SATURATION AND ISOMERIZATION OF DIETARY FATTY ACIDS INFLUENCE NUTRIENT ABSORPTION AND METABOLISM IN THE CHICKEN

by

Patrick K. Brown

Dissertation submitted to the Faculty of the

Virginia Polytechnic Institute and State University

in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

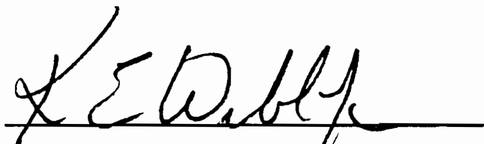
in

Animal Science
(Poultry Nutrition)

APPROVED:

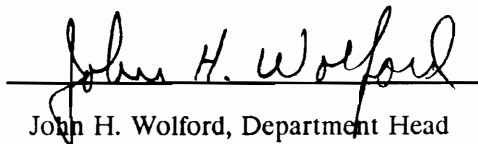
Bruce A. Watkins, Co-Chairman

Lawrence M. Potter, Co-Chairman



Kenneth E. Webb, Jr.

Walter G. Niehaus, Jr.



John H. Wolford, Department Head

July, 1991

Blacksburg, Virginia

SATURATION AND ISOMERIZATION OF DIETARY FATTY ACIDS INFLUENCE NUTRIENT ABSORPTION AND METABOLISM IN THE CHICKEN

by

Patrick K. Brown

L.M. Potter and B.A. Watkins, Chairman

Poultry Science

(ABSTRACT)

Three experiments were conducted to contrast the effect of dissimilar lipid sources on broiler chicken metabolism. In Experiment 1, the nitrogen-corrected apparent metabolizable energy values (AME_n) of soybean oil (SBO) and hydrogenated soybean oil (HSBO) were determined. Trans monoenes, present only in HSBO, comprised 41% of total fatty acids. The AME_n contents of SBO and HSBO were determined to be 8,739 and 7,657 kcal/kg, respectively.

The influence of dietary fatty acids on the lipid composition of the intestinal brush border membrane (BBM) was studied in Experiment 2. Beginning at hatch, chickens were provided isocaloric diets, identified as being either a minimal-lipid (1% 18:2n6) basal diet (ML) or one of three diets in which one-sixth of the caloric content of the basal diet was replaced by oils high in either polyunsaturates (HP), saturates (HS), or trans (HT) monoenes.

The BBM alkaline phosphatase specific activity was greater in chickens fed HT than in those fed ML, HP, and HS. The BBM concentrations of palmitic, stearic, and oleic acids were not affected by treatment. Less linoleate ($P<.06$) and linolenate ($P<.05$) were present in the BBM of chickens receiving HT and HS than in those fed ML and HP. Arachidonate was present in greater concentrations when birds were fed ML, HP, and HS than when fed HT. Trans isomers were present only in the BBM from chickens fed HT.

In Experiment 3, the effects of supplemental dietary lipids on 1) the lipid composition of intestinal tissue and 2) the in vitro absorptive rate of differing dietary nutrients were studied. Diets

similar to those in Experiment 2 were offered to broiler chicks from hatch to 28 days of age. Intestinal membrane concentrations of palmitic and oleic acids were not affected by dietary treatment. Chickens receiving the HSBO or the palm oil diet had less intestinal 18:0, 18:2n6, 18:3n3, and 20:4n6 than did those offered SBO or the control diet.

In all treatments, linoleate and oleate were absorbed at a faster rate than stearate across all treatments. The in vitro uptake rates of oleate and linoleate were not affected by dietary treatment. Stearate and glucose uptakes were reduced in chickens fed HSBO or palm oil compared to those offered either the control diet or SBO diet.

ACKNOWLEDGEMENTS

I wish to thank Dr. Bruce A. Watkins for inviting me to join the VPI&SU Poultry Science Department for my doctoral program. His interest gave me the opportunity to continue my education and is much appreciated. Sincere gratitude is extended to the Pratt Nutrition Fellowship for sponsoring my program of study.

Recognition and thanks are extended to Dr. L.M. Potter and Dr. K.R. Robbins for their insight and friendship, respectively.

Thanks are extended to committee chairmen Dr. L.M. Potter and Dr. B.A. Watkins, and to committee members Dr. W.G. Niehaus, Dr. K.E. Webb, and Dr. J.H. Wolford for their guidance throughout my program.

The backing by the faculty, staff, and students of the Poultry Science Department is appreciated. In particular, the contributions by Carol Temple toward this dissertation are gratefully remembered.

The assistance and suggestions of Jamie Matthews, Han Swinkels, Anne Wakeham, and Zaiqi Wang are valued. The generous offer by Dr. K.E. Webb for use of his centrifuges, scintillation counter, and bench space is greatly appreciated. Dr. W.G. Niehaus will be remembered for the diversity of questions answered and lessons taught.

Finally, I wish to thank James Shelton for his efforts over the past three years to illuminate the more sutile elements of VM2.

This dissertation is dedicated to my wife and children for their support, patience, and love.

TABLE OF CONTENTS

Page

ACKNOWLEDGEMENTS iv

TABLE OF CONTENTS v

LIST OF TABLES vii

LIST OF FIGURES vii

INTRODUCTION 1

REVIEW OF LITERATURE 2
History of chemical hydrogenation 2
Intestinal absorption of dietary fats 4
Metabolizable energy values of dietary nutrients 7
Biomembrane lipids 10
Membrane response to environmental stimuli 12
Essential fatty acid requirements 13
Trans geometric fatty acid isomers 13

CHAPTER I

METABOLIZABLE ENERGY VALUES OF SOYBEAN OIL
AND HYDROGENATED SOYBEAN OIL FOR BROILER CHICKS 18

Abstract 19

Introduction 20

Materials and Methods 21

Results and Discussion 24

References 26

CHAPTER II

DIETARY LIPIDS INFLUENCE COMPOSITION AND ACTIVITY OF
CHICKEN BRUSH BORDER MEMBRANES 33

Abstract 34

Introduction 35

Materials and Methods 37
Experimental diets 37

Harvesting of intestinal enterocytes 38
Marker enzymes 39

Lipid analysis 40

Results and Discussion 42

References 46

CHAPTER III

DIETARY FAT SELECTIVELY ALTERS INTESTINAL LIPID COMPOSITION AND NUTRIENT UPTAKE IN GROWING CHICKENS 53

Abstract 54

Introduction 55

Materials and Methods 57

Results and Discussion 61
Intestinal lipid composition 61
Nutrient uptake 63

References 66

CHAPTER IV

SUMMARY AND CONCLUSIONS 75

Summary and Conclusions 76

LITERATURE CITED 78

APPENDIX 85

VITA 88

LIST OF TABLES

CHAPTER I

Table | Page

1 Fatty acid composition (weight %) of soybean oil and hydrogenated soybean oil 27

2 Composition of the basal diet 28

3 Apparent metabolizable energy values of each diet 29

4 Analysis of variance for Table 3 30

CHAPTER II

1 Percent composition of diets 48

2 Fatty acid composition (weight %) of dietary lipids 49

3 Comparative enhancement of the brush border membrane marker alkaline phosphatase relative to the initial homogenate as effected by dietary treatment 50

4 Fatty acid composition (μg/mg protein) of chicken brush border membrane lipids at 28 days of age 51

CHAPTER III

1 Percent composition of diets 69

2 Fatty acid composition (weight %) of dietary lipids 70

3 Fatty acid composition (μg/mg protein) of chicken small intestine at 28 days of age 71

APPENDIX

1 Gross energy, dry matter, and crude protein content of metabolism trial test diets 86

2 Influence of dietary oil on feed intake, nitrogen intake, excreta production, and excreta energy of broiler chickens from 3 to 4 weeks of age 87

LIST OF FIGURES

CHAPTER I

Figure

1 Comparison of the nitrogen-corrected apparent metabolizable energy values for soybean oil (SBO) and hydrogenated soybean oil (HSBO). Chicks were fed diets containing 0, 10, or 20% added oils from 3 to 4 weeks of age 31

2 Extrapolation of nitrogen-corrected apparent metabolizable energy values to the 100% inclusion level for soybean oil (SBO) and hydrogenated soybean oil (HSBO). Regression lines for each oil are bound by 95% confidence limits 32

CHAPTER II

1 Schematic flow chart for the differential purification of chicken intestinal brush border membrane 52

CHAPTER III

1 Rate of lipid uptake across all dietary treatments. Chicks were fed diets which were supplemented with 6% hydrogenated soybean oil (HSBO), soybean oil (SBO), or palm oil. The contrast stearic acid vs. oleic acid and linoleic acid was significant (P<.05). 72

2 Effect of dietary lipid source in chicken intestinal tissue on the rate of stearic acid uptake (n = 8). Chicks were fed diets which were supplemented with 6% of the fat source from 0 to 28 days of age. The contrast hydrogenated soybean oil (HSBO) and palm oil vs. soybean oil (SBO) and the control diet was significant (P<.05). 73

3 Effect of dietary lipid source in chicken intestinal tissue on the rate of glucose uptake (n = 8). Chicks were fed diets which were supplemented with 6% hydrogenated soybean oil (HSBO), soybean oil (SBO), or palm oil. The contrast HSBO and palm oil vs. SBO and the control diet was significant (P<.05). 74

INTRODUCTION

The United States feed industry is the major user of by-product fats, tallows, and yellow greases in the world today. In particular, the broiler industry uses over half-a-million metric tons of these fats annually. These fats are added primarily to increase the caloric density of poultry diets, although supplemental fats may impart additional nutritive and manufacturing benefits such as increased palatability, dust reduction, and improved feed pellet binding. Almost all of these fats and greases, which typically arise from spent restaurant cooking oils, tallows, deadstock, offal, and waste greases originating from food manufacturers, contain chemically hydrogenated vegetable oils.

Chemical hydrogenation converts soybean oil, the most common substrate for the hydrogenation reaction, into a flavor and heat-stable (hardened) fat more resistant to lipid peroxidation. However, double bond isomers form when the double bond of the native oil deadsorbs from the hydrogenation catalyst and reforms as geometric and positional double bond isomers of the native oil. The effect of these isomers, particularly trans isomers, which would otherwise be foreign to a chicken's diet, have been the subject of numerous experiments. Trans fatty acids have been associated with alterations in membrane fluidity and permeability, enzyme specific activity, essential fatty acid metabolism, and the increased incidence of coronary heart disease. Consequently, since the nutritive value of a given poultry feed-grade fat is economically based on its ability to be digested, absorbed, and oxidized, and, additionally, to further understand how trans fatty acids affect membrane composition and transport, the following experiments were conducted.

Experiment 1 determined the nitrogen-corrected apparent metabolizable energy content of hydrogenated soybean oil and soybean oil in three-week old broiler chickens. Experiment 2 ascertained the effect of isocaloric-isonitrogenous diets differing in fatty acid content on enzyme activity and fatty acid composition of the intestinal brush border membrane. Experiment 3 determined the effect of supplemental dietary lipids on intestinal lipid composition and the absorptive rate of several dietary nutrients.

REVIEW OF LITERATURE

History of chemical hydrogenation

Chemical hydrogenation of vegetable oils to yield solid fats was a serendipitous discovery by the Frenchman Sabatier in 1897. Hydrogenation, the addition of hydrogen atoms to some (or all) of the double bonds in the hydrocarbon chain of unsaturated oils, was the "unsuccessful" result of his attempts to synthesize nickel carbonyl by heating ethylene in the presence of powdered nickel. Today, 88 years after the original hydrogenation patent was issued to Wilhelm Normann (1903), hydrogenation continues to provide the world's human population with an economical method to manufacture fats with melting points and consistency traits favored by consumers.

Although Normann's chemical hydrogenation process may be considered as an old process, it cannot be judged as an outmoded process. Today, essentially all commercial vegetable oil hydrogenation is still dependent on the presence of nickel catalysts, which in the presence of hydrogen gas results in the reduction of the number of double bonds in a fatty acid. However, chemical reduction of the double bonds without the concomitant formation of geometric and positional double bond isomers is basically impossible (Coenen, 1983). Briefly, isomers form because chemical hydrogenation is a two-step process whereby the double bond of the fatty acid adsorbs to the nickel catalyst and, upon deadsorption, may reform as an isomer. These products of hydrogenation can take the form of rather interesting isomers: 1) a positional isomer may result from movement of the double bond along the acyl chain; 2) geometric isomers of the double bond may develop, forming trans double bonds; and 3) isomers containing a combination of positional and geometric isomers may develop.

The Proctor and Gamble Company adapted Normann's work to produce the first commercial hydrogenated vegetable fat, Crisco, from an abundant U.S. cottonseed oil supply in 1911. Today, industrial hydrogenation provides an inexpensive method to limit the formation of peroxides in native oils, creating a flavor and temperature stable fat source for the world's human population. According

to information for 1985 (Agriculture Statistics, 1986), it is estimated that U.S. production of visible animal and vegetable fats and oils to be 6 billion kg. From this amount, approximately 52%, derived entirely from vegetable oil, undergoes hydrogenation. Of the total visible and nonvisible fat consumed in the American diet, it is calculated that 22% arises exclusively from hydrogenated soybean oil (Emken, 1981). The mean per capita intake of trans fatty acids in the United States is estimated to be approximately 9 grams per day (Hunter and Applewhite, 1986). The concentration of incorporated trans-octadecenoic fatty acids found in human liver, adipose, heart, and milk ranges from 2 to 14% of the total fatty acids present (Cook, 1981).

The United States animal feed industry, consuming 1.1 billion kg of by-product fats annually, is the largest user of waste fats and oils in the world today (Burton, 1989). These fats arise from a variety of sources: spent restaurant grease, tallow, deadstock, processing plant offal, and waste greases originating from food manufacturers. Although by-product fats will contribute essential fatty acids, these fats are fed primarily to meet the energy requirements for growth as established by the National Research Council (NRC, 1984). The energy requirements for growing broilers or turkeys cannot be met from a typical commercial diet based on corn and soybean meal without fat supplementation. Therefore, in this country, almost all diets for growing birds will contain 1 to 2% supplemental fat. Additionally, supplemental fats make several non-metabolic contributions to diet formulation: enhanced palatability, lubrication of feed milling machinery, dust control, and feed pellet binding.

In addition to creating a palatable, heat-stable fat, the hydrogenation reaction produces both geometrical and positional double bonds in the fatty acid molecule. The geometric orientation of a trans double bond differs from a cis double bond in two fundamental measurements: linearity and molecular spatial width. For example, oleic acid (cis-18:1n9), containing a single cis double bond, has a molecular spatial width of 0.72 nm and deviates from the linearity of stearic acid by 30° (Cook, 1985). Elaidic acid (trans-18:1n9), with a single trans double bond, mimics the structure of the completely saturated 18:0 species, i.e., the spatial width is 0.31 nm (versus 0.25 nm for stearate) and the acyl chain does not differ from linearity. Thus, compared to its oleic acid counterpart, elaidic acid

yields a more rigid, less soluble molecule with an increased melting point. Although the U.S. per capita consumption of trans fatty acids is estimated at over 9 grams per day (Emken, 1981), the metabolic consequences from consumption of these geometric isomers, which are otherwise produced only by the biohydrogenation of fatty acids in ruminant animals, are not entirely clear. One fact, a tenet of biochemistry, should be remembered relative to trans fatty acids: differences in the three-dimensional shape of a molecule are generally equivalent to differences in function.

The isomers resulting from hydrogenation vary according to the oil undergoing hydrogenation and the reaction conditions. Soybean oil (SBO), which accounts for 75% of all refined fats and oils in this country (Agricultural Statistics, 1986), is composed almost entirely of 18:1n9 and 18:2n6 fatty acids. As such, hydrogenation of soybean oil (HSBO) will produce C18 isomers, predominantly monoenoic isomers of oleic acid. The double bond in these isomers is generally found between carbons 6 and 14 (Gottenbos, 1983).

Intestinal absorption of dietary fats

The nutritive value for a given fat is affected mainly by its gross energy content and its ability to be digested and absorbed. The intestinal absorption of a fat is dependent upon the enzymatic hydrolysis (digestion) of the triglyceride emulsion to micellular monoglycerides and free fatty acids. Uptake of fatty acids from the bile acid micelle occurs through a monomer phase at the lumenal bulk phase-microvillous interface, with the uptake of any fatty acid species being a function of the product of its concentration and passive permeability coefficient at the microvillous surface (Westergaard and Dietschy, 1976).

The rate of triglyceride hydrolysis is affected by the affinity between the substrate triglyceride and the active site of pancreatic lipase. Factors which inhibit mobility of triglycerides within the triglyceride emulsion will limit contact with the lipase and, thus, hinder micelle formation. Micelle concentration in the intestinal lumen of growing pigs was affected by fatty acyl chain length and by the extent of chain saturation (Stahly, 1984). In contrast, in vitro results by Goller et al. (1970) indicated that triglyceride hydrolysis was relatively insensitive to the double bond configuration or

position of differing fatty acyl conformations.

Early investigations (Holmes and Deuel, 1921) determined that fats with melting points below 50°C, whether hydrogenated or nonhydrogenated, were absorbed from the small intestine at equivalent rates. Carver et al. (1955) found that the fatty acids of hydrogenated beef tallow were less well absorbed than oleic acid when both were added to practical broiler diets. Hill and Renner (1959) reported that fat digestibilities in poultry were a function of the constituent fatty acid make-up of a fat, e.g., vegetable oils, 93% digestible; fish oils, 87% digestible; and beef tallow, 67% digestible. Additionally, dietary fat absorption in poultry was positively associated with the age of the bird (Fedde et al., 1960; Renner and Hill, 1960). Freeman et al. (1968) reported stearate and palmitate were less efficiently absorbed in young pigs than unsaturated fatty acids of the same chain length. In the same experiment, the absorption of stearate and palmitate was found to increase in the presence of unsaturated fatty acids, indicating the digestibility of a fat is influenced not only by the composition of the fatty acyl substituents but also by the ratio of saturated to unsaturated fatty acids. However, Stahly (1984) reported the absorbability of short- and medium- chain fatty acids (14 carbons or less) was high regardless of the saturated to unsaturated fatty acid ratio.

There are numerous reports in the literature that quantify the in vitro and in vivo intestinal absorption rates for a variety of dietary nutrients. Studies utilizing in vitro techniques to assess intestinal absorption generally follow one of three protocols: 1) the rate of absorption by intestinal tissue sections is determined by measuring the loss from the incubating media of the nutrient in question (Walker et al., 1981); 2) the absorbance of a test molecule is estimated by the appearance of that molecule on the serosal side of the intestinal tissue (Dietschy et al., 1966); and 3) the uptake of a radiolabeled species directly into the intestinal mucosal tissue (Clark, 1971). While each of the above in vitro techniques has been utilized for quantifying the brush border uptake of a variety of test molecules, veritable determination of the unidirectional uptake of lipids across the mucosa presents problems which precludes the above conventions for lipid absorption studies. Adjacent to gut biomembranes is an unstirred water layer (UWL) which, in the small intestine, presents a solute

diffusion barrier to the cell membrane (Lukie et al., 1974). This barrier to absorption, particularly for nonpolar nutrients as lipids, has been described succinctly by Dietschy et al. (1971) as consisting of a "series of water lamellae extending outward from the cell membrane, each stirred progressively more, until they blend imperceptibly with the bulk water phase without any distinct boundary." Westergaard and Dietschy (1974), using a variety of probe molecules, reported the thickness of the unstirred water layer of rabbit jejunum to be 150 um. Wilson et al. (1971) reported that the passive absorption of micelles across the intestinal mucosa was primarily determined by the unstirred water layer. For compounds such as fatty acids and sterols, the unstirred water layer, and not the cell membrane, is the rate-limiting barrier to intestinal absorption (Sallee and Dietschy, 1973; Westergaard and Dietschy, 1974). In determining lipid absorption rates, it is necessary to correct for those solutes that adhere to the unstirred water layer but are not themselves absorbed into the mucosa. As a consequence, if the solute concentration within the adherent mucosal fluid is not corrected, the uptake rate will overestimate absorption. For example, in compounds where the mucosal uptake rate is low, equal amounts of the test molecule may be found in the adherent tier of water as are found in the mucosal tissue (Sallee et al., 1972).

