EFFECTS OF DIETARY FAT AND VITAMIN E UPON THE STABILITY OF MEAT IN FROZEN STORAGE

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ABSTRACT

EFFECTS OF DIETARY FAT AND VITAMIN E UPON THE STABILITY OF MEAT IN FROZEN STORAGE

by

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Sixteen 4-day old veal calves were allotted into four groups and fed solely on filled milk in which half of the calves received a stable saturated fat (coconut oil) and the other half an unsaturated fat (corn oil). Half of the claves on each treatment were supplemented with 500 mg d-α-tocopheryl acetate per calf per day (groups 1 and 3), whereas, the remainder were unsupplemented. The calves were slaughtered after 8 weeks and samples of kidney and omental fat and of meat (longissimus dorsi muscle) were removed, wrapped in freezer paper, frozen and stored at -18°C. The initial samples were analyzed for fatty acid composition. Samples were also analyzed for vitamin E and TBA values at 0, 1, 3 and 6 months storage.

The average slaughter weight of the calves ranged from 139 lb for group 3 to 162 lb for group 1. Carcass yield was about 53% for group 3, and approximately 60% for groups 1, 2 and 4, respectively. Average total lipid

content of the meat was below 2%, while the phospholipid content ranged from 0.75 to 0.82%. The mean level of fat in the internal lipid depots varied from 24 to 75% among groups.

The proportion of saturated fatty acids ranged from 60 to 70% in the fatty tissues and meat triglycerides from coconut oil fed calves, while the level varied from 30 to 40% for the corn oil diets. The amount of saturated fatty acids in the phospholipids (33%) was not influenced by the different rations. The distribution of monoenoic acids in the fatty tissues and meat neutral lipids varied from 23 to 34%, but showed no clear-cut pattern of distribution. The level of dienoic acid was about 28% in both the depot fats and meat triglycerides from calves fed corn oil, whereas, the level was only 5% for the same tissues from the coconut oil fed calves. The amount of linoleic acid in the phospholipids was on average about 40 and 27%, in the meat from calves fed corn oil and coconut oil diets, respectively. The level of polyenoic acids (9%) in the phospholipids from the meat was not affected by differences in treatment.

On the average the initial level of vitamin E in the meat was generally below 6 μg/g of tissue, while the initial level in the depot fats from calves fed supplemental vitamin E ranged from about 16 to 42 μg/g of tissue. Vitamin E declined steadily during storage, but the rate of decline was not the same in all tissues. In the fatty

tissues, over 60% of the initial tocopherol was still present after 3 months freezer storage. In the meat lipids, the levels of tocopherol at 3 months of storage had declined to about 40 and 25% in calves fed coconut oil and corn oil diets.

The stability of the lipid was monitored during frozen storage by the 2-thiobarbituric acid test. Up to 6 months of storage, the meat and omental tissues were stable, with a maximum TBA number of about 0.2 and 0.4, respectively. However, the kidney fat was less stable. The TBA values for kidney fat from calves fed corn oil without supplemental vitamin E had increased to the threshold level for rancidity at 6 months of freezer storage.

Results show that young calves selectively deposited dietary fats in the tissues without significant alteration. The data also demonstrated that dietary vitamin E and saturated fatty acids contribute to the stability of the tissues during frozen storage.

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DEDICATION

To the living memory of my late mother, Oiyimhebedan, who gave me all my motivation and desire in life and persevered through every imaginable problem to educate me.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDIX TABLES

LIST OF APPENDIX FIGURES

INTRODUCTION

Increasing awareness that linoleic acid tends to favor low concentrations of serum cholesterol has prompted researchers to find ways to increase the levels of PUFAS (polyunsaturated fatty acids) in the fats of meat animals. It is well recognized that the composition of the ingested fat does not appreciably affect the properties of the depot fat of ruminants, primarily due to the hydrogenation of the unsaturated fatty acids by the rumen microorganisms. To overcome this effect, ruminants have been fed encapsulated polyunsaturated oils, such as safflower oil, coated by a protein, which is hardened with formaldehyde (Scott et al., 1970). The droplets, thus protected, bypass the rumen, but are released on reaching the intestine where they are absorbed like that of non-ruminants.

Before entering the stage of rumination, calves do not hydrogenate unsaturated dietary fat, and consequently, lay down such fat like non-ruminants. Hence, in the depot fats of young calves, the dietary fat is laid down without being subject to significant changes prior to deposition. The resulting meat and meat products show marked increases in the linoleic acid content of the depot fats and

phospholipids. Since the unsaturated fats are oxidized more readily, the stability of the meat products becomes of primary interest.

Much research on the keeping quality of fats, and to a lesser extent to that of phospholipids, has been carried out on rendered or extracted lipids using such criteria as the peroxide value, the TBA number and the length of the induction period. Although work has also been done on cooked meats, less is known about the oxidative changes in the fats of meat during frozen storage.

The present study was designed to obtain knowledge about the changes in the lipids during frozen storage of meat as they occur *in situ*. Since the content of polyunsaturated fatty acids markedly influences meat stability, it was deemed necessary to determine the effect of various concentrations of linoleic acid and other PUFAS on the oxidative stability of meat in relation to changes in the level of tocopherol (vitamin E). The stability of the lipids during storage at -18°C were evaluated by use of TBA numbers (Tarladgis *et al.*, 1960).

REVIEW OF LITERATURE

Deterioration of Fats, Oils and Fat-Containing Foods

Although oxidative deterioration of lipids involving the uptake of atmospheric oxygen is common to most foods containing plant or animal tissues, the mechanism involved may vary (Tappel, 1953, 1955; Dugan, 1961; Lea, 1962). Invariably, autoxidation (uptake of oxygen) of the lipids in such foods is promoted by heat, light (especially of short wave length) and metal catalysts, especially by copper and iron (Smith and Dunkley, 1962; Waters, 1971).

There is much evidence in the literature (Dugan, 1961; Lundberg, 1961; Lea, 1961; Ingold, 1967; Waters, 1971) in support of the generally accepted oxidative mechanisms for food lipids. These include autoxidation, lipoxidase-catalyzed and hematin-catalyzed oxidation. However, the mechanisms show differences in the initiation process, activation energies and in the rates of oxidation (Tappel, 1953, 1955; Dugan, 1961; Lea, 1962). Since lipoxidase does not exist in meats (Banks, 1944; Tappel, 1952, 1953), its role will not be discussed herein.

Mechanism of Autoxidation

The currently accepted mechanism for autoxidation of unsaturated fatty acids was first elucidated by Farmer and Sutton (1943) and by Bolland and Koch (1945). They concluded that fat oxidation is initiated by the abstraction of hydrogen from a labile methylene group in the molecule of the unsaturated fatty acid (RH). A free radical (R•) is, thus, formed to which oxygen is attached to form a peroxy radical (ROO•). The peroxy radical subsequently abstracts a hydrogen from a nearby site of another unsaturated fatty acid molecule to form a hydroperoxide (ROOH) and to propagate the chain. The hydroperoxides may then decompose yielding free radicals, which can initiate new reaction chains. The steps involved in the mechanism are schematically shown below.

(I) Initiation:

$$RH \longrightarrow R^{\bullet} + H^{\bullet}$$

(II) Propagation:

$$R^{\bullet} + O_2 \longrightarrow ROO^{\bullet}$$

$$ROO^{\bullet} + RH \longrightarrow ROOH + R^{\bullet}$$

$$ROOH \xrightarrow{\text{energy}} RO^{\bullet} + {}^{\bullet}OH$$

$$RO^{\bullet} + RH \longrightarrow ROH + R^{\bullet}$$

$${}^{\bullet}OH + RH \longrightarrow H_2O + R^{\bullet}$$

Chain branching occurs in the last three reactions with

a rapid increase in the number of free radicals through autocalytic interaction.

(III) Termination:

$$2R^{\bullet} \longrightarrow R\text{-}R$$

$$2RO^{\bullet} \longrightarrow RO\text{-}O\text{-}R$$

$$2ROO^{\bullet} \longrightarrow \text{Various products}$$

According to Dugan (1961), the initiation process has an activation energy of about 45 KCal/mole, while the propagation steps have lower activation energies. The decomposition of the hydroperoxides to form free radicals, which further take part in the autocalytic chain reactions is by either thermal instability or by reaction with other materials (Farmer and Sutton, 1943).

Mabrouk and Dugan (1960) and Lundberg (1962) have demonstrated that the decomposition of hydroperoxides can take place by a monomolecular as well as by a bimolecular mechanism, as illustrated below:

Monomolecular:

$$ROOH \longrightarrow RO^{\bullet} + {}^{\bullet}OH$$

Bimolecular:

$$2ROOH \longrightarrow 2RO^{\bullet} + H_2O$$

They indicated that monomolecular decomposition takes place at a lower concentration of hydroperoxides and at higher temperatures. Mabrouk and Dugan (1960) have also reported a lower activation energy for the bimolecular than for the

monomolecular mechanism for hydroperoxide scission.

The unpleasant odors of rancid oils and fats are due mainly to aliphatic saturated and unsaturated aldehydes and acids of lower molecular weight produced by the eventual oxidative scission of the long hydrocarbon chains of lipid molecules (Mookherjee and Chang, 1963; Kimoto and Gaddis, 1970).

Hematin Compounds as Pro-Oxidants

The catalytic effect of the iron porphyrins on the oxidative deterioration of polyunsaturated fatty acids (PUFAS) was first described by Robinson (1924), who attributed the catalysis to the iron content of the molecule. Since then, a number of studies (Kendricks and Watts, 1969; Fishwick, 1970a, 1970b) on the role of heme-iron in catalytic oxidation have been reported.

According to Greene (1975), the main forms in which myoglobin (Mb) may exist in meats are as Mb, oxymyoglobin (MbO_2) and nitric oxide ferrohemochrome (NOH), respectively, in which the iron porphyrin is in the ferrous (Fe^{2+}) state. The other forms of Mb include ferrihemochrome and metmyoglobin (MetMb), both of which contain iron in the ferric (Fe^{3+}) state (Kendricks and Watts, 1969; Fishwick, 1970a, 1970b; Greene, 1975).

A number of investigators (Watts and Peng, 1947; Watts, 1954; Lewis and Wills, 1963; Tappel, 1952; Liu, 1970a, b; Liu and Watts, 1970; Greene, 1975) have indicated

that hematin compounds are involved in lipid oxidation in meat. On the other hand, Sato and Hegarty (1971) indicated that the component of cooked meat responsible for catalyzing lipid oxidation is a water soluble diffusate, hence, neither Hb, Mb, MbO_2 nor MetMb are responsible. In support of this view, Love and Pearson (1974) reported upon addition of purified MetMb and Fe^{2+} to cooked meat that only the latter compound was effective as a pro-oxidant. MetMb at levels of 1-10 mg/g of meat failed to catalyze oxidation.

Role of the Oxidation State of Iron

Considerable differences in opinion have been expressed on the mechanism of hematin-catalysis regarding the importance of the oxidation state of iron to the catalytic activity of the heme pigments. Younathan and Watts (1959), Kendricks and Watts (1969) and Greene (1975) have proposed that the Fe^{3+} hemes are the active catalysts of lipid oxidation. Evidence for this was demonstrated by showing that cooked meat developed warmed-over-flavor (WOF) during refrigerator storage more rapidly than raw meat. During cooking, the Mb of raw meat is converted to MetMb.

Smith and Dunkley (1962), Brown et al. (1963) and Hirano and Olcott (1971) found no difference in the rate of lipid oxidation catalyzed by Fe^{2+} and Fe^{3+} hemes. Consequently, Smith and Dunkley (1962) and Sato and Hegarty (1971) concluded that Fe^{2+} is the active catalytic agent

rather than the hematin-compounds.

Tarladgis (1961) reported a close relationship between lipid oxidation, spin state, ligand field, and pigment changes in animal tissue. He attributed the catalytic activity of ferric hemoproteins to the paramagnetic character of the porphyrin bound iron rather than to its oxidation state. According to Tarladgis (1961) and Fishwick (1970 b), the presence of five unpaired electrons in MetMb produces a strong magnetic field, which would favor the initiation of free radical formation from the high spin state (Fe^{3+}). Iron porphyrins in the low spin state (Fe^{2+}) as in fresh and freeze-dried meats, exhibited no catalytic activity (Fishwick, 1970a, b).

Mechanism of Hematin Catalysis

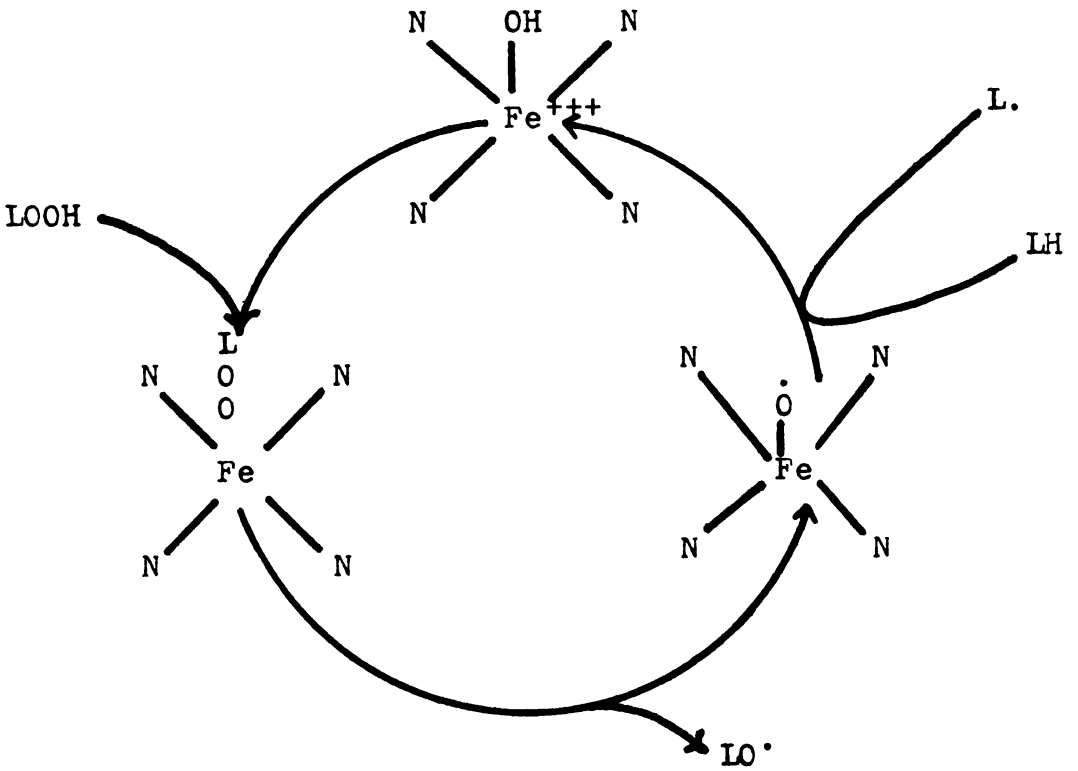
The oxidation of unsaturated fats in the presence of hematin compounds is believed to proceed by way of a chain reaction mechanism (Barron and Lyman, 1938). Banks (1944) postulated that preformed linoleate peroxide was necessary for hematin catalysis. As a consequence, Tappel (1953) proposed a theory of hematin catalysis aimed at explaining the data presented by Banks (1944). According to the theory, the mechanism of linoleate oxidation involves a direct reaction of linoleate peroxide with hematin catalysis. Propagation and termination reactions are essentially similar to the autocalytic mechanism. Hence, the critical reaction in hematin catalyzed unsaturated lipid oxidation is the

catalytic decomposition of hydroperoxides to free radicals (Tappel, 1953).

Tappel (1953) has reported the relatively low activation energy of 3.3 KCal/g mole for hematin-catalyzed oxidation. This suggests that rancidity of meat products would proceed readily at frozen storage. However, Tappel (1952, 1955) has indicated that frozen fresh meat is relatively stable despite the presence of hematin catalysts.

In a later study, Tappel (1955) attempted to broaden the knowledge of unsaturated lipid oxidation by hematin compounds and to define more precisely the mechanism of the reaction. He concluded that Mb has catalytic activity quantitatively similar to that of hemin, cytochrome c and hemoglobin. He then suggested that hematin involves the formation of a lipid peroxide-hematin compound and its subsequent decomposition into free radicals, which propagate the chain reactions with the concomittant destruction of the catalyst.

The mechanism of hematin-catalyzed unsaturated lipid peroxidation (Tappel, 1955) is shown on the following page.



$LO^{\cdot} + LH \longrightarrow LOH + L.$

$L. + O_2 \longrightarrow LOO^{\cdot}$

$LOO^{\cdot} + LH \longrightarrow LOOH + L.$

Where:

LH = Linoleic acid

LOOH = Hydroperoxide linoleate

Antioxidant Level of Hematin Compounds

Lewis and Wills (1963) demonstrated that hemoglobin, cytochrome c, hematin and tissue homogenates at high concentrations all had inhibitory effects on linoleate

oxidation. The concentration of hemoglobin necessary to show antioxidant activity increased with higher concentrations of fatty acids, which is in agreement with the results of Kendricks and Watts (1969).

Catalysis by Metal Ions

There is agreement in the literature that metals, particularly the transition elements (iron and copper), are powerful oxidative catalysts (Smith and Dunkley, 1962; Waters, 1971; Sato and Hegarty, 1971; Love and Pearson, 1974; Greene, 1975). Foods usually contain trace elements of heavy metals, which probably arise, at least in part, from the presence of metal activated enzymes or their decomposition products or else through contamination (Waters, 1971).

According to Wills (1965), inorganic iron is a particularly notorious oxidative catalyst. Love and Pearson (1974) have demonstrated the catalytic effects of Fe^{2+} as a pro-oxidant in meat. Privett and Blanck (1962) and Sato and Hegarty (1971) reported that at levels below 1 ppm, Fe^{2+} and other metals are incapable of catalyzing lipid oxidation.

A number of investigators (Privett and Blanck, 1962; Ingold, 1967; Waters, 1971) have concluded that the primary oxidative function of heavy metals, like that of hematin compounds, is to increase the rate of

hydroperoxide decomposition to free radicals. Wills (1965) reported that the catalysis of lipid peroxide formation by inorganic iron is pH-dependent. The optimum rate of peroxidation reaches a maximum close to pH 5.5 but decreases under alkaline conditions. There is strong evidence that Fe^{2+} is a more active catalyst than Fe^{3+} (Smith and Dunkley, 1962; Sato and Hegarty, 1971; Waters, 1971; Love and Pearson, 1974).

The mechanisms of metal catalysis were reviewed by Ingold (1967) and Waters (1971). The initial step in the mechanism is the donation of an electron by the Fe^{2+} to the hydroperoxide. The Fe^{2+} is, thus, oxidized to Fe^{3+} state, to be later reduced to Fe^{2+} by the decomposition products of the hydroperoxides (Ingold, 1967; Waters, 1971). This reaction is shown schematically below:

$$ROOH + M^{2+} \longrightarrow RO\cdot + \cdot OH + M^{3+} \quad (I)$$

$$ROOH + M^{3+} \longrightarrow ROO\cdot\ M^{2+} + H^{+} \quad (II)$$

$$M^{3+} + RH \longrightarrow R\cdot + M^{2+} + H+ \quad (III)$$

Metal catalysts also function as oxidation inhibitors. Smith and Dunkley (1962) and Ingold (1967) have demonstrated that above a certain critical concentration, ferrous iron and cobalt may function as inhibitors.

