May 19, 1970

To the Graduate Council:

I am submitting herewith a thesis written by Eung Bum Lee entitled "The Effects of Dietary Fats on the Fatty Acid Composition of Broiler Tissues." I recommend that it be accepted for nine quarter hours of credit in the partial fulfillment of the requirement for the degree of Master of Science, with a major in Food Technology.

M. R. Johnston
Major Professor

We have read this thesis
and recommend its acceptance:

Ivan E. McCarty

John T. Smith

Accepted for the Council

Hilton A. Smith
Vice Chancellor for
Graduate Studies and Research

THE EFFECTS OF DIETARY FATS ON THE FATTY ACID COMPOSITION OF BROILER TISSUES

A Thesis

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

---

by

Eung Bum Lee

June 1970

ABSTRACT

Male and female broilers were fed a basal diet supplemented with 3% and 13% beef tallow, and 3% and 13% peanut oil. Lipids were extracted from skin, white meat, and dark meat. The lipids were separated into neutral and phospholipid fractions, and the fatty acid compositions of the two fractions were determined using GLC for each dietary treatment. The higher levels of dietary fat produced a higher content of total lipids and neutral lipids in different tissues of broilers.

The identification of gas-liquid chromatography peaks of methyl esters of fatty acids was done using derived retention time systems and an additional system was devised and named MECL (n:2w6).

Phospholipids were generally higher in 18:0 and 20:4w6 than neutral lipids, whereas neutral lipids contained higher amounts of 18:1w9. Phospholipids showed characteristic fatty acid patterns in different tissues and dietary treatments. Each lipid fraction tended to assume the fatty acid pattern of the dietary fat treatment with neutral lipids being affected far more than phospholipids. The intermediate fatty acids of biogenesis of the w6 family of unsaturated fatty acids were observed (20:2w6, 20:3w6, 20:4w6, 22:4w6 and 22:5w6). for the w3 family fatty acids, 18:4w3, 20:5w3,

913974

22:5w3, and 22:6w6 were observed. Fatty acids, 22:2w6, 24:3w6, and 24:4w6 were occasionally observed, and these acids are believed to be derived from 22:2w6, which was contained in small amounts in the vegetable oil diet.

The two severely overlapped peaks which were usually observed in the gas-liquid chromatography of neutral lipids might contain some other compounds, other than methyl esters of fatty acids. These facts need to be checked further for the importance of fatty acid analysis using GLC.

TABLE OF CONTENTS

CHAPTER		PAGE
I.	INTRODUCTION	1
II.	REVIEW OF LITERATURE	3
	The Effects of Dietary Fats	3
	The Effects of Menhaden Oil	7
	The Effects of Protein on Fat Metabolism . . .	9
	The Effects of Cholesterol on Lipid Metabolism	11
	Fatty Acid Composition in Skeletal Muscle . .	11
	Flavor Deterioration by Autoxidation of Lipids	14
	Quantitative Analysis of Fatty Acids by GLC	17
III.	MATERIALS AND METHODS	22
	Dietary Treatments	22
	Sampling for Lipid Extraction	24
	Moisture Determination	24
	Lipid Extraction Procedure	24
	Fractionation of Extracted Lipids	26
	Methylation of Fatty Acids	29
	Gas Liquid Chromatography	30
	The Identification of GLC Peaks	31
	Equivalent Chain Length (ECL) System	33
	Linear Logarithmic Plot	34

CHAPTER PAGE

Separation Factors 36

Modified Equivalent Chain Length (MECL) System 38

Modified Equivalent Chain Length (n:2w6) System 40

IV. EXPERIMENTAL RESULTS 42

Fatty Acid Composition of Dietary Fats 42

The Effect of Dietary Fat on Moisture Content, Total Lipids, Neutral and Phospholipids . . 42

Sample GLC Peaks, Type I and II 48

The Identification of GLC Peaks of Methyl Ester of Fatty Acids 51

The Fatty Acid Composition of Neutral Lipids 66

The Fatty Acid Composition of Phospholipids 73

Analysis of Variance 79

V. DISCUSSION 82

Identification of GLC Peaks of Methyl Esters of Fatty Acids 82

Biogenesis of Polyunsaturated Fatty Acids . . 86

The Fatty Acid Composition of Neutral Lipids 89

Fatty Acid Composition of Phospholipids . . . 91

CHAPTER PAGE

The Effects of Dietary Fats on Total Lipids, and the Content of Neutral and Phospholipids 94

LITERATURE CITED 95

APPENDIXES 101

Appendix A 102

Appendix B 104

THE LIST OF ABBREVIATIONS 112

VITA 113

LIST OF TABLES

TABLE		PAGE
I.	Composition of Diets by Weight	23
II.	The Fatty Acid Composition of Beef Tallow and Peanut Oil	43
III.	The Per Cent Composition of Moisture Content, Total Lipids, Neutral Lipids, and Phospholipids of the Skin of Broilers	44
IV.	The Per Cent Composition of Moisture Content, Total Lipids, Neutral Lipids, and Phospholipids of the dark meat of Broilers	45
V.	The Per Cent Composition of Moisture Content, Total Lipids, Neutral Lipids, and Phospholipids of the White Meat of Broilers	46
VI.	The Adjusted Retention Times (Peak Top) of Methyl Esters of Fatty Acids for ECL Values, Type I GLC Peaks	54
VII.	The ECL Values from the Data of Table VI . . .	55
VIII.	The Adjusted Retention Times (Frontal Tangent) of Methyl Esters of Fatty Acid for MECL (n:2w6) Values, Type I GLC Peaks	57
IX.	The MECL (n:2w6) Values from the Data of Table VIII, Type I GLC Peaks	58
X.	The Adjusted Retention Times (Frontal Tangent) of Methyl Esters of Fatty Acids, Type II GLC Peaks	60

TABLE		PAGE
XI.	The MECL (n:2w6) Values from the Data of Table X	61
XII.	The Comparison of Adjusted Retention Times and Their Derived Values of Methyl Esters of Fatty Acid in GLC	63
XIII.	Separation Factors from the ART Data of Table VIII	67
XIV.	The Per Cent Fatty Acid Composition of Neutral Lipids in the Skin of Broilers on Different Dietary Fat	69
XV.	The Per Cent Fatty Acid Composition of Neutral Lipids in the Dark Meat of Broilers on Different Dietary Fat . . .	70
XVI.	The Per Cent Fatty Acid Composition of Neutral Lipids in the White Meat of Broilers on Different Dietary Fat . . .	71
XVII.	The Per Cent Fatty Acid Composition of Phospholipid in the Skin of Broilers on Different Dietary Fats	74
XVIII.	The Per Cent Fatty Acid Composition of Phospholipid in the Dark Meat of Broilers on Different Dietary Fats	75
XIX.	The Per Cent Fatty Acid Composition of Phospholipid in the White Meat of Broilers on Different Dietary Fats . . .	76

TABLE PAGE

XX. Total Saturated, Unsaturated, and w3 and w6 Fatty Acids of Neutral Lipids on Different Dietary Fats 77

XXI. Total Saturated, Unsaturated, and w3 and w6 Fatty Acids of Phospholipids on Different Dietary Fats 78

XXII. Analysis of Variance of Total Lipids Content in Various Tissues of Broilers 105

XXIII. Analysis of Variance of Neutral Lipids Content in Various Tissues of Broilers 106

XXIV. Analysis of Variance of Fatty Acid Composition of Skin Neutral Lipids of Broilers 107

XXV. Analysis of Variance of Fatty Acid Composition of White Meat Neutral Lipids of Broilers 108

XXVI. Analysis of Variance of Fatty Acid Composition of Skin Phospholipids of Broilers 109

XXVII. Analysis of Variance of Fatty Acid Composition of Dark Meat Phospholipids of Broilers 110

TABLE PAGE

XXVIII. Analysis of Variance of Fatty Acid Composition of White Meat Phospholipids of Broilers 111

CHAPTER I

INTRODUCTION

After James and Martin (19, 20) devised the method of gas-liquid chromatography (GLC), the analysis of fatty acids has been simplified considerably. During the past decade there have been many improvements in the analysis of fatty acids using this technique and it has produced extensive information in lipid research. The older analytical methods, such as the fractional distillation or the determination of chemical constants, are limited to the lipids which are available in large quantities. These methods are laborious, time consuming, and inaccurate as compared to the method of GLC.

It is generally believed that the lipid composition of a diet influences the fatty acid patterns of adipose tissues of animal. Although much work has been done on the depot fats, the influence of a fat diet on tissue lipids has not been studied to the same extent.

The lipids from the thigh and breast muscle, and the skin of broilers contain different amounts of total lipids and classes of lipids, and show different patterns of fatty acids. The phospholipid fraction contains more polyunsaturated and longer carbon chain length fatty acids than the neutral lipid fraction.

The determination of the fatty acid composition of tissue lipids is important to the study of flavor deterioration of meat which is believed to be caused mainly by the highly unsaturated long chain fatty acids. There is recent evidence showing the presence of highly unsaturated long chain fatty acids with 4 to 6 double bonds and more than 20 carbon atoms. They can undergo autoxidation very easily, and are mainly located in the phospholipid fraction of tissue lipids.

The greater part of the work on the effect of dietary fat on chicken has been done by either using laying hens and their eggs or the whole chicken. Little attention has been paid to the influence of dietary fats on the fatty acid composition of different classes of lipids from skeletal muscles and skin of broilers. The purpose of this study was to determine the fatty acid composition of skin, dark and white meat of broilers and their relation to the dietary fats of the rations. A method of identification of fatty acids from GLC peaks was devised.

CHAPTER II

REVIEW OF LITERATURE

The Effect of Dietary Fats

There is extensive evidence that dietary fat influences the fatty acid composition of chicken tissue.

The work of Cruickshank (10) showed that only small differences existed in the iodine number of depot fats from different tissues of laying hens. She also showed that the ingestion of unsaturated fatty acids resulted in rapid increase in unsaturation of depot fat and ingestion of saturated fatty increased the degree of saturation.

Dansky and Hill (11) reported that chickens which were fed high energy ration deposited much more fat than those fed lower energy ration. The fat content of bone, leg musle, gizzard and skin varied directly with the fat content of the total carcass, each tissue showing a characteristic fat content relative to the others. Breast muscle contained very little fat, regardless of dietary treatment.

Reiser (42, 43) studied the interconversion of unsaturated fatty acids (USFA) using laying hens and their eggs. He reported that the addition of linoleic (dienoic)

acid in the diet resulted in the deposition of dienoic, detraenoic, and pentaenoic acids in eggs. Trienoic and hexenoic acid were not increased. The addition of lenolenic acid (trienoic) resulted in an increase in all polyunsaturated fatty acids (PUSFA) having from 2 to 6 double bonds. The total lipids or different classes of lipids were unaffected by the kind or amount of fatty acids in the ration. Reiser used a spectrophotometric method to determine the number of double bonds and was unable to determine the chain length of the fatty acids. This method for determination of double bonds is not considered very accurate due to the presence of over lapping absorption spectra.

Murty et al. (37) injected hens intraperitoneally with Carbon-14 labelled acetate and found that the laying hens did not synthesize linoleic acid. Any of this nutrient which occurred in the egg must be present in the diet. This is contrary to the conclusion of their previous studies based on the presence of small amounts of linoleic acid in hens' eggs after hens received a fat-free diet for one year.

Mead (31) demonstrated by a tracer study in rats that methylene interrupted PUSFA could be synthesized from palmitoleic (16:1w7)[1], oleic (18:1w9), linoleic (18:2w6),

---

[1]See Appendix A, page 103, (1)

and linolenic (18:3w3). This study showed that double bonds were added from the carboxyl end rather than methyl end of the acid. As a consequences, the carboxyl end, and not the methyl end, changed. From oleic acid 5,8,11-eicosatrienoic acid (20:3w9) was produced as an end product. The latter acid accumulated in the tissues of fat deficient animals. 7,10,13-eicosatrienoic acid (20:3w7) and 5,8,11,14-eicosatetraenoic acid (20:4w6) were produced from palmitoleic and linoleic acid, respectively. Linolenic acid (18:3w3) led to the formation of 7,10,13,16,19-docosapentaenoic acid (22:5w3) and 4,7,10,13,16,19-docosahexaenoic acid (22:6w3).

Machlin and Gordon (23) showed that linoleic acid, but not linolenic acid, was needed for the growth of chickens (essential fatty acids) and linoleic acid could be converted to arachidonic acid (20:4w6), which also could function as an essential fatty acid. They suggested that w6 configuratin of a fatty acid was essential for the growth of a chicken. Analysis of fatty acids in tissues of chickens revealed that linoleic acid-free diets resulted in the increase of eicosatrienoic acid, while the linoleic and arachidonic acid contents are very low. Addition of linoleic acid resulted in the decrease of eicosatrienoic acid and an increase in linoleic and arachidonic acid in all tissues analyzed.

Machlin *et al*. (24) studied the fat metabolism of

laying hens using hydrogenated coconut oil and safflower oil. He concluded that the fatty acid composition of nonadipose as well as adipose tissue and eggs would reflect the fatty acid composition of the diet. Adult hens are not sensitive to deficiencies in essential fatty acids as a result of large amounts of reserve linoleic acid in adipose tissue and the slow rate of depletion of arachidonic acid.

Marion and Woodroof (25, 26) reported that GLC analysis of lipids from skin, abdominal adipose tissue, breast and thigh muscle of broilers showed that 14-, 16-, 18-, and 20-carbon fatty acids were present in all tissues. Breast muscle contained more than 10% and thigh muscle less than 10% 20:3, 20:4, 20:5, 22:5, 22:6, 24:2, 24:4, and 24:6 fatty acids. Skin and adipose tissue contained less than 1% these fatty acids. The above long chain fatty acids were mainly located in the phospholipid fraction. Each of the tissues tended to assume the fatty acid composition of dietary fats when the chickens were fed 6% corn oil, beef tallow, or mixture of 1% menhaden oil with the former two.

Marion (28) studied the influence of dietary fat on the lipids of chicken muscle. Total lipid and phospholipid (PL) levels in breast and thigh muscle were not significantly influenced by dietary fat, but each were lowered significantly with the increasing age of the chicken. PL

were generally higher in amount of stearic acid and long chain fatty acids than neutral lipids, whereas neutral lipids (NL) contained higher quantities of oleic and linoleic acids. Each lipid fraction tended to assume the fatty acid pattern of the dietary fat with NL being affected far more than PL. 20:3, 20:4, 20:5, 20:1, 24:2, 22:5 and 22:6 fatty acids were present in the PL fraction of breast and thigh muscle at about the 20% level. The NL fraction of these muscles contained about 1% 20:3, 20:4, and 20:5 fatty acids.

The work of Isaacks et al. (18) showed that the triglyceride fraction of both the blood plasma and adipose tissue of chickens more closely reflected dietary fatty acid composition than did the corresponding PL fraction.

The Effect of Menhaden Oil

Menhaden oil has been used to study the lipid metabolism in chickens as a source of long-chain PUSFA.

Edward and Marion (12) observed that the deficiency of essential fatty acids was characterised by the formation of eicosatrienoic acid in liver lipids of chickens. The feeding of corn oil caused an increase in the eicosatetraenoic acid and decrease in the eicosatrienoic acid in liver lipids, while menhaden oil did not change the content of eicosatetraenoic acid. When menhaden oil was

added to the diet, significant amounts of eicosapentaenoic acid, docosapentaenoic acid and docosahexaenoic acid were present in the liver lipids. They suggested that the presence of the long-chain fatty acids of menhaden oil inhibited the synthesis of eicosatrienoic acid from oleic acid.

Miller et al. (32) studied the lipid metabolism in broilers with purified menhaden oil which contained large amounts of higher members of the linolenic acid (w3) family. They found that the conversion of linoleic to arachidonic acid was inhibited by the effect of the linolenic (w3) family total content. The deposition of the linolenic family fatty acids in the muscle was proportionate to their content in the diet. The major fatty acid member of the w3 family in muscle lipid was 20:5 which was accompanied by somewhat less of 22:6 and considerably less of 22:5. The 20:4w3, isomer of arachidonic (20:4w6) was first found in the broiler muscle proportionate to the fatty acid in a diet.

Miller et al. (33) studied the flavor and fatty acid composition of broiler flesh after the broiler was fed moleculary distilled menhaden fatty acids. The flavor-contributing components in the menhaden oil apparently resided in the fatty acids regardless of whether the preparations were fed as the triglyceride or as the ethyl

ester. Four marine fatty acids (18:4w3, 20:4w3, 20:5w3, and 22:5w3) deposited in the flesh of the broilers, were highly correlated with the occurence of the unacceptable flavor.

The Effect of Protein on Fat Metabolism

The increased demand of the processor for broilers of uniform size and composition has stimulated the interest in the formulation of rations to give specific weights and body composition at the time of marketing.

Summer et al. (46) reported that carcass protein was increased and carcass fat was decreased in a linear manner with increasing level of dietary protein. Conversely, increasing level of dietary energy results in decreased carcass protein and increased carcass fat. While little or no improvement in weight gain was achieved by increasing the protein level beyond 20%; whereas marked changes in carcass composition were noted. Raising the protein content of the diet exerted a greater effect in lowering the fat and increasing the protein content of the female than male carcass.

Essary and Dawson (14) have demonstrated further that changes in fat deposition in the chicken are primarily in skin and adipose tissues with less marked changes occuring in muscle tissues.

Marion and Woodroof (29) showed that the chicken fed

different levels of protein and different fats were significantly different in the fatty acid composition. Feeding different fats resulted in an increased carcass deposition of the major fatty acids present in each diet. Also, birds fed high protein diets deposited less oleic and more linoleic acid in their carcass.

Marion et al. (30) studied the effect of dietary fats and proteins on lipid composition in chicken white muscle. There were significant differences between the low (16%) and high protein (24%) diets in the deposition of fatty acids in the NL and PL fractions of muscle. The content of saturated fatty acids (16:0 and 18:0) in the NL were not changed markedly by the level of protein in the diet. The deposition of USFA (16:1 and 18:1) were significantly different between the protein levels. The higher content of the above USFA were noticed in low protein diet, while the fatty acid composition of each sample was influenced by different dietary fats. In the PL fractions, the marked difference in long chain fatty acids due to the dietary fat was in the substitution of 20:5, 22:5, and 22:6 fatty acids when nenhaden oil was fed as compared to the fats of coconut oil, beef tallow, and safflower oil. No marked difference was noticed in the fatty acid composition of PL due to the level of protein except 22:6 fatty acid which showed higher deposition in

low protein diet than higher one.

The Effect of Cholesterol on Lipid Metabolism

Cholesterol has been known to influence the lipid metabolism in animal. The influence of it in lipid metabolism has been studied extensively with the connection of atherosclerosis.

Machlin and Gordon (23) added cholesterol along with a mixture of methyl myristate and laurate to purified diets free of unsaturated fatty acids. The diet depressed the growth of chicken further than the diet without the cholesterol. These growth-depressing effects were largely overcome by addition of a source of linoleic acid.