The amount of lipid present in the adherent unstirred water layer may be partitioned from the quantity of lipid actually absorbed through use of a nonabsorbable marker in conjunction with a labeled fatty acid (Lukie et al., 1974). Sallee et al. (1972) proposed that an ideal marker should meet the following criteria: 1) it should be totally impervious to the cell membrane; and 2) it should be completely resistant to metabolic activity. The authors determined that the following markers met the above criteria and yielded similar estimates for the adherent mucosal fluid volume: dextran, polyethylene glycol, or inulin. A dual-labeled incubation media, e.g., [^{14}C]lipid and [^{3}H]inulin, could simultaneously remove the effect of the unstirred water layer and, simultaneously, measure the unidirectional uptake of a fatty acid.

Metabolizable energy values of dietary nutrients

The determination of the energetic value of dietary inputs, regardless of whether a particular diet is intended to maximize animal growth or minimize a potential loss is dependent on an appreciation of the energetic losses associated with the assimilation of a meal. The ability to control animal growth and production is a reflection of that same ability to accurately estimate the energetic partitioning of a diet. It is through the manipulation of the dietary inputs to which an animal is fed that protein retention may be optimized and fat deposition minimized.

The caloric density of poultry diets is expressed as a fraction of the combustible gross energy (GE) of a feedstuff. The GE may be partitioned into components which reflect the loss of energy via feces, urine, and flatus, and, in addition, the incremental heat production associated with the digestion and metabolism of a meal. The energy lost in gaseous products of fermentation (0.4% of GE) is negligible in the nonruminant and, thus, disregarded (Noblet et al., 1987). The caloric concentration of poultry feedstuffs (NRC, 1984) is generally defined as kilocalories (kcal) of apparent metabolizable energy (ME) and, preferentially, kcal of metabolizable energy corrected for nitrogen retention (ME_n). The ME_n accounts not only for the loss of energy in the feces but also for deaminated amino acids (evidenced by uric acid nitrogen) and absorbed, but incompletely utilized, organic compounds found in urine. Correcting for nitrogen retention reflects the loss from GE of absorbed energy retained as protein that cannot be fully recovered when deposited proteins are hydrolyzed to their carbon backbones.

The ME system, as devised by Anderson (1955) and Potter and Matterson (1960), increased the probability of reliably determining the energy available for maintenance and production in poultry. While there is general agreement that ME_n is basically defined by the following equation:

$$ME_n = \frac{FE - (EE + (8.22 \times N))}{FI}$$

where FE equals the combustible energy of the feed consumed, EE equals the total energy of the

excreta, N equals the grams of nitrogen retained, and FI equals the total grams of feed consumed. Currently, a standardized procedure to determine ME_n does not exist.

Hill and Anderson (1958), using the indicator method of ME_n determination, fed test diets containing chromic oxide for a 2-week adjustment period prior to a 4-day collection period. The concentration of chromic oxide in the excreta is used to indicate the quantity of excreta derived from each unit of feed. Sibbald et al. (1960) replaced a portion of a standardized diet of known ME with an equal amount of the material to be assayed. The ME of the unknown was calculated by the caloric difference between the reference diet and that of the amended diet. Sibbald and Slinger (1963) modified this procedure by again feeding a test nutrient as graded replacements in a standardized diet. Potter (1972) determined the indicator method was biased from excessive variation within the chromic oxide assays compared to variation within the gross energy assays for excreta and feed.

Sibbald (1976) introduced an expeditious ME variant, the true metabolizable energy (TME) assay, an assay that has been "widely accepted throughout the world" (Sibbald, 1983). Briefly, 30 gram samples of a test feedstuff are force-fed to fasted Leghorn roosters, followed by a 24- to 48-hour recovery of the resulting excreta. Concomitantly, the urinary and fecal energy collected for that same time period from a fasted bird corrects for the endogenous fecal and urinary energy contained in the excreta of the fed bird.

Pesti (1984) contrasted chick metabolizable energy bioassays developed by Hill and Anderson (1958) and Potter and Matterson (1960) against the TME assay developed by Sibbald (1976). Specific areas of interest were to determine the effects on metabolizable energy from: 1) the substitution of a known test nutrient into the experimental diet; and 2) the consumption of differing amounts of diet. Pesti (1984) hypothesized that, other than the age of the birds used, a fundamental difference exists in the manner in which endogenous energy losses are calculated between the former and latter assays. Since Sibbald (1976) used a famished rooster to calculate endogenous losses, it is possible that such a value would not represent the true endogenous loss from a bird fed ad libitum amounts of diet. Pesti (1984) did in fact find ME differences between the assays which he attributed to discrepancies

between the endogenous energy losses of fed and fasted birds. For example, he found that fasted birds excreted more nitrogen than fed birds due to protein catabolism from gluconeogenesis.

Similarly, other researchers have criticized the TME assay because of Sibbald's assumption that the nitrogen and energy contained in the excreta from a fasted bird accurately reflects the metabolic and endogenous losses of a fed bird (Vohra et al., 1982). Parsons et al. (1982) reported that the proportionally greater nitrogen losses from fasted birds overestimated the metabolic and endogenous losses of the fed bird. Similarly, Dale and Fuller (1982) reported increased endogenous secretion of nitrogen from fasted roosters compared to fed roosters.

Accuracy of the TME method is particularly questioned when it is used to assay fats. Sibbald and Kramer (1977) reported that 23% of the TME values for 13 different fats were greater than the respective gross energy content of the tested fats. Obviously, the TME content of an oil or any other feedstuff cannot be greater than its respective gross energy content because TME is a subdivision of gross energy. Halloran and Sibbald (1979) reported TME values which varied ($P<.05$) as a function of fat concentrations (0, 5, 10, 15%) in the diet. In contrast, Blake (1986) conducted a total excreta collection trial in which eight levels of fat (0, 5, 10, 15, 20, 50, 75, 100%) were fed to 10-week old Large White turkeys during a four-day collection period. Due to the inablility of turkeys to consume a liquid diet, dietary fat concentrations over 20% were intubated. It is noteworthy that the ME_n content of the diets was a linear function of the fat concentration over the entire range of fat additions to the basal diet. Furthermore, the use of adult roosters to estimate the TME values of dietary fats for chickens or turkeys over a wide range of bird ages has been criticized. Sell et al. (1986) reported that the ability to absorb fats increases with age in the turkey. In a subsequent experiment examining pancreatic enzyme activity from hatch through 8 days of age in turkey poults, Sell et al. (1991) reported pancreatic lipase activity was low at hatching. Pancreatic lipase activity increased only upon the concomitant increase in pancreas weight which occured as the turkeys grew. Additionally, they found that the ME_n of tallow was 6,808 kcal/kg at 2 weeks of age compared to 8,551 kcal/kg at 8 weeks of age. Paralleling this position, in the chicken, Hill and Renner (1959) reported

the ME of tallow increased from 2 to 8 weeks.

Biomembrane lipids

Allowances for a bioregulatory role for dietary fat were envisioned, beginning with the rudimentary plasma membrane model suggested by Davson and Danielli (1952). They proposed a permeable lipid bilayer membrane composed of phospholipids and surface-attached proteins, acting together to mediate protein channel transport of metabolites. While the bilayer model they proposed was eventually discredited, their proposal provided two major benefits: 1) It countered previous beliefs that dietary fat was bioinactive, i.e., fat had been considered only to be a caloric reserve and an inert structural appurtenance; and 2) it was of heuristic value to later researchers who continue to define the nature of the bilayer membrane.

The more complete fluid mosaic membrane model proposed by Singer and Nicolson (1972) consisted of a striking variety of amphipathic proteins and lipids, with the polar hydrophilic head groups of phospholipids oriented towards aqueous entities and the apolar hydrophobic regions being directed away from polarized species. Proteins secured within the bilayer membrane are anchored by van der Waals' gravitational forces between phospholipid fatty acyl tails and nonpolar regions of the polypeptide and, additionally, by electrostatic pressures between hydrophilic segments of the protein external to the bilayer and lipid polar head groups (Clandinin et al., 1985). Thus, lipids in the bilayer proposed by Singer and Nicolson (1972) support two functions: (1) they provide a matrix for the support of multi-functional proteins; and (2) they present a permeability barrier to both contain cell components and to limit solute transport across the bilayer.

Weak van der Waals' induced dipoles bind adjacent acyl chains within the membrane bilayer. The relative strength of attraction between overlapping methylene groups is a direct function of increasing chain length and inversely related to interchain distance. A rigid biomembrane results from the close-packing of saturated acyl chains. Conversely, increased solubility and flexibility of a biomembrane occurs when the lipids are composed of unsaturated fatty acyl chains. Dramatically differing fatty acid constituents impart the biomembrane with a differential ability to act as secondary

messengers, to anchor receptor proteins, or, as with the myelin plasma membrane, to remain comparatively inert. Accordingly, an adaptive mechanism is present to respond to and meet the requirements for cell survivability imposed by differing cellular environments and physiological functions.

The principal lipid classes of the eukaryotic bilayer are normally considered to be phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, cardiolipin, cholesterol, and sphingolipids. If we accept the axiom that changes in the fatty acid composition of a membrane modify its physiological characteristics, then fatty acyl chain substituents confer the membrane with the potential for immense functional variation. For example, a single cis double bond increases the cross-sectional area of the fatty acyl tails of phosphatidylcholine by as much as 23% (Morson and Clandinin, 1986). Thus, alterations in the packed associations among phospholipids, surface-attached proteins, and integral proteins can influence membrane function.

The scientific literature contains a plethora of reports that characterize the dynamics of the membrane bilayer by its fluidity, i.e., the degree of thermal motion exhibited being a function of the fatty acyl chains esterified to the constituent phospholipids (Berlin, 1989). Hence, it is generally acknowledged that less mobile membrane components, such as cholesterol or those containing saturated fatty acyl substituents, tend to decrease membrane fluidity.

Lindblom et al. (1981) directly measured the translational movement of phospholipids within a model membrane system and reported no statistical connection between the dynamic (lateral translation) and static (molecular order) characteristics of the bilayer. The authors determined the lateral diffusion coefficients for two synthesized membranes composed of either 1,2-dioleoyl-glycero-3-phosphatidylcholine or 1-palmitoyl-2-glycero-3-phosphatidylcholine, with both containing graded amounts of cholesterol (0 to 33 mole %). Incorporation of cholesterol into either membrane had no effect on phospholipid lateral diffusion rates, i.e., the expected decrease in membrane dynamics (fluidity) upon cholesterol addition did not occur. However, in a later study, the molecular order of the bilayer increased when the steroid content of the bilayer was increased (Johansson and Lindblom,

1980). The increased order was attributed to phospholipid replacement by sterol molecule.

Membrane Response To Environmental Stimuli

The ability of membrane lipids to adapt to thermal fluctuations in the environment was first recognized by Marr and Ingraham (1962) when they found that Escherichia coli altered its fatty acid composition of membrane phospholipids to survive low temperatures. Sinensky (1974) termed the adaptive response of membrane lipids to fluctuating temperatures the homeoviscous adaptation response. This response is seen as an increase in the iodine number (elevated unsaturation of fatty acids) of membrane phospholipid fatty acids in answer to decreased ambient temperatures.

An intriguing example of the homeoviscous adaptive response is demonstrated by those mammals which are able to lower their normal homeothermic body temperature to the torpid conditions of hibernation. The hibernating ground squirrel can reduce its summer-active body temperature from 37°C to a winter-torpid temperature as low as 1°C (Lyman and O'Brien, 1974). Physiologically, in the same species, the summer-active heart rate drops from 350 beats per minute to four beats per minute during hibernation and, paralleling this, its respiration rate decreases from 150 down to two breaths per minute (Johansson, 1977). How the membranes of hibernating mammals remain functional at temperatures as low as 1°C while those of obligate homeotherms fail to do so at similarly depressed temperatures is not known.

Robert et al. (1982) reported a seasonal alteration in the fatty acid composition of heart, kidney, and brain tissue in hibernating mammals. Aloia (1988) contrasted the fatty acid composition of seven organs between summer-active and winter-torpid animals. In each organ system examined there was a tendency for monounsaturated fatty acid substituents to increase (average of 41%) and for the polyunsaturated fatty acyl substituents to decrease by an average of 9% in winter-torpid animals.

The effect of diet was related to changes in the fatty acid composition of erythrocytes from ground squirrels fed diets containing either beef tallow or soybean oil for 10 weeks prior to initiation of cold temperature (Aloia, 1988). These dietary effects were further associated with changes in the following measurements of animal activity during the hibernation cycle: animals fed the soybean oil-

containing diets hibernated 35% more days and spent 65% more time in torpid states lasting from 5 to 10 days than animals fed tallow-containing diets. Thus, a clear indication is present that characteristics of the biomembrane are altered in response to environmental conditions and that the diet which an animal is fed will affect phospholipid fatty acid make-up and animal behavior.

Essential fatty acid requirements

At approximately the same time Davson and Danielli proposed their version of the fluid mosaic membrane, Burr and Burr (1929) investigated the effects of a fat-free diet fed to rats. The deleterious effects of the diet (reduced growth, dermatitis, tail necrosis, and ultimately death) were avoided by feeding linoleic acid. Animals are unable to complete the de novo synthesis of linoleic and arachidonic acids; these fatty acids, termed essential (EFA), must be supplied in poultry diets because avian species lack desaturase systems capable of introducing double bonds beyond the $\Delta 9$ carbon. Since arachidonic acid can be derived from linoleic acid via in vivo desaturases, only the latter is required as an absolute dietary component. The dietary concentration of linoleic acid required to meet the normal physiological requirements for growing chickens and turkeys is approximately 10g/kg diet, and this amount is readily available in typical corn-soybean meal poultry diets (NRC, 1984).

Determination of essential fatty acid requirements for mature turkeys and chickens is complicated by abdominal fat reserves. Requirements in leghorn-type laying chickens have been estimated to be 9g/kg diet (Balnave, 1971). Whitehead and Herron (1988) fed breeding turkey hens isocaloric-isonitrogenous diets, containing from 8.5 to 24 grams linoleate per kg diet, from 30 weeks of age (lighting) until 55 weeks of age. They reported that diets containing 11 g/kg produced the greatest number of live poults per hen.

Trans geometric fatty acid isomers

Trans unsaturated fatty acids are non-essential for normal growth and integrity of an animal. These fatty acids are incorporated into avian tissues as fatty acyl derivatives (Al-Athari and Watkins, 1988). The extent of tissue incorporation of trans fatty acids within a particular species varies as a function of the trans dietary density (Sgoutas and Kummerow, 1970). Studies by a number of authors

have demonstrated that dietary isomers of octadecenoic acid were generally incorporated, without partiality, into all lipid classes as a function of dietary concentration (Emken, 1984).

Early studies modeled to assess the health risks to rats from the consumption of hydrogenated fats failed to demonstrate any detrimental effects on growth, fecundity, or longevity (Alfin-Slater et al., 1957). A similar conclusion was published in a review by the Federation of American Societies for Experimental Biology (1985) which generally found dietary trans isomers to be an innocuous risk to health. However, the considerable number of publications and symposia devoted to the metabolic repercussions of dietary trans fatty acids suggests that significant controversy exists in the discipline.

Anderson (1967) reported that geometric isomers of oleic acid were oxidized completely in the rat, although the process was slower than for oleic acid itself. Zottor and Walker (1989) reported that trielaidin inhibited by 50% the hydrolysis of triolein when both were tested as substrates for rat mammary lipoprotein lipase. The presence of dietary trans fatty acids did not affect the utilization of dietary protein in growing rats (Islam et al., 1983). In that study, the ratio of grams liveweight animal gain to grams casein protein consumed (protein efficiency ratio) was not affected by diets containing either 7.8 or 20.1% hydrogenated soybean oil or trielaidin. Similarly, Sgoutas (1968) found that cholesterol esters of trans fatty acids were hydrolyzed at a slower rate than cis esters. The ability to metabolize trans fatty acids via β-oxidation is not surprising since the initial oxidation of the acyl-CoA moiety by an FAD prosthetic group results in the formation of 2-trans-enoyl CoA.

Fatty acid auxotrophs suffered decreased phospholipid synthesis, which inhibited cell division, when elaidic acid was added to the incubation media (Graff and Lands, 1976). Alfin-Slater et al. (1965) reported that the addition of elaidic acid, trans,trans-linoleic acid, or cis,trans-linoleic acid to EFA deficient diets exasperated the detrimental effects typical of EFA deficiency. Trans,trans-linoleic acid was found to inhibit the conversion of linoleic acid to arachidonic acid, possibly explaining the heightened EFA-deficiency symptoms that occur when trans is added to EFA-deficient diets (Beare-Rogers, 1983).

The origin of the heightened incidence of coronary heart disease common to Twentieth-century

Western populations is associated with a notorious number of antecedents. Prominent among these is the consequence of consuming a typical American diet in which fats and oils contribute roughly 40% of the total caloric intake (Grundy et al. 1985). Atherosclerotic plaques form in response to an injury to the endothelial lining of the coronary vessels, allowing a fibrin clot to develop and serve as a medium for platelet and cholesterol aggregation. The presence of saturated fats in particular, and total dietary fats and cholesterol in general, has been implicated in several distinct pathways leading to thrombus formation.

Many researchers during the past 30 years have positively associated the concentration of serum lipids, particularly cholesterol, with increased occurrences of atherosclerosis. Indeed, when non-age atherosclerotic risk factors from a series of epidemiological studies were partitioned, the concentration of serum cholesterol explained the greatest amount of variation in the findings (Jones, 1974).

It is generally agreed that elevated intakes of saturated fatty acids prompt an increase in low density lipoproteins (LDL), the lipoprotein fraction responsible for cholesterol internalization. Furthermore, a high ratio of LDL to high-density lipoprotein (HDL), the lipoprotein credited as being a cholesterol scavenger, stimulates the deposition of cholesterol in the cell but offers less opportunities for its removal.

The impact of dietary hydrogenated fats on atherosclerosis is not conclusive. Increased bleeding time, functionally indicative of a decreased likelihood for atherosclerotic deposits, resulted when rats were fed a trans,trans-linoleic acid-rich diet (Raccuglia and Privett, 1970). As an aside, the increased bleeding time may ensue as a consequence of decreased thromboxane A_2 production, resulting from the inhibition of the conversion of linoleic acid to arachidonic acid. Weigensberg and McMillan (1964) reported that rabbits developed hypercholesterolemia, but not atherosclerotic deposits when they were fed diets containing elaidic acid. When trans fatty acids replaced the coconut oil in a diet known to induce plaques in rabbits, Kritchevsky and Tepper (1968) reported increased hypercholesterolemia but not atherosclerosis. Emken (1984) reported serum cholesterol increases in only half of 14 experiments in which human subjects were given diets containing hydrogenated fats. However, Thomas et al.

(1983) found that the adipose tissue from victims of coronary heart disease contained increased concentrations of trans-18:1 and trans-16:1. The incorporation of trans fatty acids into bilayer phospholipids is known to alter desaturase and elongase activity involving cis-polyunsaturates. Pollard et al. (1979) measured the ability of the Δ9 desaturase to attack trans-monoenoic fatty acids. When five trans-isomers of oleic acid were incubated in the presence of the desaturase, there was considerable desaturation (58% of all fatty acids were further desaturated). However, desaturation did not necessarily occur at C9 or between the single double bond and the carboxyl terminus. Additionally, the desaturase was functional at loci where desaturation would create a series of conjugated double bonds. For example, Δ7-trans- or Δ11-trans-oleifins produced Δ7-trans,9-cis- and Δ9-cis,11-trans-conjugated dienes, respectively. Furthermore, desaturation of non-Δ9 cis-fatty acids by Δ6 desaturases was demonstrated.