Action of Antioxidants in Food Lipids

Antioxidants may interfere with or delay the onset of oxidative breakdown of fats and fatty foods (Blanck, 1955). Primary or phenolic antioxidants (such as tocopherols, butylated hydroxyanisole(BHA)or butylated hydroxytoluene(BHT))function by breaking the oxidative reaction chains (Shelton, 1959). In support of this viewpoint, Cort *et al.* (1974) reported that the phenolic antioxidants act as electron or hydrogen donors to quench electron mobility with subsequent interruption of free-radical chain reactions.

According to Uri (1961), the mechanism of antioxidant action is as follows:

$$ROO^{\bullet} + AH \text{ (antioxidants)} \longrightarrow ROOH + A^{\bullet}$$

The radical may be stabilized by recombination in either of two ways:

$$A^{\bullet} + A^{\bullet} \longrightarrow A_2$$

$$ROO^{\bullet} + A^{\bullet} \longrightarrow ROOA$$

This means that during autoxidation, the antioxidants are converted into dimers and other products (Uri, 1961). It is also possible that the antioxidant is oxidized directly by oxygen, as in the case of tocopherol, which is partly oxidized to tocoquinone in fats (Tappel, 1962). At the end of the induction period, the antioxidants

disappear with little being known as to their exact fate (Cort et al., 1974).

Vitamin E as a Lipid Antioxidant

The tocopherols are products of synthesis by plants, but may occur naturally in animal tissues in the non-saponifiable portion of the lipid fraction--usually together with sterols, vitamin A, vitamin K and other naturally occurring antioxidants (Mervyn and Morton, 1959; Bieri, 1969). Vitamin E is also found in association with phospholipids, occurring largely in subcellular membranes (Witting, 1975).

There appears to be some limitations in the amount of tocopherol that can be accommodated within a normal membrane (Witting, 1975). Absorption of α-tocopherol decreases inversely with increasing levels in the diet (Adams et al., 1959; Losowsky et al., 1971). On the other hand, the α-tocopherol content of most fats and oils is a log function of their polyunsaturated fatty acid (PUFA) contant (Hove and Harris, 1951).

It is believed that the level of α-tocopherol deposited in a tissue depends on the particular isomer and homologue, the dietary level, the duration of the feeding period, the particular species, the specific tissue and the amount of destruction in the diet and gut (Adams et al., 1959; Machlin, 1962; Marusich et al., 1975; Witting, 1975). The

storage of the d-form of α-tocopherol is about twice as great as for the dl-form and the relative efficiency of absorption of the tocopherols in decreasing order is: alpha, beta, gamma and delta, respectively (Machlin, 1962; Parkhurst et al., 1968).

The stability of tocopherol in the diet depends on the amount of peroxidizable lipid, the temperature, the length of storage, the concentration of trace metal catalysts, the concentration of hydroperoxides and the protective effect of other antioxidants (Adams et al., 1959; Parkhurst, 1968; Witting, 1975). It is suggested (Moore and Sharman, 1959; Machlin, 1962) that destruction of tocopherol in the gut may be considerable when PUFAS are ingested concurrently with the tocopherols.

Species Differences in the Ability to Deposit Dietary Tocopherols in Tissues

All unsaturated lipids require stabilization, hence the need for vitamin E supplementation in feeds to stabilize the resultant animal products (Burr et al., 1946). The only lipid antioxidant, which is stored in appreciable amounts by animals, is α-tocopherol (Barnes et al., 1943; Lundberg et al., 1946). However, marked differences have been reported in the ability of domesticated species to deposit dietary tocopherol in their tissues. The ability of poultry to store dietary α-tocopherol has been confirmed (Criddle and Morgan, 1947; Kummerow et al., 1948; Mecchi

et al., 1953, 1956a; Webb et al., 1973, 1974; Marusich et al., 1975). Mecchi et al. (1956a) and Marusich et al. (1975) reported a higher level of α-tocopherol in the tissues of chickens than in turkeys fed the same diet. They also showed that turkeys require a higher level of supplemental α-tocopherol to delay the onset of rancidity than chickens.

There is little agreement on the ability of the pig to store dietary vitamin E. Watts et al. (1946) suggested that the pig could not store vitamin E at a level of practical value, probably because of inefficient absorption. However, Astrup (1973) reported absorption and deposition of vitamin E in the pig. He also indicated that the dietary vitamin E improved the taste, flavor and the oxidative stability of the meat.

Deposition of dietary α-tocopherol is reported to be very inefficient (Watts, 1946; Caravaggi and Wright, 1969). Caravaggi and Wright (1969) reported that sheep fed α-tocopheryl acetate excreted virtually all of it in the feces in 4 days. Based on these results, they recommended intramuscular administration. However, Buchanan-Smith et al. (1969) demonstrated positive absorption and increased tissue levels of α-tocopherol in sheep upon oral administration.

The ability of the calf to deposit dietary tocopherol has been well documented (Decker and Hill, 1947; Eaton et al., 1958; Adams et al., 1959; Poukka and Oksanen,

1972; Ellis et al., 1974). Similar results have also been obtained with mature steers (Kimoto et al., 1974).

Nevertheless, little has been done in supplementing ruminants with vitamin E, presumably because of their ability to alter the lipid fraction of feeds in the rumen (Shorland et al., 1957). Caravaggi et al. (1968, 1969) have suggested that vitamin E is more efficiently deposited in tissues of ruminants by intramuscular injection of α-tocopheryl acetate. They concluded that the real difference between the digestive and injection routes is due to lack of absorption rather than rumen hydrolysis. However, intramuscular administration of α-tocopheryl acetate in calves and mature steers was found to be inefficient (Blaxter et al., 1953; Adams et al., 1959).

Vitamin E Activity and Product Stability

The principal effect of an antioxidant is the neutralization of the free radical in the first stage of the autoxidative chain reaction (Uri, 1961). Thus, the reaction of the antioxidant and the free radical involves the transformation of the antioxidant into the unstable free radical form, which easily undergoes irreversible changes (Dam, 1953; Cort et al., 1974; Witting, 1975).

Michaelis and Wollman (1949) demonstrated that semiquinone is the free radical form of vitamin E. They theorized that α-tocopherol stabilizes lipids through formation of a semiquinone structure. Although the in vitro

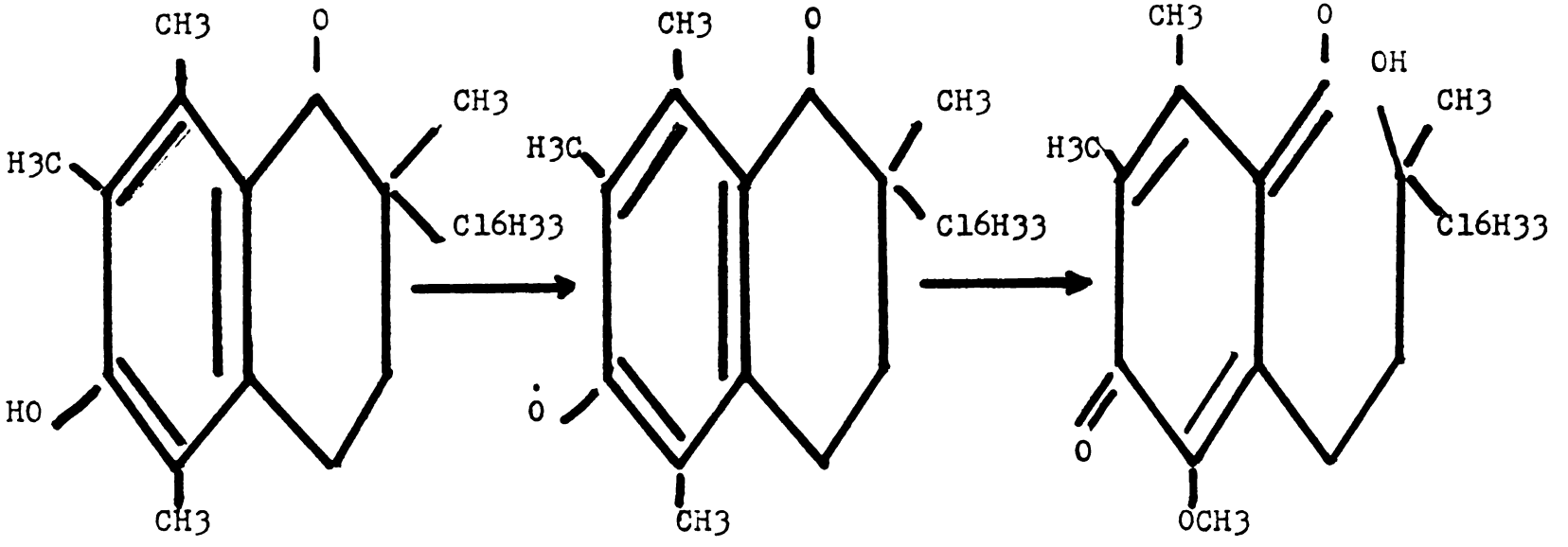
antioxidant effect of the four known tocopherols, increases in order of alpha, beta, gamma and delta, the activity *in vivo* increases in the opposite order (Dam, 1953; Parkhurst *et al.*, 1968).

Alpha-tocopherol is regarded as the lipid antioxidant of nature, and this is believed to be its most important function (Poukka and Oksanen, 1972; Witting, 1975). The chemical basis of the action of vitamin E is its combination with free radical intermediates of lipid oxidation and lipid peroxides, thus inhibiting further lipid peroxidation (Tappel, 1962).

Tappel (1962) has proposed that α-tocopherol is oxidized to a number of products in hematin-catalyzed, peroxidizing linoleic acid. The reactions involved are shown on the following page.

A number of studies have demonstrated the beneficial antioxidant activity of α-tocopherol in improving the stability of carcass lipids of poultry (Criddle *et al.*, 1947; Mecchi *et al.*, 1953; Webb *et al.*, 1973, 1974; Marusich *et al.*, 1975), of veal and beef (Lundberg, 1944; Ellis *et al.*, 1974; Kimoto *et al.*, 1974), of mutton (Caravaggi and Wright, 1969) and of pork (Astrup, 1973). Nevertheless, some workers regard α-tocopherol as a poor antioxidant, particularly in products containing highly unsaturated fatty acids (Lips, 1947; Witting *et al.*, 1964; Witting, 1969; Benedict *et al.*, 1974; Witting, 1975).

The dietary requirement for vitamin E increases with



A = α-tocopherol; B_1 = Intermediate compound and B_2 = α-tocopheryl quinone, the main product from lipid peroxidation.

increasing amounts of unsaturated fatty acids, which suggests that it plays an important dietary role as an antioxidant (Kimoto et al., 1974; Witting, 1975; Marusich et al., 1975). Alpha-tocopherol has an optimum concentration for a minimum rate of oxygen uptake, and increasing the level beyond this optimum results in an increased rate of oxygen uptake (Parkhurst et al., 1968; Witting, 1975).

The level of α-tocopherol decreases with aging of meats (Adams et al., 1959). Hence, fresh meat products having high levels of linoleic acid require more α-tocopherol in the lipid fraction to enhance their stability (Kimoto et al., 1974; Marusich et al., 1975). Alpha-tocopherol is destroyed five times as rapidly by the linolenate as by linoleate (Lips, 1957; Witting, 1969). As the degree of unsaturation in the fatty acids increases, the induction period decreases and the fatty tissues eventually become rancid, despite the presence of α-tocopherol (Kimoto et al., 1974; Ellis et al., 1974; Witting, 1975).

At low concentrations, α-tocopherol functions as an antioxidant, but at high concentrations may become a prooxidant (Chipault, 1961). The minimum formation of peroxide-free radical initiation occurs at a concentration of about 1-3 μmole of α-tocopherol per g fat (Witting, 1975). Witting (1975) suggested that an increase in tocopherol concentration results in increased peroxide formation through free-radical initiation, an increased rate of autocatalysis and an increased rate of destruction.

The Composition of Animal Fats

Lipids in meat, poultry and fish are often classified as depot or adipose tissue and as intramuscular or tissue lipids (Watts, 1962; Love and Pearson, 1971). The depot fats are largely localized as subcutaneous deposits, although large quantities may be present in the thoracic and abdominal cavities and between the muscles as intermuscular deposits.

The triglycerides are the principal components of adipose tissue (Watts, 1962) and are deposited largely as fat globules localized within the individual cells. On the other hand, tissue lipids are an integral part of various cellular structures, which include the cell wall (Kono and Colowick, 1961), the mitochondria (Holman and Wildmer, 1959) and the sarcoplasmic reticulum (Newbold et al., 1973). Although adipose tissue is deposited in a fairly consistent pattern, it is influenced by species, diet, environment, sex and other factors (Deuel, 1955).

Influence of Diet

Although species differences in the composition of depot fat may be related to the composition of the diet, dietary influences within a species can be controlled (Shorland, 1952). The data presented by Ellis and Isbell (1926 a, b) have clearly demonstrated the influence of diet upon some measures of carcass firmness and the proportion of different fatty acids in the depot fat of the pig. It

is well established that soft pork results when a high level of corn oil is fed to hogs, and that the resulting fat is more susceptible to autoxidation (Ellis, 1933). Hence, the composition of the depot fats of non-ruminants tends to reflect that of the dietary fat. On the other hand, in ruminants the depot fats are not influenced to any extent by diet (Reiser, 1951; Shorland et al., 1957). In order for ruminants to be directly responsive to dietary unsaturated fats, it is necessary to by-pass the rumen by means of a duodenal fistula (Ogilvie et al., 1961) or otherwise protect the dietary fat from the action of rumen microorganisms (Cook et al., 1970; Scott et al., 1971).

However, calves before entering the stage of rumination do not hydrogenate unsaturated dietary fat, and consequently, lay down such fat like non-ruminants (Holmberg et al., 1956). Recent studies (Poukka and Oksanen, 1972; Wright et al., 1974; Ellis et al., 1974; Kimoto et al., 1974) have confirmed the monogastric behavior of the young calf.

Fatty Acid Composition of Animal Fats

Natural fats are composed mainly of the straight chain even numbered carbon fatty acids, typically containing 16 and 18 carbon atoms (Dugan, 1971). Animals tend to be more uniform in their fatty acid composition than those of plants, though the range of fatty acids encountered is still very wide (Hansen et al., 1958).

The most abundant and widespread fatty acid in animal fat is oleic (octadec-cis-9-enoic) acid. Other unsaturated fatty acids, which are prominently distributed (though not so uniformly) include linoleic (octadec-cis-9-cis-12-dienoic) and palmitoleic (hexadec-cis-9-enoic) acid (Hilditch and Williams, 1964). Of the saturated fatty acids, palmitic (hexadecanoic) acid is the most prominent, and like oleic acid, it is seldom absent in any of the natural animal fats (Hilditch and Williams, 1964).

In animals, the endogenous fat contains about 25% palmitic acid, the remaining fatty acids being mainly oleic with minor amounts of stearic, myristic and palmitoleic (Shorland, 1952). When animals have access to dietary fat, the fatty acids may be reflected in the composition of the depot fat. Animal fats frequently contain linoleic acid and are often accompanied by linolenic (octadec-cis-9-cis-12-cis-15-trienoic) acid (Gunstone, 1967). By elongation and desaturation, these acids provide the C_{20} and C_{22} polyunsaturated fatty acids of animal phospholipids (Gunstone, 1967; Poukka and Oksanen, 1972).

It has been established that minor amounts of odd numbered fatty acids, especially of saturated C_{15} and C_{17} as well as Pentadec-cis-9-enoic and heptadec-cis-9-enoic acids, and branched chain fatty acids occur in animal fats, including those from ruminants (Shorland, 1962). Shorland et al. (1957) concluded that hydrogenation of fatty acids by rumen microorganisms results in diversification of the

fatty acid composition of the dietary unsaturated fatty acids. They also demonstrated that linolenic acid may be saturated to give high levels of stearic acid and of trans and positional isomers of oleic and linoleic acid not found elsewhere in natural fats.

The Phospholipid Content in Animal Tissues

Many studies (Hornstein et al., 1961; Watts, 1962; Kinsella, 1972) have shown that the phospholipids are integral parts of the cellular membranes and may be present in tissues as phospholiproteins. The phospholipids comprise a relatively constant proportion (< 1%) in most animal fats and contain a high content of polyenes (Watts, 1962; El-Gharbawi and Dugan, 1965; Hornstein et al., 1967; Turkki, 1967; O-Keefe et al., 1968; Keller and Kinsella, 1973; Body and Shorland, 1974).

There is broad similarity in the composition of phospholipids in the tissues of a variety of mammals and birds (Ansell and Hawthorn, 1964; Body et al., 1966). More is known about the pattern of phospholipid distribution in the tissues of sheep than in other species. Body et al. (1966) reported the following pattern of phospholipid distribution in the total tissues of maternal and fetal sheep: phosphatidyl choline (PC) - 45%; phosphatidyl ethanolamine (PE) - 25%; sphingomyelin - 11%; phosphatidyl serine (PS) - 7%; phosphatidyl inositol (PI) - 4% and all others - 8%.

According to Gunstone (1967), each type of phospholipid

tends to have its own characteristic fatty acid composition. He reported that in animal tissues, PE is notably rich in polyunsaturated C_{20} and C_{22} fatty acids, which are derived from dietary linoleic and linolenic acids. Body and Shorland (1974) reported that the amounts and kinds of PUFAS recorded in the PE fractions may vary with the level and ratios of linoleic and linolenic acid in the diet, as well as by the conditions of the analysis.

Hornstein et al. (1961) first reported that 20:4, ω6 was the only polyunsaturated components besides 18:2, ω6 in the phospholipids from pork and beef muscle. Later, Hornstein et al. (1967) reported that beef muscle phospholipids included 22:6, ω3; 22:3, ω6 and 22:4, ω6 in addition to 20:4, ω6. This discrepancy appears to be the result of using poorer analytical procedures in the earlier study. In recent studies, Body and Shorland (1974) have shown that the level of PUFAS from the PE fraction of the rumen and abomasum of fetal and maternal sheep ranged from 17-43% of the total fatty acids. In comparison, the levels of PUFAS in PC were 7-25%, and in spingomyelin, 1-4% (Body and Shorland, 1974). The main PUFA components (Body and Shorland, 1974) in PE were 20:4, ω6 and 22:5, ω3, with lesser amounts of 20:5, ω3, 22:6, ω3, 18:2, ω6 and 18:3, ω3, respectively.

Relationship of Fatty Acid Composition to Autoxidative Stability of Meats

The autoxidative stability of meats depends on the degree of unsaturation (Ellis et al., 1974; Kimoto et al., 1974). Consequently, meats, such as pork and poultry, which have high levels of PUFAS, are very susceptible to oxidation in frozen storage (Ellis, 1933; Watts, 1962). Intracellular lipids contain a higher percentage of phospholipids, have higher PUFA levels than the depot fats and are believed to be more susceptible to autoxidation (Govendarajan et al., 1973).