Chung et al. (8) reported that the iodine number of egg yolk and liver lipids of chicken was increased when cholesterol was consumed.

The recent work of Chung et al. (9) showed that the fatty acid composition of thigh and abdominal lipids of chickens were unaffected by the addition of cholesterol on different fat diets. Cholesterol when fed with corn oil decreased the proportions of stearic and palmitic acids and increased the proportions of oleic and linoleic and in egg yolk lipids as compared to the diet of corn oil alone.

Fatty Acid Composition in Skeletal Muscle

A spectrophotometric method was used to determine the

number of double bonds in a fatty acid with a limited success.

Reiser (42, 43) and Chang and Watts (6) reported the presence of fatty acids containing 5 and 6 double bonds in the egg yolk and meat tissue of chicken using a spectronic method.

The analysis of fatty acids in skeletal muscle has been done with considerable success using GLC.

Marion and Woodroof (26) showed that the majority of the fatty acids found in skin, breast, and thigh muscle of broilers were in the 16- and 18-carbon series. The fatty acids of 20-, 22-, and 24-carbon series with various unsaturation (2 to 6) were found in the breast muscle at the level of more than 12% and in the thigh muscle at less than 10%. The skin contained less than 1% the fatty acids of 20-carbon series containing 3 to 5 double bonds. They did not, however, identify the methyl end group (w) of the individual fatty acids.

Miller et al. (32, 33) determined the fatty acid composition of muscle lipids of broilers fed different dietary fats. They demonstrated that the fatty acid composition could be changed by different dietary fats. The content of USFA of the w3 series in broiler muscle lipids was changed from less than one per cent for a basal diet to more than 30% for purified menhaden oil

supplement. These w3 series of fatty acids are 18:3, 18:4, 20:4, 20:5, 22:4, and 22:6. They also reported the presence of 20:1, 20:2, 20:3, 22:3, and 22:4 fatty acids in minor quantities.

Katz et al. (21) determined the fatty acid composition of NL and PL from four tissues (dark and white muscle, skin and depot fat) of broilers. They observed that muscle tissue contained relatively larger amounts of PL than did skin and depot fats. Some 22 different fatty acids were found in the PL compared to 18 fatty acids in the NL. Approximately 95% the NL consisted of 16:0, 16:1, 18:0, 18:1, and 18:2 fatty acids; whereas, the PL fraction contained about 65% these fatty acids. The PL fraction of the meat tissues contained about 28% long chain fatty acids (20-, 22-, and 24-carbon series with 1 through 6 double bonds), while the NL contained about 1% these fatty acids. Arachidonic acid was found to be one of the major fatty acids in the PL fraction.

Marion (28) studied the effect of dietary fat on the lipids of chicken muscle. He noticed that the total lipids of breast muscle were about 1% while 0.5% were PL. The thigh muscle contained about 2% of the total lipids of which 0.5% were PL. Neither the amount nor the type of dietary fat significantly influenced the content of total lipids and PL in breast and thigh muscles. In general agreement

with Katz et al. (21), he observed that PL contained higher levels of stearic acid and long-chain PUSFA, and lower levels of oleic and linoleic acids than NL. Each dietary fat with NL being affected far more than PL.

Peng and Dugan (39) determined the composition and structure of PL in chicken muscle tissues. They used silicic acid column chromatography to separate individual PL. They observed that the PL content of thigh muscle was higher than that of the breast muscle. Phosphatidyl choline and phosphatidyl ethanolamine were found in relatively greater amounts than phosphatidyl serine and sphingomyelines. The PUSFA are located primarily at the -position and the saturated fatty acids at -position.

Marion et al. (30) demonstrated that cephalin and lecithin fatty acids from the white muscle of broilers could be changed by the dietary fatty acids. A 5% menhaden oil diet showed marked dectease in the arachidonic acid content and increase of 22:5 and 22:6 fatty acid in the cephalin and lecithin fatty acids compared to the diets of 5% coconut, beef tallow, and safflower oil diets.

Flavor Deterioration by Autoxidation of Lipids

The autoxidation of lipids produces volatile compounds such as ketones, aldehydes, alcohols, and some other compounds. These volatile compounds contribute to the off-flavor of food products. It has been known that PUSFA

undergo oxidation faster than monounsaturated fatty acids (44).

The presence of 5 and 6 double bonds fatty acids were reported by Chang and Watts (6) in poultry meat by a spectrometric method. They suggested that these fatty acids might be the cause of flavor deterioration of poultry meat upon storage. The presence of these PUSFA were later confirmed by GLC in total lipids from chicken muscle (26, 32), and in NL and PL fractions (21, 28, 39, 30).

Lea and Swaboda (22) showed that the odor of oxidation products of fatty acids was much easier to detect in water than in an oil medium. This suggests that although the total lipid level in muscle tissue may be low in comparison to skin and adipose tissue, the degree of fatty acid oxidation in muscle tissue may be quite important in determining the quality of broiler meat.

Younathan and Watts (49) reported that the PL rather than NL fraction of pork was responsible for the rancidity when heating muscle tissue.

Hornstein et al. (16) showed that the PL fraction of pork and beef meat underwent autoxidation faster than the neutral fraction. The cephalin fractions from both of the samples produced strong fish odors.

Marion and Woodroof (25) reported that the development of rancidity in broiler tissues during storage, as measured

by a 2-thiobarbituric acid (TBA) method was not influenced by dietary fat treatments. Also no appreciable change in fatty acid distribution during the development of rancidity could be detected by GLC.

El-Gharbawl and Dugan (13) studied the stability of lipids during storage of freeze-dried raw beef and showed that PL oxidized first, and the neutral lipid oxidized later. They, in disagreement with Marion and Woodroof (25), noticed that there was about a 10% loss of arachidonic acid in the PL fraction after six months of storage at room temperature under nitrogen gas, whereas the USFA disappeared more gradually in the NL of dried beef.

In reversing his previous findings (25), Marion and Woodroof (29) observed that TBA values could be influenced by the dietary treatments. They determined the TBA values on breast muscle and skin after storage of carcasses for 12 days at 2°C. The values were significantly higher in carcasses from high protein or menhaden oil diets. Diets containing coconut oil or beef tallow produced carcasses with lower TBA values.

Marion et al. (30) demonstrated that the 22-carbon series fatty acids with 4 or 5 double bonds in cephalin and lecithin, and linoleic acid in neutral lipids and lecithin in white muscle of broilers were positively correlated with TBA values. They also noticed that the

TBA values measuring oxidative rancidity in meats were fairly reliable since it was positively related to the concentration of the unsaturated fatty acids in meat tissues lipids.

Miller et al. (33) observed the deposition of four marine fatty acids (18:4w3, 20:4w3, 20:5w3, and 22:5w3) in the flesh of broilers when fed refined menhaden oil at a 5% level. These fatty acids were highly correlated with the occurrence of the unacceptable flavor.

Quantitative Analysis of Fatty Acids by GLC.

The work of James and Martin (19) showed that it was possible to analyze the volatile fatty acids using GLC. They improved this technique considerably by methylating the fatty acids and by using a more sensitive detector system for the gas emerging from the column (20). They were the first to show that plotting logarithm of retention volume (or relative retention volume)[2] against the number of carbon atoms in the molecule for the members of a homologous series fatty acids produced a straight line. In this way it was possible to identify an acid on the basis of time emergence alone.

Farquhar et al. (15) introduced the relative retention time (relative to 18:0)[2]. They published the

---

[2]See Appendix A, page 103, (2)

RRT for various fatty acids, and also reported that the logarithm of the RRT showed a straight line relationship with the number of carbon atoms in the fatty acid chain. The only difference between the retention time and retention volume is that the former is expressed in time to elute a sample, and the latter is expressed as the volume of a carrier gas. These two values showed almost identical results when to identify unknown fatty acids.

Woodford and van Cent (48) introduced the carbon number system to express the retention times of fatty acids and based the system on the retention times of straight-chain saturated fatty acids. The retention times of the saturated straight-chain esters, at least four of which are usually present in natural mixtures, were plotted against the chain length on a semilogarithmic paper and the best straight line joining them was constructed. A value corresponding to the retention time of any other peak can then be read off from the abscissa of the graph. This value would give the chain length of the hypothetical saturated straight-chain esters which would be eluded at that point. The carbon number was a characteristic of the particular esters on a particular stationary phase.

Miwa et al. (35) introduced the equivalent chain length (ECL) system which had the same principle as the

former authors' carbon number system. They stated that this ECL value was independent of operating conditions such as column temperature, carrier gas flow rate, proportion of stationary phase, and column dimentions. The slopes of the curve varied with changes in column temperature, but the ECL value remained constant. This value was dependent on the kind of stationary phase and carrier gas. They stated that a combination of two values, one for the polar and the other for non-polar stationary phase, were sufficient to characterize most of fatty acids.

Miwa (36) reviewed the method of identification of peaks in GLC for fatty acids. He reported that the ECL values were accurate only in regions of the curve where a linear relationship existed between the logarithm of retention time and the number of carbon atoms in the homologous series.

Ackman (1) reported that the identification of peaks in GLC appeared needlessly complex. The basic misapprehension was that the USFA could be structurally correlated through the corresponding saturated acids, the number of double bonds, and their position relative to the carboxyl group. He showed that there was a satisfactory relationship among the USFA themselves, independent of the saturated fatty acids. The relationships were based on the chain length, the number of double bonds, and the position

of the latter relative to the terminal methyl group. He showed that when USFA were grouped according to the number of double bonds and the same end carbon chains, it was possible to draw a straight line between the carbon number of fatty acids and logarithm of RRT (relative to oleic acid). The lines representing the each group (the same number of double bonds and the same w values with varying chain length) were parallel to the line joining mono-unsaturated fatty acids with a same w9 value. Thus he suggested that line representing the n:1w9 was better than the straight chain saturated fatty acids (35) to determine ECL values of PUSFA. He named this system modified ECL (MECL).

Ackman (2) showed that it was possible to use the separation factors (15) derived from the modified ECL system as a means tentative identification of USFA. When the acid structures were appropriately grouped by the end carbon chain, it was feasible to identify unknowns or predict retention times either between acids of one chain length and different numbers of double bonds, or between acids of one chain length and the same number of double bonds in different positions.

Ackman and Burger (3) stated that the MECL system, or separation factor procedures could be applied to the provisional identification of structurally normal PUSFA. Oleic, linoleic, linolenic, and the normal saturated fatty

acids were used as a primary standard. These standard acids did not give satisfactory starting points for the identification of longer-chain PUSFA. He suggested the use of cod liver oil as a secondary reference standard for the provisional identification of long-chain PUSFA. He reported that the composition of the major components of cod liver oil was sufficiently constant so that the components in question might be recognized visually in most instances. The presence of 16-, 18-, 20-, and 22-carbon monounsaturated fatty acids provided a good base line for the linear logartithmic plot. The presence of the 5,8,11,14,17-eicosapentaenoic acid, 7,10,13,16,19-docosapentaenoic, and 4,7,10,13,16,19-docosahexaenoic acid provided good starting points for the application of the separation factors in these series of acids.

Hofstetter *et al.* (17) determined the ECL values on 79 methyl esters of USFA and on 7 ethyl esters by GLC. His observations were in general agreement with Ackman (1).

CHAPTER III

MATERIALS AND METHODS

Dietary Treatments

Cross-bred (Vantress X Arbor Acre 50) broiler type male and female broilers were used in this experiment. The broilers were fed four different dietary fats for 8 weeks. The composition of the diets are shown in Table I. The starter diets BR45 and BR46 contained 3% beef tallow and 3% peanut oil, respectively. The finisher diets BF12 and BF13 consisted of 13% beef tallow and 13% peanut oil, respectively. All the other feed components were kept constant.

In treatment one, the broilers were fed the BR45 diet for 8 weeks, and in treatment two they were fed the BR46 diet for 8 weeks. In treatment three they were fed the BR45 diet for 5 weeks followed by the finisher diet BF12 to 8 weeks. The fourth treatment consisted of feeding the BR46 diet for 5 weeks and the finisher diet BF13 for the remaining 3 weeks.

These broilers were raised by Shash, B. M. (45). He reported that during the seventh week of age, the birds were attacked by a disease, Avian nephritis-nephrosis (Gumboro). He showed that dietary fat affected the characteristics of the body fat as measured by the melting point, saponification number, peroxide value, and iodine

TABLE I

COMPOSITION OF DIETS BY WEIGHT

Ingredients	Starter Diets		Finisher Diets	
	BR45	BR46	BF12	BF13
1. Yellow corn	51.95	51.95	51.95	51.95
2. Alfalfa meal	2.50	2.50	2.50	2.50
3. Fish meal anchovie	5.00	5.00	5.00	5.00
4. Corn gluten meal	3.00	3.00	3.00	3.00
5. Animal fat	3.00	----	13.00	----
6. Ground limestone	1.20	1.20	1.20	1.20
7. Defl. rock phosphate	1.00	1.00	1.00	1.00
8. Salt	0.48	0.48	0.48	0.48
9. Manganese sulfate	0.02	0.02	0.02	0.02
10. Vitamin mix	0.6	0.6	0.6	0.6
11. Coccidiostat	0.05	0.05	0.05	0.05
12. DL-methionine	0.40	0.40	0.40	0.40
13. L-lysine	0.80	0.80	0.80	0.80
14. Peanut meal, 50%	30.00	30.00	30.00	30.00
15. Peanut oil	----	3.00	----	13.00
Total	100.00	100.00	110.00	110.00
Calculated and determined analysis:				
Crude protein % (determined)	26.0	26.0	23.6	23.6
Productive energy cal./lb. (calculated)	1007.0	1007.0	1179.0	1179.0
C:P ratio	38.88	38.88	50.08	50.08

Source: Shah, B. M. 1965. Growth and quality of broilers from peanut oil--peanut meal diets. M. S. Thesis. The University of Tennessee.

number. He did not observed any significant differences between treatment values for flavor and tenderness of either roasted white or dark meat by triangle taste test.

Sampling for Lipid Extraction

At 8 weeks of age the birds were slaughtered and dressed, and stored at -20°C. Three broilers were picked from each dietary treatment and sex. Samples of skin, breast and thigh muscles were excised from each bird. The samples from the three birds were put together and mixed well. If they were not used immediately, they were put into a plastic bag and stored at -20°C. for later lipid extraction.

Moisture Determination

Three portions of 3-5 g. of each sample were placed in weighed aluminum moisture pans. The weight of wet sample was determined by the increment of weight after the transfer of the sample on an analytical balance.

The pans were placed in a drying oven at 100±3°C. for 18 hours. After removal from the oven, the pans were cooled for one hour in a desiccator and weighed. Moisture content was expressed as per cent of the original sample weight.

Lipids Extraction Procedure

The duplicate lipid extractions of each samples were carried out by the method of Ostrander and Dugan, Jr. (38).

A sample of approximately 50 g. for meat or 25 g. for skin was weighed in a piece of aluminum foil on an analytical balance. The sample was transferred to a blender. The weight of the sample was determined by subtracting the weight of the aluminum foil from the total. About four square inches of filter paper were added with the sample to aid in filtration.

The sample was blended with 130 ml. of absolute methanol for five minutes after the head space of the bottle was flushed with nitrogen gas.

Sixty-five milliliters of reagent grade chloroform was added and the mixture blended for 5 minutes. After the addition of 65 ml. of 2.3% zinc acetate, blending was continued for 10 seconds. The head space of the bottle was flushed with nitrogen gas each time.

The extraction mixture was poured into a Buchner funnel and filtered with suction. The residue and the filter paper were transferred to the blender bottle and the funnel was wiped with a piece of filter paper. One hundred ml. of chloroform was added to the residue and filter paper and the mixture was blended for 3 minutes. The mixture was transferred to the funnel into which a new filter paper had been placed and filtered again. Fifty milliliters of chloroform were used for rinsing the blender bottle and washing the solids into the funnel.

The head space of the blender bottle was flushed with nitrogen gas each time.

The filtrate was transferred with a small amount of chloroform to a 500 ml. separatory funnel, and two distinct layers were formed. The upper layer contained the methanol-water mixture and the bottom layer contained the chloroform which contained the lipid.

The chloroform layer was separated from the methanol-water mixture, and the volume was recorded. After thorough mixing, two 10 ml. aliquots were taken and put into weighed 50 ml. beakers to determine the total lipid content of each sample. Most of the solvent was allowed to evaporate at 70°C. in the oven. The samples were dried further to constant weight under reduced pressure in a vacuum desiccator. The per cent of total lipids in each samples was calculated.

The lipid containing chloroform from the duplicate samples was pooled, and the solvent was evaporated using a rotary evaporator. The extracted lipids were transferred with chloroform to a 50 ml. Erlenmeyer flask, after which the flask was flushed with nitrogen, stoppered, and stored at -20°C. untill the sample was ready for fractionation.

Fractionation of Extracted Lipids

Fractionation was carried out by the slurry method

described by Murty *et al.* (37). Duplicate fractionations were carried out for each sample.

The total lipids from the duplicate samples were diluted with chloroform in a 100 ml. graduated cylinder to a volume of about 60 ml. The volume was adjusted so that 25 ml. would contain at least 0.1 g. of PL fraction from each sample for the analysis of fatty acids by GLC. The volume of the mixture was recorded. Two portions of 2 ml. aliquots of sample were pipetted to preweighed 50 ml. beakers, evaporated, and weighed for the calculation of the weight of sample.

The 25 ml. aliquot was transferred to 50 g. silicic acid in a 500 ml. glass stoppered Erlenmeyer flask and 150 ml. of reagent grade chloroform was added. The flasks were shook on a wrist-action shaker for 10 minutes after the head space of the flask was flushed with nitrogen gas. Silicic acid, Mallinckrodt AR 100 mesh was activated by heating at 110°C. for 20 hours and was cooled for one hour in a desiccator prior to use.

The slurry was transferred quantitatively to a sintered glass funnel and filtered with suction. Four 50 ml. portions of chloroform were used for washing the silicic acid in the funnel. During each washing the suction was released and the silicic acid and chloroform were stirred well. The volume of the filterate, contain-

ing the nonphosphorous lipids, was recorded and two 25 ml. aliquots were transferred to weighed 50 ml. beakers. Solvent was evaporated from these subsamples and their weights were calculated from the weight of the NL fraction.

The filtrate was transferred quantitatively to a 1000 ml. round bottom flask and the solvent evaporated on a rotary evaporator under reduced pressure. The recovered NL was stored in a 50 ml. beaker at -20°C. after being flushed with nitrogen and covered with aluminum foil for later methylation.