In recent work, Watkins (1988) found that feeding high levels of trans-18:1 to male broiler chickens resulted in reduced concentrations of 20:3(n-6) and 20:4(n-6) in liver and heart phospholipids. Concomitantly, these decreases in n-6 fatty acids were compensated by an increase in n-3 fatty acids. Moreover, in comparison to triglyceride fatty acid composition, it was found that trans-18:1 isomers were selectively incorporated into the phospholipids contained in heart and liver tissues.

Mahfouz et al. (1980) found that certain isomers of trans-18:1 inhibited the Δ5-, Δ6-, and Δ9-desaturases of rat liver microsomes. These isomers were effective inhibitors of the desaturases based on the location of the double bond, with no single isomer being an effective inhibitor of all three desaturases. Svensson (1983) reported that rats fed diets containing 20% hydrogenated herring oil exhibited a significant decrease in Δ6- and Δ5-desaturase activity in liver microsomes compared to those fed a diet containing 20% peanut oil. Cook (1981) established that trans,trans-octadecenoic acids were inhibitors of Δ9- and Δ6-desaturation and elongation of fatty acids from brain homogenates and microsomes of 10-day-old rats. Rosenthal and Doloresco (1984) noted that elaidic acid was a formidable inhibitor of Δ5 desaturation in human skin fibroblasts, while trans-vaccenate (11 trans-

18:1) was without effect. Moreover, they reported that the position of the double bond determined whether a fatty acid was an efficacious inhibitor of desaturation. They found, in a series of trans fatty acids tested, all n9 trans acids were desaturase inhibitors while n7 trans acids were irresolute Δ5 inhibitors.

Clearly, the trans-bonds produced during the chemical hydrogenation of native polyunsaturates yield isomers which are capable of altering the metabolism of fatty acids and their derivatives. Today, however, it is inaccurate to describe these isomers as being foreign or unnatural; they have become indigenous throughout the food chain, prominently affecting the diets of both humans and poultry.

CHAPTER I

METABOLIZABLE ENERGY VALUES OF SOYBEAN OIL AND HYDROGENATED SOYBEAN OIL FOR BROILER CHICKS

ABSTRACT

The nitrogen-corrected apparent metabolizable energy values (AME_n) of soybean oil (SBO) and hydrogenated soybean oil (HSBO) were determined using 120 male broiler chickens between 3 and 4 weeks of age. Total amounts of saturates and monosaturates were higher but polyunsaturates lower in the HSBO than in SBO. Trans monoenes were present only in HSBO at 41% of the total fatty acids. Test diets were prepared by adding the oils at the expense of a 23% crude protein, corn-soybean meal basal diet at levels of 10 and 20%. Each of the five diets (basal and 4 test diets) was fed ad libitum to 4 pens of six chicks per pen for seven days.

A linear increase in both the gross energy and in the metabolizable energy content of the diets was obtained by the addition of varying levels of either SBO or HSBO. The AME_n contents of SBO and HSBO were determined to be 8,739 and 7,657 kcal/kg, respectively. These values represent 92.4 and 80.9% of their respective gross energy values.

INTRODUCTION

Broiler chickens fed a traditional corn-soybean meal diet are offered diets supplemented with a feed-grade fat, when prices are favorable, to boost the metabolizable energy content of the diet to the minimum levels specified by the National Research Council (NRC, 1984). Within the United States feed industry, which itself is the largest user of by-product fats in the world (1.1 billion kg/year), the domestic broiler industry alone consumes 465 million kg of fats annually (Burton, 1989). These fats arise from a variety of sources: spent restaurant grease, tallow, deadstock, offal, and waste greases originating from food manufacturing.

According to statistical data (Agriculture Statistics, 1986) detailing American synthesis of edible animal and vegetable fats, annual production it is estimated to be six billion kilogram (kg) per year. Of this amount, approximately 52%, derived entirely from vegetable oils, undergoes chemical hydrogenation. Vegetable oils are hydrogenated to prevent the formation of lipid peroxides. The process creates an economical, heat and flavor stable fat source for the world's human population. However, while the hydrogenation process does produce peroxide-resistant saturates and monosaturates from susceptible polyunsaturated fatty acids, geometric and positional double bond isomers of the native oil form during the hydrogenation process. These isomers alter the chemical and physical characteristics of the native oil. Since differences in the three-dimensional shape of a compound are generally equivalent to differences in its biological functions, an experiment was designed to determine the effect of chemical hydrogenation on the nitrogen-corrected apparent metabolizable energy (AME_n) content of soybean oil.

MATERIALS AND METHODS

A total of 120 3-week old male broiler chicks, hatched October 2, 1989, were used in a 1 x 2 x 2 augmented factorial design to determine the AME_n of soybean oil[1] (SBO) and hydrogenated soybean oil[2] (HSBO). Test diets were prepared by adding the oils (Table 1) at the expense of a 23% crude protein, corn-soybean meal basal diet (Table 2) at inclusion levels of 10 and 20%. The five diets (basal and four test diets) and water were provided ad libitum to four replicate pens of six chicks per pen during the total collection metabolism trial. From hatch to 21 days of age all pens of chickens were fed the same basal diet. Feed efficiency and weight gain were determined for each pen to verify that there were no differences in either measure prior to beginning the metabolism trial.

A 2-day period to acclimate the chicks to the test diets immediately preceded the beginning of the excreta collection. Following the acclimization period, the quantities of test diets were fed from 0600 hours on day 1 until 0600 hours on day 6. The excreta collection period began at 0600 hours on day 1 and continued for five separate 24-hour periods. Dander and feathers were mechanically blown from the collection trays according to the method of Sibbald (1986). Excreta was collected on a daily basis from aluminum foil-lined collection trays, weighed, dried to a constant weight in a 60°C oven, air equilibrated, and again reweighed. Daily dried excreta was pooled on a pen basis, ground to pass through a 1-mm screen, and a representative subsample (~ 100 g) was stored in glass jars at -20°C until analyzed. Diets and excreta were assayed for gross energy (kcal/g) by combustion in a Model 1241 Parr adiabatic bomb calorimeter[3], and nitrogen was assayed by Kjeldahl (Association of Official Analytical Chemists, 1984). The AME_n value for each of the five diets was calculated from the following equation (Blake, 1986):

---

[1]Imperial® oil, Lot 1025, Bunge Edible Oil Corp., Kankakee, IL 60901.

[2]Fan Fry®, Lot 1088, Bunge Edible Oil Corp., Kankakee, IL 60901.

[3]Parr Instrument Co., Moline, IL 61265.

$$ME_n = \frac{GE}{g\ diet} - \left[\left(\frac{\left(\frac{kcal}{g\ excreta} * g\ excreta\ collect\right)}{g\ diet\ consume}\right) + \left(8.22 * \frac{g\ nitrogen\ retain}{g\ diet\ consume}\right)\right]$$

Regression coefficients for AME_n values for each oil were estimated by the following multiple regression model:

$$Y = \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3$$

where Y equals AME_n for a given diet, and X_1, X_2, and X_3 equal the fractional inclusion rate of HSBO, SBO, and basal diet into the experimental diets, respectively. Standard errors and confidence limits were calculated about the regression estimates. Validity of regression coefficients, i.e., linearity of response and proof of a common intersection at zero level of oil inclusion for lines of slope β_1 and β_2, was verified (Table 4) using Finney's slope ratio assay (Finney, 1978).

Each oil was assayed in duplicate for fatty acid composition (Table 1). Briefly, 400 mg of each oil were weighed into a 20 x 150mm KIMAX® brand glass tube (phenolic cap with teflon liner). Eight ml of chloroform : methanol (2:1, v/v) was added to each tube, followed by vigorous vortexing. One-half ml was then transferred to a 13 x 100mm PYREX● brand glass tube (phenolic cap with teflon liner) for methylation. An internal standard consisting of 2 mg of heptadecenoic acid[4] (17:1n7) was added prior to methylation to calculate the quantities of each fatty acid relative to the internal standard. The lipid fraction was saponified (400 µl .5 N NaOH in methanol) and methylated (400 µl BF_3, 14% (w/v) in methanol) according to Metcalfe et al. (1966).

Fatty acid composition (weight percent) of the oils was quantified using a Hewlett-Packard 5890 gas chromatograph equipped with a Hewlett-Packard 3393A integrator[5]. Individual fatty acids in the samples were identified by retention times of analogous fatty acid standards purchased from Nu-Chek-Prep. Geometric and positional isomers of octadecanoic acid were partitioned using argentation thin-layer

---

[4]Nu-Chek-Prep, Inc., Elysian, MN 56028.

[5]Hewlett-Packard Co., Avondale, PA 19311.

chromatography (Dudley and Anderson, 1975).

RESULTS AND DISCUSSION

For the period preceding the metabolism trial, from hatch to 3-weeks of age, there were no differences in either body weight or feed conversion among the 20 pens used in the trial. This would be expected as all chicks were fed the identical starter diet. The AME_n values for each replicate diet, i.e., each of four replicate diets at each type and level of oil addition to the basal diet, are listed in Table 3. These values are plotted in Figure 1, together with the predicted regression coefficients (Finney, 1978) for the regression of AME_n on dietary oil concentration.

The determined AME_n value for the basal diet (2,875 kcal/kg) agreed well with the NRC (1984) estimate for this diet (2,883 kcal/kg). Convergence for both regression lines (regression of AME_n on dietary oil content for each dietary oil) occurred at this point (zero oil incorporation). Convergence at this point validates both regression equations down to the zero level of oil incorporation. Additionally, non-intersection of the regression lines outside the zero level of zero oil inclusion is demonstrated in Table 4.

Experimental determination of the AME_n values of SBO over the past 35 years has generated an extensive number of papers with which the NRC (1984) bases its estimates of nutrient composition. The SBO AME_n value which was obtained in this trial, 8,739 kcal/kg, compares favorably with the SBO AME_n estimate from the NRC (1984) of 8,800 kcal/kg. The AME_n value for HSBO (7,657 kcal/kg) was less ($P<.05$) than the AME_n value for SBO. Similarly, Hill and Anderson (1959) and Matterson et al. (1965) reported reduced AME_n values for nondescript hydrogenated vegetable oils.

The predicted regression lines for each oil type at dietary additions rates from 0 to 100%, together with the 95% confidence limits about each line are presented in Figure 2. The confidence limits graphically indicate the loss of precision which occurs as we extrapolate outside the region of oil substitution into the basal diet. Potter (1972) reported that even at a 20% fat substitution rate into a basal diet, generally the highest rate used in the appraisal of the metabolizable energy of a fat, a range of up to 544 kcal/kg of oil could be expected. Moreover, dietary oil substitutions into a basal diet at levels

considerably less than 20% may account for literature citations claiming AME_n estimates greater than the gross energy content of a fat (Sibbald and Kramer, 1977).

The results of the current study indicate a significant decrease in the AME_n content of HSBO when compared to SBO. Considering the extensive amounts of hydrogenated vegetable oils which eventually enter broiler feeds as by-product yellow greases, the presence of such isomers should be considered when formulating poultry diets.

REFERENCES

Agricultural Statistics, 1986. US Government Printing Office. Washington, DC.

Association of Official Analytical Chemists, 1984. Official Methods of Analysis, Association of Official Analytical Chemists. Washington, DC.

Blake, J.P., 1986. Methodology for evaluating the digestibility and metabolizable energy of poultry feedstuffs. Doctoral disseration. Virginia Polytechnic Institute and State University. Blacksburg, Virginia.

Burton, F., 1989. Restaurant grease: how much is out there? Render. 18:10-16.

Dudley, P.A., and R.E. Anderson, 1975. Separation of polyunsaturated fatty acids by argentation thin layer chromatography. Lipids. 10:113-114.

Finney, D.J., 1978. Slope ratio assays. Pages 148-166 in: Statistical Methods in Biological Assay. Hafner Publishing, New York.

Hill, F.W., and R. Renner, 1959. Energy values and other properties of fats in poultry nutrition. Proc. Cornell Nutr. Conf., pp. 11-15.

Matterson, L.D., L.M. Potter, M.W. Stutz, and E.P. Singsen, 1965. The metabolizable energy of feed ingredients for chickens. The University of Connecticut. Research Report 7:3-11.

Metcalfe, L.D., A.A. Schmitz, and J.R. Pelka, 1966. Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. Anal. Chem. 38:514-515.

National Research Council, 1984. Nutrient Requirements of Poultry. 8th ed. National Academy Press, Washington, DC.

Potter, L.M., 1972. The precision of measuring metabolizable energy in poultry feedstuffs. Feedstuffs. 44:28-40.

Sibbald, I.R., 1986. Research Branch Contribution 86-4E. Animal Research Centre. Agriculture Canada. pp114.

Sibbald, I.R., and J.K.G. Kramer, 1977. The true metabolizable energy values of fats and fat mixtures. Poultry Sci. 56:2079-2086.

TABLE 1. *Fatty acid composition (weight %) of soybean oil and hydrogenated soybean oil*

Fatty acid	Dietary lipids	
	HSBO	SBO
16:0	11.13	11.08
18:0	12.75	3.99
cis-18:1	30.53	25.98
trans-18:1	41.28	ND
trans-18:2n6	2.83	ND
18:2n6	1.31	51.64
18:3n6	ND	ND
18:3n3	ND	5.95
20:0	.17	.37
20:1n9	ND	.24
22:0	ND	.33
Total saturates	23.88	15.86
Total monosaturates	71.81	25.98
Total polyunsaturates	4.24	58.00

ND = Not detected, levels below .01%.
HSBO, hydrogenated soybean oil; SBO, soybean oil.

TABLE 2. *Composition of the basal diet*

Ingredient	Amount (g/kg)
Ground yellow corn (4-02-935)	592.28
Dehulled soybean meal (5-04-612)	356.75
Dicalcium phosphate (6-01-080)	16.32
Calcium carbonate (6-01-069)	14.32
DL-Methionine	2.34
Vitamin premix[1]	10.00
Mineral premix[2]	8.00
Calculated analysis:	
Protein	230
Calcium	10.0
Phosphorus (available)	4.5
Total sulfur amino acids	9.3
ME_n, (kcal/kg)	2,883

[1]Provides per kilogram of diet: vitamin A palmitate, 4.0 mg; cholecalciferol, .4 mg; vitamin E (DL-α-tocopherol), 80mg; vitamin K (menadione), 1 mg; riboflavin, 3.6 mg; pantothenic acid, 10 mg; niacin, 27 mg; 9 μg vitamin B_{12}; choline, 1500 mg; folic acid, .55 mg; thiamin, 1.8 mg; pyridoxine, 3 mg; biotin, 400 μg; ethoxyquin (66%), 200 mg.

[2]Provides per kilogram of diet: iodized NaCl, 4 g; $MnSO_4 \cdot H_2O$, 1814.6 mg; ZnO, 50 mg; $FeSO_4 \cdot 7H_2O$, 400 mg; $CuSO_4 \cdot 5H_2O$, 31.5 mg; KIO_3, .3 mg; Na_2SeO_3, .32 mg.

TABLE 3. *Apparent metabolizable energy values of each diet*

Observation	Proportional incorporation rate of dietary oils			ME_n
	Basal	HSBO	Soybean oil	
1	1.0	0	0	2,885
2	1.0	0	0	2,722
3	1.0	0	0	2,905
4	1.0	0	0	2,895
5	.9	.1	0	3,296
6	.9	.1	0	3,361
7	.9	.1	0	3,371
8	.9	.1	0	3,335
9	.8	.2	0	3,896
10	.8	.2	0	3,810
11	.8	.2	0	3,786
12	.8	.2	0	3,856
13	.9	0	.1	3,520
14	.9	0	.1	3,547
15	.9	0	.1	3,539
16	.9	0	.1	3,471
17	.8	0	.2	4,063
18	.8	0	.2	4,011
19	.8	0	.2	4,003
20	.8	0	.2	3,999

HSBO, Hydrogenated soybean oil.
ME_n, Nitrogen-corrected apparent metabolizable energy.

TABLE 4. *Analysis of variance for Table 3*

Source of variation	df	Sum of squares	Mean square
Regression	2	3,290,346	
Basal diet	1	7,638	7,638
Intersection	1	11,834	11,834
Between treatments	4	3,309,818	
Error	15	39,714	2,648
Total	19	3,349,532	

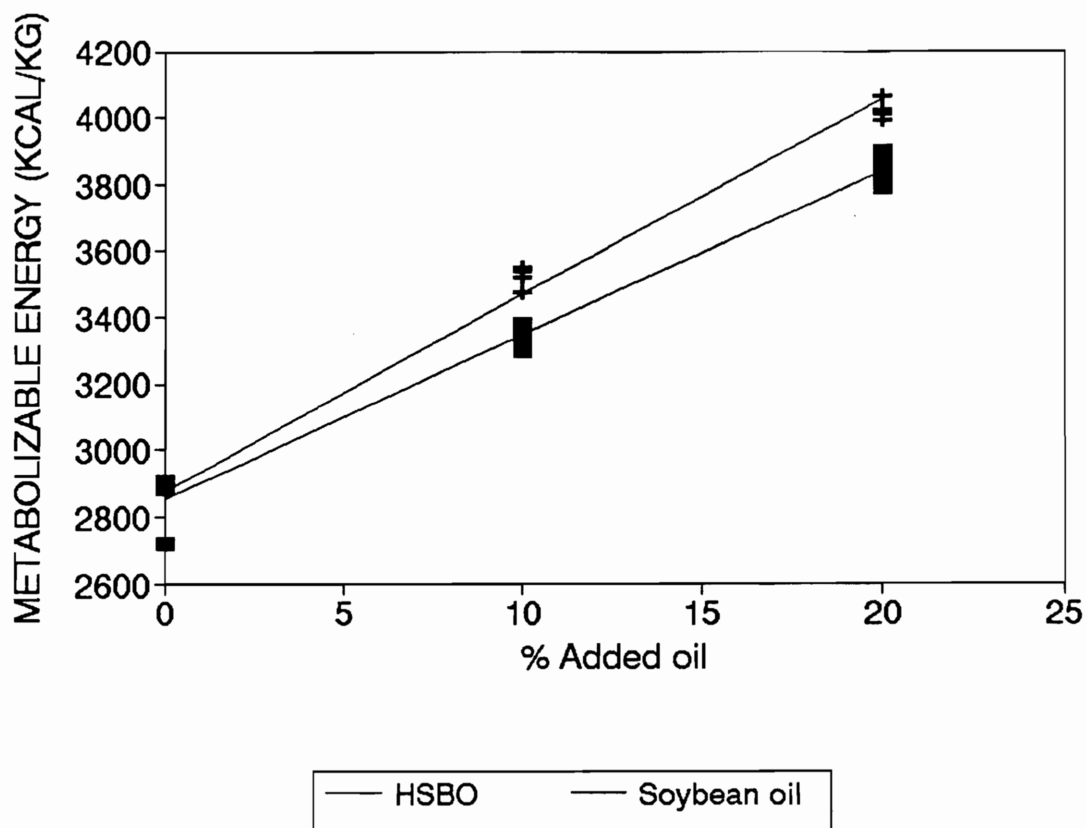


FIGURE 1. Comparison of the nitrogen-corrected apparent metabolizable energy values for soybean oil (SBO) and hydrogenated soybean oil (HSBO). Chicks were fed diets containing 0, 10, or 20% added oils from 3 to 4 weeks of age.

