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ALPHA-LINOLENIC ACID METABOLISM IN THE EGG AND THE DEVELOPING CHICK EMBRYO

by

© GEETHA CHERIAN

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

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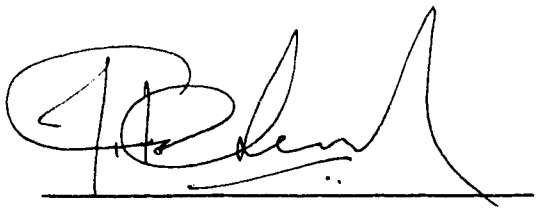

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P. E. Wainwright

Date: DECEMBER 07, 1992

To my beloved parents, Mr and Mrs T.M Cherian

Mathilumkal, Kerala, India

With all my love

I dedicate this thesis.

ABSTRACT

The importance of longer chain n-3 polyunsaturated fatty acids (PUFA) in the development of brain tissue was studied. Experiments were conducted, to develop an avian model for studying long chain n-3 PUFA metabolism in the maternal/fetal/neonatal system, to determine the extent to which the laying hen's dietary n-3 fatty acids could enrich yolk n-3 PUFA composition, to investigate the net transfer of n-3 fatty acids from the egg by the developing chick, to examine any selective transfer of n-3 PUFA by the developing chick, and to investigate the effect of yolk fatty composition on the cholesterol metabolism of the hatched chick. The second part of the thesis investigates the potential of using n-3 fatty acid enriched egg as a source of long chain PUFA for the developing infant through breast milk.

Eggs were enriched with n-3 fatty acids by feeding diets containing ground flax or canola seeds into diets for laying hens. Diets containing flax seed increased ($P<.05$) the total n-3 fatty acids to 10.8% compared with 1.6% in a regular egg. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) constituted 1.9% in the n-3 PUFA enriched eggs. The brain of chicks hatched from n-3 PUFA enriched eggs contained higher DHA in the phosphatidylethanolamine (PE) fraction with a concomitant reduction in arachidonic acid. The chick embryo preferentially utilized DHA and arachidonic acid from the yolk lipids. The levels of C20 and C22 PUFA in the hatched chicks were higher than their original amount in the yolk lipids. The chicks hatched from n-3 PUFA enriched eggs showed reduced plasma and liver cholesterol compared with chicks from n-6 fatty acid enriched eggs which also had lower plasma cholesterol and accumulation of cholesterol in liver. These experiments demonstrated the role of maternal

dietary n-3 fatty acids in modulating the DHA content of chick brain and the cholesterol content of the progeny.

Consumption of two n-3 PUFA eggs per day for six weeks increased C20 and C22 n-3 PUFA in breast milk without a reduction in arachidonic acid. Analysis of the breast milk phospholipids indicated preferential deposition of 20:4 n-6 in the sn-2 and DHA in the sn-1 position. A survey of the fatty acid compositon of infant formulas showed an absence of C20 and C22 PUFA. The chicken egg yolk with proper manipulation of the laying hen's diet could be an excellent dietary source of n-3 fatty acids for the developing brain. The lipids extracted from n-3 fatty acid enriched egg would seem ideal as a natural lipid alternative to be incorporated into formulas to achieve an improved fatty acid composition that would reflect the fatty acid composition of breast milk.

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Finally honour be to God who made all this possible.

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Chapter 1. Introduction

The brain contains high amounts of lipids. These lipids are structural and not energetic. Brain lipids are rich in n-3 polyunsaturated fatty acid (PUFA) such as docosahexaenoic acid (22:6 n-3, DHA) which can be derived from the precursor α-linolenic acid (18:3 n-3, LNA). Across mammalian species levels of DHA are similar in the brain despite wide variations in diets, which suggests an essential role for DHA in this tissue (Crawford et al., 1989). The rapid accumulation of DHA in the brain tissue of pre-natal and suckling mammals during periods of brain cell division and growth (Clandinin et al., 1980ab) suggests that the provision of DHA or the precursor LNA through maternal diet may be necessary for optimum synthesis of structural lipids, growth and functional development.

Maternal, fetal and neonatal nutrition with respect to n-3 fatty acid metabolism in brain tissue has been studied extensively in mammals (Walker, 1967; Sinclair and Crawford, 1972; Crawford et al., 1989; Connor et al., 1990). Nevertheless, the origin of long chain n-3 fatty acids in the brain tissue of the fetus and the neonate remains uncertain. In mammals, lipids from maternal circulation pass through the placenta before reaching the fetus. The placenta may transfer, synthesize or selectively transport certain fatty acids to the fetus which creates a problem in evaluating the contribution of maternal diet in fetal n-3 PUFA nutrition. Considering the importance of n-3 PUFA in the growth and development of brain tissue and central nervous system, a more 'controlled' animal model, avian system (hen-egg-embryo-chick) which separates the embryo from the

metabolic intervention namely the placenta, was adopted to study n-3 fatty acid metabolism in the maternal-fetal-neonatal system.

1.1. Studies on Mammals

In mammals, developing embryos are continously nourished by lipids of the maternal circulation through the placenta. The placenta facilitates the passage of nutrients and acts as a reservior of lipid supply to nourish the fetus. In rats, for example, up to 50% of the fatty acids of fetal circulation is derived from maternal circulation through the placenta (Coleman, 1986). A five fold increase of fetal and placental DHA compared with the DHA content of maternal serum has been reported in rats (Chen et al., 1992). The higher DHA might be due to greater conversion of precursor as indicated by the increased Δ-6 desaturase activity in the placenta (Noble, 1980,1985; Wahle, 1974).

In humans, placental transfer of preformed DHA from maternal blood to the fetus is a major source of fetal brain DHA (Carlson and Salem, 1991). This selective transfer may cause significant reduction of DHA in maternal plasma during late gestation (Holman, 1991). Furthermore, preferential uptake of DHA over precursors has been reported in the rat and the chick (Anderson and Connor, 1988; Anderson et al., 1990; Cherian and Sim, 1992a). Recently, it has been suggested that fetal liver may convert LNA to DHA which is secreted in the blood as lipoprotein and incorporated selectively to the brain tissue (Bazan, 1990). Therefore, the brain DHA level in the mammalian fetus is influenced by maternal diet as well as the enzymatic capacity in the placental and fetal liver to synthesize DHA from the precursor LNA. Thus, in mammals during prenatal period, the placental contribution creates difficulty in understanding the role of maternal diet

as well as the origin of longer chain n-3 fatty acids in the brain tissue of the progeny.

1.2. Avian models

Chickens are monogastric animals. As such, the body fat composition or the yolk fat composition can be altered readily by dietary manipulation. Further, chickens are a unique experimental animal in that they provide an automatic daily biopsy (the egg) without causing pain or sacrificing the animal.

Many similarities exist between mammalian and avian species in the supply and demand of long chain PUFA during developmental period (Noble and Cocchi, 1989). In an average egg, lipids comprise 5-6 gm are contained in the yolk. Thus egg lipids constitute a major nutritional component and serve the purpose of sustaining and nourishing the developing chick embryo (Allen and Mackey, 1982; Noble, 1986; Noble and Cocchi, 1990). Therefore, the importance of yolk lipids for the development of the chick embryo is unequivocal (Romanoff, 1960; Noble and Moore, 1966).

Because fat composition can be manipulated by diet, the egg is a unique research tool. In oviparous species, the developing embryo is absolutely dependent on nutrients stored in the egg yolk. Once a fertilized egg is incubated, lipid rich yolk is the only source of energy and also supplies n-3 fatty acids for the structural membranes of the developing embryo. Thus, the developing chick is in a nutritionally 'controlled' environment not influenced by maternal supply of fatty acids or other nutrients through the placenta as in mammals. This biologically 'self contained' model allows close relationship between nutritive substances and their physiological utilization. Thus, the laying hen and

the chick can be useful model for studying the effect of maternal dietary n-3 fatty acids on the brain tissue composition and the lipid metabolism of the progeny (Cherian and Sim, 1991; 1992b).

Furthermore, as incubation takes only 21 days, the time span involved in raising multi-generation progeny with varied n-6 to n-3 ratio or severe n-3 fatty acid deficiency situation can be easily reduced. Raising multi-generation of progeny with n-3 fatty acid deficiency has been reported to produce increased pup mortality in rodents (Guesnet et al., 1986). Addressing the importance of n-3 fatty acids in brain and behavioral development, Wainwright (1992), reported that experiments carried out with fewer pups taken as expermental unit may not be an accurate measure of functional parameters.

Upon hatching, the newly hatched chick can be used to study the effect of maternal laying hen diet or neonatal dietary (chick diet) n-3 fatty acid modification on brain tissue composition, lipid metabolism and behavioral changes in the progeny. In view of the preference of brain tissue for long chain n-3 fatty acids, and the possible involvement of the long chain n-3 fatty acids in events related to brain and visual development in infants, the information obtained from the n-3 PUFA enriched egg and the chick (avian model) appears to be valuable in understanding the role of maternal diet in the n-3 PUFA nutrition of progeny.

1.3. Essentiality of n-3 Fatty Acids

1.3.1. Sources

There are two sources of n-3 fatty acids, land (terrestrial) source represented by α-linolenic acid (LNA, 18:3 n-3) and marine source represented by eicosapentaenoic acid (EPA, 20:5 n-3), docosapentaenoic acid (DPA, 22:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3) (Figure 1.1). The nomenclature, n-3 or (omega-3), is based on the location of the first double bond counting from the methyl end of the fatty acid molecule.

In nature, LNA is predominantly seen in the chloroplast of green leafy vegetables and also in seed oils such as flax, canola and soy. Phytoplankton synthesize EPA and DHA as the major n-3 fatty acids (Moreno et al., 1979) and as phytoplankton are at the bottom of the marine food chain, all other forms of marine life eventually become enriched with these n-3 fatty acids. Linolenic acid and its long chain derivatives are important components of animal and plant cell membranes. In mammals and birds, the n-3 fatty acids are distributed selectively among lipid classes. LNA is found in triglycerides, in cholesteryl esters and in very small amounts in phospholipids, while the longer chain metabolites are found in cholesteryl esters and phospholipids (Innis, 1991). DHA, like EPA can be derived only from direct ingestion or by synthesis from dietary LNA.

1.3.2. Metabolism

To understand the effects of n-3 fatty acids on growth and development, it is essential that we understand the metabolism of LNA to its longer chain fatty acids, particularly DHA. Dietary LNA is converted to EPA and DHA through a series of steps involving desaturation and

subsequent chain elongation. The Δ-6 desaturase is regarded as the rate-limiting enzyme in long chain n-3 and n-6 fatty acid syntheis because it controls the entry of the 18 carbon fatty acids into the desaturation-elongation sequence. The desaturase enzymes are located mainly in the microsomal fraction of the liver. The major pathway for formation of long chain n-3 PUFA commences with Δ-6 desaturation at carbon 6-7 of 18:2 n-6 and 18:3 n-3, followed by further desaturation at carbon 5-6 by Δ-5 desaturase, and at carbon 4-5 by Δ-4 desaturase (Brenner et al., 1981; Cook, 1985). This pathway gives rise to endogenous formation of a series of 20 and 22 carbon chain fatty acids such as 20:4 n-6, 22:4 n-6, 22:5 n-6 from 18:2 n-6 and 20:5 n-3, 22:5 n-3 and 22:6 n-3 from 18:3 n-3 (Figure 1.2).

The efficacy of LNA in synthesizing C20 and C22 n-3 fatty acids depends on factors such as concentration of n-6 fatty acids and ratio of n-6 to n-3 fatty acids, because similar desaturase enzymes are involved in the synthesis of fatty acids of the n-6 family (18:2 n-6) to form longer chain n-6 fatty acids (Figure 1.2). Thus, competitive inhibition of the enzymes will occur depending on which substrate is present in the highest concentration. Therefore, the ratio or balance between n-6 to n-3 fatty acids is important for the optimum synthesis of longer chain C20 and C22 PUFA. A dietary ratio of 4:1 to 10:1 has been recommended by Health and Welfare Canada (1990). This is in contrast to the ratio of 25:1 to 50:1 in the typical Western diet (Kinsella, 1990; Simopoulos, 1991).

1.3.3. Brain Development.

Research in the area of nutrition and brain development has been dominated by concepts concerning protein or protein calorie malnutrition (Morgane et al., 1978; Smart, 1986 1990). However, 50 to 60% of the dry weight of the brain is lipid (Sastry, 1985), and a large proportion of this comprises PUFA of the C20 and C22 series (Sinclair, 1975). Crawford et al. (1981) suggested that linoleic acid, LNA and their longer chain metabolites may be of great significance to brain development. The n-3 fatty acid present in the brain tissue is DHA; the desaturated, chain elongated metabolites of the precursor LNA, and is primarly the most abundant lipid component of the cerebral cortex (O'Brien and Sampson, 1965; Crawford et al., 1976), retina (Anderson, 1979) testis and sperm (Poulos et al., 1975) in both mamalian and avian species (Noble and Cocchi, 1989). DHA is predominant in the ethanolamine fraction, and arachidonic acid, the major long chain n-6 fatty acid, is prominent in the inositiol fraction of brain and retina (Anderson et al., 1974; Sastry, 1985; Kishimoto, 1969, O'Brien and Sampson, 1965; Wheeler, et al., 1975). Despite variations in species, a similarity in the levels of DHA (Figure 1.3) observed among various animals, including man, suggesting an essential role of DHA in brain tissues (Crawford et al., 1976).

The periods in development when brain growth proceeds at a high rate, exceeding that of other organs, is known as the brain growth spurt (Dobbing and Sands, 1979). In general, the growth spurt includes dendritic development, formation of synaptic contacts, proliferation of the myelin-forming oligodendroglia and the synthesis of myelin. In the human brain, the last intrauterine trimester is the most active period of brain tissue

growth and DHA accumulation (Martinez et al., 1974; Clandinin et al., 1980ab). During pre-natal life, the accretion of n-3 fatty acid in the human brain is of quadratic type, the increase being most rapid towards the end of gestation (Martinez, 1991). This increase in brain DHA accretion continues early life and plateaus by two years of age (Martinez, 1991) (Figure 1.4) by which time 90% of human brain growth is complete (Dobbing and Sands, 1979).

In infants, the postnatal supply of DHA is from mother's milk or an appropriately balanced diet substitute. Human breast milk provides 0.2 to 0.3 % DHA (Jensen et al., 1992), the content of which is dependant on dietary habits, with up to 1.4% in women consuming marine foods (Innis and Kuhnlein, 1988). However, infant formulas do not contain DHA because fat sources are of vegetable origin. Maternal supplementation of n-3 fatty acids has been reported to increase the DHA content of milk in both humans and rodents (Harris et al., 1984a; Yeah et al., 1990; Wainwright et al., 1992). This supplementation would clearly elevate DHA intake as indicated by the increase in red blood cell (RBC) and plasma DHA of breast milk-fed infants compared with those fed formulas (Carlson, 1986). Similar observations were reported in piglets fed sow milk vs piglets fed prepared formula (Innis, 1992). Therefore, feeding of formulas with no DHA and wide ratios in n-6 and n-3 fatty acids may not support normal biochemical development of the brain when present formulas are consumed as the only source of PUFA in the diet (Arbuckle and Innis, 1992a; Hrboticky et al., 1990). Therefore, the supply of DHA to infants fed these formulas depends on the ability to synthesize longer chain n-6 or n-3 fatty acids from C18 precursors. The ability to synthesize longer chain PUFA depends on the

formula content of the precursor, the balance between 18:2n-6 and 18:3n-3, and the desaturase activity in the infant's liver tissue. In a recent study Arbuckle et al (1992b) stated that an n-6 to n-3 ratio of 4:1 is adequate for normal levels of C20 and C22 n-3 and n-6 PUFA in the brain tissue of piglets. Considering the n-6 to n-3 fatty acid ratio in the western diet of 25:1 to 50:1 (Kinsella, 1990; Simopoulos, 1991), it is logical to assume that infants fed on mother's milk may not receive adquate DHA. The activity of the desaturase enzyme is reported to be limited in newborns (Arbuckle and Innis, 1992b) and in premature infants (Putnam et al., 1982; Carlson et al., 1988). Thus a neonate born prior to term has lower DHA in the brain, and unless it is supplied in the diet and therefore is at risk of becoming deficient in DHA.

Deficiency of DHA can affect the n-3 fatty acid content of brain cellular and subcellular fractions such as synaptosomes and microsomes. Variation in the n-3 PUFA content of structural membranes may affect membrane functions by altering membrane fluidity, physico-chemical properties of the membranes such as receptor activity, the activity of membrane bound enzymes such as Na^+, K^+ ATPase and 5'-nucleotidase (Bourre et al., 1989). Dyer and Greenwood (1991) postulated that alteration in the fatty acid composition of brain cellular membranes might lead to behavioural abnormalities. Thus dietary depreviation of DHA imposed during early life may lead to anatomical as well as functional deformities.

Studies conducted on rodents (Walker, 1967; Lamptey and Walker 1976) reported that restriction of n-3 fatty acids interferes with normal visual function and learning difficulties in the offspring. These researchers evaluated several aspects of behaviour in rats fed safflower oil for two

generations. Levels of DHA in pup brain tissue phospholipids were reduced up to 90% compared with the controls fed soy oil, resulting in reduced exploratory behaviour and difficulties with black/white discrimination. These results were in agreement with Enslen et al. (1991) and Yamamoto et al. (1987) who observed lower exploratory behaviour and reduced color discrimination in rat pups from n-3 fatty acid deficient dams. Connor et al. (1991) reported that n-3 fatty acid deficiency in rhesus monkeys resulted in impaired vision, abnormal electroretinograms and polydypsia. In primates, the abnormal electroretinograms induced by n-3 fatty acid deficiency were irreversible.

Improved visual acuity in breast-fed versus formula-fed infants has been related to the increased dietary availabilty of n-3 fatty acids through breast milk. Increasing the DHA content of infant formulas resulted in improved visual acuity similar to that of breast milk fed infants (Carlson, 1992). Maternal supplementation of n-3 fatty acids has been reported to increase eye-opening, and sensory motor development in mice pups (Wainwright et al., 1991).

1.3.4. Health Effects

Apart from the role of n-3 fatty acids in growth and development, their function in the control of diet-related disease is an area of immense interest. The importance of n-3 fatty acids in health and disease became apparent in the pioneering studies carried out by Bang and Dyerberg (1972) and Dyerberg et al (1975). These researchers (Bang et al., 1980a) noted the low prevalence of cardiovascular disease in Eskimos, who eat mostly fish, a high source of n-3 fatty acids. Despite very high intakes of fat, these populations had low total serum cholesterol, low density and

very low density lipoprotein (LDL, VLDL) levels and high levels of high density lipoprotein (HDL) levels (Bang et al., 1980b). Furthermore, prolonged bleeding times and depressed platelet aggregation were also reported in these populations (Dyerberg et al., 1978; Dyerberg and Bang, 1979; Dyerberg, 1983). The high intake of n-3 fatty acids and low incidence of coronary heart disease in the populations stimulated great interest in the health benefits of n-3 fatty acids, which represents one of the most exciting chapters in nutrition research.