The silicic acid mixture remaining in the funnel was transferred back to the 500 ml. flask with 150 ml. of absolute methanol and the head space of the flask was flushed with nitrogen gas. The mixture was shaken on a wrist action shaker for 10 minutes. The slurry was filtered into a clean flask and 150 ml. of methanol in two portions were used for washing. The filtrate, containing PL, was transferred into a 1000 ml. round bottom flask and the solvent was evaporated on a rotary evaporator. The recovered PL was transferred quantitatively with the aid of redistilled petroleum ether (about 50 ml.) to a 100 ml. cylinder to measure the volume.

Two 10 ml. aliquots were pipetted into weighed 50 ml. beakers to determine the PL fraction. The calculation

of the yield was the same as for the neutral lipid fraction. The extracts were transfered into 50 ml. beakers, flushed with nitrogen, covered, and stored at -20°C. for later methylation of fatty acids. The yields of the PL and NL were calculated as a per cent of total lipids recovered.

Methylation of Fatty Acids

It is common practice to lower the boiling point of fatty acids by methylation for analysis using GLC. The methylation was done in 2 to 3% of sulfuric acid in methanol (v;v). Methylation was carried out on each NL and PL fraction from the previous step. This resulted in duplication fatty acid analysis for each sample. Approximately 0.1 g. of NL was transferred from the stored stock to a 20 ml. test tube and 3 ml. of 2-3% sulfuric acid in methanol solution was added.

Petroleum ether was evaporated from the remaining PL fraction by a stream of nitrogen gas in a test tube containing the sample. To the same test tube, 3 ml. of 2 to 3% sulfuric acid in methanol solution was added. Three small glass beads were added to each test tube to prevent bumping. The test tubes were placed in a water bath at $75\pm5^{\circ}C$. The test tubes were shaken occasionally to mix the methanol and lipids. There were losses of methanol by evaporation and absolute methanol was added whenever

it was needed to keep the volume constant. The completion of the methylation was observed when the oil droplets had disappeared and a clear solution resulted after shaking. Six milliliters of petroleum ether and 3 ml. of distilled water were added in each test tube and they were mixed thoroughly. The top petroleum ether layer which contained methylated fatty acids was transferred to a 5 ml. bottle with a screw cap. The petroleum ether was evaporated by a stream of nitrogen gas and the sample was stored at -20°C. for GLC.

Gas Liquid Chromatography

The GLC instrument used was Perkin-Elmer model 810, dual columns equipped with flame ionization detectors. Twelve per cent DEGS was coated on 100-80 mesh chromosorb and packed in a glass tube (6 mm. I.D. x 6 feet). The average operating column temperature was 185°C. The temperature of the injection port and detector was 220°C. and 225°C., respectively. A slightly higher temperature was used in the detector than the injection port to prevent any possible condensation in the detector. The column temperature was raised to 220°C. after completion of each run to flush out any remaining high boiling compound in the column.

In previous work a column temperature of 185°C. showed the most satisfactory resolution on this sample system. A

column temperature of 160°C. was suitable for fatty acids under 18-carbons, but showed poor peak formation with the higher acids. More than two hours was required to complete a chromatogram at 160°C. A column temperature of 220°C., on the other hand, produced better peak formation with the higher PUSFA, but this temperature produced severe overlapping with shorter chain fatty acids. Programmed temperature was tried, but this procedure made the quantitation and identification of each peak difficult especially with higher chain length acids. It has been reported that the DEGS polar stationary phase showed the best resolution in separating the PUSFA, and that this stationary phase was very stable up to 225°C. The carrier gas used was purified Argon gas.

The Identification of GLC Peaks

The provisional identification of GLC peaks was done by the comparison of logarithms of retention time of the methyl esters of the fatty acid and their derived values. The derived values used were the ECL values of Miwa et al. (35), MECL values of Ackman (1), Linear logarithmic plot (1), and separation factors (2). The MECL system was modified by the author and named the MECL (n:2w6). All of the above methods were used to identify the GLC peaks of methyl ester of fatty acid.

The measurement of retention times of GLC peaks may be accomplished one of several ways. The most common one is to measure the peak top. The measurement of the half width of a peak produced almost the same results as did the peak top method. The measurement of the frontal tangent of a peak produced less errors, especially in the higher PUSFA, which tended to have flatter peaks with isothermal column temperatures. The peak top measurement of a retention time was used to obtain ECL values to compare with the ECL values reported by Hofstetter and Holman (17), who used the peak top method. For the MECL values, the frontal tangent method was used to measure the retention times. Ackman (1) reported that the Frontal tangent method gave a better linear logarithmic plot.

All the retention times were adjusted (substracted) from the initial solvent peak, which was approximately the void volume (or time) of a column. These adjusted retention times (ART) gave better linear logarithmic plots (1). The ART was used to express the retention time of peaks thgoughout this study.

The primary standard of methyl esters of fatty acids was purchased from the Hormel Institute, the University of Minnesota. The standard consisted of 16:0, 16:1w7, 18:0, 18:1w9, 18:2w6, 18:3w3, 20:1w9, 20:4w6, and 22:1w9 methyl ester of fatty acids. The primary standard was run every-

day to locate the above acids in sample GLC. The peaks of 18:3w3 and 20:1w9, and 20:4w6 and 22:1w9, were overlapping. It was easy to locate the peaks of 16:0, 16:1w7, 18:0, 18:1w9, 18:2w6, 18:3w3, and 20:4w6 in sample GLC by referring to the primary standard.

1. Equivalent Chain Length (ECL) System

The ECL system was first proposed by Miwa et al. (34), and was essentially the same as the Carbon Number system of Woodford and van Cent (48).

The ECL values were determined by the following method. The ART of the saturated fatty acids was plotted against the chain length on semilogarithmic paper and a straight line joining them was constructed. A value corresponding to the retention time of any other peak could then be read off from the abscissa of the graph. This value gave the chain length of the hypothetical saturated esters which could be eluted at that point.

Since the primary standard was run everyday, it was easy to locate the peaks of the 16:0 and 18:0 acids. The 20:0 acid was located by the linear logarithmic plot. Frequently when the ART of 16:0, 18:0, and 20:0 from the same GLC was used for the linear logarithmic plot, some of the ART of 16:0 would not fit on the line joining 18:0 and 20:0. In this case the line representing 18:0 and 20:0 was used to read the ECL values. More accurate ECL

values were produced when the saturated acid base line was constructed using the ART values from within a sample GLC, rather than using the ART values of primary standard, or the mean values obtained from several similar sample GLC runs made on the same day or different days. The conditions of each run were never the same, and consequently produced a slight difference in ART for each run. The difference in ART was so great from one run to the other that it was not possible to obtain accurate ECL values from the mean values of several sample GLC runs. All the ECL values were obtained using a self standard of saturated acids within a sample GLC. The ECL values so obtained were compared with the ECL values reported by Hofstetter and Holman (17). They reported ECL values obtained on four stationary phases, including DEGS. They used various pure methyl esters of USFA.

2. Linear Logarithmic Plot

The logariths of retention times of methyl esters of saturated acids were known to form a straight line when they were plotted on semilogarithmic paper against the number of carbon atoms in the fatty acids (1). The incorporation of the logarithm of retention time of the USFA to the line saturated acids was not very successful. The main reason was due to the use of the nomenclature of organic acids, which count the double bond position

from the carboxyl end of an acid. Fatty acids were classified according to the number of double bonds, and their position counting from carboxyl end. For example, palmitoleic (9-hexadecenoic) and oleic (9-octadecenoic) acids all belong to one family. The author noted that it was impossible to draw parallel straight lines with saturated acids when the USFA were classified according to the nomenclature of organic acids.

Ackman (1) reported that there was a basic misapprehention that the USFA could be structurally correlated through the corresponding saturated acids, the number of double bonds, and their position relative to the carboxyl group. He showed that there was satisfactory relationship among the USFA themselves. He classified the USFA in several families which were defined by the same number of double bonds and the same end carbon chain counting from the last double bond with varying numbers of carbon atoms in fatty acids. For example, 18:1w9, 20:1w9, and 22:1w9 belong to the family of n:1w9, and they produced a straight line on the linear logarithmic plot. The line representing each USFA family was parallel to each other. The line representing the saturated acids was not parallel with the lines of the USFA. The basic theory behind this linear logarithmic plot was the influence of the end carbon chain on the boiling point of the acid. Shorter end carbon chain

acids had higher boiling points when they had the same number of double bonds and chain length (Figure 1).

With this linear logarithmic plot, it was possible to predict the retention time of other acids within the same family of acids. This linear logarithmic plot also served to confirm the peaks identified through an ECL or MECL (n:2w6) system.

3. Separation Factors

Separation factors were devised at the same time as the MECL system by Ackman (2). The basic theory for this system is based on the linear logarithmic plot. The separation factors were the ratios of retention times of different unsaturated acids after they were classified by the number of double bonds and w (omega) values. Ackman classified the separation factors into the several types. Type I included the same chain length acids with the same w values, varying only with the number of double bonds. Type II included the esters of fatty acids of the same chain length with varying number of double bonds and different end carbon chains (w values). Type III included acids of the same chain length, same number of double bonds, and different end carbon chains. Another separation factor used included the acids with the same number of double bonds and same w values, but different carbon atoms

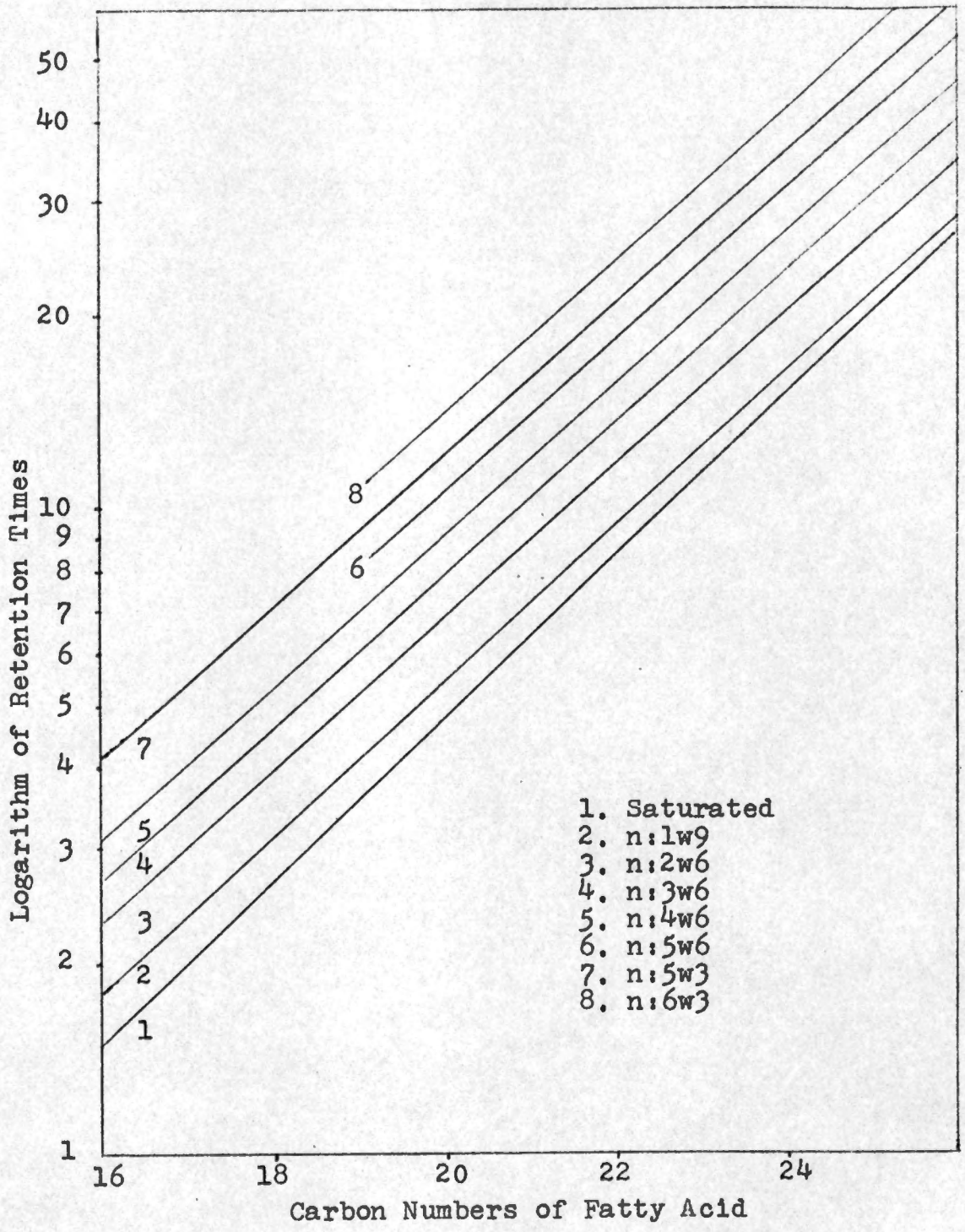


Figure 1. An example of Linear Logarithmic Plot

in the fatty acids. All these separation factors were shown to be influenced by the GLC column temperature (1), and possibly by the different kinds of polar stationary phases.

The separation factors obtained from various sample GLC runs showed some differences. Thus the separation factors that were needed for a linear logarithmic plot were calculated from within a sample GLC run rather than using the values from other sources, such as the values from the primary standard or an average values of the everyday runs.

4. Modified Equivalent Chain Length (MECL) System

The MECL system was proposed by Ackman (1). The basis of MECL are the formation of straight parallel lines in the linear logarithmic plot among the USFA families when the acids were grouped according to the number of double bonds and end carbon chain. Ackman showed that the saturated acid line was not parallel with the USFA family lines and the change of the slopes of saturated and USFA lines were not the same as the GLC column temperature changes. A different set of ECL values was produced as the column temperature was changed.

Ackman (1) suggested using the line of n:1w9 to read off retention time of other acids, and he named this system the modified equivalent chain length (MECL) system. Theo-

retically this MECL system should give better results than an ECL system, because the lines representing USFA families parallel each other. In practice, it was difficult to get the retention time of 20:1w9 and 22:1w9 which were needed to produce a straight line of the n:1w9 acids family for the MECL system. The peaks of 20:1w9 and 22:1w9 were overlapping with the 18:3w3 and 20:4w6, respectively. As stated before, it was sound practice to get the retention time of acids within a sample GLC to obtain the derived retention values. The ART of 20:1w9 and 22:1w9 could not be determined accurately, because it was not possible to get the separated peaks of 20:1w9 and 22:1w9 from 18:3w3 and 20:4w6, respectively. Approximate, but not accurate, ART of these acids, however, could be obtained from the 18:3w3 and 20:4w6 by calculating the ratio of the two overlapping peaks. The base line of the n:1w9 acid family for MECL was drawn referring to the points of 16:1w7, 18:1w9, 18:3w3, and 20:4w6. The n:1w9 line obtained was used with considerable success, but it required lots of experience with this technique.

Since an accurate n:1w9 line was difficult to obtain, another system was devised using the line of n:2w6, and this system is defined in the next section.

5. Modified Equivalent Chain Length (n:2w6) System

The ECL of fatty acids can be influenced by GLC column temperatures, and by kind of polar stationary phase. MECL values have a sounder theoretical basis, but this system is more difficult to use in a single GLC run due to the overlapping peaks of 20:1w9 and 22:1w9 with 18:3w3 and 20:4w6, respectively. Thus a good base line of n:1w9 could not be obtained. The difficulties with the n:1w9 line prompted the author to use the line of the n:2w6 acid family. 18:2w6 was present in every sample, and 20:2w6 was observed in at least half of the sample GLC runs. These two acids belong to the family of n:2w6 acids and by using these two points to give a straight line, it was possible to read off the ART of other acids. When 20:2w6 was not present or did not form a good peak, it was possible to locate the ART of 20:2w6 through the separation factor. It was found that the separation factors should come from the same w value USFA groups within a sample GLC run. For example, 20:4w6 and 22:4w6 were present in every sample in good peak formation, and the separation factors between them were calculated, this value was used to get the ART of 20:2w6 from 18:2w6.

A linear logarithmic plot was constructed using RRT published by Ackman (3) from cod liver oil. The line representing n:1w9 acids did not parallel with lines of

w6, and w3 acid families. However, the w6 and w3 acid family lines were parallel among themselves. Ackman reported that 20:1w9, and 22:1w9 might be contaminated with some other acids. The formation of unparallel lines between n:1w9 and other PUSFA should be checked further with pure compounds, because most of the derived retention times for the provisional identification of methyl ester of fatty acids are based on the sound linear logarithmic plot.

The difference between the MECL and MECL (n:2w6) system is the former, used the base line of n:1w9 and the latter used the base line of n:2w6. The procedure to obtain MECL (n:2w6) values is the same as the ECL procedure except each procedure used a different base line to read off the ART of other acids. The linear logarithmic plot showed that 20:2w6 are closer to the lines of higher PUSFA than the lines of n:1w9 and saturated acids. This would provide more accurate derived ART for higher PUSFA than the other methods. MECL (n:2w6) values, however, might be less accurate on the fatty acids having less than 18-carbon atoms.

CHAPTER IV

EXPERIMENTAL RESULTS

Fatty Acid Composition of Dietary Fats

Table II shows the fatty acid composition of beef tallow (BT) and peanut oil (PO). The major differences in individual fatty acids are found in 16:0, 18:0, and 18:2w6. The 22:2w6 and 20:5w3 fatty acids are present as minor fatty acids in PO and BT, respectively. PO contains approximately 13% saturated acids while BT contains 45% saturated acids. The percentages of saturated acids of PO and BT provide good referrence points. PO contained about 30% 18:2w6 while the BT contained approximately 5%. The difference in 18:2w6 content may provide an indication of linoleic acid family metabolism.

The 18:1w9 and 18:3w3 percentages are about the same in both BT and PO, and therefore metabolism of n:1w9 and n:3w3 acids could not be compared using these fatty acids. 22:2w6 is found in PO, but not in BT, and this fact is important in following the metabolism of 22:2w6.

The Effect of Different Dietary Fat on Moisture Content, Total Lipids, Neutral and Phospholipids

Table III, IV and V show the per cent composition of moisture content, total lipids, NL, and PL of the skin, white, and dark meat of broilers.

TABLE II

THE FATTY ACID COMPOSITION OF BEEF TALLOW AND PEANUT OIL

Fatty Acid	Beef Tallow	Peanut Oil
14:0	2.10%	--%
15:0	tr	--
16:0	25.42	10.69
16:1w7	3.36	tr
16:2w7	tr[2]	--
18:0	19.36	1.84
18:1w9	40.28	49.51
18:2w6	5.26	29.55
20:0	tr	0.94
18:3w3	1.38	1.63
20:2w6	0.27	--
20:3w6	--	3.67
20:4w6	0.27	tr
22:2w6	--	1.87
20:5w3[1]	1.15	--
22:3w6	0.50	--
22:4w6	--	--
22:5w6	--	--
22:5w3	--	--
22:6w3	0.24	0.50
Total Saturated	44.84	13.50
Total Unsaturated	55.16	86.50

1/ May contain other fatty acid.

2/ tr means less than 0.10%.