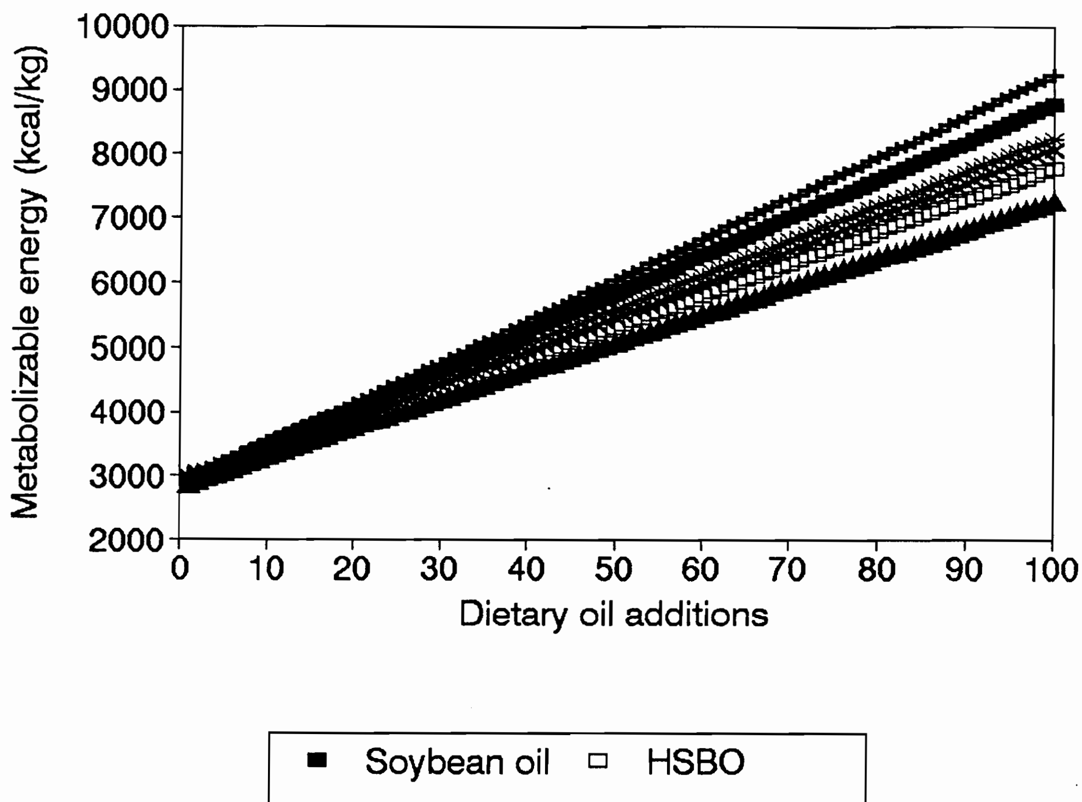


FIGURE 2. Extrapolation of nitrogen-corrected apparent metabolizable energy values to the 100% inclusion level for soybean oil (SBO) and hydrogenated soybean oil (HSBO). Regression lines for each oil are bound by 95% confidence limits. HSBO = 2875 + 4782 (x);
SBO = 2875 + 5864 (x).

CHAPTER II

DIETARY LIPIDS INFLUENCE COMPOSITION AND ACTIVITY OF CHICKEN BRUSH BORDER MEMBRANES

ABSTRACT

Influence of dietary lipids on the fatty acid composition of intestinal brush border membrane (BBM) from fasted 28-day-old male broiler chicks was studied. At hatch, chicks were provided isocaloric nutritionally-adequate diets, identified as being either a minimal-lipid (1% 18:2n6) basal diet (control) or one of three diets which substituted one sixth of the caloric content of the basal diet with oils high in polyunsaturates (SBO), saturates (palm oil), or in trans-monoenes (HSBO). Body weight gain or feed consumption were not affected by dietary treatments. The BBM purity was verified by a 7.5-fold alkaline phosphatase enrichment, while enhancement of the basolateral membrane marker Na/K adenosine triphosphatase was limited to a 1.8-fold increase. The BBM alkaline phosphatase specific activity was greater ($P<.05$) in chicks fed HSBO than in other treatments. Concentrations of 16:0, 18:0, and 18:1 were not affected by dietary treatment, but less 18:2n6 ($P<.06$) and 20:4n6 ($P<.05$) were present in the BBM of chicks fed HSBO than in those fed all other treatments. Trans isomers were present only in the BBM from chickens fed HSBO. Results demonstrate that varying the concentrations of dietary fatty acids will alter the fatty acid composition and alkaline phosphatase activity of the BBM.

INTRODUCTION

The brush border membrane (BBM) of the small intestine is the first absorptive barrier encountered by the vast majority of dietary nutrients utilized by the chicken. Insomuch as the fluid mosaic membrane model proposed by Singer and Nicolson (1972) consists of a remarkable variety of amphipathic proteins and lipids, binding into a cohesive biomembrane via electrostatic and van der Waals forces, it is hypothesized that differences in the lipid composition of the chicken BBM may affect its functional characteristics. These functional differences would arise due to three physical and metabolic characteristics of membrane lipids: 1) they provide a structured matrix for protein anchoring; 2) they confer a selective permeability barrier upon the membrane; and 3) they potentially serve as bioactive lipids.

Trans fatty acids result from the chemical hydrogenation of vegetable oils. In this country we hydrogenate approximately 3.1 billion kg of vegetable oils annually to provide a flavorable and temperature-stable fat source for the nation's human population (Agricultural Statistics, 1986). Many of these fats, which would otherwise be atypical components of a nonruminant's diet, become poultry feedstuffs as inedible-for-human-consumption waste fats and greases. Since the domestic broiler industry alone utilizes 0.5 billion kg of by-product yellow greases annually (Burton, 1989), there is considerable opportunity for consumption of hydrogenated vegetable oils by poultry (Al-Athari and Watkins, 1988a).

There has been considerable nutritional interest in the biological effects of trans fatty acids in recent years. Kritchevsky (1982) reported that rabbits fed a diet containing up to 6% trans fatty acids were hyperlipidemic but no more atherogenic than rabbits fed a similar diet supplemented with 6% olive oil. Trans geometric isomers were found to be preferentially incorporated into the liver and heart of rats fed a partially hydrogenated fat compared to total lipid concentrations in the serum, adipose tissue, testes, and adrenals (Reichwald-Hacker, 1979). Lanser and Emken (1987) reported that in egg yolk lipids of laying hens there was discrimination against incorporation of trans-18:1Δ10

into phospholipid and neutral lipids compared to cis-18:1Δ9 assimilation. Instead, the trans isomer was preferentially oxidized relative to the cis isomer or, alternately, was retroconverted to a 16:1 isomer (most likely trans-16:1Δ8).

The functions of the small intestine may be subdivided into three general functions: 1) completion of the digestive processes initiated in the ventriculus and proventriculus; 2) absorption of nutrients by the BBM; and 3) passage of unabsorbed digesta and xenobiotics to the large intestine. As such, the mucosal lining itself undergoes extremely rapid turnover, being vulnerable to incursion by pancreatic enzymes, physical abrasion, and bacterial/viral agents. Despite these facts, few studies have characterized the response of the brush border membrane in broiler chickens to dietary changes in fatty acids. Accordingly, the objective of this experiment was to determine if isocaloric-isonitrogenous diets differing in fatty acid content would influence the enzyme activity and fatty acid composition of intestinal BBM when fed to chickens from hatch to 28 days of age.

MATERIALS AND METHODS

Experimental diets

Eighty newly hatched male broiler chicks, hatched May 1, 1990, were randomly and equally distributed among 16 pens in a Petersime[1] starter battery. Each pen was randomly assigned to one of four isocaloric-isonitrogenous dietary treatments (Table 1): 1) a minimal oil-containing control diet; or 2) the control diet with hydrogenated soybean oil (HSBO), palm oil or soybean oil replacing one sixth of the total dietary metabolizable energy (500 kcal starch-derived energy/kg diet) with an isocaloric combination of each respective oil and cellulose. All diets contained sufficient linoleic acid to meet the National Research Council's (NRC, 1984) dietary requirement for essential fatty acid in growing broilers. The fatty acid compositional analysis of the four dietary lipid sources is given in Table 2. The respective diets and water were given ad libitum throughout the experiment. Feed consumption and weight gain were monitored during the 4-week growth period.

Experimental design

The four dietary treatments were replicated over four pens, being designed as a completely randomized experiment with 16 experimental units. Analysis of variance, using the general-linear-model procedure (SAS Institute, 1985), was used to test for treatment effects according to the following linear model:

$$y = \mu + T_i + e_{ij}$$

where y_{ij} = an individual observation; μ = overall mean value for the population; T_i = the ith dietary treatment effect (i = 1,2,3,4); and e_{ij} = a random error component associated with each observation. Treatment differences were tested using nonorthogonal single degree-of-freedom linear contrasts and the Student-Newman-Keuls multiple range procedure (Bishop and Lentner, 1986).

---

[1]Petersime Incubator Co., Gettysburg, OH.

Harvesting of intestinal enterocytes

Beginning on day-28 and continuing for four successive days, four birds from each of the four treatments were randomly selected and killed by cervical dislocation. The birds were laparotomized, and the small intestine, extending distally from the pancreatic ducts to the ileal-cecal junction, was quickly removed to an ice-cold porcelain tray. (Membrane purification was conducted either on ice or at 4°C unless stated to the contrary). Extraneous fat was removed and the intestinal segment was opened longitudinally and rinsed in three successive basins filled with an ice-cold solution of 300 mM mannitol and 12 mM tris base buffer (the mannitol buffer, pH adjusted to 7.4). Each segment was then placed in an incubation buffer containing 1 mg hyaluronidase/ml, 1 mg bovine serum albumin/ml, 120 mM NaCl, 20 mM tris base, 1 mM $MgCl_2$, and 3 mM K_2HPO_4 (pH adjusted to 7.4) for 20 minutes at 37°C. Following the hyaluronidase incubation, the intestinal strip was again placed on an ice-cold porcelain tray for harvesting of the enterocytes. The luminal surface of each segment was gently scraped with a glass slide to remove the mucosal lining. The harvested scrapings were placed in a 50-ml glass centrifuge tube and stored on ice until later processing that same morning. Hyaluronidase buffer was removed from the enterocytes by twice resuspending the scrapings in equal volumes of mannitol buffer and centrifuging[2] (4°C) at 4,500 x g for 12 minutes. The rinsed scrapings were then placed in a Whirl-Pak[3] bag, frozen in liquid nitrogen, and stored at -70°C until further purification.

The brush border membranes were purified according to Wilson and Webb (1990). Thawed tissue samples were placed in 50-ml glass tubes, wrapped in an ice jacket, and homogenized in 8 volumes (w/v) of mannitol-succinate buffer (see below), using a polytron[4] tissue homogenizer equipped with a 20-mm probe for 15 seconds at setting six. This buffer, the mannitol-succinate buffer, contained 5 mM $MgCl_2$, 150 mM mannitol, 10 mM tris base, 30 mM succinate, 5 mM KH_2PO_4, and

---

[2]J2-21 centrifuge, Beckman Instruments, Inc., Palo Alto, CA 94304

[3]American Scientific Products, Columbia, MD 21045

[4]Pt 10/35, Brinkman Instruments, Westburg, NY 11590

.1 mM $MnCl_2$ (pH adjusted to 7.4). The homogenate was then gently stirred in an ice bath for 30 minutes.

Following the incubation, the BBM were purified by differential centrifugations (Figure 1). The homogenate was centrifuged at 8,700 x g for 12 minutes, followed by centrifugation of the ensuing supernatant at 31,000 x g for 15 minutes. The resulting pellet was then resuspended in mannitol-succinate buffer by amalgamation with twelve strokes of a Kontes teflon tissue homogenizer[5]. The homogenate was again placed in an ice-bath and gently stirred for 30 minutes, followed by centrifugation at 8,700 x g for 12 minutes. The resulting supernatant fluid was spun at 31,000 x g for 15 minutes to aggregate the BBM into a pellet (P4). The P4 pellet was placed in a Whirl-Pak bag, frozen in liquid nitrogen, and stored at -70°C for future lipid compositional and marker enzyme analysis.

Marker Enzymes

Alkaline phosphatase (EC 2.1.3.1) specific activity was used to verify the enrichment of the BBM. Specific activity of duplicate samples was measured with an enzyme assay kit[6], with the units of activity expressed as that amount of enzyme which will produce one μmole of p-nitrophenol per mg protein • min^{-1}. Protein concentration for each fraction was measured by the micro bicinchoninic acid protein assay[7].

Contamination of the BBM by basolateral membrane components was measured by the activity of the transport protein sodium-potassium adenosine triphosphatase (EC 3.6.1.3; Na/K ATPase). The Na/K ATPase specific activity of duplicate samples was measured by the assay of Jørgenson (1975) as modified by Han Swinkels (personal communication). Liberated orthophosphate was measured by the malachite green assay of Hess and Derr (1975) as modified by Han Swinkels (personal

---

[5]0.0035-0.0040 inch tolerance, Kontes Scientific Glassware, Vineland, NJ.

[6]Kit 246, Sigma Chemical Co,. St. Louis, MO.

[7]Bicinchoninic acid assay, Pierce Chemicals, Inc., Rockford, IL 61105.

communication). Briefly, ATPase specific activity of a membranous tissue, expressed as μg orthophosphate liberated by the ester hydrolysis of adenosine triphosphate to adenosine diphosphate ($ATP + H_20 \rightarrow ADP + P_i^{-2}$) per mg of membrane protein, was determined in the presence and absence of ouabain. Ouabain, a digitalis glycoside, inhibits the activity of Na/K ATPase, but not other transport ATPase proteins, such as Ca^{+2}/Mg^{+2} ATPase or K^+/H^+ ATPase. Thus, the Na/K ATPase specific activity of a membrane can be discerned by the difference in specific activity between two ATP-containing media: one with ouabain and one without. Protein content was determined by the micro bicinchoninic acid assay.

Lipid analysis

The lipid composition of BBM tissue was based on duplicate tissue samples, each containing 15 mg of protein. Fatty acids were extracted from BBM tissue by the method of Folch et al. (1957). Tissue samples were placed in 50-ml PYREX® glass homogenization tubes[8]. Seven milliliters of methanol was added to each tube, followed by homogenization for 10 seconds at setting six in a polytron tissue homogenizer equipped with a 20-mm probe. Fourteen milliliters of chloroform were added to this solution and further homogenized for an additional 10 seconds at setting six. The homogenate was then filtered through a #40 Whatmann filter[9] into a 20 x 150 mm KIMAX® glass extraction tube[10] (phenolic cap with teflon liner). Twelve milliliters of chloroform:methanol (2:1, v/v) were added to the original PYREX® homogenization tube and blended for 10 seconds to rinse the probe and tube of any remaining lipid. This solution was then filtered through the #40 Whatmann filter into the KIMAX® extraction tube. After filtration, 8.5 ml of 0.88% KCl were added to each tube and shaken on a test tube shaker for 5 minutes. The samples were placed in a refrigerator overnight, whereupon, the solvent-aqueous phases separated, and the aqueous phase was

[8]No. 8240, Fisher Scientific, Raleigh, NC 27629.

[9]No. 1440-125, Whatman Paper Ltd., Maidstone, England.

[10]No. 14-930D, Fisher Scientific, Raleigh, NC 27629.

removed by vacuum aspiration the following morning.

The chloroform:methanol:lipid extract phase was evaporated under nitrogen to a volume of approximately 3 ml and transferred by Pasteur pipette to a 13 x 100 mm PYREX® glass tube (phenolic cap with teflon liner). An internal standard of 600 μg of heptadecenoic acid[11] (17:1n7) was added to the extract. This solution was evaporated to near dryness under a constant stream of nitrogen. The lipid fraction was saponified (200 μl 0.5 N NaOH) and methylated (200 μl BF_3; 14% in methanol) according to Metcalfe et al. (1966).

Fatty acid composition of the extracted lipids was determined using a Hewlett-Packard[12] 5,890A chromatograph equipped with a DB225 fused silica capillary column[13]. Individual fatty acids in the samples were identified by retention times of analogous fatty acid standards purchased from Nu-Chek-Prep. Geometric isomers of octadecaenoic acid were separated on Silica Gel G[14] plates by argentation thin-layer chromatography according to the method of Dudley and Anderson (1975).

---

[11]Nu-Chek-Prep, Inc., Elysian, MN 56028.

[12]Hewlett-Packard, Avondale, PA 19311.

[13]J & W Scientific, Folsom, CA 95630-4714.

[14]No. 4410-222, Whatman Paper Ltd., Maidstone, England.

RESULTS AND DISCUSSION

There were no significant differences in the average 28-day body weight (825 g) of the chicks due to dietary treatments. Similarly, average feed efficiency at 28 days (0.60-kg body weight per kg diet consumed) was not affected by dietary treatments. Both these events were not unexpected since the diets were isocaloric and isonitrogenous, differing in the source of the dietary oil but not the caloric content. Compensation for the reduced energy content of the diet was necessary to equalize growth and feed efficiency since the nitrogen-corrected metabolizable energy of HSBO was only 88% that of soybean oil (Experiment 1) when fed to 3-week-old male broiler chickens. Supporting this, Al-Athari and Watkins (1988b) reported growth depression in male broiler chicks fed 5% dietary HSBO compared to chicks fed either 5% soybean oil or 5% spent restaurant grease. Clearly, in this study, as long as the reduced energy densities of the HSBO and palm oil were recognized when formulating the diets, there were no detrimental effects to either body weight gain or feed efficiency in 28-day-old broilers from consumption of diets rich in HSBO.

The purity of the BBM was verified by a 7.5-fold increase in the specific activity of alkaline phosphatase, the BBM enzyme marker, in the P4 pellet compared with its activity in the original homogenate. In contrast, enrichment of Na/K ATPase increased only 24% as much as alkaline phosphatase activity when comparing homogenate and P4 specific activity. Dietary treatments failed to affect the ratio of P4:homogenate alkaline phosphatase enrichment (Table 3). However, alkaline phosphatase specific activity was increased ($P<.05$) in both the initial homogenate and the P4 fraction when chickens were fed HSBO compared to chickens receiving the control, palm, or soybean oil diets (Table 3). This affect on enzyme activity was not ($P>.05$) correlated with either body weight gain or feed efficiency.

Other researchers have reported alterations in membrane function in response to changes in dietary lipid composition. Morson and Clandinin (1986) reported increased activity of glucagon-stimulated adenylate cyclase in hepatic plasma membrane in response to increased membrane

concentration of linoleic and linolenic acids. This response was further correlated with dietary lipid content. McMurchie et al. (1987) indicated that catecholamine-stimulated adenylate cyclase activity of the rat heart was increased by the intake of lipids, particularly by saturates associated with stimulating increased cholesterol content of the heart membrane. Zuniga et al. (1989) reported a 1.8-fold increase in the specific activity of 5'-nucleotidase in hepatic plasma membrane from rats fed diets containing 10% n-3 polyunsaturated fatty acids compared to those receiving either 10% dietary corn or coconut oils. Unquestionably, dietary lipids, many of which are readily incorporated into membrane lipids, alter membrane-associated functions.

From Table 4, it is clear that dietary lipids were readily incorporated into the intestinal mucosal membrane over the 4-week feeding period. Significant differences in the fatty acid composition of the brushed border resulted from dietary treatments, leading to differences in the concentration of 16:1, 17:0, cis-18:1, trans-18:1 isomers, trans-18:2n6, 18:3n3, 20:2n6, 20:3n6, 20:4n6, 22:5n6, and 22:6n3 (Table 4).