Changes in Neutral Lipids and Phospholipids During Frozen Storage

It is commonly assumed that tissue lipids are quite stable during freezer storage (Cadwell et al., 1960; Keskinel et al., 1964; Witte et al., 1970; Kimoto et al., 1974). Several workers (Lea, 1957; Younathan and Watts, 1959, 1960) have suggested that negligible changes occur in total lipids during frozen storage of raw beef, because the predominant neutral lipids oxidize slowly compared to the phospholipids. However, recent studies (Keller and Kinsella, 1973; Kimoto et al., 1974) indicate that the oxidation of neutral lipids may be a factor in the deterioration of beef carcasses during freezer storage.

According to Kimoto et al. (1974) microbial growth does not occur in meats stored below -9°C. They further

stated that polar lipids are more stable than the neutral lipids in frozen storage. In contrast, other investigators (Sulzbacher and Gaddis, 1968; Keller and Kinsella, 1973) have suggested that the triglycerides from adipose tissue are the primary cause of meat deterioration during freezer storage.

Muscle phospholipids are believed to be the major contributors to oxidative deterioration of cooked meats (Younathan and Watts, 1960; Love and Pearson, 1974) and of freeze-dried meats (El-Gharbawi and Dugan, 1965; Chipault and Hawkins, 1971). Lea (1957), Caldwell et al. (1960), and Greene (1971) have reported that breakdown of phospholipids during frozen storage of raw meats results in rancidity and browning. However, other researchers (Keskinel et al., 1964; Evans et al., 1967; Terrel et al., 1968) have reported negligible changes in the fatty acids of the phospholipids in beef during freezer storage. On the other hand, Keller and Kinsella (1973) have reported major changes in the fatty acids of phosphatidyl choline (lecithin), more especially in arachidonic acid.

Lipolysis of phospholipids during freezer storage has been implicated in oxidative degradation of bovine, fish and chicken muscle (Awad et al., 1968; Bosund and Ganrot, 1969). The recent findings of McMurry and Magee (1972) that phospholipases occur in mammalian tissues and may release fatty acids from phosphoglycerides could lend support to this view.

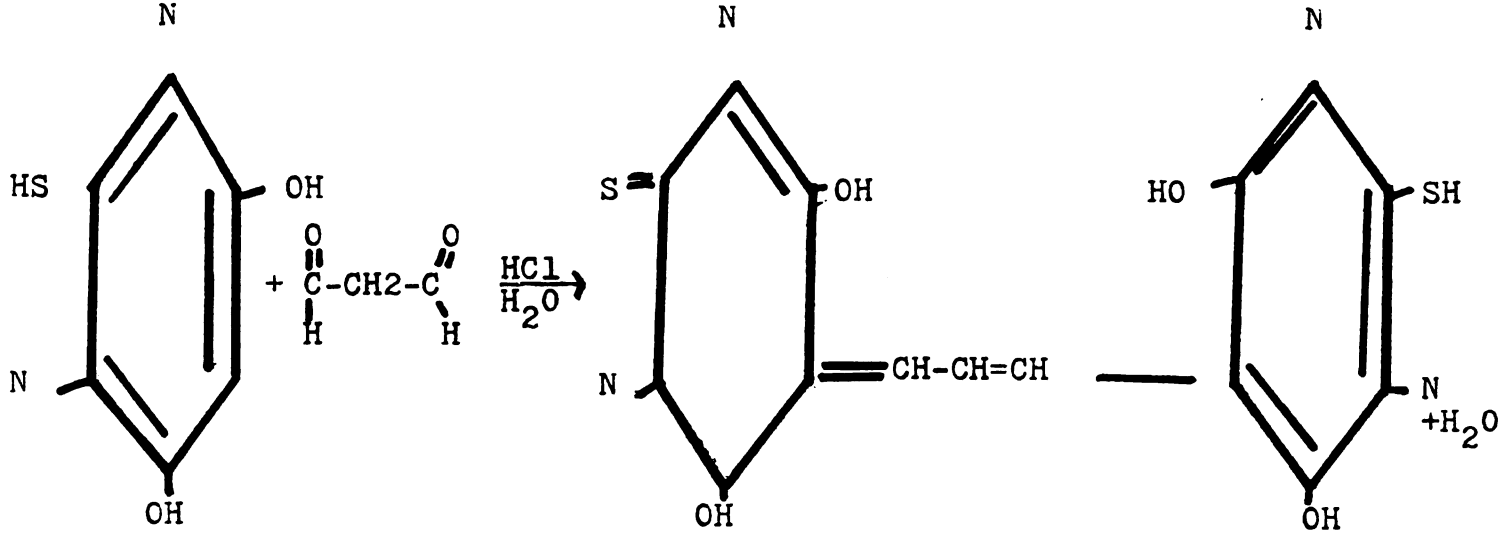
The 2-thiobarbituric Acid (TBA) Test as a Measure of Meat Rancidity

Malonaldehyde (MA) and similar substances occur in foods as decomposition products of oxidizing unsaturated fatty acids, and in the presence of water, exist mainly as the non-volatile, bound enolate anion (Kwon and Watts, 1964; Kwon *et al.*, 1965). The reaction of MA with TBA, which has been a useful index for measuring rancidity in foods was first reported by Kohn and Liversedge (1944).

According to Sinnhuber *et al.* (1958), the principal reactant is MA, a water soluble substance formed or released upon heating the sample in an acid medium. However, Tarladgis *et al.* (1964) have demonstrated that MA can be measured without the acid treatment. The red pigment obtained in the reaction occurs as a consequence of the condensation of two moles of TBA with one mole of MA (Sinnhuber *et al.*, 1958). The intensity of color is a measure of MA concentration, which has been organoleptically correlated with rancidity (Zipser *et al.*, 1964; Kwon and Watts, 1964; Kwon *et al.*, 1965).

According to Sinnhuber *et al.* (1958), the proposed TBA reaction is as shown on the following page.

Kwon *et al.* (1965) have claimed that the reactions of MA and 2-thiobarbituric-acid-reactive-substances (TBRS) in moist foods are similar to the reaction of the pure compounds. However, Slaslaw and Waravdeckar (1965), from TLC studies of extracts of irradiated fatty acids claimed



TBA MA Heat TBA Chromagen

that none of the TBRs was MA. Apparently, both MA and other aldehydes (especially 2,4-alkadienals and to some degree 2-alkenals) are capable of producing the red pigments with maximum absorbance at about 530 nM (Marcuse and Johanssen, 1972).

MA as an Index of Lipid Oxidation in Foods

The formation of MA as a product of lipid oxidation is generally accepted as the basis of the TBA test (Marcuse and Johanssen, 1972). Numerous techniques have been used in applying the TBA test to assay for MA in foods. A number of investigators (Turner et al., 1954; Yu and Sinnhuber, 1957) have heated the macerated food directly with an immiscible solvent. Others have applied the test to a metaphosphoric or trichloroacetic extract of the food (Tappel and Zalkin, 1959) or to a distillate from the acidified food (Sidwell et al., 1955; Tarladgis et al., 1960).

All modifications of the method employ acid-heat treatment of the food (Kwon and Watts, 1964). Where distillation is employed to separate the MA from other food constituents, maximum volatilization (even of free preformed MA) would not be expected at pH values above 3.0 (Kwon and Watts, 1964). This is because the volatile, hydrogen-bonded ring compound formed undergoes progressive ionization as the pH increases from 3.0 to 6.5 (Tarladgis et al., 1960; Kwon and Watts, 1964).

A major feature of the TBA test is the fact that the

acid reagent can be applied directly to food lipids without prior extraction of the fat (Lea, 1962). In order to obtain high correlations between TBA values and rancidity (Lea, 1962; Kwon and Watts, 1964; Zipser et al., 1964; Kwon et al., 1965; Pearson, 1968) it is necessary to use moist foods, especially animal tissues and dairy products (Patton and Kurtz, 1951).

According to Kwon and Watts (1964), in some cases lipid oxidation in dehydrated foods may be far advanced with little or no accumulation of MA, since the MA would be in the volatile, metal-chelated form. Pearson (1968) pointed out that the TBA test apparently measures the deterioration in both the extractable and non-extractable lipids. However, he further reported that relatively high TBA values may be found in some fresh samples, and yet in advanced stages of rancidity, the TBA values may actually fall to zero or remain constant after reaching a maximum value.

Wills (1964; 1966) observed that the presence of Fe^{2+} and Fe^{3+} in small concentrations markedly increased TBA values. Presumably the Fe^{3+} readily forms a colored complex with an organic compound on heating the oxidized lipid with TBA (Wills, 1964). She has, therefore, recommended the addition of EDTA to complex the Fe^{3+} during the blending process.

The presence of ascorbic acid in the distillate can also result in high TBA values (Wills, 1966). It has also been reported that little or no color is produced

by oxidized linoleic or oleic acids, but oxidized linolenic and arachidonic acids give an intense color reaction (Wills, 1964; 1966); hence, the TBA values for rancidity in foods may be somewhat empirical (Lea, 1962).

EXPERIMENTAL

Materials and Methods

Solvents

All solvents, chemicals and reagents were of analytical grade.

Animals and Rations

Four groups of 4-day old Holstein bull calves with four in each group were used in this experiment. They were supplied and reared to the completion of the experiment by the Department of Dairy Science at Michigan State University.

The calves were fed with colostrum and whole milk during the first week of life. After 1-week of age, the calves were fed with milk replacer (filled milk) for 57 days, at the end of which time they were slaughtered. In groups 1 and 2, the fat was supplied by 15% coconut oil. In groups 3 and 4, the fat was supplied by 15% corn oil. In addition, groups 1 and 3 received 500 mg d-α-tocopheryl acetate/calf/day, whereas, groups 2 and 4 were unsupplemented. The rate of feeding was varied with age as shown in Table 1.

Table 1. Levels of Coconut Oil and Corn Oil Filled Milk Fed to the Experimental Calves.

Days of Age	Amount Fed/Calf/Day		
0-3	Colostrum - ad lib		
4-7	Whole Milk - 4 lb		
	Warm Water (qts)	Milk Replacer (lb)	
		Coconut Oil (groups 1 and 2)[a]	Corn Oil (groups 3 and 4)[b]
7-14	2.0	0.5	0.5
15-21	2.3	0.65	0.65
22-28	2.5	0.90	0.90
29-35	3.5	1.3	1.3
36-42	4.0	1.7	1.7
43-49	4.5	2.1	2.1
50-56	5.5	2.5	2.5
57-63	6.0	3.0	3.0

a) Groups 1 and 3 were supplemented with 500 mg d-α-tocopheryl acetate per calf per day.

b) Groups 2 and 4 were unsupplemented.

The filled milks were prepared by Milk Specialties Company, Dundee, Illinois. Except for the source of fat (coconut or corn oil), the rations were identical and contained the ingredients shown in Table 2.

Vitamin E (d-α-tocopheryl Acetate)

The vitamin E acetate was donated by Eastman Kodak Company of Rochester, New York. The vitamin was dissolved in hydrogenated vegetable oil containing minimal quantities of linoleic acid to give 250 mg d-α-tocopheryl acetate (w/v) per ml of oil. The calves in groups 1 and 3 were given a dosage of 2 ml/calf/day of vitamin E-oil, which was thoroughly mixed with the milk ration before feeding. The vitamin E concentrate was made up weekly and stored at room temperature.

Statistical Treatment

Statistical analysis was calculated using STAT SERIES developed by the Michigan State University Agricultural Experiment Station and run on Control Data Corporation (CDC) 6500 computer. Analysis of variance was carried out for fatty acids, TBA numbers and vitamin E levels. Standard deviation, correlation and regression coefficients were calculated using a Cognito 1016 PR programmable electronic printing calculator (Cognito 1016 PR, Smith Corona Merchant, 299 Park Avenue, New York).

Table 2. Composition of Filled Milk.

Ingredients	Percent Composition
Whey - dried	49.2
Non-fat dry milk	25.0
Lactalbumin - dried (55% protein)	10.0
Oil (coconut or corn oil)	15.0
Calcium carbonate	0.625
MSU vitamin-mineral premix	0.175
Total	100.00

The premix provided the following nutrients per lb of product:

Vitamin A	16.250 I.U.
Vitamin D_3	5.000 I.U.
Riboflavin	4 mg
Panthotenic acid	5 mg
Niacin	18 mg
Vitamin B_{12}	7 µg
Thiamine	2 mg
Folic acid	1.25 mg
Choline	85 mg
Magnesium	141 mg

Extraction of Total Muscle Lipid

The procedure for extraction of total lipid from muscle tissue was a modification of the technique described by Folch et al. (1957). After removing all visible fat and connective tissue, the weighed samples were homogenized in a Waring blender and extracted three times with 2:1 (v/v) chloroform-methanol mixture. The weight of the extracted tissue varied from 60 to 90 g. The extract and tissue residue were then transferred to a medium grade sintered glass funnel and filtered under vacuum. The filtrate was collected in a 500 ml graduated Erlenmeyer flask. The homogenizer and the residue in the funnel were washed with an additional volume of chloroform-methanol and filtered. The final extract was quantitatively transferred into a 1000 ml separatory funnel and 10% by volume of distilled water was added and thoroughly mixed. The mixture was allowed to separate into two phases until the interface was clear. The lower phase was transferred to a 500 ml volumetric flask and evaporated in a vacuum Rotavapor-R (Buchi, Switzerland) at 20-30°C. The upper layer was similarly evaporated, but usually contained a negligible level of fat.

When the volume of the total lipid extract was reduced to 10 to 20 ml, the extract was quantitatively transferred to a previously tared 100 ml volumetric flask by washing with an additional quantity of chloroform-methanol.

The final extract was further evaporated until it reached a constant weight. The weight of the residue (lipid) was then obtained by difference.

Separation of Phospholipid and Neutral Lipids

The phospholipid was separated from the total lipid using the method of Choudhury et al. (1960). This method involves separation on activated silicic acid,in which neutral lipids are preferentially removed by washing with chloroform. The phospholipid combines with the activated silicic acid and is solubilized and extracted with methanol.

A weighed amount of silicic acid (20-25 g) was activated for at least 12 hours by drying in a 100°C oven. The lipid sample was then quantitively transferred to a 125 ml Erlenmeyer flask containing the activated silicic acid. The contents were shaken for at least 10 minutes and allowed to settle. The mixture was then thoroughly stirred and filtered through a sintered glass funnel under vacuum. The silicic acid was washed six times with 50 ml portions of chloroform. The filtrate and washings were combined and evaporated using the Rotavapor-R as described previously.

The phospholipid fraction was determined by washing the silicic acid residue with six 50 ml portions of methanol. The filtrate and washings were combined and evaporated to a constant weight using the Rotavapor-R. The combined weight of the phospholipid and neutral lipid was closely equivalent to the initial level of total lipid. The fat

samples were kept in teflon stoppered test tubes and stored at -18°C until removed for fatty acid analysis within a one week period.

Rendering of Depot Fat

Fatty tissues (1-10 g) were transferred to a Thomas Teflon Pestle Tissue grinder (Thomas Company, Philadelphia, Pennsylvania) in a steam bath. The fat was ground while being rendered and extracted with a 2:1 (v/v) chloroform-methanol mixture. The extract was dried over anhydrous sodium sulfate, filtered and washed with an additional volume of chloroform through glass wool into a previously tared 100 ml beaker. The rendering and extraction procedure was continued until the residue was completely free of any lipid material. The solvent was partially evaporated on a steam bath and the extract was evaporated to a constant weight in a vacuum oven.

Preparation of Methyl Esters

The technique for preparation of methyl esters was a modification of the method described by Morrison and Smith (1964). A total of 2.5 ml of 14% Boron trifluoride-methanol (Bf3-MEOH) was added to 100-200 mg of lipid material in a 20 x 150 mm test tube containing 1 ml of benzene. The tube was sealed with teflon-lined screw caps and heated in a steam bath for 40 minutes. After cooling the sample to room temperature, the esters were extracted by adding 2 volumes of hexane and then 1 volume of water.

The mixture was shaken vigorously in a Vortex-Genie mixer until both layers were clear. An aliquot of the upper layer was transferred to a 5 ml graduated volumetric flask and dried with about 0.3 g of anhydrous sodium sulfate.

Analysis of Fatty Acid Composition of the Triglycerides and Phospholipids

Chromatographic analysis of methyl esters was performed using a Beckman-GC-4-Gas Chromatograph equipped with a hydrogen flame detector. The glass column, 6 ft x 2 mm, (i.d.) was packed with 10% (w/w) diethylene glycol succinate (DEGS) on 100/120 mesh supelcoport (Supelco, Inc.). The column had been previously cleaned, silanized and packed under suction. The column oven temperature was 100°C, the injection port was maintained at 210°C and the detector at 185°C. The helium carrier gas flow rate was adjusted to 40 ml/minute or 60 ml/minute, depending on the separation achieved. The flow rates of hydrogen and oxygen were 30 ml/minute and 300 ml/minute, respectively. Varying quantities of sample (0.5-5 µl) were injected.

The emerging peaks were identified by comparing retention times to those of standard mixtures of known fatty acid methyl esters. Peak areas were calculated by multiplying peak height times peak width at half-height and the percentages of the total fatty acids were determined.

Determination of Tocopherol in Meat and Fatty Tissues

The tocopherol content of the meat and fatty tissues was determined by the spectrophotometric method of Erickson and Dunkley (1964). The procedure involves the extraction of tocopherol from the tissues using ethanol and hexane, followed by a separation of the extract from interfering compounds by silicic acid chromatography. The tocopherol level in the purified extract was determined spectrophotometrically after reacting with 4,7 diphenyl-1-10-phenanthroline.

Duplicate meat samples (3-5.0 g) and fat samples (1-3.0 g) were homogenized in a Virtis homogenizer (Virtis Research Equipment, Gardiner, New York) and transferred to a 50 ml centrifuge tube. Distilled deionized water was added to bring the volume to 10 ml. A total of 15 ml of absolute ethanol and 1 ml of 1 N HCl was added and mixed thoroughly. The tubes were heated in a water bath for 5 minutes at 60°C with intermittent mixing. Then 10 ml of hexane were added while still warm and the mixtures were shaken for 20 minutes in a Vortex-Genie manual shaker (Fisher Scientific Industries, Massachusetts). The samples were centrifuged for 5 minutes at 2,000 x G. The upper hexane layer was removed by pipet and dried over 0.5 g of anhydrous sodium sulfate.

Removal of interfering substances from the tocopherol was accomplished by column chromatography. Glass tubes (9-10 mm, i.d.) were cut to size and melted to the appropriate dimension with a Bunsen burner. Each glass

column was fitted with glass wool and packed with 2 g of activated silicic acid which had been previously heated for 12-16 hours at 100°C. The columns were washed with 5 ml of hexane and 5 ml of the extracted tocopherol extract were added immediately. The extract was allowed to drain into the column, after which it was rinsed into the silic acid by adding 4 ml of benzene. The eluent was collected in a 10 ml volumetric flask and was made up to volume by adding an additional 1 ml of benzene.

