases the deficiency of essential fatty acids (EFA) of phospholipids in plasma membranes (Holman, 1960). SFA and MUFA are not EFA because they can be readily synthesized from other nutrients in the diet such as glucose and amino acids (Nawar, 1985; Simopoulos, 1994). On the other hand, ω -3 and ω -6 FA are EFA because they can't be synthesized (Simopoulos, 1994). Linoleic acid (C18:2 ω 6) and alpha linolenic acid (C18:3 ω 3) are essential for growth and development and are usually obtained from plants (Holman et al., 1982; Simopoulos, 1994). In addition, docosahexaenoic acid (DHA) or C22:6 ω 3 is important for normal visual and cerebral function (Holman et al., 1982; Simopoulos, 1994). The C18:2 ω 6 is converted to arachidonic acid (C20:4 ω 6) (Simopoulos, 1994). The C18:3 ω 3 is converted to eicosapentaenoic acid (EPA) or C22:5 ω 3 and DHA through a series of desaturation and elongation steps, and the major sources of EPA and DHA are fish and poultry products in the United States (Raper et al., 1992; Simopoulos, 1994)

In the U.S., more than one third of the calories are provided by fat; more than half of total fats are supplied from animal products, and approximately half of the animal fat is saturated fat (NRC, 1988; Weiss, 1983). Saturated fat intake from animal sources may

contribute to many human health problems, such as coronary artery disease, hypertension, diabetes, arthritis, psoriasis, ulcerative colitis, and cancer (Simopoulos, 1994). Heart disease is the major cause of death (40% annual death) in the U.S. (Gallagher and Allred, 1992a; Harper, 1983). The initial stage of heart disease is diagnosed as coronary artery disease (20-25% annual death) (Gallagher and Allred, 1992a). In coronary artery disease, plaques form in the wall of blood vessels, and reduce both the size of the blood vessel and blood flow (Gallagher and Allred, 1992a). Cholesterol is a prominent part of those plaques (Gallagher and Allred, 1992a; Kuller, 1983). Saturated fats from animal sources raise blood cholesterol, and may contribute to the formation of plaques (Gallagher and Allred, 1992a; Kuller, 1983). Coronary artery disease is not recognized until the blood vessel becomes sufficiently constricted to decrease the nutrient and oxygen supply to a section of the heart or to other parts of the body (Gallagher and Allred, 1992a; Kuller, 1983). Dietary guidelines have recommended decreasing saturated fat intake for lowering serum cholesterol levels and substituting PUFA for SFA; fat intake 30% or less of total energy with 10% from SFA, MUFA, and PUFA (Nawar, 1985).

The FA and lipids participate in many aspects of cellular metabolism that affect cholesterol levels, platelet aggregation, prostaglandin and leukotriene metabolism, and other cellular functions (Shepherd and Packard, 1992). In the digestion of fat, the triglyceride must initially be emulsified as small droplets; bile acids aid in this emulsification (Gallagher and Allred, 1992b). The bile acids are produced in the liver from cholesterol, stored in the gallbladder, and secreted into the small intestine when fats arrive there from the stomach (Shepherd and Packard, 1992). The emulsified triglycerols

can be broken into a glycerol and three FA in the small intestine by an enzyme from the pancreas (Gallagher and Allred, 1992b). These glycerols and FA are absorbed into the intestinal mucosal cell, where the FA are used to remake a triglyceride (Gallagher and Allred, 1992b). This new triglyceride can't move from the mucosal cell without adding protein because of its insolubility in blood or any other water based body fluids. When a protein is added to triglyceride, it is called a chylomicron (Nawar, 1985). The chylomicron enters the lymph system instead of the blood (Shepherd and Packard, 1992). The blood supply from the intestinal area first goes to the liver so that nonlipid material goes through the liver before it goes to the rest of the body (Shepherd and Packard, 1992). Chylomicrons that carry fat bypass the liver (Shepherd and Packard, 1992). The triglyceride in the chylomicron can be removed by heart, kidney, and muscle tissues, and ATP is produced (Shepherd and Packard, 1992). However, much of it is taken out of the blood and stored in fat cells (Gallagher and Allred, 1992b).

According to density, the blood serum lipoproteins are classified into four main categories: chylomicrons, very low density lipoproteins (VLDL), low density lipoproteins (LDL), and high density lipoproteins (HDL) (Nawar, 1985). Chylomicrons are the largest and the least dense of the lipoprotein particles, whereas HDL are the smallest and most dense (Nawar, 1985). All of the lipoproteins contain cholesterol (Nawar, 1985). The LDL are present in the highest proportion of the lipoproteins, but the VLDL contain the largest amount of cholesterol (Nawar, 1985). The HDL have the largest amount of phospholipids of all lipoproteins in the blood (Nawar, 1985).

Clinical studies have indicated a positive correlation between high cholesterol or

triacylglycerol levels in the blood and the risk of coronary heart disease by atherosclerosis (Gallagher and Allred, 1992a; Kuller, 1983). While blood cholesterol levels above 250 mg/dL are considered a danger signal for a heart attack, the ratio of HDL to LDL may be even more important (Gallagher and Allred, 1992a; Kuller, 1983). The HDL to LDL ratio should be 2.6 or higher no matter what blood cholesterol levels are (Kuller, 1983; Mattson, 1983). However, dietary guidelines from the American Heart Association (AHA) still recommend total cholesterol reduction via diet (Gallagher and Allred, 1992a). There are essentially two ways to modify a diet to reduce the blood cholesterol level: reduce total calorie intake and change the intake of saturated relative to unsaturated fats (Gallagher and Allred, 1992a). The AHA has recommended a ratio of 1:1:1 of saturated: monounsaturated: polyunsaturated fats intake in the dietary fats; this ratio shows 5 to 10% decrease in blood cholesterol levels for many people (Gallagher and Allred, 1992a; Nawar, 1985).

DIGESTION TRACT AND DIGESTION OF DIETARY LIPIDS IN RUMINANTS

The mature ruminant's stomach has four different compartments: rumen, reticulum, omasum, and abomasum. The abomasum is the gastric stomach, whose function is similar to the one stomach nonruminant. The omasum, a complex multilayered compartment, functions to absorb water (Baldwin, 1984). About 85% of the total stomach volume consists of the rumen and reticulum, which serve as habitats for a large and diverse population of anaerobic microorganisms somewhat analogous to a continuous

culture system (Baldwin, 1984). In these compartments, a fairly continuous supply of nutrients is provided; the temperature is 39 to 40°C; pH is 5.5 to 7.0; and the ionic strength remains consistent (Hungate, 1988). Fermentation products also are removed, and feed residence time is compatible with rate of microbial fermentation (Hungate, 1966).

Digestion of Unprotected Dietary Lipids in Ruminants

Dietary lipids, triacylglycerols and phospholipids are hydrolyzed to glycerol and FA in the rumen (Baldwin, 1984; Jenkins, 1994). Glycerol is fermented by microbes to form the volatile FA (acetic, propionic, butyric, and valeric) and carbon dioxide and methane (Baldwin, 1984). Energy trapped as ATP during fermentation is used to maintain the rumen microbes and to support microbial growth (Baldwin, 1984). The dietary PUFA are largely hydrogenated to SFA and trans FA by the microbes. In ruminants, with a lipid intake containing 66% PUFA from plant sources, only 4.4% PUFA were found in duodenal lipid contents (Ward et al., 1964). Adding USFA to the ruminants' lipid supplement may cause digestive disturbances because of their antimicrobial effects and inhibition of ruminal fermentation (Jenkins, 1993). Ruminant diets containing more than 2 to 4% added lipid from plant oils also are likely to depress fiber digestion in the rumen (Jenkins, 1994). Thus, hydrogenation of dietary FA and the low fat content (2-6%) of ruminant diets are the reasons why the fats in animal products are highly saturated (Jenkins, 1994).

Because most of the dietary FA are hydrogenated by rumen microbes, three different types of lipids are transferred to the duodenum of ruminants; these duodenal

lipids include dietary lipid that escapes microbial transformation, dietary lipid after microbial transformation, and microbial lipid (Jenkins, 1994). In hay-fed ruminants, only 5% of the total duodenal lipid is that dietary lipid, which contains USFA and is not transformed (Fotouhi and Jenkins, 1992; Hartfoot and Hazlewood, 1988; Wu et al., 1991). The remainder is transformed dietary lipid (60%) and microbial lipid (35%), both of which contain mainly SFA. Approximately 15% of the transformed dietary lipid and microbial lipid fractions are the intermediates of biohydrogenation such as trans FA isomers and branched-chain FA in the microbial lipids. The trans FA, which are formed by incomplete biohydrogenation of dietary PUFA, are deposited into the subcutaneous fat (5 to 10%) in ruminants (Jenkins, 1994). Up to 20% of total microbial FA consist of branched-chain FA, which are synthesized by ruminal bacteria from isobutyrate, isovalerate, and 2-methylbutyrate, end products of carbohydrate fermentation (Emmanuel, 1978). The major branched FA in ruminal bacteria is Anteiso C15:0 (Emmanuel, 1978; O'Kelly and Spiers, 1991). In pasture-fed sheep, the subcutaneous fat contains up to 12% branched FA, mainly with a single methyl branch at carbon number 4 (Smith et al., 1979).

In the digestion of dietary FA, bile salts are essential for fat absorption (Harrison and Leat, 1972; Heath and Morris, 1963). The most critical phenomenon of increasing the digestibility of FA is their dispersion by the detergent action of bile salts of the protonated fatty acids on the surfaces of food particles entering the duodenum (Scott and Lough, 1971; Smith and Lough, 1976). This detergent action is promoted by several factors. The low pH (2.5 to 3.5) of the proximal duodenum assures that FA are protonated, and the high proportion of taurocholic acids in the bile salts assure their

solubility at low pH (Harrison and Leat, 1975; Moore and Christie, 1984). In addition to the detergent action, other factors also affect the digestion of FA. Increased concentrations of phosphatidylcholine resulted in increased solubility of SFA, while higher levels of phosphatidylethanolamine were related positively to the increased solubility of both SFA and USFA (Smith and Lough, 1976). Increasing lecithin infusion (900 g/d of crude lecithin) into the abomasum increased fat absorption in lactating cows (Grummer et al., 1987), whereas 14 or 28% inclusion of lecithin in a dietary fat supplement did not improve the digestibility of hydrogenated fat in steers (Jenkins, 1990). The FA composition of duodenal lipids also can influence the digestibility of FA. The digestibility of SFA decreased with increasing chain length, but increasing unsaturation in FA increased digestibility (Andrew and Lewis, 1970; Steele and Moore, 1968). The decrease in digestibility with increasing fat intake was caused by the lower digestibility of C18:0 (Weisbjerg et al., 1992). The C18:0 is less digestible and C16:0 is more digestible than the average FA mixture presented to the ruminant small intestine (Weisbjerg et al., 1992).

Digestion of Protected Dietary Lipids in Ruminants

A high level of SFA is the distinctive characteristic of fats in ruminants because of biohydrogenation of dietary FA and the low fat content (2-6%) of ruminant diets (Jenkins, 1994). Any attempt to increase the unsaturation level in fats in ruminants by feeding higher levels of dietary unsaturated lipids fails because they undergo biohydrogenation, keeping the SFA level high in duodenal lipids (Dawson et al., 1974; Hawke and Silcock, 1969). The types of diet fed to a ruminant, however, can affect the composition of the

duodenal lipids other than the polyunsaturated fat content. Feeding high energy grain diets diminished the biohydrogenation capability of ruminant microbes by reducing the number of lipolytic bacteria (Kemp et al., 1981; Latham et al., 1972). Particle size of the diet also alters microbial fermentation because fewer bacteria adhere to the small particle size (Gerson et al., 1988). However, these efforts can increase the level of elaidic acid (trans C18:1) but not the level of PUFA such as linoleic (C18:2) and linolenic (C18:3) acids (Palmquist and Schanbacher, 1991). Both C18:2 and C18:3 are EFA for animal and human health (Simopoulos, 1994). These two PUFA can be obtained from plant or grain sources (Weiss, 1983). The grain diet of ruminants contains predominantly C₁₈ PUFA (C18:2 and C18:3). Unfortunately, the C18:2 and other USFA are biohydrogenated, and most of the C18:2 are converted to C18:0 and isomers of C₁₈ MUFA (Hartfoot et al., 1973; Kellens et al., 1986). The C18:0 and other FA isomers are transported to the small intestine, where they are digested and absorbed. These absorbed FA are precursors for the synthesis of plasma triglycerides, which are incorporated into the lipids of milk and adipose tissue (Barry et al., 1963; Garton, 1967; Heath and Morris, 1963). Only very small amounts of the dietary C₁₈ PUFA escape biohydrogenation and are found in the triglycerides isolated from plasma, milk, and body fats of ruminants.

The main idea of protecting dietary lipids is to increase PUFA in ruminant fat through dietary PUFA escaping biohydrogenation in the rumen and being directly transported into the circulatory system (Ashes et al., 1979; Cook et al., 1972a; Cuitum et al., 1975; Newbold et al., 1973; Scott et al., 1971). The PUFA containing oil droplets can be protected against biohydrogenation in the rumen by encapsulation within a layer of

formaldehyde-treated casein, which resists proteolysis in the rumen (Cook et al., 1972a; Scott et al., 1971). Then the formaldehyde-protein complex is hydrolyzed in the acidic secretions of the abomasum, thus making the oil available for digestion and absorption from the small intestine (Cook et al., 1972a; Scott et al., 1971).

Protective Chemical Agents from Ruminal Hydrogenation

Formaldehyde (CH_2O) has been used to protect dietary lipids against ruminal biohydrogenation due to its ability to bind proteins. The proportion of C_{18} PUFA has been increased up to 20-30% in milk, plasma, and depot fats through the embedding of sunflower/safflower oils in a matrix of formaldehyde treated casein and feeding to ruminant animals (Cook et al., 1972b; Lough et al., 1992; Scott et al., 1971;).

Unfortunately, formaldehyde is not allowed in animal feed in the United States because of its toxicity. Epidemiological studies (Blair et al., 1985) showed that formaldehyde causes a wide range of health problems in humans and animals. Exposure to formaldehyde vapors may intensely irritate mucous membranes, the ingestion may cause severe abdominal pain, hematemesis, hematuria, proteinuria, anuria, acidosis, vertigo, coma, and death (Anonymous, 1983).

Acetaldehyde ($\text{C}_2\text{H}_4\text{O}$) and diacetyl ($\text{C}_4\text{H}_6\text{O}_2$) have been used as alternate protective agents instead of formaldehyde (Dje, 1994; Newberry, 1990), because both acetaldehyde and diacetyl are able to bind proteins and are nontoxic compounds. Newberry (1990) successfully protected soybean oil in a diacetyl treated spray dried soybean protein-casein complex from biohydrogenation when incubated with rumen fluid

in vitro. Dje (1994) fed a soybean-casein-oil gel complex treated with formaldehyde, acetaldehyde, or diacetyl to ewes and showed that levels of C18:2 in the blood stream were increased ($P < 0.05$) by 41% (formaldehyde), 42% (acetaldehyde), and 29% (diacetyl) compared to a basal (control) diet. Acetaldehyde has a molecular weight of 44.05 g, a density of 0.788 g/mL, a boiling point of 21°C, a melting point of -123.5°C, and a distinctive pungent odor (Anonymous, 1983). The oral LD₅₀ of acetaldehyde is 1930 mg/kg in rats (Anonymous, 1983). Acetaldehyde is produced by lactic acid bacteria: Lactobacillus, Lactococcus, Leuconostoc, and Streptococcus (Cogan and Jordan, 1990) and yeasts: Candida species (Collings et al., 1991). Diacetyl (2,3-butanedione), a yellowish-green liquid with a quinone odor, has a molecular weight of 86.09 g, a density of 0.990 g/mL, and a boiling point of 88 °C (Anonymous, 1983). The oral LD₅₀ of diacetyl is 1580 mg/kg in rats (Anonymous, 1983). The 2,3-butanedione is an important flavor component in foods such as butter, vinegar, coffee, and other foods, and is produced by bacteria (Anonymous, 1983). Both acetaldehyde and diacetyl compounds are generally recognized as safe (GRAS) components by the Food and Drug Administration (FDA) in the U.S.

LIPID AND PROTEIN COMPOSITION OF SOYBEAN

The soybean is utilized primarily as a source of protein and oil (Weiss, 1983). Soybean crops are used as forages, foods, and vegetable oil sources, and the U.S. is the world's leading producer and exporter of soybeans (Weiss, 1983). In 1992, about 27% of

vegetable oils used worldwide was soybean oil obtained from 60 million metric tons of soybeans produced in world (Anonymous, 1993). Ninety percent of the vegetable oils are used as margarines, shortenings, salad oils, and frying oils (Friedt and Luhs, 1992). Soy protein products, by-products of soybean oil production, are an excellent source of protein for humans and animals and have a high lysine content but a limited amount of methionine (Salunkhe et al., 1992). Only a small percentage of the soybean protein by-products (2 to 3%) are used directly as human foods; most of the soybean protein by-products are converted to animal feeds (Anonymous, 1992). The majority (>80%) of the soybean protein products used in human food are in the form of soy flours and grits added to bakery and confectionery products (Anonymous, 1992). However, soy protein products are used also as fermentation media in processed meats and for carriers of spices and flavors (Rakes, 1992).

Soybeans generally contain 40% protein, 20% lipid, 17% cellulose and hemicellulose, 7% sugars, 6% crude fiber, 6% ash, and 5% moisture (Weiss, 1983). The percentage of each component differs among varieties and the different environments in which they are grown (Weiss, 1983). The major components of FA in soybean oil are listed in Table 1 (USDA, 1984). The C18:2 (44-62%) is the most abundant FA. The lipids present in soybean are composed of 88.1% neutral lipids (triglycerides, free FA, sterols, and sterol esters), 9.8% phospholipids, and 1.6% glycolipids (Salunkhe et al., 1992). In addition, tocopherols also are contained in crude soybean oil, including 200ppm alpha (α)-, 980ppm gamma (γ)-, and 500ppm delta (δ)-tocopherols (Wan, 1991).

Table 1 -- Fatty acid composition of soybean oil^a

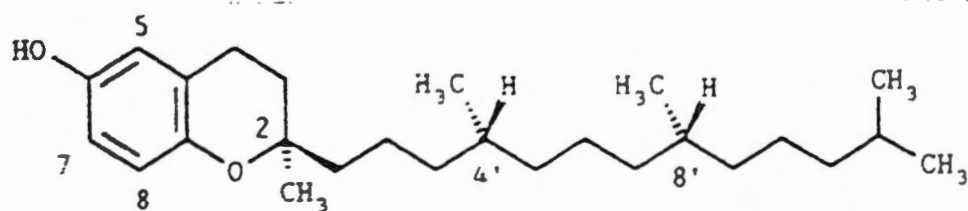
Fatty acid	Concentration (%)
Saturated (SFA)	14.93
14:0	0.11
16:0	10.82
18:0	4.00
Monounsaturated (MUFA)	24.37
16:1	0.21
18:1	23.95
20:1	0.21
Polyunsaturated (PUFA)	60.71
18:2	53.57
18:3	7.14

^aUSDA, 1984.

