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Dietary fat effects on egg yolk lipid composition and hepatic lipogenic enzymes

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DIETARY FAT EFFECTS ON EGG YOLK LIPID COMPOSITION AND HEPATIC LIPOGENIC ENZYMES

by

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DEDICATION

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ABSTRACT

Menhaden oil, corn oil, olive oil or animal fat (1, 3, 5 or 8% of diet) effects on egg yolk fatty acids and hepatic lipogenic enzymes were studied in 35 week-old laying hens. Except for significantly lowered egg weights by menhaden oil, performance was unaffected.

Yolk saturated fat remained constant (33-38% of yolk fat) regardless of fat source. Maximum ω-3 fatty acid incorporation (.31 g/yolk) was reached with a deposition efficiency of 36.3% by feeding 5% menhaden oil. Yolk lipid contained .3% linolenic (C18:3ω3), 1.0% eicosapentaenoic (C20:5ω3), and 3.8% docosapentaenoic (C22:5ω3) acids. Maximum ω-6 fatty acid incorporation (1.65 g/yolk) occurred with 17% efficiency when 8% corn oil was fed, primarily by increasing linoleic acid to 25.5%. Yolk oleate was inversely related to dietary linoleate.

Only the highest menhaden oil levels significantly affected lipogenic enzymes: fatty acid synthetase and ATP citrate lyase (EC 4.1.3.8) activities were inhibited, while glucose-6-phosphate dehydrogenase (EC 1.1.1.49) activity was 2-3 times that of hens fed the basal diet. Malic enzyme (EC 1.1.1.40) activity was not significantly influenced by dietary fat.

CHAPTER 1

INTRODUCTION

The average consumption of eggs in the United States has steadily decreased during the last 30 years mainly because of the high cholesterol content (200-300 mg/egg). Cholesterolemia is a risk indicator for cardiovascular disease which is one of the major diet related health problems in the United States.

Recently, ω-3 fatty acids with proposed beneficial effects in lowering serum cholesterol are being used to treat and prevent atherosclerosis. Current research indicates that marine oils rich in ω-3 fatty acids affect tissue lipid composition as well as plasma lipids (Goodnight et al., 1982; Nassar et al., 1986; Rand, Hennissen and Hornstra, 1986; Huang, McAdoo and Horrobin, 1987; Hulan et al., 1987; Johnson and Berdanier, 1987; Philbrick et al., 1987; Rogers et al., 1987; Sinclair et al., 1987; Yu and Sim, 1987; Hulan, 1988; Nwokolo, Zhiang and Sim, 1988). Few studies have reported the effects of marine oils on ω-3 fatty acid incorporation in egg yolk (Yu and Sim, 1987; Hulan, 1988; Nwokolo et al., 1988), but none have compared the effects of different dietary fats and marine oils on fatty acid composition of egg yolk. The apparent nutritional importance of ω-3 fatty acids makes it desirable to define conditions under which their content in egg yolk can be modified. That is the purpose of the present study.

Fatty liver hemorrhagic syndrome (FLHS) is a metabolic disease in laying hens. The main symptom of this disease is a large amount of fat

accumulated in the liver. FLHS once caused a great economic loss in the poultry industry and many factors were found to be connected to this disease including several vitamins, minerals, fat, temperature, type of housing, hormones, bird age, feed ingredients, drug, force feeding, and energy (Roland, Farmer and Marple, 1985). Fat has been reported to be effective in inhibiting hepatic lipogenesis (Haghighi-Rad and Polin, 1982; Donaldson, 1985; Herzberg and Rogerson, 1988a). Weigand, Rao and Reiser (1973) reported that rate of fatty acid synthesis is regulated not only by the level, but also by the type of dietary fat. Triscari, Hamilton and Sullivan (1978) proposed that the rate of fatty acid synthesis is inversely proportional to the concentration of unsaturated dietary fat.

Some studies have been conducted to investigate the effect of dietary fats on lipogenesis, while the present study provides the only data comparing dietary fish oil, corn oil, olive oil, and animal fat at different dietary levels on the inhibition of lipogenesis in laying hens.

CHAPTER 2

LITERATURE REVIEW

Polyunsaturated Fatty Acids (PUFA)

Cardiovascular disease (CVD) is one of the major diet related health problems in the United States. Atherosclerosis and thromboembolic complications along with peripheral vascular disease affect approximately 4 million people at a cost of $78.6 billion annually (USDHEW, 1979). The profound blood triglyceride-lowering effect of fish oil is now an established fact that has clinical significance and therapeutic potential for coronary heart disease and hypertriglyceridemia (Goodnight et al., 1982; Rand et al., 1986; Koh, 1987; Lewis, 1987; Rogers et al., 1987). Polyunsaturated fatty acids (PUFA) have long been considered essential to proper physiological function of humans and animals.

Rogers et al. (1987) reported a significant fall in serum triglyceride and diastolic blood pressure in sixty healthy male volunteers taking a fish oil supplement. A beneficial effect of fish oil on the cardiovascular risk profile was confirmed in this study. Bruckner et al. (1987) observed that fish oil increases peripheral capillary blood cell velocity in humans, and suggested that this effect was possibly caused by the alteration of vascular tone through changes in the eicosanoid ratios. Rand et al. (1986) reported that platelet membrane fluidity, measured by fluorescence polarization using platelets labelled with 1,6-diphenyl-1,3,5-hexatriene, was significantly increased upon feeding of sunflowerseed-oil (mainly ω-6 PUFA). The increase in platelet membrane

fluidity may be associated with a reduction in arterial thrombosis tendency. Høstmark, Lystad and Haug (1987) observed that rats fed a low dose (approximately 4% of dietary energy) of cod liver oil (mainly ω-3 PUFA) significantly increased osmotic resistance of red blood cells over those fed vegetable oil.

No clear biochemical mechanism for the triglyceride-lowering effect of PUFA has been found. Although observations of decreased hepatic secretion of very low density lipoprotein (VLDL) in humans and rats, diminished hepatic fatty acid synthesis in rats, and increased total hepatic fatty acid oxidation in rats with fish oil treatment all suggest that primary actions take place in liver (Goodnight et al., 1982). However, Yamazaki, Shen and Schade (1987) suggested that fish oil significantly lowered rat serum triglyceride by increasing peroxisomal β-oxidation activity. Field, Albright and Mathur (1987) reported that changes in membrane fatty acid saturation induced by dietary manipulation enhanced the regulation of HMG-CoA (3-hydroxy-3-methylglutaryl-CoA) reductase, the rate limiting enzyme of cholesterol synthesis, and acyl-CoA:cholesterol acyltransferase (ACAT) activities in the liver and intestine of rabbits. They concluded that regulation of these key enzymes of cholesterol metabolism is most likely related to the degree of fat saturation and not necessarily to the specific class of polyunsaturates within the membrane.

Several studies have demonstrated the presence of large amounts of ω-3 fatty acids in the phospholipid fractions (phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine and phosphatidylinositol) of rat brain, sciatic nerve and retina, indicating that ω-3 fatty acids may

play a role in the proper functioning of the nervous system (Philbrick et al., 1987). Dietary fish oils have been shown to inhibit development of mammary, pancreatic, intestinal and prostatic tumors in experimental animals, and are postulated to act through changes in the production of prostaglandins, leukotrienes and related compounds involved in immune responses (Carroll, 1986). PUFA existing in the membrane of tissue cells influence the fluidity of the membrane, and that changes the metabolism of prostaglandin and leukotriene metabolites and results in altered activity of membrane ion pumps and cellular responses (Lewis, 1987; Philbrick et al., 1987).

The major families of PUFA are omega-3 (ω-3), omega-6 (ω-6) and omega-9 (ω-9) fatty acids. Omega-9 fatty acids have little physiological significance. The major ω-3 fatty acids in nature are linolenic acid (C18:3ω3), eicosapentaenoic acid (EPA; C20:5ω3), and docosahexaenoic acid (DHA; C22:6ω3). Their major sources are marine oils and partially from vegetable oils. The major ω-6 fatty acids are linoleic acid (C18:2ω6) and arachidonic acid (C20:4ω6) which exist mainly in vegetable oils. The ω-3 and ω-6 families are regarded as essential fatty acids (EFA) because they cannot be synthesized de novo in the body, and are not metabolically interconvertable.

Linoleic acid is the obligatory precursor of arachidonic acid which serves as a principle substrate for prostaglandin synthesis. In mammals, linoleic acid (C18:2ω6) is metabolized along a variety of pathways, one of which is its conversion to γ-linolenic acid (GLA; C18:3ω6) by delta-6-desaturase. GLA is rapidly elongated to dihomo-γ-linolenic acid (DGLA; C20:3ω6) and subsequently desaturated by delta-5-

desaturase to arachidonic acid (AA; C20:4ω6). There is substantial evidence that PUFA, mainly linoleic, and more effectively its metabolites GLA and AA, are capable of lowering plasma cholesterol levels (Goodnight et al., 1982; Huang et al., 1987; Lewis, 1987; Siguel and Maclure, 1987).

While evidence has shown the benefits of PUFA consumption, there may be potential risks associated with increased intakes. Possible harmful effects of PUFA consumption include enhanced formation of cholesterol gallstones, stimulation of carcinogenesis, increased vitamin E requirement, promotion of obesity, and increased cholesterol absorption. Uncontrolled consumption of large amounts (15-50 ml/day) of fish liver oils (such as cod liver oil) should be discouraged because of the high levels of vitamin A and D they contain. Ingestion of 4 to 8 g/day of ω-3 fatty acids derived from fish oil has been associated with a low mortality rate from cardiovascular disease (Goodnight et al., 1982).

Fatty liver hemorrhagic syndrome (FLHS)

Fatty liver hemorrhagic syndrome (FLHS), a metabolic disease in laying hens, was first described by Couch (1956). Hens with this syndrome are heavier than nonaffected ones. Their combs are enlarged, which is believed to be indicative of a hormone imbalance, and their livers are enlarged, fatty, friable and with some hemorrhagic spots on them. Hens with FLHS show a threefold increase in serum cholesterol and an elevated serum calcium and phosphorus level (Harms et al., 1982). The signs of this condition are a 2 to 50% decrease in egg production, loss of appetite, and death (Harms et al., 1982). Many studies have been conducted to find a solution to this syndrome. Maijering (1979) reported several factors including several vitamins, minerals, fat, temperature,

type of housing, hormones, bird age, feed ingredients, drugs, force feeding, energy, aflatoxins and several other factors associated with FLHS. Obviously, FLHS is a complex problem (Roland et al., 1985).

Klasing et al. (1988) examined the effects of dietary pyruvic acid on FLHS in genetic FLHS susceptible Single Comb White Leghorn (SCWL) birds. In experiment 1, pullets were fed either a corn-milo based diet or the basal diet with 5% pyruvic acid for 8 weeks. Pyruvic acid decreased liver weight, percent liver fat, and total liver fat by 23, 37 and 54%, but did not affect feed intake or egg production. In experiment 2, hens exhibiting mortality from FLHS were fed either basal or 5% pyruvic acid diet for 3 weeks. They observed that pyruvic acid decreased liver weight and total liver fat by 9 and 27%, but did not influence feed intake or egg production. They concluded that the inclusion of 5% pyruvic acid in the diet decreased the deposition of fat in the liver of pullets developing FLHS, and promoted the loss of hepatic fat in hens exhibiting the disease.

Haghighi-Rad and Polin (1982) reported that lipid (corn oil) at 4% of the diet had an alleviating effect on FLHS. Polin and Wolford (1976) reported that various types of diets and sources of energy fed in excess could induce FLHS. Force-feeding has been shown to cause an elevation of liver fat (Ivy and Nesheim, 1973), whereas restricted feeding has the opposite effect (Wolford and Polin, 1974). These conditions are related to energy balance. Haghighi-Rad and Polin (1982) concluded that FLHS is obesity in which estrogen exerts an aggravating role in reducing the hemorrhages and hepatic lipogenesis, and the obesity arises from hens in positive energy balance.

Several reports from Akiba's laboratory have shown, with laying hens, that development of fatty livers and hemorrhages is accelerated by feeding a corn-soybean meal diet, and prevented or reduced by feeding diets containing fish meal, alfalfa meal, fermentation by-products or wheat (Akiba, Jensen and Mendonca, 1983a). Patel, McGinnis and Pubols (1981) reported that the factor present in some cereal grains which reduced liver fat in laying hens could be a pectin-like polysaccharide. Some contradictions are found in these reports. Polin and Wolford (1977) reported that injecting growing chicks with estradiol markedly increased liver lipids and resulted in liver hemorrhages similar to those seen in laying hens with FLHS. Pearce and Johnson (1986), however, failed to induce FLHS in the laying hen by estradiol administration.

Glycolysis is a main supplier of substrates for fatty acid synthesis. A high dietary intake of carbohydrate may be expected to cause a marked increase in the fat content of the liver. In practical rations for laying hen diets, about 70 to 80% of the energy is from carbohydrate, 2 to 4% from fat, and the remainder from protein (Haghighi-Rad and Polin, 1982). The amino acids should be balanced for the hen's requirements.

Jensen et al. (1976) suspected that fatty liver syndrome in laying hens was related to geographical location as it is rarely observed in certain commercial egg production areas, but frequently in others. He obtained water samples collected from commercial egg production farms in Georgia with or without a history of fatty liver syndrome, and observed that water samples from farms with a history of fatty liver syndrome had significantly more calcium, magnesium, strontium, sodium, iron and barium

than water samples from farms reporting no significant problem with fatty liver syndrome.