The eluent was thoroughly mixed and pipetted in 3 ml volumes into three separate test tubes. In a stepwise manner, 0.5 ml of 6×10^{-3} M banthophenanthroline (4,7-Diphenyl-1-10-phenanthroline) solution was added and mixed. This was followed immediately by addition of 0.5 ml of 1.0×10^{-3} M ferric chloride solution. After allowing 2 minutes for color development, 0.5 ml of 0.1 M orthophosphoric acid was added and mixed thoroughly. The addition of reagents was carried out in a darkened environment to prevent photo reduction of ferric ions. Thereafter, exclusion of light was no longer necessary. These reagents were prepared in absolute ethanol and were stored at 5°C in an amber bottle. Only freshly prepared ferric chloride solution was used for the determinations.

The absorbance was determined at 534 nM using a Gilford spectrophotometer, against a 3 ml blank of benzene treated in the same manner as the sample. Tocopherol content was calculated using the equation:

$$\text{g tocopherol/g lipid} = \frac{\text{Absorbance at 534 nM}}{0.032 \text{ (g fat/ml in original extract)}}$$

TBA Analysis for Lipid Oxidation of Meat and Depot Fat

The steam distillation method of Tarladgis et al. (1960) was used to analyze for thiobarbituric reactive material. The distillation apparatus consisted of a 250 ml round bottom flask, which was attached to a Friedrick condensor with a three-way connecting tube. Electric heating mantles were used as the source of heat.

A duplicate 10 g sample of meat or fat was homogenized with 50 ml of distilled, deionized water for 2 minutes in a Virtis homogenizer at low speed. The homogenate was transferred quantitatively into a 250 ml round bottom flask by washing with 47.5 ml of distilled, deionized water. The pH of the meat or fat slurry was lowered to 1.5 by the addition of 2.5 ml of 4 N HCl. Boiling chips were added and a small amount of Dow antifoam was sprayed into the flask to prevent foaming. The slurry was steam distilled using the highest setting on a Powerstat (The Superior Electric Company, Bristol, Connecticut), until 50 ml of distillate was collected.

The distillate was mixed and 5 ml were transferred to a 50 ml test tube. Then 5 ml of TBA reagent (0.02 M 2-thiobarbituric acid in 90% glacial acetic acid) were added. The tubes were stoppered and the contents mixed. The tubes were heated in a boiling water bath for 35 minutes. After cooling to room temperature for 10 minutes, absorbance was

read at 538 nM using a Gilford spectrophotometer against a blank containing only distilled, deionized water and TBA reagent. Absorbance readings were multiplied by a factor of 7.8 (Tarladgis *et al.*, 1960). TBA values are expressed as mg malonaldehyde per 1000 g sample.

RESULTS AND DISCUSSION

Analysis of Experimental Diets

The filled milks were stored at room temperature and analyzed at the termination of the feeding experiment. Analysis was carried out for vitamin E level, fat and dry matter content. The ration containing coconut oil contained 26 μg/g of vitamin E, 80% dry matter and 7% fat, while the corn oil ration had a vitamin E level of 322.6 μg/g, a dry matter content of 70% and 9% fat.

The diets were also analyzed for fatty acid composition as shown in Table 3. The data show that the diet with coconut oil contained appreciable quantities of saturated C8:0 to C14:0 fatty acids, whereas, the corn oil ration contained low levels of the saturated fatty acids but high levels of C18 unsaturated fatty acids.

Liveweight, Carcass Weight and Carcass Yield of Veal Calves

Liveweights were taken shortly before slaughter. Carcass weight was determined after envisceration, both before and after removal of the hide. The carcass was chilled for a period of 24 hours at about 33°F. Carcass yield was determined by expressing the carcass weight (cold) as a proportion of the liveweight. Table 4 shows the mean values

Table 3. The Fatty Acid Composition of the Two Experimental Diets Determined by Gas Liquid Chromatography.[a]

Formula	C8:0	C10:0	C12:0	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	Total
Coconut Oil Ration	6.29	5.14	60.46	14.75	12.97	0	3.01	4.54	2.84	0	100.0
Corn Oil Ration	0	0	0.37	0.66	14.72	0.40	3.59	29.40	50.65	0.20	99.99

a) Values are given as weight percent.

for these parameters.

Average liveweight ranged from 139 lb. for group 3 to 162 lb. for group 1. There was great variability in liveweight, and hence, in carcass weight and yield. The variation in liveweight was probably due to the fact that some of the animals were very unthrifty, especially on the corn oil diet. Seven of the original calves died during the early phases of the experiment and had to be replaced. Thus, some of the animals were fed at different times. The resulting differences in environmental factors may have affected growth rate. Unsatisfactory weight gains, poor physical condition and deaths occurring in calves fed rations containing highly unsaturated vegetable fat have been reported by Adams et al. (1959).

The lowest carcass yield amounted to an average of 53.30% for group 3, which was significantly less than any of the other group. Groups 1, 2 and 4 had carcass yields of 63.03, 58.60 and 61.13%, respectively, with none of these groups varying significantly from each other.

Fat Content of the Meat and Depot Fat

The total fat content of the Longissimus dorsi was, on average, below 2% and is shown in Table 5. There was a tremendous variation in group 3, probably as a result of variability in growth rate. In contrast to mature beef, the flesh of veal is low in fat (Moulton and Lewis, 1940). The data of Post et al. (1972) as quoted by Wrenn et al. (1973) indicate

Table 4. Live Weight, Carcass Weight, and Carcass Yield of Veal Calves.

	Treatments	Number of Animals	Mean Live Weight at Slaughter[a] (lb)	Mean Carcass Weight [b] (lb)	Mean Carcass Yield[c] (%)
1.	Coconut oil + Vitamin E	4	162 ± 26.9	99.5 ± 21.2	61.03 ± 3.3
2.	Coconut oil - Vitamin E	4	160.5 ± 16.2	94.43 ± 14.6	58.60 ± 3.2
3.	Corn oil + Vitamin E	4	139.5 ± 48.1	76.63 ± 39.0	53.30 ± 6.9
4.	Corn oil - Vitamin E	4	143.5 ± 27.0	88.56 ± 21.8	61.13 ± 5.1

a) Off feed for 18 hours.

b) Hide off.

c) Carcass yield = $\frac{\text{Cold carcass weight}}{\text{Slaughter weight}}$ X 100.

that the intramuscular fat content ranges from 0.67% for 6-8 week old calves to 4.77% in mature cattle. Thus, the data from the present study are consistent with the values in the literature.

It is known that intramuscular lipid increases with age and weight, but the phospholipid fraction per gram of muscle appears to be relatively constant (Link et al., 1967). Table 5 shows the level of phospholipids, which was relatively constant and generally below 1%. This is consistent with the observations of other workers (Watts, 1962; Hornstein et al., 1967; Turkki, 1967; Body and Shorland, 1974), who found but little variation in the composition of the phospholipids in muscle.

The levels of depot fat (kidney and omental) are also shown in Table 5. There was considerable variation in the level of fat deposited (24-75%), with the minimum level for the calves on the corn oil diet. It is well known that the level of adipose tissue increases with age and weight, and that it is influenced by species, diet, environment, sex and other factors (Deuel, 1955).

Fatty Acid Composition

The average fatty acid composition and the standard deviations for kidney fat, omental fat and meat lipids from calves on the various diets are shown in Tables 6, 7, 8 and 9, respectively.

The proportion of linoleic acid (C18:2) was essentially the same

Table 5. Fat Content of Longissimus dorsi, Kidney Fat and Omental Fat.[a]

	Treatments	Mean Total fat (%) of Longissimus dorsi	Mean Total Phospholipid Content of Longissimus dorsi	Total fat Kidney fat	Total fat Omental fat
1.	Coconut oil + Vitamin E	1.66 ± 0.39	0.84 ± 0.09	74.47 ± 15.1	69.55 ± 2.2
2.	Coconut oil - Vitamin E.	1.44 ± 0.20	0.76 ± 0.09	66.08 ± 14.3	68.33 ± 5.7
3.	Corn oil + Vitamin E	1.76 ± 1.10	0.75 ± 0.07	23.6 ± 29.9	27.28 ± 33.3
4.	Corn oil - Vitamin E	1.45 ± 0.26	0.82 ± 0.08	73.18 ± 10.2	44.23 ± 27.5

a) Values are given as weight percent.

in the kidney, omental and meat triglycerides from the calves in groups 3 and 4, and ranged from approximately 26 to 30% as shown in Tables 6, 7 and 8, respectively. Ellis et al. (1974) have reported similar observations, although the level of C18:2 was only 12 to 15% in their study of calves fed diets high in linoleic acid. The values obtained in the present investigation are also higher than those reported by Wrenn et al. (1973) for calves fed milk fat high in linoleic acid.

The levels of C18:2 in the kidney, omental and meat triglycerides from calves on treatments 1 and 2 ranged from 4 to 8% as shown in Tables 6, 7 and 8, respectively. Ellis et al., (1974) reported a level of about 5% linoleic acid in a commercial sample of veal.

Vitamin E supplementation appeared to have a slight influence on the amount of C18:2 in the various tissues. Increased amounts of linoleic acid were found in nearly all the lipid fractions of all tissues from vitamin E supplemented animals. However, the differences in the level of C18:2 between vitamin E enriched and nonsupplemented diets were not statistically significant. Poukka and Oksanen (1972) have reported decreased levels of C18:2 in some tissues of vitamin E deficient calves. In the present study, the rations not supplemented with additional vitamin E were naturally rich in vitamin E. This is borne out by the fact that the coconut oil and corn oil rations contained 26.0 and 322.6 μg/g of vitamin E, respectively.

Appendices 1, 2, 3 and 4 show representative chromatograms

from different animals in groups 1, 2, 3 and 4, respectively. There was no significant difference in the fatty acid composition of animal tissues from treatments 1 and 2 and of treatments 3 and 4. However, there were some significant differences between the coconut oil (groups 1 and 2) and corn oil diets (groups 3 and 4). The level of oleic acid (C18:1) was about 30% in the tissues of calves on corn oil diets, while the average value of the coconut oil treatments was 23% (Tables 6, 7 and 8).

The amount of palmitic acid (C16:0) in the depot fat and in the neutral lipids of meat from calves on the coconut oil diet was about 30% (Tables 6, 7 and 8). However, the amount of C16:0 in the phospholipids from meat of calves on the coconut oil ration amounted to 17% (Table 9). In treatments 3 and 4 (corn oil rations), the level of palmitic acid in the depot fats was 20% (Tables 6 and 7), whereas, it was 17% in the meat neutral lipids and 13% in the meat phospholipids (Tables 8 and 9).

The depot fats and meat neutral lipids contained on the average 10% stearic acid (C18:0) for both the coconut and corn oil diets (Tables 6, 7 and 8). Likewise, the phospholipid from the meat contained a mean value of 13% stearic acid on both rations. The kind of oil did not seem to affect the level of stearic acid in the tissues. However, vitamin E supplementation slightly increased the level of stearic acid in the tissues of animals fed both diets.

The high level of lauric acid (C12:0) in the coconut

oil ration (Table 3) was not reflected by its level in the omental, kidney and meat triglycerides from calves fed this ration (groups 1 and 2). The level of lauric acid in these tissues ranged from 5 to 8% (Tables 6, 7 and 8). On the other hand, the level of myristic acid (C14:0) in the depot fats and meat triglycerides from calves in groups 1 and 2 greatly increased, ranging from approximately 18 to 23% (Tables 6, 7 and 8). However, vitamin E supplementation of the coconut oil ration decreased the amounts of lauric and myristic acids in the depot fats and meat triglycerides from calves on this ration (group 1).

Fatty Acid Composition of the Phospholipids

The fatty acid composition of the phospholipids from the longissimus dorsi muscle are shown in Table 9. The level of C18:2 comprised slightly over 40% of the fatty acids in the phospholipids from calves on the corn oil diets. Kimoto et al. (1974) reported a slightly lower level of C18:2 in the meat phospholipids from calves fed safflower oil. The level of C18:2 in the phospholipids of the meat from groups 1 and 2 (coconut oil diets) was approximately 27%. This is in good agreement with the data for commercial veal samples reported by Kimoto et al. (1974).

The levels of C16:1, C18:1 and C20:3 in the phospholipids were significantly higher for meat from calves on coconut oil diets than for those on corn oil rations (Table 9). However, the level of C20:4 was not affected by the differences

Table 6. Mean Fatty Acid Composition of Kidney Fat.[a]

	Treatment	C12:0	C14:0	C14:1	C16:0	C16:1	C18:0	C18:1	C18:2
1.	Coconut oil + Vitamin E	5.26 ± 1.0	19.21 ± 2.2	1.02 ± 0.61	30.54 ± 3.0	1.72 ± 0.33	11.48 ± 0.92	23.71 ± 3.89	7.00 ± 3.10
2.	Coconut oil - Vitamin E	7.41 ± 3.4	24.58 ± 1.90	0.96 ± 0.19	29.43 ± 1.10	1.76 ± 0.32	8.93 ± 1.71	22.03 ± 2.66	4.90 ± 3.52
3.	Corn oil + Vitamin E	1.18 ± 0.51	5.91 ± 1.40	0.0	18.95 ± 2.69	1.75 ± 0.85	13.59 ± 4.11	32.84 ± 4.11	25.77 ± 3.86
4.	Corn oil - Vitamin E	1.15 ± 0.28	5.72 ± 1.10	0.15 ± 0.23	19.69 ± 3.0	1.88 ± 0.91	11.48 ± 0.86	30.88 ± 0.86	29.05 ± 2.37

a) Values are given as weight percent.

Table 7. Mean Fatty Acid Composition of Omental Fat.[a]

	Treatment	C12:0	C14:0	C14:1	C16:0	C16:1	C18:0	C18:1	C18:2
1.	Coconut oil + Vitamin E	5.92 ± 1.80	23.19 ± 2.60	1.20 ± 0.26	31.09 ± 3.02	1.35 ± 0.49	9.93 ± 0.82	21.22 ± 2.45	6.08 ± 1.97
2.	Coconut oil - Vitamin E	8.38 ± 3.44	23.38 ± 5.04	1.48 ± 0.62	31.06 ± 4.50	2.26 ± 0.54	8.47 ± 2.25	20.14 ± 4.18	4.44 ± 2.39
3.	Corn oil + Vitamin E	0.95 ± 0.34	5.23 ± 0.37	0.17 ± 0.12	20.41 ± 2.92	1.26 ± 0.58	12.76 ± 1.96	30.65 ± 4.48	28.57 ± 3.54
4.	Corn oil - Vitamin E	1.29 ± 0.35	7.16 ± 2.27	0.64 ± 0.44	23.63 ± 3.76	1.84 ± 0.35	8.9 ± 2.20	29.49 ± 1.54	26.95 ± 4.81

a) Values are given as weight percent.

Table 8. Mean Fatty Acid Composition of Glycerides in Longissimus Dorsi.[a]

Fatty Acids	Treatment 1 Coconut oil + Vitamin E	2 Coconut oil no Vitamin E	3 Corn oil + Vitamin E	4 Corn oil, no Vitamin E
C10:0	0	0.29 ± 0.58	1.63 ± 32.6	0.81 ± 1.62
C12:0	4.68 ± 1.44	6.03 ± 1.37	0.59 ± 0.41	0.99 ± 0.55
C14:0	18.65 ± 2.78	22.69 ± 3.02	5.66 ± 1.78	5.76 ± 2.09
C14:1	1.89 ± 0.62	1.64 ± 0.18	0.15 ± 0.23	0.45 ± 0.31
C15:0	0	0	2.15 ± 2.08	1.94 ± 3.30
C16:0	28.49 ± 1.82	28.39 ± 2.43	16.69 ± 2.04	17.29 ± 3.04
C16:1	2.49 ± 0.74	2.88 ± 0.90	1.57 ± 0.40	2.93 ± 0.43
C18:0	9.3 ± 1.63	8.09 ± 1.41	10.33 ± 2.84	10.25 ± 3.89
C18:1	26.30 ± 2.93	23.71 ± 2.42	30.79 ± 3.33	30.62 ± 2.13
C18:2	8.0 ± 3.33	6.28 ± 2.39	29.99 ± 6.37	26.94 ± 2.46
C18:3	0	0	0	0.53 ± 0.81

a) Values are given as weight percent.

in the rations. Kimoto et al. (1974) have also presented data showing that differences in dietary fat did not affect the content of C20:4 in the phospholipids.

Tables 10 and 11 summarize the fatty acid composition of kidney, omental and meat triglycerides including meat phospholipids. The data show that there was a significantly higher level of saturated fatty acids (60 to 70%) in the fatty tissues and meat triglycerides from calves fed coconut oil rations than those fed corn oil rations (30 to 40%). The level of saturated fatty acids in the phospholipids was about 33% and was not influenced by the different rations.

The amount of monoenoic fatty acids ranged from about 23 to 34% in the kidney, omental and meat neutral lipids from calves in groups 1, 2, 3 and 4. The distribution of monoenoic acids in the fatty tissues and meat triglycerides from calves in the various treatments seemed random. In the phospholipids, however, meat from the calves in groups 1 and 2 (coconut oil) contained a higher level of monoenoic acid than meat from calves on the corn oil rations (groups 3 and 4).

There was a significantly higher level of dienoic acid in the fatty tissues and meat neutral lipids from calves on treatments 3 and 4 (about 28%) than for those on treatments 1 and 2 (about 5%). In the phospholipids, the amount of dienoic acid greatly increased to about 40% in the meat from calves fed corn oil rations, while the level was about 27% in the meat tissues of calves fed the coconut oil diet.

Table 9. Mean Fatty Acid Composition of Phospholipids of Longissimus Dorsi.[a]

Fatty Acids	Treatment 1 Coconut oil + Vitamin E	2 Coconut oil no Vitamin E	3 Corn oil + Vitamin E	4 Corn oil, no Vitamin E
C12:0	0.85 ± 0.48	0.43 ± 0.15	0	0.12 ± 0.24
C14:0	4.51 ± 1.26	2.51 ± 0.69	0.36 ± 0.27	1.0 ± 0.49
C14:1	0.27 ± 0.32	0	0	0.22 ± 0.43
C15:0	2.18 ± 1.38	3.86 ± 0.90	3.6 ± 0.50	3.95 ± 1.41
C16:0	17.37 ± 5.37	17.33 ± 3.52	12.66 ± 2.46	14.72 ± 4.37
C16:1	1.31 ± 0.25	3.27 ± 1.19	0.13 ± 0.26	0.13 ± 0.26
C17:0	0	0.14 ± 0.27	0.25 ± 0.30	0.27 ± 0.32
C18:0	11.91 ± 1.33	11.41 ± 1.61	14.45 ± 1.76	13.12 ± 3.04
C18:1	23.65 ± 5.15	26.09 ± 5.55	14.50 ± 1.36	16.53 ± 1.70
C18:2	26.12 ± 4.19	27.19 ± 7.68	43.67 ± 1.37	40.44 ± 0.60
C18:3	0.16 ± 0.32	0	0	0.37 ± 0.73
C20:2	0.10 ± 0.20	0.10 ± 0.18	0.51 ± 0.59	0.55 ± 0.68
C20:3	1.68 ± 0.28	1.53 ± 0.97	0.51 ± 0.36	0.40 ± 0.27
C20:4	9.81 ± 2.05	7.76 ± 5.5	9.37 ± 2.46	8.18 ± 2.23

a) Values are given as weight percent.