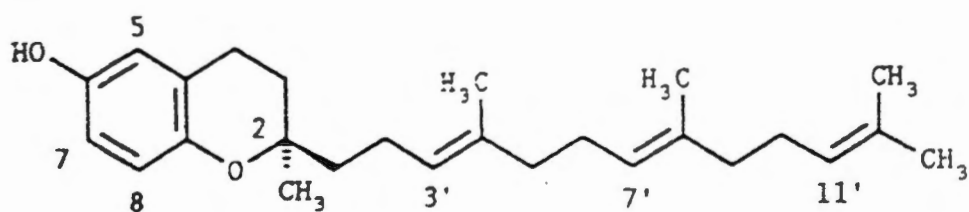
STRUCTURE, PROPERTY AND METABOLISM OF VITAMIN E

Vitamin E, an antioxidant, has been fed to livestock to prevent several deficiencies: necrotizing myopathy, fetal death, and resorption, especially in lambs (Roeder et al., 1996). In addition, vitamin E also improves the animal health and animal product quality by enhancing the immune system and delaying the discoloration of red meat (Chew, 1995; Roeder et al., 1996; Schaefer et al., 1995). All of the naturally producing chemical forms of vitamin E are shown in Figure 1 (Machlin, 1991). Vitamin E consists of eight compounds, which are divided into two groups, tocopherols and tocotrienols (Van Niekirk, 1988). Each of these eight compounds has a 6-chromanol ring structure and side chain (Machlin, 1991). Tocopherols have phytol side chains and tocotrienols have double bonds at 3', 7', and 11' positions in the side chain (Machlin, 1991). Four isomers, α (5, 7, 8), β (5, 8), γ (7, 8) and δ (8), differ in the number and the location of methyl groups on the chromanol ring in both tocopherols and tocotrienols (Machlin, 1991).

Vitamin E (tocopherols) is almost completely soluble in fat and oil but not soluble in water. In the presence of oxygen, tocopherols are oxidized to tocopheroxide, tocopheryl quinone, or tocopheryl hydroquinone, as well as dimers and trimers (Machlin, 1991). The oxidation is accelerated by exposure to light, heat, alkali, and the presence of iron and copper salts (Machlin, 1991). In the absence of oxygen, tocopherols are stable in heat and alkali, and are unaffected by acids at temperatures up to 100°C (Machlin, 1991). The isolated natural tocopherols are not very stable and undergo significant loss during storage (Machlin, 1991). However, during storage of vegetable oils, the loss of natural



Tocopherol



Tocotrienol

Trivial names		
Position of Methyl groups	Tocopherols	Tocotrienols
5, 7, 8	α -tocopherol	α -tocotrienol
5, 8	β -tocopherol	β -tocotrienol
7, 8	γ -tocopherol	γ -tocotrienol
8	δ -tocopherol	δ -tocotrienol

Figure 1 -- Formulas of eight members of tocopherol and tocotrienol series (Machlin, 1991).

tocopherols is usually minimal, even though appreciable loss occurs during cooking of foods (Machlin, 1991). The α -tocopherol is the most abundant and biologically active form of vitamin E (Bauernfeind, 1980; Kasperek, 1980). The only naturally occurring stereoisomer of α -tocopherol is designated *RRR*- α -tocopherol (formerly known as *d*- α -tocopherol or α -tocopherol), but the synthetic ones are designated as all-*rac*- α -tocopherol (Machlin, 1991). In meat products, α -tocopherol accounts for almost all vitamin E activity, but in vegetable seed oils, other isomers occur in substantial quantities (Bauernfeind, 1980). In soybean oil, α -tocopherol comprises of only 8-10% of the total tocopherols present but still represents the major portion of the biological activity (Bauernfeind, 1980).

Many early researchers recognized that α -tocopherol could function as an antioxidant in vivo (Dam, 1957; Olcott and Matill, 1941). Free radicals are generated by a number of enzymatic and nonenzymatic reactions occurring in cells, then they spontaneously react with oxygen to make oxygen radicals (Machlin and Gabriel, 1987). The antioxidant activity of vitamin E is associated with a number of small molecules: reduced glutathione, ascorbic acid, bilirubin, and uric acid, as well as with several enzymes: superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase (Machlin and Gabriel, 1987). All of these work together to defend the cell against the damaging effects of the oxygen radicals. In addition, by enhancing the immune response, vitamin E may play a role in the prevention of cancer, cardiovascular disease, cataracts, and Parkinson's disease in humans (Horwitt, 1974; Kayden and Nair, 1972; Moustgaard and Hyldgaard, 1971).

Tocopherols are absorbed through the lymphatic system, where they are transported as a lipoprotein complex (Gallo-Torres, 1980). The absorption efficiency is enhanced by the simultaneous digestion and absorption of dietary lipids. Medium-chain triglycerides particularly enhance absorption, whereas PUFA are inhibitory (Gallo-Torres, 1980). Both bile and pancreatic juice are necessary for maximal absorption of vitamin E (Gallo-Torres, 1980). Vitamin E is apparently absorbed as a lipid-bile micelle, together with free FA, monoglycerides, and other fat-soluble vitamins (Baker et al., 1980; Mathias et al., 1981). Vitamin E penetrates the epithelial cells by diffusion processes through the apical plasma membrane of the absorptive cells (Baker et al., 1980; Mathias et al., 1981). Absorption of vitamin E is maximal in the median portion of the small intestine but vitamin E is not absorbed in the large intestine (Baker et al., 1980; Mathias et al., 1981). High correlation exists between the total lipid or cholesterol in the serum and the tocopherol level (Chow, 1975; Clevidence and Lehman, 1989; Kayden et al., 1973). The vitamin is most concentrated in membrane rich cell fractions: the mitochondria and microsomes (Murphy and Mavis, 1981). The existence of proteins in the cytosol that specifically bind α -tocopherol, along with the cytosolic binding proteins, apparently facilitate the transport of tocopherol into mitochondrial and microsomal membranes (Murphy and Mavis, 1981). Although γ -tocopherol is absorbed and deposited in tissues readily, it is retained only to a limited degree in the comparison of α -tocopherol. Lucy (1978) proposed that the phytyl side chain of α -tocopherol may interact with the fatty acyl chain of polyunsaturated phospholipids, particularly arachidonate. It is generally assumed that α -tocopherol is oriented with the chromanol head group toward the surface of the membrane near the

phosphate region of the phospholipid and with the hydrophobic phytol tail buried within the hydrocarbon region in the membranes (Burton and Ingold, 1986). Adipose tissue, liver, and muscle represent the major storage deposits of the vitamin E (Machlin and Gabriel, 1982). The rate of depletion of tocopherol upon its withdrawal from the diet varies considerably: first being depleted the fastest from plasma and liver, then skeletal and heart muscle, and finally adipose tissue (Machlin and Gabriel, 1982).

THE EFFECTS OF GENETIC AND ENVIRONMENTAL FACTORS ON LAMB FLAVOR AND FAT COMPOSITION

In the U.S., consumers still prefer beef or pork rather than lamb, due to lamb's unpleasant or unacceptable flavor, lack of product availability, and the high percentage of fat in lamb (Romans et al., 1994; Sink and Caporaso, 1977). Flavor is an important aspect of the quality and acceptability of all red meats: beef, pork, and lamb (Ford and Park, 1980; Theunissen et al., 1979). Many previous meat researchers have been interested in increasing market weight through changing domestic animals' genetic base, feeding periods, and implementation of new diets and management systems. In addition, researchers have focused on producing leaner meats with less saturated fat and more unsaturated fat. These genetic and environmental changes influence meat flavor, such as that of lamb. The most important genetic factor influencing flavor is species (Sink, 1979), while feed source is the most important environmental factor (Shahidi and Rubin, 1986).

The Genetic Factors: Breed, Gender, Slaughter Weight and Age

From the previous research, it is very difficult to find discernible trends on the effects of breed, gender, and slaughter weight and age on the lamb flavor. Apparent inconsistencies of reported results are likely due to the interactions in slaughter weight and age and breed, gender, or diet.

Effects of the breed on lamb flavor are inconsistent. No flavor difference was detected by the sensory panelists in cooked meat from lambs of three different breeds: Columbia, Hampshire, and Targhee (Fox et al., 1962). However, Cramer et al. (1970a) observed a statistical difference ($P < 0.05$) in mutton flavor among different breeds of sheep, which was caused by the fineness of wool among breeds. Cramer et al. (1970b) failed to find different fat characteristics in the subsequent study; however, the flavor difference among sheep breeds or a correlation of mutton flavor intensity and fineness of wool was due to the wide range of wool grades.

The effects of gender on the flavor of lamb are inconsistent and inconclusive. Several researchers in the 1960s found no differences in flavor of meat between rams and wethers nor any palatability problems in ram meat (Batcher et al., 1969; Fox et al., 1962; Garrigus et al., 1962; Gates et al., 1964; Pattie et al., 1964). However, other researchers observed that the meat from rams possessed more intense flavor (Cramer et al., 1970a; Crouse et al., 1981) or less acceptable flavor (Kemp et al., 1972; Misock et al., 1976) than the meat from wethers or ewes. Batcher et al. (1969) also found a gender effect on lamb flavor, but only in the high fat containing (33%) lamb patties. Interaction between gender and slaughter weight has also been observed on flavor of lamb meat. Misock et al. (1976)

and Crouse et al. (1981) reported that meat from heavy weight market ram (carcass weight from 45 to 72kg) had less preferable flavor than that from wether; the chilled ram carcasses had an ammonia or staggy odor (Crouse et al., 1981).

The effects of slaughter weight and age on the flavor of lamb also were inconclusive. Trained sensory panelists found that the intensity of lamb flavor decreased as live weight and age increased (Weller et al., 1962), and lamb flavor became more desirable as the live weights of lambs increased from 35 to 54 kg for ewes and wethers (Kemp et al., 1976). However, the sensory panel did not observe any lamb flavor difference as carcass weight of rams and ewes increased from 62 to 76 kg. Other researchers also have observed that flavor from meat of lighter weight ram lambs (41 kg) was more desirable than that of heavier ram lambs (68 kg) (Field et al., 1978; Paul et al., 1964). Paul et al. (1964) also observed that the lamb flavor intensity increased with increasing carcass fatness.

The Environmental Factor: Feed Sources

Different types of pasture grass affected the flavor of meat from grazing lambs. Field et al. (1983) found that meat from lambs on rape pasture had off-flavors while Spurway (1972) found off-flavors in meat from lambs grazing vetch pasture. However, lambs grazing alfalfa pastures produced meat with no flavor problems (Hight et al., 1972) and had more acceptable flavor than meat from lambs grazing other types of pasture (Nixon, 1981). In New Zealand and Australia, lambs on alfalfa pasture at least 3 wk prior to slaughter produced meat with more intense flavor than meat produced by lambs on

other types of pasture (Nicol and Jagusch, 1971).

The form and energy level of the diet also affect lamb flavor. Dried alfalfa hay had no effect on meat flavor when fed to lambs (Ford and Park, 1980; Paul et al., 1964). However alfalfa pellets, which are a high energy level diet, produced meat with more desirable lamb flavor (Crouse et al., 1978). Several researchers agree that a more concentrated high energy diet produces lamb meat with more desirable flavor than does a less concentrated low energy diet (Kemp et al., 1981; Locker, 1979; Summers et al., 1978; Vimini et al., 1984).

Inclusion of grains or oilseeds in the diet of lambs also affects the flavor of the meat. For example, lambs fed a barley containing diet produced meat with less desirable lamb flavor than a diet containing no barley (Purchase et al., 1979; Wong et al., 1975a).

Lamb chops from lambs fed soybean and corn had a more intense, less desirable, musty flavor than chops from lambs fed alfalfa and corn (Crouse et al., 1983). Both of these latter diets were isocaloric.

Dietary effects on lamb flavor volatiles also have been investigated. Compared to meat from lambs finished on a corn diets, meat from lambs finished on clover pasture had very high levels of 2,3-octanedione (108 vs <0.05 ppm), and 3-hydroxyoctan-2-one (14.8 vs <0.05 ppm), and relatively high concentrations of diterpenoids: phyt-1-ene (1.27 vs 0.52 ppm), phyt-2-ene (8.58 vs 0.82 ppm), phytane (0.26 vs <0.05 ppm), and neophytadiene (4.97 vs 0.39 ppm) in 300 mg of the ovine adipose tissue (Suzuki and Bailey, 1985). These diterpenoids apparently formed from the fermentation of phytol or chlorophyll by rumen microorganisms (Body, 1977). In addition, meat from clover-fed

lambs had higher levels of heptanal, octanal, and 2-nonenal than meat from corn-fed lambs. In contrast, fat from corn-fed lambs had a higher concentration of γ -dodecalactone than that from clover fed lambs (Suzuki and Bailey, 1985).

The composition of lamb fat also can be altered by different diets. Feeding high energy grain diets to lambs resulted in modified FA composition of the fat and changed the fat texture from a hard to a soft, oily texture (Field et al., 1978; Miller et al., 1967; Miller and Rice, 1967). Feeding the grain diet to lambs resulted in high proportions of relatively low-temperature-melting methyl-branched chain and odd-numbered FA and low proportions of high-temperature-melting SFA, C16:0 and C18:0 (Busboom et al., 1981; Duncan et al., 1974; Garton et al., 1972; Miller et al., 1980). Miller et al. (1980) proposed that increased propionate from the concentrated diets increased the synthesis of low-temperature-melting, methyl branched chain FA and decreased the synthesis of high-temperature-melting, even-numbered SFA at the tissue level. Methylmalonyl-CoA, a metabolite of propionate, not only incorporates into methyl-branched chain FA but also inhibits the incorporation of malonyl-CoA into the saturated even-numbered FA (Cardinale et al., 1970).

Lipid supplements, made by encapsulating the oil droplets in a formaldehyde-treated protein coat, protect USFA from the biohydrogenation action of rumen microorganisms. Feeding such supplements to lambs produced meat containing up to 20% C18:2 and changed meat flavor (Ford and Park, 1980; Lawrie, 1981; Sink, 1979; Sink and Caporaso, 1977). The flavor of meat from these lambs was less desirable because of elevated levels of cis- γ -dodec-6-enolactone (sweet) and trans, trans-deca-2,4-

dienal (oily) (Ford and Park, 1980). The natural source and state of the protected lipid supplement also can influence the flavor of lamb; the different fat sources gave different flavors to lamb, and the spray drying process assisted in developing the sweet and oily flavor (Park et al., 1978). When lambs were fed each of two protected lipid supplements: sunflower seed-casein or safflower oil-casein (each treated with formaldehyde), meat from lambs fed a sunflower diet had tenfold levels of γ -dodec-6-enolactone (1-2 ppm) than did meat from lambs fed a safflower seed diet (Ford et al., 1976; Park et al., 1976). In addition, the levels of n-hexanal, hept-2-enal, oct-2-enal n-pentanol, and n-butanol were elevated in meat produced on the two protected lipid diets. These components plus trans, trans-deca-2,4-dienal, and cis- γ -dodec-6-enolactone, which were isolated from distillation of the lamb meat, are common products of the C18:2 oxidation in the lipid system (Park et al., 1976).

FATTY ACID COMPOSITIONS OF LAMB

The lipids in muscles are mainly triglycerides of FA, and the major compositional characteristic of the fat is its FA composition. The FA compositions difference among animals are mainly due to the species difference. The position of the muscle within the body, the diet of the animal, its weight, fatness, age, and gender also can vary the FA compositions (Rossell, 1992; Sinclair and O'Dea, 1987). Lipids from muscles near skin tend to be more unsaturated (Rossell, 1992). Animals with higher amounts of body fat tend to have more unsaturated lipids. Beef, mutton, and pork do not differ greatly in

degree of unsaturation; pork tends to be more unsaturated and mutton is somewhat less saturated than beef fat (Rossell, 1992; Sinclair and O'Dea, 1987). The FA composition of mutton tallow is shown in Table 2 (USDA, 1979), and the major three FA are C16:1 (21.5%), C18:0 (19.5%), and C18:1 (40.6%). The composition of FA from different parts of a sheep's carcass is shown in Table 3 (Enser, 1991; Sinclair and O'Dea, 1987). In most cases, the myristic (C14:0) acid is 2%, but in intramuscular fat (lean meat with all visible fat removed), the C14:0 rises to 5%. The C16:0 varies from 17 to 26%, depending on the type of muscles. However, the most significant variation is in the C18:0; it varies from 3% fat in legs and ears, to 11% in rump fat, 21% in fat from chest, 33% in mesenteric (between intestines), and 34% perinephric (kidney) fats. Therefore, the rate of individual FA deposition is dependent on the location of the muscle; the lower melting point fats are deposited near the skin (mobilizable structure) and the higher melting point fats consisted of internal fats (rigid structure) (Enser, 1991; Rossell, 1992; Sinclair and O'Dea, 1987).

CHEMICAL COMPOSITION OF LAMB FLAVOR

The aroma of cooked lamb or mutton meat is distinctive and different from that of any other meat. Early research workers found that the meaty aroma of red meat developed during cooking through degradation and reactions of compounds in fatty tissues (Howe and Barbella, 1937) and muscle fibers (Crocker, 1948) or could be produced by heating meat juices and fibers (Kramlich and Pearson, 1958). The most recent explanation of developing the meaty flavor is that the flavor of meat is attributed to

Table 2 -- Fatty acid composition of mutton tallow^a

Fatty acid	Concentration (%)
Saturated (SFA)	44.8
14:0	3.8
16:0	21.5
18:0	19.5
Monounsaturated (MUFA)	39.9
16:1	2.3
18:1	37.6
Polyunsaturated (PUFA)	7.8
18:2	5.5
18:3	2.3

^aUSDA, 1979.

Table 3 -- Composition of fats from different sites^a of sheep carcasses

	Major fatty acids (mass %)					
	14:0	16:0	18:0	18:1	18:2	18:3
Perinephric (kidney)	2	26	34	30	1	1
Mesenteric (between intestines)	2	24	33	32	1	1
Chest	2	26	21	42	1	1
Rump	2	23	11	53	1	1
Leg	1	17	3	69	1	1
Ear	1	17	3	64	2	2
Intramuscular (all visible fat removed)	5	24	14	38	4	2

^aSinclair and O'Dea, 1987; Enser, 1991.

the complex mixture of compounds produced by heating a heterogeneous system containing nonodorous precursors, and it is composed of volatile compounds with odor properties, nonvolatile compounds with taste and tactile properties, and potentiators and synergists (Moody, 1983; Shahidi and Rubin, 1986). Furthermore, the undesirable lamb flavors probably are derived from water-soluble components of adipose tissue and thermal degradation of muscle components (Sink, 1979; Wasserman, 1979).