Roland et al. (1985) reported that feeding a low calcium diet to birds caused increased body weight, liver weight, liver hemorrhagic score and reduced production depending upon the strain. They believed that calcium deficiency is a possible cause of, or is at least related to, FLHS. It was further proposed that calcium is necessary for blood clotting which can be related to the capillary hemorrhages observed in FLHS. Previous studies reported calcium to be effective as a hypolipemic agent. A low calcium diet induced secretions of estrogen. Thus, there is a relationship of calcium and fat metabolism to serum estrogen levels.

Hepatic Lipogenesis

FLHS is a complex problem. In order to control FLHS in poultry, many studies have been carried out on the regulation of hepatic lipogenesis.

Adipose tissue is a major site of fatty acid synthesis in rats as well as other mammalian species (Nelson et al., 1987). However, O'Hea and Leveille (1969) have shown that 90 to 95% of the total fatty acid synthesis in poultry occurs in the liver. Saadoun and Leclercq (1987) reported that the liver is the main organ responsible for de novo lipogenesis in chickens and extrahepatic lipogenesis is poorly influenced by nutritional state.

Lipogenesis which mainly forms palmitate from acetyl-CoA has been found in cytosol fraction in many tissues, including liver, kidney, brain, lung, mammary gland and adipose tissue. Its cofactor requirements include NADPH, ATP, Mn^{2+} and HCO_3^- (as a source of CO_2). Fatty acid synthesis is

catalyzed by fatty acid synthetase which is a multienzyme complex. The equation for the overall synthesis of palmitate from acetyl-CoA and malonyl-CoA is shown below:

$$CH_3CO*S*CoA + 7\ HOOC*CH_2CO*S*CoA + 14\ NADPH + 14\ H^+ \longrightarrow$$

$$CH_3(CH_2)_{14}COOH + 7\ CO_2 + 6\ H_2O + 8\ CoA*SH + 14\ NADP^+$$

The oxidative reactions of the hexose monophosphate shunt (HM shunt) are the chief source of the hydrogen required for the reductive synthesis of fatty acids. Glucose-6-phosphate dehydrogenase, which is a NADP-dependent enzyme, catalyzes the dehydration of glucose-6-phosphate to 6-phosphogluconate. Its activity has been used as an indicator of HM shunt activity. It is significant that tissues which possess an active HM shunt are also the tissues specializing in active lipogenesis, ie. liver, adipose tissue and the lactating mammary gland. Other sources of NADPH include the extramitochondrial isocitrate dehydrogenase reaction (probably not a substantial source) and the reaction that converts malate to pyruvate catalyzed by the "malic enzyme" (NADP malate dehydrogenase). Acetyl-CoA, the main building block for fatty acids, is formed from carbohydrate via the oxidation of pyruvate within the mitochondria. However, acetyl-CoA does not diffuse readily into the extramitochondrial compartment, the principal site of fatty acid synthesis. The activity of extramitochondrial ATP citrate lyase (citrate cleavage enzyme), like the "malic enzyme", increases in the well-fed state, closely paralleling the activity of the fatty acid synthesizing system. The acetyl-CoA formed by decarboxylation of pyruvate in mitochondria then was condensed with oxaloacetate to form citrate, as part of the citric acid cycle. This is followed by the translocation of citrate into the extramitochondrial

compartment, where in the presence of CoA and ATP, it undergoes cleavage to acetyl-CoA and oxaloacetate catalyzed by ATP citrate lyase. The acetyl-CoA is then available for malonyl-CoA formation and synthesis to palmitate. The activities of fatty acid synthetase, ATP citrate lyase, glucose-6-phosphate dehydrogenase and malic enzyme will be used as an indicator to estimate the activity of hepatic lipogenesis in this study.

Investigations have demonstrated that liver lipid deposition, hepatic lipogenesis and enzyme activity in caged laying hens were accelerated by feeding a corn-soybean (CS) meal diet (Maurice, Jensen and Tojo, 1979), and reduced by supplements of distillers' dried grains with solubles, brewer's dried grains, brewer's yeast, soy mill feed, alfalfa meal, fish meal, and wheat bran or wheat when included in diets isonitrogenous and isoenergetic with the CS diet (Akiba and Jensen, 1983). Donaldson's (1985) data indicated that calorie-protein ratio affects body fat by increasing lipogenic activity as the ratio is increased. Rosebrough, Steele and Frobish (1982a) reported that increasing sulfur amino acid from a suboptimal level to an optimal level decreased *in vitro* lipogenesis, whereas increasing lysine levels increased lipogenesis. Increments of well-balanced proteins appeared to decrease lipogenesis by providing more favorable substrates for lean tissue growth (Rosebrough *et al.*, 1982b). They also found that two apparently glucogenic precursors, lactate and alanine, enhanced lipogenesis. Enhanced hepatic lipogenesis and liver fat deposition can also be observed by increasing dietary metabolic energy with increased dietary carbohydrate (Tanaka, Ohtani and Shigeno, 1983), keeping hens under higher temperatures (Griffith *et al.*, 1969; Schexnailder and Griffith, 1973; Akiba *et al.*, 1983b), feeding 2.5

ppm or more aflatoxin (Hamilton and Garlich, 1971), feeding a barley-based diet rather than a corn-based diet (Qureshi et al., 1980a), feeding a corn-soy-based diet rather than a wheat-soy-based diet (Maurice and Jensen, 1979), or housing in cages (Griffith et al., 1969). Hepatic lipogenesis is lowered by the addition of pantethine to corn-soy-based diet (Hsu et al., 1987), dietary cellulose (Cherry and Jones, 1982), feeding iodinated casein or thyroid powder (Wolford, 1971), and substituting menhaden fish meal for soybean meal (Akiba and Jensen, 1983). Hepatic lipogenesis is not only influenced by the amount of carbohydrate, but also by the type. Diets rich in simple sugars have been shown to induce higher level of hepatic lipids in laboratory animals and man compared with diets containing complex carbohydrates (Maurice and Jensen, 1979). In summary, the regulation of hepatic lipogenesis is related to genetics, dietary factors, hormones and environment. The mechanisms by which these factors regulate and interact with others need more work for elucidation.

It is well-known that dietary fat can inhibit hepatic lipogenesis (Herzberg and Rogerson, 1988a,b). Donaldson (1985) observed increased growth with increasing dietary fat in balanced diets, which is a reflection of greater total energy and nutrient intake. He suggested that the increased growth may result from improved palatability or increased nutrient density as fat is added to the diet. Mateos and Sell (1980) have proposed two mechanisms explaining the advantages of fat calories: 1) a synergism may exist between saturated and unsaturated fatty acids and 2) fat may increase digestibility of other dietary components. Maiorino et al. (1986) reported that added animal fat improved the absorption of the

residue fat in carriers and unsaturated fatty acids in the carrier improved the utilization of added fat. Yeh, Leveille and Wiley (1970) noticed that efficiency of feed utilization for weight gain was improved by increasing the dietary fat (corn oil) level from 2 to 10% but decreased when the fat content was increased to 20% of the diet.

Lipogenic enzymes and rates of fatty acid synthesis in rat and mouse liver appear to be regulated not only by the level of dietary fat but also by the type of fat (Weigand et al., 1973). Yeh et al. (1970) reported that increasing the level of dietary fat (corn oil) from 2 to 10% depressed the specific activities of both malic enzyme and the ATP citrate lyase by about 50%. A further increase in the dietary fat level to 20% resulted in specific activities of malic enzyme and ATP citrate lyase which were only 14 and 28%, respectively, of the activity observed in chicks fed the 2% corn oil diet. Weigand et al. (1973) observed that addition of 15% cocoa butter to a fat-free diet was necessary to achieve a degree of inhibition of rat liver fatty acid synthetase comparable to that obtained with 2.5% safflower oil supplementation. Clarke, Romsos and Leveille (1977a) reported that supplementing 3% C18:2 methyl esters to the fat-free diet significantly inhibited the activities of fatty acid synthetase and acetyl-CoA carboxylase, whereas comparable amounts of absorbed C18:0 methyl esters exerted no inhibitory action on the activity of either enzyme. Rosebrough and Steele (1985) found high fat diet (47% of total calories) decrease ($P<.05$) in vitro lipogenesis in hens. The hens fed the low fat diet (8% of total calories) had heavier ($P<.05$) livers that contained more ($P<.05$) lipid than those of hens fed the high fat diet. Johnson and Berdanier (1987) reported that feeding menhaden oil

(5%) resulted in lower enzyme activity and fatty acid synthesis than feeding corn oil, beef tallow or hydrogenated coconut oil. Wilson, Hays and Clarke (1986) reported a 50% lower rate of fatty acid biosynthesis caused by high level of linoleate safflower oil diet supplementation than did by comparable levels of animal fat or palmitate. Polyenoic acids such as linoleate, arachidonate, linolenate and eicosapentaenoate are more effective inhibitors of de novo fatty acid biosynthesis than are saturated or monounsaturated fatty acids (Herzberg, 1983).

Saturated fat was not absorbed well and that influenced the result. Triscari et al. (1978) found that intragastrically administered fatty acyl ethyl esters were well absorbed; however, fatty acid synthesis was decreased by three consecutive daily doses of C18:1 or C18:2 (5 g/kg), but not by C18:0. A single daily administration of C18:0, C18:1 or C18:2 was ineffective in altering lipogenic rates. They made some suggestions from their data: 1) Fatty acid synthesis responds selectively to C18:0, C18:1 and C18:2. 2) The inhibition of fatty acid synthesis by unsaturated fatty acids is time dependent. 3) The rate of fatty acid synthesis is inversely proportional to the concentration of unsaturated dietary fat. Clarke et al. (1977b) reported the apparent absorbabilities of C16:0 and C18:0 to be 40 and 35%, while values for C18;1, C18:2 and C18:3 were 88, 87 and 89%, respectively. Because of the poor absorbability of C16:0, more C16:0 was added to the diet to make comparable amounts of fatty acid absorbed. They fed the rats with methyl esters of C16:0, C18:0, C18:1, C18:2 or C18:3 fatty acids, respectively, and found that C18:2 and C18:3 were able to reduce the rate of hepatic fatty acid synthesis and the

activities of hepatic fatty acid synthetase and malic enzyme, while C16:0 and C18:0 had no depressive effect on hepatic fatty acid synthesis.

Some investigators have suggested that the decrease in liver lipogenesis with increasing dietary fat is caused by the concomitant decrease in carbohydrate intake, rather than by a specific effect of the fat. However, in other studies where carbohydrate intake was controlled, a specific effect of dietary polyunsaturated fat was still observed. Clarke et al. (1977b) fed rats with different methyl esters of fatty acids. Their experimental design was such that the average daily intake of carbohydrate was identical among rats of different treatments within an experiment. The data demonstrated that C18:2 and C18:3 specifically inhibited rat liver fatty acid synthesis independent of carbohydrate intake. Carrozza et al. (1979) made a small increase in fat content of the diet which does not significantly affect glucose intake, and fed to rats. He observed that the diet clearly depressed the activities of glucose-6-phosphate dehydrogenase (G6DP), 6-phosphogluconate dehydrogenase (6PGD), malic enzyme (ME), ATP citrate lyase (ATP-CL), acetyl-CoA carboxylase (ACX) and fatty acid synthetase (FAS). It was suggested that the depression of the above enzyme activities caused by the presence of fat is chiefly due to the fat per se rather than to the decrease in glucose intake.

Fatty Acid Composition and Egg Yolk

Average consumption of eggs in the United State has steadily decreased during the last 30 years. Part of this is due to a changing lifestyle in which eating the traditional breakfast meal has declined. However, much of the decline may be attributed to concern among the

medical community and the general public that dietary cholesterol may attribute to the incidence of coronary heart disease (Waldroup et al., 1986). Although this idea is not so conclusive, this concern has undoubtedly influenced egg consumption. Eggs contain protein of a high biological value, nearly all the vitamins and minerals, and a preferential saturated : unsaturated fatty acid ratio of 1:2, plus 200-300 mg of cholesterol (Menge et al., 1974). The American Heart Association recommends that daily intake of cholesterol be limited to less than 300 mg (Newsweek, 1988).

Many studies have investigated the deprivation of cholesterol from the yolk and/or increasing the polyunsaturated fatty acids in the yolk. Hargis's (1988) review on modifying egg yolk cholesterol in the domestic fowl, concluded that cholesterol content of egg yolk is increased by increased dietary cholesterol and fats. Dietary fiber and certain drugs have been demonstrated to reduce yolk cholesterol concentrations but only marginally. Although selection studies have indicated sufficient genetic variability in yolk cholesterol to make a genetic selection program possible. Selection for lower egg cholesterol has not been successful or has resulted in only modest (5-7%) reductions in egg cholesterol levels with an associated decrease in egg production. The inability to markedly reduce yolk cholesterol concentrations is possibly due to natural selection pressures to maintain a certain level of cholesterol in the egg for use by the developing embryo. Currently, there is little evidence to suggest that a commercially significant reduction in egg cholesterol content is going to occur by conventional nutritional manipulation or by genetic selection program (Hargis, 1988).

It is now well established that the fatty acid composition of egg yolk depends to a large extent on the type of dietary fat fed. The total percentage of saturated fatty acids in egg fat remains relatively constant at between 30 and 35% regardless of the type of fat fed. An increase in the amount of dietary polyunsaturated fatty acids will result in an increase in polyunsaturated fatty acids in the egg. Most of this increase, however, is in linoleic acid at the expense of oleic acid. Highly saturated fat products added to feeds increase oleic acid and decrease linoleic acid contents of the yolk fat. There is a limit to the percentage of linoleic acid that can be deposited in the egg. With soybean oil or safflower oil, regardless of the level used, it is not possible to increase the linoleic acid content of the yolk fat above 40% (Summers, Slinger and Anderson, 1966).