TABLE III

THE PER CENT COMPOSITION OF MOISTURE CONTENT, TOTAL LIPIDS, NEUTRAL LIPIDS, AND PHOSPHOLIPIDS OF THE SKIN OF BROILERS

Treatment[1]	Moisture[2] Contents	Total Lipids[3] Wet Wt.	Total Lipids[3] Dry Wt.	Neutral[3] Lipid	Phospho-[3] Lipid
3% BT, Male	49.04	31.53	61.87	98.09	1.91
3% BT, Female	52.85	40.00	73.87	98.73	1.26
13% BT, Male	48.18	31.72	61.22	98.76	1.26
13% BT. Female	49.35	42.74	83.38	98.88	1.12
3% PO, Male	42.40	29.91	51.93	95.28	4.70
3% PO, Female	52.86	41.47	87.97	97.72	2.29
13% PO, Male	52.30	37.17	77.93	98.38	1.62
13% PO, Female	51.20	40.80	83.60	98.67	1.33

1/ BT means beef tallow and PO means peanut oil.

2/ Means of three samples

3/ Means of four samples

TABLE IV

THE PER CENT COMPOSITION OF MOISTURE CONTENT, TOTAL LIPIDS, NEUTRAL LIPIDS, AND PHOSPHOLIPIDS OF THE DARK MEAT OF BROILERS

Treatment[1]	Moisture[2] Contents	Total Lipids[3]		Neutral[3] Lipids	Phospho-[3] Lipids
		Wet Wt.	Dry Wt.		
3% BT, Male	20.88	2.06	2.60	78.86	21.14
3% BT, Female	24.18	2.49	3.28	76.28	23.73
13% BT, Male	24.30	3.14	4.14	83.91	16.09
13% BT, Female	24.52	2.88	3.82	82.42	17.58
3% PO, Male	22.80	2.30	2.98	75.39	24.61
3% PO, Female	20.06	2.65	3.49	80.59	19.42
13% PO, Male	24.73	3.56	4.73	84.05	15.95
13% PO, Female	23.27	2.74	3.57	78.36	21.63

1/ BT means beef tallow and PO means peanut oil.

2/ Means of three samples

3/ Means of four samples

TABLE V

THE PER CENT COMPOSITION OF MOISTURE CONTENT, TOTAL LIPIDS, NEUTRAL LIPIDS, AND PHOSPHOLIPIDS OF THE WHITE MEAT OF BROILERS

Treatment[1]	Moisture[2] Contents	Total Lipids[3]		Neutral[3] Lipids	Phospho-[3] Lipids
		Wet Wt.	Dry Wt.		
3% BT, Male	25.26	.94	1.26	50.10	49.90
3% BT, Female	25.12	.95	1.27	53.08	47.04
13% BT, Male	26.37	1.09	1.48	57.63	42.37
13% BT, Female	27.10	1.24	1.70	54.56	45.44
3% PO, Male	25.07	.91	1.21	39.99	60.01
3% PO, Female	26.32	1.22	1.66	53.28	46.72
13% PO, Male	25.88	1.20	1.62	54.79	45.21
13% PO, Female	27.17	1.18	1.62	44.92	55.08

1/ BT means beef tallow and PO means peanut oil.

2/ Means of three samples

3/ Means of four samples

The skin contained on the average about 73% total lipids (dry weight basis), with 98% of the total lipids as NL and the remaining 2% as PL. The skin of female broilers contained more total lipids than the skin of the male, in all treatments. Higher levels of total lipids were detected in the skin when the broilers were fed higher dietary fats.

The white meat contained on the average about 1.5% total lipids (dry weight basis), and the total lipid content increased slightly when the broilers were given higher levels of dietary fats. The total lipids of white meat consisted of about 51% NL and 49% PL.

The dark meat contained on the average about 3.5% total lipids (dry weight basis), with 80% of the total lipids as NL and 20% as PL. The total lipid content of the dark meat increased when the amount of fat was increased in the diet.

In comparing the effects of added dietary fats on the lipid composition of the skin, white, and dark meat of male and female broilers, there appeared to be some difference in the NL and Pl content with dietary fat treatments. This study showed the content of NL in the skeletal muscles could be increased by the higher level of dietary fat, consequently decreasing the proportion of PL. This is contradictory to the findings of Marion (28) who reported the dietary fat could not change the proportion of

NL and PL in skeletal muscles.

The Sample GLC Peaks, Type I and Type II

The sample GLC runs were grouped into two types, namely, Type I and Type II. A representative sample GLC of Type I is shown in Figure 2. Type I showed very good separation among the fatty acids, and showed four very distinctive peaks of 22:4w6, 22:5w6, 22:5w3, and 22:6w3. The 20:5w3 peak was overlapped with the 22:2w6 peak and these peaks were present in some samples of Type I sample GLC runs. The overlapping of 20:5w3 and 22:2w6 produced and unsymmetrical peak, and this peak sometimes included 22:3w6 when a smaller amount of it was present, otherwise 22:3w6 showed a separated peak. Most of the Type I GLC runs were observed in samples of PL fractions, while none of the NL fractions showed a GLC Type I run. The differences between Type I and Type II sample GLC runs were, Type I has very good separation of PUSFA including the four distinctive peaks of 22:4w6, 22:5w6, 22:5w3, 22:6w3, and Type II showed none of the four distinctive peaks observed in Type I. The Type II runs showed two overlapping peaks. The first overlapping peak (peak I) contained 20:5w3, 22:2w6, and 22:3w6. When 22:4w6 was present in small quantities, it was included in the first peak, and produced a skewed peak with considerable tailing. 22:5w6 peaks were

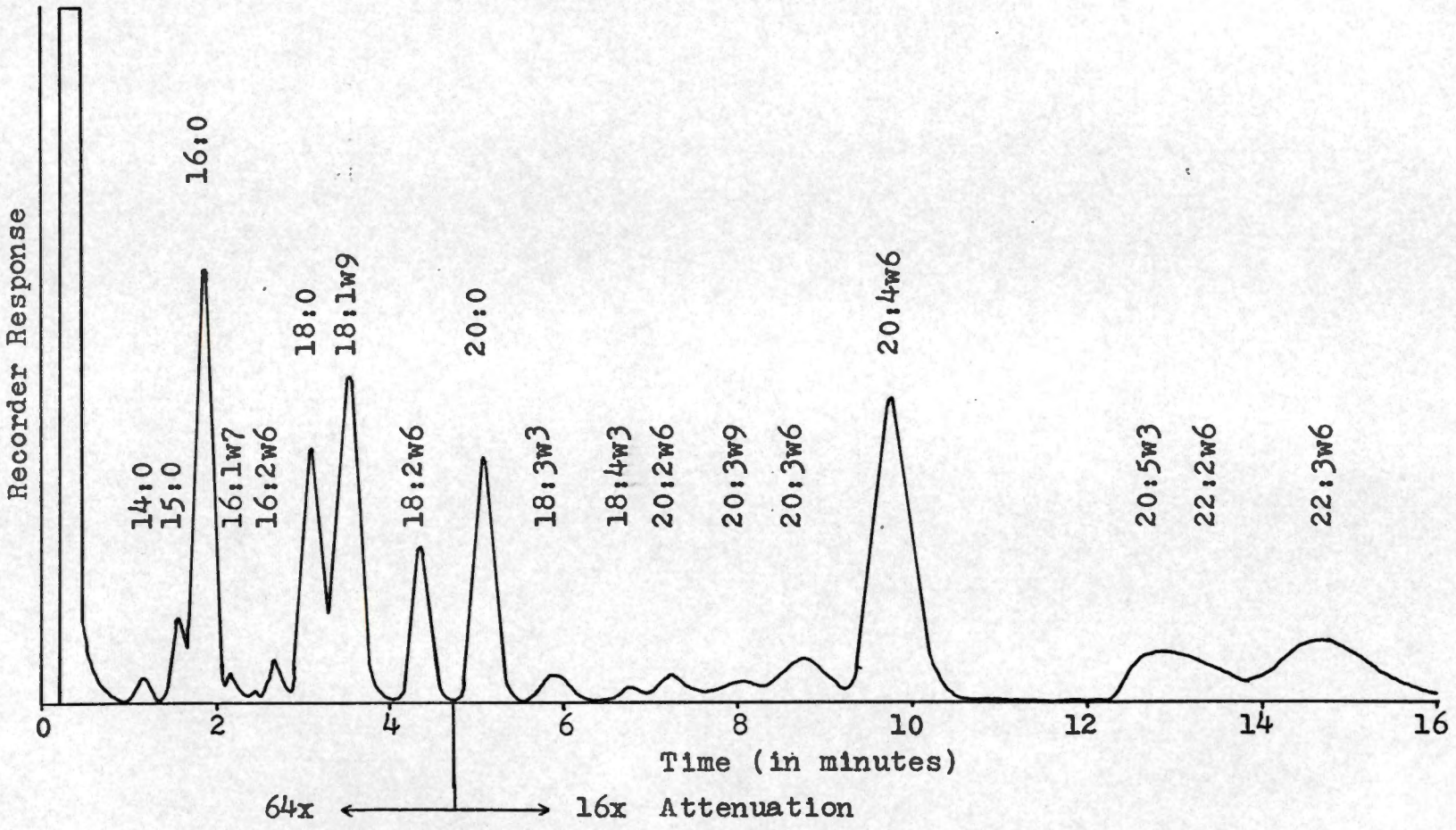


Figure 2. GLC Peaks of Type I Samples

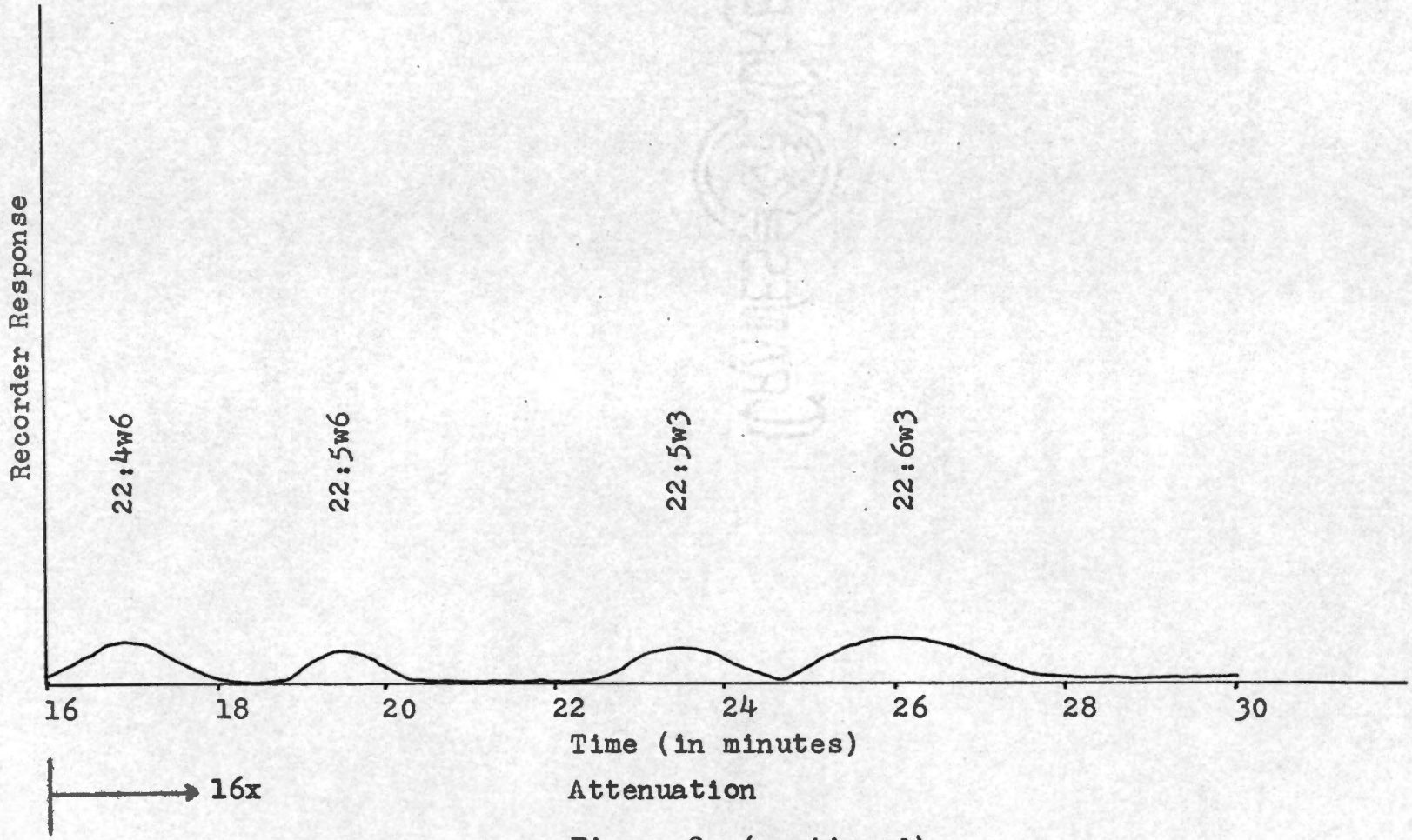


Figure 2. (continued)

not present in most of the Type II runs. The second overlapped peak (peak II) contained 22:5w3, 22:6w3, and possibly 24:3w6, with the 22:6w3 peak usually the dominating peak. The 24:4w6 peak was observed after the second overlapping peak, and was completely separated from this second peak. The first and second overlapping peaks, usually showed flat and skewed peaks, thus it was not practical to separate the individual fatty acids in each overlapping peak. For this reason, the individual PUSFA contained in each of the two overlapping peaks were not separated, but the per cent composition of the combined PUSFA in each overlapping peak was reported. There were many Type II sample GLC runs which showed distinctive peaks within the two overlapping peaks. Thus the possible identity of the acids contained in these two peaks could be ascertained. The ART and their MECL (n:2w6) from Type II sample GLC runs are reported later in this thesis. A representative Type II sample GLC run is shown in Figure 3. The overlapped peaks are separated by a dotted line to show the individual peaks.

The Identification of GLC Peaks of Methyl Ester of Fatty Acids

Table VI shows the ART of Type I sample GLC runs. The ART was measured by the peak top method. All of the samples are from the PL fractions. Table VII shows the ECL values

Figure 3. GLC Peaks of Type II Samples

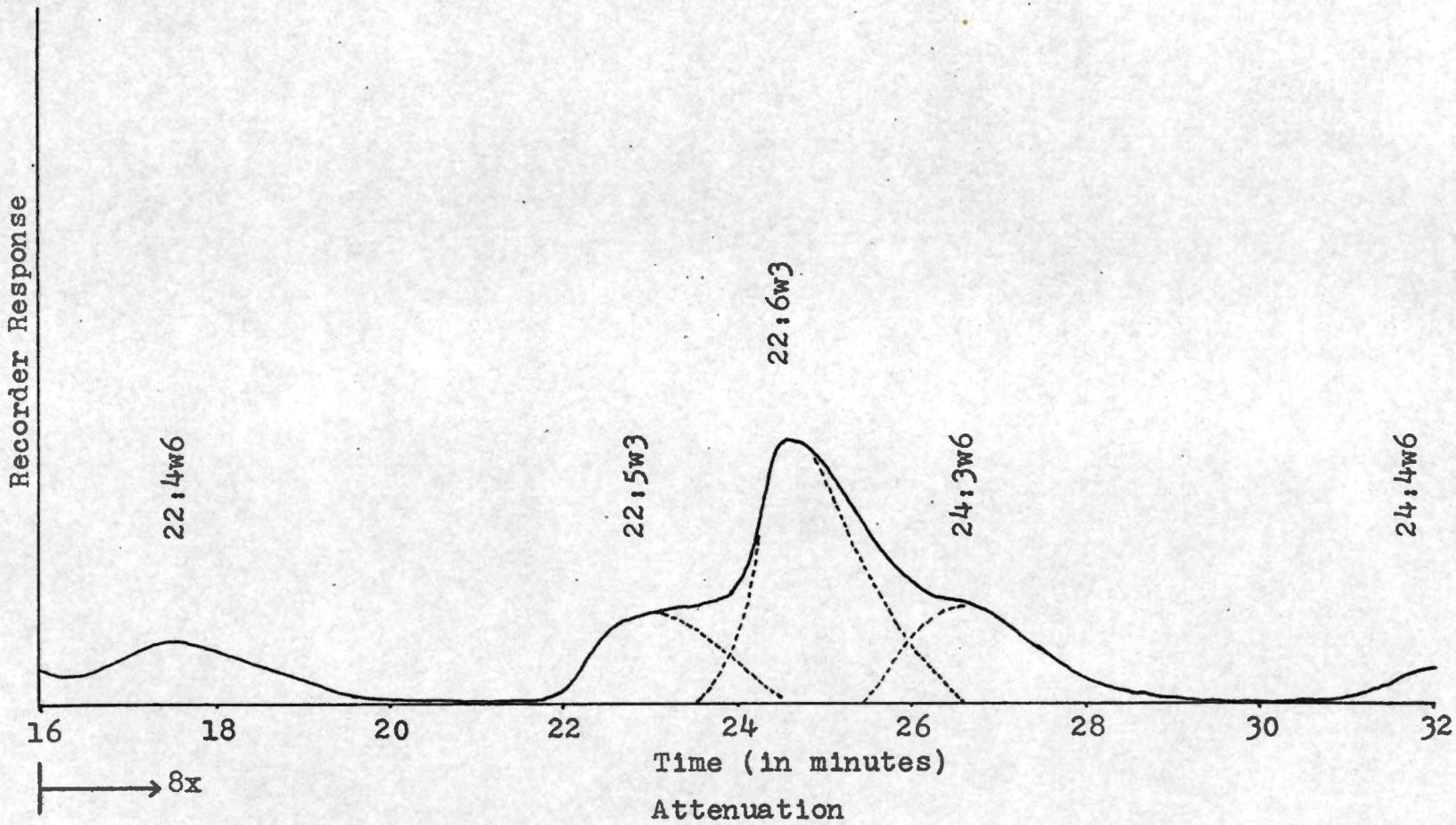


Figure 3. (continued)

TABLE VI

THE ADJUSTED RETENTION TIMES (PEAK TOP) OF METHYL ESTERS OF FATTY ACIDS FOR ECL VALUES TYPE I GLC PEAKS

Fatty Acids	Sample Number (1)[1/]	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
14:0	0.82	0.80	0.88	0.90	0.92	0.96	0.99	1.00	0.97	0.86
15:0	1.38	1.22	1.41	1.42	1.38	1.40	1.45	1.40	1.39	1.38
16:0	1.60	1.54	1.66	1.66	1.60	1.64	1.59	1.64	1.64	1.63
16:1w7	1.86	1.81	1.89	--	1.91	1.96	1.87	1.94	1.95	1.96
16:1w7	2.38	2.32	2.44	2.44	2.40	2.42	2.37	2.46	2.41	2.46
18:0	2.78	2.72	2.86	2.86	2.81	2.86	2.77	2.90	2.83	2.90
18:1w9	3.24	3.16	3.34	3.34	3.30	3.32	3.25	3.36	3.29	3.36
18:2w6	4.08	4.00	4.20	4.18	4.16	4.22	4.09	4.20	4.11	4.20
20:0	4.82	4.70	4.90	4.90	4.96	4.98	--	--	--	--
18:3w3	5.52	5.42	5.68	5.72	5.68	5.74	5.52	5.76	5.67	5.72
20:2w6	6.98	6.82	7.16	7.18	7.14	7.33	6.97	7.26	7.21	7.30
20:3w6	8.24	8.02	8.42	8.46	8.42	8.63	8.19	8.51	8.47	8.50
20:4w6	9.34	9.20	9.66	9.68	9.64	9.82	9.41	9.70	9.61	9.72
20:5w3	12.46	12.06	12.76	12.82	--	--	12.77	12.16	--	13.00
22:3w6	13.92	13.86	14.50	14.42	--	--	--	--	--	--
22:4w6	16.06	16.04	16.70	17.72	16.76	17.22	16.27	16.81	16.81	17.10
22:5w6	18.46	18.46	19.24	19.32	19.26	19.78	18.77	19.36	19.40	19.72
22:5w3	21.36	21.26	22.14	22.22	22.16	23.78	19.95	23.58	22.27	22.76
22:6w3	24.46	24.56	25.54	25.74	25.46	26.23	24.78	27.66	25.75	26.20
24:4w6	--	--	--	--	--	--	--	--	--	--

1/ The retention times were expressed in minutes.