The longer bleeding tendency observed in the Eskimos led to further research on the modulation of eicosanoid biosynthesis by n-3 fatty acids. Eicosanoids are a group of metabolites derived from arachidonic acid (20:4 n-6) and EPA which include prostaglandins, prostacyclins, thromboxanes, leukotrienes, lipoxins and hydroxy fatty acids (Marcus, 1978). The discovery in 1979 that the eicosanoids derived from EPA have different biological properties than those derived from arachidonic acid (n-6 fatty acid) stimulated further research on the nutritional aspect of n-3 fatty acids (Needleman et al., 1979). Arachidonic acid is the precursor of the 2-series of prostanoids (prostaglandins and thromboxanes) and of leukotrienes of the 4-series. The precursor of arachidonic acid is linoleic acid, the major PUFA in western diets. EPA and DHA are precursors of the prostanoids of the 3-series and leukotrienes of the 5-series (Figure 1.5).

Competition between the two families of PUFA occurs in prostaglandin formation. EPA competes with arachidonic acid for prostaglandin and leukotriene synthesis at the cyclooxygenase and lipoxygenase level. Feeding EPA enriched diets leads to: 1) a decrease in production of

prostaglandin E_2(PGE_2) metabolites, 2) decrease in thromboxane A_2, a potent platelet aggregator and vasoconstrictor, 3) a decrease in leukotriene B_4 formation, an inducer of inflammation and a powerful inducer of leukocyte chemotaxis, 4) an increase in thromboxane A_3 a weak platelet aggregator and a weak vasoconstrictor, 5) an increase in prostacyclin PGI_3, an active vasodialator, 6) an increase in leukotriene B_5, a weak inducer of inflammation and a weak chemotactic agent (Weber et al., 1986; Lewis et al., 1986; Lorenz et al., 1983).

The imbalanced regulation of eicosanoid formation from arachidonic acid in selected cell is a common element of several health disorders. Evidence suggests that n-3 fatty acids slow the speed of eicosanoid formation. Excess EPA may compete for access to the oxygenases, decrease the amplification of hydroperoxides, inactivate the oxygenase and form alternate eicsanoids (Lands, 1986)

N-3 fatty acids also protect against atherosclerosis, a degenerative disease of arteries which leads to coronary heart disease (CHD), by increasing the formation of prostacyclin which makes the endothelial surface less thrombogenic, and blocks the production of thrombxane A_2 (Castelli et al., 1990). The reduction in the formation of thrombi and the increase in the fibrinolytic activity of plasma will also inhibit the growth of atheroma and prevent vasal occlusure (Leaf and Webber, 1988). EPA administration has effects on the metabolism of leukotrienes. In place of the 4-series of leukotrienes from arachidonic acid, a 5-series is produced from EPA (Prescott, 1984). Modification of leukotrienes and therefore, leukocyte function, by EPA feeding may be an additional mechanism through which EPA might inhibit thrombosis and CHD.

Diets rich in n-3 fatty acids lower serum cholesterol concentrations by improving receptor mediated LDL uptake (Kritchevsky, 1988; Balasubramaniam et al., 1985; Harris et al., 1989) and enhancing cholesterol excretion via bile acids, as well as decreasing VLDL production by the liver (Beyen and Katan, 1985). There are several mechanisms by which n-3 fatty acids lower VLDL levels. Suppression of both hepatic triglyceride and apolipoprotein B synthesis by fish oil has been demonstrated in humans (Harris et al., 1984b). N-3 fatty acids have also been reported to reduce the size (Leaf and, Webber, 1988) and synthesis (Harris et al., 1983; Illingworth et al., 1984) of LDL. Dietary fish oil supplementation has also been associated with reduction in triglycerides (Phillipson et al., 1985). The mechanism by which dietary n-3 fatty acids lower plasma triglyceride might involve: 1) inhibition of triglyceride synthesis in the liver (Wong et al., 1984), 2) suppression of apoprotein B production (Nestle et al., 1984), and 3) chanelling of polyunsaturated fatty acids towards oxidation and ketogenesis rather than triglyceride synthesis (Beyen and Katan, 1985).

The anti-inflammatory effects of n-3 fatty acids are mediated when EPA is metabolized by the 5-lipoxygenase pathway to a compound analogous to the metabolites of 20:4 n-6. EPA is converted sequentially to leukotriene A_5. These intermediate in the pathway may be metabolized enzymatically to either LTB_5 or LTC_5. Conversely, 20:4 n-6 is further metabolised through a series of enzymatic conversions to form leukotriene B_4, leukotriene C_4, leukotriene D_4, leukotriene E_4 (Figure 1.5). These leukotrienes are potent proinflammatory mediators; LTB_4 promotes degranulation, aggregation, and endothelial adherence. LTC_4 and LTD_4 cause arteriolar constriction, LTC_4, LTD_4 and LTE_4 mediate vascular permeability and constriction of non

vascular airway smooth muscle (Samuelson, 1983; Kremer et al., 1987, 1989). Thus in addition to the traditional role in growth and development, n-3 fatty acids play a major role in diet-related diseases.

1.4. Objectives of this Thesis

The objectives of the first part of this thesis were to develop a unique research model for studying the importance of n-3 PUFA in the maternal\fetal\neonatal system. To achieve this, we utilized a 'self-contained' model, the chicken egg. As chickens are monogastric animals, the diet of the laying hen (maternal diet) can be manipulated with different n-3 fatty acid sources to create a n-3 fatty acid rich environment in the egg. When a fertilized egg is incubated, the yolk n-3 fatty acids are the sole source of n-3 fatty acids for the developing chicken embryo. Thus, this biologically 'self contained' model allows a close correlation between nutritive substances and their physiological utilization (Figure 1.6). Therefore, a series of experiments were conducted to develop the avian model and to study the role of maternal (laying hen) dietary n-3 fatty acids on the enrichment of yolk lipids with n-3 fatty acids, to investigate the net transfer of n-3 fatty acids from the egg to the developing embryo, to examine whether there is any preferential transfer of n-3 fatty acids during the developmental (fetal) period, to investigate if there is any conversion of the precursor 18:3 n-3 by the developing chick, and to examine the effect of maternal (egg) n-3 fatty acids on the cholesterol metabolism of the hatched chick. Information derived from these studies can be useful not only in understanding the role of maternal diet in modulating the n-3 fatty acid content of brain tissue in the progeny but also useful in designing animal

products of improved nutritional value with a lower n-6 to n-3 fatty acid ratio to meet the infant's need through maternal supply (breast milk) or infant formulas. The second part of the thesis explores the possibility of delivering egg n-3 fatty acids to the developing infant through breast milk. The influence of n-3 fatty acid modified egg consumption on enriching human breast lipid fatty acids is investigated. Further, the potential of using n-3 fatty acid enriched egg yolk lipid as an alternate natural biological source of lipid in infant formula preparation is addressed.

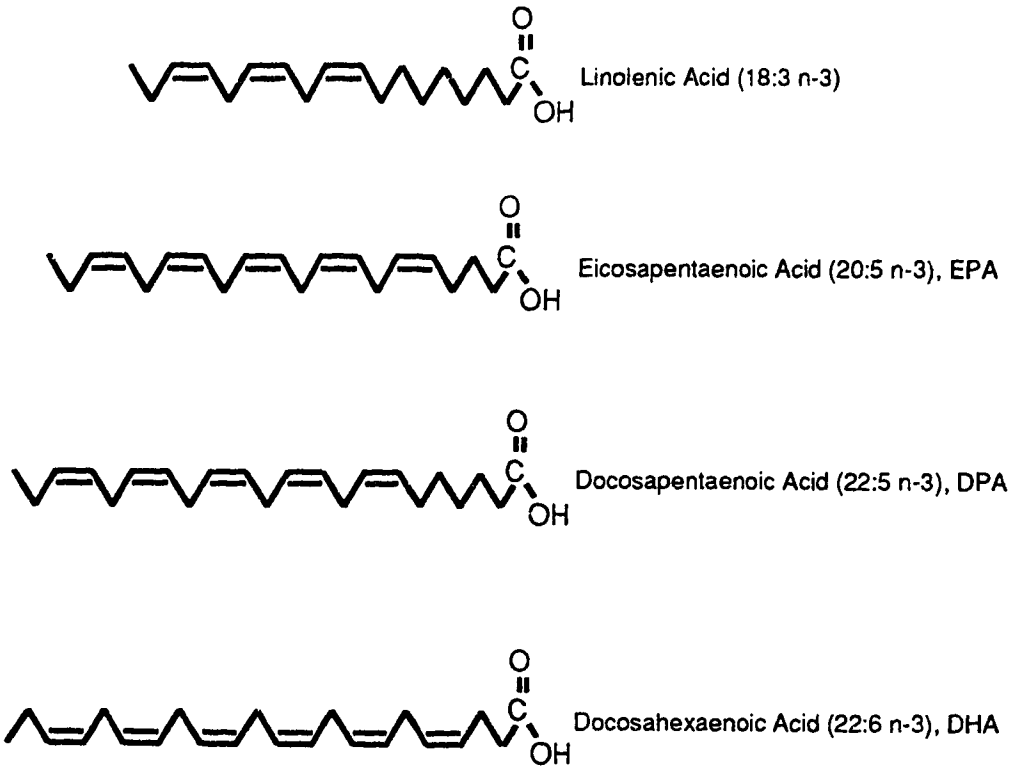

Figure 1.1. Structure of the major n-3 fatty acids

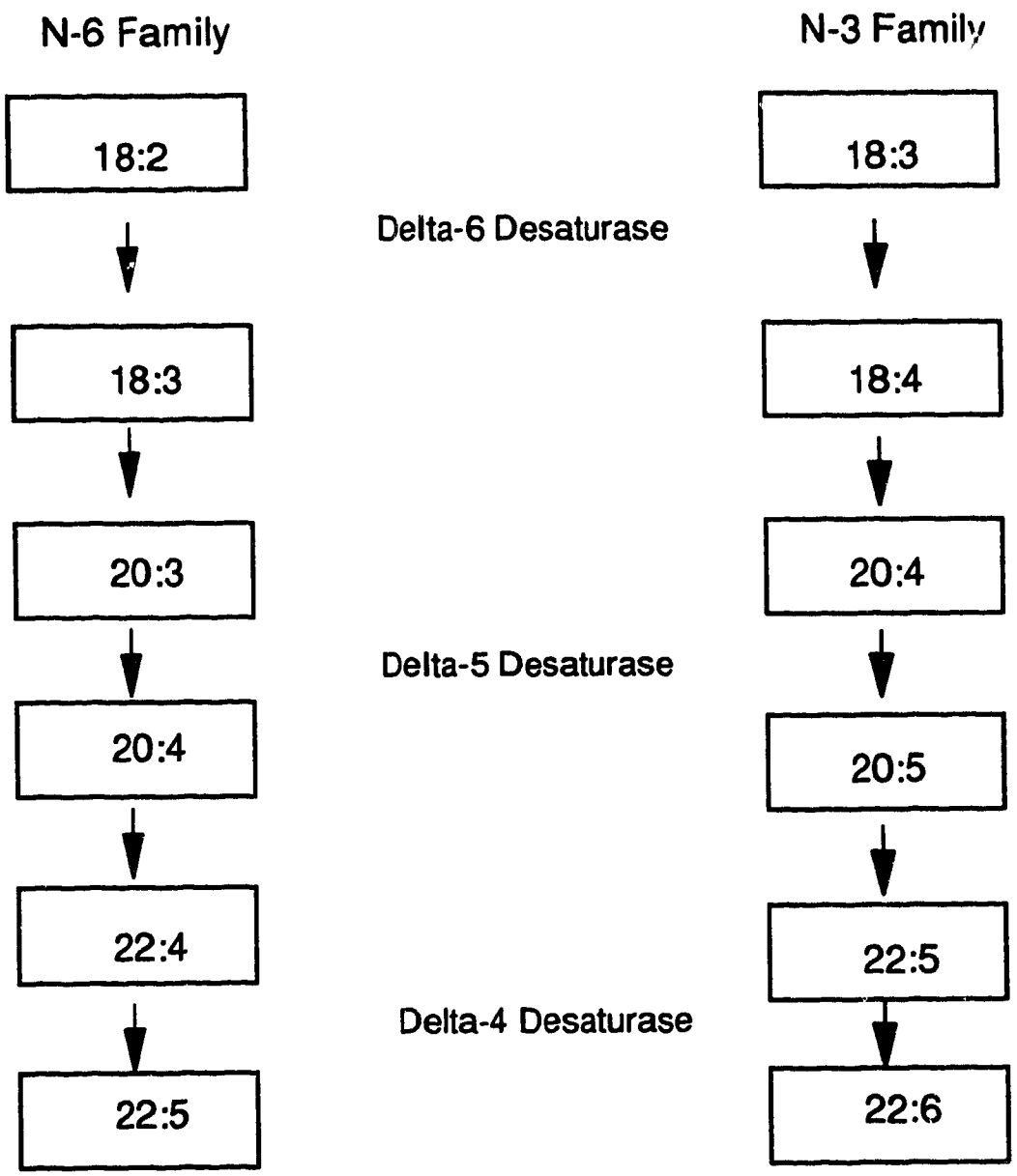

Figure 1.2. Schematic diagram of the major pathways of n-6 and n-3 fatty acid metabolism

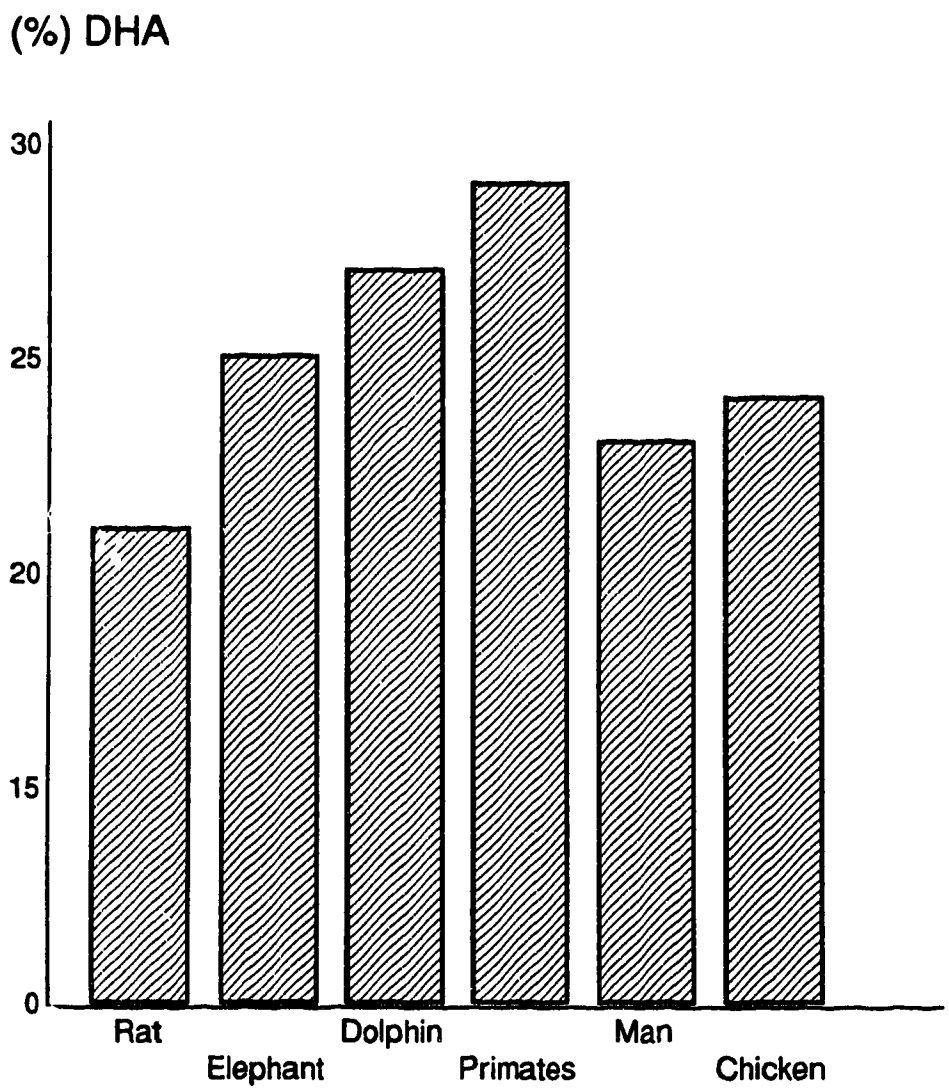

Figure 1.3. Comparison of the docosahexaenoic acid content (%) in the brain phosphatidylethanolamine fraction of different species