Neutral, Acid and Basic Volatile Compounds in Cooked Lamb

In the neutral fraction of volatiles of cooked lamb meat are found the carbonyl compounds responsible for the mutton flavor: C_2 to C_{10} n-alkanals, C_5 to C_{10} 2-alkanones and 2-methylcyclopentanone (Jacobson and Koehler, 1963). Based on gas chromatographic and mass spectrometric (GCMS) analysis and olfactory evaluation, ten aldehydes: C_5 - C_9 alkanals; 2-octenal; 2,4-heptadienal; 2,4-heptadienal (isomer); 2,4-decadienal and 2,4-decadienal (isomer), three ketones: 2-nonanone; 2-dodecanone; and 2-tridecanone, and one lactone: γ -octalactone, were identified as the main mutton odor contributors (Caporaso et al., 1977; Dimick et al., 1966).

In the acidic fraction of the flavor volatiles of cooked lamb meat, branched medium-chain acids (4-methyloctanoic and 4-methylnonanoic acids) and unsaturated C_8 - C_{10} acids seemed to have the characteristic mutton odor (Brennand and Lindsay, 1982; Wong et al., 1975b). In the basic fraction of the volatiles, 12 alkylpyridines, 12 alkylpyrazines, and 2 alkyl thiazoles were isolated from roasted lamb fat (Buttery et al., 1977). Pyridines may contribute to the undesirable mutton odor, but alkylpyrazines are generally associated with

a pleasant roasted food aroma. Pyridines probably arise from the thermal reactions of 2, 4-dienals and NH_3 , with adipose tissue being the major source of the carbonyls through autoxidation of PUFA (Buttery et al., 1977).

Nonvolatile Compounds in Cooked Lamb

Several nonvolatile components exist in lamb meat and may contribute to meaty flavor by themselves or as precursors of flavor volatiles. These include: glucose, fructose, and ribose (Macy et al., 1964); peptides and phosphotases (Batzer et al., 1960); glycoprotein (Batzer et al., 1962); nucleotides, nucleosides, and amino acids (Gorbatory and Lysakovskaya, 1980; Macy et al., 1970a); carnosine, glutathione, taurine, and urea (Macy et al., 1970a); creatine and creatinine (Macy et al., 1970b); and thiamin (Schutte, 1976). These compounds are in the water-soluble fraction of cooked lean meat.

Other Volatile Compounds in Cooked Lamb: Cyclic and Sulfur

Thermal degradation of certain nonvolatiles, water-soluble substances in meat and fat extracts produce components of mutton flavor. These thermal degradation products are mostly aromatic or heterocyclic compounds, and contain oxygen, nitrogen, or sulfur (Chang and Peterson, 1977; Ho, 1980; Nixon et al., 1979; Wasserman, 1979). Oxygen containing heterocyclics, such as furans, furanones, and pyrans, can be derived from the Amadori rearrangement of the sugars; furans are formed by the reaction of glucose and cysteine or cystine (Mabrouk, 1976; Schutte, 1976). Two furan derivatives, 4-hydroxy-5-methyl-3(2H)-furanone and 4-hydroxy-2,5,-dimethyl-3(2H)-furanone, were formed in this

manner in the neutral fraction of volatile flavor from roasted beef (Min et al., 1979). In addition, nitrogen-containing heterocyclics, such as pyrroles, thiazoles, oxazoles, pyridines, and pyrazines, could be formed from the reaction of sugar and amino acids (Koehler et al., 1969; Mabrouk, 1976; Wang and Odell, 1973).

Hydrogen sulfide (H_2S) can be produced from all protein containing foods upon heating, and may affect the flavor of meat. During the cooking of lamb, H_2S (10%) is released from the decomposition of glutathione in the initial heating; the later source of H_2S production (90%) is cysteine and cystine (Cramer and Pepper, 1965; Kunsman and Riley, 1975). Cooked lamb contains higher sulfur levels than beef (Cramer and Pepper, 1965; Kunsman and Riley, 1975). The recommended daily intake of sulfur is 1.0-1.4 g in mature lambs (NAS /NRC, 1968) because of wool production. Hydrogen sulfide from cysteine and cystine reacts with glucose to produce thiophenes, thiazoles and thiazolines (Mabrouk, 1976), with saturated aldehydes to produce thianes, oxathianes, niazines, thiolanes and mercaptoalkyl sulfides, and with unsaturated aldehydes to produce aliphatic methylthio (Boelens et al., 1974). In addition, thiamin is also a precursor of sulfur containing flavor compounds (Schutte, 1976).

Volatile Flavor Compounds Identified from Ovine Subcutaneous Adipose Tissue

The constituents of mutton odor come from the neutral and acidic (4-methyloctanoic acid) fractions of cooked lamb (Caporaso et al., 1977; Nixon et al., 1979; Wong et al., 1975a,b). Most of the flavor volatiles are from the neutral fraction of ovine adipose tissue extracts. Fifty one compounds (Appendix A) have been identified in the neutral

fraction of ovine adipose tissue extracts by GCMS analysis: 26 aldehydes, 12 ketones, 6 lactones, 4 alcohols, and 3 cyclic components (Caporaso et al., 1977). Alkanals, alkenals, and alkadienals represent the oxidized flavor in foods (Forss, 1972) and are usually formed by the oxidation of the PUFA, such as linoleic, linolenic, and arachidonic. However, these FA are available in only small amounts in lamb meat (Ziegler et al., 1976). The C₅-C₉ alkanals represent green, oily, fatty, or tallowy flavors. The C₁₀-C₁₂ alkanals have citrus or orange peel flavors (Badings, 1970). All 2,4-alkadienals from C₇-C₁₀ have fatty or deep-fried odors; trans-2-cis-4-heptadienal and trans-2-cis-4-decadienal have sour and sweet odor, respectively, while the odors of trans-2,cis-4 dienals and trans-2,trans-4 dienals (C₇-C₁₀) were described as rancid and fatty, respectively (Hoffmann, 1962; Hoffmann and Meijboom, 1968). According to Arctander (1969), several ketones and lactones have certain flavor characters: 2-heptanone (spicy), 2-nonanone (fatty), 2-decanone (citrus), 2-undecanone (fruity), 2-dodecanone (waxy), 2-tridecanone (oily), γ -heptalactone (sweet), γ -octalactone (oily), γ -nonalactone (musk), and γ -decalactone (fruity). As stated previously, the flavor of lamb meat is affected by the genetic (breed, gender, slaughter weight and age) and environmental (feeding sources) factors. The derived flavor volatiles from cooked lamb meat can be divided into neutral, acid, basic, cyclic and sulfur compounds.

Previous researchers suggested that the oxidized flavor (neutral volatile compounds) is the major contributor of lamb meat/mutton flavor, moreover the oxidized flavor comes from the oxidation of PUFA in lamb meat. Therefore, the flavor changed by the increasing PUFA in lamb meat has to be investigated.

CHAPTER III

MATERIALS AND METHODS

PREPARATION OF PROTECTED SOYBEAN OIL SUPPLEMENTS

Dried, ground whole soybean seeds (28-50 mesh) were obtained from Tyler Feed Service Inc., (Madisonville, TN). Sodium caseinate from bovine milk was purchased from Erie National Food (Erie, IL). Soy lecithin, an emulsifier, was purchased from a local market in Knoxville, TN, and reagent grade acetaldehyde and diacetyl were purchased from Sigma Chemical Co., (St. Louis, MO).

A batch of supplement was prepared as follows. Water was poured into a steam kettle (Groen M.F.G., Chicago, IL) and heated to 80°C. Sodium hydroxide was added and mixed with heated water to adjust the pH to 11.0. Sodium caseinate and soy lecithin were added to the mixture in the steam kettle. The mixed solution was divided into 4 equal size portions. One portion of the mixture solution was transferred into a meat grinder (Cryovac, Inc., Duncan, SC), dried ground full fat soybeans were added and ground and mixed with the solution for 30 min. Acetaldehyde was added into the mixture, and the mixture ground and mixed for 1-2 min. This procedure was repeated for the other batch, except diacetyl was added to the mixture after being ground and mixed. Treated soybean supplements were cooled at room temperature and ground in a Hobart Model 84142 grinder (Hobart, Co., Troy, OH) through a 0.625 cm screen. Ground supplements

were packed in plastic bags and stored no longer than three weeks at 5°C until used.

EXPERIMENTAL DESIGN AND INGREDIENTS OF FEEDING SUPPLEMENTS

Eighteen weaned feeder lambs, of mixed breed were assigned in a completely random design to one of three feeding treatments: (1) the control supplement containing ground soybeans without additive; (2) the acetaldehyde supplement containing ground soybeans treated with acetaldehyde; (3) the diacetyl supplement containing ground soybeans treated with diacetyl. Six lambs from each treatment were assigned to two pens of three lambs each, and the two pens were fed the same dietary supplement. The pens were located at the Cherokee Farm of The University of Tennessee Agricultural Experiment Station.

All 18 lambs were dewormed and given appropriate vaccinations prior to the start of feeding. An adjustment period (14 days) was used to adapt the lamb to new feeding facilities and to allow recovery from the stresses associated with weaning. The total diet contained a basal diet (Appendix B) plus a supplement treatment, which was fed for 9 weeks. The percentage of each ingredient in the three dietary supplements (control, acetaldehyde, and diacetyl) are shown in Table 4; the control supplement had ground, full-fat soybeans at the same level of soybeans present in the treated supplements.

Table 4 -- Ingredients of different diets fed to experimental lambs

Ingredient	Supplements		
	Control (%)	Acetaldehyde (%)	Diacetyl (%)
Cracked corn	63.3	63.3	63.3
Alfalfa pellets	20.0	20.0	20.0
Rolled oats	5.0	0.5	5.0
Mineral Salt	0.6	0.6	0.6
Limestone	0.4	0.4	0.4
Antibiotic	0.05	0.05	0.05
Soybean (48% CP ^a)	10.0	0.0	0.0
AC ^b treated soybeans	0.0	10.0	0.0
DA ^c treated soybeans	0.0	0.0	10.0

^aCrude protein.

^bAcetaldehyde.

^cDiacetyl.

CHEMICAL COMPOSITION OF FEEDING SUPPLEMENTS

The concentrations of moisture, fat, and protein in each feeding supplement (control, acetaldehyde, and diacetyl) were determined by the following methods.

Moisture Content

The moisture content in each supplement (control, acetaldehyde, and diacetyl) was determined by the AOAC (1995) method 4.1.02. Two (2.0000) gram of each supplement was used, and then the procedure described later under the moisture section in chemical composition of LD muscle and fat samples was followed.

Fat Content

The total lipid content in each supplement was estimated by a modified procedure of Melton et al. (1979). Ten (10.00) gram samples of the control supplement (ground whole soybeans) were extracted, but 20.00 g samples of the acetaldehyde treated supplement and the diacetyl treated supplement were extracted. The procedures described later under the total lipid content section in chemical composition of LD muscle and fat samples then were followed.

Protein Content

The protein content of each supplement was determined by a modified AOAC (1995) Kjeldahl method 4.2.05. Two (2.0000) gram of each ground supplement was

delivered into a 250-mL digestion tube. Two Kjeltabs and 15.0 mL of sulfuric acid (93-98% H₂SO₄) were added in the digestion tube.

Digestion of protein in supplements. A Kjeltec 1015 digester (Tecator, Inc., Herndon, VA) was preheated to 420°C before the digestion tubes were placed on the digestion unit. The digestion tubes with the prepared samples were put into the digester tube stand, and the exhaust manifold was fitted on the top of the tube stand. The stand with the digestion tubes and exhaust manifold was placed in the preheated digester (420°C). The sample in each digestion tube was digested for 3 to 5 min with maximum air flow through the exhaust manifold, and then the air flow was adjusted until fumes were just contained. After the digestion (45 min) of samples, air flow through the exhaust manifold was increased and the stand with the digestion tubes and exhaust manifold was removed from the digester, which was placed in the cooling unit.

Distillation of digested protein in supplements. A Kjeltec 1026 distillation unit (Tecator, Inc., Herndon, VA) was prepared as follows: (1) the levels of alkali and water in the unit were filled; (2) the power switch was turned on; (3) the cold water tap was turned on; (4) the STEAM button was pressed; (5) the WATER button was pressed two lines to make sure that the tubing was completely filled with water; (6) the ALKALI, DELAY, AND STEAM times were set to 2, 0.2, and 3.6 min, respectively; (7) the safety door was opened and the AUTO/MAN button was pressed for automatic operation. Finally, an aliquot (25 mL) of saturated boric acid and 1-2 drops indicator solution (phenolphthalein) were delivered into a 125-mL Erlenmeyer receiving flask. After the digested samples in the digestion tubes were cooled, the cooled digestion tube and a receiving flask were

placed in the distilling unit. The distillation continued until the STEAM lamp was on steady and beeper sounded. Both the digestion tube with residues and the receiving flask were removed from the distilling unit, and the solution in the receiving flask was further analyzed.

Titration and calculation of digested and distilled protein in supplements. The solution in the receiving flask was titrated with 0.1 N HCL standard to a steel gray or nearly colorless endpoint, and the volume of HCL used was recorded. The percent concentration of protein was calculated by following formula:

$$P_{\text{Protein}} (\%) = \frac{[(14.0)(\text{mL HCl sample} - \text{mL HCl blank})(0.1 \text{ N HCl})(6.25)]}{(\text{g sample})} \times 100$$

FEEDING TREATMENTS AND PERFORMANCE OF LAMBS

All 18 feeder lambs were individually weighed on two consecutive days at the beginning of the feeding, and then weighed at 14 day intervals to measure the weight gained by each lamb after the adjustment period (14 days). The weight of feed eaten per day in each treatment group of lambs was measured throughout the feeding period, and the amount of daily feed offered per lamb was adjusted to assure the experimental lambs were on full feed during the 9 week feeding period. Lambs were weighed individually

each week, and an average weekly weight gain (kg) by each lamb was calculated across the feeding period. From the data of feed intake and the chemical composition of the three feed supplements, an average daily supplement intake (g), fat intake (g), and level of each C18 unsaturated fatty acid (oleic or C18:1, linoleic or C18:2, and linolenic or C18:3) intake were estimated. In addition, tocopherol (alpha, gamma, and delta tocopherol) daily intake (mg) per lamb also was estimated.

LAMB SLAUGHTER AND SAMPLING

After the 9 week feeding period, lambs were transported from Cherokee Farm to the Food Science and Technology Department abattoir at The University of Tennessee, Knoxville. The lambs were slaughtered under USDA inspection. The hot carcass weight was determined for each lamb, and the carcass was stored at 5 to 6 °C for 48 hr prior to collection of other carcass data.

Four different types of fat (back, kidney, intramuscular, and intermuscular fat) were collected from each lamb for fatty acid analysis. Approximately 100 g each of back (over the pelvic area) and kidney fat were obtained, put into separate Whirl-Pak polyethylene bags (NASCO Inc., Fort Atkinson, WI), sealed, and stored at -18°C until powdered. The longissimus (LD) muscle from the 5th to the 7th rib was collected from both sides of each lamb carcass, and the fat on the outside of the LD muscle was trimmed for the intermuscular fat sample. The marbling of the LD muscle was the intramuscular fat sample. The intermuscular fat and LD muscle samples were packed in separate

polyethylene bags, sealed, and stored at -18°C until powdered. Each of four types of samples were frozen in liquid nitrogen and powdered by a Waring Blender in a 0.9-L stainless blending jar. Each powdered sample was stored separately less than 3 months in a sealed polyethylene bag at -18°C until analyzed.

The 12th rib loin chop from each side of each carcass was obtained for flavor volatile analysis and tocopherol analysis in the LD muscle. The two loin chops were vacuum packaged in a single barrier plastic bag (Cryovac, Inc., Duncan, SC) using a Koch Vacuum Packaging Machine (Koch Supplies Inc., Kansas City, MO). The packages were stored at -18°C approximately 6 months before being analyzed.

CHEMICAL COMPOSITION OF LD MUSCLE AND FAT SAMPLES

The moisture content and the total lipid content of the LD muscle from the 5th to 7th rib of each lamb were determined. The total lipid content in the back, kidney and intermuscular fat were analyzed also. The fatty acid compositions of total lipids of the back, kidney and intermuscular fat samples and of the nonpolar and polar lipids from the LD were measured. The methods used for all the analyses were as follows.

Moisture Content

The moisture content in the LD muscle was determined by the AOAC (1995) method 4.1.02. A sample (2.0000 g) of the frozen, powdered the LD muscle was spread evenly in a dry, preweighed aluminum dish (50 mm diameter x 40 mm deep) with a lid.

The wet sample and dish were dried in a NAPCO Model 5831 vacuum oven (NAPCO Science Co., Tualatin, OR) at 95-100°C under 100 mm Hg pressure for 6 hr or more to a constant weight. The dried samples were cooled in a desiccator under vacuum and reweighed. Percentage moisture in the sample was determined from weight lost during drying.

Total Lipid Content

Total lipids were extracted and quantified by a modified procedure of Melton et al. (1979). The modification was the addition of butylated hydroxytoluene (BHT) at 0.12-g / 915-ml CHCl_3 used for the extraction. Five (5.0000) gram samples of back, kidney, and intermuscular fat were extracted, but 50.00-g powdered LD muscle sample was extracted. Each sample was homogenized in a high speed Waring blender with 130 ml of methanol for 5 min. Sixty five milliliter of BHT- CHCl_3 (0.12-g BHT/915-mL CHCl_3) solution was added to the homogenized sample and it was blended for 5 min. Then another 65-mL of BHT- CHCl_3 was added and the mixture blended for 20 sec. Finally, 65-mL of an aqueous solution containing 1.5-g zinc acetate was added, and the mixture blended for an additional 10 sec. The homogenate was filtered through a Whatman #1 filter paper (11.0-cm diameter) in a Buchner funnel with suction into a filter flask. The solid residue with the filter paper was reblended with 100-mL BHT- CHCl_3 solution for 5 min, and then filtered through the Whatman filter paper into the filter Flask. The blender jar was rinsed with 75-mL BHT- CHCl_3 solution which was filtered into the filter flask. All of filtrate was poured into a 500-ml graduated cylinder. The filter flask was rinsed with 25-mL

methanol, which was added to the filtrate in the cylinder. The filtrate in the graduated cylinder was flushed with nitrogen gas, tightly covered with plastic film and stored at 5 to 6°C for 24 hr. The volume of chloroform layer (bottom layer) in the graduated cylinder was recorded. The cylinder contents were poured into a 500-mL separatory funnel and allowed to separate for 2 hr at 5 to 6°C. The chloroform layer (bottom) was transferred into a 500-mL round bottom flask which was flushed with nitrogen gas, covered and stored at 5 to 6°C for further analysis.