Fatty acid composition of yolk fat is under the influence of both fatty acid synthesis, which tends to be inhibited by dietary fat, and fatty acids of dietary origin. The fatty acid synthetic patterns of the liver control the pattern of lipid incorporation into egg yolk under low fat diet. Whereas, when high fat diet is applied, the major fatty acid source for egg yolk is from dietary origin rather than from biosynthesis because of the inhibited lipogenesis. However, the changes in fatty acid composition of yolk fat could not be entirely accounted for the quantitative reduction in triglyceride synthesis and the fatty acid pattern in dietary fat when hens were fed this fat source. The discrepancy may be due to metabolic conversions of dietary fatty acids before egg yolk triglyceride incorporation or to invalid assumptions about

the use of acetate incorporation data to access triglyceride synthesis (Naber and Biggert, 1987).

Yu and Sim (1987) fed a diet containing 8% fat which was 0, 20, 50, 80 or 100% of salmon oil blended with animal tallow to 37-week-old SCWL laying hens. They analyzed egg yolk lipids daily and found that maximum incorporation of ω-3 polyunsaturated fatty acids in eggs was reached on day 8 with levels of eicosapentaenoic acid (C20:5 ω-3) and docosahexaenoic acid (C22:6 ω-3) at 1.2 and 5.4% of yolk lipids, respectively (with the 100% fish oil diet). Percentages of lipid classes in yolks were unaffected by different levels of fish oil. Omega-3 fatty acids were preferentially incorporated into phospholipids. No significant differences in feed consumption, egg laying efficiency, or egg weight among treatments were observed.

Hulan (1988) used 4 dietary treatments with 0, 4, 8 and 12% herring meal (HM) in diet to feed SCWL laying hens and determined the effects of feeding different levels of HM on performance and ω-3 fatty acid content of egg yolk lipids. He reported that feeding up to 12.0% HM had no effect ($P>.05$) on age at sexual maturity, egg production, egg specific gravity or Haugh Units, but increasing levels of HM in the diets resulted in lower ($P<.05$) liver weights and a linear decrease in egg weight ($P<.05$). Additions of HM to the diets resulted in a linear increase in ω-3 fatty acids (eicosapentaenoate, docosapentaenoate and docosahexaenoate), and a decrease in ω-6 polyunsaturated fatty acids and cholesterol of egg yolk lipids.

Nwokolo *et al*. (1988) fed barley and full-fat canola seed (FFCS) pelleted in ratios of 80:20, 70:30, 60:40 and 50:50 to SCWL hens at 40%

dietary level for 10 months. They found that feeding FFCS, a good source of linolenic acid, was associated with a depression in liver content of lipids and cholesterol, and serum concentration of total and high density lipoprotein (HDL) cholesterol. Cholesterol content of eggs was increased. However, there was a linear relationship ($P<.01$) between dietary linolenic acid and the content of some ω-3 fatty acids (linolenic acid and docosahexaenoic acid) in liver, serum and eggs. It is a contradict that Hulan (1988) reported an inverse relationship between the dietary ω-3 fatty acids level and the cholesterol content of the egg yolk.

Polyunsaturated fatty acids of yolks were increased by highly unsaturated dietary fat. However, unsaturated fat feeding at high levels significantly increases egg yolk cholesterol concentration (Naber, 1976). Supplementing a laying diet with 20% coconut oil or safflower oil produced a significant (but not marked) increase in yolk cholesterol, whereas soy sterols were without effect (Menge et al., 1974). It has been reported that the cholesterol content of egg yolk is increased by the addition of certain vegetable oils, some surface active agents, bile acids and diethylstilbestrol to the diet (Keshavarz, 1976). It has been also documented the cholesterol content of yolk is reduced by increasing dietary levels of protein, inclusion of oathulls, pectin gums and scleroglucan, phytosterol, saponins, cholestyramine, aromatic hepaenes, nicotinic acid, and large amounts of vitamin A to the diet (Keshavarz, 1976). Waldroup et al. (1986) confirmed that addition of the hypocholesteremic agent Probucol to the laying hen diet at levels up to 1% significantly reduces egg yolk cholesterol by 5 to 7% without impairment of the performance of laying hens. Vargas and Naber (1984) indicated that

feeding various sources of dietary fiber to laying hens with proper adjustment of the total nutrient density of the diet causes a significant decrease in egg yolk cholesterol with no change in egg production when compared with the basal diet.

The 30% reduction in yolk cholesterol level observed by Sim and Bragg (1977) is encouraging, but not significant in practical terms to the industry. A minimum decrease of 50% in yolk cholesterol to levels ranging from 100 to 150 mg cholesterol per egg would potentially be of benefit commercially (Hargis, 1988).

CHAPTER 3

EXPERIMENTAL PROCEDURE

Eighty 35 week-old SCWL Dekalb XL laying hens were fed diets containing added fat levels of 0, 1, 3, 5 and 8% each of menhaden oil, corn oil, olive oil and animal fat using a 4 × 5 × 2 factorial design for two 28-day periods. A basal diet (0% added fat), formulated by linear program to meet all National Research Council (NRC, 1984) nutrient recommendations except metabolizable energy, was used in each dietary fat series as a control (Table 1). Hens were housed two per cage (61.0 × 45.7 cm) in an open house with an average ambient temperature of 24.7 C, ranging from an average minimum of 16.2 C to an average maximum of 33.1 C, over the experimental period. Feed and water were supplied ad libitum and light was provided on a 16 h light:8 h dark daily cycle. Egg numbers and feed consumption were recorded daily for each replicate, and body weights were determined initially and at the end of each period.

Feed and excreta samples for each replicate were collected during the last 14 days of the first period for laboratory analyses for chromium oxide, gross energy, total fat, fatty acids, crude protein and amino acids. Excreta samples were oven dried at 80 C and allowed to equilibrate to laboratory conditions prior to analysis. All eggs laid during the last 14 days of the first period for each replicate were weighed and saved for yolk fatty acid analysis. Eggs were stored at 7.2 C prior to analysis. The fatty acid composition of each supplemental fat was also determined.

Nutrient utilization was evaluated using the chromium oxide marker technique (Edwards and Gillis, 1959). The gross energy contents of feed and excreta were determined by bomb calorimetry using an adiabatic oxygen bomb calorimeter (model 1241, Parr Instrument Co., Moline, IL) in order to calculate the metabolizable energy of the experimental diets. Crude protein (N x 6.25) contents of feed and excreta were estimated from total nitrogen determined by nesslerization of microkjeldahl digests (Association of Official Analytical Chemists, 1980).

Total fat determinations were carried out by chloroform:methanol (2:1, v/v) extraction. Fatty acid methyl esters were prepared from the chloroform:methanol extracted fat of feed, excreta and egg yolk and from samples of each supplemental fat using 12% (w/v) boron trifluoride as described by the American Oil Chemists' Society (AOCS, 1972). Fatty acids were determined by the method of Ackman (1969) using a gas chromatograph (model GC-8A, Shimadzu Scientific Instruments, Inc., Columbia, MD) equipped with an integrator (model SP4270, Spectra-Physics, Mountain View, CA). Separation of the fatty acid methyl esters was achieved using a borosilicate glass wide bore capillary (30 m × .75 mm I.D.) column with Supelcowax 10 bonded phase (1.0 μm film) packing (Supelco, Inc., Bellefonte, PA) at a column temperature of 230 C with nitrogen as the carrier gas and detector and injector temperatures of 300 C. Fatty acids were identified by comparison of retention times with those of standard fatty acid methyl ester mixtures (Supelco, Inc., Bellefonte, PA) chromatographed under the same conditions, and were calculated from integrated peak areas as percent of total fat. Analyzed fatty acid compositions of the supplemental fats is shown in Table 2.

Hydrolysis of feed and excreta for amino acid analyses was accomplished with 6 N HCl in an autoclave (16 to 18 h) on samples containing sodium thioglycolate (mercaptoacetic acid sodium salt, w/w) to preserve methionine (Veronese et al., 1974). Cystine was converted to cysteic acid by performic acid oxidation (Schram, Moore and Bigwood, 1954; Hirs, 1956) prior to the acid hydrolysis described above, but carried out in the absence of sodium thioglycolate. Amino acids were determined by high performance liquid chromatographic (HPLC) separation of pre-column o-phthalaldehyde derivatives at room temperature (Lindroth and Mopper, 1979; Jones, Pääbo and Stein, 1981). An HPLC (model SP8000B, Spectra-Physics, Mountain View, CA) equipped with a fluorescence detector (model 157, Beckman Instruments, Inc., Berkeley, CA), an autoinjector (model 725, Micromeritics Instrument Corp., Norcross, GA), and a reversed phase 3 μm C-18 spherical silica (10 cm × 4.6 mm I.D.) column (Microsorb, Rannin Instrument Co., Inc., Emeryville, CA) was employed in the determination of all amino acids except cysteine. Cysteic acid assays were carried out using a second HPLC system (model 421A system controller, two model 110B solvent delivery modules, and model 210A sample injection valve, Beckman Instruments, Inc., Berkeley, CA) equipped with an integrator (model SP4270, Spectra-Physics, Mountain View, CA), a fluorescence detector (Spectro/Glo Fluorometer, Gilson Medical Electronics, Middelton, WI) and a reversed phase 5 μm C-18 spherical silica (25 cm × 4 mm I.D.) column (Bio-Sil ODS-5S, Bio-Rad Laboratories, Richmond, CA).

Activities of four hepatic lipogenic enzymes, fatty acid synthetase (FAS), glucose-6-phosphate dehydrogenase (D-glucose-6-phosphate:$NADP^+$ 1-oxidoreductase, EC 1.1.1.49; G6PD), ATP citrate (*pro-3S*)

lyase (ATP:citrate oxaloacetate-lyase, EC 4.1.3.8; ATP-CL) and malate dehydrogenase (decarboxylating)($NADP^+$) (L-malate:$NADP^+$ oxidoreductase (oxaloacetate-decarboxylating), EC 1.1.1.40, malic enzyme), were measured in birds from the 0, 5 and 8% added fat dietary groups during the first two weeks of the second period. Since it is known that some lipogenic enzymes and fatty acid synthesis show considerable variation throughout the daily 24 h cycle (Carrozza et al., 1979), birds were sacrificed by cervical dislocation at 0830 to 0930 h each day. A portion of the liver was rapidly removed and homogenized in 10 ml of cold (4 C) buffer (pH 7.0) containing .15 M potassium chloride, 4 mM magnesium chloride, 4 mM 2-mercaptoethanol and 4 mM N-acetyl cysteine (Herzberg and Rogerson, 1988). Following centrifugation at 100,000 × *g* for 30 min at 4 C in an ultracentrifuge (model L8-70M, Ti70.1 rotor, Beckman Instruments, Inc., Palo Alto, CA), the supernatant fractions were removed and used for quantification of enzyme activities. Protein concentration of the supernatants was determined using the Folin phenol reagent as described by Lowry et al., 1951.

FAS was determined from the rate of malonyl-CoA-dependent NADPH oxidation by the spectrophotometric method of Arslanian and Wakil (1975). G6PD was measured from the rate of glucose-6-phosphate-dependent reduction of NADP (Bergmeyer, Gawehn and Grassl, 1974). ATP-CL was determined by the method of Yeh et al. (1970), and malic enzyme was determined from the rate of malate-dependent NADP reduction (Yeh et al., 1970). Enzyme assays were conducted at room temperature using a kinetics spectrophotometer (Ultrospec model 4053, Pharmacia LKB, Piscataway, NJ). Enzyme activities

were expressed as μmoles of substrate converted to product per min per mg protein which is equal to International Units per mg protein (IU/mg).

Data were subjected to analysis of variance and linear regression analysis (Steel and Torrie, 1960).

Table 1. Composition of basal diet

Ingredient	Percent
Ground milo	61.89
Soybean meal	22.38
Dehydrated alfalfa meal	5.00
Ground limestone	7.59
Dicalcium phosphate	1.31
DL-methionine	.18
Salt	.35
Vitamin mix[1]	1.00
Trace mineral mix[2]	.10
Chromium oxide	.20
Calculated nutrient composition	
Protein, %	17.53
Metabolizable energy, kcal/g	2.67
Fat, %	2.02
Total sulfur amino acids	.65
Calcium, %	3.40
Phosphorus, %	.58

[1]Supplied the following per kg of diet: 3965 IU vitamin A, 615 ICU vitamin D_3, 1.8 mg riboflavin, 11.2 mg niacin, 4.5 mg calcium pantothenate, 5.3 μg vitamin B_{12}, 2.2 IU d-α-tocopherol acetate, .9 mg menadione sodium bisulfite, 372 mg choline chloride, and 50 mg ethoxyquin.

[2]Supplied the following (ppm): 20 Fe, 60 Zn, 60 Mn, 4 Cu, and 1 Mo.