TABLE VII

THE ECL VALUES FROM THE DATA OF TABLE VI[1/]

Fatty Acids	Sample Number (1)[2/]	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
14:0	14.00	13.60	13.64	13.72	14.02	14.04	14.02	14.20	14.60	13.76
15:0	15.20	15.08	15.38	15.40	15.48	15.04	15.66	15.42	15.40	15.42
16:0	16.00	15.98	16.00	16.00	15.98	16.00	16.00	16.00	16.00	16.00
16:1w7	16.52	16.50	16.48	--	16.72	16.62	16.60	16.58	16.62	16.58
16:2w7	17.47	17.42	17.40	17.40	17.42	17.38	17.32	17.48	17.40	17.42
18:0	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00
18:1w9	18.54	18.56	18.56	18.56	18.56	18.40	18.56	18.52	18.52	18.50
18:2w6	19.38	19.42	19.42	19.40	19.36	19.38	19.38	19.20	19.24	19.28
20:0	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
18:3w3	20.48	20.52	20.54	20.56	20.48	20.50	20.44	20.40	20.38	20.32
20:2w6	21.32	21.36	21.52	21.52	21.30	21.48	21.28	21.22	21.24	21.16
20:3w6	21.94	21.96	22.00	22.02	21.88	21.98	21.86	21.78	21.82	21.80
20:4w6	22.40	22.56	22.52	22.52	22.36	22.42	22.36	22.24	22.26	22.14
20:5w3	23.44	23.46	23.54	23.60	--	--	23.44	23.02	--	23.15
22:3w6	23.84	23.98	24.02	24.00	--	--	--	--	--	--
22:4w6	24.48	24.50	24.54	24.56	24.30	24.48	24.30	24.02	24.26	24.04
22:5w6	24.86	25.02	25.02	25.02	24.82	25.00	24.83	24.64	24.76	24.52
22:5w3	25.40	25.54	25.60	25.62	25.34	25.68	25.04	25.34	25.22	25.02
22:6w3	25.90	26.10	26.10	26.10	25.80	26.02	25.82	25.90	25.76	25.50
24:4w6	--	--	--	--	--	--	--	--	--	--

1/ Table VI, page 54.

2/ These values were correspond to the data of Table VI, page 54.

of the corresponding samples from Table VI. The ECL values were determined by the self standard. They show relatively accurate ECL values. For Type I sample GLC runs, the ECL system was employed successfully, providing that the ART of the saturated acids came from within the same GLC run.

Table VIII shows the ART measured by the frontal tangent method using the same samples as in Table VI, page 54, Table IX shows the MECL (n:2w6) value obtained from the ART of corresponding samples in Table VIII. The MECL (n:2w6) values show more accurately the values of the fatty acids of 18-carbon or more.

The sample numbers used in Table VI, VII, VIII, and IX, page 54, 55, 57, and 58, respectively, indicate the following source of the samples: 1 and 2 represent the dark meat of male broilers on a 13% peanut oil (PO) diet, 3 and 4 represent the white meat of male broilers on a 13% PO diet, 5 and 6 represent the skin of female broilers on a 3% PO diet, 7 and 8 represent the white meat of female broilers on a 3% PO diet, 9 represents the dark meat of male broilers on a 3% beef tallow (BT) diet, and 10 represents the white meat of male broilers on a 3% BT diet. The ART of the above samples was chosen using the best peak formation of 20:2w6 found in the sample GLC runs. The ART of 20:2w6 is needed to draw a straight line of n:2w6 along with the ART of 18:2w6 to read off the MECL (n:2w6) values for other acids.

TABLE VIII

THE ADJUSTED RETENTION TIMES (FRONTAL TANGENT) OF METHYL ESTERS OF FATTY ACIDS FOR MECL (n:2w6) VALUES

Fatty Acids	Sample Number[1] (1)[2]	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
14:0	0.72	0.68	0.74	0.74	0.88	0.90	0.67	--	0.69	0.68
15:0	1.28	1.22	1.28	1.32	1.30	1.30	1.24	1.26	1.29	1.28
16:0	1.48	1.44	1.50	1.50	1.52	1.52	1.45	1.48	1.51	1.52
16:1w7	1.76	1.70	1.78	1.80	1.88	1.88	1.83	1.78	1.87	1.84
16:2w7	2.24	2.18	2.28	2.32	2.30	2.28	2.27	2.24	2.29	2.30
18:0	2.58	2.54	2.66	2.66	2.68	2.68	2.57	2.62	2.67	2.72
18:1w9	3.04	2.96	3.12	3.10	3.14	3.14	3.01	3.06	3.09	3.14
18:2w6	3.84	3.76	3.96	3.92	3.96	4.00	3.83	3.86	3.90	3.96
20:0	4.58	4.46	4.74	4.72	4.74	4.76	--	--	--	--
18:3w3	5.26	5.06	5.34	--	5.40	5.46	5.17	5.28	5.37	5.38
20:2w6	6.60	6.46	6.80	6.82	6.98	7.02	6.57	6.78	6.95	6.94
20:3w6	7.76	7.56	7.94	7.96	8.08	8.18	7.77	7.88	8.07	7.96
20.4w6	8.86	8.68	9.14	9.14	9.32	9.32	8.89	9.06	9.17	9.24
20:5w3	11.86	11.46	12.28	12.24	--	--	--	11.22	--	12.40
22:3w6	13.20	12.80	13.74	13.52	--	--	--	12.38	--	--
22:4w6	15.20	15.26	15.88	15.82	16.34	16.34	15.40	15.98	16.05	16.26
22:5w6	17.50	17.46	18.34	18.32	18.84	18.88	17.77	18.68	18.67	19.06
22:5w3	20.60	20.36	21.54	21.43	22.18	21.68	20.67	21.68	21.41	21.70
22:6w3	23.20	23.26	24.14	24.22	25.30	24.68	23.77	23.88	24.67	25.10
24:4w6	--	--	--	--	--	--	--	--	--	--

1/ The sample numbers were correspond to the Table VI, page 54.

2/ These retention times were expressed in minutes.

TABLE IX

THE MECL (n:2w6) VALUES FROM THE DATA OF TABLE VIII[1/]
TYPE I GLC PEAKS

Fatty Acids	Sample Number (1)[2/]	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
14:0	11.80	11.74	11.70	11.94	12.70	12.74	12.00	--	12.00	11.90
15:0	13.96	13.82	13.82	14.02	14.00	14.04	13.92	14.02	14.14	14.00
16:0	14.46	14.44	14.40	14.50	14.64	14.60	14.47	14.60	14.68	14.60
16:1w7	15.10	15.04	15.04	15.14	15.40	15.38	15.30	15.28	15.42	15.30
16:2w7	16.00	15.98	15.96	16.08	16.10	16.02	16.08	16.14	16.16	16.06
18:0	16.52	16.56	16.52	16.58	16.62	16.48	16.54	16.62	16.70	16.68
18:1w9	17.14	17.10	17.22	17.13	17.20	17.16	17.10	17.16	17.20	17.20
18:2w6	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00
20:0	18.64	18.62	18.66	18.66	18.64	18.62	--	--	--	--
18:3w3	19.14	19.08	19.10	--	19.08	19.08	19.10	19.10	19.10	19.08
20:2w6	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
20:3w6	20.62	20.58	20.57	20.54	20.53	20.52	20.58	20.52	20.52	20.46
20:4w6	21.08	21.10	21.10	21.06	21.02	21.00	21.12	21.00	20.96	21.00
20:5w3	22.18	22.12	22.20	22.10	--	--	--	21.76	--	--
22:3w6	22.56	22.58	22.60	22.48	--	--	--	22.10	--	22.04
22:4w6	23.10	23.20	23.16	23.04	23.00	23.00	23.18	23.00	22.90	23.00
22:5w6	23.62	23.68	23.70	23.56	23.52	23.53	23.70	23.58	23.42	23.58
22:5w3	24.20	24.28	24.30	24.12	24.10	24.00	24.26	24.08	23.90	24.05
22:6w3	24.66	24.76	24.72	24.58	24.54	24.44	24.78	24.42	24.40	24.54

1/ Tale VIII, page 57.

2/ These values were correspond to the data of Table VIII, page 57.

The 20:2w6 was usually present in small amounts (about 0.5%), and these quantities did not give a sharp enough peak to obtain an accurate measurement of retention time for the fatty acid. The addition of a small amount of 20:2w6 to an unknown sample would provide a good peak for 20:2w6. 18:2w6 is usually present in good quantities in all biological samples, thus, the ART of 18:2w6 and 20:2w6 should give a good line of n:2w6 to read off the MECL (n:2w6) values for the unknown peaks. The best standard MECL (n:2w6) values can be obtained using a sample of known composition, such as cod liver oil as suggested by Ackman (3), and run at the same GLC conditions as the unknown sample. The MECL (n:2w6) values reported in Table IX, page 58, for the Type I sample runs showed very satisfactory results, especially on the higher PUSFA.

Table X shows the ART from the Type II sample runs. The Type II samples for Table X were chosen using the sample runs having the best peak formation of 20:2w6 for MECL (n:2w6) values, and some shoulder formations in the overlapped peaks I and II. The individual fatty acids within each of the overlapped peaks was determined manually. The overlapped peak I contained 20:5w3, 22:2w6, and 22:3w6. The overlapped peak II contained 22:5w3, 22:6w3, and possibly 24:3w6. The overlapped peaks were separated manually into individual fatty acids, and their ART reported in Table X. Table XI shows the MECL (n:2w6) values obtained using the correspond-

TABLE X

THE ADJUSTED RETENTION TIMES (FRONTAL TANGENT) OF METHYL ESTERS OF FATTY ACIDS. TYPE II GLC PEAKS

Fatty Acid	Sample Number (1) 1/	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
14:0	0.86	0.86	0.86	--	0.76	0.73	0.94	0.80	0.98	0.85
15:0	--	--	--	--	1.14	1.20	1.38	1.14	1.28	--
16:0	1.50	1.52	1.54	1.64	1.37	1.44	1.60	1.34	1.50	1.53
16:1w7	1.84	1.84	1.84	1.94	1.66	1.70	1.98	2.08	2.24	1.74
16:2w7	--	--	--	--	2.12	2.20	2.45	--	--	--
18:0	2.68	2.70	2.74	2.92	2.47	2.58	2.85	2.44	2.64	2.73
18:1w9	3.14	3.14	3.18	3.36	2.88	3.00	3.32	2.86	3.06	3.15
18:2w6	4.02	4.00	4.08	4.30	3.66	3.80	4.22	3.68	3.86	4.01
20:0	4.74	4.74	4.84	5.06	4.30	4.70	5.02	--	--	--
18:3w3	5.36	5.34	5.46	5.66	4.90	--	5.72	--	--	5.45
18:4w3	--	--	--	--	6.10	--	6.88	--	--	6.65
20:2w6	7.04	7.06	7.14	7.48	6.30	6.70	7.33	6.44	6.70	7.05
20:3w6	8.29	8.36	8.44	9.14	--	8.10	8.72	--	--	8.35
20:4w6	9.44	9.40	9.54	10.04	8.70	8.82	9.48	8.78	8.94	9.35
20:5w3	12.02	11.70	12.84	12.38	10.56	10.68	12.16	10.56	10.90	11.67
22:2w6	12.70	12.30	13.40	13.00	11.00	11.50	12.40	--	--	--
22:3w6	14.20	14.06	14.64	15.64	12.70	12.50	13.80	--	--	13.65
22:4w6	16.30	16.26	16.74	--	--	14.90	16.98	14.80	14.78	15.75
22:5w6	--	--	--	--	16.80	--	--	--	--	--
22:5w3	22.54	21.48	22.70	23.54	20.00	18.80	22.20	19.14	19.78	21.95
22:6w3	23.44	23.70	24.54	25.64	21.76	22.00	25.00	22.04	22.42	23.55
24:3w6	25.40	25.30	26.40	27.30	24.00	23.60	27.50	--	--	--
24:4w6	31.44	30.82	32.54	33.16	28.40	28.00	--	28.48	28.90	31.85

1/ Retention times expressed in minutes.

TABLE XI

THE MECL(n:2w6) VALUES FROM THE DATA OF TABLE X[1/]

Fatty Acid	Sample Number (1)[2/]	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
14:0	12.50	12.60	12.42	--	12.30	12.20	12.60	12.60	13.10	12.60
15:0	--	--	--	--	13.90	14.10	14.08	13.92	14.08	--
16:0	14.44	14.54	14.50	14.46	14.50	14.50	14.50	14.46	14.50	14.52
16:1w7	15.20	15.26	15.14	15.08	15.16	15.10	15.30	--	--	15.10
16:2w7	--	--	--	--	16.00	16.02	16.00	16.00	16.00	16.00
18:0	16.52	16.62	16.58	16.54	16.60	16.60	16.56	16.58	16.60	16.60
18:1w9	17.12	17.15	17.10	17.10	17.16	17.14	17.10	17.12	17.14	17.12
18:2w6	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00
20:0	18.58	18.62	18.58	18.56	18.60	18.76	18.62	--	--	--
18:3w3	19.00	19.00	19.02	18.98	19.04	--	19.10	--	19.30	19.08
18:4w3	--	--	--	--	19.84	--	19.76	--	--	19.80
20:2w6	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
20:3w6	20.56	20.60	20.60	20.72	--	20.72	20.70	--	--	20.62
20:4w6	21.04	21.00	21.02	21.20	21.12	21.02	21.06	21.06	21.10	21.02
20:5w3	21.92	21.80	22.10	21.82	21.82	21.70	21.70	21.72	21.84	21.80
22:2w6	22.08	22.00	22.20	21.94	22.00	22.00	21.94	--	--	22.02
22:3w6	22.50	22.44	22.52	22.70	22.40	22.30	22.34	--	--	22.38
22:4w6	--	22.96	23.02	--	--	22.86	23.10	22.90	22.98	22.90
22:5w6	--	--	--	--	23.50	--	--	--	--	--
22:5w3	24.16	23.94	24.12	24.20	24.14	23.76	24.10	23.80	24.04	24.08
22:6w3	24.30	24.30	24.40	24.62	24.44	24.32	24.46	24.30	24.50	24.32
24:3w6	24.54	24.53	24.74	24.60	24.72	24.60	24.80	--	--	24.48
24:4w6	25.34	25.24	25.42	25.42	25.40	25.20	--	25.20	25.32	25.40

1/ Table X, page 60.

2/ These values were correspond to the data of Table X, page 60.

ing ART values in Table X, page 60. The ECL system was tried on the Type II sample GLC runs, but showed very poor results.

The Type II samples used in Table X and XI, page 60 and 61, respectively, are from the following samples. The sample numbers used in the above two Tables indicate the following source of the samples: 1 represents the NL from the dark meat of male broilers on a 13% PO diet, 2 and 3 the dark meat, and 4 white meat, 5 represents the NL from the dark meat of female broilers on a 3% PO diet, 6 represents the PL from the dark meat of female broilers on a 13% PO diet, 7 and 8 represent the PL from the skin and the white meat of male broilers on a 13% BT diet, respectively, and 9 and 10 represent the NL from the white meat of male broilers on a 3% BT diet.

Table XII is the summary of GLC peaks identification procedure used. The mean values of ART measured by the peak top method (Table VI, page 54) and by the frontal tangent method (Table VIII, page 57) are compared. This shows the difference in ART obtained by the two methods, especially of the higher PUSFA. The relative retention time (18:1w9) of cod liver oil fatty acids as reported by Ackman (3) are compared with the RRT (18:1w9) derive from Table VIII, page 57. This comparison RRT shows that there are great differences in RRT (18:1w9) of higher PUSFA. The

TABLE XII

THE COMPARISON OF ADJUSTED RETENTION TIMES AND THEIR DERIVED VALUES OF METHYL ESTERS OF FATTY ACIDS IN GLC

Fatty Acid	ART 1/		RRT(18:1w9) 2/		ECL 3/		MECL(n:2w6) 4/		
	PT	FT	St'd	Sample	St'd	Sample	St'd	Type I	Type II
14:0	0.91	0.74	0.23	0.24	--	13.91	12.54	12.06	12.43
15:0	1.38	1.28	0.32	0.42	--	15.35	13.62	13.98	14.02
16:0	1.62	1.42	0.45	0.48	--	16.00	14.70	14.54	14.49
16:1w7	1.82	1.81	0.55	0.59	16.55	16.57	15.34	15.24	15.17
16:2w7	2.40	2.27	0.66	0.74	17.50	17.41	15.93	16.05	16.00
18:0	2.83	2.64	0.86	0.84	18.00	18.00	16.77	16.58	16.58
18:1w9	3.30	3.08	1.00	1.00	18.51	18.53	17.24	17.16	17.13
18:2w6	4.14	3.90	1.27	1.27	19.30	19.35	18.00	18.00	18.00
20:0	4.88	4.67	1.63	1.52	20.00	20.00	18.80	18.64	18.62
18:3w3	5.64	5.30	1.73	1.72	20.40	40.46	19.00	19.09	19.07
20:2w6	7.14	6.79	2.37	2.20	21.36	21.34	20.00	20.00	20.00
20:3w6	8.39	7.92	2.80	2.57	22.13	21.90	20.64	20.54	20.65
20:4w6	9.58	9.08	3.19	2.95	22.43	22.37	20.69	21.04	21.06
20:5w3	12.58	11.14	4.37	3.70	23.45	23.38	21.96	21.89	21.82
22:2w6	--	--	--	--	--	--	21.00	--	21.02
22:3w6	14.18	13.14	5.18	4.27	23.94	23.96	22.48	22.39	22.45
22:4w6	16.75	15.85	5.93	5.15	24.58	24.35	22.96	23.06	22.96
22:5w6	19.18	18.36	6.90	5.96	24.97	24.85	23.44	23.59	23.50
22:5w3	22.15	21.32	8.10	6.92	25.38	25.38	23.93	24.14	24.03
22:6w3	25.64	24.22	9.46	7.86	26.03	25.90	24.42	24.58	24.40
24:4w6	--	--	--	--	26.50	--	25.06	--	25.29

TABLE XII (continued)

1/ ART means adjusted retention time. PT means the retention times were measured by the peak top method, and FT by the frontal tangent. PT is the mean value from Table VI, page 54, and FT is the mean value from Table VIII, page 57.