Dietary treatments failed to influence the membrane content of either 16:0 or 18:0. Of particular interest, chickens fed the diet rich in 16:0 (palm oil diet) had membrane concentrations of 16:0 no different ($P>.05$) than dietary treatments containing much less palmitic acid. In spite of the fact that the palm oil diet was supplemented with approximately 32 times as much 16:0 as the minimal oil diet, the membrane concentration of palmitate did not differ between the treatments. The invariable amounts of 16:0 and 18:0 may reflect the tenet that acyltransferases preferentially place saturated fatty acids at the sn-1 position of acylglycerols and phospholipids, indicating a homeostatic mechanism for preservation of membrane structure.

Feeding HSBO increased the 16:1n7 concentrations of BBM compared to birds receiving SBO, palm oil and the control diet. Similarly, Al-Athari and Watkins (1988b) reported that diets containing an abundance of HSBO prompted increased liver 16:1n7 concentration compared to chicks receiving SBO. Geometric isomers of oleic acid were readily incorporated into the lipids extracted from the BBM. Since the de novo synthesis of trans fatty acids by avian species and plants is not possible,

isomers containing the trans double bonds were not present in chickens fed diets free from trans fatty acids. Chicks receiving the palm oil diet had increased concentrations of cis-18:1 fatty acids compared to those receiving other dietary treatments.

The levels of 18:2n6 were lower ($P<.06$) in the BBM from chickens fed HSBO or palm oil versus those fed SBO or the control diet. Concomitantly, a product of 18:2n6 metabolism, 20:4n6, was lower in BBM from chicks fed HSBO than in chicks fed the other diets. For HSBO fed birds, this may reflect the relative abundance of 18:2n6 available for desaturation, elongation, and desaturation to 20:4n6 since the ratio of product:substrate (20:4n6/18:2n6) did not differ among the HSBO, SBO, and control dietary treatments (control, 0.19; SBO, .16; HSBO, .16). Other researchers (Watkins, 1988; Rogel and Watkins, 1987) have reported decreased tissue and subcellular concentrations of 20:4n6 in response to feeding hydrogenated vegetable oils to chicks, a drop attributed to inhibitory affects of the HSBO itself. In this study, while there were no differences in the amount of 18:2n6 present between chicks fed palm oil or HSBO supplements, decreased amounts of 20:4n6 were present in the HSBO treatments compared to the palm oil treatments. This certainly indicates the potential for inhibition by trans isomers in the conversion of 18:2n6 to 20:4n6.

Birds receiving the palm oil supplement experienced an increased conversion (20:4n6/18:2n6 = .28) of 18:2n6 to 20:4n6 compared to chicks receiving other treatments. Similarly, Zevenbergen and Haddeman (1989) reported that rodent diets rich in saturates promoted increased arachidonic acid levels compared to diets containing abundant unsaturated fatty acids. Furthermore, they reported that trans fatty acids did not directly influence enzymes involved in eicosanoid synthesis in rats receiving adequate linoleic acid but with trans fatty acids supplying 20% of the dietary energy. Instead, they established that prostacyclin production by aortic segments was a linear function of arachidonic acid concentration in aortic phospholipids.

Linoleic acid, having no endogenous precursors, must be present in the diet of avian species. While the essential fatty acid (EFA) requirement in the broiler has been set at 1% by the NRC (1984), the dietary requirement may be affected by other dietary components. For example, saturated

fats and cholesterol have been shown to increase the linoleate requirement (Takasugi and Imai, 1966). A deficiency of essential fatty acids (linoleic acid) is usually correlated with a corresponding increase in 20:3n9. This metabolite of oleic acid, often termed the deficiency triene, results when insufficient amounts of linoleate or arachidonate prompt the desaturation ($\Delta 6$), elongation, and desaturation ($\Delta 5$) of oleic acid, creating 20:3Δ5,8,11. In this study, where all treatments received sufficient amounts of linoleic acid, there were no overt signs of EFA deficiency. However, Al-Athari and Watkins (1988b) reported a purified diet (EFA-deficient) supplemented with 5% HSBO heightened indices of essential fatty acid deficiency in chick liver microsomes compared to chicks receiving the same basal diet supplemented with equal amounts of other fats (spent restaurant grease or a saturated fat).

In summary, the substitution of one sixth of the caloric content of an isocaloric-isonitrogenous basal diet with 500 kcal of energy supplied by either HSBO, SBO, or palm oil failed to influence body weight gain or feed conversion efficiency of male broiler chicks from hatch to 28 days of age. These treatments had a significant effect on BBM alkaline phosphatase specific activity and fatty acid profiles of chick BBM. Feeding diets rich in HSBO decreased the BBM concentration of arachidonic acid. Finally, chickens receiving the HSBO diet failed to demonstrate observable signs of EFA deficiency.

REFERENCES

Agricultural Statistics, 1986. United States Government Printing Office. Washington, DC.

Al-Athari, A.K., and B.A. Watkins, 1988a. Chromatographic analysis of 18:1 isomers in blended feed-grade fats used in poultry diets. Poultry Sci. 67:307-312.

Al-Athari, A.K., and B.A. Watkins, 1988b. Distribution of trans and cis fatty acid isomers in chicks fed different fats. Poultry Sci. 67:778-786.

Bishop, T., and M. Lentner, 1986. Experimental Design and Analysis. Valley Book Company. Blacksburg, VA.

Burton, F., 1989. Restaurant grease: how much is out there? Render. 18:10-16.

Dudley, P.A., and R.E. Anderson, 1975. Separation of polyunsaturated fatty acids by argentation thin layer chromatography. Lipids. 10:113-114.

Folch, J., M. Lees, and G.H. Sloane-Stanley, 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497-509.

Hess, H.H., and J.E. Derr, 1975. Assay of inorganic and organic phosphorus in the 0.1-5 nanomole range. Anal. Biochem. 63:607-613.

Jørgenson, P.L., 1975. Techniques for the study of steroid effects on membranous (Na^+ + K^+)-ATPase. Methods in Enzymology. 36:434-439.

Kritchevsky, D., 1982. Trans fatty acid effects in experimental atherosclerosis. Federation Proc. 41:2813-2817.

Lanser, A.C., and E.A. Emken, 1987. Comparitive deposition of trans-10- and cis-9-octadecenoates in egg and tissue lipids of the laying hen. J. Agric. Food Chem. 35:248-252.

McMurchie, E.J., G.S. Patten, J.S. Charnock, and P.L. McLennan, 1987. The interaction of dietary fatty acid and cholesterol on catecholamine-stimulated adenylate cyclase activity in the rat heart. Biocim. Biophys. Acta. 898:137-153.

Metcalfe, L.D., A.A. Schimitz, and J.R. Pelka, 1966. Rapid preparation of fatty acid esters from lipids

for gas chromatographic analysis. Anal. Chem. 38:514-515.

Morson, L.A., and M.T. Clandinin, 1986. Diets varying in linoleic acid content alter liver plasma membrane lipid composition and glucagon-stimulated adenylate cyclase activity. J. Nutr. 116:2355-2362.

National Research Council, 1984. Nutrient Requirements of Poultry. National Academy of Science. Washington, DC.

Reichwald-Hacker, I., K. Ilsemann, and K.D, Mukherjee, 1979. Tissue specific incorporation of positional isomers of dietary cis- and trans-octadecenoid acids in the rat. J. Nutr. 109:1051-1056.

Rogel, A.M., and B.A. Watkins, 1987. Liver subcellular fatty acid profiles of chicks fed diets containing hydrogenated fats and varying linoleate levels. Lipids. 22:637-642.

SAS Institute, 1985. SAS User's Guide: Statistics, Version 5 Edition, SAS Inst. Inc. Cary, NC.

Singer, S.J., and G.L. Nicolson, 1972. The fluid mosaic model of the structure of cell membranes. Science. 1275:720-731.

Takasugi, Y., and Y. Imai, 1966. Distribution of lipids in subcellular fractions of fatty livers. J. Biochem. 60:191-197.

Watkins, B.A., 1988. Influences of biotin deficiency and dietary trans-fatty acids on tissue lipids in chickens. Br. J. Nutr. 61:99-111.

Wilson, J., and K.E. Webb, 1990. Simultaneous isolation and characterization of brush border and basolateral membrane vesicles from bovine small intestine. J. Anim. Sci. 68:583-590.

Zevenbergen, J.L., and E. Haddeman, 1989. Lack of effects of trans fatty acids on eicosanoid biosynthesis with adequate intakes of linoleic acid. Lipids. 24:555-563.

Zuniga, M.E., B.R. Lokesh, and J.E. Kinsella, 1989. Disparate effects of dietary fatty acids on activity of 5'-nucleotidase of rat liver plasma membrane. J. Nutr. 119:152-160.

TABLE 1. *Percent composition of diets*

Ingredient	Control Diet	Control + SBO	Control + Palm	Control + HSBO
Soybean meal	42.9	42.9	42.9	42.9
DL-Methionine	.3	.3	.3	.3
Dicalcium phosphate	1.9	1.9	1.9	1.9
Limestone	1.3	1.3	1.3	1.3
Vitamin premix[1]	1.0	1.0	1.0	1.0
Mineral premix[2]	.8	.8	.8	.8
Glucose	10.0	10.0	10.0	10.0
Safflower oil	1.1	1.1	1.1	1.1
Corn starch	40.5	26.8	26.8	26.8
Cellulose	.2	8.2	7.9	7.4
SBO	.	5.7	.	.
Palm oil	.	.	6.0	.
HSBO	.	.	.	6.5
Nutrient analysis:				
Analyzed CP, %	20.6	21.2	21.3	21.8
Analyzed Fat, %	1.7	7.4	7.7	8.2
Ca, %[3]	1.0	1.0	1.0	1.0
Available P, %[3]	.45	.45	.45	.45
TSAA[3]	.93	.93	.93	.93
ME_n, kcal/kg[3]	3,000	3,000	3,000	3,000

HSBO, hydrogenated soybean oil; SBO, soybean oil; TSAA, total sulfur amino acids.

[1]Provides per kilogram of diet: vitamin A palmitate, 4.0 mg; cholecalciferol, .4 mg; vitamin E (DL-α-tocopherol), 80mg; vitamin K (menadione), 1 mg; riboflavin, 3.6 mg; pantothenic acid, 10 mg; niacin, 27 mg; 9 μg vitamin B_{12}, choline, 1500 mg; folic acid, .55 mg; thiamin, 1.8 mg; pyridoxine, 3 mg; biotin, 400 μg; ethoxyquin (66%), 200 mg.

[2]Provides per kilogram of diet: iodized NaCl, 4 g; $MnSO_4 \cdot H_2O$, 1814.6 mg; ZnO, 50 mg; $FeSO_4 \cdot 7H_2O$, 400 mg; $CuSO_4 \cdot 5H_2O$, 31.5 mg; KIO_3, .3 mg; Na_2SeO_3, .32 mg.

[3]Based on National Research Council (1984) tables.

TABLE 2. *Fatty acid composition (weight %) of dietary lipids*

Fatty Acid	Dietary lipid source			
	Safflower	HSBO	Palm	SBO
12:0	ND	ND	.39	ND
14:0	ND	ND	1.23	ND
16:0	7.17	11.13	43.06	11.08
18:0	2.52	12.75	4.56	3.99
18:1	13.08	30.53	40.80	25.98
t-18:1	ND	41.28	ND	ND
t-18:2n6	ND	2.83	ND	ND
18:2n6	77.06	1.31	9.61	51.64
18:3n3	ND	ND	ND	5.95
20:0	.17	.17	.35	.37
20:1n9	ND	ND	ND	.24
22:0	ND	ND	ND	.33

HSBO, hydrogenated soybean oil; SBO, soybean oil.
ND = Not detected, levels below .01% of fatty acid methyl esters present.

TABLE 3. *Comparitive enhancement of the brush border membrane marker alkaline phosphatase relative to the initial homogenate as effected by dietary treatment*

	Specific activity[1]		
Dietary treatment	Homogenate[3]	Final pellet[3]	Enrichment[4]
Minimal oil	.64	5.21	8.14
SBO	1.03	6.38	6.19
Palm oil	1.05	7.78	7.41
HSBO[2]	1.65	8.74	5.30
SEM	.22	.86	1.14

[1]Specific activity, one unit of activity is defined as that amount of enzyme which will produce one micromole of p-nitrophenol per minute under the conditions of the assay.

[2]HSBO, Hydrogenated soybean oil; SBO, soybean oil.

[3]The contrast HSBO vs. all other dietary treatments was significant ($P<.05$).

[4]Enrichment of specific activity of final pellet:homogenate. The effect of dietary treatment was not significant.

TABLE 4. *Fatty acid composition (ug/mg protein) of chicken brush border membrane lipids at 28 days of age*

Fatty Acid	Dietary lipids				Pooled SEM
	Control	HSBO	Palm	SBO	
15:0	1.45	.78	ND	1.42	.24
16:0	45.56	32.54	45.62	36.52	5.41
16:1n7	1.97[b]	3.42[a]	.26[c]	ND[c]	.32
17:0	1.42[b]	.63[a]	.62[a]	1.22[b]	.21
18:0	50.28	40.23	45.44	47.58	6.33
18:1[d]	27.11	23.95	34.92	21.94	4.13
t-18:1[f]	ND[b]	53.22[a]	ND[b]	ND[b]	7.30
t,t-18:2n6[f]	ND	1.95	ND	ND	0.90
18:2n6	75.29	55.41	59.23	75.45	9.27
18:3n3[e]	2.04[b]	1.75[b]	1.28[b]	2.95[a]	0.28
20:0	.50	.65	.61	.47	.26
20.2n6	.62	.44	.16	ND	.19
20:3n6	2.18[a]	1.05[ab]	1.47[ab]	.66[b]	.35
20:4n6[f]	14.28	9.20	16.61	12.28	1.96
22:4n6[f]	2.17	.71	1.99	1.77	.38
22:5n6	.85[a]	ND	ND	2.05[b]	.17
22:6n3[f]	2.01[ab]	.93[b]	1.87[ab]	3.09[a]	.45

HSBO, hydrogenated soybean oil; SBO, soybean oil.

ND = not detected, levels below .01% of fatty acid methyl esters present.

[a-c]For individual fatty acids, values not sharing a common superscript letter were significantly different by SNK ($P < .05$).

[d]The contrast palm oil diet vs. all others was significant ($P < .05$).

[e]The contrast palm and HSBO vs. control and SBO was significant ($P < .05$).

[f]The contrast HSBO vs. all others was significant ($P < .05$).

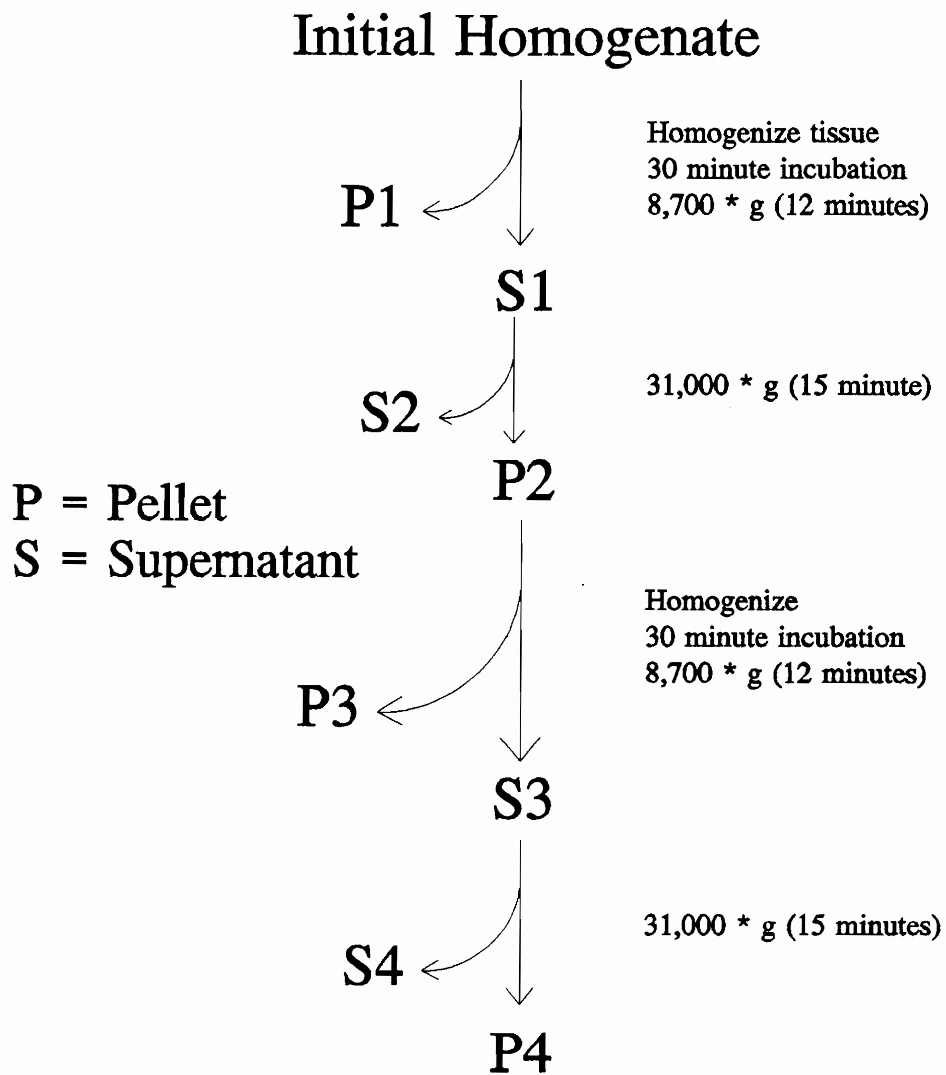


FIGURE 1. Schematic flow chart for the differential purification of chicken intestinal brush border membrane.

CHAPTER III

DIETARY FAT SELECTIVELY ALTERS INTESTINAL LIPID COMPOSITION AND NUTRIENT UPTAKE IN GROWING CHICKENS

ABSTRACT

The effects of supplemental dietary lipids on 1) the lipid composition of intestinal tissue and 2) the in vitro absorptive rate of differing dietary nutrients were studied. Four dietary treatments consisting of 1) a nutritionally-adequate, minimal oil-containing diet (negative control) and 2) the control diet with 6% of the carbohydrate being replaced by either hydrogenated soybean oil (HSBO), soybean oil (SBO), or palm oil. Feeding the experimental diets did not affect body weight. After being fed the experimental diets for 4 weeks, a 30 cm length of mid-small intestine was removed for fatty acid compositional analysis and to determine the in vitro uptake rate for stearic acid, linoleic acid, linolenic acid and glucose.

Results indicate that the fatty acid composition of the intestinal tissue does not necessarily reflect the make-up of dietary lipids. Intestinal concentrations of palmitic and oleic acid were not affected by dietary treatment. Chickens receiving the HSBO or the palm oil diet had less intestinal 18:0, 18:2n6, 18:3n3, and 20:4n6 than did those offered SBO or the control diet.

Linoleate and oleate were absorbed at a faster rate than stearic acid across all treatments, indicating preferential absorption of fatty acids possessing a less lipophilic physical character. The in vitro uptake rates of oleate and linoleate were not affected by dietary treatment. Stearate and glucose uptake was reduced in chickens fed HSBO or palm oil compared to those offered either the control diet or SBO.

INTRODUCTION

Supplementation of poultry diets with dietary fats and oils has become an accepted convention to increase the metabolizable energy content of diets to levels established by the National Research Council (NRC, 1984). The effects of these supplements, virtually all of which are composed of a diverse blend of waste fats and oils containing significant amounts of hydrogenated vegetable oils (Al-Athari and Watkins, 1988), on the intestinal transport of nutrients in the broiler chicken is not known.