The total lipid content in the each sample was determined as follows. For each sample, two clean 50-ml beakers were dried in an oven (100°C) for 30 min, allowed to cool to room temperature in a desiccator and weighed. Ten milliliter of the sample chloroform layer was pipetted into each beaker, and the chloroform was evaporated under a hood overnight. Each beaker containing a lipid residue was dried in the 100°C oven for 30 min, cooled to room temperature in the desiccator and weighed. The total lipid content of each sample was calculated from the following equations:

$$\text{Total lipid (\%)} = \frac{(Y)(Vol.)}{(10 \text{ mL})(W)} \times 100$$

where:

Y = weight (grams) of lipid in 10-mL chloroform;

Vol. = total volume (mL) of chloroform layer, and

W = sample weight (gram) extracted.

The chloroform extract in the 500-mL round bottom flask was concentrated under

vacuum at 55°C in a rotary evaporator. The residue was dissolved in 25-mL chloroform, transferred into a 25-mL Erlenmeyer flask, flushed with nitrogen gas, tightly covered and stored in a freezer (-18°C) for further analysis.

Fatty Acid Analysis

The fatty acid composition of the total lipids extracted from the back, kidney, and intermuscular fat of each lamb was determined. The total lipids extracted from the LD muscle of each lamb were separated into nonpolar and polar lipid fractions prior to analysis of the fatty acid composition of each lipid fraction.

Lipid content in concentrated extracts. The weight of total lipids per 1.00 mL of concentrated extract from each back, kidney, and intermuscular fat sample and from the LD muscle of each lamb was determined. An aliquot (1.00 mL) of each concentrated extract was pipetted into each of two dry preweighed, 50-mL beakers and was dried overnight under a hood. Each beaker and residue were dried at 100°C for 30 min prior to being cooled in a desiccator and weighed. The average weight (g) of total lipids per milliliter of each concentrated extract was calculated.

Fractionation of LD total lipids. A volume of each concentrated extract containing 0.5000 g of total lipid from each LD muscle was pipetted into a 125-mL Erlenmeyer flask, and the contents dried under vacuum at 55°C on a rotary evaporator. The residue was dissolved in 10-mL hexane:diethyl ether, 92:8, v/v (HEX:DEE). A Bond Elute 5-g silica cartridge (Phenomenex, Inc., City State) was conditioned by elution of 10 mL of HEX:DEE through it under vacuum. The 10.00 mL of HEX:DEE containing 0.5000-g

total lipids was passed through the silica cartridge under vacuum with the eluent collected in a 125-mL Erlenmeyer flask. The nonpolar lipids were eluted completely by four 10-mL portions of HEX:DEE with all of the eluent collected in the Erlenmeyer flask. The polar lipids (phospholipids) were eluted from the silica cartridge by 40-mL methanol with the eluent collected in a second 125-mL flask. An aliquot (1 mL) of 0.01% BHT in methanol was added to the polar fraction to hinder oxidation.

Preparation of fatty acid methyl esters (FAME). FAME were prepared according to AOCS (1983). For each back, kidney, and intermuscular fat sample, a volume of the concentrated extract containing exactly 100 mg total lipids was pipetted into a 125-mL flask and dried under vacuum at 55°C in a rotary evaporator. An aliquot (4 mL) of sodium hydroxide (0.5 N) in methanol and a few glass boiling beads were added to the flask. The flask was attached to a condenser and its contents boiled under reflux for 10 min to saponify the lipids. Boron trifluoride (14%) in methanol (5.0 mL) was added to the flask and allowed to react for 2 min to esterify the fatty acids. Pentane (7.00 mL) was added through the condenser to the flask and the flask contents heated under reflux for 1 min. The flask was removed from the heat, cooled and separated from the condenser. The pentane layer containing the FAME was floated in the neck of the flask by adding saturated sodium chloride (NaCl) solution. The FAME layer (top layer) was collected by pipet and transferred into a vial. A small amount of anhydrous sodium sulfate (Na_2SO_4) was added to the vial to absorb the residual water from the FAME. The vial was flushed with nitrogen, sealed with a cap and stored at -18°C until analyzed by gas chromatography. Part (100 mg) of the nonpolar lipids and all of the phospholipids

fraction from each LD muscle were derivatized to FAME in the same way as described for the total lipids. The FAME of both nonpolar lipids and phospholipids from each LD muscle were stored in nitrogen flushed, sealed vials at -18°C until analyzed.

Gas chromatographic analysis of fatty acids. The FAME were analyzed with a Shimadzu Model GC-9A gas chromatograph equipped with an automatic injection system Model AOC-9 (Shimadzu Corporation, Kyoto, Japan). A 0.25-mm i.d. by 30-m long fused silica SP-2330 capillary column (Supelco, Inc., Bellefonte, PA) was used to separate the methyl esters, which were detected with a flame ionization detector (FID). The injection temperature was 250°C, and the column temperature was programmed from 130°C to 220°C at 2°C/min. Helium was the carrier gas with a flow rate at 50 mL/min, and a split ratio of 30:1. The area of the peak corresponding to individual FAME were recorded using a Shimadzu data processor, Chromatopac Model CR501, interfaced with an IBM personal computer. All chromatograms were stored using the Chromatopac Data Achieve Utility version 3.1 software (Shimadzu Scientific Instruments, Inc. Columbia, MD). Different fatty acid methyl ester standards were analyzed under the same conditions as the sample methyl esters to determine the retention times of individual FAME. The identification of sample individual FAME was achieved by matching the retention time of the sample peak with that of the standard peak. Quantitative analysis of the sample FAME was done as follows. Standards containing known weight percentages of individual FAME, present in levels similar to those in the samples, were analyzed by GC, and the correction factors relative to C16:0 were calculated according to the AOCS (1983) method for fatty acid analysis. The area of individual sample FAME was corrected using

its correction factor (Appendix C). The relative weight percentages of the different fatty acids in each sample were then calculated using their corrected areas (AOCS, 1983).

TOCOPHEROL ANALYSIS IN BACK FAT AND LD MUSCLE SAMPLES

The levels of α - and γ -tocopherols in the back fat sample and in the LD muscle sample from the 12th rib of each lamb were determined. From the concentrated total lipid extract of each sample, an aliquot containing exactly 1.0000 g of total lipids was pipetted into a 125-mL Erlenmeyer flask, and the flask contents dried under vacuum at 55°C on a rotary evaporator. The residue was dissolved in 4.00-mL hexane and the sample was filtered through a Nylon 0.45 mm disk using a disposable syringe (Becton Dickinson and Co., Rutherford, NJ). The filtered solution was collected in a brown bottle, which was flushed with nitrogen, sealed, and stored at -18°C until high performance liquid chromatography (HPLC) analysis.

The α - and γ -tocopherols were analyzed by the method of Carpenter (1979) on a Waters' HPLC equipped with a U6K injector, a Model 510 pump, and a Shimadzu Fluorescence HPLC Monitor RF-530 (Shimadzu Corporation, Kyoto, Japan). A 4 mm x 30 cm μ -Porasil column (10 μ m silica, from Phenomenex) with a mobile phase of 1.5 % isopropyl alcohol (IPA) in hexane at a flow rate of 1.5 mL/min, was used to separate the tocopherols. Tocopherol peak areas were measured by a Shimadzu Model C-R6A Chromatopac data processor, after tocopherols were detected at an excitation wavelength of 295 nm and emission wavelength of 330 nm.

Both α - and γ -tocopherol standards (Sigma Chemical Co., St. Louis, MO) were analyzed under the same conditions as samples. Linear calibration curves between HPLC peak areas and amounts of tocopherol analyzed were prepared for each tocopherol. Both α - and γ -tocopherols were identified in each sample from its HPLC chromatogram by matching retention times with those of the standards. The concentration of each tocopherol was calculated in each back fat samples ($\mu\text{g/g}$ total lipids) and each LD muscle sample ($\mu\text{g/g}$ lean tissue).

FLAVOR VOLATILE EXTRACTION AND ANALYSIS

A loin chop from the 12th rib area of one side of each lamb was broiled to an internal temperature of 75°C on a electric broiler (Farberware Co., Yonkers, NY). The LD muscle was removed from the broiled chop, trimmed of outer fat and weighed.

Flavor volatiles were extracted from the cooked LD muscle portion by the steam distillation extraction (SDE) method of McLeod and Cave (1975) as modified by Melton et al. (1993) in the following manner. A trimmed, broiled LD muscle sample (26.4 to 89.9 g) was blended with 650-mL hot (60 to 70°C) HPLC grade water and 0.2 mL of each of two internal standards: methyl laurate (1.00 mg/mL) and methyl myristate (1.00 mg/mL) (Sigma Chemical Co., St. Louis, MO). The mixture was blended in a 1.9-L glass container for 5 min on grind speed of an Osterizer commercial blender (Appliance & Electric Service Co., Knoxville, TN). The blended sample was transferred to a 1-L round bottom flask with an inlet side tube extending near to the bottom center of the flask. The

side tube was attached to a nitrogen supply, the flask was attached to the SDE apparatus and was immersed in a 128°C silicone oil bath. A 125-mL Erlenmeyer flask containing 45-mL CH_2Cl_2 as the volatile extracting solvent was attached to the SDE apparatus and CH_2Cl_2 heated to boiling on a corning PC 35 hot plate (Corning Glass Works Inc., Corning, NY) at a setting of 2. Nitrogen gas was bubbled at a slow rate through the homogenized sample mixture and passed over a dry-ice-ethanol cold trap and out of SDE system, bubbling through HPLC grade water. Dry ice was added to the cold trap every 15-20 min to maintain its temperature at -60°C. From the point that the flask containing the muscle homogenate was inserted into the hot oil bath and the heat was applied to the CH_2Cl_2 , volatiles were extracted 2.5 hr. It required 30 min heating for the flavor volatiles from the food homogenate to be converted to a gaseous vapor which was extracted by the vaporized CH_2Cl_2 . After extraction and cooling of the flavor extract, 1 mL of pyrogallol solution (0.1 mg/mL methanol) was added to each extract to prevent oxidation of the volatiles.

The extract was dried with anhydrous sodium sulfate and concentrated to 0.2 mL by the gas entrainment method of MacLeod and Cave (1975) as described by Melton et al. (1993). The concentrated extract was stored under nitrogen in a sealed glass vial at -18°C until analyzed.

Qualitative Analysis of Lamb Meat Flavor Volatile by GCMS

Positive identification of each treatment group flavor volatile was made after analysis by gas chromatography and mass spectrometry (GCMS). A volume of 0.1 mL of

each extracted solution in the same treatment group (0.6 mL) was delivered into a collection tube and reconcentrated to 0.2 mL by the gas entrainment method of MacLeod and Cave (1975) as described by Melton et al. (1993). The reconcentrated extract was analyzed with a Shimadzu Model QP1000 mass spectrometer and GC-9A gas chromatograph (Shimadzu Corporation, Kyoto, Japan) equipped with a carbon dioxide cryogenic unit. A 60-m long (two 30-m columns joined by butt connector) by 0.25 mm i.d. fused silica SP-2330 column (Supelco, Inc., Bellefonte, PA) was used to separate the flavor volatiles. The injection temperature was 250°C and the column temperature was programmed from 25°C to 50°C at 10°C/min, and then from 50 to 230°C at 3°C/min and held at 230°C for 25 min. Mass spectra of the flavor volatiles in each treatment group were obtained at a gain 4.5, an ion source temperature 250°C, and an energy level of 70 eV with a splitless mode of injection. Positive identification of a flavor volatile component in a sample was determined by matching the sample mass spectrum and retention time of with those a known compound (standard) analyzed under the same GCMS conditions. The retention time of the volatile from GC-FID analysis of the sample also was the same as that of the known compound (standard) from GC-FID analysis used for quantitative analysis described later.

For some volatiles, tentative identification was made by matching its sample peak mass spectrum with that of a known compound through a computerized search of a mass spectra library. A tentative identification was a match of spectra with a similarity index of 70 or greater. Also, a peak in any sample GC chromatogram with the same retention time as that of a known volatile was tentatively analyzed as that volatile.

Quantitative Analysis of Lamb Meat Flavor Volatile by GC

Each flavor volatile extract from the individual lamb meat sample was analyzed with a Shimadzu Model GC-9A gas chromatograph (Shimadzu Corporation, Kyoto, Japan), which was equipped with the same column as described for GC-MS. The volatiles were detected with a flame ionization detector (FID). The injection temperature was 250°C and the column temperature was programmed from 50°C to 230°C at 3°C/min. Helium was used as the carrier gas with a flow of 50 mL/min with a 30:1 split. The area of each peak corresponding to individual flavor volatile was recorded by a Shimadzu data processor, a Chromatopac Model CR501 interfaced with an IBM personal computer. All chromatograms were stored by the Chromatopac Data Archive Utility version 3.1 software (Shimadzu Scientific Instruments, Inc. Columbia MD). The volatiles in the sample were identified by different flavor volatile standards injected into the column under the same conditions as the sample flavor volatile to determine and compare the retention time of each individual flavor volatile from GCMS data. Quantitative analysis of the sample flavor volatile was done as follows. The relative weight percentages of the different flavor volatile in each sample were then calculated from the GC chromatogram using the corrected areas.

STATISTICAL ANALYSIS

Statistical analyses were performed using the general linear model (GLM) method (SAS Institute, Inc., 1985) for each dependent variable. The dependent variables included

levels of moisture and lipids in the LD muscle, total lipids contents in the different fat samples (intermuscular, back fat, and kidney fat), concentration of individual fatty acids in nonpolar and polar LD lipids and each fat sample, tocopherol levels in back fat and LD muscle and levels of each volatile in cooked LD muscle. Each of the dependent variables including each fatty acid level in the nonpolar and polar lipids of the LD muscle and the fat samples, were analyzed statistically as a function of treatment ($n=3$) or type of dietary supplements (control, acetaldehyde, and diacetyl) fed to the lambs as shown in Table 5. Significant differences among treatments for least-squares means for the levels of each dependent variable were separated by the PDIFF option (SAS Institute Inc., 1985). Standard errors of the treatment least-squares means for each dependent variable were obtained also.

The level of each fatty acid also was analyzed as a function of treatment, sample site and their interaction as shown in Table 6. Significant differences among sample sites for least-squares means of the concentration of each fatty acid were identified by the PDIFF option as described previously. Standard errors for the sample site least-squares means also were obtained.

For the flavor volatiles, the concentration of each volatile was analyzed by GLM as a function of treatment as shown in Table 5. Least-square means and the standard error of the mean were obtained for volatile concentrations in each treatment. Significantly different treatment least-square means were determined by orthogonal comparisons as shown in Table 7.

Table 5 -- Analysis of variance for dependent variables as a function of treatment or the type of supplements (control, acetaldehyde or diacetyl) fed to the lambs

Source	Degree of freedom
Treatment	2
Error	15
Corrected Total	17

Table 6 -- Analysis of variance for each fatty acid level as a function of treatment, in different fat sampling sites (LD nonpolar and polar lipids, intermuscular, kidney, and back fats) and their interaction

Source	Degree of freedom
Treatment (T)	2
Sites (S) ^a	4
T x S	8
Error	75
Corrected Total	89

^aSome fatty acids were present in only 2 or 3 sites reducing degrees of freedom for sites, T x S, error, and total sources accordingly.

Table 7 -- Orthogonal comparisons for separation of differences among supplements for flavor volatile concentrations

Comparison	Supplement (Treatment)		
	Control	Acetaldehyde	Diacetyl
C1	+2	-1	-1
C2	0	+1	-1

CHAPTER IV

RESULTS AND DISCUSSION

CHEMICAL COMPOSITION OF FEEDING SUPPLEMENTS

The percentage concentrations of moisture, fat, and protein in three different feeding supplements (control, acetaldehyde, and diacetyl) are presented in Table 8. On a dry matter basis, the supplements had similar protein levels (48.6 to 50.0%) and fat levels (15.5 to 17.4%).

PERFORMANCE OF LAMBS

The average weekly weight gain of the lambs fed each supplement during a 9 wk feeding study are presented in Table 9. No differences in weekly weight gain ($P>0.05$) existed among the lambs fed the different supplements. Lambs ($n=18$) weighed an average of 35.0 ± 7.3 kg at the beginning of the feeding trial and 49.7 ± 7.0 kg at slaughter or after being fed a supplement and basal diet for 9 weeks.

The average amount of each supplement eaten daily by a single lamb is also given in Table 9. The estimated amounts of dietary fat intake, intakes of tocopherol (alpha, gamma, and delta) and intakes of C18 unsaturated fatty acids from each supplement are presented also in Table 9. Besides the supplement, lambs fed the control supplement

Table 8 -- Chemical composition^a of different soybean supplements

Chemical Composition	Supplements		
	Control ^b	Acetaldehyde	Diacetyl
Moisture ^a (%)	10.63	61.12	60.70
Protein ^a (%)	43.40	19.43	19.29
Fat ^a (%)	15.42	6.71	6.82

^aWeight % wet basis.

^bGround full fat soybeans plus casein (4.2 to 1 ratio).

Table 9 -- Means for weight gain of lambs fed different supplements, supplement intake and estimated intakes of fat, tocopherols and unsaturated C18 fatty acids from supplements eaten by lambs during feeding trial

Item	Supplements		
	Control	Acetaldehyde	Diacetyl
Weight gain (kg/lamb/week)	1.68	1.80	1.73
Supplement intake (g DM ^a /lamb/day)	199.42	206.06	208.30
Supplement fat intake (g/lamb/day)	34.41	35.57	36.14
<u>Tocopherols isomer intake^b</u>			
Alpha-tocopherol (mg/lamb/day)	3.46	3.91	3.97
Gamma-tocopherol (mg/lamb/day)	30.27	31.34	31.80
Delta-tocopherol (mg/lamb/day)	9.32	9.56	9.71
<u>C18 fatty acid intake^b from supplement</u>			
Oleic acid (C18:1) (g/lamb/day)	6.91	7.11	7.20
Linoleic acid (C18:2) (g/lamb/day)	18.69	19.32	19.63
Linolenic acid (C18:3) (g/lamb/day)	2.59	2.68	2.74

^a Dry matter basis; control supplement was only the dry matter found in the ground full-fat soybeans and casein but not the other components found in the other supplements.

^b Calculated intake based on average composition in crude soybean oil.

consumed daily 0.99 kg of coarse ground corn basal diet (Appendix B) and 0.33 kg of hay; lambs fed the acetaldehyde supplement consumed daily 0.996 kg coarse ground corn diet and 0.363 kg hay; and lambs fed the diacetyl supplement consumed daily 1.00 kg coarse ground corn and 0.373 kg hay.

CHEMICAL COMPOSITION OF LD MUSCLE AND FAT SAMPLES IN LAMBS FED DIFFERENT DIETARY SUPPLEMENTS

The average moisture levels of the LD muscles of lambs fed different dietary supplements are presented in Table 10. There were no differences in the moisture contents in the LD muscle of lambs fed different dietary supplements ($P > 0.05$).