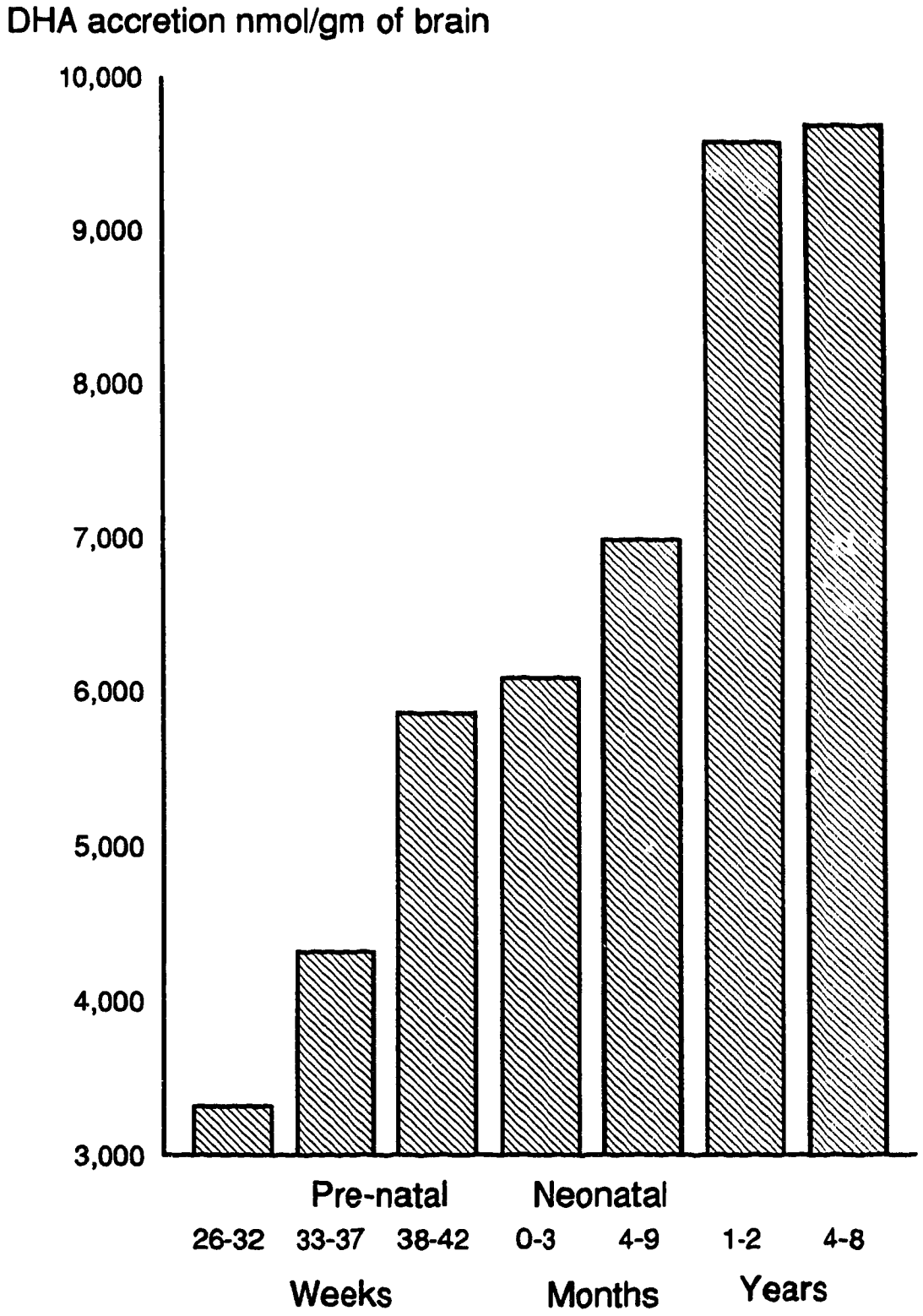

Figure 1.4. Docosahexaenoic acid accretion during pre and post natal life

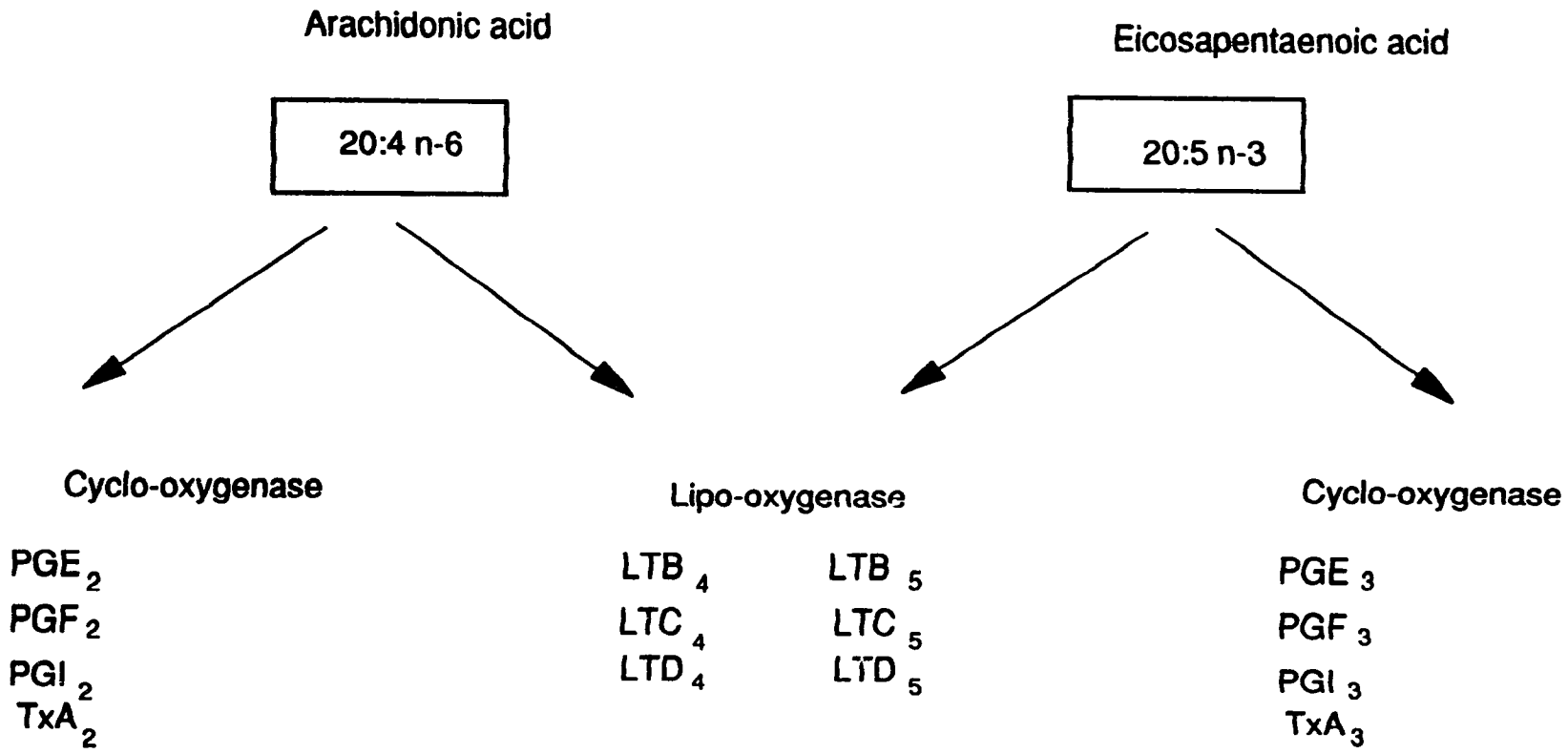

Figure 1.5. **Pathways of eicosanoid biosynthesis**

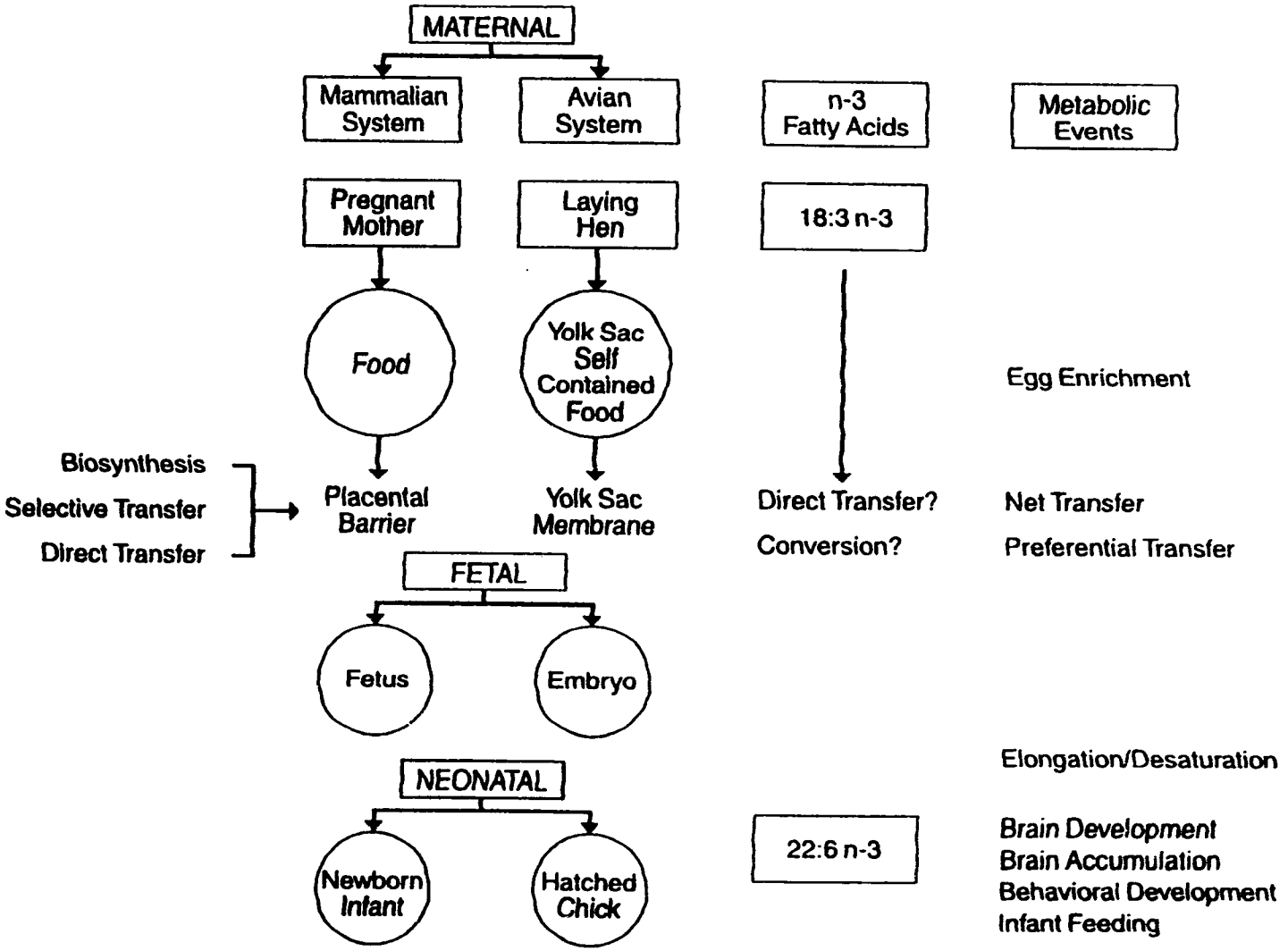

Figure 1.6 Experimental Model to Study n-3 PUFA Metabolism for Maternal/Fetal/Neonatal System

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Chapter 2. Role of Maternal Diet on the Hatched Chick

In order to examine the effect of of maternal (yolk) n-3 fatty acids on the developing chick, the egg yolk lipids have to be enriched with n-3 fatty acids. Thus in the first experiment, we incorporated into the diet of laying hens oil seeds varying in their αlinolenic acid content to study the changes in egg lipids, and to use the avian model to study n-3 fatty acid metabolism.

2.1. Effect of Feeding Full Fat Flax and Canola Seeds to Laying Hens on the Fatty Acid Composition of Eggs, Embryos, and Newly Hatched chicks[1]

2.1. Introduction

Flax and canola have been recognised as rich plant sources of α linolenic acid, the parent fatty acid of longer omega-3 fatty acids. Studies carried out with poultry have confirmed that LNA from oil seeds can be converted to longer chain omega-3 fatty acids, such

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[1] A version of this paper has been published. G. Cherian and J.S. Sim, 1991. Poultry Science 70:917-922.

as EPA, DPA, and DHA through an elongation and desaturation pathway, thus enriching the egg yolk with omega-3 fatty acids (Sim, 1990). The beneficial effects of omega-3 fatty acids, such as their anti-thrombotic as well as anti-inflammatory properties, have been established by clinical and epidemiological studies (Herold and Kinsella, 1986). Research concerning omega-3 fatty acids is of continued interest because studies have shown that long-chain polyunsaturated fatty acids accrue in the developing brain of pre-natal and suckling mammals and are essential for the synthesis of complex structural lipids and appropriate maturation of a variety of physiological processes (Clandinin and Van Aerde, 1990).

Research has shown the egg yolk lipid composition is influenced by dietary fat (Balnave, 1970). Once a fertilized egg is incubated, the constituents of the yolk are the sole source of nutrients to support the growth of the embryo (Lanser, 1979). Thus, the laying hen, the egg, and the newly hatched chick model would be very useful in studying the effects of laying hen diet on the fatty acid profile of the egg yolk, embryo and the newly hatched chick. The present study was conducted as a preliminary investigation to determine the incorporation of omega-3 fatty acid from the laying hen diet into the egg, and into the developing progeny. Such incorporation might improve chick growth, health, development, and also serve as an experimental tool to understand omega-3 fatty acid metabolism in pre-and post-natal chick tissues.

Flax and canola seeds, both rich in LNA, contain 42% fat and 23% crude protein, respectively, and are relatively inexpensive and practical to feed. The natural antioxidants in the full fat seeds protect the polyunsaturated fatty acids better than the extracted oil. Thus, if fully utilised, flax and canola seeds should serve as an excellent dietary source of energy, protein, and omega-3 fatty acids.

2.2. Materials and Methods

Animals and Diets

Forty Single Comb White Leghorn (SCWL) laying hens were housed individually in cages (30 x 40 cm) and were assigned randomly to four test diets (Table 2.1). Fatty acid compositions of the diets are shown in Table 2.2. Twenty SCWL roosters were also housed individually in cages (30 x 40 cm), provided a breeding diet *ad libitum*, and trained twice a week for semen collection. The eggs were collected daily, and the omega-3 fatty acid profile of the yolk was monitored. The hens were artificially inseminated twice on consecutive days with .05 mL of pooled semen from the roosters. Fertilized eggs (n=200), collected for a week after a plateau of omega-3 fatty acid incorporation was reached (within 2 wk), were incubated in a forced air incubator. The eggs were incubated at 37 C with relative humidity of 55% and turned once per h for 18 days. They were then transferred to hatchers with temperature at 37.2 C and relative humidity 55% for 3 days. Newly hatched chicks from

laying hens fed diets containing ground flax (FXC), ground canola (CAC), or wheat and soy bean meal (WSC) were killed by decapitation.

Sample Collection and Analyses.

Five eggs from each dietary treatment were collected on Day 0, 5, 10, and 15 after feeding the experimental diets. The eggs were weighed, broken, and the yolks were separated and weighed. On Days 15, 17, 19, and 21, ten (10) embryos from each treatment group were weighed, killed by decapitation, and the brain tissue separated, weighed, and immediately kept frozen at -30 C until analyzed. On Day 21, ten (10) newly hatched chicks from each treatment group were killed by decapitation, and blood (5 mL) and brain tissue were collected. Blood was centrifuged at 3000 rpm in a Beckman centrifuge and the plasma separated. Total lipids were extracted from the yolk, brain, and plasma with chloroform-methanol (2:1 vol/vol) by the method of Folch et al. (1957). Lipid extracts were methylated (Metcalfe et al., 1961) and analysed for fatty acids by using a gas chromatograph equipped with a SP-2330 fused-silica capillary column (30 m by .25 mm id). The gas chromatograph was programmed to start at 180 C for 10 min and to increase at 5 C/min to 195 C. Injector and detector temperatures were set at 230 C, and helium was used as a carrier gas at a flow rate of 1.5 mL/min.

Statistical analyses of data were done by one-way ANOVA using the SPSS-X (Statistical Package for Social Sciences, 1986). Significant differences among treatment means were determined using

the Student-Newman-Keuls multiple range test (Steel and Torrie, 1980).

2.3. Results

Egg Yolk Omega-3 Fatty Acid Content

Significant (P<.05) incorporation of omega-3 fatty acid in the egg yolk was obtained by feeding 8 or 16% ground flax or 16% ground canola to laying hens (Table 2.3). The omega-3 fatty acid content reached a plateau around Day 15. At this time, the total omega-3 fatty acids in the egg yolk were 7.82, 10.75, 4.15, and 1.64% of the total fatty acids for 8 and 16% flax, 16% canola, and the control group, respectively. The predominant omega-3 fatty acid in the egg yolk was LNA; however, increased concentrations of EPA, DPA and DHA were observed in the yolk of laying hens fed ground flax or canola seed. Feeding 8 or 16% ground flax or 16% ground canola resulted in similar increases in linoleic acid (LA, 18:2 n-6) content as compared with the control. There was also a significantly lower concentration of arachidonic acid (AA, 20:4n-6) in the FXC and CAC groups. The AA content of FXC group was lower than the AA content of CAC group.

Fatty Acid Composition in Chick Plasma

The egg yolk lipid composition had a significant (P<.05) effect upon the plasma fatty acid profile of newly hatched chicks (Table 2.4). Significantly higher concentrations of myristic acid (14:0)

and lower concentrations of palmitic acid (16:0) were recorded in the plasma of FXC and CAC groups. The plasma of FXC group had a significantly lower concentration of oleic acid (18:1 n-9). The total omega-3 fatty acids in the plasma of the FXC group was 12.45 and 12.97% compared to 1.95% in the WSC group. Except for DHA (1.95%), other omega-3 fatty acids were not detectable in the WSC group. Of the individual omega-3 fatty acids in the FXC group, LNA increased the most. However, in the CAC group, the DHA increase was most pronounced. Concurrent with the increase in omega-3 fatty acids, there was a significant decrease in the concentration of AA in the FXC group. Total saturated fatty acids (SAT) were not affected by treatments. Total monounsaturated fatty acids (MUFA) were significantly lower and total polyunsaturated fatty acids (PUFA) higher in the FXC groups than in the other groups.

Embryo and Chick Brain Fatty Acid Profile

The omega-3 fatty acid compositions of the developing and the newly hatched chick brain tissue are presented in Table 2.5. Significantly ($P<.05$) higher levels of omega-3 fatty acids were present in the brain tissue of the FXC and CAC groups compared with the WSC group. The predominant fatty acid in this tissue was DHA. However, there was a significant ($P<.05$) increase in DPA, EPA, and a relatively small increase in LNA in the FXC and CAC groups as compared with the control group.

2.4. Discussion

In the present study we have demonstrated that the total omega-3 fatty acid content of egg yolk can be enriched through the diet of the laying hen. α-Linolenic acid is the precursor for formation of EPA, DPA, and DHA through the desaturation elongation pathway (Anderson et al., 1989). Linolenic acid was the only source of omega-3 fatty acid in the diet. However, egg yolks from hens fed 8 or 16% ground flax and 16% ground canola were enriched with EPA, DPA, and DHA. Therefore, the laying hen can convert LNA to its elongated omega-3 fatty acids, and deposit these into the egg yolk.

Upon incubation of fertilized eggs, the yolk lipids are the sole supply of lipids for the developing embryo (Lanser, 1979). The omega-3 fatty acid enriched egg resulted in a larger pool of omega-3 fatty acids for the developing embryo. This relative enrichment was reflected in the plasma fatty acid profile of the newly hatched chick. The higher levels of LNA, EPA, DPA, and DHA in the plasma of FXC and CAC chicks indicate there is an efficient conversion mechanism by the developing embryo for the desaturation and chain elongation of LNA. Lower levels of AA in the plasma of FXC chicks deserve further attention. The present study also indicated that higher levels of LNA in the enriched egg yolk appeared to inhibit AA bio-synthesis from LA in the developing embryo. Arachidonic acid is synthesized from LA through an elongation and desaturation pathway, which is shared by both omega-3 and omega-6 families. It has been

reported (Brenner et al., 1969) that LNA competes with C18:2 n-6 for the Δ-6 desaturase enzyme where LNA is the preferred substrate over LA thus limiting the synthesis of AA from LA. Significantly lower levels of monounsaturated fatty acids were obtained in the plasma of FXC group; however, this group had the highest level of polyunsaturated fatty acids (PUFA) (both omega-3 and omega-6).