Table 10. Summary of Average Fatty Acid Composition of Kidney and Omental Fats.[a]

	Kidney Fat			
	Coconut Oil Diet		Corn Oil Diet	
Type of Acid	+ Vitamin E	- Vitamin E	+ Vitamin E	- Vitamin E
% Saturated	66.5	70.4	39.6	38.0
% Monoenoic	26.5	24.8	34.6	32.9
% Dienoic	7.0	4.9	25.8	29.1
% Polyenoic	0	0	0	0
	Omental Fat			
% Saturated	70.0	71.7	39.4	33.80
% Monoenoic	23.8	23.9	23.1	32.0
% Dienoic	6.1	4.4	28.6	27.0
% Polyenoic	0	0	0	0

a) Values given are in weight percent.

Table 11. Summary of Average Fatty Acid Composition of Meat Lipids.[a]

	Longissimus dorsi Triglycerides			
	Coconut Oil Diet		Corn Oil Diet	
Type of Acid	+ Vitamin E	- Vitamin E	+ Vitamin E	- Vitamin E
% Saturated	61.1	65.5	37.1	37.0
% Monoenoic	30.7	28.0	32.5	27
% Dienoic	8.0	6.3	30	27
% Polyenoic	0	0	0	0
	Meat Phospholipids (Longissimus dorsi)			
% Saturated	36.8	35.5	31.3	33.2
% Monoenoic	25.2	31.9	14.5	17.0
% Dienoic	26.2	27.3	44.2	41.0
% Polyenoic	11.7	9.3	9.9	9.9

a) Values are given in weight percent.

The level of polyenoic acid was about 9% in the phospholipids of the meat and was the same for all treatments.

Representative meat samples from groups 3 and 4 were analyzed for possible changes in the fatty acid composition at 6 months of storage. No changes in the fatty acid composition of neutral and meat phospholipids were found to occur as a result of freezer storage.

The Tocopherol Content in the Meat and Fatty Tissues

Influence of Storage Time on the Stability of Vitamin E in Longissimus dorsi.

The levels of tocopherol in the meat were determined for fresh samples and again after 1 and 3 months of storage as shown in Table 12. In the coconut oil fed animals, vitamin E supplementation did not greatly improve the amount in the meat tissues. At the end of 1 month, the levels of vitamin E in both groups 1 and 2 had declined to about 78%, but at the end of 3 months, the levels in group 2 (coconut oil - vitamin E) was only 54% of the initial value, whereas, there was little change in group 1 (coconut oil + vitamin E).

The initial levels of vitamin E in treatments 3 and 4 (corn oil diets) were 5.19 and 3.44 μg/g of tissue (Table 12), respectively. The level of vitamin E at the end of 1 month represented about 70% of the initial level for each treatment, but at the end of 3 months the levels had declined

to about 42 and 25%, respectively. Thus, supplementation of corn oil with vitamin E influenced the level and stability of vitamin E in the meat.

Figure 1 shows the effect of storage time on the stability of vitamin E in the meat. The rate of decline in the meat from calves fed coconut oil diets + vitamin E (group 1) was very gradual ($b = -0.27$), whereas, the decline was rather sharp ($b = -0.97$) in the meat from calves fed corn oil diets + vitamin E (group 3). Thus, both storage and the type of oil in the diet significantly ($P < 0.01$) influenced vitamin E stability in the tissues.

Adams *et al.* (1959) have reported that aging of meat results in the loss of tocopherol. The greater loss of vitamin E in meat from the corn oil fed calves supports the contention that tocopherols are very unstable in storage, especially in products containing highly unsaturated fatty acids, which is in agreement with reports by Lips (1947, Keating *et al.* (1965), and Witting (1975). A significant interaction ($P < 0.05$) between diets and length of storage indicates that the stability of vitamin E in the meat behaved differently according to the kind of oil used. Thus, vitamin E was more stable in meat from calves fed coconut oil.

The low level of tocopherol obtained in *longissimus dorsi* muscle is in agreement with the values reported by Kimoto *et al.* (1974). They found 4.4 and 6.6 μg/g of tissue of vitamin E in meat from calves fed safflower oil supplemented with 486 mg of α-tocopheryl acetate for 10

Table 12. Mean Tocopherol (Vitamin E) Levels in Longissimus Dorsi as Influenced by Storage Time.[a]

Storage Period in Months	Longissimus Dorsi			
	Coconut Oil		Corn Oil	
	+ Vitamin E	- Vitamin E	+ Vitamin E	- Vitamin E
Initial Level	3.29 ± 2.6	5.12 ± 0.9	5.19 ± 2.16	3.44 ± 0.75
1 Month	2.58 ± 2.3	3.98 ± 1.1	3.59 ± 1.73	2.43 ± 0.47
3 Months	2.55 ± 2.2	2.75 ± 1.7	2.17 ± 0.61	0.86 ± 0.62

a) Values are expressed in μg/g of lipid.

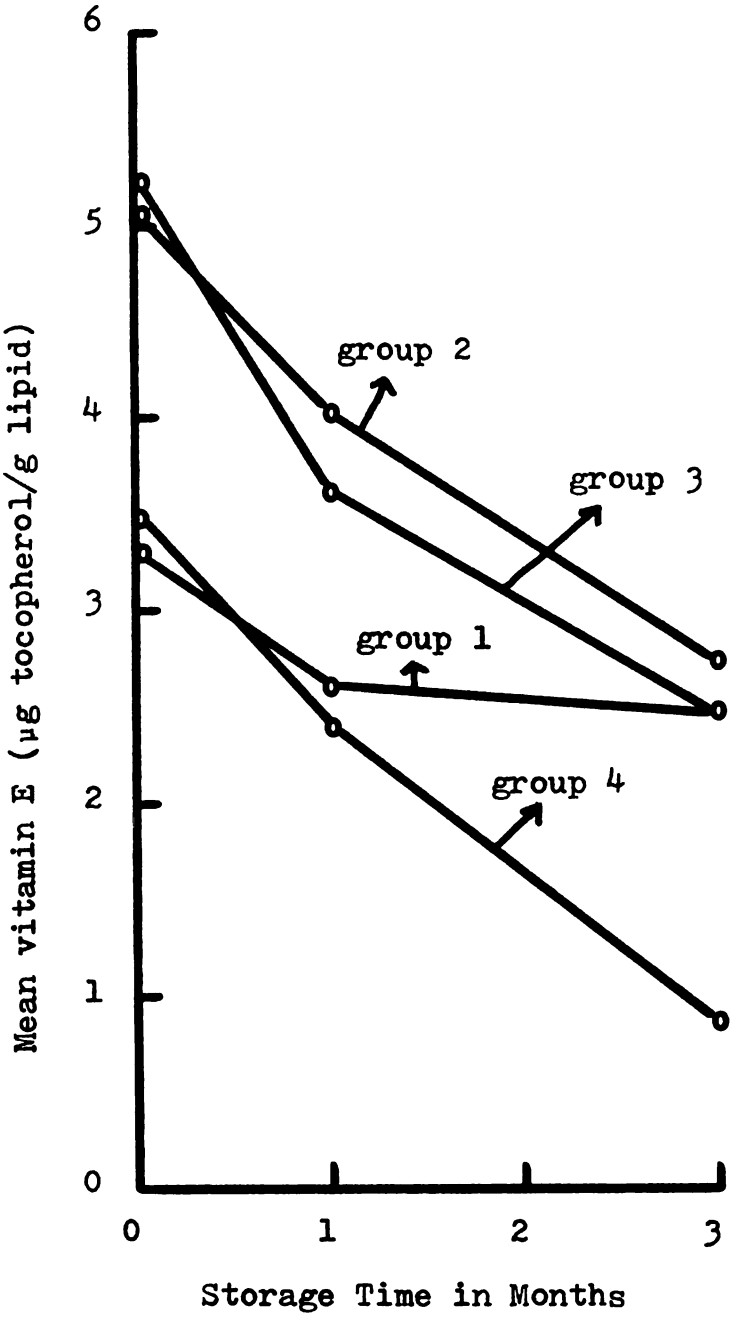


Figure 1. Changes in tocopherol level of longissimus dorsi during freezer storage. group 1 = coconut oil + vitamin E; group 2 = coconut oil - vitamin E; group 3 = corn oil + vitamin E; group 4 = corn oil - vitamin E.

weeks and then fed unprotected and protected oil for another 8 weeks, respectively.

Tocopherol Content of Kidney Fat

The tocopherol level in the kidney fat was monitored over a period of 6 months freezer storage as shown in Table 13. The initial levels were 43.68, 19.09, 16.43 and 18.30 μg/g/lipid for groups 1, 2, 3 and 4, respectively. Supplemental vitamin E feeding markedly increased its level in the fatty tissues of the coconut oil fed group. At any given storage time, there was a significant ($P < .05$) difference in the tocopherol content between groups 1 and 2; however, groups 2, 3 and 4 did not differ significantly.

Tocopherol supplementation of the corn oil diet (group 3) did not improve retention (Table 13). Three of the four calves in this treatment were unthrifty, and had unsatisfactory growth rates (Appendix Table 1). The fat content of perinephric fatty tissues for this group was only about 24% (Table 5) compared to 75 and 68% for groups 1 and 2 (coconut oil), respectively. The low level of vitamin E retained by treatment 3 may be due to the low level of fat in the tissues since tocopherol is a fat soluble vitamin. Moreover, some of these calves had diarrhea which may have caused a marked decrease of vitamin E in the blood plasma. Thomas and Okamoto (1955) have reported that diarrhea causes the loss of vitamin E from the plasma. The variability in this group is illustrated by calf No. 371, which had a slaughter

Table 13. Mean Tocopherol (Vitamin E) Levels of Kidney Fat as Influenced by Storage Time.[a]

Storage Period in Months	Coconut Oil		Corn Oil	
	1	2	3	4
	+ Vitamin E	- Vitamin E	+ Vitamin E	- Vitamin E
0	43.68 ± 16.89	19.09 ± 3.6	16.43 ± 12.0	18.30 ± 9.3
1	37.05 ± 13.6	14.27 ± 2.0	13.95 ± 13.0	14.09 ± 6.2
3	26.16 ± 10.7	11.99 ± 2.9	11.04 ± 15.7	10.60 ± 4.7
6	13.95 ± 6.2	4.98 ± 3.3	4.87 ± 8.8	7.68 ± 5.7

a) Values are expressed in μg/g of lipid.

weight of 210 lb, a high fat content and a high initial tocopherol level of 34 µg/g of tissue.

The rate of vitamin E loss in kidney fat with storage was generally similar for all treatments as shown in Figure 2. At 1 month of storage, the tocopherol retained was over 75% of the initial value for all treatments. At 3 months, the level retained was about 60% for all treatments, but at 6 months, the level varied between 26 and 43% for all treatments. A faster and significantly greater rate of decline ($b = -4.9$) was observed for treatment 1 than for treatment 3 ($b = -1.88$). Analysis of variance of the factors that influence vitamin E stability in kidney fat indicated a highly significant storage effect ($P < 0.01$). The differences in vitamin E retention due to the type of oil in the diet were also statistically significant ($P < 0.05$). There were also significant ($P < 0.05$) interactions between vitamin x oil, and of oil x vitamin x storage, which indicated the effects were not always in the same direction.

The levels of tocopherol found in kidney fat from calves on treatment 1 are similar to values reported by Ellis et al. (1974), who fed calves safflower oil supplemented with vitamin E for a period of 10 weeks followed by feeding a protected safflower oil diet for another 8 weeks.

Tocopherol Content of Omental Fat

In Table 14, the stability of tocopherol in omental fat during 6 months freezer storage is shown. The initial levels

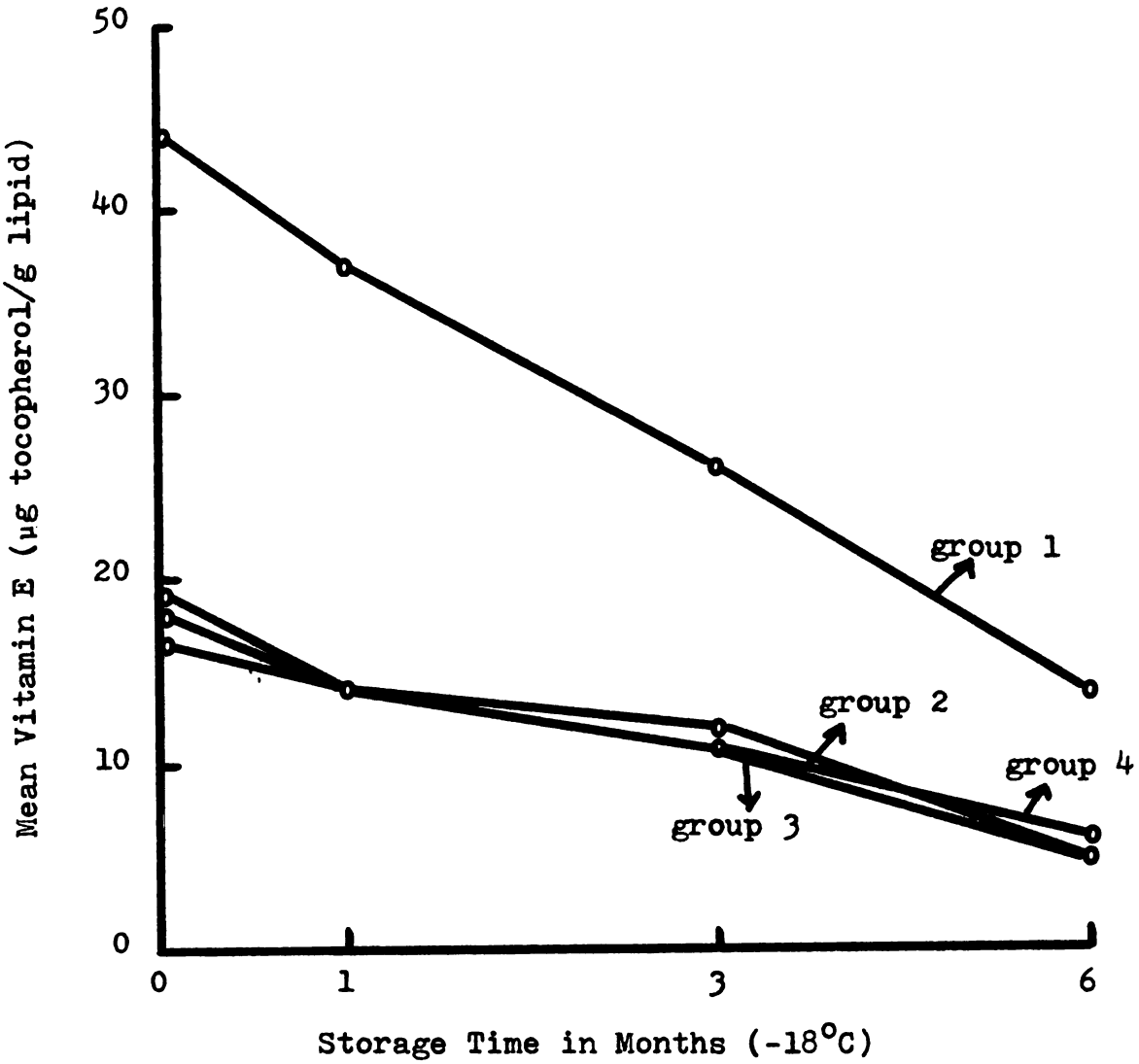


Figure 2. Changes in tocopherol level of kidney fat. group 1 = coconut oil + vitamin E; group 2 = coconut oil - vitamin E; group 3 = corn oil + vitamin E; group 4 = corn oil - vitamin E.

were 41.09, 14.95, 22.96 and 23.46 μg/g of tissue for treatments 1, 2, 3 and 4, respectively. As in the case of kidney fat, vitamin E supplementation of the coconut oil diet significantly ($P < 0.01$) increased its level in omental fat. There was a significant ($P < 0.05$) interaction between vitamin E supplementation and the type of oil in the diet. Supplemental vitamin E in the corn oil diets did not significantly influence the content of tissue vitamin E, probably due to the environmental factors that have been previously discussed.

Figure 3 shows the decline in vitamin E levels in omental fat during storage. As with kidney fat, there was a significant decline during storage ($P < 0.01$). The rate of vitamin E disappearance in the omental fat of group 1 was much slower ($b = -2.7$) than that of kidney fat ($b = -4.9$). The regression coefficient for the disappearance of vitamin E in group 3 (corn oil diet) was $b = -1.95$. At the end of 1 month's storage, the levels of vitamin E were from 72 to 87% of the initial value. At 3 months, the levels were from 62 to 74% and at 6 months, residual levels of vitamin E were 58, 51, 46 and 47% for treatments 1, 2, 3 and 4, respectively.

The data obtained in this study indicate that the initial levels of tocopherol in kidney and omental fat were essentially similar on the same treatment. Ellis *et al.* (1974) did not report any significant differences in the tocopherol level in the kidney and omental fats of calves. In the present study, however, there was a significantly

Table 14. Mean Tocopherol (Vitamin E) Levels of Omental Fat as Influenced by Storage Time.[a]

Storage Period in Months	Coconut Oil		Corn Oil	
	1	2	3	4
	+ Vitamin E	- Vitamin E	+ Vitamin E	- Vitamin E
0	41.09 ± 10.0	14.95 ± 2.6	22.96 ± 16.4	23.46 ± 6.7
1	35.83 ± 3.6	10.42 ± 2.1	18.82 ± 16.0	19.22 ± 6.2
3	30.55 ± 4.5	10.00 ± 1.5	14.26 ± 16.4	15.84 ± 5.2
6	24.04 ± 10.1	7.57 ± 3.0	10.74 ± 16	11.15 ± 6.9

a) Values are expressed in μg/g of lipid.