Table 2. Fatty acid composition of supplemental fats

Fatty acid	Menhaden oil	Corn oil	Olive oil	Animal fat
	(% of total fat)			
C14	9.03	---	.07	3.41
C14:1	.37	---	---	---
C14:2	---	---	---	1.03
C15	.52	---	---	.70
C15:1	---	---	---	---
C15:2	.21	---	---	.28
C16	18.57	11.82	16.33	26.48
C16:1	11.94	.33	4.10	4.13
C16:2	.74	---	.13	.85
C17	2.39	.11	---	1.52
C17:1	2.18	---	---	.15
C18	4.78	1.81	.17	16.10
C18:1	12.70	27.16	54.87	40.14
C18:2ω6	1.62	57.44	19.50	3.47
C19	.49	---	---	.32
C18:3ω6	1.20	.85	1.23	.17
C18:3ω3	3.54	---	---	.15
C20	---	.26	.94	.41
C20:1	1.62	---	.90	.43
C20:2	.13	---	.48	.26
C20:3ω6	.21	---	.34	---
C20:4ω6	.84	---	.23	---
C20:5ω3	14.49	.22	---	---
C22:1	.66	---	---	---
C22:2ω6	---	---	---	---
C21:5ω3	.64	---	---	---
C22:4ω6	.18	---	.21	---
C22:5ω3	.30	---	---	---
C24	1.97	---	---	---
C22:6ω3	8.58	---	.49	---
C22:5ω6	---	---	---	---
C24:1	---	---	---	---
Saturated FA	37.78	14.00	17.51	48.94
Monounsaturated FA	29.49	27.49	59.87	44.85
Polyunsaturated FA	32.73	58.51	22.61	6.20
ω3 FA	27.51	.22	.49	.15
ω6 FA	4.07	58.29	21.51	3.64

CHAPTER 4

RESULTS AND DISCUSSION

Effects of diets varying in fat level and source on the performance of laying hens

Productive Performance

Egg production rate, egg mass output, feed conversion, feed intake and body weight change were not significantly influenced by dietary fat level or source (Table 3). However, the feed intake of hens fed the 8% menhaden oil diet was apparently lower than that of birds fed the other experimental diets. It is suggested that flavor may be one of the reasons causing decreased feed intake. The comparatively low egg production rate, egg mass output, body weight change, and high feed conversion also accompanied the decreased feed intake.

Yeh et al. (1970) reported that the efficiency of feed utilization for weight gain was improved by increasing the dietary fat (corn oil) level from 2 to 10%, but decreased when the fat content was increased to 20% of the diet. Donaldson (1985) also noticed that growth increased with increasing dietary fat in a balanced diet. Reid (1985) concluded that fat supplementation improved performance and feed conversion by means of its "extra-caloric" and "extra-metabolic" effects. He reported that fats exerted their "extra-caloric" and "extra-metabolic" effects by increasing nutrient availability in dietary ingredients and by improving energetic efficiency. However, the failure of added fat to improve performance in

this study was probably due to the short duration of the experiment (28 days).

Egg Weight

Egg weight was significantly influenced by dietary fat source, but not by fat level. Eggs laid by hens fed menhaden oil were lighter ($P<.05$) than those laid by hens fed either corn oil or olive oil, but were not different in weight from those laid by hens fed animal fat (Table 4).

The egg weight data obtained during this experiment did not support the report of Sell, Angel and Escribano (1987) who found that inclusion of supplemental fats in laying hen diets frequently results in an increase in egg weight. The effect was greatest during 30 to 34 wk of age, and essentially disappeared by 38 wk of age. But our data did support Hulan's (1988) report that feeding up to 12% herring meal, which contains a high level of ω-3 fatty acids, resulted in a linear decrease in egg weight ($P<.05$). Several reports have indicated that there is no effect of supplemental fat on egg weight. The reasons for disparities between these observations are not known (Sell et al., 1987). Sell et al. (1987) proposed several factors which might influence the results; among these are metabolizable energy concentrations of diets, dietary metabolizable energy:nutrient ratios, fat level tested, and the ages of the laying hens during periods of egg weight evaluation. They also suggested that increases in egg weight were related to greater intakes of linoleic acid.

Dry Matter Digestibility

The dry matter (D. M.) digestibility averaged over fat type was not significantly affected by fat level (Table 5). Significant differences were found among the different fat sources. Animal fat produced the highest D. M. digestibility. The olive oil diets were second in digestibility, and the menhaden oil and corn oil treatments had the lowest digestibilities. Combined effects of fat level and source on D. M. digestibility were significant (Table 5).

Metabolizable Energy

Metabolizable energy of the diets was increased by increasing the dietary fat level; however, the difference was not significant. The metabolizable energy content of the diets also varied with fat source. It was significantly high in animal fat and olive oil diets, but low in corn oil and menhaden oil diets (Table 6). Metabolizable energy intake of laying hens was significantly high at 8% olive oil and animal fat treatments. Significantly low metabolizable energy intake was observed at 8% menhaden oil treatment which was mainly a result of low feed consumption. However, the other treatments were not different in metabolizable energy intake (Table 7).

Fat Retention

Increasing dietary fat level from 0 to 8%, regardless of the fat source, significantly improved fat retention in laying hens. The fat source, as well as the fat level, significantly influenced fat retention. Menhaden oil as the dietary fat source produced the highest fat retention,

dietary olive oil caused the second highest fat retention, and corn oil and animal fat treatments resulted in the lowest (Table 8).

Since the experimental basal diet contains about 2% fat, which Pincludes both saturated and unsaturated fatty acids, the improved fat retention resulted from fat supplementation can be explained by Maiorino et al. (1986) who reported that added animal fat improved the absorption of fat in carrier feedstuffs. Reid's (1985) study, which reported that utilization of saturated fats was improved to a greater degree in the presence of vegetable oil by the interactions between unsaturated and saturated fatty acids, also supported this explanation. However, the reason why menhaden oil as the dietary fat source resulted in a higher fat retention than both corn oil and animal fat in this study was not clear.

Amino Acid Retention

Lysine and threonine retentions were significantly high with olive oil and animal fat treatments, and significantly low with menhaden oil and corn oil treatments. However, they were not significantly influenced by the dietary fat level (Tables 9 and 10). Retention of total sulfur amino acids (methionine plus cysteine) was significantly influenced by both fat level and source (Table 11), with the highest retention being obtained with animal fat, the lowest retention with olive oil supplementation, and intermediate retentions with corn oil and menhaden oil treatments. No consistent change was observed under the influence of the fat level.

Lysine, sulfur amino acids and threonine are the amino acids which are most difficult to supply in proper amounts from dietary proteins in poultry diets. Plant proteins, the major protein source for poultry diets, are particularly deficient in lysine. So, special attention was

paid to the retention of these critical amino acids. Amino acid retention was reported to be improved by dietary fats (Reid, 1985), however, the mechanisms by which fats improved amino acid retention remained unknown.

Nitrogen Retention

Increasing the fat level from 0 to 5% did not produce any significant difference in nitrogen (N) retention. But when the fat was added at 8%, the mean of N retention over fat type decreased significantly. The low N retention at 8% menhaden oil treatment was responsible for the significant decrease of the mean. Low feed intake was probably associated with the low value at 8% menhaden oil treatment. No differences were found between different fat sources (Table 12).

Generally speaking, the performance of the laying hens was not influenced by dietary fat level or source in this experiment. In this study, the length of the experimental period may play an important role in regulating the performance under the influence of dietary fats. However, more work is needed to elucidate the effect of fish oil on egg weight and feed intake.

Effects of dietary fats on lipogenic enzyme activities

Fatty Acid Synthetase (FAS)

Increasing the dietary fat level of menhaden oil, corn oil, olive oil and animal fat from 5 to 8% resulted in a decrease in the activity of FAS, but the differences were not significant (Table 13). FAS activity was significantly depressed to 68 and 42% of the activity of the control treatment by 5 and 8% menhaden oil feeding, respectively. However, no significant difference was found by the other fat source treatments at

either 5 or 8% fat level. Fat source, but not fat level, significantly influenced FAS activity in this case (Table 13).

ATP Citrate Lyase (ATP-CL)

The enzyme activity showed a similar pattern of changes to FAS. It was decreased to 56 and 52% of the activity of the control treatment by 5 and 8% menhaden oil feeding, respectively. Corn oil, olive oil and animal fat supplementation did not significantly affect the activity of ATP-CL (Table 13).

Glucose-6-Phosphate Dehydrogenase (G6PD)

The activity of G6PD was not significantly influenced by dietary corn oil, olive oil or animal fat supplementation at levels of 5 and 8%. However, it was significantly increased to about two (216%) and three (322%) times the activity of the control treatment by 5 and 8% menhaden oil treatments, respectively. Contrary to the effect of menhaden oil on FAS and ATP-CL, the activity of G6PD was increased rather than decreased by fat supplementation at levels of 5 and 8% (Table 13).

Malic Enzyme

No consistent pattern of change or statistically significant difference was observed in the activity of this enzyme caused by fat supplementation. Table 13 illustrates the non-significant influence on malic enzyme activity by fat supplementation.

Fatty acid synthesis and activities of some lipogenic enzymes showed considerable change throughout the daily 24 h cycle (Carrozza et al., 1979). This experiment provided cycle consistency by measuring the enzyme activities during a constant period of time each day to reduce the

time-induced variation of enzyme activity. The results of this study showed the activities of FAS and ATP-CL were not significantly influenced by the fat level, but by the fat source. Menhaden oil is the only fat source exerting inhibitory effect on these two enzymes, which is not completely in accordance with the literature. Herzberg and Rogerson (1988) showed that the lipogenic enzymes, FAS, ATP-CL, malic enzyme and G6PD, as well as *in vivo* lipogenesis, exhibited the same pattern of effects caused by diets with the addition of 10% corn oil, animal fat or marine oil. Activities of these lipogenic enzymes decreased in the order: animal fat > corn oil > marine oil. The invalid effect of dietary fats on the activity of malic enzyme and the stimulating effect on G6PD activity shown in this study contradicted the reports of Yeh *et al*. (1970) and Herzberg and Rogerson (1988). However, the effect of dietary fats on malic enzyme corresponded with Goodridge's (1969) findings. Goodridge (1969) reported that growing chicks (7 to 28 days of age) fed diets containing 15% corn oil or forced-fed corn oil had rates of hepatic fatty acid synthesis comparable to those of control chicks fed a low fat diet. He also found that malic enzyme activity was not affected by fat feeding. Yeh *et al*. (1970) fed growing chicks diets containing 2 to 20% corn oil and found that both hepatic lipogenesis and enzyme activity (malic enzyme and ATP-CL) in chicks were decreased. Not only do both enzymes respond to alterations in fatty acid synthesis, but also their activities change in a parallel fashion. They suggested that activity of malic enzyme can probably be used to estimate the activity of ATP-CL under their experimental condition, but the activities of these two enzymes might be disassociated under certain circumstances. However, in the present study,

the activities of both malic enzyme and ATP-CL did not respond to the corn oil feeding (up to 8%). Their difference in responding to menhaden oil feeding showed an inconsistent pattern of change by fat supplementation. The reasons for the discrepancies between Goodridge's (1969) findings and those of Yeh et al. (1970) are not clear. It is also difficult to explain the differences existing between our findings and previous ones, since the effects of these supplemental fats on lipogenic enzyme activities are not prominent in this experiment. Low sensitivity of the enzyme assay might be one reason. A paradigm of starvation-refeeding can be used to induce a higher rate of hepatic fatty acid synthesis in future studies (Johnson and Berdanier, 1987). Other factors, including the ingredient and metabolizable energy content of the diet, the length of the experiment period, the age and the breed of the hens, resulted in different experimental protocols. However, menhaden oil supplementation significantly lowered the activities of FAS and ATP-CL at 5 and 8% fat levels in this study, which agrees with Johnson and Berdanier's (1987) report that feeding menhaden oil (5%) resulted in lower enzyme activities and fatty acid synthesis than feeding corn oil, beef tallow or hydrogenated coconut oil. Akiba et al. (1983a) also concluded that fish meal can be used to prevent FLHS by inhibiting hepatic lipogenesis and by lowering the activities of lipogenic enzymes. Polyenoic acids such as linoleate, arachidonate, linolenate and eicosapentaenoate are more effective inhibitors of de novo fatty acid biosynthesis than are saturated or monounsaturated fatty acids (Herzberg, 1983). Wilson et al. (1986) reported a 50% lower rate of fatty acid biosynthesis in high level linoleate safflower oil diet supplementation than in comparable level

animal fat or palmitate diets. Triscari et al. (1978) found the rate of fatty acid synthesis is inversely proportional to the concentration of unsaturated dietary fat. The data shown in this study do not agree with those statements since corn oil, a high polyunsaturated fatty acid (58.51%) containing oil, at a 5 or 8% level, did not show any significant effect on enzyme activity. Whereas, menhaden oil which contains lower polyunsaturated fatty acid (32.73%) than corn oil showed inhibitory effect on enzymes. However, the finding that the enzyme activities at 8% dietary fat level are lower than those at 5% ($P>.05$) supports Triscari et al.'s (1978) study. It showed, in this study, that menhaden oil has a greater inhibitory effect on lipogenesis-related enzymes than corn oil, olive oil and animal fat, which means not only the degree of saturation, but also the location of double bonds played an important role in the regulation of hepatic lipogenesis. Therefore, a presumption can be made based on the result of this study that ω-3 fatty acids are more effective than ω-6 fatty acids in inhibiting hepatic lipogenesis. However, further study will be required for understanding the mechanisms of lipogenesis regulation.

Many methods were used for measuring the hepatic lipogenesis, including in vivo and in vitro approaches. In vivo methods measure the incorporation of tritiated water, [^{14}C]alanine, [^{14}C]acetyl-CoA, acetate-1-^{14}C or glucose-U-^{14}C into fatty acids. The in vitro methods measure the disappearance or the increasing of NADH or NADPH in the reaction by spectrophotometer or the incorporation of the isotopes substrate into the product by scintillation counter. The radioisotope method of in vitro approaches is very reliable for assaying either crude or purified enzyme

systems, while the spectrophotometric method is useful in determining the activities of purified preparations (Methods in Enzymology, 1975). In this study, crude enzyme preparations are used for spectrophotometric method. We should realize that substrate concentrations under in vivo condition are usually much smaller than the ones used in enzyme kinetics assay. Therefore, the activity we obtained from in vitro study is not exactly the same activity existing in the body.