Polyunsaturated fatty acids apparently inhibit Δ-9 desaturase activity, resulting in the formation of oleic acid (18:1 n-9) (Garg et al., 1988). This inhibition, however, was not evident in the CAC group, which had a significantly ($P<.05$) higher omega-6:omega-3 fatty acid ratio. Therefore, it seems that omega-6:omega-3 fatty acid ratio is an important criteria in generating higher levels of oleic acid. Monounsaturated fatty acids have been a subject of interest due to their possible hypolipidemic and antithrombotic effects (Grundy, 1987). If these effects exist, then eggs from canola-fed hens would be more beneficial than eggs from flax-fed hens even though hens fed flax had higher omega-3 fatty acids.

The individual and total omega-3 fatty acids in brain tissue of the developing and newly hatched chick were influenced by maternal dietary treatment. The predominant fatty acid in this tissue was DHA. Irrespective of the omega-3 fatty acid level of the egg, the substantial accretion of DHA in the brain of control chicks, indicates the importance of DHA and its involvement in the growth and maturation of the developing chick brain. The degree of elongation and desaturation of LNA to its long chain metabolites

such as EPA, DPA, and DHA, varied between tissues; brain tissue had the highest incorporation of DHA, but LNA increased more in the plasma and yolk. Hence, the additional LNA in the diet or in the yolk either may not have been equally available to all the tissues, or its desaturation and chain elongation were not equal in the tissues examined. Regardless of the LNA content of the enriched egg, identical levels of DHA in the brain tissue of pre-and post-hatch FXC and CAC chicks suggest that the developing chick brain has a saturation point for the uptake of DHA, and beyond that threshold, elongation and desaturation of additional LNA may be either inhibited or limited. It is not known whether the additional DHA in the brain tissue of WSC chicks came from egg yolk LNA or DHA, or was transported through circulating plasma, or synthesised in the brain tissue.

In the mammalian system [illegible] accrues selectively in the developing fetal brain from the materna[illegible]ver through the placenta during cell division (Crawford et al., 1976). Similarities between mammals and avians in PUFA supply between the mother and those accumulated by the embryo have been reported (Nobel and Cocchi, 1989). Considering the importance of maternal dietary n-3 fatty acids in modulating the brain tissue n-3 fatty acid content of the progeny, it will be important to assess the net transfer of fatty acids from maternal sources (yolk). Further studies using the avian model will be needed to understand the role of yolk n-3 fatty acids in providing n-3 PUFA to the newly hatched chick. This is addressed in the work which follows.

Table 2.1. Composition of Laying Hen Diets

Ingredients	Control	8% Flax	16% Flax	16% Canola
	(%)			
Barley	7.0	17.5	21.6	22.1
Wheat	41.0	26.0	30.0	21.5
Soybean meal	19.6	18.0	12.0	15.0
Starch	17.0	15.1	5.0	10.0
Animal tallow	3.0	3.0	3.0	3.0
Flax seeds	---	8.0	16.0	---
Canola seeds	---	---	---	16.0
Dicalcium phosphate	2.0	2.0	2.0	2.0
Limestone	7.9	7.9	7.9	7.9
Iodized salt	.3	.3	.3	.3
DL-methionine	.1	.1	.1	.1
Vitamin-mineral mix[1]	2.0	2.0	2.0	2.0
Vitamin B_6 supplement	.1	.1	.1	.1
Calculated composition				
CP, %	16.8	16.8	16.8	16.8
ME, kcal/kg	2,900.0	2,900.0	2,900.0	2,900.0
Calcium, %	3.6	3.6	3.6	3.6
Total phosphorus, %	.83	.83	.83	.83
Phosphorus available, %	.61	.61	.61	.61
Lysine, %	.78	.78	.78	.78
Methionine + cystine, %	.60	.60	.60	.60

[1]The layer vitamin-mineral mix supplied per kilogram of diet the following: vitamin A, 8000IU; vitamin D_3, 1,200 ICU; vitamin E, 5 IU; riboflavin, 4 mg; calcium pantothenate, 6 mg; niacin, 15 mg; vitamin B_{12}, 10 μg; choline chloride, 100 mg; biotin, 100 μg; DL-methionine, 500 mg; manganese sulfate, .4 g; zinc oxide, .1 g.

Table 2.2.

Total lipid (%) and fatty acid composition of lipids from laying hen diets

Fatty acid[1](%)	Control	8% Flax	16% Flax	16% Canola
16:0	17.98	11.43	10.97	9.47
18:0	10.40	6.27	5.78	5.07
18:1	43.40	32.38	30.69	54.75
20:1	.39	.30	.29	.58
18:2 n-6	22.20	17.83	12.09	20.55
20:4 n-6	.93	.61	.50	1.32
18:3 n-3	3.28	30.91	40.30	7.59
Σ Lipid	4.64	5.16	5.21	6.21
Σ SAT	28.38	17.70	16.25	15.54
Σ MONO	43.79	32.68	30.98	55.33
Σ PUFA	26.41	49.35	52.89	29.46
Σ n-6	23.13	18.44	12.59	21.87
Σ n-3	3.28	30.91	40.30	7.59

[1]SAT = saturated fatty acid; MONO = monounsaturated fatty acid; PUFA = polyunsaturated fatty acids.

Table 2.3.

Total lipid and fatty acid composition of the egg yolks collected from laying hens fed diets containing 8 or 16% ground flax or 16% ground canola seeds

	Laying Hen Diet				
Fatty acid	Control	8 % Flax	16 % Flax	16 % Canola	SEM
	---------- (% of total fatty acids) ----------				
18:2 n-6	9.43[b]	13.13[a]	13.04[a]	12.37[a]	0.28
20:4 n-6	1.41[a]	.94[c]	.83[d]	1.26[b]	0.01
18:3 n-3	.62[d]	5.79[b]	8.76[a]	2.37[c]	0.19
20:5 n-3	.00[b]	.12[a]	.15[a]	.11[a]	0.01
22:5 n-3	.00[c]	.29[a]	.30[a]	.18[b]	0.11
22:6 n-3	1.02[b]	1.42[a]	1.54[a]	1.49[a]	0.04
Σ n-6	10.84[b]	14.07[a]	13.87[a]	13.63[a]	0.23
Σ n-3	1.64[d]	7.82[b]	10.75[a]	4.15[c]	0.19

[a-d]Means with no common superscripts within the same row are significantly different ($P<.05$).

SEM = Standard error of the mean

Table 2.4. Effect of feeding full fat and canola seeds to laying hens on the plasma n-6 and n-3 fatty acid composition of newly hatched chicks

	Laying Hen Diets				
Fatty acid[1](%)	Control	8% Flax	16% Flax	16% Canola	SEM
14:0	0.00[b]	.34[a]	.35[a]	.29[a]	0.01
16:0	18.86[a]	16.75[b]	16.80[b]	16.75[b]	0.23
18:0	10.49[a]	11.11[a]	10.98[a]	10.93[a]	0.15
16:1	1.90[a]	1.25[b]	1.30[b]	1.77[a]	0.17
18:1	42.63[b]	32.15[a]	32.45[a]	43.10[b]	0.34
18:2 n-6	16.42[a]	[illegible]	17.53[a]	14.24[b]	0.19
20:4 n-6	6.86[a]	3.71[b]	3.64[b]	6.10[a]	0.12
18:3 n-3	.00[c]	4.59[a]	4.71[a]	1.81[b]	0.11
20:5 n-3	.00[c]	1.09[b]	1.30[a]	1.00[b]	0.13
22:5 n-3	.00[c]	3.09[a]	3.18[a]	1.00[b]	0.11
22:6 n-3	1.95[b]	3.68[a]	3.78[a]	3.01[a]	0.17
5R SAT	29.36[a]	28.20[a]	28.13[a]	27.97[a]	0.37
Σ MONO	44.53[a]	33.00[b]	33.75[b]	44.87[a]	0.44
Σ PUFA	26.11[b]	33.74[a]	34.14[a]	27.16[b]	0.44
Σ n-6	23.28[a]	21.29[a]	21.17[a]	20.34[b]	0.24
Σ n-3	1.95[c]	12.45[a]	12.97[a]	6.82[b]	0.15
n-6:n-3	8.23[a]	1.71[c]	1.63[c]	2.98[b]	0.09

[a-d]Means with no common superscripts within the same row are significantly different ($P<.05$).

[1] SAT = saturated fatty acid; MONO = monounsaturated fatty acid; PUFA = polyunsaturated fatty acids. SEM = Standard error of the mean

Table 2.5.

Effect of feeding full fat flax and canola seeds to laying hens on the omega-3 fatty acid content of pre-and post-hatch chick brain.

Days pre hatch	Fatty acid (%)	Laying Hen Diets				
		Control	8% Flax	16% Flax	16% Canola	SEM
15	18:3 n-3	.20[b]	.45[a]	.34[a]	.34[a]	0.04
	20:5 n-3	.29[c]	1.09[a]	1.62[a]	.72[b]	0.11
	22:5 n-3	.61[c]	1.58[a]	1.63[a]	.87[b]	0.07
	22:6 n-3	17.03[b]	21.97[a]	22.23[a]	20.44[a]	0.37
17	18:3 n-3	.28[b]	.50[a]	.49[a]	.43[a]	0.02
	20:5 n-3	.20[c]	1.03[a]	1.62[a]	.77[b]	0.23
	22:5 n-3	.52[c]	.88[b]	1.32[a]	.72[b]	0.06
	22:6 n-3	15.53[c]	20.49[b]	23.28[a]	21.02[b]	0.49
19	18:3 n-3	.23[b]	.50[a]	.69[a]	.43[a]	0.10
	20:3 n-3	.22[c]	1.47[a]	1.62[a]	.73[b]	0.05
	22:5 n-3	.41[c]	1.38[a]	1.47[a]	.72[b]	0.06
	22:6 n-3	15.46[b]	23.14[a]	23.16[a]	22.11[a]	0.32
21	18:3 n-3	.37[b]	.53[a]	.69[a]	.47[b]	0.11
	20:5 n-3	.19[c]	1.49[a]	1.49[a]	.73[b]	0.12
	22:5 n-3	.41[c]	1.60[a]	1.69[a]	.73[b]	0.08
	22:6 n-3	15.37[b]	23.14[a]	23.62[a]	22.11[a]	0.34

[a-c]Means with no common superscripts within the same row are significant at ($P<.05$).

SEM = Standard error of the mean

2.5. References

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Chapter 3. Net Transfer and Incorporation of Essential Fatty Acids into Developing Chick Embryos[1]

3.1. Introduction

In the previous chapter we observed that eggs high in LNA greatly enriched the tissue lipids of their chicks with long chain metabolites of LNA such as EPA, DPA, and DHA, along with a concomitant reduction of arachidonic acid (Chapter 2) (Cherian and Sim, 1991). This enrichment indicates that developing embryos not only sequester the preformed long chain metabolites of LNA but are also converting yolk LNA to longer chain metabolites. Nevertheless, the origin of long chain PUFA in the developing embryo is not clearly understood. Upon incubation of a n-3 or n-6 fatty acid enriched egg, the lipids of the yolk are the sole supply of n-3 or n-6 fatty acids for the developing embryo and subsequently the chick. Therefore, it is possible to determine the net transfer of n-3 or n-6 PUFA from yolk to the developing embryo and quantify the changes in the lipid composition of the progeny.

In view of the significant impact of the maternal diet on the fetal accretion of PUFA, it will be important to assess the net synthesis or transfer of these fatty acids or the appropriate amount of precursor for accretion. In the present study, the relative contribution of maternal (yolk) n-3, n-6, or n-9 fatty acids to embryo assimilation and/or synthesis of these fatty acids during development of the chick embryo was

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1 A version of this paper has been accepted for publication.

G. Cherian and J. S. Sim, 1992. Poultry Science (in press).

examined quantitatively.

3.2. Materials and Methods

Fatty Acid Modulation

Eighteen Single Comb White Leghorn laying hens were housed individually under 16 h light in cages (30 x 40 cm). The hens (six for each of the diets) consumed ad libitum one of the three different test diets containing: 1. ground sunflower seeds high in C18:1 n-9 (n-9 monounsaturated fatty acid, MUFA), 2. flax seeds high in LNA (n-3 PUFA) or 3. sunflower seeds high in C18:2 n-6 (n-6 PUFA) (Table 3.1). The flax seeds and linoleic-rich sunflower seeds were purchased from local feed producers, and oleic acid-rich sunflower seed was donated[2]. The fatty acid composition of the diets are shown in Table 3.2. The hens were artificially inseminated and the fertilized eggs were collected after 3 wk on the experimental diets and incubated as described in Chapter 1 (Cherian and Sim, 1991). Six eggs from each test diet were unincubated as controls for analysis.

Sample Collection

The newly hatched chicks (six per diet) were killed by decapitation within 1 h after hatching. The brain and liver tissue of the chicks were separated and weighed. The yolk sac was also removed and weighed. Total lipid was extracted from the egg yolk (unincubated), brain, liver, carcass, and yolk sac of the newly hatched chick with chloroform:methanol (2:1, vol/vol) according to the method of Folch et al. (1957). Percentage lipid contents were determined gravimetrically for all tissues. Lipid extracts were dried under nitrogen and were methylated using boron trifluoride methanol according to the method of Metcalfe and Pelka (1961).

The fatty acid methyl esters of total lipids were recovered with hexane and quantified by an automated gas chromatograph[3] equipped with an on column injector using DB-23 fused silica capillary column[4] (30 m x .25 mm inside diameter). The gas chromatograph was programmed to start at 180 C for 10 min and to increase at 5 C/min to 195 C. Injector and detector temperatures were set at 230 C, and helium was used as the carrier gas at a flow rate of 1.5 mL/min. A Hewlett-Packard[5] Series 3353 laboratory automation system was used to integrate peak areas. Fatty acid methyl esters were identified by comparison with retention times of authentic standards. Quantification of fatty acids in the total lipids were carried out using behenic acid (C22:0) as an internal standard[6]. Fatty acid values are expressed either as milligrams per egg or as weight percentages.

For data reported on the whole chick, the values for the carcass, brain, and liver were added together. Percentage incorporation of a fatty acid by the chick was defined as the amount of that fatty acid recovered in the newly hatched chick divided by the amount of that fatty acid present in the unincubated egg. This calculation accounts for neither in vivo synthesis nor fatty acid oxidation for energy by the developing embryo. Therefore, 'percentage incorporation' should be considered the balance between anabolic, catabolic, and transport processes.

The data for the effect of diet on the percentage incorporation of n-9, n-6 and n-3 fatty acids in the egg and chick were analysed by one-way analyses of variance, using the General Linear Model (GLM) procedure (SAS, 1985). Effects of dietary treatments and days of incubation (0, 21) on the

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[2]SVO Enterprises, Cleveland, OH 44094

fatty acid composition of the yolk and yolk sac were analyzed by two-way ANOVA. Significant differences among treatment means were determined using Student-Neuman-Keuls multiple range test at the 5% probability level (Steel and Torrie, 1980).

3.3. Results

The fatty acid composition of the laying hen diets provide 65.3, 45.8, and 61.1% of n-9, n-3, or n-6 fatty acids, respectively (Table 3.2). The n6:n3 ratio of the diets were 18.5, .51, and 43.6 for the MUFA, n-3 PUFA, and n-6 PUFA, diet respectively.

Total lipid and fatty acid composition of egg yolk

Incorporation of n-9, n-3, or n-6 fatty acids in the egg yolk lipids reflected the dietary fatty acid source (Table 3.3). The n-3 PUFA diet enriched egg yolk lipids with n-3 fatty acids, mainly LNA at 392.3 mg per egg. The content of long chain metabolites of LNA such as EPA, DPA, and DHA were at 10.6, 20.4 and 104.8 mg per egg, respectively. Similarly, the n-6 PUFA diet enriched egg yolk lipids mainly with C18:2 n-6 at 1,434.6 ± 28.9 mg per egg. Incorporation of oleic acid-rich sunflower seed in the hen's diet increased deposition of MUFA such as C18:1 n-9 and C20:1 n-9 in the yolk lipids. Although there were large differences in the fatty acid composition of egg yolk lipids, no difference was observed in the total lipid content of the egg yolk.

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[3]Model 3700, Varian Associates Inc., Sunnyvale, CA 94089

[4]Supelco Canada Ltd., Oakville, ON, L6K 3V1, Canada.

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

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

The total lipids in the yolk were 6.2, 6.3, and 6.4 g per egg for MUFA, n-3 PUFA, and n-6 PUFA eggs, respectively.

Total lipid and fatty acid incorporation into chick carcass

Irrespective of the fatty acid composition of yolk lipids, chicks incorporated a higher percentage of total n-6 fatty acids than either saturated fatty acid or MUFA (Table 3.3). The higher levels of MUFA in the eggs from hens fed n-9 MUFA diet did not cause any change in the percentage of incorporation of MUFA into the progeny. As total n-3 fatty acids in the egg increased, the percentage incorporation of these fatty acids to the progeny tissues tended to decrease. The higher level of LNA in the eggs from n-3 PUFA diet did not cause any change in the percentage incorporation of LNA. When the levels of n-3 fatty acids were low in the egg (MUFA and n-6 PUFA groups), the percentage incorporation of n-3 fatty acids increased significantly. Similarly, the presence of LA in n-6 PUFA eggs (approximately 2.5 times that of n-3 PUFA or MUFA eggs) did not lead to an increase in the percentage incorporation of total n-6 fatty acids to the chick tissues. Total n-6 fatty acids in the egg were 12.8, 729.5, and 1,556.7 mg in the MUFA, n-3 PUFA and n-6 PUFA groups, respectively. The incorporation of these PUFA to the newly hatched chick tissues were 29.4, 31.8, and 31.9%, respectively.

In general, altering the essential n-6 or n-3 fatty acid composition of the egg resulted in a reciprocal effect on the percentage incorporation of these fatty acids into the progeny (Table 3.3). This was particularly pronounced in the case of AA and DHA. Feeding flax seed resulted in an incorporation of AA at 46.4 mg compared with 103.8 mg into egg yolk by feeding the n-6 PUFA diet seed to laying hen. However, no difference was

observed in the percentage incorporation of this fatty acid in both cases, irrespective of their original amount in the yolk lipids. Similarly, the egg yolk from hens fed the n-6 PUFA diet had the lowest level of DHA at 21.6 mg as compared with 104.8, and 39.8 mg for eggs from hens fed the n-3 PUFA or MUFA diet. However, the percentage incorporation of DHA was 72.2% for the n-6 PUFA group and 33.4 and 49.0% for the n-3 PUFA and MUFA diet, respectively. The level of C22:4 n-6 (MUFA diet), C22:5 n-6 (n-3 PUFA diet) C20:5 n-3 (MUFA and n-6 PUFA) and C22:5 n-3 (MUFA and n-6 PUFA), the long chain metabolites of LA and LNA, were present in higher quantity than originally present in the egg for MUFA, n-3 PUFA resulting in an incorporation rate higher than 100%, or were present even in their absence in the yolk lipids. In general, the developing chick embryo incorporated more long chain polyunsaturated fatty acids of the n-3 and n-6 series. The total lipid content of the chick carcass was not affected by yolk fatty acid composition.