The transport of nutrients across a membrane is influenced by lipid components. Thomson et al. (1986) reported that rats fed diets supplemented (20%, w/w) with saturated fats had altered intestinal uptake of leucine, glucose, and differing fatty acids. These same diets reduced mean villus height, width, thickness, surface area, cell size, villus density, and mucosal surface area in the jejunum and ileum. In a related study, Thomson et al. (1989) associated a reduced intestinal uptake of cholesterol in rats fed a diet supplemented (20%, w/w) with a combination of eicosapentaenoic/docosahexaenoic acid (EPA/DHA) and hydrogenated beef tallow with reduced intestinal uptake of dietary cholesterol.

Membrane lipids furnish the polar/nonpolar surface charge densities necessary to accommodate the noncovalent anchoring of tertiary membrane proteins. In addition to noncovalent interactions, recent research has discovered examples of proteins strongly bound to membrane lipids by covalent forces (Futerman et al., 1985). The lipid attachment site appears to specifically involve the inositol group of phosphatidylinositol (PI). A subsequent membrane phospholipid function recently identified by biochemists was that the hydrolysis of membrane PI by ligand-activated phospholipase C is part of a signal-transducing mechanism (Nishizuka, 1984). These important membrane lipid functions, together with literature citations indicating ready dietary alteration of membrane and tissue phospholipids (Keelan et al., 1986; Watkins, 1988), indicates the potential for a multitude of consequences to metabolism via manipulation of dietary lipids. Additionally, in Experiment 2,

alterations in the fatty acid content of diets fed to broiler chickens from hatch to 4 weeks of age was demonstrated to affect lipid composition and enzymatic activity of intestinal brush border membranes. Accordingly, this study was undertaken to establish the effects of supplemental dietary lipids on: 1) the lipid composition of intestinal tissue; and 2) the absorptive rate of several dietary nutrients.

MATERIALS AND METHODS

Sixty-four male broiler chicks, hatched November 6, 1990, were equally and randomly divided among 16 pens in a Petersime[1] starter battery. Four replicates of each dietary treatment (Table 1) were represented in the 16 experimental units. The four dietary treatments were: 1) a negative control diet, containing the minimal amount of safflower oil[2] to satisfy NRC requirements for 18:2n6; and 2) the control diet with six percent of the carbohydrate being replaced by one of three dietary lipids: a) an oil rich in trans fatty acids (hydrogenated soybean oil, HSBO[3]); b) a saturated fat source (palm oil)[4]; or c) an oil rich in polyunsaturates (soybean oil, SBO)[5]. Chicks were provided ad libitum amounts of diet and water from hatch through the sampling period (initiated at 28 days of age).

At 28 days, and continuing for four successive days, one bird from each treatment (four birds/day) was randomly selected, killed by cervical dislocation, and laporotomized. Based on work by Hurwitz et al. (1973) and Sturkie (1986) indicating the jejunum was the site of maximal lipid uptake, a 30-cm length of small intestine, taken equidistant from the midpoint of the small intestine, was removed and rinsed with ice-cold saline. Adherent fat was removed prior to opening the intestine along its mesenteric border. The mucosal surface was washed gently with cold saline to remove visible mucus and digesta.

Intact intestinal sections from four chickens per dietary treatment were excised from each washed segment and kept on ice until frozen at -20°C for later lipid analyses. Lipids were extracted according to the method of Folch et al. (1957). Saponification (400 µl of 0.5 N NaOH in methanol) and methylation (400 µl BF_3; 14% in methanol) followed the procedure of Metcalfe et al. (1966).

---

[1]Petersime Incubator Co., Gettysburg, OH.

[2]Hollywood Oil Co., Los Angeles, CA.

[3]Fan Fry®, Lot 1088, Bunge Edible Oil Corp., Kankakee, IL, 60901.

[4]RBD Palm Oil, Lot 48059, Kraft Ingredients Corp., Memphis, TN 38101.

[5]Imperial Oil®, Lot 1025, Bunge Edible Oil Corp., Kankakee, IL 60901.

Fatty acid profiles for each sample were ascertained using a Hewlett-Packard[6] Model 5,890A gas chromatograph equipped with a Model 3,893A integrator and outfitted with a DB 225 fused silica capillary column[7]. Fatty acid concentrations of entire intestinal segments were expressed as μg/mg tissue protein. Tissue protein content was assayed by the micro bicinchoninic protein assay[8].

Circular intestinal pieces (diameter = 7/16 inch, 1.1 centimeter) were cut from the excised segment and mounted as a flat disk (mucosal surface exposed) in incubation chambers (Westergaard and Dietschy, 1974). The prepared incubation chambers were placed in a preincubation media (40°C and pH 7.4) containing oxygenated (95% O_2:5% CO_2) Krebs-bicarbonate buffer (Umbreit et al., 1972) for 10 minutes.

The concentration of the three lipid probes in each incubation solution was .1 mM (stearic acid, 2.84 mg/dl; oleic acid, 2.82 mg/dl; linoleic acid, 2.80 mg/dl). Using sterile techniques and solutions, 1.50 μCi [^{14}C]-stearate[9], 1.20 μCi [^{14}C]-oleate[10], and 1.01 μCi [^{14}C]-linoleate[11], plus the appropriate amount of each non-labeled lipid probe to equal a final lipid concentration equivalent to .1 mM was dissolved in chloroform-methanol (2:1, v/v) in an incubation beaker. The chloroform-methanol phase was then evaporated under nitrogen to ensure complete removal of the lipid-carrier solvents (chloroform-methanol or ethanol). Fifty ml of 40 mM taurodeoxycholic acid in Krebs-bicarbonate buffer (with Ca^{++} omitted and pH 7.4) was then added to the incubation beaker, sealed with parafilm, stirred with a magnetic stirbar for 2 hours, and then further diluted with 50 ml Krebs-bicarbonate buffer (Ca^{++} omitted and pH 7.4) to give a final volume of 100 ml. The solution was

---

[6]Hewlett-Packard Co., Avondale, PA 19311.

[7]J & W Scientific Company, Rancho Cordova, CA.

[8]Bicinchoninic acid assay, Pierce Chemicals, Rockford, IL 61105

[9]American Radiolabeled Chemicals, Inc., Lot number 89721, St. Louis, MO 63146.

[10]American Radiolabeled Chemicals, Inc., Lot number 8961, St Louis, MO 63146.

[11]E.I.du Pont Co., Lot number 2687-073, Wilmington, DE 19898.

then gassed with 95% O_2:5% CO_2 for 5 minutes, sealed with parafilm, and gently stirred in a 40°C waterbath overnight. The next morning the solution was again gassed for 2 hours at 40°C and, if necessary, the pH was adjusted to 7.4. Thirty µCi of [^{3}H]-inulin[12] was added to each beaker to measure the adherent mucosal fluid volume before beginning each assay.

One hundred milliliters of a 10 mM glucose incubation solution (180 mg/dl) in Krebs-bicarbonate buffer was prepared to measure intestinal uptake of glucose. To each incubation beaker, 3.00 µCi [^{14}C]-glucose[13] plus an appropriate amount of non-labeled glucose to bring the final glucose concentration to 10 mM was added immediately prior to beginning the assay. Thirty µCi [^{3}H]-inulin were added to measure the adherent fluid volume.

After preincubation, the chambers were transferred to a water bath containing the incubation beakers (40°C), and gassed with 95% O_2:5% CO_2. The media was stirred at 630 rpm using spinfin magnetic stirbars[14] to reduce the effective resistance of the intestinal unstirred water layer (Thomson et al., 1987). After precisely 6 minutes, nutrient uptake was terminated by removing the chamber from the incubation beaker and rinsing the exposed tissue with ice-cold saline for 5 seconds. The exposed tissue disks were then cut from the incubator with a sharpened steel punch (diameter = 7/16 inch, 1.1 centimeter) and gently blotted on moistened filter paper. Two tissue disks per incubator were then transferred to individual, tared, glass scintillation vials and dried overnight in a 75°C oven. After weighing, the tissue disks were solubilized with 1 ml of 1.25 N NaOH. Neutralizer scintillation cocktail[15] was added, and radioactivity for [^{3}H]- and [^{14}C]-channels was determined using an external standard source to correct for quench[16] (H number) as calculated from ^{14}C and ^{3}H quench curves.

---

[12]E.I. du Pont Co., Lot 2704-112, Wilmington, DE 19898.

[13]E.I. du Pont Co., Lot 2649-140, Wilmington, DE 19898.

[14]Model S8314-24, Baxter Scientific Products, McGaw Park, IL.

[15]Neutralizer, Number 11174, Research Products International, Mount Prospect, IL 60056.

[16]Model LS 7500, Beckman Instruments, Fullerton, CA 92634.

The volume of the adherent mucosal fluid (AMF) was calculated by dividing the concentration of the AMF marker in the tissue by its concnetraiton in the incubation media. The quantity of probe found in this volume is subtracted from the total mass of probe molecule in the tissue slice. This subtraction yields the probe mass actually absorbed through the brush border membrane and was described by (Sallee et al., 1972):

$$J = \frac{P - (AMF \times R)}{P_s \times \min \times mg} \times 100$$

where J = nmoles probe absorbed/minute/100mg of dry tissue,

P = dpm of AMF marker in tissue,

P_s = dpm/nmole of the probe molecule in the incubation media,

and R = (dpm probe/µl IM) ÷ (dpm AMF marker/µl IM).

RESULTS AND DISCUSSION

The fatty acid composition of the four dietary lipids are listed in Table 2. All diets contained equal amounts of the basal diet, which was supplemented with 18:2n6-rich safflower oil to conform to NRC requirements for linoleic acid. The HSBO was the only dietary lipid source found to contain positional and trans geometric fatty acids. Isomers of 18:1 monoenes were identified as the unresolved peak at 10.94 minutes immediately following the 18:1n9 peak at 10.70 minutes. Thus, 18:1n9 and the positional cis-isomers of octadecenoic acid are described as total cis-18:1 fatty acids. Similarly, geometric isomers of trans-18:1 were not partitioned from elaidic acid by the chromatographic approach used in this experiment; all trans-18:1 isomers were amalgamated into a single trans-18:1 class.

Intestinal lipid composition

In the present experiment, dietary lipids were incorporated into the lipids of the small intestine of four-week old male broilers (Table 3). This supports a similar conclusion made by Fritsche et al. (1991) and Watkins (1988) pertaining to a variety of chicken tissues. These experiments indicated the fatty acid composition of dissimilar classes of tissues (heart, liver, serum, thymus, and bursa of Fabricius) mirrored the lipid content of the diet.

Dietary oils did not affect the concentration of palmitic acid (16:0, expressed as μg fatty acid/mg tissue protein) within the small intestine. In Experiment 2, a similar 16:0 response was seen in the fatty acid profile of chicken brush border membranes in spite of feeding diets with vastly different amounts of palmitic acid. These results indicate the existence of a homeostatic mechanism to maintain equivalent tissue concentrations of palmitic acid despite greatly differing dietary concentrations of the fatty acid.

Intestinal tissue concentrations of stearic acid (18:0) were affected by dietary treatments. Chickens fed either palm oil or HSBO contained less 18:0 than those fed either SBO or the control diet. This dietary effect could be the result of feedback inhibition of acetyl-SCoA carboxylase by

palmitic and stearic acids (Wakil et al., 1983).

Concentrations of cis-18:1 fatty acids were not affected by dietary treatments. Oleic acid (18:1n9), which is readily synthesized in animal systems, is generally considered to be the principal depot fatty acid in the chicken. As such, it would not be unexpected that the de novo synthesis of oleic acid, particularly when a surfeit of dietary energy is available to furnish acetyl-SCoA substrate, would result in equivalent tissue concentrations of 18:1 regardless of dietary oil concentration within the diet.

The presence of geometric isomers was dependent on the presence of HSBO in the diet. These trans-containing isomers, all derived from the exogenous dietary source, were found only in chickens fed the HSBO diets. The presence of trans-isomers found in the brush border membrane (Experiment 2) and in this study reinforces work by Emken (1981) which demonstrated a self-limiting concentration of trans-isomers in human tissues from subjects consuming HSBO.

There was a dramatic decrease in intestinal content of linoleic and arachidonic acids in chickens fed palm oil or HSBO. Since all diets contained equal amounts of the safflower oil-supplemented basal diet (NRC, 1984), with the minimal-oil control diet containing the least amount of 18:2n6, altered linoleate metabolism by the supplemental palm oil and HSBO may be responsible for decreased 18:2n6 incorporation into intestinal tissue. Previous research has demonstrated trans-isomers of 18:1 inhibit the desaturation of linoleic acid by rat liver microsomes (Mahfouz et al., 1980). Lawson et al. (1983) reported suppression of arachidonate from both positional and geometric isomers of octadecenoates in the rat, with the greatest inhibition by geometric isomers.

Levels of the n-3 fatty acid α-linolenic acid (18:3n3) were decreased in chickens fed palm oil or HSBO compared to those receiving the control or SBO diets. Analogous results were reported by Garg et al. (1990). They found decreased serum concentrations of 18:3n3 ensued in rats fed hydrogenated beef tallow (HBT) compared to those receiving fish oil or linseed oil alone or in combination with HBT. Similarly, in this study, the concentration of the 18:3n3 metabolite docosahexaenoic (22:6n3) acid paralleled that of 18:3n3, i.e., chickens fed palm oil or HSBO had

reduced concentrations of 18:3n3 ($P<.06$) compared to chickens fed SBO or the control diet. Phetteplace and Watkins (1989) reported practical broiler diets supplemented with oils rich in n-3 fatty acids and their metabolites were readily incorporated into chicken tissues. These fatty acids, when supplied from fish oil, have been associated with beneficial health consequences in humans.

Nutrient uptake

In all in vitro uptake studies, the bulk phase was stirred at 630 rpm to reduce the effective resistance of the unstirred water layer. Intestinal tissue slices taken from the mid-small intestine were incubated with one of four radioactive solutions to measure the uptake of glucose, stearate, oleate, or linoleate over a 6-minute time period in response to dietary treatments. Uptake rates of [^{14}C]-probes was determined after correcting radioactivity for probe molecules present in the adherent fluid layer but not absorbed by the enterocyte (Westergaard and Dietschy, 1974). Uptake rates were expressed as nanomoles of probe molecules absorbed per 100 mg of dry tissue per minute (nmol·100 $mg^{-1}·min^{-1}$).

Across all dietary treatments, linoleic acid and oleic acids were absorbed at a faster rate (1.115 and 1.058 nmol·100 $mg^{-1}·min^{-1}$, respectively) than stearic acid (.722 nmol·100 $mg^{-1}·min^{-1}$; Figure 1). Thomson and Dietschy (1981) recounted in their review of nonruminant lipid absorption that long chain fatty acids solubilized in bile acid micelles infiltrate the mucosal membrane in molecular form (not as an intact micellular entity). Thus, molecular passage through the aqueous unstirred water layer would be enhanced by hydrophilic physical traits. Moreover, the addition of double bonds to long chain fatty acids was shown to reduce their lipophilic properties (Sallee, 1978). These facts, based on the hydrophilic/lipophilic character of a molecule, could explain preferential absorption of unsaturated long chain fatty acids of a particular chain length over the comparable saturate. Moreover, Westergaard and Dietschy (1976) reported the solubility of fatty acids in Krebs-bicarbonate buffer at 37°C decreases by a factor of 2.32 for each methylene group added to the chain, with a concomitant decrease in membrane permeability to fatty acids.

The rate of uptake of oleate and linoleate into intestinal tissue was not affected by dietary

treatment. Others have reported a lack of dietary lipid effects on the intestinal uptake of various lipids. Thomson et al. (1988) reported that diets rich in n-3 fatty acids (eicosapentaenoic acid and docosahexaenoic acid) did not influence intestinal absorption of cholesterol in rats. They concluded that decreased serum cholesterol levels resulting from consumption of n-3 fatty acids would not likely result from alterations in intestinal absorption of cholesterol. Thomson et al. (1987) reported that diets supplemented with saturates (16:0 and 18:0), oleic acid, linoleic acid, or linolenic acid influenced the absorption of only one fatty acid (linoleic acid) out of five tested lipid probes in rats (Thomson et al., 1987).

The chickens fed palm oil or HSBO had decreased rates of absorption of stearic acid than did chickens fed either the control diet or SBO (Figure 2). Johnson and Borgström (1964) first identified fatty acid absorption as being a passive process related linearly to lipid concentration, but enzymatically independent based on their observation that the uptake of oleic acid was not affected by heat inactivation or metabolic inhibitors. In the present experiment, interference with the passive uptake of stearate may have resulted from increased cytosolic concentration of 18:0 or 18:0-mimicking trans geometric isomers, forming an osmotic barrier to uptake. This concentration gradient could amass due to enzymatic hindrance by these nutrients to oxidation or biosynthesis into intestinal triglyceride portomicron vessels. Since glycerol or a glycerol precursor such as glucose is essential for triacylglyceride formation, these fatty acids may accumulate because of insufficient glucose concentrations, resulting from reduced glucose uptake (see below).

The intestinal uptake of glucose, which is mediated by an active process, was altered by dietary fatty acid concentration (Figure 3). There were several intriguing single-degree-of-freedom linear contrasts which evolved from this work. First, and most significant ($P<.005$), chickens fed diets supplemented with HSBO or palm oil had decreased glucose uptake rates compared to chickens receiving the control diet or supplemental SBO. Thomson et al. (1986) indicated similar results when a diet high in saturated fats (20%, w/w) was fed to adult rats, inducing a reduction in jejunal uptake of glucose. Second, and significant at $P<.03$, was that oil supplementation in general decreased

intestinal absorption of glucose.

Since glucose absorption into the small intestine must occur against a concentration gradient, uptake is mediated by a class of Na^+-dependent, lipophilic, membrane transporter proteins (Kasanicki and Pilch, 1990). In muscle cells, glucose transport is initiated by the insulin-stimulated exocytosis of the transporter from the cytosolic space to the cell membrane (Simpson and Cushman, 1986), with an analogous increase in the rate of glucose absorption of up to 30-fold (Kasanicki and Pilch, 1990).

Insulin secretion from the pancreatic β-cell is partitioned into three groups according to their mode of action: 1) nutrient metabolites; 2) hormones; and 3) neural secretions (Wolheim and Sharp, 1981). There are, of course, differences in the degree to which different nutrient metabolites, particularly amino acids and glucose, are able to prompt insulin release. However, glucose , because of its ability for direct incorporation and metabolism within the β-cell itself, is the primary nutrient stimuli for insulin release. Thus, diets containing significant amounts of lipids could be hypoinsulinemic and explain reduced glucose uptake. Finally, factors increasing plasma membrane fluidity (saturated fatty acids) have been shown to influence glucose absorption (Pilch et al., 1980). In the current study, diets supplemented with HSBO or palm oil would mimic these effects, perhaps explaining decreased glucose absorption from chicks fed these diets.

REFERENCES

Al-Athari, A.K., and B.A. Watkins, 1988. Chromatographic analysis of 18:1 isomers in blended-feed grade fats used in poultry diets. Poultry Sci. 67:307-312.

Emken, E.A., 1981. Metabolic aspects of positional monosaturated fatty acids isomers. J. Am. Oil Chem Soc. 58:278-283.

Folch, J., M. Lees, and G.H. Sloane-Stanley, 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497-509.

Fritsche, K.L., N.A. Cassity, and S.C. Huang, 1991. Effect of dietary fats on the fatty acid compositions of serum and immune tissues in chickens. Poultry Sci. 70:1213-1222.

Futerman, A.J., M.G. Low, K.E. Ackermann, W.R. Sterman, and I. Silman, 1985. Identification of covalently bound inositol in the hydrophobic membrane-anchoring domain of Torpedo acetylcholinesterase. Biochem. Biophys. Res. Commun. 129:312-317.

Garg, M.L., A.B.R. Thomson, and M.T. Clandinin, 1990. Interactions of saturated, n-6 and n-3 polyunsaturated fatty acids to modulate arachidonic acid metabolism. J. Lipid Res. 31:271-277.