2/ RRT means relative retention time (18:1w9). Standard RRT are from Ackman (3), and sample RRT are the mean values from Table VIII, page 57.

3/ The standard ECL values are from the data of Hofstetter and Holman (17), and sample ECL values are the mean values from Table IX, page 58.

4/ The standard MECL (n:2w6) values were determined using the date of Ackman (3). The sample (Type I) MECL (n:2w6) are the mean values from Table IX, page 58, and sample (Type II) are the mean values from Table XI, page 61.

feasibility of using RRT (18:1w9) is questioned, since the RRT does not give any idea of the peak identification in GLC. Reporting ART rather than the RRT might save time, and unnecessary rounding off of the RRT values. The ECL values from the data of Hofstetter and Holman (17) are referred to as the standard ECL values. The mean ECL values reported in Table VII, page 63, are compared with the standard ECL values. It has been found that using a self standard of saturated acids within a GLC run increases the accuracy of the ECL values, rather than using the ART of saturated acids from the primary standard or the average ART values of several GLC runs. The principle of using the self standard was extended to the MECL (n:2w6) system. The reliability of the ECL values was very good in Type I samples, however, they were useless in Type II samples. Since the ECL values could not be used for Type II sample GLC runs, another method, namely the MECL (n:2w6), was devised. This system proved very satisfactory on PUSFA. The MECL (n:2w6) values reported in Table XII, page 63, were determined using RRT (18:1w9) values reported by Ackman (3), using a linear logarithmic plot. In Table XII, page 63, the standard MECL (n:2w6) values are reported, along with the mean values of MECL (n:2w6) for Type I sample GLC from Table IX, page 58, and for Type II sample GLC from Table XII, page 63, show very consistant values among the individual

fatty acids, and on the fatty acids of 18-carbon atoms or higher. The RRT (18:1w9) values reported by Ackman (3) were obtained using a 20% DEGS stationary phase, and a column temperature of 170°C. The ECL values reported by Hofstetter and Holman (17) were obtained using a 20% DEGS stationary phase, and a column temperature of 226°C. Even though the operating conditions for the standard values of RRT (18:1w9) and ECL of fatty acids were different from the sample GLC runs, the derived retention times showed very good agreement between standard and the sample values.

Table XIII shows the separation factors obtained using the ART of Table VIII, page 57. Ackman (1) showed that the separation factors could be changed by operational conditions such as column temperature. It has been found by the author that more accurate separation factors could be obtained using ART values within a GLC run rather than from the primary standard or the average ART values of several GLC runs. The separation factors obtained using ART values can serve as a secondary check to confirm the ecl and MECL (n:2w6) values.

The Fatty Acid Composition of Neutral Lipids

Tables XIV, XV, and XVI show the per cent fatty acid composition of NL from different tissues of skin, dark meat, and white meat of every dietary fat treatment.

The fatty acid compositions of skin NL are reported in

TABLE XIII

SEPARATION FACTORS FROM THE ART DATA OF TABLE VIII[1/]

Type of SF[2/] and Source	Sample numbers[3/] 1	2	3	4	5	6	7	8	9	10
Type I SF										
22:6w3 22:5w3	1.13	1.14	1.12	1.13	1.14	1.14	1.15	1.10	1.15	1.16
22:5w6 22:4w6	1.15	1.14	1.15	1.16	1.16	1.15	1.15	1.17	1.16	1.17
22:4w6 22:3w6	1.15	1.19	1.16	1.17	--	--	--	--	--	--
20:4w6 20:3w6	1.14	1.15	1.15	1.15	1.15	1.14	1.14	1.15	1.14	1.16
20:3w6 20:2w6	1.18	1.17	1.17	1.17	1.16	1.17	1.18	1.16	1.16	1.15
Type II SF										
22:6w3 22:5w6	1.33	1.33	1.32	1.32	1.34	1.31	1.34	1.28	1.32	1.32
22:5w3 22:4w6	1.36	1.33	1.36	1.36	1.36	1.33	1.34	1.36	1.33	1.33
20:5w3 20:4w6	1.34	1.32	1.34	1.34	--	--	--	1.24	--	1.34
18:3w3 18:2w6	1.37	1.35	1.35	--	1.36	1.37	1.35	1.37	1.38	1.36
18:3w3 18:1w9	1.73	1.71	1.71	1.72	1.74	1.72	1.7	1.73	1.74	1.71
18:2w6 18:1w9	1.26	1.27	1.27	1.26	1.26	1.27	1.27	1.26	1.27	1.26

TABLE XIII (continued)

Type of SF[2] and Source	Sample numbers[3] 1	2	3	4	5	6	7	8	9	10
Type III SF										
22:5w3 22:5w6	1.18	1.17	1.17	1.17	1.18	1.15	1.16	1.16	1.15	1.14
Other SF										
20:0 18:0	1.76	1.76	1.78	1.78	1.77	1.78	--	--	--	--
18:0 16:0	1.74	1.76	1.77	1.77	1.76	1.76	1.77	1.77	1.77	1.79
22:4w6 20:4w6	1.72	1.76	1.74	1.73	1.75	1.75	1.73	1.76	1.75	1.76
22:5w3 20:5w3	1.74	1.78	1.75	1.75	--	--	--	1.73	--	1.75
20:2w6 18:2w6	1.72	1.72	1.72	1.74	1.76	1.76	1.72	1.76	1.78	1.75

1/ Table VIII, page 57.

2/ SF stands for separation factors.

3/ Sample numbers are correspond to Table VIII, page 57.

TABLE XIV

THE PER CENT FATTY ACID COMPOSITION OF NEUTRAL LIPIDS IN THE SKIN OF BROILERS ON DIFFERENT DIETARY FATS

Fatty Acid	3% Beef Tallow		13% Beef Tallow		3% Peanut Oil		13% Peanut Oil	
	Male	Female	Male	Female	Male	Female	Male	Female
14:0	8.89	1.16	1.74	2.35	1.38	0.77	0.41	0.91
15:0	0.99	0.40	--	0.69	--	--	--	--
16:0	24.33	23.95	23.57	22.77	21.63	18.82	15.49	18.78
16:1w7	6.44	6.15	4.84	6.44	3.73	3.92	1.73	4.35
16:2w7	--	0.40	--	--	--	--	--	--
18:0	7.79	7.16	9.42	6.54	5.50	5.32	4.97	6.15
18:1w9	40.06	42.15	44.06	47.04	41.99	40.50	46.09	40.37
18:2w6	16.38	17.65	12.04	13.04	23.43	25.34	29.49	20.84
20:0	--	0.18	0.13	--	0.34	0.46	0.35	0.29
18:3w3	0.82	1.41	1.11	1.14	1.03	1.40	1.34	1.26
20:2w6	--	0.10	0.60	--	tr	0.11	--	tr
20:3w6	tr	0.29	--	--	tr	0.24	0.25	tr
20:4w6	tr	--	1.10	--	tr	0.26	0.16	0.43
Peak I[1]	--	--	2.01	--	1.24	2.86	--	2.16
Peak II[2]	--	--	0.95	--	1.07	--	--	4.70
24:4w6	--	--	--	--	--	--	--	0.16

1/ Peak I may contain 20:5w3, 22:2w6, 22:3w6, and 24:4w6.

2/ Peak II may contain 22:5w3, 22:6w3, and 24:3w6.

TABLE XV

THE PER CENT FATTY ACID COMPOSITION OF NEUTRAL LIPIDS IN THE DARK MEAT OF BROILERS ON DIFFERENT DIETARY FATS

Fatty Acids	3% Beef Tallow		13% Beef Tallow		3% Peanut Oil		13% Peanut Oil	
	Male	Female	Male	Female	Male	Female	Male	Female
14:0	0.59	1.41	1.61	0.58	0.75	0.47	0.56	
15:0	--	tr	0.18	0.63	0.40	--	--	
16:0	15.94	23.70	24.98	9.33	17.01	13.04	15.30	
16:1w7	2.02	3.65	5.49	1.50	3.05	2.14	1.98	
16:2w7	--	--	--	--	--	--	--	
18:0	4.91	8.19	7.07	2.19	4.92	3.75	4.46	
18:1w9	45.52	36.84	46.85	15.96	34.06	41.95	42.40	
18:2w6	49.23	8.04	9.52	8.87	19.78	24.69	24.21	
20:0	0.19	0.12	0.62	--	--	0.24	0.14	
18:3w3	1.09	0.84	0.74	0.24	1.15	0.97	0.75	
20:2w6	tr	0.30	--	--	tr	--	tr	
20:3w6	0.22	--	--	--	0.15	0.14	0.15	
20:4w6	0.24	0.32	0.63	1.04	1.12	0.54	0.54	
Peak I[1]	--	6.92	2.38	19.55	6.59	4.40	2.61	
Peak II[2]	--	9.71	--	29.40	8.09	7.04	5.39	
24:4w6	--	--	--	11.92	2.89	0.67	1.41	

1/ Peak I may contain 20:5w3, 22:2w6, 22:3w6, and 24:4w6.

2/ Peak II may contain 22:5w3, 22:6w3, and 24:3w6.

TABLE XVI

THE PER CENT FATTY ACID COMPOSITION OF NEUTRAL LIPIDS IN THE WHITE MEAT OF BROILERS ON DIFFERENT DIETARY FATS

Fatty Acid	3% Beef Tallow		13% Beef Tallow		3% Peanut Oil		13% Peanut Oil	
	Male	Female	Male	Female	Male	Female	Male	Female
14:0	1.48	1.73	2.23	1.57	0.97	0.98	0.34	0.34
15:0	tr	0.34	--	0.72	0.90	0.98	0.10	--
16:0	13.07	20.02	21.33	23.37	15.31	17.99	13.80	11.00
16:1w7	3.19	5.01	3.51	5.02	1.76	2.54	0.55	0.62
16:2w7	--	--	--	--	0.59	--	--	--
18:0	2.37	5.58	6.57	6.65	3.99	5.62	2.99	3.18
18:1w9	18.40	30.13	29.98	43.44	24.94	32.70	27.59	28.48
18:2w6	10.44	10.15	10.26	11.37	12.88	14.23	13.07	14.57
20:0	--	0.11	0.22	2.92	0.10	--	--	0.18
18:3w3	0.21	0.84	tr	1.14	0.26	1.21	0.87	0.55
20:2w6	tr	--	--	--	tr	--	--	--
20:3w6	tr	tr	0.24	--	--	--	--	tr
20:4w6	2.11	0.40	1.12	0.68	0.98	0.48	0.31	0.47
Peak I[1]/	19.39	14.67	11.05	0.88	12.27	8.91	6.71	9.14
Peak II[2]/	26.01	11.01	11.32	2.30	13.92	7.93	22.58	22.36
24:4w6	2.59	--	1.26	--	11.11	6.33	11.14	9.06

1/ Peak I may contain 20:5w3, 22:2w6, 22:3w6, and 24:4w6.

2/ Peak II may contain 22:5w3, 22:6w3, and 24:3w6.

Table XIV, page 69. The PO diet shows lower levels of 18:0, and higher levels of 18:2w6 and total w3 and w6 fatty acids. The 18:1w9 contents were about the same (40%) in every dietary treatment. The effect of fatty acid composition of dietary fats are reflected in the skin NL. Statistical analysis did not show any effects between the sexes.

Table XV, page 70, shows the fatty acid composition of dark meat NL of different dietary fat treatments. The effects of the dietary fats (on the dark meat NL) are about same as in skin NL.

Table XVI, page 71, shows the effect of the fatty acid composition of white meat NL on different dietary treatments. Compared to the BT diet, the content of 16:0 and 18:0 decreased, and the content of 18:2w6 increased. The total saturated fatty acid content was lowered and the total USFA and total w3 and w6 acids were increased by the PO diet.

The fatty acid composition of skin, dark meat, and white meat NL reflect the effects of dietary fatty acids. The higher levels of BT diet showed higher levels of 18:0 in the NL of different tissues, and the higher levels of PO diet showed higher levels of 18:2w6 in the NL fraction of different tissues. Total USFA and total w3 and w6 fatty acid content in the NL fractions of the three tissues was increased by the PO diet, while the total USFA were decreased by the PO diet. The AF contained about 45% saturated fatty

acids of which half were 18:0, but the NL fractions from the tissues of the broiler fed higher BT dietary treatment contained less than 10% of 18:0 acid. This may suggest a certain selectivity of fatty acids in the lipids of broilers.

The 22:2w6 fatty acids (about 2%) which were present in PO showed in the dark and white meat NL. The 24:3w6 and 24:4w6, which are presumed to be derived from 22:2w6, were occasionally observed in the meat lipids.

The Fatty Acid Composition of Phospholipids

The effect of different dietary treatments on the fatty acid composition of PL from skin, dark meat, and white meat are reported on Table XVII, XVIII and XIX, respectively. The fatty acid patterns in PL are quite different from those of the NL. The PL contained higher levels of 18:0, 20:4w6, 22:4w6, 22:5w6, 22:5w3, and 22:6w3 fatty acids than the NL, and showed more variety of fatty acids in sample GLC runs than the NL. The 18:1w9 were lowered significantly in the PL. The statistical analysis shows that dietary fatty acids influence the fatty acid composition of PL of skin and dark meat. The higher PUSFA reported in the PL could have originated from the lower acids such as 18:2w6 and 18:3w3.

The summary of the three groups of fatty acids, total saturated, total unsaturated, and w3 and w6 families of fatty acids from three tissues and different dietary treatments is reported in Table XX and XXI for NL and PL, respectively.

TABLE XVII

THE PER CENT FATTY ACID COMPOSITION OF PHOSPHOLIPID IN THE SKIN OF BROILERS ON DIFFERENT DIETARY FATS

Fatty Acid	3% Beef Tallow		13% Beef Tallow		3% Peanut Oil		13% Peanut Oil	
	Male	Female	Male	Female	Male	Female	Male	Female
14:0	1.00	0.46	0.26	0.75	0.34	0.43	1.53	0.32
15:0	1.92	2.30	1.31	--	1.64	2.94	1.65	2.73
16:0	25.18	22.11	15.27	22.95	26.67	24.04	20.21	18.71
16:1w7	4.03	2.02	0.52	3.69	1.60	1.16	0.68	0.63
16:2w7	2.08	3.86	2.01	--	2.48	4.12	2.00	4.78
18:0	15.89	14.86	8.07	12.93	15.01	15.23	12.95	14.62
18:1w9	26.91	24.24	12.20	36.06	21.46	20.43	20.45	18.61
18:2w6	10.53	9.96	5.36	11.30	9.33	10.54	8.71	9.33
20:0	3.00	0.48	0.32	0.33	0.33	0.60	4.78	2.01
18:3w3	1.12	0.68	0.52	0.79	0.71	0.79	0.64	1.19
20:2w6	0.75	0.32	--	--	1.08	0.36	0.44	1.70
20:3w6	0.51	0.86	0.32	0.29	1.41	1.35	1.31	0.95
20:4w6	5.28	11.72	8.18	7.54	9.75	12.53	9.05	10.30
20:5w3 1/	0.73	0.23	22.08	tr	4.83	--	2.70	8.37
22:4w6	0.81	1.85	2.21	1.57	2.45	2.86	4.72	5.31
22:5w6	1.50	0.92	--	0.27	--	0.70	3.34	--
22:5w3	0.25	1.44	9.24	0.29	--	0.50	0.54	1.89
22:6w3	0.38	1.63	11.98	1.17	--	0.95	0.58	--
24:4w6	0.86	--	--	--	--	--	3.78	--

1/ This may contain other acids.

TABLE XVIII

THE PER CENT FATTY ACID COMPOSITION OF PHOSPHOLIPID IN THE DARK MEAT OF BROILERS ON DIFFERENT DIETARY FATS

Fatty Acid	3% Beef Tallow		13% Beef Tallow		3% Peanut Oil		13% Peanut Oil	
	Male	Female	Male	Female	Male	Female	Male	Female
14:0	0.67	0.90	0.16	0.36	tr	tr	2.52	2.25
15:0	4.67	4.47	3.70	--	5.50	6.10	4.39	2.49
16:0	24.04	22.01	16.83	19.49	18.60	18.98	16.22	20.98
16:1w7	0.90	0.84	0.42	0.85	0.54	0.82	0.24	0.57
16:2w7	1.43	1.41	1.40	0.51	1.37	1.91	1.53	1.02
18:0	22.94	21.05	16.73	20.08	17.47	14.79	18.18	20.60
18:1w9	23.55	19.00	18.68	22.68	18.10	17.27	15.48	17.90
18:2w6	12.98	15.20	13.00	12.08	15.65	14.73	14.72	16.47
20:0	--	--	tr	--	tr	0.27	2.01	--
18:3w3	0.52	0.32	0.37	0.41	0.29	0.21	0.53	0.42
20:2w6	0.28	0.18	0.12	0.29	0.31	0.36	0.62	0.52
20:3w6	0.52	0.72	0.38	0.71	0.41	0.69	0.60	0.43
20:4w6	4.19	7.82	6.92	11.11	10.29	9.83	10.12	9.31
20:5w3 1/	tr	0.18	9.30	0.21	1.21	1.26	1.25	--
22:4w6	1.58	2.11	1.18	3.51	4.12	4.09	1.92	3.63
22:5w6	0.32	0.59	0.49	2.60	1.68	1.70	4.13	1.94
22:5w3	0.64	0.93	6.33	1.95	1.32	2.03	1.86	0.64
22:6w3	0.87	1.60	--	2.76	3.09	3.40	1.51	1.32
24:4w6	--	--	3.43	--	--	--	2.22	--

1/ This may contain other acids.