Effects of dietary fats on fatty acid composition of the yolk

The predominant fatty acids in egg yolks from hens fed the basal diet were oleic acid (42.3%), palmitic acid (28.3%), linoleic acid (11.9%), stearic acid (7.5%) and palmitoleic acid (6.6%). Monounsaturated fatty acids consisted of about 49% of the total fat. PUFA made up 15% of the total fat (14% ω-6 fatty acids). It is not surprising that these fatty acids, except palmitoleic acid, are so rich in yolks because they were also high in the basal diet with 26, 19, 41 and 2.5% of oleic, palmitic, linoleic and stearic acids, respectively. The low content (.79%) of palmitoleic acid in the basal diet and the high content of it in yolks suggests that the mechanisms which regulate fatty acid incorporation are not simply of dietary origin.

Addition of dietary fats significantly influences the fatty acid composition but not the degree of saturation of the yolk lipids. The saturated fatty acids range from 33 to 37% of total yolk lipids in all dietary treatments (Tables 14a-d). This agrees with the findings of Summer et al. (1966). But it is quite different from the observations of Shaddad et al. (1985) where 48% saturated fat was found in yolks from White Leghorn birds that were fed a control diet. Palmitic acid and

stearic acid are the two major fatty acids in saturated fraction, ranging from 69 to 79% and 19 to 26% of the saturated fat, respectively. The reason why the saturated fraction remains so consistent is that the percentage of the main saturated fatty acid, palmitic acid, in yolks is not influenced by dietary fats. Most of the other saturated fatty acids are significantly influenced by dietary fats without substantial influence on the saturated fraction.

Yolk monounsaturated fat is significantly influenced by the fat source but not by the fat level (Tables 14a-d). It is highest in olive oil and animal fat treatments with an average level of 52 and 51%, respectively. It is lowest in corn oil treatment with an average level of 42%. Menhaden oil treatment results in an average level of 47%. These observations can be easily explained by the high monounsaturated fat contents in olive oil (60%) and animal fat (45%) and the comparatively low contents in menhaden oil (29%) and corn oil (27%). Oleic acid and palmitoleic acid are the two major fatty acids making significant changes in monounsaturated fat concentration in yolk lipids. Oleic acid makes up 42% yolk lipids with basal diet treatment, being increased to 51 and 48% by 8% olive oil and 8% animal fat treatments, and being decreased to 37 and 34% by 5% menhaden oil and 8% corn oil treatments, respectively. Palmitoleic acid makes up 6.5% yolk lipids with basal diet treatment, being increased to 7.5% by 5% menhaden oil treatment, and being decreased to 2.9, 4 and 5%, respectively, by 8% dietary corn oil, olive oil and animal fat.

Addition of dietary fats significantly affects the polyunsaturated content of yolk lipids (Tables 14a-d). The polyunsaturated fat of yolks

is increased from 15 to 19 and 28% by 5% menhaden oil and 8% corn oil treatments, respectively, and is decreased to 12 and 10% by 8% olive oil and 8% animal fat treatments, respectively. The fat levels do not influence the polyunsaturated fat content of yolks in menhaden oil treatment, but do make an increase in the polyunsaturated fat content of yolk lipids with corn oil treatment and a decrease with olive oil and animal fat treatments. By analyzing the ω-3 and ω-6 fatty acid content of polyunsaturated fraction, it is observed that only menhaden oil treatment made a significant increase in ω-3 fatty acids concentration of yolks, and this increase was from .6% (basal treatment) to 5.1% (5% menhaden oil treatment). The maximum ω-3 polyunsaturated fatty acids incorporation was reached by adding 5% menhaden oil with the levels of linolenic acid (C18:3ω3), EPA (C20:5ω3) and DPA (C22:5ω3) at .26, 1 and 3.8% of yolk lipids, respectively, and the data remained steady at 8% fat treatment. An additional .27 g of ω-3 fatty acids was incorporated into yolk fats by increasing dietary menhaden oil level from 0 to 5%. This is different from Yu and Sim's (1987) report that the maximum incorporation of ω-3 PUFA into eggs was reached with levels of EPA and DHA (C22:6ω3) at 1.2 and 5.4% of yolk lipids by 8% salmon oil treatment. Addition of menhaden oil does not influence the total ω-6 fatty acids of yolks; whereas corn oil significantly increases its incorporation from 14 (basal treatment) to 28% (8% corn oil treatment). The two-fold increase is mainly attributed to the increased incorporation of linoleic acid which is 12% of yolk lipids in basal treatment and increased to 25% by adding 8% corn oil to the basal diet. The total ω-6 fatty acids of yolk lipids is significantly decreased from 14 to 11 and 9%, respectively, by olive oil and animal fat

treatments. Linoleic acid accounts for the decrease. The increase in linoleic acid is at the expense of oleic acid. On the other hand, a decrease in linoleic acid is accompanied by an increase in oleic acid in yolk lipids. Apparently, linoleic acid might have an inhibitory or antagonistic effect on oleic acid incorporation in yolk lipids. How antagonism works is not clear. The inhibition of the desaturation process from stearic acid to oleic acid is suggested. However, monounsaturated fatty acids and polyunsaturated fatty acids tend to be replaced by each other in yolk lipids depending on the supply of fatty acids. This agrees with Summer et al. (1966). An additional .78 g of ω-6 fatty acids were incorporated into yolk fat by increasing dietary corn oil level from 0 to 8%.

In summary, menhaden oil made a significant change on egg yolk fatty acid composition by increasing ω-3 fatty acid incorporation with a mild reduction in monounsaturated fat content and without significantly affecting the saturated fraction of yolk lipids. Corn oil which contains high polyunsaturated fat (mainly ω-6 fatty acids) resulted in decreased monounsaturated and increased polyunsaturated fats of yolks. On the other hand, yolk oleic acid and palmitoleic acid were replaced by dietary linoleic acid under a corn oil diet. Olive oil and animal fat dietary treatments both resulted in increased monounsaturated and decreased polyunsaturated fats of yolks to a similar extent. Although these two dietary fats are different in the degree of saturation (animal fat is almost three times as saturated as olive oil) and fatty acid composition, their dietary effects on the fatty acid incorporation pattern of yolks are similar. The results can be explained by the fact that olive oil has both

higher monounsaturated and polyunsaturated fat (mainly linoleic acid) than animal fat, so the incorporation of monounsaturated fat into yolks under olive oil treatment is inhibited by polyunsaturated fat to a greater extent than under animal fat treatment. This explains the reason why the amount of monounsaturated fat incorporation of yolks in olive oil and animal fat treatments are similar. The results can also be explained by the fact that part of the dietary saturated fatty acids in animal fat are desaturated and metabolically converted to mono- or polyunsaturated fats before they are incorporated into the tissues. That is why the amount of saturated fat incorporation in yolks is similar between olive oil and animal fat dietary treatment. Dietary fatty acids can be elongated, shortened, desaturated or metabolized by the other processes before they are incorporated into the tissue. It is demonstrated by the increase of docosapentaenoic acid (C22:5ω3) content of yolk lipids in hens fed menhaden oil in this study, since there is very low content of this fatty acid in dietary menhaden oil. Dietary fatty acids exert their effect on fatty acid composition of yolk lipids not only by providing the fatty acid sources but also by inhibiting hepatic lipogenesis. The major fatty acids provided by hepatic lipogenesis for yolk lipids are mainly palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0) and oleic acid (C18:1) when dietary fat is low. Fatty acid composition of yolk lipids mainly depends on the dietary source under high fat diet, since hepatic lipogenesis is inhibited by dietary fats. The results of this study showed that the changes in fatty acid composition of yolk fats are not parallel with that of dietary fats. Naber and Biggert (1989) proposed that nonhepatic depot fat stores in the hens may moderate dietary influences on liver and egg

yolk composition. Leclercq (1975) reported that hens mobilize about 1.6 g of their adipose lipid stores daily, which is transported to both the liver and ovary where it could alter fatty acid composition of these tissues. In conclusion, dietary fats may play an important role in regulating the fatty acid composition of yolk lipids; however, it can not be completely counted as the only reason. The patterns of fatty acid synthesis and the activity of lipogenesis, the *in vivo* biological conversions and the velocity of stored fat breakdown can also alter the fatty acid composition of tissues.

Table 3. Effects of fat source and level on laying hen performance

Added fat	Egg production	Egg mass output	Feed conversion	Feed intake	Body weight change
	(%)	(g/day)	(kg/doz)	(g/day)	(g/day)
Menhaden oil					
0%	89.3	51.1	1.49	110.5	-2.03
1%	88.4	49.4	1.39	101.3	1.01
3%	82.1	44.9	1.38	93.2	-1.01
5%	86.6	48.6	1.39	100.3	-3.04
8%	44.2	34.5	2.32	77.1	-8.11
Corn oil					
0%	90.2	50.4	1.42	106.4	0.00
1%	91.1	57.0	1.48	112.5	0.00
3%	68.8	39.0	1.83	95.3	2.03
5%	88.4	51.1	1.42	104.4	-1.01
8%	91.1	53.9	1.27	96.3	3.04
Olive oil					
0%	87.5	52.1	1.53	111.5	2.03
1%	88.4	51.5	1.42	104.4	1.01
3%	83.0	47.8	1.37	94.3	1.01
5%	83.9	49.9	1.41	98.3	2.03
8%	90.2	53.8	1.41	106.4	5.07
Animal fat					
0%	79.5	43.5	1.44	95.3	0.00
1%	79.5	47.2	1.46	96.3	4.05
3%	86.6	52.0	1.43	102.4	1.01
5%	84.8	48.4	1.40	99.3	4.05
8%	94.6	53.6	1.31	103.4	1.01
Significance	NS	NS	NS	NS	NS

Table 4. Effects of fat source and level on egg weight

Fat source	Added fat (%)					Means
	0	1	3	5	8	
	(g)					
Menhaden oil	57.4	55.8	54.8	56.1	56.0	56.0[Z]
Corn oil	55.8	62.6	57.1	57.9	59.2	58.5[Y]
Olive oil	59.5	58.2	57.6	59.5	59.7	58.9[Y]
Animal fat	54.8	59.7	60.1	57.2	56.6	57.7[YZ]
Means	56.9	59.1	57.4	57.7	58.1	

[Y,Z]Means with no common superscripts differ significantly ($P<.05$).

Table 5. Effects of fat source and level on dry matter digestibility

Fat source	Added fat (%) 0	1	3	5	8	Means
	(%)					
Menhaden oil	72.9^{abcde}	71.8^{bcdef}	71.3^{cdef}	72.1^{abcdef}	67.2^{g}	71.0^{Z}
Corn oil	69.5^{fg}	69.3^{fg}	69.1^{fg}	71.3^{def}	73.2^{abcde}	70.5^{Z}
Olive oil	70.5^{ef}	70.4^{ef}	74.1^{abcd}	74.6^{ab}	73.9^{abcd}	72.7^{Y}
Animal fat	74.9^{a}	73.9^{abcd}	73.6^{abcd}	74.7^{ab}	74.3^{abc}	74.3^{X}
Means	71.9	71.4	72.0	73.2	72.1	

$^{a-g}$Means with no common superscripts differ significantly ($P<.05$).

$^{X-Z}$Means within the column with no common superscripts differ significantly ($P<.05$).

Table 6. Dietary metabolizable energy values

Fat source	Added fat (%) 0	1	3	5	8	Means
	(kcal/g)					
Menhaden oil[1]	2.97^{ef}	2.87^{fgh}	3.02^{de}	3.13^{cd}	3.17^{bc}	3.03^{YZ}
Corn oil[1]	2.71^{i}	2.83^{ghi}	2.99^{e}	3.15^{c}	3.33^{a}	3.00^{Z}
Olive oil[1]	2.76^{hi}	2.84^{ghi}	3.13^{cd}	3.32^{a}	3.32^{a}	3.07^{XY}
Animal fat[1]	2.94^{efg}	2.97^{ef}	3.06^{cde}	3.28^{ab}	3.39^{a}	3.13^{X}
Means	2.85^{D}	2.88^{D}	3.05^{C}	3.22^{B}	3.30^{A}	

$^{a-i}$Means with no common superscripts differ significantly ($P<.05$).

$^{A-D}$Means within the row with no common superscripts differ significantly ($P<.05$).

$^{X-Z}$Means within the column with no common superscripts differ significantly ($P<.05$).

[1]Metabolizable energy (kcal/g) of fat sources: menhaden oil = 6.7; corn oil = 11.3; olive oil = 11.6; animal fat = 9.7.

Table 7. Effects of fat source and level on metabolizable energy intake

Fat source	Added fat (%)					Means
	0	1	3	5	8	
	(kcal/day)					
Menhaden oil	327.8^{ab}	290.7^{bc}	281.3^{bc}	313.7^{ab}	244.6^{c}	291.6
Corn oil	288.8^{bc}	318.6^{ab}	285.2^{bc}	328.9^{ab}	320.4^{ab}	308.4
Olive oil	307.1^{ab}	296.1^{bc}	295.0^{bc}	325.9^{ab}	353.3^{a}	315.5
Animal fat	280.1^{bc}	286.2^{bc}	312.8^{ab}	325.7^{ab}	349.9^{a}	311.0
Means	301.0	298.0	293.6	323.6	317.0	

$^{a-c}$Means with no common superscripts differ significantly ($P<.05$).

Table 8. Effects of fat source and level on fat retention

Fat source	Added fat (%) 0	1	3	5	8	Means
	(%)					
Menhaden oil	58.1^{ghi}	64.0^{fg}	77.6^{bcd}	79.5^{bcd}	88.8^{a}	73.6^{Y}
Corn oil	40.1^{k}	63.1^{fgh}	76.6^{cde}	80.7^{bcd}	85.2^{ab}	69.1^{Z}
Olive oil	55.8^{hi}	50.6^{ij}	80.4^{bcd}	80.9^{abcd}	83.5^{abc}	70.2^{YZ}
Animal fat	44.7^{jk}	69.2^{ef}	73.9^{de}	74.9^{de}	79.3^{bcd}	68.4^{Z}
Means	49.7^{D}	61.7^{C}	77.1^{B}	79.0^{B}	84.2^{A}	

$^{a-k}$Means with no common superscripts differ significantly ($P<.05$).