Hurwitz, S., A. Bar, M. Katz, C. Sklain, and P. Budowski, 1973. Absorption and secretion of fatty acids and bile acids in the intestine of the laying fowl. J. Nutr. 103:543-547.

Johnson, J.M., and B. Borgström, 1964. The intestinal absorption and metabolism of micellar solutions of lipids. Biochim. Biophys. Acta. 84:412-423.

Kasanicki, M.A., and P.J. Pilch, 1990. Regulation of glucose-transporter function. Diabetes Care. 13:219-227.

Keelan, M., M.T. Clandinin, K. Walker, and A.B.R. Thomson, 1986. Dietary fat saturation alters rat brush border membrane phospholipid fatty acid composition. Clin. Invest. Med. 9:A54-61.

Lawson, L.D., E.G. Hill, and R.T. Holman, 1983. Suppression of arachidonic acid in lipids of rat tissues by dietary mixed isomeric and cis and trans octadecenoates. J. Nutr. 113:1827-1835.

Mahfouz, M., S. Johnson, and R.T. Holman, 1980. The effect of isomeric trans-18:1 acids on the

desaturation of palmitic, linoleic and eicosa-8,11,14-trienoic acids by rat liver microsomes. Lipids. 15:100-107.

Metcalfe, L.D., A.A. Schmitz, and J.R. Pelka, 1966. Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. Anal. Chem 38:514-515.

National Research Council, 1984. Nutrient Requirements of Poultry. National Academy Press. Washington, DC.

Nishizuka, Y., 1984. Turnover of inositol phospholipids and signal transduction. Science. 225:1367-1370.

Phetteplace, H.W., and B.A. Watkins, 1989. Effects of various n-3 lipid sources on fatty acid compositions in chicken tissues. J. Food Comp. Anal. 2:104-117.

Pilch, P.F., P.A. Thompson, and M.P. Czech, 1980. Coordinate modulation of D-glucose transport activity and bilayer fluidity in plasma membranes from control and insulin treated cells. Proc. Natl. Acad. Sci. 77:915-918.

Sallee, V.L., F.A. Wilson, and J.M. Dietschy, 1972. Determination of unidirectional uptake rates for lipids across the intestinal brush border. J. Lipid Res.13:184-192.

Sallee, V.L., 1978. Fatty acid and alcohol partitioning with intestinal brush border and erythrocyte membranes. J. Membr. Biol. 43:187-201.

Simpson, I.A., and S.W. Cushman, 1986. Hormonal regulation of mammalian glucose transport. Annu. Rev. Biochem. 55:1059-1089.

Sturkie, P.D., 1986. Avian Physiology. 4th edition. Springer-Verlog. New York, New York.

Thomson, A.B.R., and J.M. Dietschy, 1981. Intestinal lipid absorption: major extracellular and intracellular events. Page 1147 in: Physiology of the Gastrointestinal Tract. L.R. Johnson, ed. Raven Press, New York.

Thomson, A.B.R., M. Keelan, M.T. Clandinin, and K. Walker, 1986. Dietary fat selectively alters transport properties of rat jejunum. J. Clin. Invest. 77:279-288.

Thomson, A.B.R., M. Keelan, M. Garg, and M.T. Clandinin, 1987. Spectrum of effects of dietary

long-chain fatty acids on rat intestinal glucose and lipid uptake. Can. J. Physiol. Pharmacol. 65:2459-2465.

Thomson, A.B.R., M. Keelan, M. Garg, and M.T. Clandinin, 1988. Dietary effects of ω3-fatty acids on intestinal transport function. Can. J. Physiol. Pharm. 66:985-992.

Thomson, A.B.R., M. Keelan, M. Garg, and M.T. Clandinin, 1989. Influence of dietary fat composition on intestinal absorption in the rat. Lipids. 24:494-501.

Umbreit, W.W., R.H. Burris, and J.F. Stauffer, 1972. Manometric & Biochemical Techniques. Burgess Publishing Company. Minneapolis, MN.

Wakil, S.J., J.K. Stoops, and V.C. Joshi, 1983. Fatty acid synthesis and its regulation. Annu. Rev. Biochem. 52:537-579.

Watkins, B.A., 1988. Influences of biotin deficiency and dietary trans-fatty acids on tissue lipids in chickens. Br. J. Nutr. 61:99-111.

Westergaard, H., and J.M. Dietschy, 1974. Validation of a chamber that allows measurement of both tissue uptake rates and unstirred layer thickness in the intestine. Gastroenterology. 67:652-661.

Westergaard, H., and J.M. Dietschy, 1976. The mechanism whereby bile acid micelles increase the rate of fatty acid and cholesterol uptake into the intestinal mucosal cell. J. Clin. Invest. 58:97-108.

Wolheim, C.B., and G.W.G. Sharp. 1981. Regulation of insulin release by calcium. Physiol. Rev. 61:914-973.

TABLE 1. *Percent compostition of diets*

Ingredient	Control	SBO	Palm	HSBO
Soybean meal	47.12	47.12	47.12	47.12
DL-Methionine	.25	.25	.25	.25
Dicalcium phosphate	1.8	1.8	1.8	1.8
Limestone	1.29	1.29	1.29	1.29
Vitamin premix[1]	1.0	1.0	1.0	1.0
Mineral premix[2]	.8	.8	.8	.8
Safflower oil	1.23	1.23	1.23	1.23
Corn starch	46.51	40.51	40.51	40.51
SBO	.	6.0	.	.
Palm oil	.	.	6.0	.
HSBO	.	.	.	6.0
Calculated nutrient analysis[3]				
Crude protein, %	23	23	23	23
Calcium, %	1.0	1.0	1.0	1.0
Available P, %	.45	.45	.45	.45
TSAA, %	.93	.93	.93	.93

HSBO, hydrogenated soybean oil; SBO, soybean oil; TSAA, total sulfur amino acids.

[1]Provides per kilogram of diet: vitamin A palmitate, 4.0 mg; cholecalciferol, .4mg; vitamin E (DL-α-tocopherol), 80mg; vitamin K (menadione), 1 mg; riboflavin, 3.6 mg; pantothenic acid, 10 mg; niacin, 27 mg; vitamin B_{12}, 9 μg vitamin B_{12}, choline, 1500 mg; folic acid, .55 mg; thiamin, 1.8 mg; pyridoxine, 3 mg; biotin, 400 μg; ethoxyquin (66%), 200 mg.

[2]Provides per kilogram of diet: iodized NaCl, 4 g; $MnSO_4 \cdot H_2O$, 1814.6 mg; ZnO, 50 mg; $FeSO_4 \cdot 7H_2O$, 400 mg; $CuSO_4 \cdot 5H_2O$, 31.5 mg; KIO_3, .3 mg; Na_2SeO_3, .32 mg.

[3]Based on National Research Council (1984).

TABLE 2. *Fatty acid composition (weight %) of dietary lipids*

Fatty Acid	Dietary lipid source			
	Safflower	HSBO	Palm	SBO
12:0	ND	ND	.39	ND
14:0	ND	ND	1.23	ND
16:0	7.17	11.13	43.06	11.08
18:0	2.52	12.75	4.56	3.99
18:1	13.08	30.53	40.80	25.98
t-18:1	ND	41.28	ND	ND
t-18:2n6	ND	2.83	ND	ND
18:2n6	77.06	1.31	9.61	51.64
18:3n3	ND	ND	ND	5.95
20:0	.17	.17	.35	.37
20:1n9	ND	ND	ND	.24
22:0	ND	ND	ND	.33

HSBO, hydrogenated soybean oil; SBO, soybean oil.
ND = Not detected, levels below .01% of fatty acid methyl esters present.

TABLE 3. *Fatty acid composition (ug/mg tissue protein) of chicken small intestine at 28 days of age*

Fatty Acid	Dietary lipids				Pooled SEM
	Control	SBO	Palm	HSBO	
14:0	ND	ND	.33[a]	.11[b]	.03
15:0	ND	ND	.11	.09	.02
16:0	13.94	11.87	13.42	6.57	3.13
16:1n7	2.38	.53	1.10	.51	.81
17:0	.20[ab]	.26[a]	.11[b]	.12[b]	.03
18:0[1]	8.42	8.84	5.49	5.80	1.07
18:1	15.40	14.08	18.89	11.25	5.23
t-18:1[2]	ND[b]	ND[b]	ND[b]	3.39[a]	.88
18:2n6[1]	16.65	29.32	9.99	10.29	3.64
t,t-18:2n6[2]	ND[b]	ND[b]	ND[b]	.43[a]	.05
18:3n3[1]	.54	2.03	.32	.36	.27
18:3n6[1]	.15	.16	.05	.02	.05
20:0	.08	.17	.10	.07	.03
20:1n9	.11	.17	.15	.04	.07
20:2n6[1]	.08	.32	.03	.03	.05
20:3n6	.41	.24	.25	.31	.06
20:4n6[1]	3.08	2.66	1.74	1.58	.36
22:4n6[1]	.59	.49	.23	.21	.08
22:6n3	.29	.35	.11	.12	.09

HSBO, hydrogenated soybean oil; SBO, soybean oil.
ND = Not detected, levels below .01% of fatty acid methyl esters present.
[ab]For individual fatty acids, unless otherwise denoted by contrasts, values not sharing a common superscript letter were significantly different by SNK ($P < .05$).
[1]The contrast palm and HSBO vs. control and SBO was significant ($P < .05$).
[2]The contrast HSBO vs. all others was significant ($P < .05$).

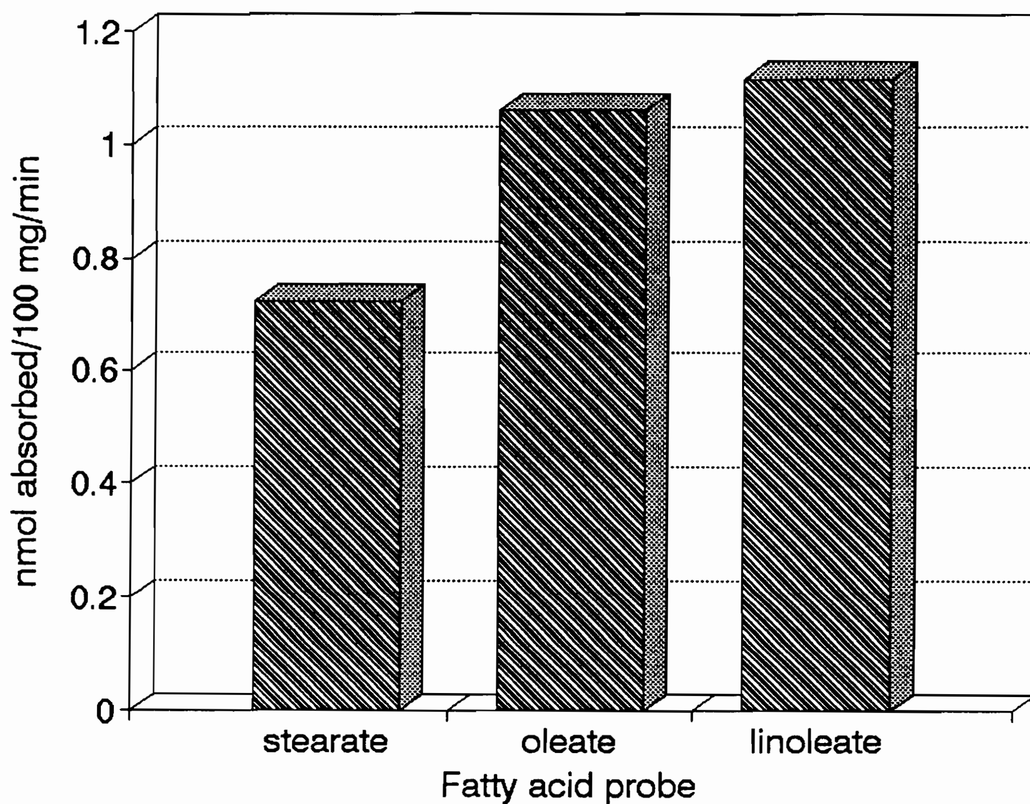


FIGURE 1. Rate of lipid uptake across all dietary treatments. Chicks were fed diets which were supplemented with 6% hydrogenated soybean oil (HSBO), soybean oil (SBO), or palm oil. The contrast stearate vs. oleate and linoleate was significant ($P<.05$).

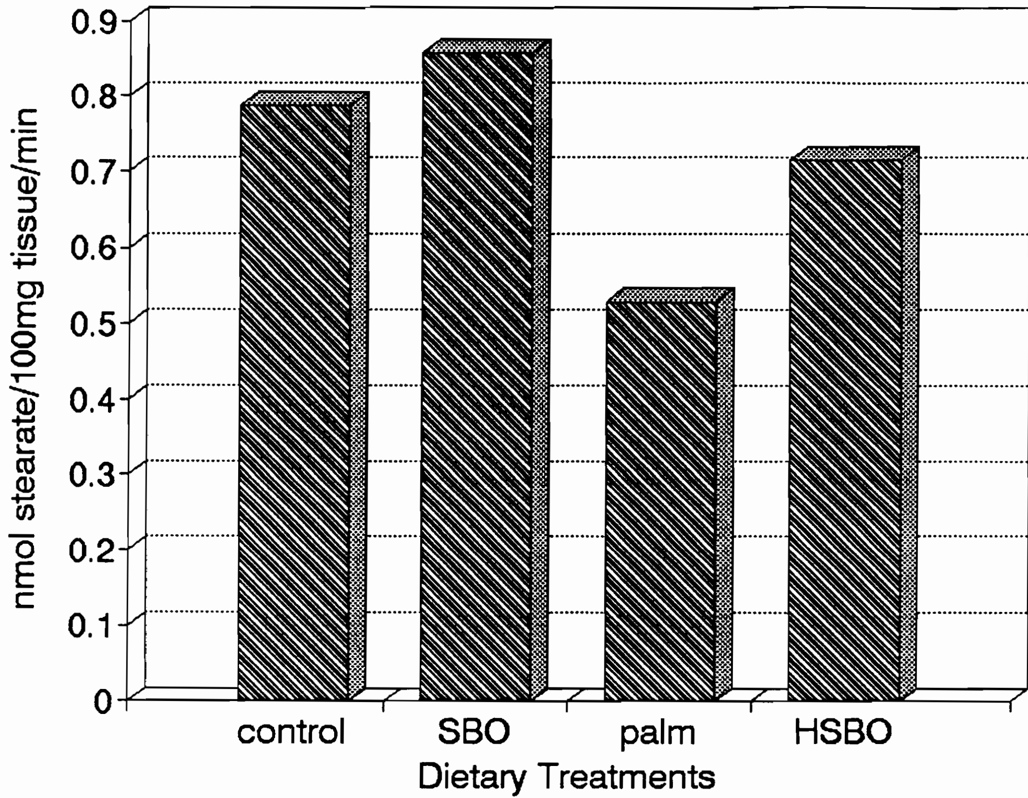


FIGURE 2. Effect of dietary lipid source on the rate of stearic acid uptake (n = 8). Chicks were fed diets which were supplemented with 6% of the fat source from 0 to 28 days-of-age. The contrast hydrogenated soybean oil (HSBO) and palm oil vs. soybean oil (SBO) and the control diet was significant (P<.05).

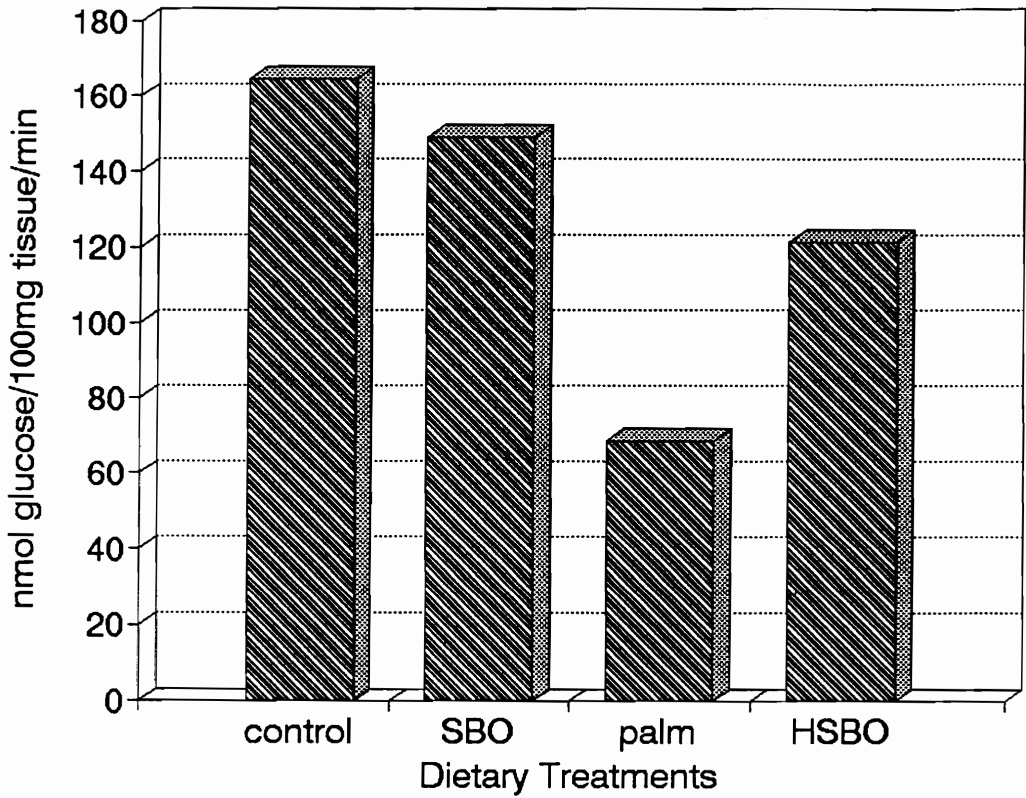


FIGURE 3. Effect of dietary lipid source in chicken intestinal tissue on the rate of glucose uptake (n = 8). Chicks were fed diets which were supplemented with 6% hydrogenated soybean oil (HSBO), soybean oil (SBO), or palm oil. The contrast HSBO and palm oil vs. SBO and the control diet was significant ($P<.05$).

CHAPTER IV

SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSIONS

In Experiment 1, the nitrogen-corrected metabolizable energy values of soybean oil and hydrogenated soybean oil were determined. Trans monoenes were present only in HSBO at 41% of the total fatty acids present in the sample. The AME_n for each oil increased as a linear function of dietary lipid concentration. This differs from some reports which indicated an "extra-caloric" effect upon AME_n from increased dietary concentrations of dietary lipid, such that the AME_n of an oil could exceed its gross energy content. The AME_n contents of SBO and HSBO were determined to be 8,739 and 7,657 kcal/kg oil, respectively. These values represent 92.4 and 80.9% of their respective gross energy values.

Results from the second experiment indicate the isocaloric substitution of dietary carbohydrate with HSBO, SBO, or palm oil failed to influence body weight or feed efficiency from the period from hatch to 4-weeks of age. Alkaline phosphatase specific activity was greatest in chicks fed HSBO compared to those fed any other dietary treatment. Intestinal brush border concentrations of 16:0, 18:0, and cis-18:1 were not affected by diet. However, less 18:2n6 ($P<.06$) and 20:4n6 ($P<.05$) were present in the brush border of chicks given HSBO than in those fed all other treatments. Trans isomers of 18:1 were incorporated into the brush border membrane at greater concentrations than were found in intact intestinal segments of chickens offered similar diets.

In Experiment 3, the concentration of cis-monoenes of either 16 or 18 carbons were not affected by the dietary treatments. Chickens receiving the HSBO or the palm oil diets had less intestinal 18:0, 18:2n6, 18:3n3, and 20:4n6 than did those given SBO or the control diet. Across all dietary treatments, 18:1n9 and 18:2n6 were preferentially absorbed by intestinal slices compared to 18:0 during a 6-minute in vitro uptake assay. The uptake rates of 18:1n9 and 18:2n6 were not affected by dietary treatment. However, 18:0 and glucose intestinal uptake was reduced in chickens fed HSBO or palm oil compared to chickens fed either the control diet or SBO.