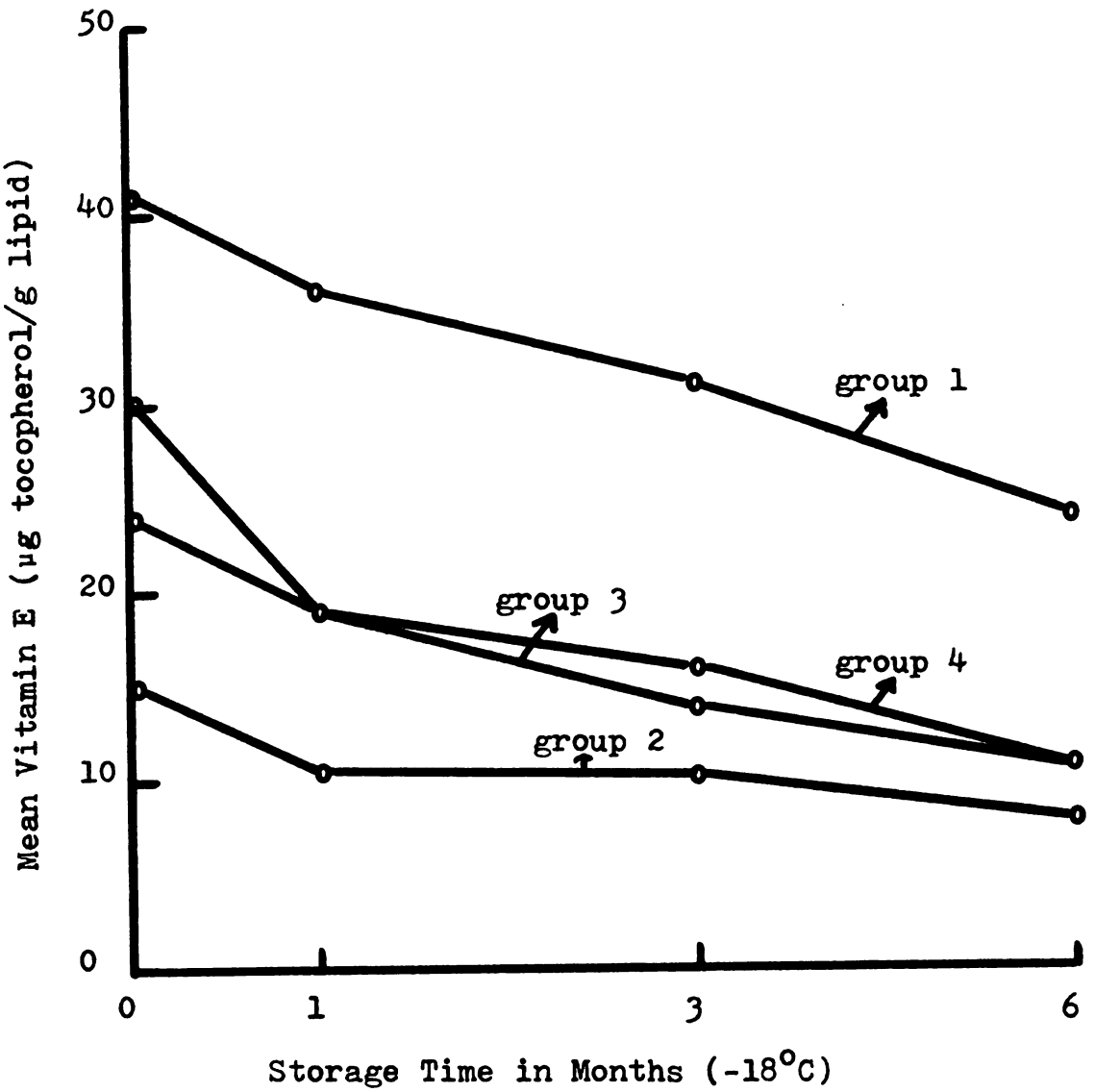


Figure 3. Changes in tocopherol level of omental fat. group 1 = coconut oil + vitamin E; group 2 = coconut oil - vitamin E; group 3 = corn oil + vitamin E; group 4 = corn oil - vitamin E.

stability, it is important to be able to predict the stability of tocopherol in frozen animal tissues. This is more important as a result of the ever-growing intercontinental trade in frozen meat and dairy products.

The relationship between length of storage and the level of vitamin E follows a simple linear regression equation:

$$y = a + bx$$

where y = dependent variable, a = intercept, b = slope, and x = independent variable.

This regression equation can be used to predict storage stability of vitamin E. Thus, it is possible to predict with some degree of confidence the corresponding mean of y, for a given value of x. We can also predict what a single observed value of y would be for a given x, but with less confidence. Hence, the point estimate for either the mean or a single value is:

$$\hat{y} = a + bxo$$

where $\hat{y}$ is the predicted value corresponding to a given value xo, and xo = storage time.

The standard error of $\hat{y}$ $(Se\hat{y}) = \pm Mse \sqrt{(1/n + \frac{(xo - \bar{x})^2}{\leqslant x^2})}$

where: Mse = standard error of mean, n = number of treatments, xo = storage time, and $\bar{x}$ = mean of treatment. The standard error for a given value depends upon xo.

Lipid Oxidation in Animal Tissues

Lipid Oxidation in Meat

The data for TBA numbers of longissimus dorsi muscle are shown in Table 15. The relationships among the treatment groups are illustrated in Figure 4. Up to 6 months of freezer storage, all treatments were stable since the maximum TBA number was below 0.3. According to Watts (1962), the threshold for rancidity detection in meats occurs at a TBA value between 1.0 and 2.0.

There were,however, differences in the relative rates of fat oxidation among the treatments (Figure 4). Analysis of variance indicated a highly significant ($P < 0.01$) effect of vitamin E on the rate of oxidation in meat. Storage time also had a highly significant ($P < 0.01$) effect on the rate of lipid oxidation. However, differences in the type of oil did not seem to have any significant effect on the stability of the meat as determined by TBA values. One major point of interest is that after 6 months of freezer storage, oxidation was still below the induction stage (Figure 4) as indicated by the low regression coefficient.

Lipid Oxidation in Depot Fats

TBA values for lipid oxidation in kidney and omental fats are shown in Table 16. The relative rates of lipid oxidation among the treatment groups are illustrated in Figures 5 and 6, respectively. Analysis of variance

Table 15. Mean Thiobarbituric Acid (TBA) Number of Longissimus Dorsi as Influenced by Storage Time.[a]

Storage Period in Months	Longissimus Dorsi			
	Coconut Oil		Corn Oil	
	1 + Vitamin E	2 - Vitamin E	3 + Vitamin E	4 - Vitamin E
0	0.04 ± 0.03	0.04 ± 0.02	0.00 ± 0.0	0.06 ± 0.04
1 Month	0.08 ± 0.02	0.12 ± 0.01	0.07 ± 0.02	0.12 ± 0.03
3 Months	0.11 ± 0.02	0.16 ± 0.03	0.11 ± 0.03	0.18 ± 0.07
6 Months	0.11 ± 0.03	0.17 ± 0.04	0.14 ± 0.04	0.21 ± 0.01

a) TBA values are given in mg/1,000 g of tissue.

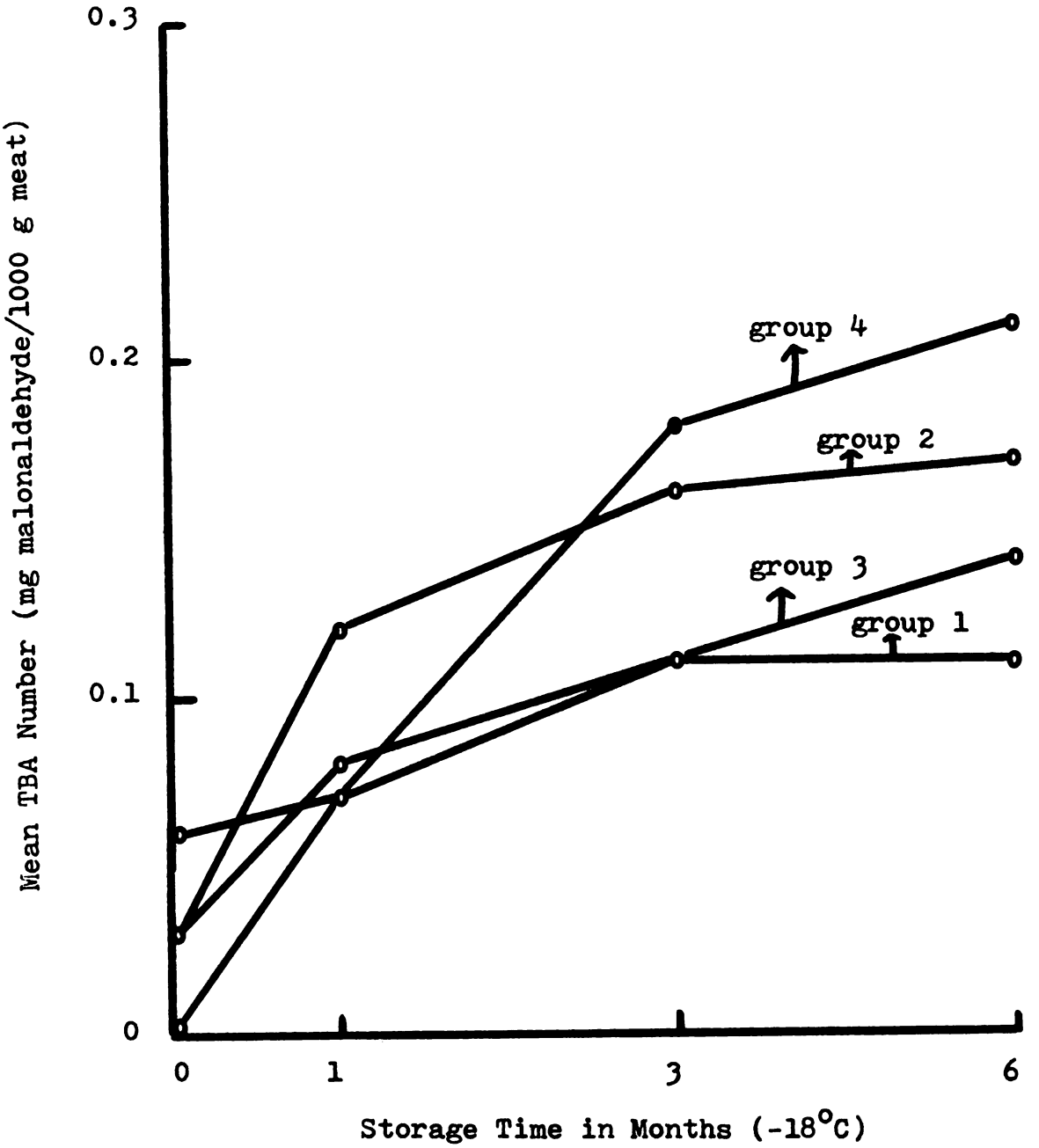


Figure 4. Changes in TBA numbers of longissimus dorsi as influenced by length of freezer storage. group 1 = coconut oil + vitamin E; group 2 = coconut oil - vitamin E; group 3 = corn oil + vitamin E; group 4 = corn oil - vitamin E.

indicated a higher rate of lipid oxidation in kidney fat than in omental fat. In addition, the type of oil used in the ration also significantly influenced ($P < 0.01$) the rate of lipid peroxidation in kidney fat (Table 16). The rate of oxidation of omental fat was influenced less by the type of oil in the diet than was kidney fat.

The rapid disappearance of vitamin E from the kidney fat was apparent and is related to increased oxidation. On the other hand, vitamin E was more stable in omental fat during storage, and greatly improved its stability. There was also a strong interaction ($P < 0.05$) between the type of oil in the diet and the level of vitamin E on the rate of oxidation in kidney fat, with the corn oil without added vitamin E being oxidized most rapidly.

There were no significant differences in the levels of PUFAS between the kidney and omental fat (Tables 6 and 7). Thus, the overriding factor influencing the rate of oxidation seems to be the level of available vitamin E. This correlates with a similar report by Lea (1953) showing that chicken fat was more stable than turkey fat, although both fats have similar fatty acids. The difference has been attributed to variations in tocopherol content. Ellis *et al.* (1974) have reported that the oxidative stability of rendered depot fats was inversely related to the C18:2 content if the tocopherol levels were similar. The data from the present study essentially confirm the results of Ellis *et al.* (1974). Hence, adipose tissue may not be

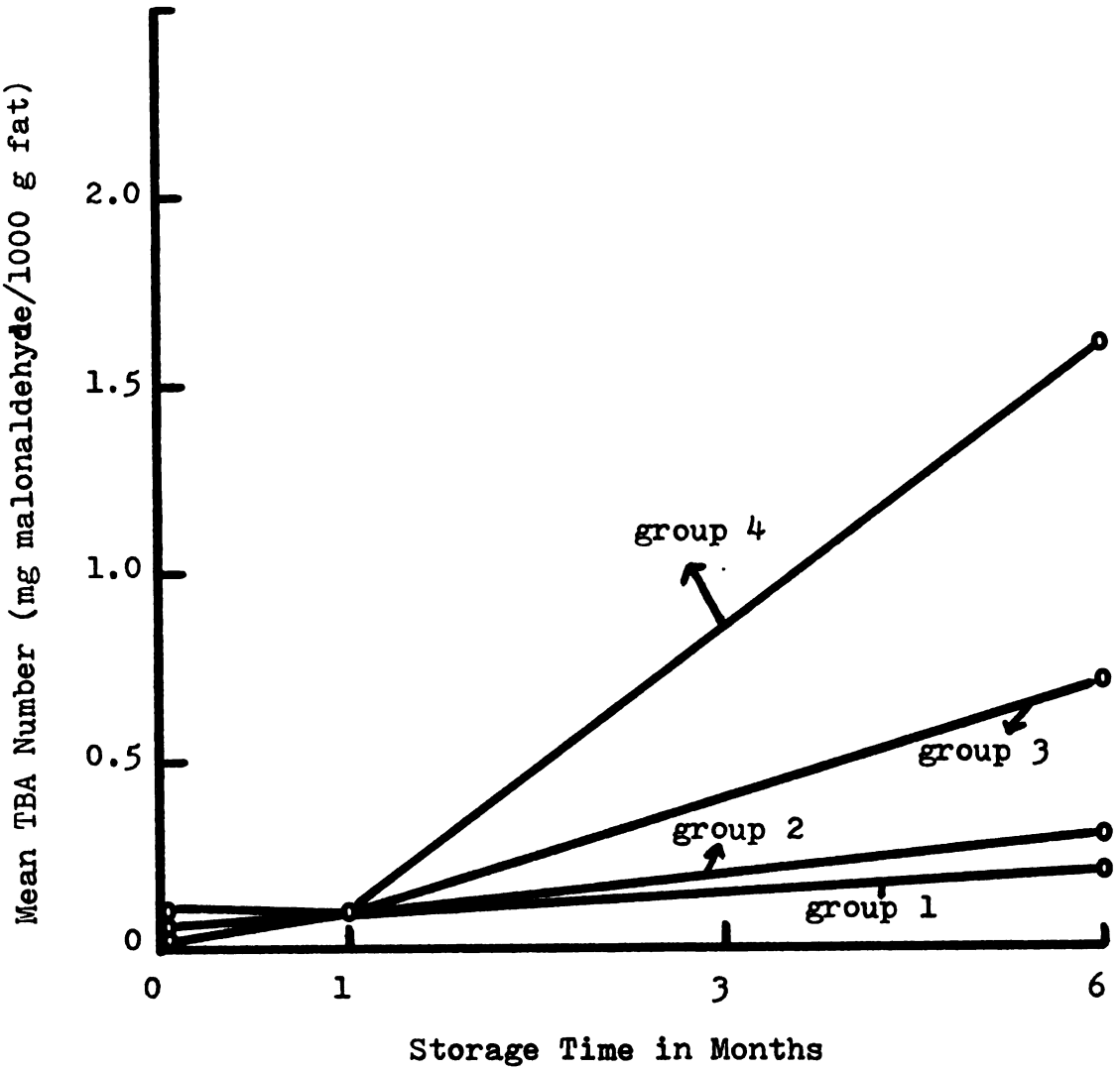


Figure 5. Changes in TBA numbers of kidney fat during freezer storage (-18°C). group 1 = coconut oil + vitamin E; group 2 = coconut oil - vitamin E; group 3 = corn oil + vitamin E; group 4 = corn oil - vitamin e.

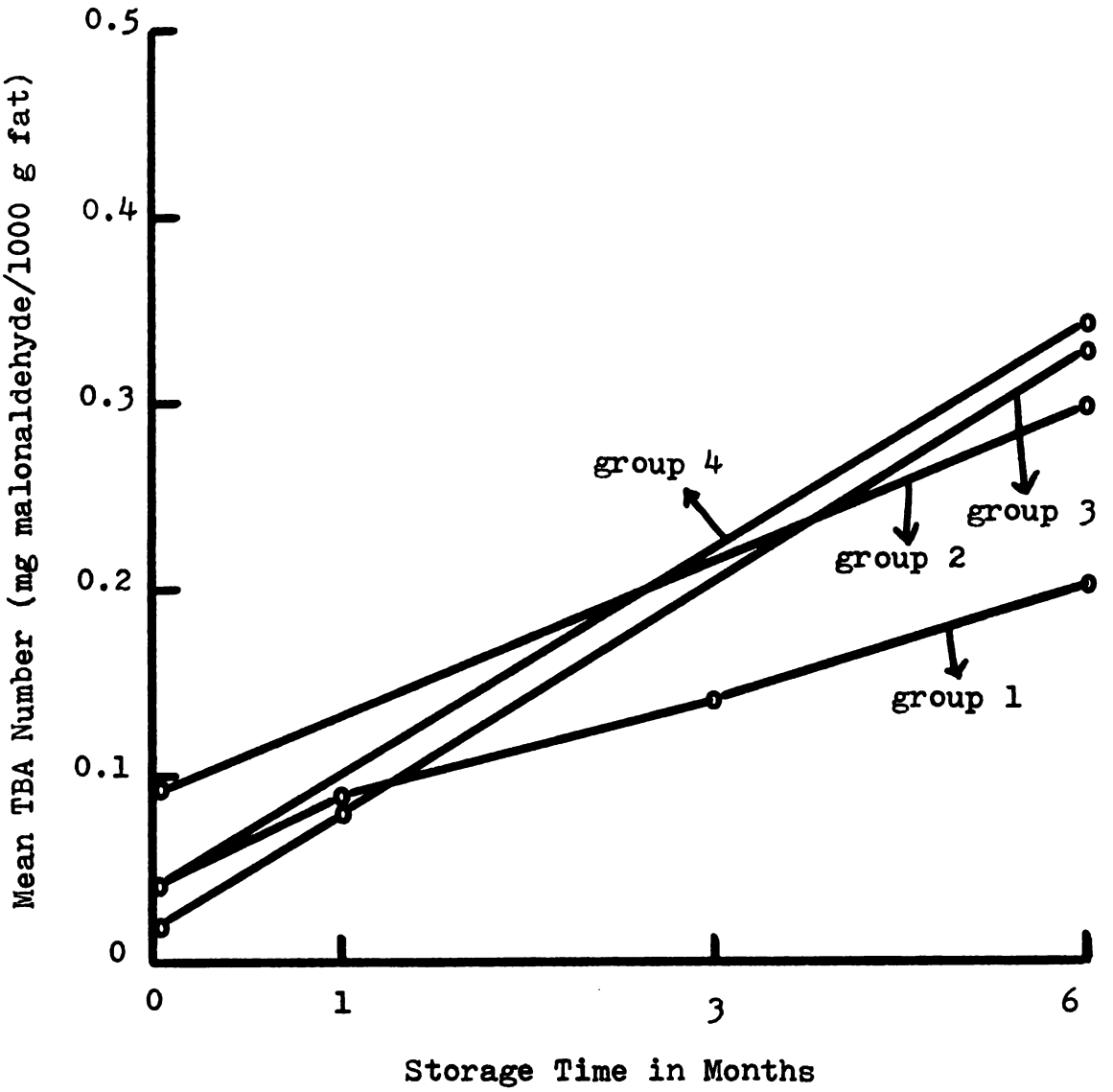


Figure 6. Changes in TBA numbers of omental fat during freezer storage (-18°C). group 1 = coconut oil + vitamin E; group 2 = coconut oil - vitamin E; group 3 = corn oil + vitamin E; group 4 = corn oil - vitamin E.