$^{A-D}$Means within the row with no common superscripts differ significantly ($P<.05$).

^{Y,Z}Means within the column with no common superscripts differ significantly ($P<.05$).

Table 9. Effects of fat source and level on lysine retention

Fat source	Added fat (%)					Means
	0	1	3	5	8	
	(%)					
Menhaden oil	81.4	79.6	81.4	74.8	74.3	78.3[Z]
Corn oil	73.1	71.8	77.9	76.7	75.8	75.1[Z]
Olive oil	76.5	82.2	86.9	84.1	89.3	83.8[Y]
Animal fat	78.7	81.0	85.3	83.6	87.4	83.2[Y]
Means	77.4	78.6	82.9	79.8	81.7	

[Y,Z]Means within the column with no common superscripts differ significantly ($P<.05$).

Table 10. Effects of fat source and level on threonine retention

Fat source	Added fat (%)					Means
	0	1	3	5	8	
	(%)					
Menhaden oil	83.9	84.3	85.9	74.4	90.5	83.8[Z]
Corn oil	82.4	89.6	90.9	86.4	86.8	87.2[YZ]
Olive oil	86.4	87.2	92.1	89.4	91.6	89.3[Y]
Animal fat	91.0	87.3	90.7	90.7	91.5	90.2[Y]
Means	85.9	87.1	89.9	85.2	90.1	

[Y,Z]Means within the column with no common superscripts differ significantly ($P<.05$).

Table 11. Effects of fat source and level on sulfur-containing amino acid (methionine + cysteine) retention

Fat source	Added fat (%) 0	1	3	5	8	Means
	(%)					
Menhaden oil	91.2^{abcde}	$89.6^{abcdefg}$	91.5^{abcd}	91.1^{abcde}	82.8^{i}	89.2^{Y}
Corn oil	88.6^{bcdefg}	88.5^{defg}	88.3^{efg}	87.8^{fg}	86.6^{gh}	88.0^{YZ}
Olive oil	88.6^{cdefg}	84.0^{hi}	90.5^{abcdef}	87.3^{g}	87.9^{fg}	87.6^{Z}
Animal fat	90.5^{abcdef}	88.6^{cdefg}	91.7^{a}	91.6^{abc}	91.6^{ab}	90.8^{X}
Means	89.7^{A}	87.7^{B}	90.5^{A}	89.4^{A}	87.2^{B}	

$^{a-i}$Means with no common superscripts differ significantly ($P<.05$).

$^{A-B}$Means within the row with no common superscripts differ significantly ($P<.05$).

$^{X-Z}$Means within the column with no common superscripts differ significantly ($P<.05$).

Table 12. Effects of fat source and level on N retention

Fat source	Added fat (%) 0	1	3	5	8	Means
	(%)					
Menhaden oil	45.6^{ab}	37.5^{bc}	37.0^{bc}	45.9^{ab}	10.0^{d}	35.2
Corn oil	33.7^{c}	35.9^{bc}	35.2^{c}	37.2^{bc}	34.0^{c}	35.2
Olive oil	36.1^{bc}	33.3^{c}	37.2^{bc}	43.2^{abc}	37.1^{bc}	37.4
Animal fat	48.4^{a}	41.6^{abc}	36.9^{bc}	38.5^{abc}	36.9^{bc}	40.5
Means	40.9^{A}	37.1^{A}	36.6^{A}	41.2^{A}	29.5^{B}	

$^{a-d}$Means with no common superscripts differ significantly ($P<.05$).
^{A,B}Means within the row with no common superscripts differ significantly ($P<.05$).

Table 13. Effects of fat source and level on hepatic lipogenic enzyme activities in laying hens

Enzyme	Basal diet	Menhaden oil		Corn oil		Olive oil		Animal fat	
		5%	8%	5%	8%	5%	8%	5%	8%
	(IU/mg protein)								
Fatty acid synthetase	33.6^{ab}	22.9^{bc}	14.2^{c}	43.6^{a}	31.8^{ab}	44.7^{a}	33.8^{ab}	36.0^{ab}	30.8^{ab}
Glucose-6-P dehydrogenase[1]	17.5^{c}	37.9^{b}	56.4^{a}	17.7^{c}	22.1^{c}	17.5^{c}	20.3^{c}	18.3^{c}	20.9^{c}
Malic enzyme[2]	59.8	66.2	42.3	73.0	51.7	62.1	69.8	77.7	52.1
ATP citrate lyase[3]	42.6^{a}	24.0^{b}	22.0^{b}	42.7^{a}	42.2^{a}	32.4^{ab}	42.7^{a}	47.2^{a}	39.6^{a}

$^{a-c}$Means within a row with no common superscripts differ significantly ($P<.05$).

[1]Glucose-6-phosphate dehydrogenase (EC 1.1.1.49)

[2]EC 1.1.1.40

[3]EC 4.1.3.8

Table 14a. Fatty acid composition of yolks from hens fed menhaden oil

Fatty acid	Added menhaden oil (%)				
	0	1	3	5	8
	(% of total yolk fat)				
C14:0	.4[b]	.5[b]	.8[b]	1.0[a]	.9[a]
C14:1	.1[c]	.1[bc]	.2[ab]	.2[a]	.1[bc]
C16:0	28.2	27.2	27.5	26.5	24.1
C16:1	6.4	6.6	7.5	7.5	6.3
C17:0	.1[e]	.2[d]	.4[c]	.5[b]	.7[a]
C17:1	.1[d]	.2[cd]	.2[bc]	.3[b]	.5[a]
C18:0	7.5	7.5	8.1	8.1	8.9
C18:1	43.0[a]	40.4[a]	36.9[b]	37.1[b]	41.9[a]
C18:2ω6	11.3	13.1	12.5	11.3	9.9
C18:3ω6	.5	.5	.9	1.0	.6
C18:3ω3	0.0[b]	.1[b]	.2[a]	.3[a]	.2[a]
C20:0	.2	.2	.2	.3	.2
C20:2	.2	.2	.1	.1	.1
C20:4ω6	1.4[a]	.9[b]	.5[c]	.5[c]	.5[c]
C20:5ω3	0.0[c]	.2[c]	.5[b]	1.0[a]	1.0[a]
C22:2ω6	.1	.1	.1	.1	.1
C22:4ω6	.1[c]	.2[bc]	.4[ab]	.7[a]	.5[a]
C22:5ω3	.5[c]	1.9[bc]	3.0[ab]	3.8[a]	3.5[ab]
Saturated FA	36.3	35.5	37.0	36.3	34.8
Monounsaturated FA	49.6[a]	47.3[ab]	44.8[b]	45.0[b]	48.8[a]
Polyunsaturated FA	14.0	17.2	18.3	18.7	16.4
ω3 FA	.6[c]	2.2[bc]	3.7[ab]	5.1[a]	4.7[a]
ω6 FA	13.3	14.8	14.5	13.6	11.6

[a-d]Means within a row with no common superscripts differ significantly (P<.05).

Table 14b. Fatty acid composition of yolks from hens fed corn oil

Fatty acid	Added corn oil (%)				
	0	1	3	5	8
	(% of total yolk fat)				
C14:0	.4	.4	.3	.3	.3
C16:0	27.3	28.2	26.7	25.7	25.2
C16:1	6.3[a]	5.0[b]	4.0[c]	3.9[c]	2.9[d]
C17:0	.1	.1	.2	.1	.3
C17:1	.2	.1	.2	.1	.2
C18:0	7.3	7.8	8.0	8.1	8.9
C18:1	42.9[a]	40.0[a]	35.8[b]	34.8[b]	33.8[b]
C18:2ω6	12.6[c]	15.4[c]	21.6[b]	24.0[ab]	25.5[a]
C18:3ω6	.4	.4	.3	.3	.3
C20:0	.2	.2	.2	.2	.2
C20:2	.3[b]	.3[b]	.4[a]	.4[a]	.4[a]
C20:4ω6	1.3	1.4	1.7	1.6	1.7
C22:2ω6	.1	.1	.1	.1	.1
C22:5ω3	.6	.5	.5	.4	.4
Saturated FA	35.4	36.7	35.4	34.5	34.9
Monounsaturated FA	49.3[a]	45.2[b]	40.0[c]	38.7[c]	36.8[c]
Polyunsaturated FA	15.3[c]	18.1[c]	24.5[b]	26.8[ab]	28.3[a]
ω3 FA	.6	.5	.5	.4	.4
ω6 FA	14.5[c]	17.3[c]	23.7[b]	26.0[ab]	27.5[a]

[a-d]Means within a row with no common superscripts differ significantly (P<.05).

Table 14c. Fatty acid composition of yolks from hens fed olive oil

Fatty acid	Added olive oil (%)				
	0	1	3	5	8
	(% of total yolk fat)				
C14:0	.4	.4	.3	.3	.3
C16:0	29.0[a]	26.6[b]	25.9[b]	25.8[b]	26.4[b]
C16:1	6.6[a]	5.9[ab]	5.3[bc]	4.6[cd]	4.0[d]
C17:0	.1	.2	.1	.1	.1
C17:1	.1	.2	.1	.1	.1
C18:0	8.0[a]	7.3[ab]	6.7[bc]	6.2[c]	6.6[bc]
C18:1	40.8[d]	44.6[c]	47.5[b]	50.0[ab]	50.8[a]
C18:2ω6	12.2	11.8	11.5	10.5	9.6
C18:3ω6	.5	.5	.3	.3	.2
C20:0	.2[c]	.3[a]	.3[ab]	.3[a]	.2[bc]
C20:2	.3	.3	.2	.2	.1
C20:4ω6	1.3	1.4	1.4	1.2	1.3
C22:5ω3	.6[a]	.6[a]	.5[ab]	.4[bc]	.3[c]
Saturated FA	37.6[a]	34.7[b]	33.3[bc]	32.7[c]	33.5[bc]
Monounsaturated FA	47.5[c]	50.7[b]	52.9[ab]	54.7[a]	55.0[a]
Polyunsaturated FA	14.8	14.6	13.8	12.6	11.5
ω3 FA	.6[a]	.6[a]	.5[ab]	.4[bc]	.3[c]
ω6 FA	14.0	13.7	12.1	12.1	11.1

[a-d]Means within a row with no common superscripts differ significantly (P<.05).

Table 14d. Fatty acid composition of yolks from hens fed animal fat

Fatty acid	Added animal fat (%)				
	0	1	3	5	8
	(% of total yolk fat)				
C14:0	.4[b]	.4[b]	.6[a]	.6[a]	.6[a]
C15:0	.1[c]	.1[c]	.1[b]	.1[ab]	.1[a]
C16:0	28.6	27.5	27.5	27.7	27.0
C16:1	6.9	6.4	6.3	6.2	5.0
C17:0	.1[d]	.2[cd]	.3[bc]	.4[b]	.7[a]
C17:1	.2[d]	.3[c]	.4[b]	.5[b]	.7[a]
C18:0	7.0	7.8	7.3	7.5	7.8
C18:1	42.5	43.5	43.2	46.1	47.9
C18:2ω6	11.7[a]	11.5[a]	11.7[a]	8.6[b]	8.0[b]
C18:3ω6	.4[a]	.4[a]	.4[a]	.3[b]	.2[b]
C20:0	.2	.2	.2	.2	.3
C20:2	.2	.2	.3	.3	.2
C20:4ω6	1.4	1.2	1.2	1.1	1.1
C22:5ω3	.6	.5	.5	.5	.4
Saturated FA	36.2	36.1	36.1	36.4	36.5
Monounsaturated FA	49.5	50.1	49.9	52.8	53.5
Polyunsaturated FA	14.3[a]	13.8[a]	14.0[a]	10.8[b]	10.0[b]
ω3 FA	.6	.5	.5	.5	.4
ω6 FA	13.5[a]	13.1[a]	13.3[a]	10.0[b]	9.3[b]

[a-d]Means within a row with no common superscripts differ significantly (P<.05).

CHAPTER 5

CONCLUSIONS

The addition of menhaden oil, corn oil, olive oil, or animal fat to laying hen diets at levels of 1, 3, 5 or 8%, respectively, resulted in non-significant improvement in performance based on egg production rate, egg mass output, feed intake, feed conversion, and body weight change (Table 5). Fat retention was significantly improved by increasing the dietary fat level (Table 4). Among the tested fat sources, menhaden oil produced the highest fat retention, corn oil and animal fat resulted in the lowest. Egg weight was significantly influenced by the fat source but not by the fat level. The eggs laid by the hens which had menhaden oil as a dietary fat source were significantly lighter than those laid by hens fed with dietary corn oil and olive oil ($P<.05$), but not by hens fed with dietary animal fat (Table 12). However, contrary to reports in the literature, fat supplementation did not increase egg weight.

The activities of lipogenic-related enzymes, including fatty acid synthetase (FAS), glucose-6-phosphate dehydrogenase (G6PD), and ATP citrate lyase (ATP-CL), were significantly influenced by fat supplementation. FAS activity in menhaden oil treatment was inhibited to 68 and 42% of the activity in basal diet treatment by 5 and 8% menhaden oil feeding, respectively. The activity of ATP-CL was decreased to 56 and 52% of the activity in basal diet treatment by 5 and 8% menhaden oil feeding, respectively. The activity of G6PD, contrary to the report of Carrozza et al. (1979), was not inhibited but rather enhanced by 5 and 8%

menhaden oil feeding to two and three times the activity in basal diet treatment, respectively. Corn oil, olive oil and animal fat supplementation did not significantly influence these enzymes. The activity of malic enzyme was not significantly affected by fat supplementation. The parallel relationship among the activity levels of FAS, ATP-CL, G6PD and malic enzyme caused by fat supplementation as reported in the literature was not seen in this study (Figures 1a-d).