Experiments 2 and 3 demonstrate ready incorporation of trans monoenes into intestinal

tissues of those fed HSBO. Fatty acid concentrations in intestinal slices and brush border membranes did not necessarily reflect respective fatty acid concentrations in the diet. For example, there were consistent quantities of 16:0 and cis-18:1 found regardless of the concentration in the diet. However, feeding the chicks HSBO affected the concentrations of both 18:2n6 and 20:4n6 in intact intestinal slices as well as brush border preparations. Nonetheless, despite 1) the incorporation of trans isomers into intestinal tissues, 2) heightened enzymatic activity of alkaline phosphatase, and 3) reduced concentrations of the bioactive lipids there were no detrimental effects to body weight or feed efficiency associated with chickens fed HSBO. Additionally, no overt signs of essential fatty acid deficiency were apparent. Since all diets contained adequate amounts of linoleic acid this would emerge as a reasonable response. However, there are accounts in the literature where HSBO supplements have heightened indices of essential fatty acid deficiency.

The reduced intestinal uptake of glucose in response to dietary oils in general and HSBO and palm oil supplements in particular poses potential applications. Pre-starter diets for day-old turkey poults and broiler chicks are generally of the highest quality with regards to protein quality and quantity. These diets are usually supplemented with by-product fats, fats which are not completely digested due to limited amounts of pancreatic-biliary secretions and an immature brush border microvilli. Pre-starter diets containing reduced amounts of supplemental oils, particularly those not containing long chain saturates or trans monoenes, could prove beneficial towards enhancing glucose uptake. Alternatively, medium chain fatty acids, which are taken-up even in the absence of bile salts, could replace less digestible animal-vegetable fat blends to provide a readily absorbable fat source in pre-starter diets.

LITERATURE CITED

Agricultural Statistics, 1986. US Government Printing Office. Washington, DC.

Al-Athari, A.K., and B.A. Watkins, 1988. Distribution of trans and cis fatty acid isomers in chicks fed different fats. Poul. Sci. 67:778-786.

Aloia, R.C., 1988. Lipid, fluidity, and functional studies of the membranes of hibernating mammals. Pages 1-40 in: Physical Regulation of Membrane Fluidity. R.C. Aloia, C.C. Curtain, and L.M. Gordon, ed. A.R. Liss, Inc., New York.

Alfin-Slater, R.B., Wells, A.F., Aftergood, and H.J. Deuel, 1957. Nutritive value and safety of hydrogenated vegetable fats as evaluated by long-term feeding experiments with rats. J. Nutr. 63:241-261.

Alfin-Slater, R.B., R.S. Morris, H. Hansen, and J.F. Proctor, 1965. Effects of nonessential fatty acids on essential fatty acid deficiency. J. Nutr. 87:168-72.

Anderson, D.L., 1955. The energy value of poultry feed. Pages 5-10 in: Proc. Cornell Nutrition Conference. Ithaca, N.Y.

Anderson, R.L., 1967. Oxidation of the geometric isomers of Δ9-octadecenoic acid by rat liver mitochondria. Biochim. Biophys. Acta. 144:18-24.

Belanve, D., 1971. The contribution of absorbed linoleic acid to the metabolism of the mature laying hen. Comp. Biochem. Phys. 40A:1097-1105.

Beare-Rogers, J.L, 1983. Trans- and positional isomers of common fatty acids. Adv. Nutr. Res. 5:171-200.

Berlin, E., 1989. Effect of saturation of dietary lipids on insulin receptors and membrane fluidity in rabbit erythrocytes. Nutr. Rep. Inter. 39:367-381.

Blake, J.P., 1986. Methodology for evaluating the digestibility and metabolizable energy of poultry feedstuffs. Ph.D. Dissertation. Virginia Polytechnic Institute and State University. Blacksburg, Virginia.

Burton, F., 1989. Restaurant grease: how much is out there? Render. 18:10-16.

Carver, D.S., E.E. Rice, R.E. Gray, and P.E. Mone, 1955. The utilization of fats of differing melting points added to broiler feed. Poultry Sci. 34:544-546.

Clandinin, M.T., C.J. Field, K. Hargreaves, L. Morson, and E. Zsigmond, 1985. Role of diet fat in subcellular structure and function. Can. J. Physiol. Pharmacol. 63:546-556.

Clark, B.S., 1971. The uptake of oleic acid by rat small intestine: a comparison of methodologies. J. Lipid Res. 12:43-55.

Coenen, J.W.E., 1983. Hydrogenation of edible oils and fats. Proc. 16th ISF Congress, Budapest, Hungary.

Cook, H.W., 1981. The influence of trans-acids on desaturation and elongation of fatty acids in developing brain. Lipids. 16:920-926.

Cook, H.W., 1985. Fatty acid desaturation and chain elongation in eukaryotes. Pages 181-212 in: Biochemistry of Lipids and Membranes. D.E. Vance and J.E. Vance, ed. Benjamin/Cummings Co. Menlo Park, CA.

Dale, N.M., and H.L. Fuller, 1982. Endogenous energy losses of fed versus fasted roosters. Poultry Sci. 61:898-901.

Davson, H., and J.F. Danielli, 1952. The Permeability of Natural Membranes. 2nd edition. Cambridge University Press. London, England.

Dietschy, J.M., V.L. Sallee, and F.A. Wilson, 1971. Unstirred water layers in intestine: rate determinant of fatty acid absorption from micellar solutions. Gastroenterology. 61:932-934.

Dietschy, J.M., H.S. Salomon, and M.D. Siperstein, 1966. Bile acid metabolism. I. Studies on the mechanisms of intestinal transport. J. Clin. Invest. 45:832-846.

Emken, E.A., 1984. Nutrition and biochemistry of trans and positional fatty acid isomers in hydrogenated oils. Ann. Rev. Nutr. 4:339-376.

Emken, E.A., 1981. Metabolic aspects of positional monounsaturated fatty acid isomers. J. Am. Oil Chem. Soc. 58:278-283.

Fedde, M.R., P.E. Waibel, and R.E. Burger, 1960. Factors affecting the absorbability of certain dietary fats in the chick. J. Nutr. 70:447-452.

Federation of American Societies for Experimental Biology, 1985. Health Aspects of Dietary Trans Fatty Acids. Federation of American Societies for Experimental Biology. Bethesda, MD.

Freeman, C.P., D.W. Holme, and E.F. Annison, 1968. The determination of the true digestibilities of interesterified fats by young pigs. Br. J. Nutr. 22:651-660.

Goller, H.J., D.S. Sguotas, I.A.I. Ismail, and F.D. Gunstone, 1970. Dependence of sterol ester hydrolase activity on the position of ethylenic bond in cholesteryl cis-octadecenoates. Biochemistry. 9:3072-3076.

Gottenbos, J.J., 1983. Biological effects of trans fatty acids. Pages 375-390 in: Dietary Fats and Health. E.G. Perkins and W.J. Visek, ed. American Oil Chemists' Society. Champaign, IL.

Graff, G., and W.E. Lands, 1976. A shift from phospholipid to triglyceride synthesis when cell division is inhibited by trans fatty acids. Chem. Phy. Lipids. 17:301-14.

Grundy, S.M., R. Arky, G.A. Bray, W.V. Brown, and N.D. Ernst, 1985. Coronary risk factor statement for the American public. Atherosclerosis. 5:678A-682A.

Halloran, H.R., and I.R. Sibbald, 1979. Metabolizable energy values of fats measured by several procedures. Poultry Sci. 58:1299-1307.

Hill, F.W., and D.L. Anderson, 1958. Comparison of metabolizable energy of grain and grain products for chickens. Poultry Sci. 39:573-579.

Hill, J.W., and R. Renner, 1959. Energy values and other properties of fats in poultry nutrition. Pages 11-15 in: Proc. Cornell Nutr. Conf.

Holmes, A.D., and H.J. Deuel, 1921. Digestibility of some hydrogenated oils. Am. J. Physiol. 54:479-488.

Hunter, J.I., and T.H. Applewhite, 1986. Isomeric fatty acids in the U.S. diet: levels and health perspectives. Am. J. Clin. Nutr. 44:707-717.

Islam, M.N., J.L. Schlitzer, and N.B. Islam, 1983. Effect of trans fatty acids on protein utilization and

serum cholesterol. J. Food Sci. 48:100-103.

Johansson, L.B.A., and G. Lindblom, 1980. Orientation and mobility of molecules in membranes studied by polarized light spectroscopy. Q. Rev. Biophys. 13:63-118.

Johansson, B.W., 1977. Heart and circulation in hibernators. Pages 346-355 in: Mammalian Hibernation III. Fisher, K.C., A.R. Dawe, C.P. Lyman, E. Schonbaum, and E.E. South, ed. Oliver and Boyd, London.

Jones, R.J., 1974. Role of dietary fat in health. J. Am. Oil Chem. Soc. 51:251-254.

Kritchevsky, D., and S.A. Tepper, 1968. Experimental atherosclerosis in rabbits fed cholesterol-free diets: influence of chow components. J. Atheroscler. Res. 8:357-369.

Kritchevsky, D., 1982. Trans fatty acid effects in experimental atherosclerosis. Fed. Proc. 41:2813-2817.

Lindblom, G., L.B.A. Johansson, and G. Arvidson, 1981. Effect of cholesterol in membranes. Biochemistry. 20:2204-2209.

Lukie B.E., H. Westergaard, and J. M. Dietschy, 1974. Validation of a chamber that allows measurement of body tissue uptake rates and unstirred layer thicknesses in the intestine under conditions of controlled stirring. Gastroenterology. 67:652-661.

Lyman, C.P., and R.C. O'Brien, 1974. A comparison of temperature regulation in hibernating rodents. Am. J. Physiol. 227:218-223.

Mahfouz, M.M., S. Johnson, and R.T. Holman, 1980. The effect of isomeric trans-18:1 acids on the desaturation of palmitic, linoleic and eicosa-8,11,14-trienoic acids by rat liver microsomes. Lipids. 15:101-107.

Marr, A.G., and J.L. Ingraham, 1962. Effect of temperature on the composition of fatty acids in Escherichia coli. J. Bacteriol. 84:1260-1269.

Morson, L.A., and M.T. Clandinin, 1986. Diets varying in linoleic and linolenic acid content alter liver plasma membrane lipid composition and glucagon-stimulated adenylate cyclase activity. J. Nutr. 116:2355-2362.

National Research Council, 1984. Nutrient Requirements of Poultry (8th Ed.). National Academy

Press, Washington, DC.

Noblet, J., T. Bikawa, and J. LeDividich, 1987. Interaction between energy level in the diet and environmental temperature on the utilization of energy in growing pigs. J. Anim. Sci. 65:717-29.

Normann, W., 1903. British patent application no. 1515. October, 1903.

Parsons, C.M., L.M. Potter, and B.A. Bliss, 1982. True metabolizable energy corrected to nitrogen equilibrium. Poultry Sci. 61:2241-2246.

Pesti, G.M., 1984. Influence of substitution method and of food intake on bioassays to determine metabolizable energy with chickens. Br. Poultry Sci. 25:495-504.

Pollard, M.R., F.D. Gunstone, A.T. James, and L.J. Morris, 1979. Desaturation of positional and geometric isomers of monenoic fatty acids by microsomal preparations from rat liver. Lipids. 15:306-314.

Potter, L.M., and L.D. Matterson, 1960. Metabolizable energy of feed ingredients for the growing chick. Poultry Sci. 39:781-782.

Potter, L.M., 1972. The precision of measuring metabolizable energy with Leghorn roosters. Feedstuffs. 44:28, 29 and 40.

Raccuglia, G., and O.S. Privett, 1970. Influence of dietary trans,trans-linoleate on hematologic and hemostatic properties of rat blood. Lipids. 5:85-89.

Renner, R., and F.W. Hill, 1960. The influence of corn oil, lard and tallow by chickens of various ages. Poul. Sci. 39:849-854.

Robert, J., D. Montaudon, L. Dubourg, G. Rebel, J. Miro, and B. Canguilhem, 1982. Changes in lipid composition of the brain cellular membranes of an hibernating mammal during its circannual rhythm. Comp. Biochem. Physiol. 71B:409-416.

Rosenthal, M.D., and M. A. Doloresco, 1984. The effects of trans fatty acids on fatty acyl Δ5 desaturation by human skin fibroblasts. Lipids. 19:869-874.

Sallee, V.L., F.A. Wilson, and J.M. Dietschy, 1972. Determination of unidirectional uptake rates for

lipids across the intestinal brush border. J. Lipid Res. 13:184-192.

Sallee, V.L., and J.M. Dietschy, 1973. Determinants of intestinal mucosal uptake of short- and medium-chain fatty acids and alcohols. J. Lipid Res. 14:475-484.

Sgoutas, K.S., and F.A. Kummerow, 1970. Incorporation of trans fatty acids into tissue lipids. Am. J. Clin. Nutr. 23:1111-1119.

Sgoutas, K.S., 1968. Hydrolysis of synthetic cholesterol esters containing trans fatty acids. Biochim. Biophys. Acta. 164:317-326.

Sibbald, I.R., J.D. Summers, and S.J. Slinger, 1960. Factors affecting the metabolizable energy content of poultry feeds. Poultry Sci. 39:544-556.

Sibbald, I.R., and S.J. Slinger, 1963. A biological assay for metabolizable energy in poultry feed ingredients together with findings which demonstrate some of the problems associated with the evaluation of fats. Poultry Sci. 42:313-325.

Sibbald, I.R., 1976. A bioassay for true metabolizable energy in feedingstuffs. Poultry Sci. 55:303-308.

Sibbald, I.R., and J.K.G. Kramer, 1977. The true metabolizable energy values of fats and fat mixtures. Poultry Sci. 56:2079-2086.

Sibbald, I.R., 1983. The T.M.E. system of feed evaluation. Animal Research Centre Technical Bulletin 1983-20E. Agriculture Canada. Ottawa, Canada.

Sell, J.L., A. Krogdahl, and N. Hanyu, 1986. Influence of age on utilization of supplemental fats by young turkeys. Poultry Sci. 65:546-554.

Sell, J.L., C.R. Angel, F.J. Piquer, E.G. Mallarino, and H.A. Al-Batshan, 1991. Developmental patterns of selected characteristics of the gastrointestinal tract of young turkeys. Poultry Sci. 70:1200-1205.

Sinensky, M., 1974. Homeoviscous adaptation--A homeostatic process that regulates the viscosity of membrane lipid in Escherichia coli. Proc. Natl. Sci. 71:522-528.

Singer, S.J., and G.L. Nicolson, 1972. The fluid mosaic model of the structure of cell membranes. Science. 175:720-731.

Stahly, T.S., 1984. Use of fats in diets of growing pigs. Pages 313-331 in: Fats in Animal Nutrition. Wiseman, J., ed. Butterworths. London.

Svensson, L., 1983. The effect of dietary partially hydrogenated marine oils on desaturation of fatty acids in rat liver microsomes. Lipids. 18:171-178.

Thomas, L.H., J.A. Winter, and R.G. Scott, 1983. Concentrations of trans unsaturated fatty acids in the adipose body tissue of descendants dying of ischemic heart disease compared with controls. J. Epidemiol. Commun. Health. 57:22-24.

Vohra, P., D.B. Chami, and E.O. Oyawoye, 1982. Determination of metabolizable energy by a fast method. Poultry Sci. 61:766-769.

Walker, P.S., H.P. Van Krey, J.A. Cherry, and P.B. Siegel, 1981. Jejunal glucose absorption in selected high and low weight lines of domestic fowl. J. Nutr. 111:1894-1899.

Watkins, B.A., 1988. Influences of biotin deficiency and dietary trans fatty acids on tissue lipids in chickens. Br. J. Nutr. 61:99-111.

Weigensberg, B.I., and G.C. McMilllan, 1964. Lipids in rabbits fed elaidinized olive oil and cholesterol. Exp. Mol. Pathol. 3:201-214.

Westergaard, H., and J.M. Dietschy, 1974. Delineation of the dimensions and permeability characteristics of the two major diffusion barriers to passive mucosal uptake in the rabbit intestine. J. Clin. Invest. 54:718-732.

Westergaard, H., and J.M. Dietschy, 1976. The mechanism whereby bile acid micelles increase the rate of fatty acid and cholesterol uptake into the intestinal mucosal cell. J. Clin. Invest. 58:97-108.

Whitehead, C.C., and K.M. Herron, 1988. Fatty acid requirements of breeding turkeys. Br. Poultry Sci. 29:761-768.

Wilson, F.A., V.L. Sallee, and J.M. Dietschy, 1971. Unstirred water layers in intestine: rate determinant of fatty acid absorption from micellar solutions. Science. 174:1031-1033.

Zottor, B.A.K., and B.L. Walker, 1989. Relative hydrolysis of trans- and cis-triglyceride by rat mammary lipoprotein lipase. Nutr. Res. 9:679-683.

APPENDIX

TABLE 1. *Gross energy, dry matter, and crude protein content of metabolism trial test diets*

Diet	Gross energy[1]	Dry matter[2]	Crude protein[3]
Basal	3,941	90.0	24.0
HSBO, 10%	4,505	91.5	21.2
HSBO, 20%	5,005	92.0	19.2
SBO, 10%	4,505	91.0	20.9
SBO, 20%	5,045	92.0	18.8

HSBO, hydrogenated soybean oil: SBO, soybean oil.
[1]Gross energy = kcal/kg diet.
[2]Percent dry matter.
[3]Percent crude protein.

TABLE 2. *Influence of dietary oil on feed intake, nitrogen intake, excreta production, and excreta energy of broiler chickens from 3 to 4 weeks of age*

Diet	Dry matter feed intake (grams)	Total nitrogen intake (grams)	Excreta dry matter production (grams)	Gross energy of dry matter excreta (kcal/kg)
Basal	2,395	102.2	723	3,877
Basal	2,245	95.7	771	3,933
Basal	2,430	103.6	719	3,876
Basal	2,874	122.6	853	3,905
HSBO, 10%	2,846	105.7	870	4,320
HSBO, 10%	2,333	86.7	683	4,268
HSBO, 10%	2,893	107.4	824	4,325
HSBO, 10%	2,902	107.8	861	4,309
HSBO, 20%	2,692	90.1	692	4,692
HSBO, 20%	2,784	93.2	768	4,705
HSBO, 20%	2,362	79.1	659	4,748
HSBO, 20%	2,774	92.9	739	4,689
SBO, 10%	2,739	100.6	726	4,079
SBO, 10%	2,916	107.1	747	4,106
SBO, 10%	2,783	102.2	732	4,028
SBO, 10%	2,853	104.8	787	4,114
SBO, 20%	2,739	89.6	688	4,246
SBO, 20%	2,647	86.5	683	4,353
SBO, 20%	2,589	8436	681	4,419
SBO, 20%	2,684	87.8	680	4,483

HSBO, hydrogenated soybean oil; SBO, soybean oil.

VITA

Patrick K. Brown was born in Oak Park, Illinois on September 24, 1953 to Patrick F. and Dorothy F. Brown. He entered Auburn University in Auburn, Alabama in 1971 and graduated in December, 1975 with a Bachelor of Science degree in Agriculture Science. Following graduation, he was employed by Hudson Farms in Pike Road, Alabama until 1979. At that time, he purchased a farm in Grady, Alabama and produced market hogs until 1986. He entered the University of Tennessee that year and received a Master of Science degree in Animal Science in August, 1988. Doctoral studies began at VPI&SU in 1988 and were completed under the direction of Drs. B.A. Watkins and L.M. Potter in 1991.

Patrick K. Brown