Total lipid and fatty acid disappearance from the yolk sac

Uptake of fatty acids by the developing chick embryo was studied further by examining the fatty acid composition of residual yolk sac lipids (Table 3.4). Regardless of the fatty acid composition of the yolk, no differences were observed in the total lipids remaining in the yolk sac after 21 days of incubation. The total lipids in the yolk sac were 1.2, 1.3, and 1.3 g for the n-9 MUFA, n-3 PUFA, and n-6 PUFA diets, respectively. Among the n-3 fatty acids, DHA showed a significant decrease in the yolk sac lipids relative to the egg yolk in all the diets. In the n-3 PUFA eggs, C22:5 n-3 was significantly reduced. No differences were observed in the concentrations of C20:5 n-3 and C18:3 n-3 in all the eggs.

In the case of n-6 fatty acids, the level of C18:2 n-6 was significantly reduced in the yolk sac of n-6 PUFA group. However, the level of C20:4 n-6 was reduced in all the eggs.

3.4. Discussion

The present study indicates an adaptive mechanism by the developing chick embryo for maximum utilization of n-6 and n-3 fatty acids. In view of the requirement of DHA for proper development and synthesis of structural lipids in membranes (Bordoni et al., 1986; Noble and Cocchi, 1989), the laying hens fed a diet deficient in n-3 fatty acids (n-6 PUFA) maximized the uptake of DHA. The developing chick embryo responded to the limited supply in the reserve (yolk) by increasing the percentage of incorporation of these essential fatty acids into the tissues, which might be due to the limited catabolism of these fatty acids to produce energy.

Irrespective of the C20:4 n-6 content of the eggs in the n-3 PUFA and n-6 PUFA diets, the percentage incorporation of C20:4 n-6 was not different in the chicks hatched from these treatments. Therefore, n-3 PUFA chicks may have metabolically 'conserved' this fatty acid whereas the n-6 PUFA group may have catabolized more of this fatty acid for energy, retaining lesser amounts for incorporation. Thus in the chicks from the n-3 PUFA group, a sparing response was noted for n-6 fatty acid when the yolk content of AA was reduced.

The role of egg yolk fatty acid composition in percentage incorporation of essential fatty acids in the chick embryo was reported by Lin et al. (1991). These researchers reported that LNA in the egg yolk did not result in any meaningful increase in brain DHA. Compared with moderate levels of LNA in the MUFA group, chicks hatched from LNA-rich n-3 PUFA diet

incorporated DHA into the brain and liver tissue of the hatched chicks. This enrichment was specifically in the phosphatidylethanolamine fraction of the brain and liver tissue lipids (Chapter 4, Cherian and Sim, 1992a). The efficacy of LNA in raising the level of long chain metabolites of LNA such as EPA, DPA, and DHA has been questioned in both human (Dyerberg et al., 1980) and animal studies (Anderson et al., 1989). In chickens, long chain n-3 PUFA in tissues and in egg yolk increases when LNA is fed (Phetteplace and Watkins, 1989; Farrell and Gibson, 1990; Ajuyah et al., 1991). Eggs from hens fed full fat flax seed incorporated higher DHA in the brain tissue compared with chicks from hens fed soy or sunflower seeds (Cherian and Sim, 1991). Diets containing n-3 fatty acid enriched egg yolk (predominantly LNA) caused significant enrichment of EPA, DPA, and DHA but lowered arachidonic acid, consequently lowering the n6:n3 PUFA ratios particularly in the phospholipid fraction of rat hepatic tissue (Jiang and Sim, 1992).

The presence of C22:4 n-6, C22:5 n-6, C20:5 n-3, and C22:5 n-3 in the progeny were higher than their original amount in the yolk lipids. This indicates that requirements for these fatty acids are not satisfied by yolk lipids. These fatty acids are produced by chain elongation and desaturation of LA and LNA (Dyerberg, 1986), indicating that the developing embryos are not only sequestering the preformed long chain metabolites, but converting yolk LA or LNA to long chain PUFA. As developing embryos assimilate the yolk lipids during incubation, the synthesis of C20 and C22 PUFA from C18 precursors at the yolk sac membrane has been demonstrated by the identification of Δ-6 desaturation activity and conversion of LA to AA in the chick embryo (Noble and Shand, 1985).

Therefore, this increased absorption rate or synthesis from the precursor is presumably geared towards higher requirement of these PUFA during cell division and growth. Of the various fatty acids present in the yolk sac, C20:4 n-6 and C22:6 n-3 concentrations decreased. This suggests that the developing embryos preferentially utilized these fatty acids. A preferential absorption of PUFA from yolk phospholipids was observed by Isaacs et al. (1964); Noble and Moore (1964); and Lin et al. (1991).

The importance of n-6 and n-3 PUFA for the development of the central nervous system in both mammalian and avian species is well documented (Noble and Cocchi, 1989; Innis, 1991). In the present study, the high ratio of n-6:n-3 fatty acids in the eggs from n-6 PUFA diet led to the least amount of DHA incorporation in the brain tissue of the progeny. Very high n-6:n-3 ratio (>150:1) in the diets has been reported to cause depletion of DHA and retinal abnormalities in animals (Neuringer et al., 1988) and reduced electroretinographic response in infancy (Uauy et al., 1990). Calculating the % energy provided by the n-3 fatty acids in the n-6 PUFA egg a value of 0.5 % kcal is obtained (assuming that 9 cal/gm of fatty acid and 65.9 kcal per egg) (Pennington, 1989). Therefore, the requirement of n-3 fatty acids for the developing embryo may be higher than 0.5 kcal%, and the energy derived from the n-3 fatty acid enriched-egg comes to 1.52 kcal. In human milk, n-3 fatty acids represents 0.7 to 1.3% of calories (Gibson and Kneebone, 1981), which corresponds to the calories provided by the enriched egg to the developing chick embryo. Considering the unique fatty acid profile, consumption of two n-3 PUFA enriched eggs may provide up to 1057 mg of n-3 fatty acids, with 210 mg being DHA. The inadequacy of infant formulas and weaning foods for infants

in meeting their n-3 and n-6 PUFA content is well documented (Jensen et al., 1978; Jackson and Gibson, 1989; Simopoulos and Salem, 1991). Thus, the n-3 fatty acid enriched egg would be useful as weaning food for infants fed with infant formulas which do not have any DHA, and also as a natural lipid source to be included in infant formula preparations.

Table 3.1. Composition of laying hen diets

Ingredients and analyses	Laying hen diets[1]		
	N-9 MUFA	N-3 PUFA	N-6 PUFA
	-------(%)-------		
Wheat	61.3	66.9	58.7
Soybean meal	8.0	5.3	7.6
Flax seed (FL-3)	0.0	15.0	0.0
Sunflower seed (SF-9)	18.0	0.0	0.0
Sunflower seed (SF-6)	0.0	0.0	21.0
Limestone	8.3	8.3	8.3
Calcium phosphate	1.9	2.0	1.9
Salt	0.3	0.3	0.3
DL-methionine	0.1	0.1	0.1
Layer premix[2]	2.1	2.1	2.1
Calculated analyses:			
CP,	15.0	15.0	15.0
ME, kcal/kg	2,732	2728	2700
Ether extracts,	6.2	6.0	5.9
Calcium,	3.6	3.6	3.6
Available P	.5	.5	.5

[1]MUFA = monounsaturated fatty acid;
PUFA = polyunsaturated fatty acid.

[2]Supplied the following per kilogram of diet : vitamin A, 8,000 IU; cholecalciferol, 1,200 ICU; vitamin E, 5 IU; riboflavin, 4 mg; calcium pantothenate, 6 mg; niacin, 15 mg; vitamin B_{12}, 10μg; choline chloride, 100 mg; biotin, 100 μg; selenium, .1 mg; DL-methionine, 500 mg; manganese sulphate, .4 g; zinc oxide, .1 g.

Table 3.2. Major fatty acids of laying hen diets[1]

Fatty acid	Laying hen diets		
	n-9 MUFA	n-3 PUFA	n-6 PUFA
	---------- (%) ----------		
C16:0	6.9	8.2	9.7
C18:0	3.8	2.8	4.7
C16:1	0.5	0.6	0.8
C18:1	65.3	19.4	22.0
C18:2(n-6)	22.2	3.5	61.1
C18:3(n-3)	1.2	45.8	1.4
Total SAT	10.7	11.0	14.4
Total MUFA	65.8	20.0	22.8
n-6:n-3	18.5	0.5	43.6

[1]MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; SAT = saturated fatty acid;

Table 3.3. Effect of diet on the incorporation (incorp.) of n-6 and n-3 fatty acids from egg into the newly hatched chick[1,2]

Fatty acid	MUFA diet			N-3 PUFA diet			N-6 PUFA diet			SEM[3]
	Egg Yolk	Chick	% Incorp.	Egg Yolk	Chick	% Incorp.	Egg Yolk	Chick	% Incorp.	% Incorp
	---- (mg) ----			---- (mg) ----			---- (mg) ----			
SAT	1,634.8^{y}	428.0	26.8	1,863.9^{x}	488.4	22.7	1,613.5^{y}	446.8	26.6	1.3
MUFA	3,259.4^{x}	689.7	21.2	2,623.2^{y}	576.7	24.7	1,772.6^{z}	460.9	22.2	1.4
18:2	581.1^{z}	162.3	27.9	683.1^{y}	205.3	28.9	1,423.3^{x}	392.7	29.6	1.8
20:4	109.6^{x}	39.6	36.2^{b}	46.4^{y}	25.4	54.9^{a}	103.8^{x}	49.7	48.0^{a}	3.4
22:4	4.1^{y}	7.9	193.5^{a}	0.0^{z}	1.9	---[4]	9.5^{x}	8.0	84.2^{b}	3.0
22:5	18.0^{x}	6.3	32.7^{b}	0.0^{y}	5.1	---	20.1^{x}	9.6	47.8^{a}	4.1
Sum n-6	712.8^{y}	216.1	29.4	729.5^{y}	237.7	31.8	1,556.7^{x}	460.0	31.9	2.1
18:3	14.2^{y}	5.4	38.7^{a}	392.3^{x}	92.9	25.6^{b}	17.2^{y}	4.6	26.4^{b}	2.8
20:5	0.0^{y}	.1	---	10.6^{x}	6.3	59.4	0.0^{y}	.1	---	
22:5	0.0^{y}	1.7	---	20.4^{x}	7.0	34.3	0.0^{y}	.2	---	
22:6	39.8^{y}	19.5	49.0^{b}	104.8^{x}	35.0	33.4^{c}	21.6^{z}	15.6	72.2^{a}	2.5
Sum n-3	54.0^{y}	26.7	57.2^{a}	528.4^{x}	141.2	26.7^{c}	39.3^{z}	20.5	52.2^{b}	1.8

$^{a-c}$ Incorporation values within a row with no common superscripts differ significantly (P<.05).
$^{x-z}$ Egg yolk fatty acids within a row with no common superscripts differ significantly (P<.05).
[1]Chick=chick carcass plus brain plus liver.
[2]MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid.
[3]SEM for the incorporation value
[4]Indicates incorporation values not reported as the egg yolk did not contain those fatty acids.

Table 3.4. Effect of diet on the essential fatty acid composition and total lipids of egg yolk and chick yolk sac contents[1,2]

Fatty acid	MUFA diet		N-3 PUFA diet		N-6 PUFA diet	
	Egg	Yolk Sac	Egg	Yolk Sac	Egg	Yolk Sac
	---- (%) ----		---- (%) ----		---- (%) ----	
C18:2 n-6	9.9 ± .7[b]	11.0 ± .2[a]	11.4 ± .2	12.7 ± .1	28.5 ± .6	26.8 ± 1.1
C20:4 n-6	1.8 ± .0[b]	1.1 ± .1[a]	.8 ± .0	.7 ± .0	2.2 ± .0[a]	1.7 ± .1[b]
C22:4 n-6	.0 ± .0[b]	.2 ± .0[a]	.0 ± .0[b]	.1 ± .0[a]	.2 ± .0[a]	.2 ± .0[b]
C22:5 n-6	.3 ± .0[b]	.7 ± .4[a]	.0 ± .0[b]	.2 ± .4[a]	.4 ± .0	.3 ± .0
C18:3 n-3	.3 ± .0	.3 ± .0	7.2 ± .1	7.2 ± .4	.3 ± .0	.3 ± 0
C20:5 n-3	.0 ± .0	.0 ± .0	.2 ± .0	.3 ± .1	.0 ± .0	.0 ± .0
C22:5 n-3	.0 ± .0	.0 ± .0	.3 ± .0[a]	.2 ± .0[b]	.0 ± .0[b]	.3 ± .0[a]
C22:6 n-3	.7 ± .0[a]	.3 ± .1[b]	1.8 ± .1[a]	.8 ± .1[b]	.4 ± .0[a]	.3 ± .1[b]
Lipids[3]	6.2 ± .3[a]	1.2 ± .1[b]	6.3 ± .2[a]	1.3 ± .1[b]	6.4 ± .3[a]	1.3 ± .2[b]

Data presented as Means ± S.E

[2]MUFA = monounsaturated fatty acid, PUFA = polyunsaturated fatty acid

[1,2ab]Means for egg and yolk sac within diet type with no common superscripts are significant ($P < .05$)

[3]Grams per egg or yolk sac

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Chapter 4. Preferential Accumulation of n-3 Fatty Acids in the Brain of Chicks from Eggs Enriched with n-3 Fatty Acids.

4.1. Introduction

The previous chapters demonstrated the importance of the fatty acid composition of the maternal diet (n-9, n-3, and n-6) in modulating the fatty acid content of the yolk and the tissues of the hatched chick. Furthermore, in Chapter 3 we also observed that the chick embryo incorporated higher longer chain n-3 and n-6 fatty acids and retained less of these fatty acids in the yolk sac. This suggests that the developing embryos preferentially utilized these fatty acids. A selective absorption of PUFA from yolk phospholipids has been reported previously (Isaacs et al. 1964; Noble and Moore, 1964; Lin et al. 1991.

The importance of the n-3 family of PUFA for the development of the central nervous system in both mammalian and avian species is unequivocal (Holman, 1971; Mead, 1971; Noble and Cocchi, 1989). Among these fatty acids, docosahexaenoic acid (DHA, C22:6 n-3) has provoked the greatest interest because it is one of the major fatty acids in the membrane phospholipids of the brain, where it functions in the maintenance of membrane fluidity, permeability, ion conduction, and release of acetylcholine (for reviews, see Johnston, 1979; Menon and Dhopeshwarkar, 1982; Neuringer et al., 1988). Large amounts of DHA are required by the brain during the late gestational and early postnatal periods when cellular differentiation, active synaptogenesis, and membrane biogenesis

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[1] A version of this paper has been published. G. Cherian and J. S. Sim, Poultry Science. 1992. 71:1658-1668.

takes place (Clandinin et al., 1980). Deficiency of n-3 fatty acids has been reported to cause impaired learning defects (Lamptey and Walker, 1976), visual abnormalities and polydipsia in nonhuman primates (Connor et al., 1984; Neuringer et al., 1984; Reisbick et al., 1990). Docosahexaenoic acid must be supplied by dietary intake of C22:6 n-3 or by synthesis from precursor αlinolenic acid (LNA, C18:3 n-3).

In mammals, including humans, dietary LNA is reported to be a less efficient precursor of brain DHA (Anderson et al., 1990; Lin et al., 1991). Recently, we reported that eggs high in LNA greatly enriched the brain and liver lipids of the progeny with the long chain metabolites of LNA such as eicosapentaenoic acid (EPA, C20:5 n-3), docosapentaenoic acid (DPA, C22:5 n-3), and DHA (Chapter 1, Cherian and Sim, 1991). During development of the chicken embryo, higher levels of Δ-6 desaturase activity resulting in the synthesis of C20 and C22 PUFA from C18 precursor in the liver and yolk sac membrane was reported by Noble and Shand (1985). This increased synthesis of long chain PUFA is presumably geared towards higher need of these fatty acids during cell division and growth.

Considering the importance of n-3 PUFA in brain development, we investigated whether the developing chick brain has any preferential transfer of DHA from maternal sources. Therefore, we compared the magnitude of changes in the fatty acid composition of phosphatidylethanolamine (PE) and phosophatidylcholine (PC) fraction of brain and liver tissue of newly hatched chicks from eggs enriched with n-3, n-6, or n-9 fatty acids.

4.2. Materials and Methods

Fatty Acid Modulation

Eighteen Single Comb White Leghorn laying hens were housed in cages (30 x 40 cm) and were assigned to one of the three test diets containing: 1. ground sunflower seeds high in oleic acid (C18:1 n-9), (SF-9), 2. ground flax seeds high in LNA (FL-3) or 3. regular ground sunflower seeds high in LA, (SF-6) (Table 4.1). The fatty acid compositions of the diets are shown in Table 4.2. The hens were artificially inseminated and the fertilized eggs were incubated as previously described (Chapter 2; Cherian and Sim, 1991).

Sample Collection and Analyses

At Day 23 of feeding, eggs from hens fed the test diets were collected. The eggs were weighed, broken open and the yolk separated from the white. For each yolk sample, about 1.0 g of yolk was weighed. On the day of hatching, newly hatched chicks were weighed and killed by decapitation, and the liver and brain tissue were harvested. The yolk sac attached to the hatched chick was also separated. An incision was made on the yolk sac and the contents were collected.