Table 16. Mean TBA Numbers of Kidney and Omental Fat.[a]

		Kidney Fat		Omental Fat	
		Storage Period		Storage Period	
	Treatment	Initial Level	Level at 6 Months	Initial Level	Level at 6 Months
1.	Coconut oil + Vitamin E	0	0.20 ± 0.02	0.04 ± 0.06	0.20 ± 0.10
2.	Coconut oil, no Vitamin E	0.09 ± 0.05	0.31 ± 0.09	0.09 ± 0.05	0.29 ± 0.09
3.	Corn oil + Vitamin E	0.01 ± 0.02	0.73 ± 0.61	0.02 ± 0.04	0.39 ± 0.32
4.	Corn oil, no Vitamin E	0.04 ± 0.06	1.59 ± 0.36	0.08 ± 0.05	0.40 ± 0.01

a) TBA values are given in mg/1,000 g of tissue.

homogeneous in bovine animals as shown by the differing rates of lipid oxidation in kidney and omental tissues. Ingle et al. (1972 a, b) have reported that the internal adipose tissues (kidney and omental) were most active in younger animals (lambs and calves) and possess considerably greater activity than that of the subcutaneous depots. However, they also reported that lipogenesis and lipolysis are more active in the perinephric than in omental fat, which is in agreement with the results from the present study.

SUMMARY AND CONCLUSIONS

It was found that young calves selectively deposit dietary fats in the tissues without significant changes in the fatty acid profile. Coconut oil markedly increased the levels of myristic and palmitic acids as well as the level of saturated fatty acids in the depot and tissue lipids. Corn oil increased the level of polyunsaturated fatty acids (PUFAS), especially the level of linoleic acid, in the depot fats, meat triglycerides and in the meat phospholipids. The level of arachidonic acid in the meat phospholipids was fairly constant on both diets.

Supplementation of coconut oil diets with vitamin E significantly elevated its level in the depot fats, but had less effect upon the meat lipids. Vitamin E supplementation of the corn oil ration did not significantly improve the level retained in the depot fats or meat lipids. Vitamin E declined steadily during frozen storage. The rate of vitamin E loss in storage was most rapid in kidney fat followed by the meat lipids, and omental fat, in that order. Losses of about one-third of the tocopherols occurred in the depot fats within 3 months of storage at -18°C. Thereafter, a rapid rate of decline was observed, especially in the kidney

fat.

By 6 months storage, the TBA values had increased to the threshold level for rancidity in the kidney fat from calves fed corn oil without supplemental vitamin E. However, TBA values were below threshold levels in the omental fat and meat tissues, regardless of whether they were derived from calves fed vitamin E or not. The poor stability of kidney fat is theorized to be related to its faster lipid turnover rate and a higher rate of metabolic activity.

Results indicate that the fatty acid composition of the ration can alter the fatty acid profile in veal tissues. The data also show that dietary vitamin E and saturated fatty acids contribute to meat stability during frozen storage.

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APPENDIX

Appendix Table 1. Calves: Weights at Salughter (lb).

Number	Slaughter Date	Slaughter Weight	Carcass Weight With Hide	Carcass Weight Without Hide
Coconut oil + Vitamin E				
350	6- 3-75	162	110	98
367	7-30-75	182	127	115.5
373	7-30-75	180	130	114.5
454	11- 4-75	124	87	70
Coconut oil, no Vitamin E				
354	6-17-75	148	93.5	81.0
355	6-17-75	152	100.5	88.5
368	7-30-75	158	99	93.2
369	7-30-75	184	130	115.0
Corn oil + Vitamin E				
342	6- 3-75	121	66.5	57
349	6- 3-75	102	63	53
351	6- 3-75	125	74	64
371	7-30-75	210	152.5	132.5
Corn oil, no Vitamin E				
348	6- 3-75	108	67.5	58
364	7-30-75	144	104	90.5
365	7-30-75	174	126	109
372	7-30-75	148	109	96.8

Appendix Table 2. Veal Lipids: Longissimus dorsi.

Calf Number	Weight of Sample (g)	Dry Matter (g)	Total Weight of Fat (g)	% Fat (Total)	Weight of Triglycerides (g)	Weight of Phospholipids (g)	% Phospholipids
350	70.0	12.5	1.3039	1.8627	0.5930	0.5927	0.8467
367	75.5	14.1	1.5409	2.0493	0.8359	0.7013	0.9289
373	71.5	13.6	0.8126	1.1365	0.1264	0.6293	0.8801
454	82.5	15.7	1.3122	1.5905	0.6197	0.5840	0.7079
354	70.0	13.4	1.0086	1.4408	0.4426	0.4848	0.6926
355	71.0	13.3	0.8253	1.1624	0.2024	0.4937	0.6954
368	70.0	17.5	1.0622	1.5174	0.4720	0.6035	0.8621
369	70.0	12.9	1.1376	1.6251	0.4778	0.5699	0.8141
342	70.0	11.5	1.0790	1.5414	0.4519	0.5096	0.728
349	64.0	10.6	0.7224	1.1288	0.1868	0.4797	0.7495
351	70.0	11.5	0.6756	0.9651	0.1238	0.4858	0.694
371	70.0	14.3	2.3723	3.389	1.8306	0.5920	0.8457
348	70.8	11.5	0.8093	1.1431	0.1993	0.5572	0.7870
364	70.0	13.1	1.0967	1.5667	0.4623	0.6452	0.9217
365	71.0	13.3	1.2075	1.725	0.4136	0.5169	0.7280
372	70.0	11.3	0.9515	1.3593	0.3671	0.5828	0.8326

Appendix Table 3. Veal Lipids: Kidney Fat.

Number	Weight of Sample (g)	Weight of Residue (g)	Total Weight of Fat (g)	Percent of Fat (a)	Fat Content by Calc. (g)	% Fat (b)	% Fat (Mean)
Coconut oil + Vitamin E							
350	5.0	0.34	3.62	72.4	3.52	70.4	71.4
367	5.0	0.08	4.90	98.0	4.65	93	95.5
373	5.0	0.28	3.37	67.4	3.78	75.6	71.5
454	5.0	0.42	2.78	55.6	3.17	63.4	59.5
Coconut oil, no Vitamin E							
354	5.0	0.504	2.52	50.4	2.81	56.2	53.3
355	5.0	0.23	3.40	68.0	4.0	80	74.0
368	5.0	0.41	2.26	45.2	3.22	64.4	54.8
369	5.0	0.14	3.83	76.6	4.39	87.8	82.2
Corn oil + Vitamin E							
342	2.5	0.509	0.046	1.84	0.30	6.0	3.92
349	2.5	0.87	0.24	9.6	----	9.6	9.6
351	2.5	0.80	0.32	12.8	----	12.8	12.8
371	5.0	0.291	3.07	61.4	3.74	74.8	68.1
Corn oil, no Vitamin E							
348	5.0	0.15	4.54	90.8	4.35	87	87
364	5.0	0.30	3.52	70.4	3.70	74	72.2
365	5.0	0.30	3.39	67.8	3.70	74	70.9
372	5.0	0.22	2.26	45.2	4.0	80	62.6

Appendix Table 4. Veal Lipids: Omental Fat.

Number	Weight of Sample (g)	Weight of Residue (g)	Total Weight of Fat (g)	Percent of Fat (a)	Fat Content by Calc. (g)	% Fat (b)	% Fat (Mean)
Coconut oil + Vitamin E							
350	7.5	0.315	4.56	60.8	6.13	81.73	71.3
367	7.0	0.458	5.02	71.1714	5.01	71.57	71.4
373	9.5	0.636	6.40	67.368	6.24	65.68	68.6
454	1.3	0.0930	0.84	64.6154	0.9	69.23	66.9
Coconut oil, no Vitamin E							
354	6.2	0.418	3.31	53.3871	4.33	69.84	61.6
355	7.0	0.250	4.48	64.0	5.9	84.28	74.2
368	6.3	0.467	2.98	47.3016	5.3	84.13	65.7
369	8.5	0.385	5.36	63.0588	6.83	80.35	71.8
Corn oil + Vitamin E							
342	5.0	0.539	0.2956	5.912	----	5.91	5.9
349	5.09	0.487	1.1386	22.772	----	22.77	22.8
351	7.0	0.709	0.333	4.7571	----	4.76	4.80
371	10.0	0.445	7.036	70.36	8.07	80.7	75.6
Corn oil, no Vitamin E							
348	5.0	0.572	0.27	5.4	----	5.4	5.4
364	8.0	0.969	3.28	41.0	3.79	47.38	44.2
365	7.5	0.535	4.45	59.33	5.17	68.93	64.1
372	6.5	0.505	4.19	64.4615	4.31	64.81	63.2

Appendix Table 5. Veal Vitamin E: µg/g lipid.

Treatment and Sample Number	Kidney Fat	Omental Fat	Kidney Fat	Omental Fat	Kidney Fat	Omental Fat	Kidney Fat	Omental Fat
	Initial Value		1 Month		3 Months		6 Months	
	Vit. E	Vit. E	Vit. E	Vit. E	Vit. E	Vit. E	Vit E.	Vit. E
Coconut oil + Vitamin E								
350	38.75	40.35	34.8	37.03	16.22	23.78	13.20	12.40
367	35.54	32.70	28.64	32.0	22.0	33.2	20.54	30.54
373	31.76	35.95	27.89	33.95	25.2	32.4	8.11	29.19
454	68.65	55.38	56.85	40.32	41.2	32.8	13.95	24.04
Coconut oil, no Vitamin E								
354	24.05	18.60	14.0	8.4	12.7	9.5	9.73	3.78
355	16.94	12.57	16.15	8.92	15.16	8.11	4.32	6.49
368	15.94	14.86	11.56	12.76	8.11	11.6	4.8	10.27
369	19.46	13.78	15.36	11.61	12.0	10.8	10.81	9.73
Corn oil + Vitamin E								
342	7.33	16.80	4.62	13.51	0.00	1.89	0.00	0.00
349	11.45	10.93	7.69	7.60	2.16	7.84	0.00	4.05
351	12.69	16.95	9.6	11.54	8.0	8.92	1.35	4.32
371	34.24	47.16	33.90	42.64	34.0	38.4	18.11	34.59
Corn oil, no Vitamin E								
348	7.45	13.56	5.38	8.92	4.62	8.65	0.00	1.08
364	28.46	25.41	19.50	21.48	14.59	15.41	13.6	13.6
365	22.97	27.57	17.54	23.76	9.2	19.6	7.84	12.70
372	14.32	27.29	13.95	22.73	14.0	19.73	10.0	17.20

Appendix Table 6.

Veal Longissimus dorsi TBA Value: mg/1000 g Tissue

Veal Longissimus dorsi Vitamin E: µg/g Lipid

Treatment and Sample Number	Initial Value	1 Month	3 Months	6 Months	Initial Value	1 Month	3 Months
	TBA	TBA	TBA	TBA	Vit.E	Vit.E	Vit.E
Coconut oil + Vitamin E							
350	0.00	0.10	0.133	0.140	6.56	5.32	5.0
367	0.04	0.06	0.08	0.08	4.03	3.55	3.24
373	0.07	0.07	0.09	0.10	1.77	1.45	1.35
454	0.055	0.09	0.12	-(.13)	0.81	⊖	⊖
Coconut oil, no Vitamin E							
354	0.03	0.13	0.195	0.22	6.45	5.48	4.65
355	0.02	0.12	0.13	0.13	4.84	4.19	3.63
368	0.06	0.10	0.15	0.18	4.68	3.38	1.35
369	0.03	0.13	0.16	0.16	4.52	2.90	1.35
Corn oil + Vitamin E							
342	0.00	0.09	0.14	0.16	3.30	2.0	1.29
349	0.00	0.06	0.10	0.12	3.76	2.47	2.42
351	0.00	0.09	0.12	0.094	5.65	4.11	2.26
371	0.00	0.04	0.07	0.19	8.06	5.81	2.70
Corn oil, no Vitamin E							
348	0.03	0.07	0.10	0.21	3.45	2.8	1.29
364	0.04	0.13	0.26	0.20	2.42	1.77	1.35
365	0.11	0.125	0.17	0.23	4.19	2.74	0.81
372	0.07	0.14	0.19	0.20	3.71	2.42	0.00

Appendix Table 7. TBA Values for Kidney and Omental Fat Tissues (mg/1000 g tissue).

Calf Number	TBA Values at 0-Month of Storage		TBA Values at 6-Months of Storage	
	Kidney Fat	Omental Fat	Kidney Fat	Omental Fat
Coconut oil + Vitamin E				
350	0.00	0.00	0.23	----
367	0.00	0.14	0.18	0.27
373	0.00	0.05	0.20	0.13
454	0.00	0.00	----	----
Coconut oil, no Vitamin E				
354	0.05	0.04	0.36	0.23
355	0.06	0.06	0.20	0.19
368	0.16	0.16	0.42	0.37
369	0.08	0.13	0.27	0.37
Corn oil + Vitamin E				
342	0.05	0.00	----	----
349	0.00	0.00	----	0.39
351	0.00	0.00	0.30	0.32
371	0.00	0.09	1.16	0.28
Corn oil, no Vitamin E				
348	0.04	0.00	----	----
364	0.13	0.12	1.17	0.34
365	0.00	0.09	1.83	0.39
372	0.00	0.09	1.77	0.42

Appendix Table 8. Veal Lipids: Fatty Acid Composition of Kidney Fat (%).

Treatment and Sample Number	Fatty Acids							
	C12:0	C14:0	C14:1	C16:0	C16:1	C18:0	C18:1	C18:2
Coconut Oil + Vitamin E								
350	4.62	16.47	1.28	34.36	1.92	11.79	23.46	6.09
367	6.63	20.29	1.73	30.46	2.10	10.14	21.11	7.55
373	5.39	21.34	0.78	26.98	1.36	12.22	20.98	10.92
454	4.65	18.74	0.30	30.35	1.50	11.75	29.27	3.45
Coconut Oil, no Vitamin E								
354	7.45	26.52	1.21	29.25	2.08	6.83	24.70	1.95
355	7.65	25.55	0.78	28.04	1.32	10.98	23.92	1.76
368	6.70	24.18	1.02	29.69	1.86	9.28	19.48	7.77
369	7.84	22.08	0.84	30.72	1.76	8.64	20.00	8.12
Corn Oil + Vitamin E								
342	1.42	7.87	0	21.12	1.34	12.48	28.97	26.75
349	0.51	4.48	0	18.39	1.07	14.15	32.17	29.22
351	1.71	5.99	0	15.36	1.59	16.48	38.64	20.23
371	1.09	5.29	0	20.91	2.98	11.25	31.59	26.89
Corn Oil, no Vitamin E								
348	0.88	4.45	0.12	19.97	1.90	11.03	30.23	31.42
364	1.05	6.57	0	17.58	1.01	14.32	31.37	28.09
365	1.12	5.12	0	17.38	1.47	12.55	31.84	30.51
372	1.53	6.73	0.48	23.85	3.13	8.02	30.07	26.19

Appendix Table 9. Veal Lipids: Fatty Acid Composition of Omental Fat (%).

Treatment and Sample Number	Fatty Acids							
	C12:0	C14:0	C14:1	C16:0	C16:1	C18:0	C18:1	C18:2
Coconut Oil + Vitamin E								
350	3.70	26.61	1.00	29.16	1.54	11.07	20.67	6.25
367	5.92	20.30	1.19	34.14	0.65	9.36	22.15	6.27
373	5.94	22.62	1.04	33.15	1.42	9.32	18.13	8.29
454	8.12	23.22	1.57	27.90	1.79	9.97	23.92	3.49
Coconut Oil, no Vitamin E								
354	6.33	23.36	1.06	34.89	2.26	9.03	20.65	2.39
355	7.52	19.29	0.85	32.35	1.70	10.97	24.97	2.34
368	13.46	30.87	2.11	24.54	2.11	5.54	14.77	6.59
369	6.19	21.47	1.91	32.46	2.99	8.32	20.18	6.45
Corn Oil + Vitamin E								
342	0.99	5.53	0.14	22.69	0.85	12.86	28.09	28.84
349	0.53	5.56	0.28	22.18	1.19	10.14	26.94	33.17
351	1.37	4.96	0	16.25	0.89	14.88	36.97	24.64
371	0.92	4.85	0.24	20.51	2.10	13.15	30.61	27.61
Corn Oil, no Vitamin E								
348	1.07	5.29	0.20	21.44	1.74	10.55	28.72	30.99
364	1.79	8.14	0.99	20.00	2.35	8.95	28.55	28.83
365	1.01	5.30	0.33	24.61	1.53	10.33	28.89	27.99
372	1.28	9.90	1.05	28.48	1.75	5.77	31.79	19.98

Appendix Table 10. Veal Lipids: Fatty Acid Composition of Longissimus Dorsi (%).

Treatment and Sample Number	Fatty Acids									
	C10:0	C12:0	C14:0	C14:1	C15:0	C16:0	C16:1	C18:0	C18:1	C18:2
Coconut Oil + Vitamin E										
350		4.89	21.78	1.82		29.88	2.30	8.83	23.28	7.20
367		4.24	20.59	2.62		28.39	3.41	7.27	25.61	7.87
373		3.06	15.93	1.12		29.73	1.62	10.06	25.98	12.49
454		6.52	17.08	1.99		25.95	2.62	11.04	30.31	4.48
Coconut Oil, no Vitamin E										
354		7.19	26.13	1.68		31.71	2.73	6.29	20.49	3.78
355		7.23	23.73	1.37		26.86	2.05	9.67	24.41	4.68
368	1.17	4.74	18.85	1.77		26.31	4.16	8.55	26.31	8.13
369		4.94	22.05	1.72		26.68	2.58	7.88	23.63	8.52
Corn Oil + Vitamin E										
342	6.53	0.94	5.15	0	0.84	17.07	1.55	10.30	28.64	28.98
349		0.74	3.43	0	4.43	14.53	1.24	7.53	28.80	39.29
351		1.67	7.56	0	3.34	15.84	1.33	14.23	30.02	26.01
371		0.87	6.50	0.48		19.34	2.14	9.27	35.71	25.68
Corn Oil, no Vitamin E										
348		0.47	2.94	0	7.11	14.68	2.91	15.81	30.51	23.86
364	3.25	1.63	7.54	0.69	0	14.84	2.97	6.85	29.80	26.03
365		0.59	5.40	0.55	0	18.79	3.44	8.61	33.58	29.04
372		1.26	7.15	0.58	0.65	20.86	2.39	9.71	28.59	28.81

Appendix Table 11. Veal Lipids: Fatty Acid Composition of Phospholipids (Longissimus dorsi).