TABLE XIX

THE PER CENT FATTY ACID COMPOSITION OF PHOSPHOLIPID IN THE WHITE MEAT OF BROILERS ON DIFFERENT DIETARY FATS

Fatty Acid	3% Beef Tallow		13% Beef Tallow		3% Peanut Oil		13% Peanut Oil	
	Male	Female	Male	Female	Male	Female	Male	Female
14:0	0.74	0.25	0.37	0.31	0.24	3.10	6.42	0.10
15:0	5.35	5.87	3.12	--	5.22	5.42	2.52	8.39
16:0	23.28	22.92	29.37	22.64	23.19	21.99	20.98	22.53
16:1w7	0.76	0.71	1.01	1.49	0.82	0.84	0.70	0.34
16:2w7	1.83	1.87	1.19	1.35	1.38	0.98	0.63	1.99
18:0	10.54	10.52	12.57	10.25	10.35	9.67	10.51	10.59
18:1w9	22.95	22.46	24.69	27.27	21.70	20.35	18.44	17.92
18:2w6	16.42	13.43	8.44	13.08	14.12	13.34	11.46	12.21
20:0	--	0.12	0.46	--	--	--	0.96	--
18:3w6	0.44	0.44	0.57	0.37	0.26	0.28	0.34	0.45
20:2w6	0.47	0.44	0.53	0.21	0.52	0.68	0.59	0.40
20:3w6	1.26	1.19	tr	1.13	0.91	0.97	0.69	0.60
20:4w6	9.03	8.89	2.55	11.98	11.63	11.06	12.24	10.17
20:5w6 1/	0.55	0.68	11.73	0.10	0.69	2.79	2.12	5.99
22:4w6	2.04	2.50	--	2.79	3.45	2.94	3.99	3.35
22:5w6	0.78	1.79	--	2.11	1.70	1.21	1.88	1.72
22:5w3	1.51	2.25	--	1.71	1.38	1.40	0.55	0.56
22:6w3	2.09	3.09	--	2.83	2.39	3.12	2.65	2.67
24:4w6	--	--	2.52	--	--	--	--	--

1/ This may contain other acids.

TABLE XX

TOTAL SATURATED, UNSATURATED, AND w3 AND w6 FATTY ACIDS OF NEUTRAL LIPIDS ON DIFFERENT DIETARY FATS

Source and Fatty Acid	3% Beef Tallow		13% Beef Tallow		3% Peanut Oil		13% Peanut Oil	
	Male	Female	Male	Female	Male	Female	Male	Female
Skin								
T.Satu[1]	34.00	31.85	34.76	31.85	28.85	25.37	21.20	26.13
T. Unsatu[2]	66.00	68.15	65.24	68.15	71.15	74.63	78.70	73.87
T. w3 & w6[3]	19.50	19.45	17.34	19.25	22.42	30.24	30.87	31.15
Dark Meat								
T. Satu	--	21.63	33.42	34.46	12.73	23.08	17.50	20.46
T. Unsatu	--	78.27	66.58	65.54	87.27	76.92	82.50	79.54
T. w3 & w6	--	30.80	26.09	23.20	69.81	39.81	38.41	35.16
White Meat								
T. Satu	16.92	27.78	30.45	35.23	21.27	25.57	17.23	14.60
T. Unsatu	83.08	72.22	69.55	64.77	78.73	74.43	82.77	85.40
T. w3 & w6	61.49	37.08	36.06	16.31	51.44	39.28	54.63	56.30

1/ T. Satu means total saturated fatty acids.

2/ T. Unsatu means total unsaturated fatty acids.

3/ T. w3 and w6 means total of w3 and w6 unsaturated fatty acids.

TABLE XXI

TOTAL SATURATED, UNSATURATED, AND w3 AND w6 FATTY ACIDS OF PHOSPHOLIPIDS ON DIFFERENT DIETARY FATS

Source and Fatty Acids	3% Beef Tallow		13% Beef Tallow		3% Peanut Oil		13% Peanut Oil	
	Male	Female	Male	Female	Male	Female	Male	Female
Skin								
T. Satu[1]	46.99	40.21	25.23	36.96	43.99	43.24	41.12	38.39
T. Unsatu[2]	53.01	59.79	74.77	63.04	56.01	56.76	58.88	61.61
T. w3 & w6[3]	19.99	29.67	60.04	23.29	30.47	31.05	35.75	37.59
Dark Meat								
T. Satu	52.32	48.43	37.42	39.93	41.57	40.14	43.32	46.32
T. Unsatu	47.68	51.57	62.58	60.07	58.43	59.86	56.68	53.63
T. w3 & w6	21.80	30.32	42.08	36.03	38.42	39.86	39.43	34.19
White Meat								
T. Satu	39.91	39.68	45.89	33.20	39.00	40.18	41.39	41.61
T. Unsatu	60.09	60.32	54.11	66.80	61.00	59.82	58.61	58.39
T. w3 & w6	34.55	35.28	27.17	36.69	37.10	37.66	38.84	38.14

1/ T. Satu means total saturated fatty acids.

2/ T. Unsatu means total unsaturated fatty acids.

3/ T. w3 and w6 means total of w3 and w6 unsaturated fatty acids.

Analysis of Variance

Analysis of variance (ANOVAR) was carried out on total lipid content and PL content for each sample source (skin, dark meat, and white meat). The results of ANOVAR are reported on Table XXII and XXIII, in Appendix B, for the total lipid content, and for the NL content in the different tissues. ANOVAR was done only on the NL content, because the NL and PL together equaled 100%. The results of the ANOVAR on the total lipid and NL content showed that diet, sex, and the combination of diet and sex showed a significant difference in every tissue analyzed. This means the higher levels of dietary fats resulted in the deposition of more total lipid and a greater proportion of NL fraction than lower levels of dietary fat treatments. The females showed higher levels of total lipid than the males.

Due to the complexity of fatty acid composition in the NL and PL, the effect of dietary fat, on individual fatty acid compositions were not analyzed statistically. The individual fatty acids were grouped into the total saturated (14:0, 15:0, 16:0, 18:0, and 20:0), total unsaturated acid (100%-total saturated acid), and total w3 and w6 families of fatty acids for the convenience of ANOVAR.

ANOVAR was then carried out for the per cent fatty acid composition on each of the above three groups. BT and PO diets showed three major differences in the fatty acid composition: BT showed a higher level of total saturated

fatty acid and a lower level of total USFA. PO contained higher levels of 18:2w6 and w6 family fatty acids. These facts led to the grouping of individual fatty acid compositions into the three groups for the ANOVAR. Since the higher n:2w6 family of fatty acids could be synthesized from 18:2w6, the grouping of w6 family fatty acids was justified. The same reason could be applied for the w3 fatty acid family.

All the ANOVAR were carried out on a 4 x 2 x 2 (or 4) factorial design, diet x sex x replications, of two way classification. The results of ANOVAR are reported in Appendix B.

Table XXIV and XXV, in Appendix B, show the results of ANOVAR on the three groups of fatty acids for skin NL, and white meat NL, respectively. This shows the dietary fat affected the fatty acid composition of NL.

The ANOVAR was carried out for the fatty acid composition of PL of every treatment, after the individual fatty acids were grouped into total saturated, total USFA, and total w3 and w6 fatty acids. The results of the ANOVAR are reported in Table XXVI, XXVII, and XXVIII, in Appendix B, for the fatty acid composition of the PL of the skin, dark meat, and white meat respectively of the different treatments.

The ANOVAR shows that the total saturated, total USFA,

and total w3 and w6 fatty acid in the skin and dark meat PL are influenced by the dietary treatment.

CHAPTER V

DISCUSSION

Identification of GLC Peaks of Methyl Esters of Fatty Acids

The identification of individual fatty acids was done only in a 12% DEGS stationary phase, and no other secondary methods, such as a hydrogenation, or an ozonolysis, was used to identify the acids further. A non-polar stationary phase such as apiezon L was not used to identify the individual fatty acids further because the long chain PUSFA frequently overlapped each other, and this made it difficult to identify those acids in the non-polar stationary phase.

The ECL or MECL (n:2w6) contained two principal components: one contributed by London force due mainly to the hydrocarbon moiety, and another caused by polar attraction of double bonds and ester groups to the liquid stationary phase. In the polar phase, these effects are additive. Another possible effect is shape of the molecule when it dissolves in the polar phase. A saturated acid would be expected to have a globular shape because the surrounding polar phase will reject the non-polar hydrocarbon moiety and force them to interact to produce a globular shape. This shape may play an important role in the determination of retention times because a globular shape may make it

easier for the molecule to get in and out of the polar stationary phase. Ackman (1) showed that the end carbon chain (w value) plays an important role in the determination of the boiling points of fatty acids. Furthermore it is generally believed that of two fatty acids having the same carbon chain and the same number of double bonds, the one with the smallest w value will have the longest retention time. Ackman (1) also showed when the logrithms of retention time of each family of fatty acids, the USFA gave straight, parallel lines. The ratios of the logarithms of the retention time among the USFA lines were constant enough to get a separation factor. It was found that the separation factors were constant within a certain limit. More accurate results could be obtained when the separation factors obtained from a sample GLC run were used only for that GLC run. The retention time of the individual fatty acids showed some deviation when the sample contained many fatty acids or a relatively large amount of a certain fatty acid compared to the retention times obtained with a few standard fatty acids. This could be due to a difference in partitioning between a dilute and concentrated sample on the stationary phase. Due to the above errors, the RRT reported by Ackman (3) using cod liver oil would be more practical.

The ECL values published by Hoffstteter and Holman (17) had two inherent errors. They express the retention times by measuring the peak top rather than the frontal tangent. The former could be influenced by the relative amount of a sample, while the latter will not change. Ackman (1) reported that the linear logarithm plots of saturated and USFA were not parallel to each other. This suggested that the expression of the retention values of PUSFA in terms of saturated acids were not on a sound basis. The slops of saturated and unsaturated acid lines in the linear logarithmic plot were not constant, rather they varied with experimental conditions. So the expression of retention times of PUSFA in terms of saturated acids on the linear logarithmic plots is not justifiable in theory. Since the retention times of USFA are influenced by the chain length, number of double bonds, and the end carbon chain of the acids, it would be a logical choice to use an USFA line of one fatty acid family which satisfies the above three factors.

The MECL system (1) which uses the line representing n:1w9 family fatty acids could not be used for the expression of the retention times of other USFA because it was found that the MECL system using the retention time of 20:1w9 and 22:1w9 had points which overlapped with other acids in the polar stationary phase. The line representing the n:2w6 family was chosen for the expression of other

USFA, because the points required to make the n:2w6 line, namely 18:2w6 and 20:2w6, are relatively free from overlapping by other acids. Using the line of n:2w6, the MECL (n:2w6) system was found to be very satisfactory especially on higher PUSFA. Occasionally some of the retention times of fatty acids in a single GLC run did not fit on a linear logarithmic plot very well. This could be due to the changing column temperature. The column temperature was raised after each run to 220°C to flush out any possible high boiling compounds, and returned to 185°C. The thermometer which is supposed to read the column temperature apparently did not function properly, because the retention time was changing all the time even with the same sample. The other possible source of error was oxidation, especially in the PUSFA. Marion (24) has reported no change of fatty acid composition even though the USFA underwent autoxidation; however, El-Gharbawi and Dugan (13) showed that PUSFA composition could be changed by autoxidation of PUSFA. The present experiments were based on Marion's (24) previous article, and rigid control of autoxidation was not carried out, even though all the samples were flushed with N_2 gas and stored under -20°C. During the extraction of lipids and consequent fractionation, the smaples were exposed to air and could have undergone free radical autoxidation.

This would be expected to be more pronounced in PL. PUSFA undergo autoxidation more easily than the less USFA, and the PL seem to have a faster rate. This may be due to the fact that the PL contain more PUSFA, and also to structural difference in the triglycerides. No evidence of this assumption has been presented in this paper.

The overlapped peak I and II observed in the Type II sample GLC runs could be some altered USFA or some other compounds. No further attempt has been made to identify any unusual compounds in the overlapped peak I and peak II.

Biogenesis of Polyunsaturated Fatty Acids

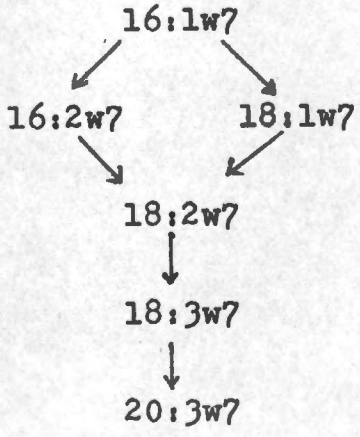
The complexity of the description of the fatty acid composition of broiler lipids, especially PL, can be simplified if the USFA are grouped according to the end carbon chain length (w value), counting from the last double bond. The normal USFA of animal origin can be classified into four families by their w values:

palmitoleic.....w7
oleic.....w9
linoleic.....w6
linolenic.....w3

The biogenesis of higher PUSFA can be achieved by an elongation of chain length and the addition of double bonds in each family of fatty acids. The interconversion of each family of USFA does not take place in animals. Palmitoleic (w7) and oleic (w9) families of fatty acids can be synthesized in animal from acetate but the linoleic

(w3) families of fatty acids are not synthesized in animals. Because of the inability of the body to synthesize w6 and w3 families of PUSFA, these acids are usually termed the essential fatty acids. The details of biogenesis of PUSFA in rats was reported by Mead (31) using tracer techniques. He reported that methylene interrupted PUSFA can be synthesized from palmitoleic, oleic, linoleic and linolenic acids. This study showed that double bonds were added from the carboxyl end rather than methyl end of the synthesized acid. As a consequence, the carboxyl end, not the methyl end, changes. The classification of fatty acids by the end carbon chain length (w value) has sound basis in the understanding of the metabolic biogenesis of PUSFA. The classification of USFA according to their w values, for the MECL system and the linear logarithmic plot by Ackman is an interesting coincidence when compared with the biogenesis of PUSFA classified according to their w values.

Palmitaleic acid can produce the following PUSFA:



Oleic acid can lead to the following PUSFA:

18:1w9
|
18:2w9
|
20:3w9

20:3w9 acid was observed in the essential fatty acid deficient animals.

Linoleic acid can produce the following PUSFA:

18:2w6
↓
18:3w6
↓
20:3w6
↓
20:4w6
↓
22:4w6
↓
22:5w6

Linolenic acid can lead to the following PUSFA:

18:3w3
↓
18:4w3
↓
20:4w3
↓
20:5w3

22:5w3
↓
22:6w3

The Fatty Acid Composition of Neutral Lipids

16:0 and 18:0 are the major saturated fatty acids in the NL of broilers. ANOVAR showed that the percentages of total saturated fatty acids were significantly lower in the PO diet than in BT diet. The percentages of 16:0 and 18:0 were higher in the BT diet than in the PO diet. This is a reflection of the dietary fat treatments.

The BT diet contained higher levels of 16:1w7 than did the PO diet, and this is reflected in the 16:1w7 and 16:2w7 content in NL.

Neutral lipids of broilers show about 40% of 18:1w9, which strongly reflects the content of 18:1w9 in the dietary treatment. Some of the NL samples contained high levels of overlapped peak I and peak II in sample GLC runs, and these overlapped peaks reduced the content of 18:1w9. The biogenesis products of 18:1w9, 20:3w9, is occasionally observed in trace amounts. This is a confirmation of the findings of Machlin and Gordon (23), who observed the disappearance of 20:3w9 when the w6 family of fatty acids were added to the diet of broilers. 20:3w9 was observed in the fatty acid composition of animals fed a diet deficient in the essential fatty acids.

The contents of the total w6 family of fatty acids was pooled together with the total w3 family of fatty acids for the ANOVAR. The result of the ANOVAR for the total w3 and w6 families of fatty acids shows that there is a significant difference between the BT and PO dietary treatments. The PO diet contained about 30% 18:2w6, while the PO diet contained only about 5% of 18:2w6. The content of 18:2w6 is largely reflected in the NL fraction of broilers on PO dietary treatments. Because of the difference in the content of 18:2w6 and constant amount of 18:3w3 in the BT and PO dietary fats, the results of the ANOVAR for the total w3 and w6 families can be interpreted as being due to the w6 family of USFA. The biogenesis products of PUSFA from 18:2w6 can not be clearly defined in NL due to the frequent formation of the overlapped peak I and peak II of sample GLC runs. Peak I and II, however, contain the following w6 PUSFA: 22:2w6, 22:3w6, 22:4w6, 22:5w6, 24:3w6, and 24:4w6. The 24 carbon chain fatty acids were reported by Marion and co-workers (25, 26, 28, 29, 30) and Katz et al. (21). They, however, did not report w values for these acids. The w values for these 24 carbon series of fatty acids are provisionally identified as w6 values, and found to be preferentially deposited in the neutral lipids, with a few exceptions deposited in PL. Miller and co-workers (32, 33) did not

report any findings of 24 carbon series of USFA.

The overlapped peak I and II of sample GLC runs was observed to be larger in the white meat neutral lipid fraction that in the skin neutral lipid fraction. It is clearly shown that 18:2w6 acid is preferentially deposited in the neutral lipid fraction of broilers even with BT diets which contained lower levels of 18:2w6.

The content of 18:3w3 is about the same in the BT and PO diets (approximately 1.5%), and about the same level of 18:3w3 is deposited in the NL fraction regardless of the dietary treatment. The biogenesis products of 18:3w3 are observed as 20:5w3 in overlapped peak I, and 22:5w3 and 22:6w3 in overlapped peak II in sample GLC runs. The higher levels of the above PUSFA are observed in the white meat NL fraction. As stated before, the overlapped peak I and II in sample GLC runs might contain some other unknown impurities. This assumption is based on the formation of severely skewed peaks in overlapped peak I and II in Type II samples.

Fatty Acid Composition of Phospholipids

The results of ANOVAR show that the total saturated fatty acid content of PL in the skin and dark meat, but not in the white meat, is significantly influenced by the dietary treatments of broilers. The PL fraction contains a considerably higher amount of 18:0 saturated

acid than is found in the NL fraction. The levels of 16:0 are generally slightly higher in the PL fraction than in the NL fraction. The higher content of 18:0 is perhaps due to the requirement of this saturated acid in the PL in alpha positions.

The content of the w7 family of USFA is considerably increased in PL fraction. Almost no 16:2w7 was observed in the NL fraction while the PL fraction contained about 1.5% of this acid. 16:2w7 is preferentially deposited in the PL fraction, and this fact is a very interesting point concerning the w7 family acid metabolism.

The content of 18:1w9 in the PL fraction is decreased compared to the NL fraction. The lower levels of 18:1w9 may be due to the selectiveness of PL to form ester linkages with fatty acids.

All the biogenesis intermediate PUSFA of the w6 family of fatty acids were observed in the PL fraction. They are 18:2w6, 20:2w6, 20:3w6, 20:4w6, 22:4w6, and 22:5w6. Most of the total w3 and w6 reported in Table XXI is of the w6 family of PUSFA. The requirement for the w6 PUsfa (essential fatty acids) was reflected by the large amount of w6 family of PUSFA in the PL fraction. Machlin and Gordon (23) suggested the w6 value of fatty acids is the part which plays an essential role. The PL

fraction contained more than 10% 18:2w6, and less than 10% 20:4w6. The increment of 20:4w6 in the PL fraction was significantly higher compared with the NL fraction.