Total lipids were extracted from feed samples, egg yolk, yolk sac contents, liver, and brain with chloroform:methanol (2:1, vol/vol) by the method of Folch et al. (1957). The lipid extract was concentrated under nitrogen at 60 C to about 100 μL and as applied on precoated silica gel G plates[2] (20 x 20 cm) that had been previously activated by heating at 120 C for 2 h. Lipid standards such as tripalmitin (TG), PC, PE[2] were mixed and applied aside the lipid extract. The plates were developed in chloroform: methanol:water (65:25:4) 10 cm from the origin (25 min), air-dried, and

then were developed in hexane and diethyl ether (4:1) for 17 cm from the origin (20 min) according to the modified method of Fried and Shapiro (1979). The plates were then air-dried, and sprayed with .1% (wt/vol) 2',7'-dichlorofluorescein[2] in ethanol. The spots corresponding to TG, PC, and PE were identified under ultraviolet light[3], and were scraped off into screw-capped tubes and were converted to fatty acid methyl esters using a mixture of boron-trifluoride, hexane, and methanol (35:20:45, vol/vol/vol) (Metcalfe et al., 1961). The fatty acid methyl esters of total lipids, TG, PC, and PE fractions were separated and quantified by an automated gas choramotography equipped with autosampler and flame ionisation detectors[4], using a 30 m x .25 mm inside diameter fused silica capillary column[5]

The initial column temperature was set at 70 C for 3 min, increased to 180 C at 30 C/min, and held for 10 min. Then the column temperature was elevated to 230 C at a temperature of 5 C/min and held at the final temperature for 3 min. Helium was used as the carrier gas at a flow rate of 3.0 mL/min. The flame ionization detector was set at 240 C. A Hewlett-Packard Series 3355[6] laboratory automation system was used to integrate peak areas. Lipid class separation and percent compositional determination was done by an Iatroscan TH-10 Mark II analyser[7] in combination with

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2 Sigma Chemical Co., St. Louis, MO 63178-9916

3 Fisher Scientific Ltd., Ottawa, ON, K1G 4A9, Canada.

4 Model 3400, Varian Assosciates, Inc., Sunnyvale, CA 94089.

5 Supelco Canada Ltd., Oakville, ON, L6K 3V1, Canada.

preccated silica gel Chromarods-SII[7]. Each sample (1 to 2 μL) was spotted on the rods using a 10 μL Hamilton syringe and developed for 11 min for three times in chloroform:methanol:water (60:30:2.75), vol/vol/vol) air-dried for 5 min in between and again developed in hexane:diethylether: formic acid (90:7.5,.75 vol/vol/vol) for 50 min and dried at 110 C for 2 min. Rods were scanned under the following conditions: hydrogen pressure, .75 kg/cm^2; air flow, 2,000 mL/min. The recorder speed was 20 cm/min at a sensitivity of .1 vs. Peak areas were calculated with Hewlett-Packard Series 3353[6] laboratory automation system. Response factors for each lipid class were determined and applied accordingly. Lipid standards[2] were used for identification.

A one-way ANOVA was used to analyze the effect of dietary treatments on fatty acid contents of total and various lipid classes. Significant differences among means were determined using Duncan's Multiple Range test (Steel and Torrie, 1980). Effects of dietary treatments and days of incubation (0, 21) on the fatty acid composition of the yolk and yolk sac were analyzed by two-way ANOVA.

4.3. Results

The diets of the laying hen were isocaloric, isonitrogenous (Table 4.1) and contained 65.3% of C18:1 n-9, 45.8% of C18:3 n-3, and 61.1% of C18:2 n-6 for SF-9, FL-3, and SF-6 diets respectively (Table 4.2). Regardless of the fatty acid composition of the diet, the weights of yolk total lipids

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

[7] T.M.A scientific supply, Mississagua, ON, L4Y 3E7, Canada.

were 6.5, 6.5 and 6.3 g for eggs from hens fed SF-9, FL-3 or SF-6 diet, respectively. Incorporating flax seed (FL-3 diet) in the laying hen diet to the deposition of total n-3 fatty acids in the egg yolk lycerides of 9.2% compared with .5 and .8% for the eggs from hens fed SF-9 or SF-6 diets, respectively. The incorporation of LNA from the FL-3 diet was predominantly in the yolk triglycerides with only a moderate increase in PC and PE (Table 4 3). In contrast, EPA, DPA, and DHA, the longer chain metabolites of LNA were exclusively deposited into yolk phospholipids. Between PC and PE, the longer chain n-3 fatty acids were concentrated in the PE fraction (Table 4.3). The eggs from the FL-3 diet incorporated EPA, DPA, and DHA at 1.7, 1.4, and 10.4% in the yolk PE with a significant reduction in arachidonic acid. Fatty acids such as C22:4 n-6, or C22:5 n-6 were not detectable in the PE fraction of eggs from the FL-3 diet. In other words, the long chain metabolites of LNA were deposited preferentially into the yolk PE fraction. While in the PE fraction of eggs from SF-9 and SF-6 diet, increased ($P < .05$) deposition of C22:4 n-6 and C22:5 n-6 was detected. After 21 days of incubation, the total lipids in the yolk sac were 1.2, 1.3, and 1.3 g for SF-6, FL-3, and SF-6 diets, respectively. The total n-3 fatty acids were significantly higher in the triglycerides and phospholipids of the yolk sacs of FL-3 chicks as compared with the yolk sacs of SF-9 and SF-6 chicks (Table 4.4). The contents of C22:6 n-3 were .3, 1.2, and .8% in the PE fraction of the SF-9, FL-3 and SF-6 yolk sacs, respectively.

The weight of the hatched chick, brain weight or the total lipids in the brain tissue did not differ among the three treatments (Figure 4.1). The brain tissue of chicks hatched from FL-3 eggs contained significantly

higher total n-3 fatty acids (predominantly DHA) compared with those hatched from SF-9 or SF-6 eggs (Table 4.5). Irrespective of the yolk fatty acid composition, the brain PC fraction contained more saturated fatty acids and less long chain PUFA, and PE had more long chain PUFA attaining up to 57% of the fatty acids. Total n-3 fatty acids in the brain PE fraction were 29.3, 41.3, and 21.9% for chicks from hens fed SF-9, FL-3 and SF-6 diets, respectively (Table 4.6). Docosahexaenoic acid was the major PUFA (36.5%) in the brain PE fraction in chicks from hens fed the FL-3 diet as compared with 28.5, 20.7, for chicks from hens fed SF-9 and SF-6 diets, respectively. This increase in DHA was followed by a concomitant reduction in arachidonic acid and an increase in the C22:6 n-3:C20:4 n-6 ratio in the brain PE of FL-3 chicks. In the total lipids of the brain, PC constituted 52.1, 44.7 and 45.2%, and PE constituted 33.1, 40.8 and 41.9% for SF-9, FL-3 and SF-6 diets, respectively (Figure 4.2).

The fatty acid compositions of liver triglycerides are shown in Table 4.8. C18:1 n-9 was the principal fatty acid in the liver triglyceride fraction. The liver tissue of FL-3 chicks had an increased ($P<.05$) level of total n-3 fatty acids as compared with SF-9 and SF-6 chicks. The total n-6 fatty acids in the liver TG fraction was significantly higher in the chicks from SF-6 diets compared with SF-9 and FL-3 diets. Consequently, the increase of n-6 fatty acids resulted in an increased n6:n3 ratio in the liver TG fraction of SF-6 chicks.

Compared with the liver TG fraction, PC and PE had more saturated and polyunsaturated fatty acids (Table 4.8). Total n-3 fatty acids were higher in the liver PC and PE fraction of the chicks from hens fed FL-3 diets as compared with SF-9 and SF-6 chicks. Concomitantly, the levels of total n-6

fatty acids were reduced (P<.05) in the PC and PE fraction of FL-3 chicks. Unlike the brain lipids, the incorporation of long chain n-3 PUFA followed a similar pattern in the PC and PE fraction.

4.4. Discussion

Results presented here indicate the maternal (yolk) fat manipulation can have very pronounced effects on the fatty acid composition of the brain tissue, which requires large amounts of n-6 and n-3 PUFA to sustain high growth (Sinclair and Crawford, 1972; Menon and Dhopeshwarkar, 1982). The major components of chick brain phospholipids were PC and PE. The greater proportion of n-3 fatty acids in the total lipids and the phospholipid classes of brain tissue of chicks hatched from FL-3 diets were accompanied by lesser proportions of n-6 fatty acids such as C18:2 n-6, C20:4 n-6, C22:4 n-6, and C22:5 n-6 and vice versa for chicks hatched from SF-6 diets. The severity of this reduction varied among the n-6 fatty acids and also among the lipid classes. For example, significantly lower proportions of C18:2 n-6 and C20:4 n-6 were observed in the brain PE fraction of chicks from the FL-3 diets, whereas in the PC fraction only C20:4 n-6 was reduced. Of interest was the considerable reduction in the percentage of C22:4 n-6 and C22:5 n-6 in the brain phospholipid of chicks from the FL-3 diet, with the decline in C22:5 n-6 being extremely pronounced. The results from the present study indicate there is a reciprocal replacement of the n-6 fatty acids by the n-3 fatty acids in the brain PE and PC fraction of chicks hatched from n-3 fatty acid enriched eggs. However, brain tissue of chicks hatched from the SF-9 diet did not show any increase in monounsaturated fatty acids, indicating a preference for long chain PUFA by the brain tissue.

The relationship between phospholipid fatty acid composition and function in brain tissue is not clearly established. An increased de novo synthesis of C22:4 n-6 from C20:4 n-6 may influence eicosanoid synthesis in these tissues (Nakagawa and Horrocks, 1986). As well, accumulation of n-3 fatty acids, especially C20:5 n-3 and C22:6 n-3 at the expense of C20:4 n-6 has been implicated in the impaired conversion of C20:4 n-6 to prostaglandins (Dyerberg and Bang, 1980). Similarly, it has been reported that neural C22:6 n-3 can be oxygenated to form compounds analogous to eicosanoids and also act as potent regulators of C20:4 n-6 metabolism (Bazan et al., 1983; Salem et al., 1986). Chronic, excess, or imbalanced eicosanoid synthesis may be conducive to excessive inflammation, thrombotic tendencies, and immune suppression (Kinsella et al., 1990). Changes in the DHA:AA ratio observed in the present study are likely to influence the cellular eicosanoid metabolism in these tissues. Also, it has been reported that DHA is important in maintaining membrane fluidity and associated functions such as calcium binding and ion conduction (King et al., 1971). Studies by Enslen et al. (1991) have shown that dietary restriction of n-3 fatty acids during development causes profound modification of the fatty acid composition of brain phospholipids and exploratory behavior in rats. The present study, focussed on examining the changes in fatty acid composition, thus, behavioral or other clinical changes were not reported.

Irrespective of the yolk fatty acid composition, total saturates (C16:0 and C18:0) contributed to 57% of the fatty acids in the PC fraction of the chick brain lipids. However, in the PE fraction PUFA contributed up to 55% of all the fatty acids, with DHA being the most predominant. Changes in

the fatty acid composition of the brain phospholipids did not alter the PC:PE ratio for chicks from FL-3 and SF-6 diets. These observations support the hypothesis that a homeostatic mechanism protects the brain membranes from significant changes in phospholipids, thus preserving the constant state of membrane fluidity (McMurchie et al., 1986). The dietary treatment did not affect the percentage of total PUFA in the brain tissue, indicating the capability of this tissue in maintaining total content of PUFA. This has been reported in other species such as the rat and the pig and may be of functional significance (Nouvelot et al., 1983; Yamamoto et al., 1987; Hrboticky et al., 1989; Enslen et al., 1991).

Unlike in the brain, no preferential incorporation of DHA in the PE fraction of the liver tissue was observed. The liver n-3 fatty acids were evenly distributed in the PC and PE fractions. The importance of the liver in supplying DHA for the nervous system has recently been reported in the rat (Scott and Bazan, 1989). A close time association is a clear association in time with the accumulation of long chain PUFA in the brain and liver lipids of suckling rats (Sinclair, 1974). The relative contribution of liver in supplying long chain PUFA in the newly hatched chick brain awaits further studies.

In the egg yolk, the enrichment of LNA was mainly in the yolk triglycerides. In contrast, EPA, DPA, & DHA, the longer chain metabolites of LNA were exclusively deposited into the yolk PE fraction, which constitutes only 24% of the yolk phospholipids compared with PC which constitutes up to 69% of the yolk phospholipids (Noble, 1987). In laying hens PE-specific incorporation of n-3 fatty acids upon feeding flax seeds was reported recently by Jiang et al., 1991.

The needs for PUFA in the developing chick embryonic tissues are twofold. The first requirement is to provide PUFA for glycerolipid synthesis in membrane formation. The second is to maintain adequate precursor for eicosanoid biosynthesis (Watkins, 1991). This demand for PUFA is evident from the fatty acid composition of the remaining yolk sac. Preferential absorption of yolk PE has been reported earlier (Noble, 1964) and is not surprising because this fraction is rich in PUFA. As incubation proceeded, the concentration of C22:6 n-3 in the yolk sac PE and PC diminished, suggesting that the developing embryo preferentially utilized those yolk phospholipids that contained a large proportion of 22-carbon fatty acids.

Table 4.1. Composition of laying hen diets[1]

Ingredients and analyses	Dietary Treatments		
	SF-9	FL-3	SF-6
	(%)		
Wheat	61.3	66.9	69.7
Soybean meal	8.0	5.3	7.6
Flax seed (FL-3)	.0	15.0	.0
Sunflower seed (SF-9)	18.0	.0	.0
Sunflower seed (SF-6)	.0	.0	21.0
Limestone	8.3	8.3	8.3
Calcium phosphate	1.9	2.0	1.9
Salt	.3	.3	.3
DL-methionine	.1	.1	.1
Layer premix[2]	2.1	2.1	2.1
Calculated analyses:			
CP%	15.0	15.0	15.0
ME, kcal/kg	2,732	2728	2700
Ether extracts%	6.2	6.0	5.9
Calcium%	3.6	3.6	3.6
Available P%	.5	.5	.5

[1]The laying hen diets contained ground flax seed (FL-3), ground high oleic acid sunflower seed (SF-9), and high linoleic acid sunflower seed (SF-6).

[2]Supplied per kilogram diet following: vitamin A, 8,000 IU; cholecalciferol, 1,200 ICU; vitamin E, 5 IU; riboflavin, 4 mg; calcium pantothenate, 6 mg; niacin, 15 mg; vitamin B_{12}, 10μg; choline chloride, 100 mg; biotin, 100 μg; selenium, .1 mg; DL-methionine, 500 mg; manganese sulphate, .4 g; zinc oxide, .1 g.

Table 4.2. Major fatty acids of laying hen diets

Fatty acid	Dietary Treatments		
	SF-9	FL-3	SF-6
	---------(%)---------		
C16:0	6.9	8.2	9.7
C18.0	3.8	2.8	4.7
C16:1	.5	.6	.8
C18:1	65.3	19.4	22.0
C18:2(n-6)	22.2	3.5	61.1
C18:3(n-3)	1.2	45.8	1.4
Total SAT	10.7	11.0	14.4
Total MUFA	65.8	20.0	22.8
Total n-6	22.2	23.5	61.1
Total n-3	1.2	45.8	1.4
Total n-6:Total n-3	18.5	.5	43.6

SAT = saturated fatty acid;

MUFA = monounsaturated fatty acid.

Table 4.3. Fatty acid composition of the yolk lipid fractions

Fatty acid	SF-9	FL-3	SF-6
		(%)	
	---------- Triglyceride ----------		
C18:3 (n-3)	.5± .0[b]	8.8 ± .6[a]	.5 ± .0[b]
Total SAT	25.6 ± 1.0[b]	29.2 ± .7[a]	29.2 ± .8[a]
Total MUFA	65.7 ± 1.2[a]	50.9 ± 1.0[b]	44.4 ± 1.0[c]
Total n-6	8.2 ± .8[b]	10.3 ± .5[b]	25.7 ± .8[a]
Total n-3	.5 ± .0[b]	9.2 ± .8[a]	.8 ± .0[b]
Total n-6:Total n-3	16.4 ± .6[b]	1.1 ± .0[c]	32.1 ± 1.0[a]
	---------- Phosphatidylcholine -------		
C18.2 (n-6)	13.9 ± .3[b]	13.0 ± .7[b]	26.5 ± 1.0[a]
C20:4 (n-6)	3.7 ± .6[ab]	1.5 ± .2[b]	4.9 ± .3[a]
C18:3 (n-3)	.5 ± .0[b]	1.9 ± .3[a]	.5 ± .0[b]
C20:5 (n-3)	---	.2 ± .0	---
C22:5 (n-3)	---	.5 ± .0	---
C22:6 (n-3)	1.6 ± .4[c]	4.2 ± .2[a]	.8 ± 1.0[b]
Total SAT	42.5 ± 1.0	44.8 ± 1.2	44.3 ± 1.0
Total MUFA	37.4 ± .9[a]	33.8 ± 1.4[b]	22.6 ± 1.0[c]
Total n-6	18.1 ± .8[b]	14.5 ± .8[c]	32.4 ± 1.3[a]
Total n-3	2.1 ± .7[b]	6.8 ± .8[a]	1.3 ± .0[c]
Total n-6:n-3	8.6 ± .9[b]	2.1 ± .3[c]	24.9 ± .2[a]
	---------- Phosphatidylethanolamine ------		
C18:2 (n-6)	9.6 ± .4[b]	9.4 ± .1[b]	19.4 ± .8[a]
C20:4 (n-6)	15.5 ± 1.0[a]	4.9 ± .8[c]	12.6 ± .8[b]
C22:4 (n-6)	.9 ± .0[b]	0.0	1.3 ± .0[a]
C22:5 (n-6)	1.9 ± .1[b]	---	2.5 ± .1[a]
C18:3 (n-3)	0.0 [b]	1.7 ± .3[a]	0.0 [b]
C20:5 (n-3)	0.0 [b]	1.7 ± .0[a]	0.0 [b]
C22:5 (n-3)	.2 ± .0[c]	1.4 ± .0[a]	.4 ± .0[b]
C22:6 (n-3)	5.4 ± .8[b]	10.4 ± .9[a]	2.4 ± .6[c]
Total SAT	43.2 ± 1.2	45.9 ± 1.3	42.0 ± 1.0
Total MUFA	23.4 ± 1.0[a]	24.9 ± .9[a]	19.6 ± 1.3[b]
Total n-6	27.9 ± 1.0[b]	14.3 ± .8[c]	35.8 ± 1.7[a]
Total n-3	5.6 ± .3[b]	15.2 ± .7[a]	2.8 ± .9[c]

[a-c]Means ± S.E within the same row with no common superscripts are significantly different ($P < .05$).
SAT = saturated fatty acids.
MUFA = monounsaturated fatty acids.