The average concentrations of total lipids in the LD muscle and at different fat depots of lambs fed different dietary supplements also are shown in Table 10. There was no difference ($P > 0.05$) in the total lipid contents in the LD muscle between the lambs fed the control supplement and those fed the two treated (acetaldehyde and diacetyl) supplements; however, lambs fed the diacetyl supplement had a lower amount ($P < 0.05$) of total lipids in the LD muscle than those fed the acetaldehyde supplement. No significant differences in the total lipid contents were found among the lambs fed different supplements for back fat, kidney fat or intermuscular fat samples (Table 10).

Results of this study differ from those published for a formaldehyde-treated, protected fat supplement for cattle. According to Garrett et al. (1976), steers fed the formaldehyde treated supplement were fatter at slaughter those fed the basal diet because

Table 10 -- Least-squares mean moisture level^a of the longissimus (LD) muscle and mean levels^{a,b} of total lipids in the LD muscle and different fat samples from lambs fed different dietary supplements

Component Sample site	Supplements		
	Control	Acetaldehyde	Diacetyl
<u>Moisture^c</u>			
LD muscle	74.28 ± 0.24	74.44 ± 0.23	74.91 ± 0.23
<u>Total lipids^c</u>			
LD muscle	3.74ab± 0.61	4.58a± 0.61	2.63b± 0.61
back fat	67.10 ± 2.25	64.50 ± 2.25	67.95 ± 2.25
kidney fat	79.76 ± 1.80	79.19 ± 1.80	79.53 ± 1.80
intermuscular fat	70.55 ± 1.17	72.28 ± 1.17	69.87 ± 1.17

^aLSmeans ± standard error (n=6).

^bLSMeans in a row followed by unlike letters are different ($P < 0.05$).

^cWeight %, wet basis.

the protected supplement contained an additional fat source. However, in the feeding study of Garrett et al. (1976), a much greater amount of the supplement, and thus the supplement fat, was fed than was fed in this study.

Fatty Acid Composition of LD Muscle Nonpolar Lipids

The composition of fatty acids in nonpolar lipids of LD muscle are shown in Table 11. The following fatty acids were identified: capric (C10:0), lauric (C12:0), myristic (C14:0), pentadecanoic (C15:0), isomer of hexadecanoic (C16:0I), palmitic (C16:0), palmitoleic (C16:1c), margaric (C17:0), margaroleic (C17:1), stearic (C18:0), oleic (C18:1), linoleic (C18:2), linolenic (C18:3), eicos-9-enoic (C20:1), arachidonic (C20:4), eicosa-5,8,11,14,17-pentaenoic (C20:5), and docosa-7,10,13,16,19-pentaenoic (C22:5) acids. These fatty acids were divided into three major groups: saturated (SFA), monosaturated (MUFA), and polyunsaturated (PUFA) fatty acids.

In the SFA (C10:0, C12:0, C14:0, C15:0, C16:0I, C16:0, C17:0, and C18:0), no differences ($P > 0.05$) in the mean concentrations of any of the fatty acids in the nonpolar lipids of lambs fed the control supplement and those fed the two treated (acetaldehyde and diacetyl) supplements were found. In the MUFA (C16:1c, C17:1, C18:1, and C20:1), differences ($P < 0.05$) were found among the concentrations of two of the fatty acids. The mean concentration of C16:1c in the nonpolar lipids of the LD muscle of lambs fed the control supplement was higher than in the same lipids of lambs fed the treated (acetaldehyde and diacetyl) supplements (Table 11). No difference existed in the C16:1c concentration of the LD nonpolar lipids of lambs fed the acetaldehyde supplement and

Table 11 -- Least-squares mean concentrations^{a,b} (weight % of fatty acid methyl esters) of fatty acids in nonpolar lipids of the longissimus muscle from lambs fed different dietary supplements

Fatty acids	Supplements		
	Control	Acetaldehyde	Diacetyl
C10:0	0.17b ± 0.01	0.20a ± 0.01	0.20a ± 0.01
C12:0	0.22 ± 0.05	0.20 ± 0.05	0.23 ± 0.05
C14:0	3.88 ± 0.30	3.55 ± 0.30	3.25 ± 0.30
C15:0	0.18 ± 0.03	0.23 ± 0.03	0.30 ± 0.03
C16:0I	0.37 ± 0.11	0.30 ± 0.11	0.50 ± 0.11
C16:0	27.50 ± 0.53	27.05 ± 0.53	26.28 ± 0.53
C16:1c	0.45a ± 0.05	0.32b ± 0.05	0.30b ± 0.05
C17:0	0.53 ± 0.03	0.57 ± 0.03	0.62 ± 0.03
C17:1	0.45 ± 0.03	0.38 ± 0.03	0.45 ± 0.03
C18:0	14.80 ± 0.61	15.38 ± 0.61	15.33 ± 0.61
C18:1	44.55 ± 0.82	43.35 ± 0.82	43.07 ± 0.82
C18:2	4.80b ± 0.48	6.37a ± 0.48	6.80a ± 0.48
C18:3	0.28b ± 0.02	0.43a ± 0.02	0.45a ± 0.02
C20:1	0.97ab ± 0.05	0.90a ± 0.05	1.07b ± 0.05
C20:4	0.65 ± 0.14	0.57 ± 0.14	0.90 ± 0.14
C20:5	0.12 ± 0.02	0.12 ± 0.02	0.13 ± 0.02
C22:5	0.12 ± 0.02	0.12 ± 0.02	0.17 ± 0.02

^aLSmeans ± standard error (n=6).

^bLSMeans in a row followed by unlike letters are different ($P < 0.05$).

those fed the diacetyl supplement. However, the lambs fed the diacetyl treatment had a higher percentage of C20:1 ($P < 0.05$) in their LD nonpolar lipids than did those fed the acetaldehyde supplement, but neither of these latter group of lambs had levels of C20:1 different ($P > 0.05$) from that present in the lamb group fed the control supplement (Table 11).

In the PUFA (C18:2, C18:3, C20:4, C20:5, and C22:5), there were significant differences in the concentrations of C18:2 and C18:3 in the nonpolar LD lipids of lambs from the different treatments (Table 11). Lambs fed the acetaldehyde or diacetyl supplements had higher levels of C18:2 and C18:3 in the nonpolar LD lipids than did lambs fed the control supplement, but there were no differences in concentrations of C18:2 and C18:3 in the LD nonpolar lipids between lambs fed the acetaldehyde and the diacetyl treatments.

Fatty Acid Composition of LD Muscle Polar Lipids

The composition of fatty acids in polar lipids of LD muscle are listed in Table 12. The following fatty acids were identified: capric (C10:0), myristic (C14:0), pentadecanoic (C15:0), isomer of hexadecanoic (C16:0I), palmitic (C16:0), palmitoleic (C16:1c), trans-9-hexadecenoic (C16:1t), margaric (C17:0), margaroleic (C17:1), stearic (C18:0), oleic (C18:1), linoleic (C18:2), linolenic (C18:3), eicos-9-enoic (C20:1), eicosa-8,11,14-trienoic (C20:3), arachidonic (C20:4), eicosa-5,8,11,14,17-pentaenoic (C20:5), docosa-7,10,13,16,19-pentaenoic (C22:5), and docosa-4,7,10,13,16,19-hexaenoic (C22:6) acids. These fatty acids were divided into three major groups: saturated polar fatty acids (SFAP),

Table 12 -- Least-squares mean concentrations^{a,b} (weight % of fatty acid methyl esters) of fatty acids in polar lipids of the longissimus muscle from lambs fed different dietary supplements

Fatty acids	Supplements		
	Control	Acetaldehyde	Diacetyl
C10:0	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00
C14:0	0.87 ± 0.06	0.98 ± 0.06	0.97 ± 0.06
C15:0	1.45 ± 0.07	1.46 ± 0.07	1.48 ± 0.07
C16:0I	6.98 ± 0.45	6.63 ± 0.45	7.07 ± 0.45
C16:0	13.45 ± 0.37	13.06 ± 0.37	13.45 ± 0.37
C16:1c	4.81 ± 0.27	4.68 ± 0.27	5.42 ± 0.27
C16:1t	1.75 ± 0.15	1.78 ± 0.15	1.82 ± 0.15
C17:0	0.20 ± 0.01	0.22 ± 0.01	0.20 ± 0.01
C17:1	0.93a ± 0.06	0.77b ± 0.09	0.68b ± 0.07
C18:0	10.16 ± 0.32	10.73 ± 0.32	10.53 ± 0.32
C18:1	17.21a ± 0.92	12.98b ± 0.92	13.10b ± 0.92
C18:2	22.10c ± 0.49	27.14a ± 0.49	25.58b ± 0.49
C18:3	0.77 ± 0.04	0.85 ± 0.04	0.80 ± 0.04
C20:1	0.50 ± 0.06	0.38 ± 0.06	0.47 ± 0.06
C20:3	0.83 ± 0.04	0.80 ± 0.04	0.77 ± 0.04
C20:4	13.10 ± 0.46	13.00 ± 0.46	12.98 ± 0.46
C20:5	0.98 ± 0.06	0.95 ± 0.06	0.91 ± 0.06
C22:5	1.80 ± 0.07	1.75 ± 0.07	1.80 ± 0.07
C22:6	0.61 ± 0.08	0.68 ± 0.08	0.58 ± 0.08
UNKS	1.43	1.23	1.35

^aLSmeans ± standard error (n=6).

^bLSMeans in a row followed by unlike letters are different ($P < 0.05$).

monounsaturated polar fatty acids (MUFAP), and polyunsaturated polar fatty acids (PUFAP).

In the SFAP (C10:0, C14:0, C15:0, C16:0I, C16:0, C17:0, and C18:0), no differences ($P > 0.05$) in the mean concentrations of any of the fatty acids in the polar lipids of lambs fed the control supplement and those fed the two treated (acetaldehyde and diacetyl) supplements were found. In the MUFAP (C16:1c, C16:1t, C17:1, C18:1, and C20:1), differences ($P < 0.05$) were found among the concentrations of two of the fatty acids. The mean concentrations of C17:1 and C18:1 in the polar lipids of the LD muscles of lambs fed the control supplement were higher ($P < 0.05$) than in the same lipids of lambs fed the treated (acetaldehyde and diacetyl) supplements (Table 12). No difference ($P > 0.05$) existed in the C17:1 and C18:1 concentrations of the LD polar lipids of lambs fed the acetaldehyde supplement and those fed the diacetyl supplement.

In the PUFAP (C18:2, C18:3, C20:3, C20:4, C20:5, C22:5, and C22:6), there was a significant difference in the concentrations of C18:2 in polar LD lipids of lambs from the different treatments (Table 12). Lambs fed the acetaldehyde or diacetyl supplements had higher levels of C18:2 ($P < 0.05$) in the polar LD lipids than did lambs fed the control supplement; moreover, the lambs fed the acetaldehyde supplement had a higher percentage of C18:2 ($P < 0.05$) in their LD muscle polar lipids than did those fed the diacetyl supplement.

Fatty Acid Composition of Intermuscular Fat Samples

The composition of fatty acids in intermuscular fat are shown in Table 13. The following fatty acids were identified: capric (C10:0), lauric (C12:0), myristic (C14:0), myristoleic (C14:1), pentadecanoic (C15:0), isomer of hexadecanoic (C16:0I), palmitic (C16:0), palmitoleic (C16:1c), margaric (C17:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2), linolenic (C18:3), eicos-9-enoic (C20:1), and arachidonic (C20:4) acids. No differences were found ($P > 0.05$) in the concentration of any of the fatty acids in the intermuscular fat among lambs fed the different dietary supplements.

Fatty Acid Composition of Kidney Fat Samples

The composition of fatty acids in kidney fat are shown in Table 14. The following fatty acids were identified: capric (C10:0), lauric (C12:0), myristic (C14:0), myristoleic (C14:1), pentadecanoic (C15:0), isomer of hexadecanoic (C16:0I), palmitic (C16:0), palmitoleic (C16:1c), margaric (C17:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2), linolenic (C18:3), eicos-9-enoic (C20:1), and arachidonic (C20:4) acids. These fatty acids were divided into three major groups: kidney saturated fatty acids (SFAK), kidney monounsaturated fatty acids (MUFAK), and kidney polyunsaturated fatty acids (PUFAK).

In the SFAK (C10:0, C12:0, C14:0, C15:0, C16:0I, C16:0, C17:0, and C18:0), there was a significant difference in the concentration of C18:0 in the kidney fat of lambs fed the control and two treated (acetaldehyde and diacetyl) supplements (Table 14). The lambs fed the acetaldehyde or diacetyl supplements had a lower percentage of C14:0 and a higher percentage of C18:0 in the kidney fat than did lambs fed the control supplements;

Table 13 -- Least-squares mean concentrations^{a,b} (weight % of fatty acid methyl esters) of fatty acids in intermuscular fat of lambs fed different dietary supplements

Fatty acids	Supplements		
	Control	Acetaldehyde	Diacetyl
C10:0	0.44 ± 0.12	0.21 ± 0.12	0.19 ± 0.12
C12:0	0.53 ± 0.12	0.28 ± 0.12	0.24 ± 0.12
C14:0	9.34 ± 2.51	4.27 ± 2.51	3.79 ± 2.51
C14:1	0.15 ± 0.04	0.07 ± 0.04	0.07 ± 0.04
C15:0	1.04 ± 0.32	0.47 ± 0.32	0.46 ± 0.32
C16:0I	0.35 ± 0.11	0.16 ± 0.11	0.14 ± 0.11
C16:0	26.18 ± 0.68	25.51 ± 0.62	24.56 ± 0.62
C16:1c	1.08 ± 0.30	0.45 ± 0.30	0.46 ± 0.30
C17:0	2.24 ± 0.71	1.02 ± 0.71	1.04 ± 0.71
C18:0	19.34 ± 0.87	20.05 ± 0.79	21.12 ± 0.79
C18:1	40.42 ± 0.72	38.73 ± 0.65	39.51 ± 0.65
C18:2	9.53 ± 2.89	6.89 ± 2.89	6.41 ± 2.89
C18:3	0.97 ± 0.31	0.73 ± 0.31	0.67 ± 0.31
C20:1	2.44 ± 0.82	0.99 ± 0.82	1.17 ± 0.82
C20:4	0.26 ± 0.08	0.17 ± 0.08	0.17 ± 0.08

^aLSmeans ± standard error (n=6).

^bLSMeans in a row followed by unlike letters are different ($P < 0.05$).

Table 14 -- Least-squares mean concentrations^{a,b} (weight % of fatty acid methyl esters) of fatty acids in kidney fat of lambs fed different dietary supplements

Fatty acids	Supplements		
	Control	Acetaldehyde	Diacetyl
C10:0	0.17 ± 0.01	0.15 ± 0.01	0.15 ± 0.01
C12:0	0.17 ± 0.03	0.17 ± 0.03	0.15 ± 0.03
C14:0	3.78a ± 0.23	3.24b ± 0.23	3.07b ± 0.23
C14:1	0.07 ± 0.02	0.09 ± 0.02	0.06 ± 0.02
C15:0	0.39 ± 0.02	0.34 ± 0.02	0.37 ± 0.02
C16:0I	0.14 ± 0.01	0.14 ± 0.01	0.14 ± 0.01
C16:0	23.90 ± 0.54	22.71 ± 0.54	22.65 ± 0.54
C16:1c	0.41a ± 0.05	0.30b ± 0.06	0.21b ± 0.06
C17:0	1.05 ± 0.08	0.86 ± 0.08	1.00 ± 0.08
C18:0	24.94b ± 0.56	27.16a ± 0.56	26.91a ± 0.56
C18:1	39.05a ± 0.60	35.43b ± 0.60	36.26b ± 0.60
C18:2	4.46b ± 0.47	7.65a ± 0.47	7.13a ± 0.47
C18:3	0.50b ± 0.04	0.85a ± 0.04	0.80a ± 0.04
C20:1	0.89ab ± 0.04	0.86b ± 0.04	1.00a ± 0.04
C20:4	0.09 ± 0.01	0.11 ± 0.01	0.12 ± 0.01

^aLSmeans ± standard error (n=6).

^bLSMeans in a row followed by unlike letters are different ($P < 0.05$).

there was no difference ($P > 0.05$) in the concentrations of C14:0 and C18:0 in the kidney fat between lambs fed the acetaldehyde or the diacetyl treatment. In the MUFAK (C14:1, C16:1c, C18:1, and C20:1), differences ($P < 0.05$) were found among the concentrations of two of these fatty acids. The mean concentrations of C16:1c and C18:1 in the kidney fat of lamb fed the control supplement were higher than in the kidney fat of lambs fed treated (acetaldehyde and diacetyl) supplements (Table 14). No differences ($P > 0.05$) existed in either C16:1c and C18:1 concentration between the kidney fat of lambs fed the acetaldehyde supplements and those fed the diacetyl supplement. However, the lambs fed the acetaldehyde treatment had a lower percentage of C20:1 in their kidney fat than did those fed the diacetyl supplement, but neither of these latter groups lambs had levels of C20:1 different ($P > 0.05$) from that present in the lambs fed the control supplement (Table 14).

In the PUFAK (C18:2, C18:3, and C20:4), there were significant differences in the concentrations of C18:2 and C18:3 in the kidney fat of lambs from the different treatments (Table 14). Lambs fed the acetaldehyde or diacetyl supplements had higher levels of C18:2 and C18:3 in the kidney fat than did lambs fed the control supplement, and there were no differences in concentrations of C18:2 and C18:3 in the kidney fats between lambs fed the acetaldehyde and those fed the diacetyl treatment.

Fatty Acid Composition of Back Fat Samples

The composition of fatty acids in back fat of lambs are shown in Table 15. The following fatty acids were identified: capric (C10:0), lauric (C12:0), myristic (C14:0),

Table 15 -- Least-squares mean concentrations^{a,b} (weight % of fatty acid methyl esters) of fatty acids in back fat of lambs fed different dietary supplements

Fatty acids	Supplements		
	Control	Acetaldehyde	Diacetyl
C10:0	0.23 ± 0.02	0.23 ± 0.02	0.25 ± 0.02
C12:0	0.21 ± 0.04	0.14 ± 0.04	0.14 ± 0.04
C14:0	4.05 ± 0.41	3.83 ± 0.41	3.84 ± 0.41
C14:1	0.11 ± 0.04	0.05 ± 0.04	0.12 ± 0.04
C15:0	0.58 ± 0.05	0.56 ± 0.05	0.57 ± 0.05
C16:0I	0.12 ± 0.02	0.11 ± 0.02	0.13 ± 0.02
C16:0	26.77 ± 0.73	27.37 ± 0.73	26.49 ± 0.73
C16:1c	0.48 ± 0.28	0.28 ± 0.31	0.76 ± 0.23
C17:0	1.70 ± 0.30	1.68 ± 0.30	1.50 ± 0.30
C18:0	17.51 ± 0.84	17.82 ± 0.84	17.04 ± 0.84
C18:1	43.09a ± 1.00	40.13b ± 1.00	41.35ab ± 0.06
C18:2	3.77b ± 0.36	6.13a ± 0.36	6.06a ± 0.36
C18:3	0.34b ± 0.04	0.61a ± 0.04	0.57a ± 0.04
C20:1	1.00 ± 0.07	0.98 ± 0.07	1.12 ± 0.07
C20:4	0.14b ± 0.01	0.19a ± 0.01	0.21a ± 0.01

^aLSmeans ± standard error (n=6).