Treatment and Sample Number	Fatty Acids						
	C12:0	C14:0	C14:1	C15:0	C16:0	C16:1	C17:0
Coconut Oil + Vitamin E							
350	1.47	5.69	0.48	2.63	23.07	1.26	0
367	0.99	5.50	0.60	2.17	19.44	1.18	0
373	0.42	3.34	0	3.61	16.01	1.11	0
454	0.50	3.49	0	0.32	10.96	1.67	0
Coconut Oil, no Vitamin E							
354	0.54	2.88	0	4.06	17.58	2.34	0
355	0.22	1.49	0	4.03	22.18	2.68	0
368	0.42	3.02	0	4.74	14.23	1.52	0.54
369	0.54	2.65	0	2.59	15.31	0	0
Corn Oil + Vitamin E							
342	1.0	0.64	0	3.27	13.13	0.53	0
349	0	0	0	4.26	11.89	0	0
351	0	0.34	0	3.71	9.86	0	0.45
371	0	0.46	0	3.15	15.75	0	0.53
Corn Oil, no Vitamin E							
348	0	0.86	0	5.93	9.85	0	0
364	0	0.70	0	3.71	13.19	0.52	0.65
365	0.48	1.74	0	2.58	20.24	0	0
372	0	0.71	0.86	3.58	15.59	0	0.41

- table continued -

Appendix Table 11. (Continued)

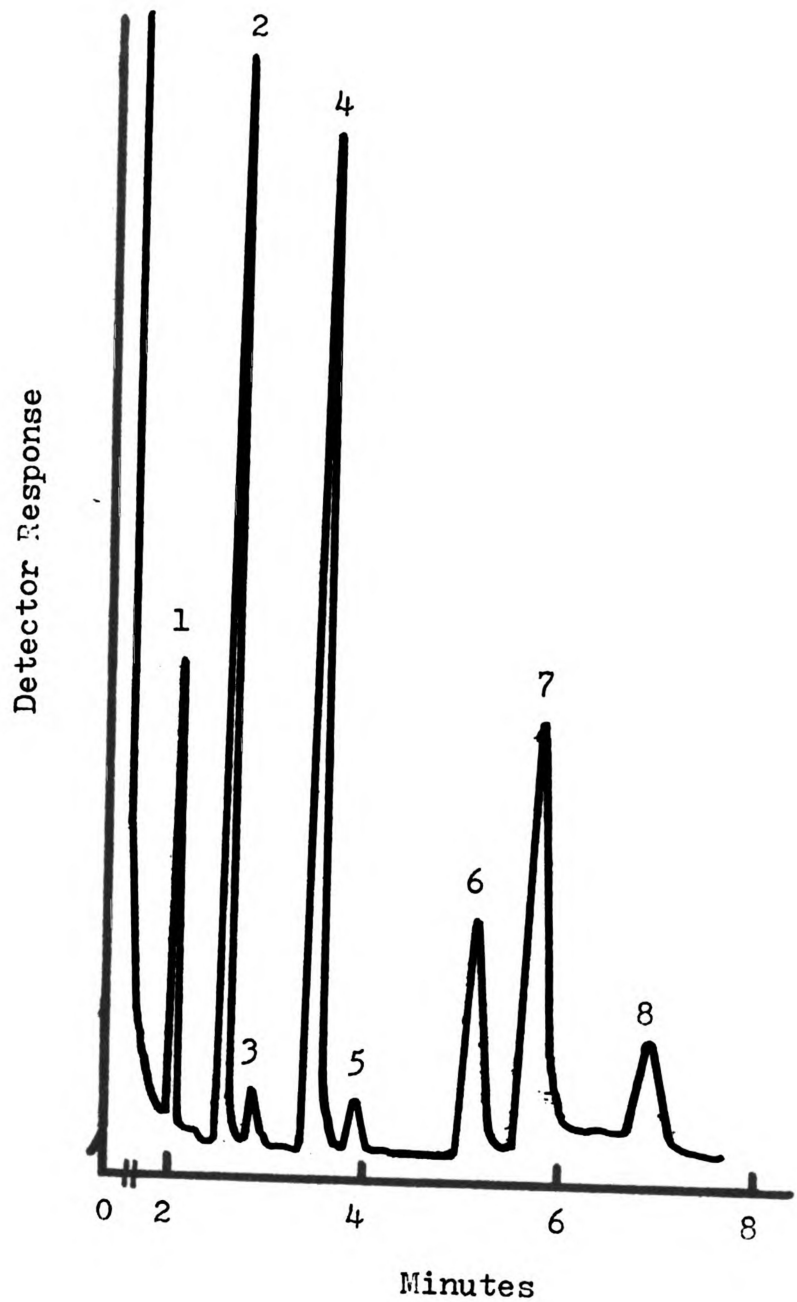
Treatment and Sample Number	C18:0	C18:1	C18:2	C18:3	C20:2	C20:3	C20:4
Coconut Oil + Vitamin E							
350	10.68	21.12	22.88	0	0	1.43	9.28
367	10.86	21.41	27.32	0	0	1.50	9.03
373	12.87	20.71	31.54	0	0	1.74	8.15
454	13.23	31.36	22.72	0.64	0.37	2.04	12.70
Coconut Oil, no Vitamin E							
354	10.37	26.78	19.93	0	0	2.52	12.98
355	12.75	33.68	22.97	0	0	0	0
368	12.81	21.85	28.46	0	0.41	2.05	9.91
369	9.69	22.04	37.41	0		1.53	8.16
Corn Oil + Vitamin E							
342	15.35	14.86	43.54	0	1.17	0.56	6.94
349	14.22	16.09	45.42	0	0	0	8.11
351	16.12	14.21	42.08	0	0	0.63	12.60
371	12.09	12.83	43.64	0	0.86	0.86	9.83
Corn Oil, no Vitamin E							
348	16.69	16.04	39.83	0	0	0.48	10.31
364	14.43	14.66	40.24	1.46	0.82	0.52	9.09
365	9.82	18.79	41.26	0	0		5.08
372	11.55	16.61	40.45	0	1.39	0.61	8.24

Appendix Figure 1. #350 Kidney fat (group 1)

Peak No.

1	=	C12:0
2	=	C14:0
3	=	C14:1
4	=	C16:0
5	=	C16:1
6	=	C18:0
7	=	C18:1
8	=	C18:2

Instrument: Beckman GC-4 chromatograph
Detector: Hydrogen flame
Column: Glass (6 ft x 2 mm i.d.)
Carrier gas flow rate: 40 ml/minute
Column temperature: 100°C
Injection temperature: 210°C
Oven temperature: 185°C
Sensitivity (attenuation) of 5×10^3

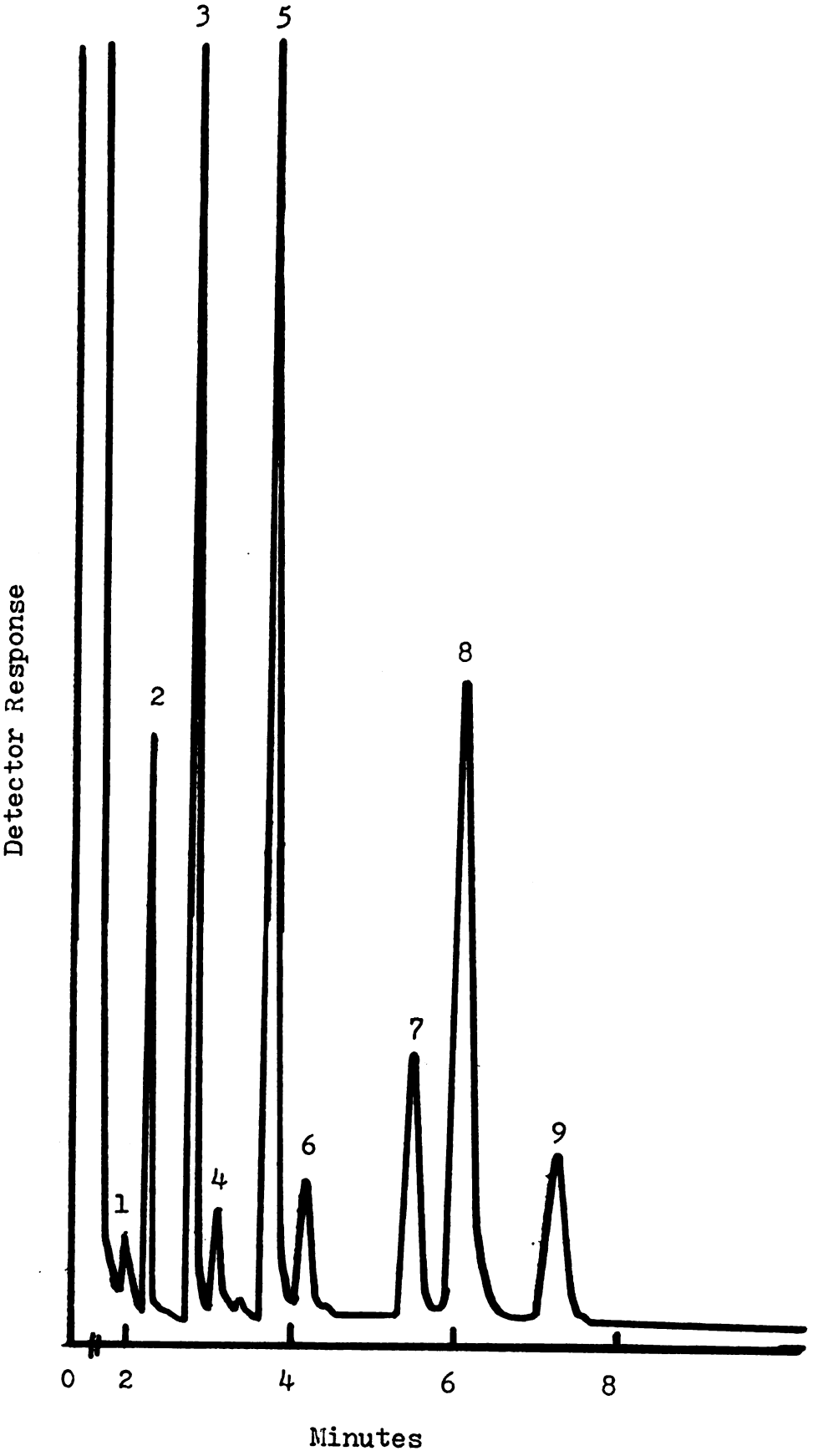

Detector Response
1
2
3
4
5
6
7
8
0
2
4
6
8
Minutes

Appendix Figure 2. #368 Longissimus dorsi (group 2)

Peak No.

1	=	C10:0
2	=	C12:0
3	=	C14:0
4	=	C14:1
5	=	C16:0
6	=	C16:1
7	=	C18:0
8	=	C18:1
9	=	C18:2

Instrument: Beckman GC-4 chromatograph
Detector: Hydrogen flame
Column: Glass (6 ft x 2 mm i.d.)
Carrier gas flow rate: 40 ml/minute
Column temperature: 100°C
Injection temperature: 210°C
Oven temperature: 185°C
Sensitivity (attenuation) of 5×10^3

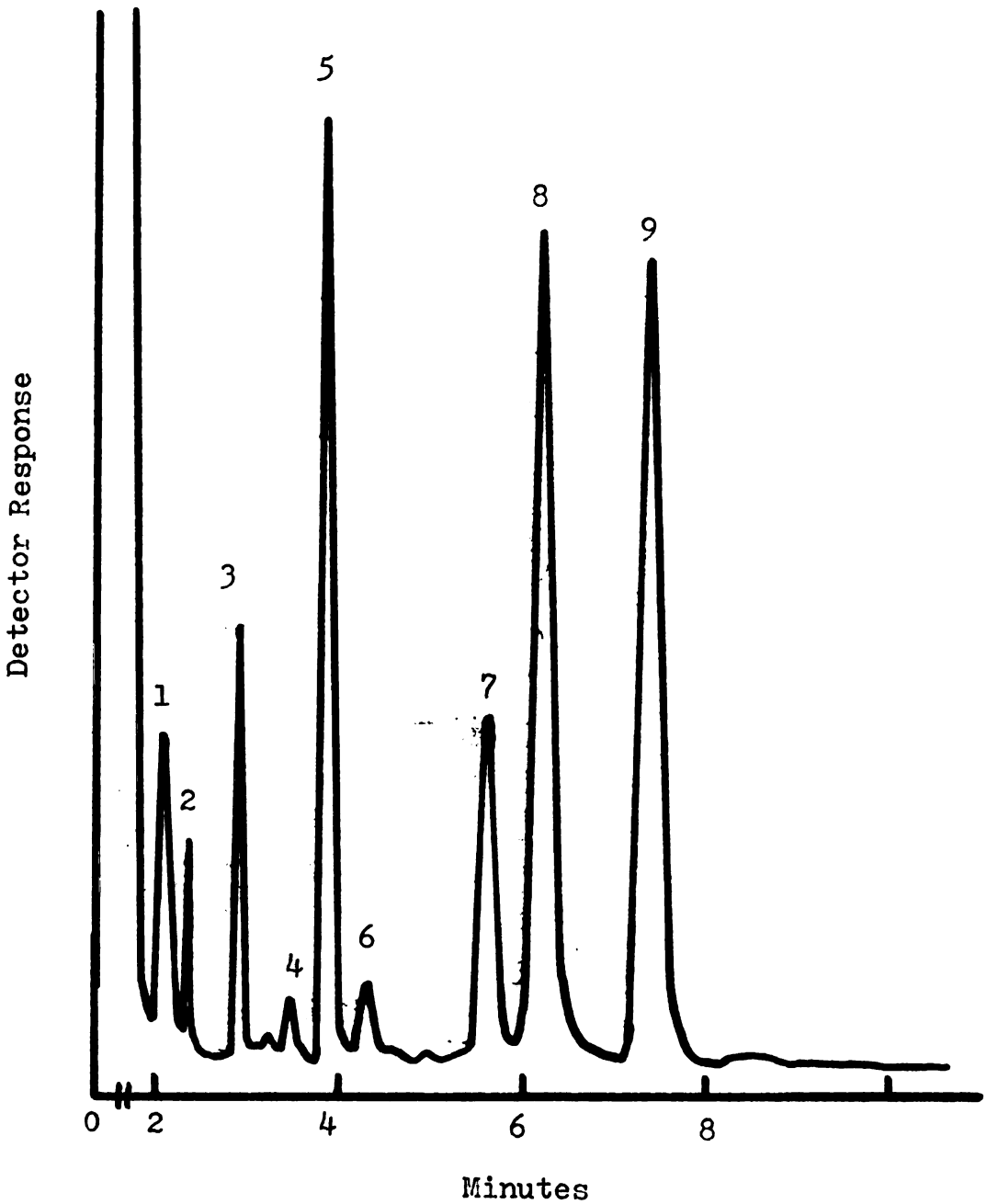

3
5
8
2
7
9
6
4
1
Detector Response
0
2
4
6
8
Minutes

Appendix Figure 3. #342 Longissimus dorsi (group 3)

Peak No.

1	=	C10:0
2	=	C12:0
3	=	C14:0
4	=	C15:0
5	=	C16:0
6	=	C16:1
7	=	C18:0
8	=	C18:1
9	=	C18:2

Instrument: Beckman GC-4 chromatograph
Detector: Hydrogen flame
Column: Glass (6 ft x 2 mm i.d.)
Carrier gas flow rate: 40 ml/minute
Column temperature: 100°C
Injection temperature: 210°C
Oven temperature: 185°C
Sensitivity (attenuation) of 5×10^3

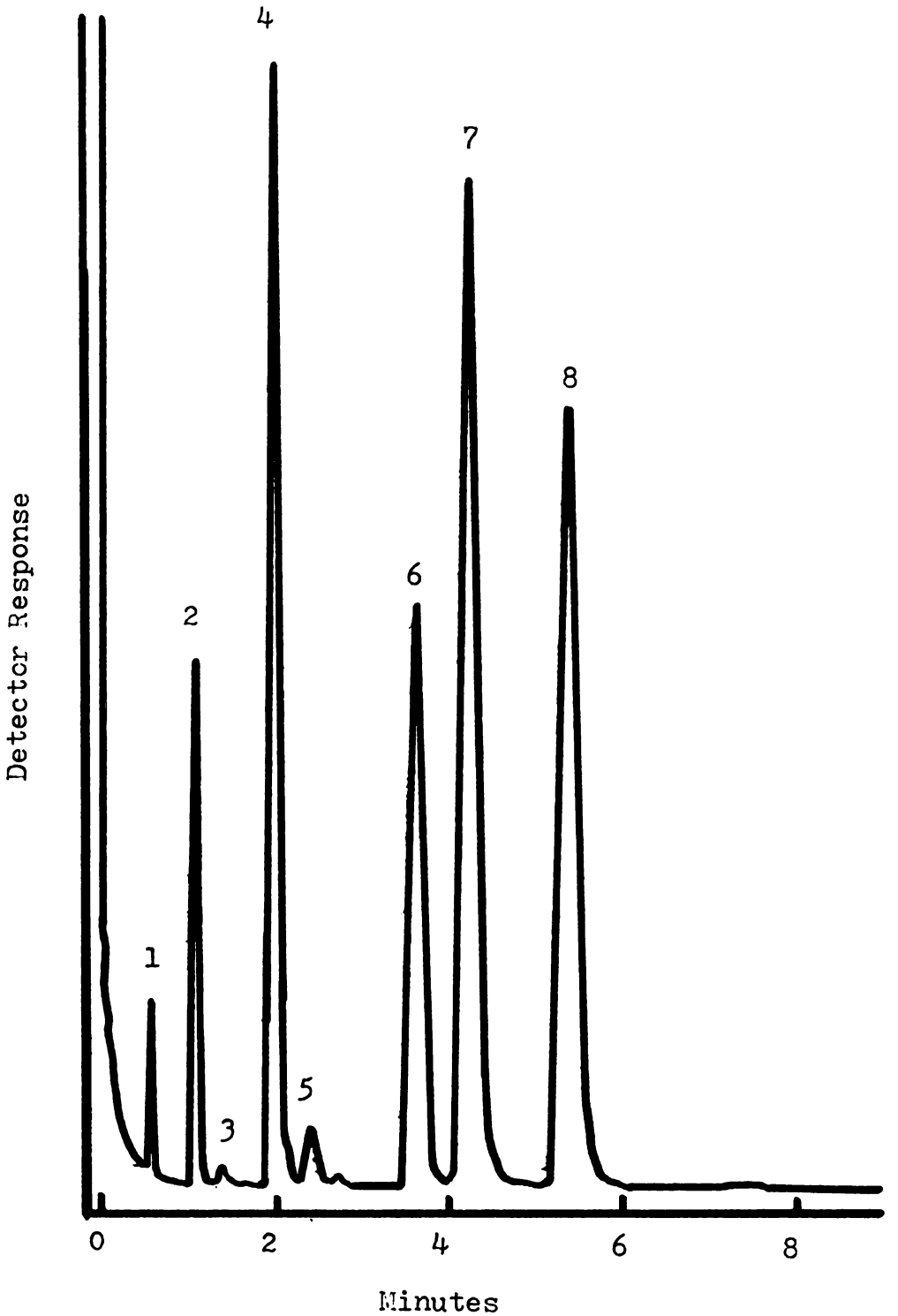

Detector Response
1
2
3
4
5
6
7
8
9
0
2
4
6
8
Minutes

Appendix Figure 4. #364 Kidney fat (group 4)

Peak No.

1	=	C12:0
2	=	C14:0
3	=	C14:1
4	=	C16:0
5	=	C16:1
6	=	C18:0
7	=	C18:1
8	=	C18:2

Instrument: Beckman GC-4 chromatograph
Detector: Hydrogen flame
Column: Glass (6 ft x 2 mm i.d.)
Carrier gas flow rate: 40 ml/minute
Column temperature: 100°C
Injection temperature: 210°C
Oven temperature: 185°C
Sensitivity (attenuation) of 5×10^3


Detector Response
1
2
3
4
5
6
7
8
0
2
4
6
8
Minutes