Table 4.4. Fatty acid composition of the yolk sac lipid fractions

Fatty acid	SF-9	FL-3	SF-6
		(%)	
	------ Triglyceride fatty acids -------		
C18:2 (n-6)	9.6 ± .5[b]	10.4 ± 1.1[b]	24.9 ± 2.1[a]
C18:3 (n-3)	.4 ± .1[b]	6.9 ± .7[a]	.6 ± .2[b]
Total SAT	25.9 ± .4[b]	30.7 ± 1.0[a]	31.3 ± .6[a]
Total MUFA	63.1 ± .4[a]	52.0 ± 1.6[b]	43.5 ± .9[c]
Total n-6	9.9 ± .6[b]	10.4 ± .4[b]	25.7 ± .5[a]
Total n-3	.5 ± .2[c]	7.1 ± 1.3[a]	.7 ± .1[c]
Total n-6:Total n-3	19.8 ± .9[b]	1.5 ± .6[c]	36.7 ± 1.0[a]
	------ Phosphatidylcholine fatty acids -------		
C18:2 (n-6)	12.7 ± .6[b]	13.3 ± .5[b]	21.5 ± .3[a]
C20:4 (n-6)	2.9 ± .3[b]	1.7 ± .0[c]	4.1 ± .3[a]
C18:3 (n-3)	.6 ± .1[b]	1.5 ± .1[a]	---
C22:6 (n-3)	.5 ± .1[b]	.9 ± .1[a]	.3 ± .0[b]
Total SAT	44.0 ± 1.3	42.3 ± 1.0	45.3 ± .6
Total MUFA	38.7 ± .4[a]	31.3 ± 1.6[b]	25.1 ± .9[c]
Total n-6	15.6 ± .5[b]	15.0 ± .4[b]	2.2[a]
Total n-3	1.1 ± .0[b]	2.4 ± 1.3[a]	.0[c]
Total n-6:Total n-3	14.2 ± 1.9[b]	6.3 ± .6[c]	85.3 ± 1.0[a]
	---- Phosphotidylethanolamine fatty acids ---		
C18:2 (n-6)	9.6 ± .5[b]	10.4 ± 1.1[b]	12.9 ± 2.1[a]
C20:4 (n-6)	5.9 ± .4	5.2 ± .3	5.1 ± .4
C22:6 (n-3)	.3 ± .1[b]	1.2 ± .7[a]	.8 ± .2[b]
Total SAT	46.6 ± 1.4	47.0 ± 1.0	46.5 ± .6
Total MUFA	36.6 ± .4[a]	33.0 ± 1.6[b]	32.8 ± .9[b]
Total n-6	15.5 ± .7[b]	15.6 ± .4[b]	18.9 ± .5[a]
Total n-3	.3 ± .0[b]	1.2 ± 1.3[a]	.8 ± .0[b]
Total n-6:Total n-3	51.7 ± 1.9[a]	13.0 ± .6[c]	23.6 ± 1.0[b]

[a-c]Means ± SE within the same row with no common superscripts are significantly different ($P < .05$), SAT = saturated fatty acid. MUFA = monounsaturated fatty acid,

Table 4.5. Major fatty acids of chick brain total lipid

Fatty acid	SF-9	FL-3	SF-6
	------- % of total fatty acids -------		
C16:0	27.9 ± .2	28.3 ± .2	28.9 ± .5
C18:0	15.5 ± .4[b]	16.7 ± .3[a]	17.4 ± .2[a]
C16:1	1.7 ± .1	1.8 ± .2	1.4 ± .2
C18:1	22.6 ± .5[a]	20.0 ± .5[b]	17.8 ± .6[c]
C18:2 (n-6)	3.2 ± .6	3.3 ± .1	3.9 ± .3
C20:4 (n-6)	9.0 ± .3[a]	6.3 ± .1[b]	9.5 ± .1[a]
C22:4 (n-6)	2.6 ± .1[b]	1.1 ± .0[c]	3.5 ± .1[a]
C22:5 (n-6)	2.0 ± .1[b]	.4 ± .1[c]	4.7 ± .2[a]
C20:5 (n-3)	.1 ± .0[b]	.9 ± .0[a]	---
C22:5 (n-3)	.6 ± .1[b]	1.3 ± .0[a]	---
C22:6 (n-3)	12.8 ± .4[b]	17.9 ± .6[a]	11.1 ± .6[c]
Total SAT	43.4 ± .5[b]	45.9 ± .3[a]	47.1 ± .5[a]
Total MUFA	24.3 ± .5[a]	22.4 ± .6[b]	19.2 ± .6[c]
Total n-6	16.8 ± .4[b]	11.0 ± .1[c]	21.6 ± 1.0[a]
Total n-3	13.5 ± .3[b]	20.7 ± .4[a]	11.7 ± .6[c]
Total n-6: n-3	1.2 ± .2[b]	.5 ± .0[c]	1.8 ± .1 [a]

[a-c]Means ± SE within the same row with no common superscripts are significantly different (P<.05).

SAT = saturated fatty acids.

MUFA = monounsaturated fatty acids.

Table 4.6. Fatty acid composition of phospholipids in the chick brain

Fatty acid	SF-9	FL-3	SF-6
		(%)	
	------- Phosphatidylcholine ------		
C18:2 (n-6)	1.5 ± .1[c]	2.3 ± .1[b]	2.7 ± .2[a]
C20:4 (n-6)	6.6 ± .1[a]	4.9 ± .0[b]	6.4 ± .6[a]
C22:4 (n-6)	1.1 ± .0[a]	.6 ± .0[b]	1.1 ± .1[a]
C22:5 (n-6)	.9 ± .1[b]	.1 ± .0[c]	1.6 ± .2[a]
C22:5 (n-3)	.2 ± .0[b]	.6 ± .1[a]	.2 ± .0[b]
C22:6 (n-3)	6.3 ± .3[b]	8.2 ± .1[a]	3.8 ± .6[c]
Total SAT	57.2 ± .3	57.7 ± .4	58.1 ± .6
Total MUFA	25.7 ± .3	24.9 ± .9	25.0 ± 1.2
Total n-6	10.1 ± .1[b]	7.9 ± .2[c]	11.8 ± 1.1[a]
Total n-3	6.7 ± .3[b]	8.8 ± .1[a]	4.0 ± .6[c]
Total n-6: n-3	1.5 ± .1[b]	.9 ± .0[c]	3.0 ± .3[a]
	------- Phosphatidylethanolamine ------		
C18:2 (n-6)	1.4 ± .3	1.6 ± .1	2.2 ± .2
C20:4 (n-6)	16.4 ± .3[a]	9.9 ± .1[b]	16.7 ± .2[a]
C22:4 (n-6)	7.8 ± .2[b]	2.6 ± .1[c]	7.3 ± .2[a]
C22:5 (n-6)	3.9 ± .1[b]	.4 ± .0[c]	8.8 ± .2[a]
C22:5 (n-3)	.8 ± .1[b]	2.6 ± .1[a]	.8 ± .1[b]
C22:6 (n-3)	28.5 ± .9[b]	36.5 ± .4[a]	20.7 ± .8[c]
Total SAT	31.5 ± .3	31.1 ± .4	31.3 ± .6
Total MUFA	12.8 ± .2	10.9 ± .7	12.8 ± .2
Total n-6	27.5 ± .5[b]	14.5 ± .3[c]	35.0 ± .5[a]
Total n-3	29.3 ± .1[b]	41.3 ± .4[a]	21.9 ± .9[c]
Total n-6: n-3	.9 ± .1[b]	.4 ± .0[c]	1.6 ± .1[a]

[a-c]Means ± SE within the same row with no common superscripts differ significantly ($P < .05$). SAT = saturated fatty acids.

MUFA = monounsaturated fatty acids.

Table 4.7. Brain and liver content of total lipids, phosphatidylcholine (PC), phosphatidylethanolamine (PE) of newly hatched chicks .

Diets	Tissue	Total Lipids (%)	PC	PE
SF-9	Brain	4.1 ± .1	52.1 ± 1.6[a]	33.1 ± 2.6[b]
FL-3		4.1 ± .2	44.7 ± 1.7[b]	40.8 ± .6[a]
SF-6		3.8 ± .1	45.2 ± 1.7[b]	41.9 ± .6a
SF-9	Liver	12.1 ± .7[a]	6.3 ± .41	3.6 ± .2[b]
FL-3		10.5 ± .4[ab]	7.9 ± .48	6.1 ± .5[a]
SF-6		9.4 ± .4[b]	8.2 ± 1.13	5.1 ± .4[a]

[a-b]Means ± SE within the same column and tissue with no common superscripts differ significantly ($P<.05$).

Table 4.8. Fatty acid composition of chick liver triglycerides

Fatty acid	SF-9	FL-3	SF-6
	(%)		
C16:0	4.5 ± .8[ab]	4.0 ± .6[b]	6.1 ± .7[a]
C18:0	2.8 ± .6[b]	4.4 ± .6[ab]	5.3 ±
C16:1	1.4 ± .2	1.4 ± .1	1.0 ± .
C18:1	79.5 ± 1.4[a]	71.3 ± 1.3[b]	67.4 ± 1.3[b]
C18:2 (n-6)	6.0 ± .2[b]	8.0 ± .3[c]	14.3 ± .6[a]
C20:4 (n-6)	1.4 ± .1[b]	1.4 ± .1[b]	2.1 ± .3[a]
C22:4 (n-6)	1.1 ± .0[a]	.6 ± .0[b]	1.1 ± .1[a]
C22:5 (n-6)	.9 ± .1[b]	.1 ± .0[c]	1.6 ± .2[a]
C18:3 (n-3)	.5 ± .1[b]	2.9 ± .4[a]	---
C22:5 (n-3)	.2 ± .0[b]	.6 ± .1[a]	.2 ± .0[b]
C22:6 (n-3)	.5 ± .1[b]	2.9 ± .4[a]	.4 ± .6[b]
Total SAT	7.6 ± 1.4[b]	8.8 ± 1.4[ab]	12.0 ± 1.4[a]
Total MUFA	80.9 ± 1.3[a]	72.7 ± 1.1[b]	68.4 ± .9[b]
Total n-6	9.4 ± .3[b]	10.1 ± .4[b]	19.1 ± .7[a]
Total n-3	1.2 ± .3[b]	6.4 ± .7[a]	.6 ± .6[b]
Total n-6: n-3	7.8 ± 1.8[b]	1.6 ± .1[c]	31.8 ± 3.9[a]

[a-c]Means ± S.E within the same row with no common superscripts are significantly different ($P<.05$).

SAT, saturated fatty acids.

MUFA, monounsaturated fatty acids.

Table 4.9. Fatty acid composition of chick liver phospholipids

Fatty Acid	SF-9	FL-3	SF-6
		(%)	
	------- Phosphatidylcholine -------		
C18:2 (n-6)	11.2 ± 1.6[b]	13.6 ± 1.1[b]	19.1 ± .1[a]
C20:4 (n-6)	10.8 ± .7[a]	6.1 ± .2[b]	11.8 ± .4[a]
C22:4 (n-6)	.3 ± .0[b]	---	1.1 ± .1[a]
C22:5 (n-6)	1.1 ± .1	---	1.7 ± .2
C22:5 (n-3)	1.1 ± .1[b]	2.6 ± .4[a]	.2 ± .0[c]
C22:6 (n-3)	6.6 ± 1.2[b]	10.1 ± .6[a]	4.1 ± .2 [c]
Total SAT	47.0 ± .9	48.7 ± .8	46.2 ± .28
Total MUFA	20.2 ± 1.9[a]	19.3 ± .9[a]	15.8 ± 1.6[b]
Total n-6	23.4 ± 1.8[b]	19.7 ± 1.3[c]	33.7 ± 1.0[a]
Total n-3	8.3 ± 1.4[b]	12.7 ± 1.0[a]	4.3 ± .3[c]
Total n-6/Total n-3	2.8 ± .9[b]	1.6 ± .3[b]	7.8 ± .3[a]
	------- Phosphatidylethanolamine -------		
C18:2 (n-6)	7.2 ± .4	7.8 ± .8	6.6 ± .3
C20:4 (n-6)	14.8 ± .9[b]	9.2 ± .5[c]	15.5 ± .3[a]
C22:4 (n-6)	.6 ± .2[b]	.2 ± .1[c]	1.4 ± .2[a]
C22:5 (n-6)	1.9 ± .2[a]	.5 ± .1[b]	2.1 ± .2[a]
C18:3 (n-3)	---[b]	1.1 ± .1[a]	---[b]
C20:5 (n-3)	---[b]	1.4 ± .2[a]	---[b]
C22:5 (n-3)	.3 ± .1[b]	1.0 ± .2[a]	.4 ± .0[b]
C22:6 (n-3)	8.1 ± .9[b]	11.3 ± 1.0[a]	4.2 ± .1[c]
Total SAT	45.5 ± .8[ab]	41.8 ± 1.3[b]	46.8 ± .6[a]
Total MUFA	20.9 ± .8	21.2 ± 1.0	23.0 ± 1.1
Total n-6	24.5 ± 1.0[a]	17.7 ± 1.5[b]	25.6 ± .3[a]
Total n-3	8.4 ± 1.1[b]	14.8 ± 1.6[a]	4.6 ± .1[c]
Total n-6: n-3	2.9 ± .4[b]	1.2 ± .2[c]	5.6 ± .1[a]

[a-c]Means ± S.E within the same row with no common superscripts are significantly different (P<.05). SAT, saturated fatty acids; MUFA, monounsaturated fatty acids.

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Chapter 5. Synthesis of Long Chain Polyunsaturated Fatty Acids by the Developing chick

5.1 Introduction

Results from Chapter 3 indicate that chicks hatched from n-3 fatty acid enriched eggs showed increased deposition of C18:3 n-3 and its longer chain C20 and C22 metabolites with a concomitant reduction in C20:4 n-6 in the liver and brain. This increase in C20 and C22 PUFA suggests that the developing embryos are not only sequestering the preformed long chain metabolites, but converting yolk 18:2 n-6 and 18:3 n-3 to long chain PUFA. These C20 and C22 polyunsaturated fatty acids are produced by chain elongation and desaturation of C18:2 n-6 or C18:3 n-3 in the liver microsomes (Dyerberg, 1986). The rate limiting enzyme in this pathway is Δ-6 desaturase (Holloway et al., 1963). Therefore, we were interested in investigating the Δ-6 desaturase activity in the chick tissues. The activity of desaturase enzyme has been shown to be influenced by dietary fatty acid composition (Garg et al., 1988). However, the role of maternal diet in modulating the desaturase activity in the progeny has not been reported. The present research investigated the incorporation of flax in the hen's diet on the yolk n-3 fatty acid composition and Δ-6 desaturase activity in the liver tissue of newly hatched chicks.

5.2. Materials and Methods

[1-^{14}C] linoleic acid (53 mCi/mmol) was purchased from Amersham[1], fatty

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[1]Amersham Canada Ltd, 1166 S. Service Road West, Oakville, ON,L6L 5T7, Canada

[2] Sigma Chemical Co, St Louis, MO 63178-9916.

acid methy ester standards and other chemicals were obtained from Sigma[2].

Egg Collection and Preparation of Microsomes

Ten (10) Single Comb White Leghorn laying hens were housed in cages and were assigned to one of the two test diets containing: 1. ground flax seed (FLAX) or a wheat soy bean diet with added animal tallow were used as control (CONTROL). The composition of the laying hens's diet and the fatty acid composition of the diet is shown in Table 5.1 and 5.2. At day 21 of feeding, the eggs were collected and incubated as described previously (Cherian and Sim, 1991). On the day of hatching the chicks (six from each treatment) were sacrificed by decapitation. The liver was excised, rinsed and homogenised in a medium containing .25M sucrose, .1 M-potassium phosphate, 1mM-EDTA, 1 mM dithiothreitiol, pH 7.2. Homogenates were centrifuged at 800g for 10 min, and the supernatant was centrifuged at 105,000g for 60 min. The microsomal pellet were suspended in a solution containing .25M sucrose and .15M KCl, pH 7.2 for measurements of δ-6 desaturase activity.

Δ-6Desaturase assy

Desaturation of C18:2 n-6 fatty acid by liver microsomes was estimated by measuring the amount of C18:3n-6 produced from [1-^{14}C 18:2 n-6] as described by Garg et al., 1988. The reaction medium contained 4μmol of ATP, 0.1μmol of CoA, 1.25μmol of NADH, 0.5 μmol of nicotinamide, 5μmol of $MgCl_2$, 62.5 μmol of NaF, 1.5μmol of glutathione, 62.5 μmol of potassium phosphate buffer, pH 7.0, and 200 nmol of [1-^{14}C] linoleic acid. The assay mixture was incubated in a shaking water bath at 37C for 20 min. The reaction was stopped using 2ml of 10% KOH in methanol. Lipids were saponified, acidified and extracted in hexane and methylated. The fatty

acid methyl esters were separated on TLC plates (Silica Gel-G impregnated with 10% $AgNO_3$). Plates were developed in hexane-diethyl ether (17:3, vol/vol). Methyl esters of C18:2 n-6 and C18:3 n-6 were spotted aside the samples. The plates were air dried and sprayed with .1% (wt/vol) 2',7' dichlorofluoresin in ethanol. The spots were made visible under ultraviolet light and the bands were scrapped off into scintillation vials and counted for radioactivity with 10 ml of ecolite by using a liquid scintillation counter. Protein was estimated according to the Bradford method (Bradford, 1976). Enzyme activity was expressed as pmol of C18:3 n-6 formed from C18:2 n-6/min per mg of microsomal protein. Total lipids were extracted from egg yolk (10) and liver microsomes (Folch et al., 1957). Lipid extracts were methylated (Metcalfe et al., 1961) and analysed for fatty acids by using a gas chromatograph as described earlier (Cherian and Sim, 1992).

Differences among treatment means were carried out by student t-test (Steel and Torrie, 1980).

5.3. Results and Discussion

Incorporating flax seeds in the laying hen diet increased the total n-3 fatty acid in the egg yolk lipids to 13.0% compared with 2.3% for the eggs from control diet. α-linolenic acid was the major n-3 fatty acid in the enriched egg at 10.0%. The longer chain metabolites of LNA such as 22:5 n-3 and 22:6 n-3 were higher ($P < .05$) in the FLAX eggs (Table 5.3). This increase in n-3 fatty acids resulted in a decrease of monounsaturated fatty acids and and lower n-6/n-3 ratio in these eggs ($P < .05$). The yolk fatty acid composition resulted in a marked change in the fatty acid composition of the liver microsomes (Figure 5.1). The chicks hatched from

FLAX eggs had higher ($P < .05$) C18:3 n-3, C22:5 n-3 and C22:6 n-3 in the liver microsomes than chicks hatched from the CONTROL which resulted in a lower n-6 to n-3 ratio. When compared with the control group, the microsomes of the FLAX group had significantly lower C16:0. No difference was observed in the level of C18:0. The total saturates were also lower ($P < .05$) in the FLAX group than in the Control. No difference was observed in the monounsaturated fatty acid of liver microsomes. Fatty acids of the n-6 group such as C18:2 n-6 and the longer chain metabolites of C18:2 n-6, C20:4 n-6, C22:4 n-6 and C22:5 n-6 were reduced ($P < .05$) in the microsomal lipids of FLAX group chicks.

The FLAX chicks had significantly lower ($P < .05$) Δ-6 desaturase activity (Table 5.5). Δ-6 Desaturase converts C18:2 n-6 and C18:3 n-3 to C18:3 n-6 and C18:4 n-3, and is the rate limiting step in the synthesis of C20:4 n-6 from C18:2 n-6 (Holloway et al., 1963). Thus a decrease in Δ-6 desaturase activity would indicate the inhibition of C20:4 n-6 formation from C18:2 n-6. This is evident from the reduced C20:4 n-6 content in liver microsomes of FLAX chicks.