^bLSMeans in a row followed by unlike letters are different ($P < 0.05$).

myristoleic (C14:1), pentadecanoic (C15:0), isomer of hexadecanoic (C16:0I), palmitic (C16:0), palmitoleic (C16:1c), margaric (C17:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2), linolenic (C18:3), eicos-9-enoic (C20:1), and arachidonic (C20:4) acids. These fatty acids were divided into three major groups: back fat saturated fatty acids (SFAB), back fat monosaturated fatty acids (MUFAB), and back fat polyunsaturated fatty acids (PUFAB).

In the SFAB (C10:0, C12:0, C14:0, C15:0, C16:0I, C16:0, C17:0, and C18:0), no differences ($P > 0.05$) in the mean concentrations of any of the fatty acids in the back fat of lambs fed the control supplement and those fed the two treated (acetaldehyde and diacetyl) supplements were found. However, in MUFAB (C14:1, C16:1c, C18:1, and C20:1) percentage of C18:1 was higher in control fed lambs than in lambs fed acetaldehyde supplement. No differences ($P > 0.05$) existed in level of C18:1 in back fat of lambs fed the diacetyl and those fed the other supplements.

In the PUFA (C18:2, C18:3, and C20:4), there were significant differences in the concentrations of C18:2, C18:3, and C20:4 in the back fat of lambs from the different treatments (Table 15). Lambs fed the acetaldehyde or diacetyl supplements had higher levels of C18:2, C18:3, and C20:4 in the back fats than did lambs fed the control supplement. However, there were no differences in concentrations of C18:2, C18:3, and C20:4 in the back fats between lambs fed the acetaldehyde and the diacetyl treatments.

Fatty Acid Levels in Different Fat Sites Affected by Feeding Treated Supplements

Since fatty acid concentrations were in weight percentages, the total concentration of all fatty acids was 100% for any one fat sampling site. A significantly higher percentage concentration for any one fatty acid in any treatment compared to another was, therefore, offset by lower percentages of one or more different fatty acids in the same sampling site. Thus, higher percentage concentrations of C18:2 and C18:3 in the nonpolar LD lipids (Table 11), the kidney fat (Table 14), and the back fat (Table 15) and of higher levels of C18:2 in the polar LD lipids (Table 12) of the lambs fed the acetaldehyde and diacetyl treated supplements generally were accompanied by lower percentages of C18:1 in the respective fat site.

Although no reports were found in which acetaldehyde or diacetyl treated lipid supplements were fed to ruminant animals to alter meat fatty acid composition, a formaldehyde treated lipid supplement containing oil high in linoleic acid was. In these reports, feeding of a formaldehyde treated-casein-safflower oil supplement to ruminant animals resulted in increased levels of C18:2 and decreased amounts of C16:0, C16:1 and C18:1 in the external (subcutaneous) and internal (kidney, omental, and perirenal) body fat of beef steers (Dinius et al., 1975; Faichney et al., 1972; Garrett et al., 1976) or lambs (Scott et al., 1971). In the formaldehyde treated supplement, the C18:2 was protected from biohydrogenation in the rumen by being encased in a protein-formaldehyde complex. Research has shown that the soybean oil in the acetaldehyde and diacetyl treated supplements in this study also is encased in a protein complex and protected from biohydrogenation in the rumen (Dje, 1994).

It is interesting to note that feeding of the formaldehyde-casein-safflower oil supplement increased C18:2 content of veal fat from its normal 3% level to approximately 12% with a simultaneous decrease in C16:0 acid content (Formo et al., 1979). This increase in C18:2 content is greater than that found in this investigation. However, the formaldehyde supplement supplied the total dietary fat requirement for the calves while in this study the treated supplement provided only part of the daily dietary fat requirement; the corn diet provided the rest. The corn in the diet contained 3.22% fat, and each lamb ate an average of 1.00 kg of the diet daily, consuming 32.2 g of fat from the corn diet/day. Each lamb consumed an average of approximately 35 g protected soybean oil from the diacetyl and acetaldehyde treated supplements (Table 9). This is most likely the reason for the lower levels of C18:2 deposited in the lamb fat from this protected oil supplements compared with that deposited in veal fat from the formaldehyde-casein-safflower oil supplement.

Comparison of Fatty Acid Levels in Different Fat Sites

Table 16 shows the fatty acid concentrations in the different fat sampling sites averaged across the different treatments. While intermuscular fat had the highest level of C10:0, and the LD polar lipids and kidney fat the lowest, C10:0 levels in LD nonpolar lipids and back fat samples were not different ($P > 0.05$) from either of these sites. The intermuscular fat had the highest levels of C12:0 and C14:0 while the LD polar lipids had no detectable C12:0 and the lowest amount of C14:0. Concentrations of C12:0 and C14:0 in the LD nonpolar lipids, the intermuscular fat and the back fat were not different ($P >$

Table 16 -- Least-squares mean concentrations^{a,b,c} averaged across supplements for different fat sampling sites from lambs fed the different supplements

Fatty acid	Fat sample site				
	LD nonpolar lipids	LD polar lipids	Intermuscular fat	Kidney fat	Back fat
C10:0	0.19ab ± 0.04	0.10b ± 0.06	0.27a ± 0.04	0.16b ± 0.04	0.24ab ± 0.04
C12:0	0.22b ± 0.04	--	0.35a ± 0.04	0.15b ± 0.04	0.15b ± 0.04
C14:0	3.56b ± 0.67	0.94c ± 0.67	5.81a ± 0.67	3.35b ± 0.74	3.90b ± 0.67
C14:1	--	--	0.11 ± 0.02	0.09 ± 0.02	0.09 ± 0.02
C15:0	0.24d ± 0.09	1.47a ± 0.09	0.66b ± 0.09	0.37cd ± 0.10	0.57bc ± 0.09
C16:0I	0.39b ± 0.12	6.89a ± 0.12	0.21b ± 0.12	0.11b ± 0.14	0.12b ± 0.13
C16:0	26.94a ± 0.65	13.33c ± 0.65	25.42a ± 0.67	22.17b ± 0.72	26.94a ± 0.65
C16:1c	0.36ab ± 0.11	--	0.67a ± 0.11	0.31b ± 0.13	0.49ab ± 0.13
C17:0	0.57cd ± 0.20	0.21d ± 0.20	1.44ab ± 0.20	0.95bc ± 0.22	1.65a ± 0.20
C17:1	0.43b ± 0.11	4.97a ± 0.11	--	--	--
C18:0	15.18d ± 0.76	10.48e ± 0.76	20.16b ± 0.79	24.43a ± 0.84	17.51c ± 0.77
C18:1	43.66a ± 1.04	14.43d ± 1.04	39.55b ± 1.07	35.44c ± 1.15	41.44ab ± 1.05
C18:2	5.98bc ± 0.80	25.03a ± 0.80	7.61b ± 0.80	5.43bc ± 0.89	5.31c ± 0.81
C18:3	0.39c ± 0.08	0.81a ± 0.08	0.78ab ± 0.08	0.60abc ± 0.09	0.50c ± 0.08
C20:1	0.98ab ± 0.21	0.45b ± 0.21	1.53a ± 0.21	0.84b ± 0.24	1.03ab ± 0.22
C20:3	--	0.80 ± 0.02	--	--	--
C20:4	0.71b ± 0.12	13.03a ± 0.12	0.20c ± 0.12	0.09c ± 0.14	0.19c ± 0.13
C22:5	0.12b ± 0.03	1.78a ± 0.03	--	--	--
C22:6	--	0.63 ± 0.04	--	--	--

^aLSmeans ± standard error (n=18).^bWeight percentage of fatty acid methyl esters.^cLSmeans in a row followed by unlike letters are different ($P < 0.05$).

0.05) from each other, but for C14:0 were intermediate in value between those present in the intermuscular fat and LD polar lipids and for C12:0 were lower than that in the intermuscular fat. For C15:0, highest levels were found in the LD polar lipids and lowest levels in the LD nonpolar lipids except for the kidney fat; intermuscular fat and back fat had the highest levels of C16:0, generally the most abundant saturated fatty acid, while LD polar lipids contained the lowest amount. Kidney fat had C16:0 concentrations between, but different ($P < 0.05$) than, those of these other fat sites. Highest concentrations of C17:0 were located in the back fat samples with the exception of intermuscular fat and lowest in LD polar lipids except for LD nonpolar lipids. Levels of C18:0 were significantly different among all fat sites with kidney fat having the highest percentage and LD polar lipids having the lowest concentration.

For the monounsaturated fatty acids, the most abundant, C18:1, was highest in concentration in the LD nonpolar lipids except for the back fat; C20:1 was greatest in level in the intermuscular fat and lowest in the LD polar lipids and the kidney fat, and C16:1c in intermuscular fat was higher than that in kidney fat and was not detected in LD polar lipids and percentages C16:1c in LD nonpolar lipids and back fat were similar to levels of C16:1c in all other sites. On the other hand, LD polar lipids had the highest percentage of C17:1 compared with LD nonpolar lipids and C17:1 was not found in any of the other fat sample sites.

In general, the LD polar lipids contained the highest levels of polyunsaturated fatty acids (C18:2, C18:3, C20:4 and C22:5) of all fat sample sites. The polyunsaturated fatty acids, C20:3 and C22:6 were found only in the LD polar lipids, while C22:5 was found

only in LD nonpolar and polar lipids and was highest in concentration in the LD polar lipids. While C18:2 was the most abundant polyunsaturated fatty acid in each fat sample site; the second most abundant fatty acid was dependent upon the fat site. For example, C18:3 was the second most abundant polyunsaturated fatty acid in the intermuscular fat, kidney fat and back fat, but C20:4 was in the LD nonpolar lipids. The acid, C18:2, was higher in level in the intermuscular fat compared with the back fat, but neither of these fat sample sites had a different level of C18:2 than the LD nonpolar lipids or the kidney fat. For C18:3, intermuscular and kidney fat sites did not have different levels from the LD polar lipid, which had a higher level of C18:3 than LD nonpolar lipids and back fat. Kidney fat did not have a different concentration of C18:3 than the LD nonpolar lipids or the back fat sample, but intermuscular fat had a higher level than these latter two sites. For C20:4, the intermuscular, kidney, and back fat sites had the lowest concentrations, which were not different from each other, and the LD nonpolar lipids had a higher amount of C20:4 than these three sites and less C20:4 than did the LD polar lipids.

In summary, the kidney fat had the lowest amounts of monounsaturated and polyunsaturated fatty acids of all fat samples followed by the external fat sites (intermuscular fat and back fat sites). The intramuscular nonpolar lipid sample generally had higher levels of the monounsaturated fatty acid, C18:1, and polyunsaturated fatty acids than the external fat sites, but lower levels than the LD polar lipids. The iodine value (centigrams iodine per g fat) is a measurement of the unsaturation in a fat and can be calculated from the fatty acid composition (Weiss, 1987). The greater the iodine value for a given fat, the more unsaturated the fat. Based on the fatty acid composition in Table 16,

the iodine values for kidney fat, back fat, intermuscular fat, LD nonpolar lipids and LD polar lipids are 45, 50, 55, 56 and 121, respectively. The fat sites could be ranked in increasing level of fat unsaturation as follows: kidney fat < back fat < intermuscular fat < LD nonpolar lipids < LD polar lipids.

Weiss (1987) reported that the depot fats, such as those surrounding the kidney, are relatively hard fats with a higher melting point than the fatty layers found in the back and belly muscles and other subcutaneous areas, which are softer and have a lower melting point. The kidney fat is the “killing” fat and the fat found in the back and belly muscles and other subcutaneous areas are “cutting” fats (Weiss, 1987). Weiss (1987) reported that for a pig, the “killing” fat had an iodine value of 57-60 while the “cutting” fat had an iodine value of 67-70; this author also reported that, like pork fat, beef and lamb fats occur as cutting and killing fats. The results of this study, generally, are in agreement with the observations of Weiss (1987) in that the lamb kidney fat was more saturated than any of the other lamb fat samples.

However, the lack of a significant difference in C18:2 content between kidney fat and the LD nonpolar lipids is in disagreement with results of several researchers who fed formaldehyde treated oil supplements to ruminant animals (Ackerson et al., 1976; Cook et al., 1970; 1972a, Dinius et al., 1975; Garret et al., 1976.). These latter researchers found that kidney fat of the ruminant animals fed the formaldehyde treated supplement had a higher level of C18:2 than the LD muscle fat. However, in agreement with this study, kidney fat of ruminant animals contained higher levels of C18:0 relative to other fat depots; kidney fat preferentially assimilated the fatty acids of chylomicron triglycerides,

which normally contain high levels of C18:0 in ruminant animals (Duncan et al., 1974). Also, to a certain degree, the location of fat depots in the animal affects the type of fatty acid deposited. The higher melting point (saturated) fatty acids tend to be deposited to a greater extent in the internal fat depots such as the kidney fat than in subcutaneous fat depots such as back fat; the lower melting point (unsaturated) fatty acids are deposited in greater abundance near the skin (Enser, 1991; Rossell, 1992; Sinclair and O'Dea, 1987).

The previous discussion does not explain, however, why the LD polar lipids were so unsaturated compared with LD nonpolar lipids. It has been well documented and reported that the fatty acids of lamb muscle phospholipids (polar lipids) contain higher levels of polyunsaturation than the lamb muscle triglycerides (nonpolar lipids). Lillard (1987) reported that lamb triglycerides contained 44.6% saturated, 50.5% monosaturated and 5.2% polyunsaturated fatty acids while lamb phospholipids had 42.2% saturated, 24.5% monounsaturated and 33.3% polyunsaturated fatty acids. In this study, the LD polar lipids contained a higher level of polyunsaturated fatty acids than the LD nonpolar lipids (42.1 versus 7.2%). Also, the LD nonpolar lipids in this study contained 47.3% saturated fatty acids, 45.0% monounsaturated fatty acids and 7.2% polyunsaturated fatty acids. The LD polar lipids in this study contained 33.4% saturated, 19.9% monounsaturated, and 42.1% polyunsaturated fatty acids. The higher levels of polyunsaturated fatty acids in the lamb LD nonpolar and polar lipids in this study compared with reported literature values are due to their increased deposition from the diacetyl and acetaldehyde treated supplements fed to the lambs.

VITAMIN E (TOCOPHEROLS) LEVELS IN TISSUES OF LAMBS FED DIFFERENT DIETARY SUPPLEMENTS

The α - and γ -tocopherol contents of back fat and the longissimus muscle of lambs fed different supplements are presented in Table 17. Lambs eating either the acetaldehyde or diacetyl treated supplement had ($P < 0.05$) higher levels of α -tocopherol in the back fat than did lambs eating the control supplement. On the other hand, compared with lambs eating the acetaldehyde treated supplement, lambs eating the diacetyl treated supplements had ($P < 0.05$) the highest level of γ -tocopherol in their longissimus muscle. Although not significant at $P < 0.05$, γ -tocopherol was higher in concentration at $P < 0.08$ in the back fat of lambs fed the diacetyl treatment than in the fat of lambs fed the acetaldehyde treatment, and α -tocopherol content was higher in concentration ($P < 0.09$) in the longissimus muscle of lambs fed the diacetyl treated supplement than in that of lambs fed the acetaldehyde treated supplement.

Recently, there have been several reports in which supplemental dietary α -tocopherol acetate has been fed to steers and heifers (Arnold et al., 1992; Garber et al., 1996; Nockels et al., 1996) and lambs (Wulf et al., 1995) to increase the concentration of α -tocopherol in the tissues. Increased levels of the vitamin increase the time period that meat remains bright red in the fresh meat retail case (5-6°C) by hindering the oxidation of oxymyoglobin to metmyoglobin (Arnold et al., 1992; Garber et al., 1996; Wulf et al., 1995). Feeding of 500 I.U. of α -tocopherol acetate to lambs daily increased the amount of α -tocopherol in the longissimus lumborum by 65%, but feeding 1000 I.U. did not

Table 17 -- Least-squares means of tocopherol levels^{a,b} in back fat and the longissimus (LD) muscle of lambs fed different dietary supplements

Site Vitamin	Supplements		
	Control	Acetaldehyde	Diacetyl
<u>Back fat</u>			
α -tocopherol ($\mu\text{g/g}$ lipid)	2.09b $\pm$ 0.24	3.00a $\pm$ 0.22	3.28a $\pm$ 0.22
γ -tocopherol ($\mu\text{g/g}$ lipid)	1.82 $\pm$ 0.25	1.30 $\pm$ 0.23	1.88 $\pm$ 0.23
<u>LD muscle</u>			
α -tocopherol ($\mu\text{g/g}$ lean)	1.47 $\pm$ 0.36	1.30 $\pm$ 0.36	2.21 $\pm$ 0.36
γ -tocopherol ($\mu\text{g/g}$ lean)	0.35ab $\pm$ 0.06	0.24b $\pm$ 0.06	0.44a $\pm$ 0.06

^aLSmeans $\pm$ standard error (n=6).

^bLSmeans in a row followed by unlike letters are different ($P < 0.05$).

increase further the level of α -tocopherol in the muscle (Wulf et al., 1995). Feeding of supplemental dietary α -tocopherol acetate (1000 to 2000 I.U of α -tocopherol) to steers and heifers for 28 up to 67 days increased levels of the α -tocopherol in the different tissues from 25 to 300% depending on the tissue analyzed, the level of the vitamin fed and the length of time fed. The tissues accumulate α -tocopherol relative to the amount and length of time the vitamin is fed (Arnold et al., 1992; Machlin, 1991).

In this study, no effort was made to feed additional tocopherol content to the lambs. However, since the acetaldehyde or diacetyl treated supplements protected the unsaturated fatty acids of the soybean oil from biohydrogenation in the rumen, they possibly could protect the oil-soluble tocopherols from degradation in rumen also. Table 9 shows in this study that lambs ingested an estimated 31.3 mg γ -tocopherol from the acetaldehyde supplement and 31.8 mg γ -tocopherol from the diacetyl treatment compared to 30.3 mg ingested daily by lambs eating the control supplement (Table 9). Results indicate the possibility that, compared with the control and acetaldehyde supplements, the diacetyl supplement protected γ -tocopherol from rumen degradation resulting in increased levels of the vitamin in the tissues. However, more research is needed to confirm this possibility.