Menhaden oil feeding at the levels of 0, 1, 3, 5 and 8% resulted in a significant ($P<.05$) linear increase in the ω-3 fatty acid content of the yolk lipids (Figure 2). Corn oil, olive oil and animal fat supplementation resulted in a non-significant change of the ω-3 fatty acid content. Omega-6 fatty acid content of yolk lipids was significantly increased from 14 to 28% by 8% corn oil feeding and decreased from 14 to 12, 11 and 9% by 5% menhaden oil, 8% olive oil and 8% animal fat supplementation, respectively. Data shown in Figure 3 demonstrate a significant linear relationship between ω-6 fatty acids content of yolk lipids and the level of dietary corn oil. Oleic acid of yolk lipids tended to be replaced by linoleic acid in the diet. Figures 4a-c illustrate a significant inverse relationship between oleic acid and linoleic acid content of yolk lipids with corn oil, olive oil and animal fat supplementation. Corn oil, the high linoleic acid containing oil (57%), caused a 9% drop in oleic acid content and a 13% increase in linoleic acid of yolk lipids. Olive oil, the high oleic acid containing oil (55%), reduced the linoleic acid content of yolk lipids by 3% and increased the oleic acid content by 10%. These findings agree with the reports in the literature.

The monounsaturated and polyunsaturated fatty acid contents of yolk lipids can be significantly influenced by dietary fat supplementation. However, the saturated portion of yolk lipids remained constant. The "good fatty acids" do incorporate into yolk lipids to a great extent. Based on the calculation, the total ω-3 fatty acid content of an egg (average 56 g/egg) was increased from .04 g (basal diet) to .31 g by 5% menhaden oil treatment; the total ω-6 fatty acid content of an egg (average 59 g/egg) was increased from .87 g (basal diet) to 1.65 g by 8% corn oil treatment. Menhaden oil ω-3 fatty acids and corn oil ω-6 fatty acids were deposited with a calculated efficiency of 36.3% (Figure 5) and 17% (Figure 6), respectively. The literature suggested that ingestion of moderate amounts (4 to 8 g/day) of ω-3 fatty acids derived from fish oil as well as limiting fat intake to less than 8% of total calories and increasing dietary linoleic acid intake to 4 to 8% of the total calories in human diet will be beneficial (Goodnight et al., 1982). The increase in ω-3 and ω-6 fatty acids in yolk lipids caused by fat supplementation is encouraging. However, it is still a rather small contribution to the recommended human diet. For the overall evaluation of the nutrition value of eggs, more research is needed to elucidate the relationship between dietary fatty acids and cholesterol content of yolk lipids in order to get an egg with maximum ω-3 fatty acids incorporation and minimum cholesterol content.

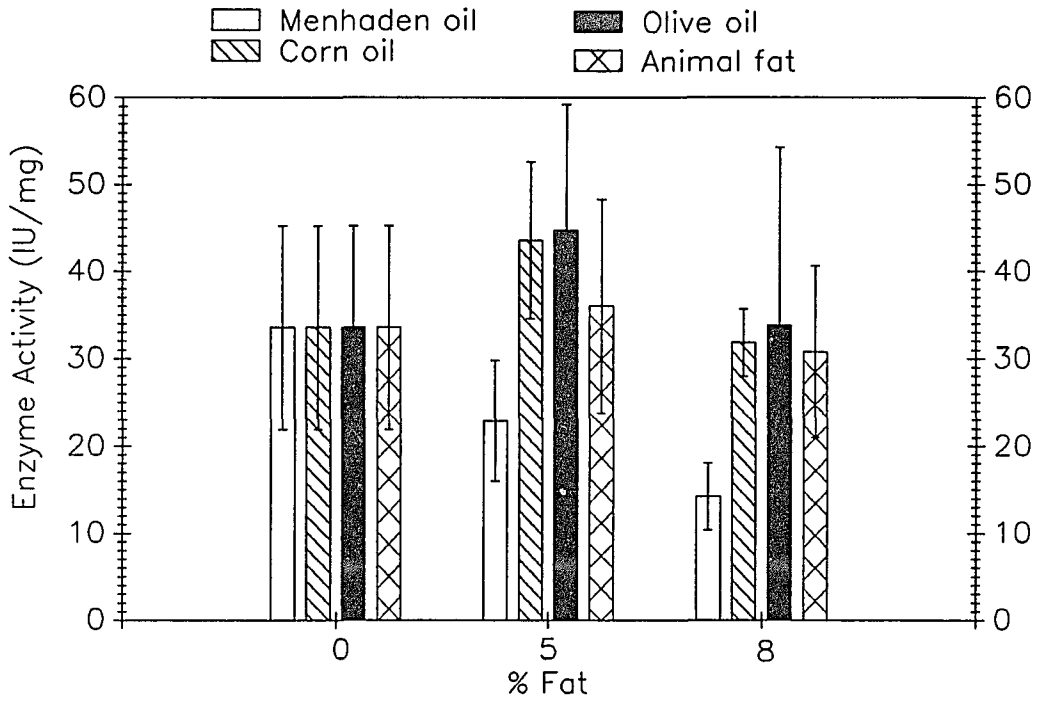

Figure 1a. Effect of diets varying in fat level and source on hepatic fatty acid synthetase activity in laying hens.

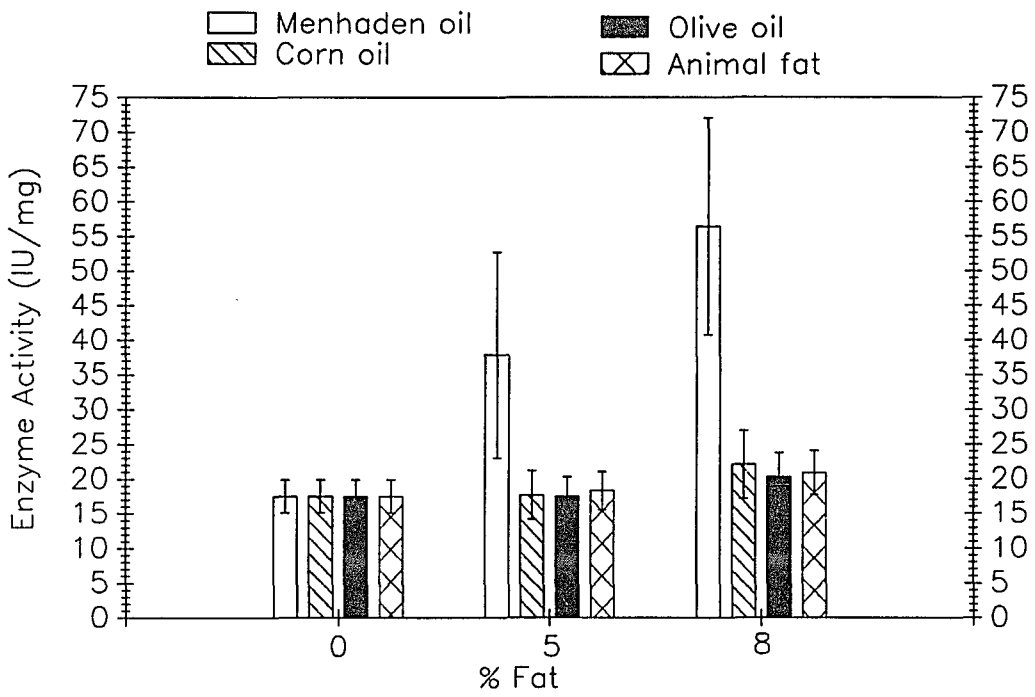

Figure 1b. Effect of diets varying in fat level and source on hepatic glucose-6-phosphate dehydrogenase (EC 1.1.1.49) activity in laying hens.

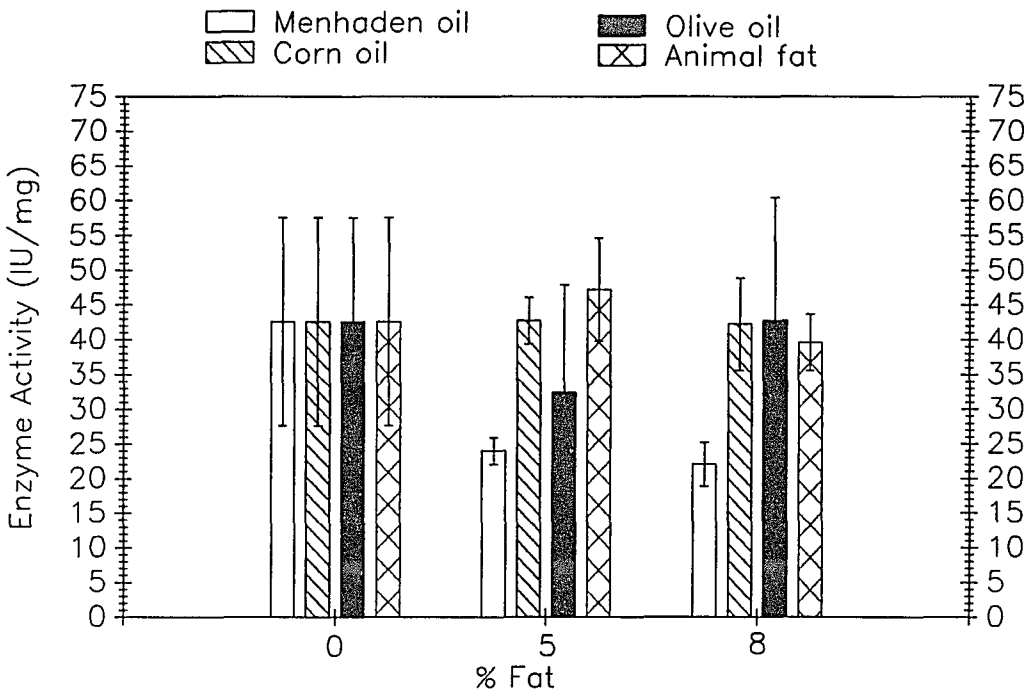

Figure 1c. Effect of diets varying in fat level and source on hepatic ATP citrate lyase (EC 4.1.3.8) activity in laying hens.

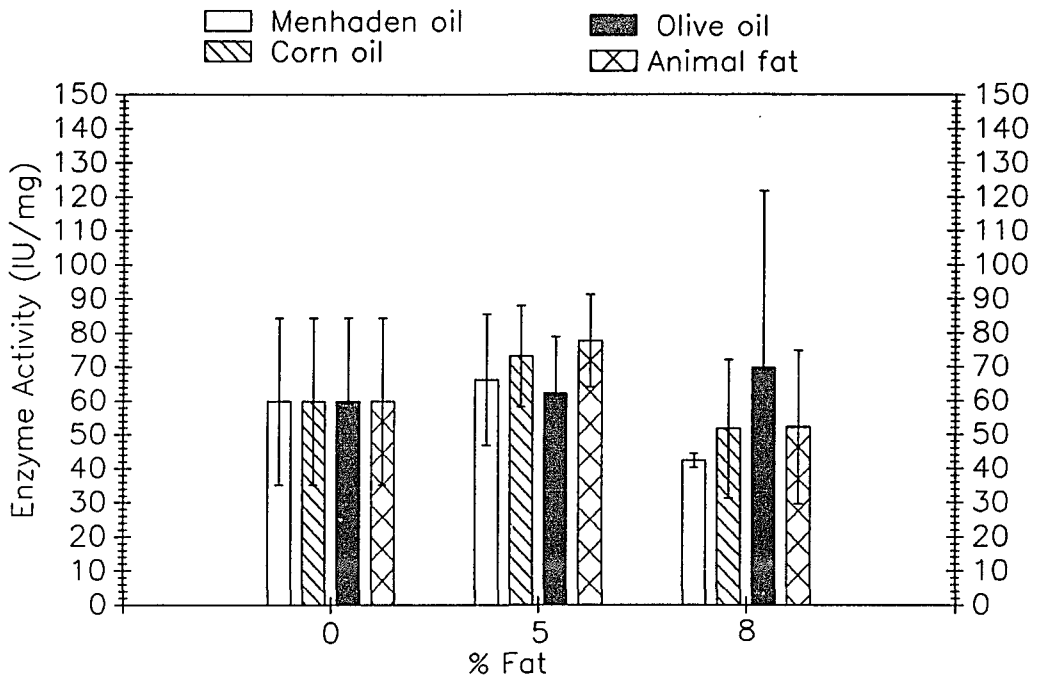

Figure 1d. Effect of diets varying in fat level and source on hepatic malic enzyme (EC 1.1.1.40) activity in laying hens.

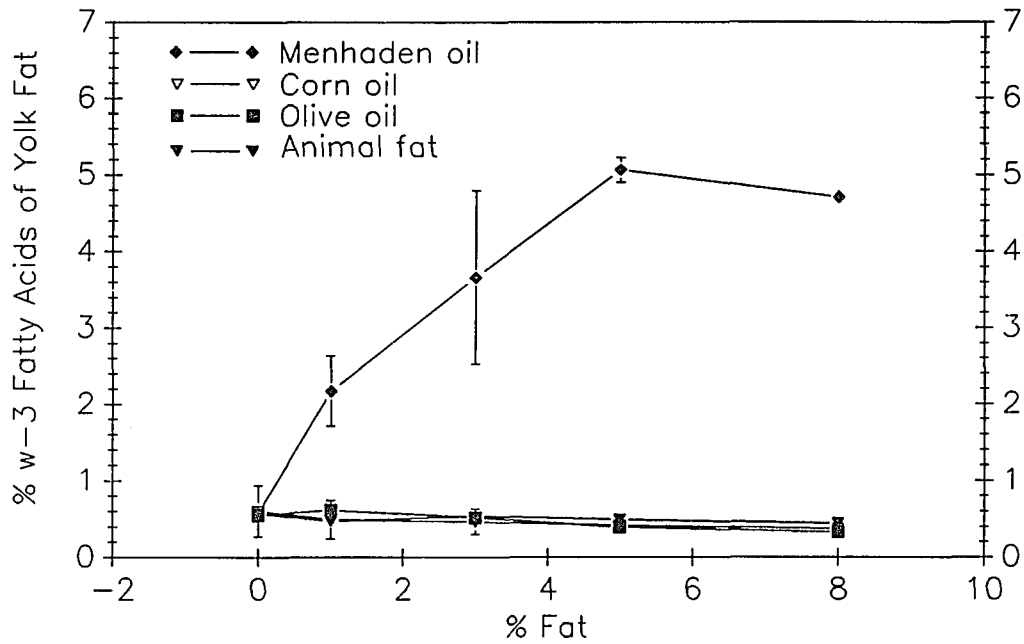

Figure 2. The effect of fat supplementation on ω-3 fatty acid content of egg yolk fat.