The content of 18:3w3 is generally less than 1% in the PL fraction, and 22:5w3 and 22:6w3 are observed in almost constant amounts as end products of the w3 family acid biogenesis. As intermediate compounds of w3 PUSFA biogenesis, 18:4w3 was occasionally seen in trace amounts, and 20:5w3 usually existed in small quantities. There are a few samples, however, which show higher levels of 20:5w3. These samples show a similarity to the Type II samples observed in the NL fraction.

Marion and co-workers (25-30) have published many papers concerning the metabolism of fatty acids in broilers; however, they never stated the w values for the individual fatty acids. Since the biogenesis of PUSFA followes the w value of USFA, all the USFA should be classified according to their w values. Miller and co-workers (32, 33) reported w values for the w3 type of acids. They used the purified w3 series of fatty acids in menhaden oil for the study of flavor deterioration of broiler muscles 20:5w3, 22:5w3, and 22:6w3 were reported by Miller and his group (32, 33), but the

22:4w6 and 22:5w6 found in this study was not reported by them. There is some possibility that some of the fatty acids reported by them could be 22:4w6 and 22:5w6. Since they did not report any retention times for the individual fatty acids, the validity of the above assumption can not be checked by the MECL (n:2w6) system.

The Effects of Dietary Fats on Total Lipid, and the Content of Neutral and Phospholipids

The results of ANOVAR for the contents of total lipids, neutral and PL in different tissues show that dietary treatments, sex, and the combination of sex and diet are significantly different within the 1% probability level for different tissues. This observation is in disagreement with the observation of Reiser (42,43) and Marion (28). The content of NL in skeletal muscle lipids is increased by the higher levels of the dietary fat treatment which makes it appear that the PL content has decreased compared with the NL content. Since PL are considered a structural component of the cell, they should be standaridized against the total nitrogen content of a sample rather than the total lipid content.

LITERATURE CITED

LITERATURE CITED

1. Ackman, R.G. 1963. Structural correlation of unsaturated fatty acid esters through graphical comparison of Gas-liquid Chromatography retention times on a poyester substrates. J. Am. Oil Chem. Soc. 40, 558-564.

2. Ackman R.G. 1963. An analysis of Separation factors applicable in the Gas-Liquid Chromatography of unsaturated fatty acid methyl esters on a polyester substrates. J. Am. Oil Chem. Soc. 40, 564-567.

3. Ackman, R.G., and R.D. Burgher. 1965. Cod liver oil fatty acid as secondary reference standard in the Gas-Liquid Chromatography of polyunsaturated fatty acids of animal origin: Analysis of dermal oil of the Atlantic leather back turtle. J. Am. Oil Chem. Soc. 42, 38-42.

4. Acosta, S.O., W.W. Marion, and R.H. Forsythe. 1966. Total lipids and phospholipids in turkey tissues. Poultry Sci. 45, 169-184

5. Barakat, W.A.R. 1967. Evaluation of fish meals and their effect on fatty acid deposition in broilers fat. M.S. Thesis. The University of Tennessee, Knoxville, Tenn.

6. Chang, I., C.L., and B.M. Watts. 1952. The fatty acid content of meat and poultry before and after cooking. J. Am. Oil Chem. Soc. 29, 334-338.

7. Carroll, K.K. 1965. Dietary fat and the fatty acid composition of tissue lipids. J. Am. Oil Chem. Soc. 42, 516-528.

8. Chung, R.A., J.C. Rogler, and W.J. Stadelman. 1965. The effects of dietary cholesterol and different dietary fats on cholesterol content and lipid composition of egg yolk and various body tissues. Poultry Sci. 44, 221-228.

9. Chung, R.A., E.Y. Davis, R.A. Munday, Y.C. Tsao, and A. Moore. 1966. Effect of sholesterol with different dietary fats on the fatty acid composition of egg yolk and various body tissues. Poultry Sci. 45, 133-139.

10. Cruickshank, E.M. 1934. Studies in fat metabolism in the fowl. 1. The composition of the egg fat and depot fat of the fowl as affected by ingestion of large amounts of different fats. Biochem. J. 28, 967-977.

11. Dansky, L.M., and F.W. Hill. 1952. The influence of

dietary energy level on the distribution of fat in various tissues of the chicken. Poultry Sci. 31, 912.

12. Edwards, H.M., Jr., and J.E. Marion. 1963. Influence of dietary menhaden oil on growth rate and tissue fatty acids of the chick. J. Nutrition 81, 123-130.

13. El-Gharbawi, M. I., and L.R. Dugan. 1965. Stability of nitrogenous compounds and lipids of freeze-dried raw beef. J. Food Sci. 30, 817-822.

14. Essary, E.D., and L.E. Dawson. 1965. Quality of fryer carcasses as related to protein and fat levels in diet. I. Fat deposition and moisture pick-up during chilling. Poultry Sci. 44, 7-23.

15. Farquhar, J.W., W. Insull, Jr., P. Rosen, W. Stoffel, and E.H. Athens. 1959. The analysis of fatty acid Mixtures by Gas Liquid Chromatography. Nutrition Rev. (Suppl.) 17, 16-17.

16. Hornstein, I., P.E. Crowe, and M. J. Heimberg. 1961. Fatty acid composition of meat tissue lipids. J. Food Sci. 26, 581-586.

17. Hofstetter, H.H., N. Sen, and R.T. Holman. 1965. Characterization of unsaturated fatty acids by Gas-Liquid Chromatography. J. Am. Oil Chem. Sci. 42, 537-540.

18. Isaacks, R.E., R.E. Davis, T.M. Ferguson, R. Reiser, and J.R. Couch. 1964. Studies on avian fat composition. I. Effect of dietary fat on the fatty acid of triglyceride and phospholipid fraction of the blood plasma and adipose tissue lipids of laying hens. Poultry Sci. 43, 105-113.

19. James, A.T. and Martin, A.P. 1956. Gas-Liquid Chromatography. The separation and identification of the methyl esters of saturated and unsaturated acids from formic to n-octadecanoic acid. Biochem. J. 63, 144-152.

20. James, A.T., and A.P. Martin. 1956. Gas-Liquid Chromatography. The separation and identification of the methyl esters of saturated and unsaturated acids from formic to n-octadecanoic acid. Biochem. J. 63, 144-152.

21. Katz, M.A., L.R.Dugan, Jr., and L.E. Dawson. 1966.

Fatty acids in neutral lipids and phospholipids from chicken tissues. J. Food Sci. 31, 717-720.

22. Lea, C. H., and P. T. A. Swaboda, 1958. The flavor of aliphadic aldehydes. Chem. Ind. 40, 1289-1290.

23. Machlin, L. J., and R. S. Gordon. 1961. Effect of dietary fatty acids and cholesterol on growth and fatty acid composition of chicken. J. Nutrition 75, 157-164.

24. Machlin, L. J., R. S. Gordon, L. Marr, and C. W. Pope. 1962. Effect of dietary fat on the fatty acid composition and tissues of hen. Poultry Sci. 41, 1340-1343.

25. Marion, J. E., and J. G. Woodroof. 1963. (a) The influence of dietary fat on the lipid composition of broiler tissues and the development of rancidity during storage. Poultry Sci. 42, 1287.

26. Marion, J. E., and J. G. Woodroof. 1963. (b) The fatty acid composition of breat, thigh, and skin tissues of chicken broilers as influenced by dietary fats. Poultry Sci. 42, 1202-1207.

27. Marion, W. W., and R. H. Forsythe. 1964. Autoxidation of turkey lipids. J. Food Sci. 29, 530-534.

28. Marion, J. E. 1965. Effect of age and dietary fat on the lipids of chicken muscle. J. Nutrition 85, 38-44.

29. Marion, J. E., and J. G. Woodroof. 1966. Composition and stability of broiler carcasses as affected by dietary protein and fat. Poultry Sci. 45, 241-247.

30. Marion, J. E., T. S. Boggess, Jr., and J. G. Woodroof. 1967. Effect of dietary fat and protein on lipid composition and oxidation in chicken muscle. J. Food Sci. 32, 426-429.

31. Mead, J. F. 1961. Synthesis and metabolism of polyunsaturated acid. Federation Proc. 20, 952-955.

32. Miller, D., E. H. Gruger, K. Leong, and G. M. Knobl, Jr. 1967. Dietary effect of menhaden-oil ethyl esters on the fatty acid pattern of broiler muscle

lipids. Poultry Sci. 46, 438-445.

33. Miller, D., E. H. Gruger, Jr., K. C. Leong, and G. M. Knoble, Jr. 1967. Effect of refined menhaden oils on the flavor and fatty acid composition of broiler flesh. J. Food Sci. 32, 342-345.

34. Mohrhauer, H., and R. T. Holman. 1963. The effect of dose level of essential fatty acid upon fatty acid composition of the rat liver. J. Lipid Res. 4, 151-159.

35. Miwa, T. K., K. Sl. Mikolajczak, F. R. Earle, and I. A. Wolff. 1960. Gas chromatographic characterization of fatty acids: Identification constants for mono- and Dicarboxylic methyl esters. Anal. Chem. 32, 1739-1742.

36. Miwa, T. K. 1963. Identification of peaks in Gas-Liquid Chromatography. J. Am. Oil Chem. Soc. 40, 309-313.

37. Murty, N. L., M. C. Williams, and R. Reiser. 1960. Non-synthesis of linoleic acid from acetate 1-C^{14} by the laying hen. J. Nutrition 72, 451-454.

38. Ostrander, J., and L. R. Dugan. 1961. A rapid quantitative lipid extraction method. American Meat Institute Foundation Bull. 50. Chicago, Ill.

39. Peng, D. Y., and L. R. Dugan, Jr. 1965. Composition and structure of phospholipids in chicken muscle tissues. J. Am. Oil Chem. Soc. 42, 536-553.

40. Pippen, E. L. 1967. Poultry flavor. In Schultz, H. W., E. A. Day, and L. M. Libbey, ed. Symposium on Food: The Chemistry and Physiology of Flavors. pp. 251-266. The AVI Publishing Co., Inc., Westport, Conn.

41. Pudelkewicz, C., J. Seufert, and R. T. Holman. 1968. Requirement of the female rat for linoleic and linolenic acid. J. Nutrition 94, 138-146.

42. Reiser, R. T. 1950. The metabolism of polyunsaturated fatty acids in growing chicks. J. Nutrition 42, 325-336.

43. Reiser, R. T. 1951. The synthesis and interconversion of polyunsaturated fatty acids by the laying hen. J.

Nutrition 44, 159-176.

44. Schultz, H. W., E. A. Day, and R. O. Sinnhuber, ed. 1962. Symposium on Food: Lipids and Their Oxidation. The AVI Publishing Co., Inc., Westport, Conn.

45. Shah, B. M. 1965. Growth and quality of broilers from peanut oil-peanut meal diets. M. S. Thesis. The University of Tennessee, Knoxville, Tenn.

46. Summers, J. D., S. J. Slinger, and G. C. Ashton. 1965. The effect of dietary energy and protein on carcass composition with a note on a method for estimating body composition. Poultry Sci. 44, 501-512.

47. Taylor, L. E. 1964. Composition of two methods in the study of muscle lipids from grain- and grass-finished beef. M. S. Thesis. The University of Tennessee, Knoxville, Tenn.

48. Woodford, F. P., and C. M. van Gent. 1960. Gas-Liquid Chromatography of fatty acid methyl esters: the "carbon-number" as a parameter for comparison of columns. J. Lipid Res. 1, 188-190.

49. Younathan, M. T., and B. M. Watts. 1960. Oxidation of tissue lipids in cooked pork. Food Res. 25, 538-542.

APPENDIXES

APPENDIX A

1. The first number indicates the chain length of carbons; the second indicates the number of double bonds. The number following w (omega) indicates the position of the first double bond, counting from the terminal methyl group according to the system used by Mohrhauer and Holman (34). This system aids in identifying the family relationship of the fatty acids and their different isomers. In this system double bonds were assumed to be cis form unless otherwise noted. If one double bond was trans form the gemetry of all double bonds were indicated. Thus trans-9-cis-12-octadecadienoic acid became 18:2w6c, 9t.

n:1w7	Palmitoleic family
n:1w9	Oleic Acid family
n:2w6	Linoleic Acid family
n:3w3	Linolenic Acid family
w3	Three end carbon chain length
w6	Six end carbon chain length
w7	Seven end carbon chain length
w9	Nine end carbonchain length

2. Relative retention time or relative retention volume was calculated using the following formular:

$$\text{relative retention time} = \frac{\text{retention time of an unknown}}{\text{retention time of a standard}}$$

To determine the above values of a series of compounds, the internal standard should be chosen approximately midway in the series in elution time or volume.

APPENDIX B

TABLE XXII

ANALYSIS OF VARIANCE OF TOTAL LIPIDS CONTENT IN VARIOUS TISSUES OF BROILERS

Source	Skin		Dark Meat		White Meat	
	DF	MS	DF	MS	DF	MS
Diet	3	20.00**	3	1.404**	3	10.16**
Sex	1	593.14**	1	0.045**	1	10.24**
Diet X Sex	3	26.28**	3	0.671**	3	4.57**
Error	24	0.43	24	0.002	24	0.16

** Statistical significance at 0.01 level of probability

TABLE XXIII

ANALYSIS OF VARIANCE OF NEUTRAL LIPIDS CONTENT IN VARIOUS TISSUES OF BROILERS

Source	Skin		Dark Meat		White Meat	
	DF	MS	DF	MS	DF	MS
Diet	3	8.94**	3	57.21**	3	124.43**
Sex	1	6.06**	1	10.42**	1	5.54**
Sex & Diet	3	2.27**	3	42.06**	3	192.92**
Error	24	0.014	24	0.46	24	0.68

** Statistical significance at 0.01 level of probability

TABLE XXIV

ANALYSIS OF VARIANCE OF FATTY ACID COMPOSITION OF SKIN NEUTRAL LIPIDS OF BROILERS

Source	Total Satu		Total Unsat		Total w3, w6	
	DF	MS	DF	MS	DF	MS
Diet	3	97.41**	3	97.41**	3	203.50**
Sex	1	0.01	1	0.00	1	0.62
Diet X Sex	3	11.56	3	11.56	3	5.81
Error	7	10.24	7	10.24	7	9.85

** Statistical significance at 0.01 level of probability

1/ Total Satu means total saturated fatty acids.

2/ Total Unsatu means total unsaturated fatty acids.

3/ Total w3, w6 means total w3 and w6 families of unsaturated fatty acids.

TABLE XXV

ANALYSIS OF VARIANCE OF FATTY ACID COMPOSITION OF WHITE MEAT NEUTRAL LIPIDS OF BROILERS

Source	Total Satu [1]		Total Unsatu [2]		Total w3, w6 [3]	
	DF	MS	DF	MS	DF	MS
Diet	3	165.05**	3	165.67**	3	591.84**
Sex	1	92.87**	1	97.97**	1	845.67**
Diet X Sex	3	24.12*	3	23.97*	3	98.38**
Error	7	5.02	7	5.03	7	6.17

** Statistical significance at 0.01 level of probability

* Statistical significance at 0.05 level of probability

1/ Total Satu means total saturated fatty acids

2/ Total Unsatu means total unsaturated fatty acids.

3/ Total w3, w6 means total w3, and w6 families of unsaturated fatty acids.

TABLE XXVI

ANALYSIS OF VARIANCE OF FATTY ACID COMPOSITION OF SKIN PHOSPHOLIPIDS OF BROILERS

Source	Total Satu 1/		Total Unsatu 2/		Total w3, w6 3/	
	DF	MS	DF	MS	DF	MS
Diet	3	80.54*	3	80.54*	3	134.00**
Sex	1	0.21	1	0.21	1	37.01
Sex & Diet	3	29.55	3	29.56	3	225.08**
Error	5	6.95	5	6.95	5	6.70

** Statistical significance at 0.01 level of probability

* Statistical significance at 0.05 level of probability

1/ Total Satu means total saturated fatty acids.

2/ Total Unsatu means total unsaturated fatty acids.

3/ Total w3, w6 means total w3 and w6 families of unsaturated fatty acids.

TABLE XXVII

ANALYSIS OF VARIANCE OF FATTY ACID COMPOSITION OF DARK MEAT PHOSPHOLIPIDS OF BROILES

Source	Total Satu 1/		Total Unsatu 2/		Total w3, w6 3/	
	DF	MS	DF	MS	DF	MS
Diet	3	103.73**	3	103.74**	3	231.40*
Sex	1	0.02	1	0.02	1	6.02
Sex & Diet	3	8.91	3	8.81	3	18.95
Error	7	12.03	7	12.03	7	47.30

** Statistical significance at 0.01 level of probability

* Statistical significance at 0.05 level of probability

1/ Total Satu means total saturated fatty acids

2/ Total Unsatu means total unsaturated fatty acids.

3/ Total w3, w6 means total w3 ans w6 families of unsaturated fatty acids.

TABLE XXVIII

ANALYSIS OF VARIANCE OF FATTY ACID COMPOSITION OF WHITE MEAT PHOSPHOLIPIDS OF BROILERS

Source	Total Satu 1/		Total Unsatu 2/		Total w3, w6 3/	
	DF	MS	DF	MS	DF	MS
Diet	3	2.63	3	2.63	3	21.36
Sex	1	9.73	1	9.73	1	10.17
Diet X Sex	3	24.06	3	24.07	3	11.87
Error	4	4.0	4	4.07	4	3.49

1/ Total Satu means total saturated fatty acids

2/ Total Unsatu mean total unsaturated fatty acids.

3/ Total w3, w6 means total w3 and w6 families of unsaturated fatty acids.

THE LIST OF ABBREVIATIONS

ANOVAR	Analysis of Variance
ART	Adjusted Retention Times
BT	Beef Tallow
DEGS	Diethylene Glycol Succinate
ECL	Equivalent Chain Length
GLC	Gas-Liquid Chromatography
MECL	Modified Equivalent Chain Length
MECL (n:2w6)	Modified Equivalent Chain Length using the line of n:2w6
NL	Neutral Lipid
PL	Phospholipid
PUSFA	Polyunsaturated Fatty Acid
PO	Peanut Oil
USFA	Unsaturated Fatty Acid
w Value	Omega value, end carbon chain length counting from the last double bond in a fatty acid.

VITA

Eung Bum Lee was born in Seoul, Korea on March 21, 1938. He attended Seoul University, Seoul, Korea, and graduated with a degree of Bachelor of Science in Agricultural Chemistry in spring 1961. He came to America in summer 1962. He was employed by Drew Chemical Corp., Boonton, N. J. as a chemist for a year ending summer 1964. In June 1964, he enrolled in the Department of Food Technology, The University of Tennessee, Knoxville, Tennessee. He was employed by the Department of Biochemistry of The University of Tennessee as a research assistant from September 1967 to April 1970.