The level of α -tocopherol in the back fat of lambs (Table 17) is less than that (5.23-5.56 $\mu\text{g/g}$ tissue) in the subcutaneous fat of beef or dairy steers fed primarily a corn diet and no supplemental dietary tocopherol acetate (Garber et al., 1996). However, the concentration of α -tocopherol in the lamb back fat was closer to the levels (2.6-3.4 $\mu\text{g/g}$ fresh tissue) in the subcutaneous fat of Holstein steers and mixed breed beef steers fed a

corn diet as reported in a another experiment (Arnold et al., 1992). No recent reports were found in which the level of α -tocopherol was reported in lamb back fat or subcutaneous fat.

The level of α -tocopherol in the longissimus (LD) muscle of lambs fed the different supplements (Table 17) is only 37 to 63% of the concentration (3.5 $\mu\text{g/g}$ lean tissue) found in the longissimus lumborum of lambs fed a predominantly corn diet and no supplemental dietary α -tocopherol (Wulf et al., 1995). The reason for this lower level may have been the long frozen storage (approximately one year at -18°C) of the longissimus muscle in an intact rib section of each lamb. Tocopherols are not very stable (Machlin, 1991). However, tocopherol concentrations do differ in concentration among muscles and adipose tissues of the same and different meat animals (Arnold et al., 1992; Garber et al., 1996; Nockels et al., 1996). For example, levels of the same vitamin (2.0-2.2 $\mu\text{g/g}$ fresh tissue) in the longissimus muscles of beef and dairy steers fed a corn diet (Arnold et al., 1992) were lower than that in the longissimus lumborum of the lambs (Wulf et al., 1995)

Levels of γ -tocopherol in the back fat and longissimus muscle of the lambs fed different supplements were less than that of α -tocopherol (Table 17). According to Machlin (1991), γ -tocopherol is absorbed and deposited in tissues readily, but it is retained only to a limited degree compared with α -tocopherol. However, no research reports were found in which diets with supplemental γ -tocopherol were fed to ruminant animals with subsequent measurement in the animal tissues. Since γ -tocopherol is a better antioxidant than α -tocopherol (Madhavi et al., 1996), the possibility exists that increased

tissue levels of the γ -tocopherol achieved through dietary supplementation would be more effective at increasing shelf-life of fresh red meat than α -tocopherol. This possibility needs to be investigated.

FLAVOR VOLATILES IN BROILED MUSCLE OF LAMBS FED DIFFERENT SUPPLEMENTS

The flavor volatiles in broiled LD muscle are listed in Table 18. Twenty-five compounds were isolated, but only 21 compounds were identified. The mass spectra of unknowns 1, 2, 3 and 4 are shown in Appendix D1, 2, 3, and 4, respectively. The identified flavor volatile compounds were divided into groups based on a common precursor fatty acid or fatty acid type: oleic (C18:1), linoleic (C18:2), linolenic (C18:3), arachidonic (C20:4) or saturated (C14:0, C15:0, C16:0, C17:0, and C18:0) (Belitz, 1987; deMan, 1990; Pokorny, 1989).

Octanal, nonanal, and t-2-decenal are formed from the oxidation of C18:1 (Belitz, 1987; deMan, 1990). However, there were no differences ($P > 0.10$) in the percent concentrations of these aldehyde flavor volatile compounds in the broiled LD muscles of lambs fed the control supplement and those fed the two treated (acetaldehyde and diacetyl) supplements or between lambs fed the acetaldehyde supplement and those fed the diacetyl supplement. Significantly higher concentrations of one or more of these volatiles in the LD muscle of lambs fed the control supplement versus those fed the treated supplements were expected, mainly because of the higher level of C18:1 in the polar lipids

Table 18 -- Least-Squares means^{a,b} of concentrations (weight%) of flavor volatiles in broiled longissimus (LD) muscle of lambs fed different dietary supplements

Flavor volatile	R.T. (min)	Supplement		
		Control	Acetaldehyde	Diacetyl
Pentanal ^c	12.845	2.40 ± 3.50	7.82 ± 3.50	6.35 ± 3.50
Hexanal ^c	15.434	4.28 ± 2.02	3.77 ± 2.02	5.02 ± 2.02
2-n-Pentyl-furan ^d	17.079	0.27 ± 0.11	0.07 ± 0.11	0.08 ± 0.11
t-2-Pentenal ^c	18.027	0.60 ± 0.40	0.30 ± 0.40	0.95 ± 0.40
Heptanal ^c	18.855	0.75 ± 0.27	0.72 ± 0.27	0.72 ± 0.27
t-2-Hexenal ^d	21.378	0.20b ± 0.20	0.72a ± 0.20	0.62a ± 0.20
Octanal ^c	22.635	0.48 ± 0.13	0.40 ± 0.13	0.28 ± 0.13
Unknown 1	25.010	1.33b ± 0.58	3.20a ± 0.58	2.37a ± 0.58
t-2-Heptenal ^c	25.764	0.67 ± 0.20	0.62 ± 0.20	0.40 ± 0.20
Nonanal ^c	26.501	1.92a ± 0.22	1.17b ± 0.22	1.23b ± 0.22
Unknown 2	28.403	8.47 ± 2.27	7.50 ± 2.27	5.93 ± 2.27
t-2-Octenal ^d	29.879	1.15a ± 0.15	0.97b ± 0.15	0.68b ± 0.15
2-Cyclohex-ene-1-one ^d	32.517	7.78 ± 2.03	4.75 ± 2.03	4.23 ± 2.03
Benzaldehyde ^d	33.336	0.72 ± 0.15	0.58 ± 0.15	0.48 ± 0.15
t-2-Nonenal ^d	33.922	0.65a ± 0.07	0.52b ± 0.07	0.31c ± 0.07
t-2-Decenal ^c	37.810	8.45 ± 0.91	7.90 ± 2.91	6.47 ± 2.91
2-Trideca-none ^d	41.486	0.93 ± 0.14	0.82 ± 0.14	0.67 ± 0.14
Tetradecanal ^d	44.791	1.58a ± 0.19	1.38b ± 0.19	0.92c ± 0.19
t,t-2,4-Un-decadienal ^c	47.936	1.87a ± 0.16	1.40b ± 0.16	1.40b ± 0.16
Unknown 3	51.150	41.70 ± 5.35	37.92 ± 5.35	42.07 ± 5.35
Hexadecanal ^d	52.414	1.08 ± 0.33	1.07 ± 0.33	1.63 ± 0.33
Continued				

Table 18 -- Continued

Flavor volatile	R.T. (min)	Supplement		
		Control	Acetaldehyde	Diacetyl
t,t-2,4-do- decadienal ^c	52.987	1.35 ± 0.30	1.47 ± 0.30	1.87 ± 0.30
Gamma-nona- lactone ^c	56.642	7.48 ± 2.16	10.17 ± 2.16	9.95 ± 2.16
Unknown 4	57.800	1.83b ± 0.24	2.18a ± 0.24	2.62a ± 0.24
2-Penta- decenal ^d	58.211	2.00 ± 0.70	2.60 ± 0.70	2.70 ± 0.70

^aLSmeans ± standard error (n=6).^bLSmeans in a row followed by unlike letters are different ($P < 0.10$).^cPositively identified.^dTentatively identified.

of the LD muscle of lambs fed the control supplement versus lambs fed the other supplements (Table 12).

Pentanal, hexanal, heptanal, t-2-heptenal, t-2-octenal, and t-2-nonenal are derived from the oxidation of C18:2 (Belitz, 1987; deMan, 1990). No differences ($P > 0.10$) were found between the percentage concentrations of the saturated aldehydes: pentanal, hexanal, and heptanal, in the broiled meat of the lambs fed the control supplement or that of lambs fed the two treated (acetaldehyde and diacetyl) supplements. However, the percentage concentration of t-2-octenal and t-2-nonenal in the broiled LD muscles of lambs fed different supplements were significantly different. Lambs fed the control supplement had higher levels ($P < 0.10$) of these latter two volatiles in their broiled LD muscles than did lambs fed the acetaldehyde or diacetyl supplements. There were no differences ($P > 0.10$) in the percentage concentrations of t-2-heptenal or t-2-octenal in the broiled meat from lambs fed the acetaldehyde supplement and those fed the diacetyl supplement. However, the level of t-2-nonenal was higher ($P < 0.10$) in the broiled LD muscle of lambs fed the acetaldehyde supplement than in that of lambs fed the diacetyl supplement. From the fatty acid analysis of LD muscle lipids, the mean concentration of C18:2 was higher in the LD muscle of lambs fed the two treated (acetaldehyde and diacetyl) supplements than in those fed the control supplement (Tables 11 and 12). Therefore, the concentrations of all volatiles derived from C18:2 should be higher in meat from lambs fed the two chemically treated supplements than in meat from lambs fed the control supplement. This was not found in this measurement of these flavor volatiles from the LD muscles of these lambs. Reasons for these discrepancies are unknown at the

present time.

The following flavor compounds are derived from the oxidation of C18:3: t-2-pentenal, t-2-hexenal, 2-n-pentylfuran (Belitz, 1987; deMan, 1990) and benzaldehyde (Pokorny, 1989). Only one difference ($P < 0.10$) was found among the concentration of these four flavor volatiles in the broiled LD muscle of lambs fed the different supplements (Table 18). The percentage concentrations of t-2-hexenal were higher in the muscle of the lambs fed either acetaldehyde or diacetyl supplement than in that of the lambs fed the control supplement. There was no difference ($P > 0.10$) in the concentrations of t-2-hexenal in the broiled LD muscle flavor volatile compounds between lambs fed the acetaldehyde and the diacetyl supplements. Perhaps the higher concentration of C18:3 in the nonpolar lipids of the LD muscle of lambs fed the treated supplements compared with that of lambs fed the control supplements (Table 11) contributed to the higher concentrations of t-2-hexenal.

Gamma-nonolactone can be formed via thermal oxidation of C18:1, C18:2 and saturated fatty acids (Pokorny, 1989). Tetradecanal, hexadecanal, and 2-tridecanone are formed also by oxidation of the saturated fatty acids. Among the concentrations of these four volatiles in the LD muscle, only that of tetradecanal was different ($P < 0.10$) among the lambs fed the different supplements (Table 18). Meat from lambs fed the control diet had higher levels of tetradecanal than did meat from lambs fed the other two supplements. However, there were no corresponding concentrations differences in the precursor saturated fatty acids (C14:0, C15:0, C16:0, C17:0 and C18:0) in the nonpolar or polar lipids of the LD muscle (Tables 11 and 12). Therefore, reasons for the higher levels of

tetradecanal in the meat of lambs fed the control supplement are not known.

The flavor volatile compounds, 2-cyclohexene-1-one, t,t-2,4 undecadienal, t,t-2,4-dodecanal, and 2-pentadecenal, are not derived from the oxidation of any fatty acid measured in the LD muscle in this study. The relative concentrations of 2-cyclohexene-1-one, t,t-2,4-undecadienal and 2-pentadecenal were not different ($P > 0.10$) among lambs fed the different supplements (Table 18). But, the level of t,t-2,4-undecadienal in the broiled LD muscle of lambs fed the control supplement was higher than in those fed the treated (acetaldehyde and diacetyl) supplements.

In the four unidentified flavor volatile compounds, the percent concentrations of unknowns 1 and 4 were significantly different in the broiled LD muscle flavor volatiles of lambs from different treatments. Lambs fed the acetaldehyde or diacetyl supplements had higher levels ($P < 0.10$) of unknowns 1 and 4 than did lambs fed the control supplement; there was no difference ($P > 0.10$) in concentrations of unknowns 1 and 4 in the broiled LD muscle flavor volatiles between lambs fed the acetaldehyde or the diacetyl treatment. The most abundant volatile, unknown 3, measured in this study unfortunately was not identified.

Park et al. (1976) found that the levels of t,t-2,4-decadienal and cis- γ -dode-6-enolactone were higher in the cooked lamb flavor volatiles from the lambs fed the formaldehyde-protected sunflower seed-casein supplement or the formaldehyde treated safflower oil-casein supplement than those fed the basal diet. Those fed the protected supplements also contained approximately 20% C18:2 in muscle lipids compared with 3 to 4% C18:2 in muscle lipids from lambs fed the basal diet. The fatty acid, C18:2, is the

precursor of both t,t-2,4-decadienal and cis- γ -dode-6-enolactone as well as n-hexanal, t-2-heptenal and 2-octenal. Neither t,t-2,4-decadienal nor cis- γ -dode-6-enolactone were detected in the broiled LD muscle flavor volatiles in this study. The levels of n-hexanal, t-2-heptenal and t-2-octenal were higher in cooked meat flavor volatiles from the lambs fed the formaldehyde-protected sunflower seed-casein or safflower oil casein than in those from lambs fed the basal diet (Park et al., 1976). These results with the volatiles from the broiled LD muscle were reversely related with those of Park et al. (1976). The levels of t-2-heptenal and t-2-octenal were lower ($P < 0.10$) in the meat from lambs fed the two protected (acetaldehyde and diacetyl) supplements than in that from the lambs fed the control supplement, and no differences ($P > 0.10$) in hexanal concentrations were found among the lambs fed the different supplements (Table 18). Perhaps the reason for these opposite results was the much lower levels of C18:2 in the muscle lipids of lamb fed the protected supplements in this study (Tables 11 and 12) compared with that in the study reported by Park et al. (1976). The predominant source of dietary lipid fed to the lambs by Park et al. (1976) was from the protected supplements. As stated previously, in this study, the protected supplements provided only about 50% of the dietary lipid to the lambs; the ground whole corn provided the rest.

CHAPTER V

SUMMARY AND IMPLICATIONS

The objectives of this research were as follows: (1) to produce dietary supplements in gel form containing high levels of linoleic acid oil from ground, full-fat soybean mixed with casein and treated with acetaldehyde or diacetyl; (2) to investigate the effect of feeding these supplements on the fatty acid composition of meat fat of the lambs; (3) to determine the effect of feeding the supplements on the levels of vitamin E in lamb meat and adipose tissue; and (4) to evaluate the effect of feeding the supplements on the flavor volatiles of broiled longissimus muscle in lambs.

Ground full-fat soybeans mixed with casein were treated with acetaldehyde or diacetyl to form a gel or left untreated as a control supplement. On a dry matter basis (DMB), the control, acetaldehyde and diacetyl supplements contained, respectively, 49.7, 50.0 and 49.1% protein and 17.2, 15.5 and 17.4% fat. Each supplement was fed to a group of six lambs from shortly after weaning until slaughter (90 days). Each lamb consumed daily a supplement (approximately 180 g DMB control or 207 g DMB acetaldehyde or diacetyl treated), 1.00 kg of a ground corn basal diet and 0.33 kg of hay.

From each lamb, the longissimus (LD) muscle (12-13th rib), back fat, kidney fat and intermuscular fat outside the LD muscle were obtained for chemical analysis. The moisture and total lipid contents of the LD muscle and the levels of total lipids in the three fat samples were determined. The LD lipids were separated into nonpolar and polar lipids.

The fatty acid compositions of the LD nonpolar and polar lipids, the back fat, the kidney fat and the intermuscular fat were analyzed. Also, the loin chop from the 12th rib of both sides of each carcass was obtained. The LD muscle from the loin chop of the 12th rib from the left side of each carcass and the back fat were analyzed for α - and γ -tocopherol contents. The 12th rib loin chop from the right side of each lamb carcass was broiled to an internal temperature of 75°C before the LD muscle was removed and extracted for flavor volatiles by simultaneous distillation extraction. The flavor volatiles were analyzed qualitatively and quantitatively using gas chromatographic-mass spectrometric analyses. The concentration of LD moisture and total lipid contents, the concentration of each fatty acid in each lipid sample, the levels of α - and γ -tocopherols in the back fat and the LD muscle, and the concentration of each flavor volatile were analyzed statistically as a function of the three supplement treatments with significantly different means among treatments identified.

Compared with lambs fed the control supplement, lambs fed the two treated (acetaldehyde or diacetyl) supplements had higher levels of ($P < 0.05$) of linoleic acid (C18:2) in the LD muscle nonpolar and polar lipids, the kidney fat and the back fat. Levels of linolenic acid (C18:3) were also higher ($P < 0.05$) in the LD muscle nonpolar lipids, kidney fat and back fat of the lambs fed the two treated (acetaldehyde or diacetyl) supplements than those fed the control supplement. The levels of oleic acid (C18:1) were lower ($P < 0.05$) in the LD muscle polar lipids and kidney fat of lambs fed the two treated supplements than those of lambs fed the control supplement. However, the levels of stearic acid (C18:0) were higher ($P < 0.05$) in the kidney fat of lambs fed the two treated

supplements when compared with that of lambs fed the control supplement. The implication of these findings is that the C18:2 and C18:3 of the soybean oil in the acetaldehyde and diacetyl treated supplements were better protected from biohydrogenation in the rumen of the lambs than in the control supplement resulting in the increased deposition of these acids in the tissue of lambs fed the treated supplements. Since the fatty acids were expressed in weight percentages where the total concentration of all fatty acids totaled 100% for any one sample, significant increases in any one fatty acid concentration results generally in decreased levels of one or more of the other fatty acids. In this case, the increased levels of C18:2 and C18:3 in the fat of the lambs fed the treated supplements resulted in decreases in the levels of C18:1 and/or C18:0. In this study, the acetaldehyde and diacetyl treated supplements supplied approximately half of the dietary fat to the lambs while the ground corn supplied the other half. Perhaps if the chemically treated supplements had supplied all of the dietary fat to the lambs, a more dramatic increase in the levels of the polyunsaturated fatty acids (C18:2 and C18:3) would have been found. Additional research is needed to investigate this possibility.

The iodine values (cg I/g lipid) of the lipids in the different fat depots of the lambs were calculated from the fatty acid composition to be 45, 50, 55, 56 and 121, respectively, for the kidney fat, back fat, intermuscular fat, LD nonpolar lipids and LD polar lipids. Since a larger iodine value shows a greater degree of unsaturation in oil/fat, fat sites increased in unsaturation from kidney fat to the LD polar lipids. This observation was in agreement, generally, with levels of unsaturation previously reported in animal fat depots. Feeding treated (acetaldehyde or diacetyl) supplements to the lambs affected the vitamin E

levels in the fat and meat depots. Compared to feeding the control supplement, feeding the treated supplements increased ($P < 0.05$) the levels of α -tocopherol in the back fat samples. However, differences in γ -tocopherol levels in tissues were found due to feeding the lambs the different treated supplements. Compared with the acetaldehyde treated supplement, feeding the diacetyl treated supplement resulted in higher levels of γ -tocopherol in the back fat ($P < 0.10$) and in the LD muscle ($P < 0.05$). Since vitamin E (particularly the γ -form) is fat soluble and is found naturally in the full-fat soybeans in the control and treated supplements, these results imply that feeding the treated supplements, particularly the one with diacetyl, appears be more efficient in depositing vitamin E in ruminant animal tissues than when it is fed unprotected in the diet. More research is needed to determine this possibility.