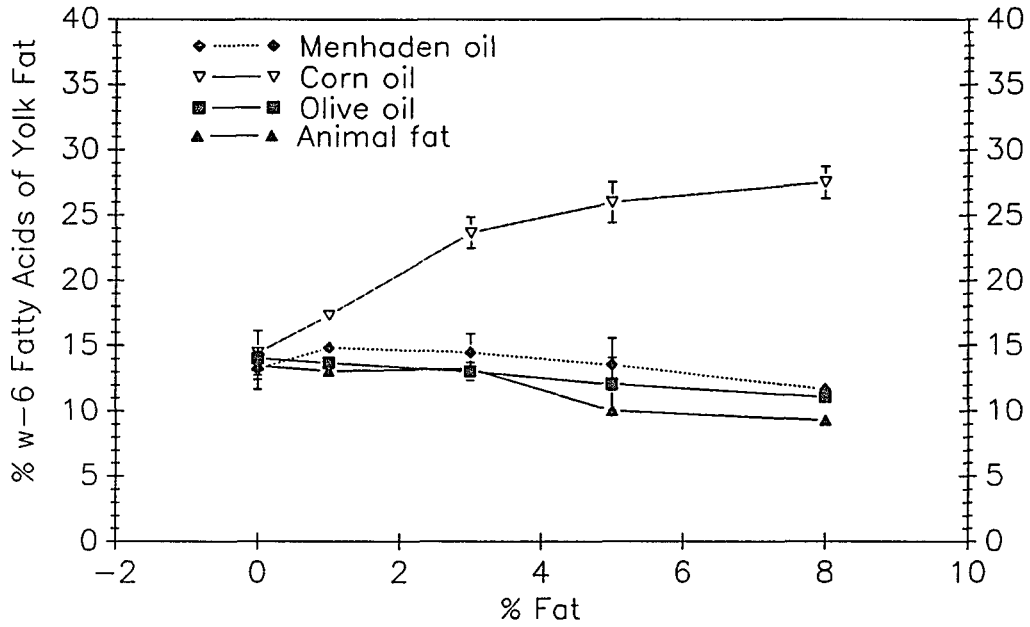

Figure 3. The effect of fat supplementation on ω-6 fatty acid content of egg yolk fat.

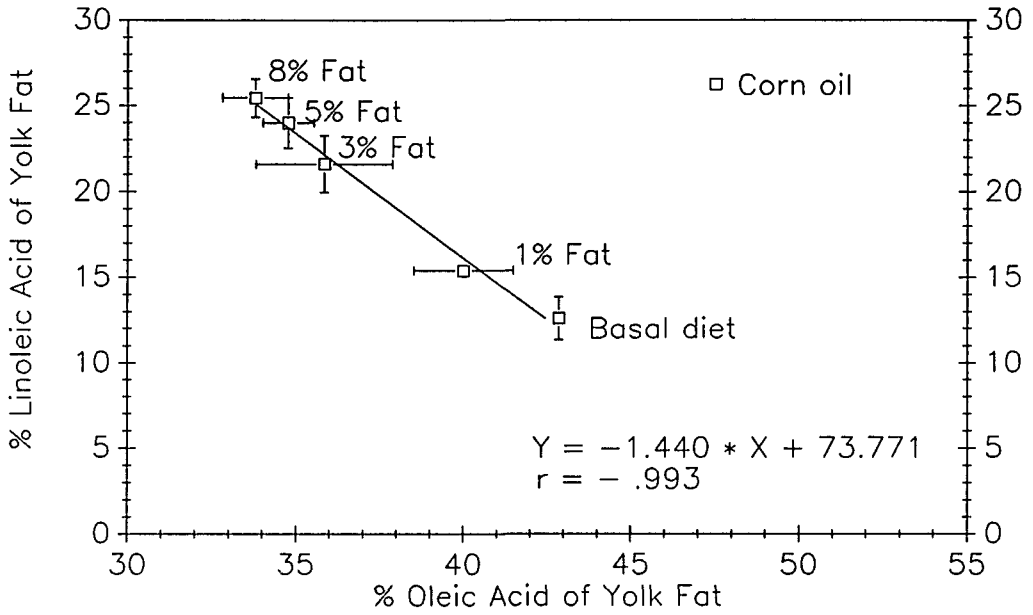

Figure 4a. The inverse relationship between oleic acid and linoleic acid content of egg yolk fat resulting from feeding corn oil to laying hens.

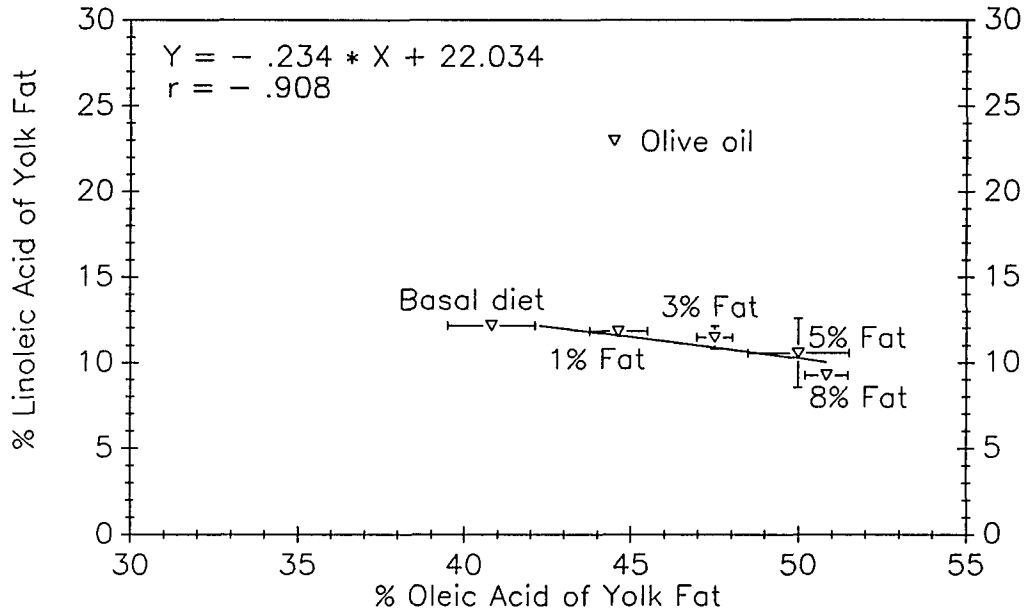

Figure 4b. The inverse relationship between oleic acid and linoleic acid content of egg yolk fat resulting from feeding olive oil to laying hens.

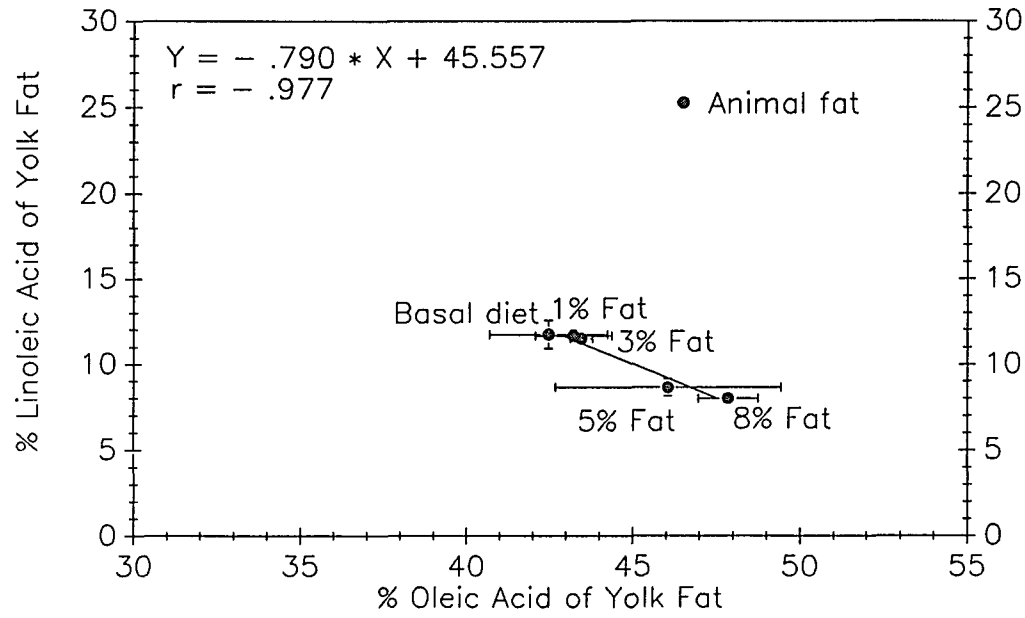

Figure 4c. **The inverse relationship between oleic acid and linoleic acid content of egg yolk fat resulting from feeding animal fat to laying hens.**

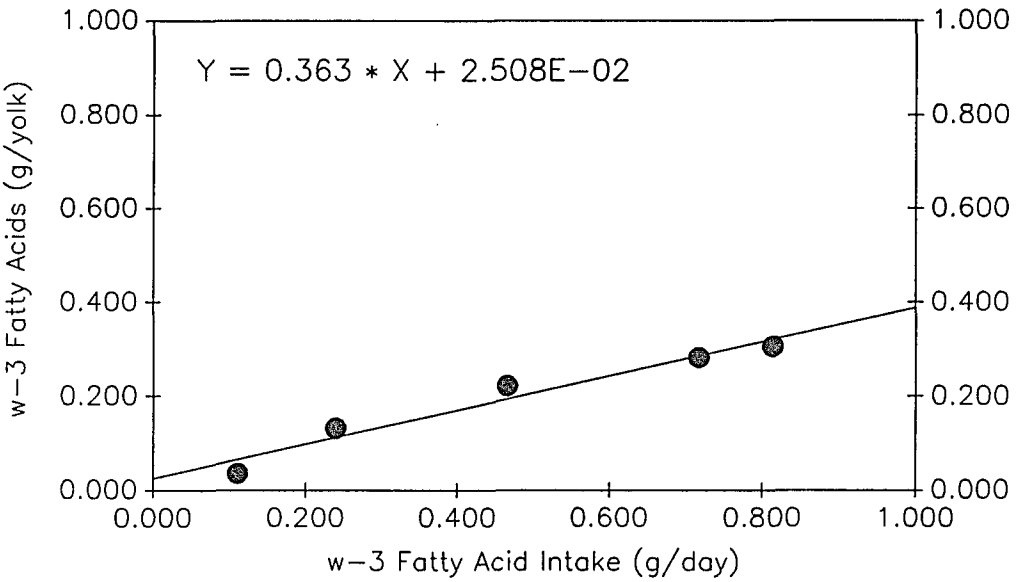

Figure 5. Efficiency of deposition of ω-3 fatty acids in egg yolk fat from dietary menhaden oil.

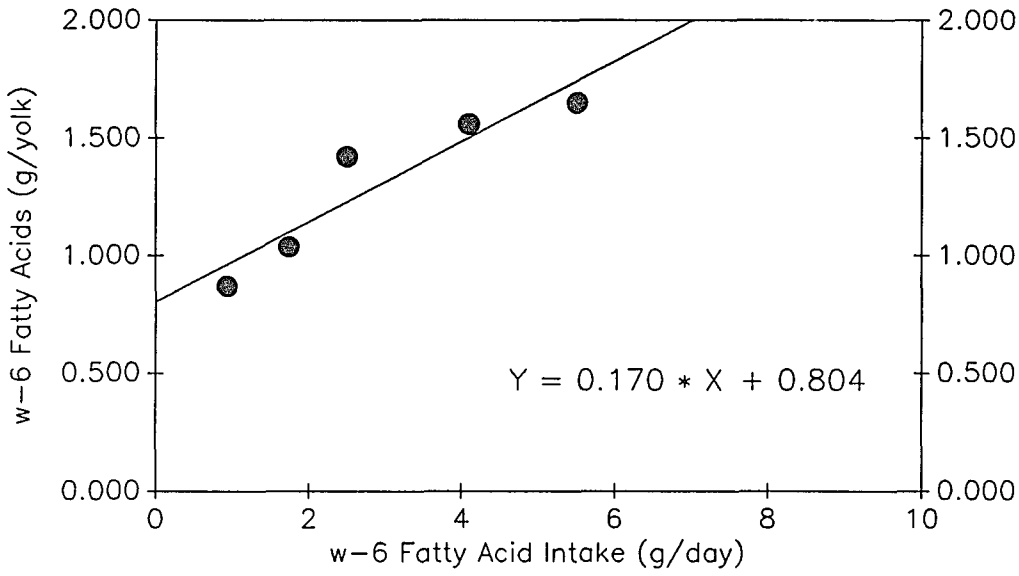

Figure 6. Efficiency of deposition of ω-6 fatty acids in egg yolk fat from dietary corn oil.

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