Twenty-five flavor volatiles were isolated from the broiled LD muscles of the lambs. Twenty-one were identified and included seven alkanals, seven 2-alkenals, two 2,4-alkadienals, 2-n-pentylfuran, 2-cyclohexene-1-one, benzaldehyde, 2-tridecanone, and gamma-nonolactone. Although some differences were found ($P < 0.10$) in the concentrations of the aldehydes derived from oxidation of specific fatty acids in the cooked LD muscle of lambs fed the different supplements, generally the greater concentration of a specific aldehyde did not coincide with its more abundant precursor fatty acid. Reasons for this finding are unknown at the present time.

Thus, dietary supplements containing ground, full-fat soybeans mixed with casein and treated with acetaldehyde or diacetyl were produced in gel form. The acetaldehyde and diacetyl treated supplements, when fed to lambs, increased levels of polyunsaturated

fatty acids, C18:2 and C18:3, deposited in the fat of the lambs. The treated supplements, particularly the one treated with diacetyl, resulted in increased levels of α - and γ -tocopherol in the LD muscle and back fat of lambs fed the supplements.

Additional research is needed, however, to maximize the levels of C18:2 and C18:3, and the different forms of vitamin E deposited in tissues when ruminant animals are fed the treated supplements. The level of fat in the supplements needs to be increased and those supplements fed to the animals as the main source of their dietary fat. Increased levels of both α - and γ -tocopherol should be incorporated into the supplements, and the efficiency of tocopherol deposition, as well as the level deposited, in the tissues of ruminant animals fed these supplements determined.

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APPENDICES

APPENDIX A

VOLATILE COMPOUNDS IDENTIFIED IN THE NEUTRAL FRACTION^a OF COOKED OVINE FAT

Volatile compounds identified in the neutral fraction^a of cooked ovine fat

Chemical Families	Volatile compounds
<u>Aldehyde</u>	Pentanal, Hexanal, Heptanal, Octanal, Nonanal, Decanal, Undecanal, Tridecanal, Tetradecanal, Pentadecanal, Heptadecanal Octadienal 2-Hexenal, 2-Hepenal, 2-Octenal, 2-Nonenal, 2-Decenal, 2-Undecenal, 2-Tridecenal, 2-Tetradecenal, 2-Pentadecenal 2,4-Heptadienal, 2,4-Heptadienal (isomer), 2,4-Decadienal, 2,4-Decadienal (isomer) 2-Methyl-2-butenal
<u>Ketones</u>	2-Heptanone, 2-Nonanone, 2-Decanone, 2-Undecanone, 2-Dodecanone, 2-Tridecanone, 2-Tetradecanone, 2-Pentadecanone, 2-Hexadecanone, 2-Heptadecanone 3-Tetradecanone 4-Methyl-2-pentanone
<u>Lactones</u>	γ -Heptalactone, γ -Octalactone, γ -Nonalactone, γ -Decalactone, γ -Tetradecalactone, γ -Butyrolactone
<u>Alcohols</u>	1-Pentanol, 1-Hexanol, 1-Heptanol, 1-Octanol
<u>Furans</u>	2- <i>n</i> -Pentylfuran
<u>Hydrocarbons</u>	Toluene, Naphthalene

^aCaporaso et al., 1977.

APPENDIX B

COMPOSITION OF BASAL CORN DIET FED TO LAMBS

Composition of basal corn diet fed to lambs

Ingredient	Composition (%)
Coarse ground corn	63.3
Alfalfa pellets	20.0
Soybean meal (48%)	10.0
Rolled oats	5.0
Trace mineral mix	0.6
Limestone	0.4
Antibiotic	0.05

APPENDIX C

CORRECTION FACTORS (CF) FOR FATTY AID METHYL ESTER STANDARDS

Correction factors (CF) for fatty acid methyl ester standards

Names	Fatty acids	Correction factors
Decanoic	10:0	1.0456
Undecanoic	11:0	1.0000
Dodecanoic	12:0	1.0052
Tridecanoic	13:0	1.0000
Myristic	14:0	0.9732
Myristoleic	14:1	1.0042
Pentadecanoic	15:0	1.1247
Palmitic	16:0	1.0000
Palmitoleic	16:1	1.0042
Margaric	17:0	1.0182
Stearic	18:0	1.0182
Oleic	18:1	1.0366
Nonadecanoic	19:0	1.0000
Linoleic	18:2	1.0266
Eicosanoic	20:0	1.1115
Linolenic	18:3	1.0064
Eicosenoic	20:1	1.0144
Eicosadienoic	20:2	0.9700
Eicosatrienoic	20:3	0.9884
Arachidonic	20:4	1.0400
Docosanoic	22:0	1.1298
Eicosapentaenoic	20:5	1.0000
Docosatetraenoic	22:4	1.0000
Docosapentaenoic	22:5	1.0000
Docosahexaenoic	22:6	1.0000

APPENDIX D

MASS SPECTRA OF FOUR UNKNOWN FLAVOR VOLATILES IN BROILED MUSCLE OF LAMBS FED DIFFERENT DIETARY SUPPLEMENTS

 FILENAME: DIACTE.03
 COMMENT: BGI INPUT: DIACTE.01

MODE: EI
 EV: 70 GAIN: 4.5
 IS TEMP: 250 SCAN SPEED: 10
 SOL TEMP: 116

DATA	R.T.	PEAK	MASS	RANGE	BASE	PEAK	TOTAL	RAW-	B.G.
1	27.0	67	41-	270	33520(	70)	150163	439-	435
		42	41-	270	33520(	70)	145945	439-	435

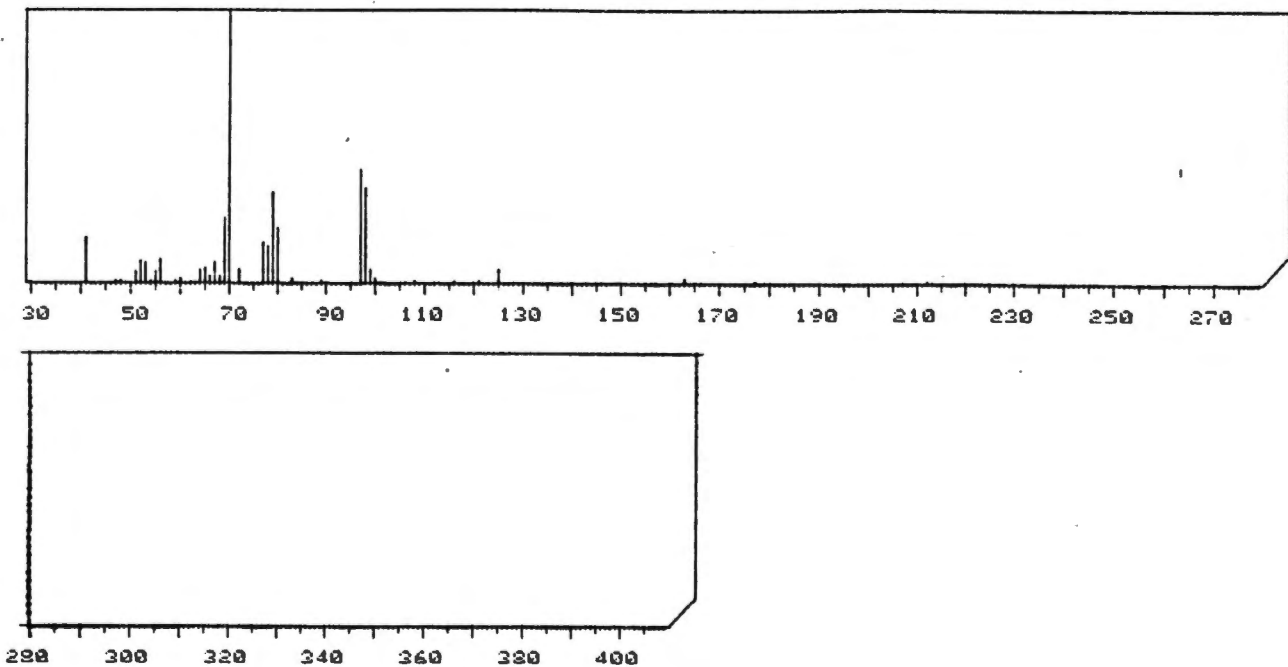


Figure D-1 -- Mass spectra of unknown No. 1 flavor volatile in broiled muscle of lambs fed different dietary supplements

 FILENAME:DIACTE.13
 COMMENT:BG2 INPUT:DIACTE.01

MODE: EI
 EI: 70 GAIN: 4.5
 IS TEMP: 250 SCAN SPEED: 10
 COL TEMP: 132

DATA	R.T.	PEAK	MASS	RANGE	BASE	PEAK	TOTAL	RAW-	B.G.
1	32.3	33	39-	332	50100%	41)	190104	534-	532
		38	40-	262	50100%	41)	176971	534-	532

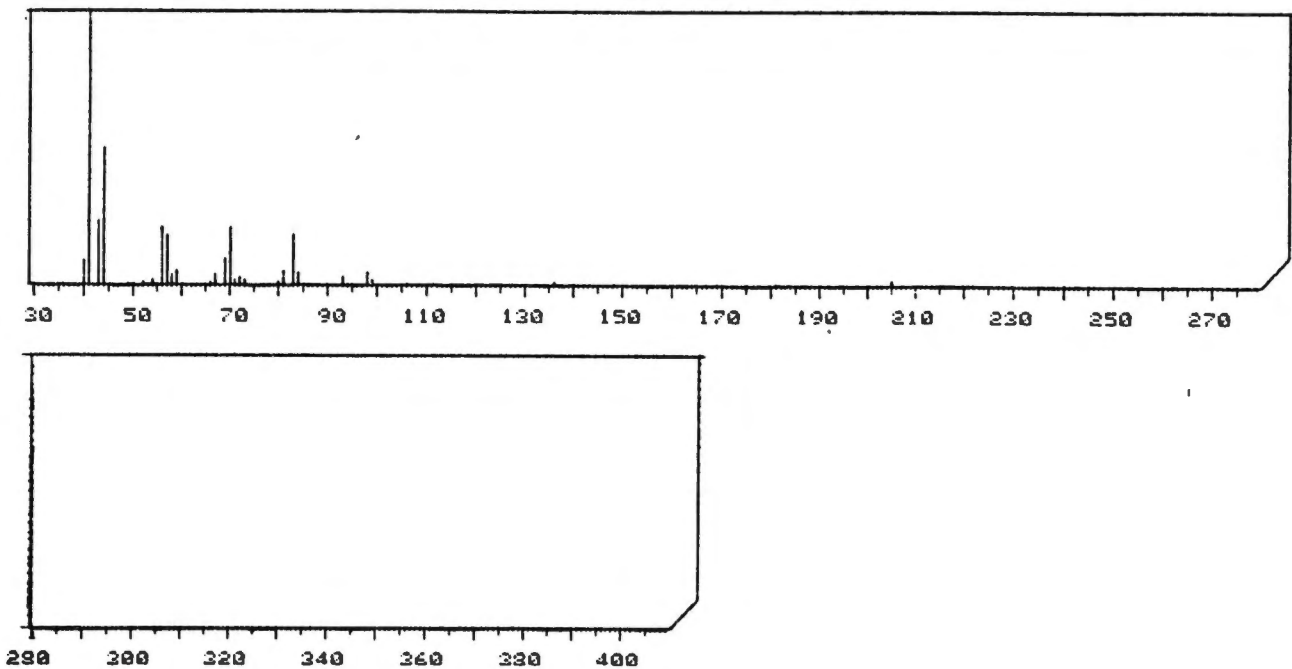


Figure D-2 -- Mass spectra of unknown No.2 flavor volatile in broiled muscle of lambs fed different dietary supplements

FILENAME: DIACTE.42
COMMENT: BGS INPUT: DIACTE.01

MODE: EI
EJ: 70 GAIN: 4.5
IS TEMP: 250 SCAN SPEED: 10
COL TEMP: 124

DATA	R.T.	PEAK	MASS	RANGE	BASE	PEAK	TOTAL	RAW-	B.G.
1	50.0	161	36-	222	402500(	45)	3235227	808-	800
		61	36-	222	402500(	45)	3238760	808-	800

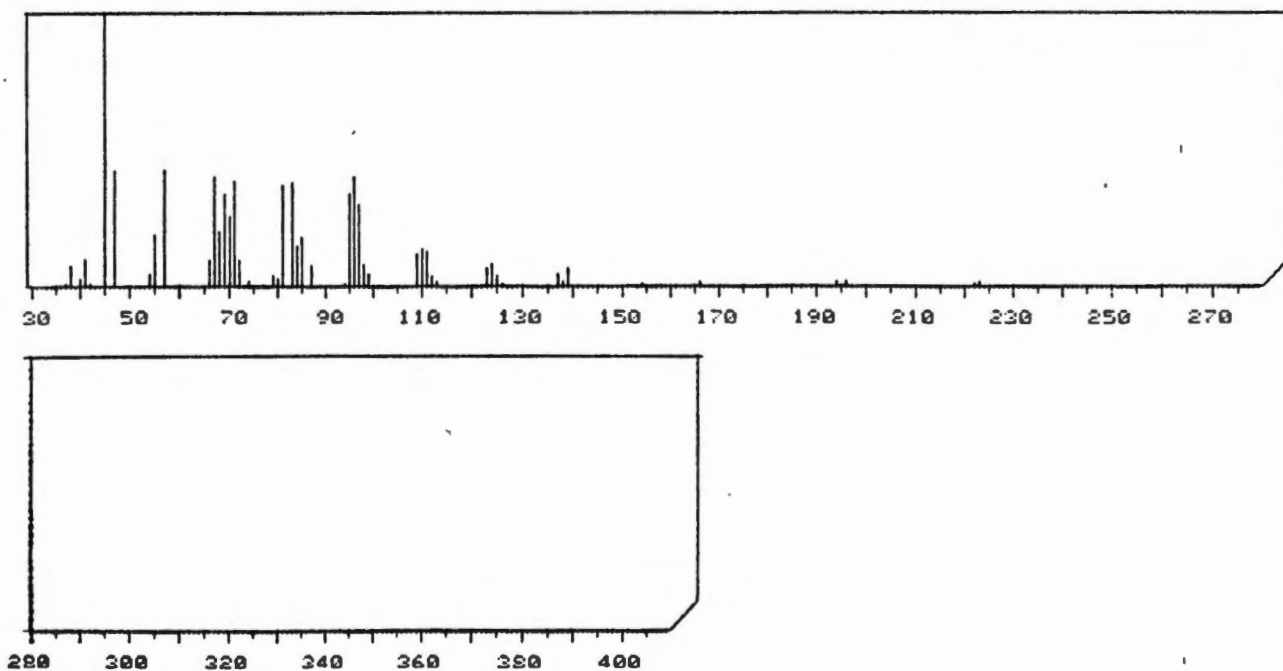


Figure D-3 -- Mass spectra of unknown No.3 flavor volatile in broiled muscle of lambs fed different dietary supplements

 FILENAME: DIACTE.43
 COMMENT: BGI INPUT: DIACTE.01

MODE: EI
 FID: 70 GAIN: 4.5
 IS TEMP: 250 SCAN SPEED: 10
 COL TEMP: 190

DATA	R.T.	PEAK	MASS	RANGE	BASE	PEAK	TOTAL	RAW-	E.G.
1	52.1	80	46-	389	76500(	46)	222938	824-	823
		84	46-	287	76500(	46)	203906	824-	823

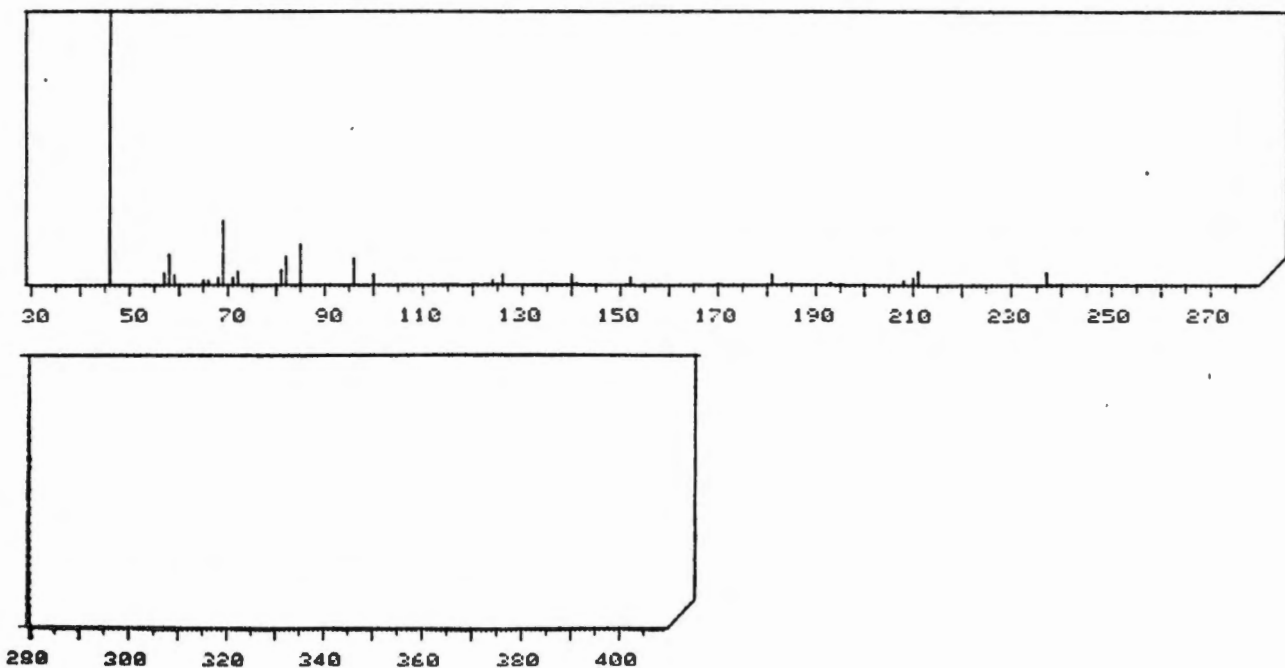


Figure D-4 -- Mass spectra of unknown No.4 flavor volatile in broiled muscle of lambs fed different dietary supplements

VITA

Jung Hoon Lee was born in Seoul, Republic of Korea on November 22, 1964, to In Seon Lee and Young Yun Kim. In 1983, he graduated from Kyung Hee High School, in Seoul, Korea. He married Chan Sook Park on July, 15 1990. One year later, he received his Bachelor of Science degree with a major in General Science at Oregon State University. In 1994, he received the second Bachelor of Science degree with a major in Food Science and Technology at the same university. In August, 1994, he started his graduate studies in the Food Science and Technology major at the University of Tennessee, Knoxville. He completed requirements for Master of Science degree in Food Science and Technology in May, 1997. He was also admitted to the doctoral program in Food Science and Technology at the same university.

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