Results from the earlier chapters indicated reduced level of C20:4 6 in the brain tissue of chicks hatched from n-3 fatty acid enriched eggs. This lower C20:4 n-6 might be due to the reduced synthesis from C18 precursor due to the inhibition of the enzymatic pathway as reported in the present experiment. The importance of the liver in supplying C20 and C22 fatty acids for the nervous system has been reported in the rat (Scott and Bazan, 1989). Also, there is a clear association in time with the accumulation of long chain PUFA in the brain and liver lipids of suckling rats (Sinclair, 1974). These results demonstrates the role of chick liver

in supplying long chain PUFA to the newly hatched chick brain and other tissues as indicated by the increased amounts of C20 and C22 fatty acids in the present experiment.

Dietary control of Δ-6 desturase activity has been reported earlier in rats (Garg et al., 1988; Brenner et al., 1989). However, the presence of n-3 fatty acid in the egg yolk and its effect on the Δ-6 desaturase activity of chick tissue has not been reported. The developing chick embryo requires PUFA for synthesis of membrane lipids and eicosanoids. This is evidenced by the increase in Δ-6 desaturase activity towards hatching in the embryonic chick liver (Noble and Shand, 1985).

Results from the previous chapters (2-5) demonstrated the role of maternal (laying hen) dietary n-3 PUFA in modulating the yolk fatty acid composition and the n-3 PUFA metabolism in the brain and liver tissue of the hatched chick. Maternal supplementation of n-3 fatty acids has been reported to increase the DHA content of milk in both humans and rodents (Harris et al., 1984; Yeah et al., 1990; Wainwright et al., 1992). This would clearly elevate DHA intake as indicated by the increase in RBC and plasma DHA of breast milk-fed infants compared with those fed formulas (Carlson, 1986). Considering the importance of n-3 fatty acids for the optimum synthesis of structural lipids, growth and functional development of the brain tissue and synthesis of eicosanoids, n-3 PUFA enriched egg may be an ideal alternative source of n 3 PUFA for pregnant and lactating women.

Table 5.1.

Composition of laying hen diets

Ingredients	Experimental diets	
	Flax	Control
	(%)	
Wheat	66.90	72.40
Soybean meal	5.30	11.80
Animal Tallow	----	3.00
Flax seeds	15.00	---
DiCal - phos	2.00	2.00
Limestone	8.30	8.30
Iodized salt	0.30	0.30
D/L-Methionine	0.10	0.10
Layer premix[1]	2.10	2.10
Pyridoxine		
Calculated composition		
Crude protein (%)	16.5	16.8
ME (kcal/kg)	2700.0	2900.0

[1]Supplied per kilogram diet the following : vitamin A, 8,000IU; vitamin D_3, 1,200 ICU; vitamin E, 5 IU; riboflavin, 4mg; calcium pantothenate, 6mg; niacin, 15 mg; vitamin B_{12}, 10 μg; choline chloride, 100 mg; biotin, 100 μg; DL-methionine, 500 mg; manganese sulfate, 146 mg; zinc oxide, 58 mg.

Table 5.2.

Fatty Acid Composition of the experimental Diets

Fatty Acid	Experimental Diets	
	Flax	Control
	------ (%)	------
C14:0	2.6	1.1
C16:0	12.5	19.2
C18:0	4.9	8.8
C16:1	1.4	1.3
C18:1	27.4	38.5
C18:2 n-6	20.9	27.3
C18:3 n-3	28.6	3.8
Total saturates	20.0	29.1
Total monounsaturates	28.8	39.8
Total n-6	20.9	27.3
Total n-3	28.6	3.8
Total n-6:Total n-3	0.7	7.2

Table 5.3. Fatty acid composition of the yolk total lipids

Fatty acid	Experimental Diets		
	FLAX	CONTROL	SEM
	------- (%) -------		
C16:0	20.8*	24.5	0.28
C18:0	10.5*	9.4	0.19
C16:1	3.3	3.7	0.22
C18:1	38.5*	47.9	0.57
C18:2 (n-6)	13.1	11.0	0.34
C20:4 (n-6)	.5	.3	0.22
C18:3 (n-3)	10.0*	.6	0.33
C20:5 (n-3)	.3	.2	0.06
C22:5 (n-3)	.7*	.1	0.09
C22:6 (n-3)	2.1*	1.2	0.11
Total Saturates	31.7*	34.6	0.37
Total MUFA	41.8*	51.6	0.50
Total n-6	13.5*	11.6	0.41
Total n-3	13.0*	2.3	0.37
Total n-6:Total n-3	1.0*	5.0	0.11

[1] The laying hen diet contained grounf flax seed (FLAX) or wheat soybean meal based (CONTROL) diet.

*indicates significantly different from control ($P < .05$).

MUFA = monounsaturated fatty acid

SEM = standard error of the mean

Figure 5.1. Fatty acid composition of the liver microsomes of the chicks hatched from n-3 fatty acid enriched (FLAX) or control (CONTROL) eggs. n=6. * indicates means are significantly different ($P<.05$).

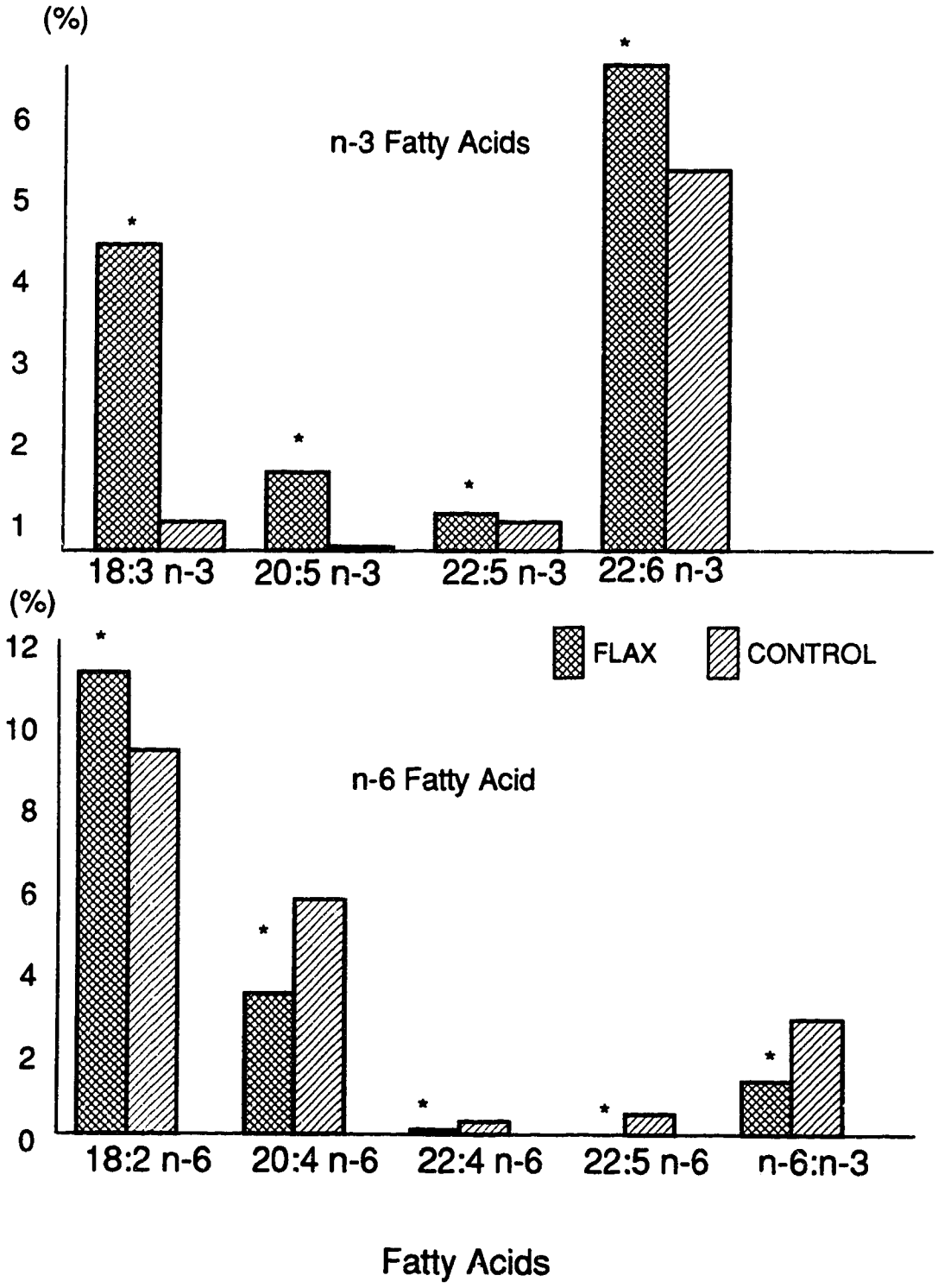
(%)
n-3 Fatty Acids
6
5
4
3
2
1
18:3 n-3
20:5 n-3
22:5 n-3
22:6 n-3
(%)
FLAX
CONTROL
n-6 Fatty Acid
12
10
8
6
4
2
0
18:2 n-6
20:4 n-6
22:4 n-6
22:5 n-6
n-6:n-3
Fatty Acids

Table 5.4.

Effect of feeding flax seeds to laying hens on the Δ-6 desaturase activity in the liver of hatched chicks.

Laying Hen Diet	Δ-6 desaturase activity (pmol/min per mg of protein)
FLAX	49.4 ± 2.7
CONTROL	82.8 ± 3.8

Values are means ± S.E for six microsomal preparations.
FLAX and CONTROL represents liver tissue of chicks hatched from hens fed diets containing ground flax or wheat soybean meal based control diets
*means are significantly different from control ($P<.05$)

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Chapter 6. Cholesterol Metabolism: Omega-3 Fatty Acid and Cholesterol Content of newly Hatched Chicks From Alpha- Linolenic Acid Enriched Eggs[1].

6.1. Introduction

Epidemiological and clinical studies have demonstrated that the type and quantity of dietary fat affects plasma cholesterol levels. For many years, the so-called cholesterol-lowering PUFA were limited to n-6 fatty acids. The hypocholesterolemic effects of n-6 PUFA has been attributed to enhanced excretion of cholesterol through bile as bile acids and as an increase in LDL-receptor mediated clearance (Paul et al., 1980; Kovanen et al., 1981). In many studies, dietary n-6 fatty acids also induced a reduction of HDL-cholesterol (Mattson and Grundy, 1985).

The past decades have witnessed an increasing interest in the health benefits of n-3 PUFA. The emphasis has been on the longer chain n-3 fatty acids such as EPA and DHA. The hypocholesterolemic effects of n-3 fatty acids from marine as well as linseed oils have been reported in epidemiological (Bang and Dyerberg, 1972; Dyerberg, 1986), clinical trials (Herold and Kinsella; Nestle, 1986; MacDonald et al., 1990 and in experimental animals (Garg et al., 1988). Another type of unsaturated fatty acids, the

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[1] A version of this chapter has been published. G. Cherian and J. S. Sim, Lipids. 1992. 27:706-710

monounsaturated fatty acids, also have been recognised for their cholesterol lowering effets (Mattson and Grundy, 1985). The quantity of dietary fat fed to animals during development has been reported to influence cholesterol metabolism in the adulthood (Riser and Sidelman. 1972). The exact mechanism for this phenomenon is not known. Recently, we (Jiang et al., 1990) reported that cholesterol metabolism in developing embryos and newly hatched chicks was influenced by the cholesterol content of the maternal diet and the yolk .

The enrichment of chicken egg with n-3 fatty acids by incorporating flax and canola seeds or their oils in laying hens diets has been demonstrated in the previous chapters and also reported in the literature (Chapter 2-5; Cherian and Sim, 1991; Sim, 1990; Farrell and Gibson, 1990; Anderson et al., 1989). During incubation of a fertile egg, the lipids of the yolk serve as an energy source and supply fatty acids for the synthesis of membrane lipids of the developing chicken embryos. Unlike the mammalian fetus, the chicken embryo is not dependent on maternal fatty acids through circulation. Maternal fatty acids can cross the placenta and may, for example, contribute up to 50% of fetal fatty acids during late gestation in the rat (Coleman, 1986). When an n-3 fatty acid enriched fertilized egg is incubated, the developing embryo is in a n-3 fatty acid rich 'contained' environment. Therefore, we hypothesized that the n-3 fatty acid rich lipid pool available to the developing chick embryo might alter the cholesterol metabolism of the hatched chick.

Therefore, the objective of the present study was to determine the extent to which dietary n-3 or n-6 fatty acids can be incorporated into egg yolk and to examine its effect on the fatty acid and cholesterol content of the progeny (hatched chick).

6.2. Materials and Methods

Fatty acid modulation.

Twenty single Comb White Leghorn (SCWL) laying hens were housed in cages (30 x 40 cm) and were assigned to one of the three test diets containing: 1. 10% ground flax seed (Flax), 10% ground canola seed (Canola) or 16% ground soybean seed (Soy bean), and were compared to birds fed 12% sunflower seed (Sunflower) serving as controls (Table 3.1). The fatty acid compositions of the diets are shown in Table 3.2. The hens were artificially inseminated on consecutive days with 0.05 mL of pooled semen sample. Fertilized eggs collected for six days after birds had been two weeks on experimental diets. The eggs were incubated in a forced-air incubator (Robbins Incubator Company, Denver, CO). The eggs were incubated at 37 C at a relative humidity of 64% and turned once per hour for eighteen days. The eggs were then transferred to hatchers and incubated for three days at 37.2 C and at a relative humidity of 64%.

Sample collection and analyses.

Eggs from laying hens fed ground flax seed, ground canola seed, ground soy bean seed and the ground sunflower seed were collected

and the yolks were separated, weighed and kept frozen till analysis. On the day of hatching, 10 newly hatched chicks from laying hens from each of the diets containing ground flax (FXC), ground canola (CAC), ground soybean seed (SBC) or groundsunflower (SFC) were weighed and killed by decapitation. Five chicks from each treatment were ground in chloroform/methanol (2:1, vol/vol) solution with a polytron (Brinkman Instruments, Westbury, NY). From the remaining five chicks, blood, brain,and liver tissues were collected. Blood samples were centrifuged at 3000 rpm in a Beckman centrifuge (Beckman Instruments, Mississauga, Ontario, Canada) and plasma was separated. Total lipids were extracted from yolk, whole chicks, liver, plasma and brain by the method of Folch et al., 1957.

Aliquots of the lipid extracts were dried under nitrogen and were converted to fatty acid methyl esters using a mixture of boron-trifluoride, hexane and methanol, (35:20:45, vol/vol/vol) (Metcalfe and Schmitz, 1961). Fatty acid methyl esters were separated and quantified by automated gas chromatography (Model 3400, Varian Associates, Inc., Sunnyvale, CA) using a DB-23 fused capillary column (30 m by 0.25 mm). The initial column temperature was set at 70 C for 3 min, increased to 180 C at 30 C/min, and held for 10 min. Next the column temperature was elevated to 230 C at a temperature of 5 C/min and held at the final temperature for 3 min. Helium was used as the carrier gas at a flow rate of 1.5 mL/min. The fatty acid results were expressed on a percentage basis. Plasma cholesterol was measured by the enzymatic method using a commercial

kit (Sigma Chemicals Co., St. Louis. MO). For liver cholesterol determination, an aliquot of the total lipid extract was dried under nitrogen along with the internal standard 5α-cholestane, and the cholesterol content was measured by gas chromatography (GC) (Fenton and Sim, 1990).

Statistics. Data were analyzed by one way analyses of variance (ANOVA) using AOV5. Significant differences between treatment means were determined with Duncan's Multiple Range Test (Steel and Torrie, 1980).

6.3. Results

The total lipid and fatty acid composition of the diets are shown in Table 6.2. The only sources of n-3 or n-6 fatty acids in the diet were 18:3 n-3 and 18:2 n-6. The total n-3 and n-6 fatty acids in the diet were 27.2, 11.4, 5.0, 3.3% and 19.8, 25.4, 37.3, and 45.0 for flax, canola, soy bean and the sunflower based diets, respectively. The fatty acid composition of egg yolk was significantly ($P < .05$) modified by the laying hen test diets (Table 6.3). Feeding ground flax seed enriched the egg yolk with LNA and its metabolites such as EPA and DHA resulting in the total incorporation of 8.29% of n-3 fatty acids compared with 2.83% for canola seed, 1.66% for soybean seed and 1.11% for ground sunflower seed. LNA was the major n-3 fatty acid in the yolk of hens fed diets containing ground flax or canola seed, although considerable amounts of EPA and DHA were found in these yolks. Conversely, incorporation

of 12% sunflower seeds in the laying hen's diet resulted in a significant ($P < .05$) increase in 18:2n-6 and arachidonic acid (20:4n-6) at the expense of oleic acid (18:1n-9) (data not shown). Consequently, this resulted in a decrease in monounsaturated fatty acid (MUFA) content and a higher total n-6/n-3 ratio in the eggs from hens fed diets containing soybean seeds. The 20:4n-6 content of eggs from the flax group was significantly ($P<0.05$) lower than the canola group at 0.82 and 1.37%, respectively.

The total lipid fatty acid composition of whole body, plasma, and liver tissue was modified by the egg yolk lipid composition (Table 6.4). Significantly ($P<.05$) higher contents of LNA and its metabolites such as EPA, DPA and DHA were found in the total body lipids, plasma lipids and liver lipids of the hatched chicks from n-3 fatty acid enriched flax, canola and soy than those hatched from n-6 fatty acid enriched sunflower group eggs. The chicks hatched from flax, canola and soy group had significantly ($P < .05$) reduced 18:2n-6 and 20:4n-6 content. In contrast, chicks hatched from n-6 fatty acid enriched eggs (sunflower group) had significantly higher ($P<.05$) levels of 18:2n-6 and its metabolites such as 20:4n-6, 22:4n-6, and 22:5n-6 in all tissues examined. The total lipid content of the whole body and the liver was not affected. Although, plasma total lipids were significantly reduced in the flax, canola when compared with chicks from sunflower group.

The brain fatty acid composition of the newly hatched chicks is shown in Table 6.5. Compared to SBC and SFC chicks, CAC and FXC

chicks had elevated concentration of EPA, DPA and DHA. The effect was most pronounced with respect to DHA which is the major polyunsaturated fatty acid in chick brain. The concentrations of 18:2n-6, 22:4n-6, and 22:5n-6 were significantly higher in the SFC chicks. In the FXC and CAC chicks, the level of 20:4n-6 in brain was reduced ($P<.05$) by the yolk fatty acid composition. The level of MUFA in the brain lipids was not affected by the yolk fatty acid composition.

The chicks hatched from hens fed diets containing flax seeds showed significantly reduced liver cholesterol levels (Figure 6.1). Cholesterol levels in the heart and brain were not affected by the yolk fatty acid composition.

6.4. Discussion

The presence of n-3 or n-6 fatty acids in the laying hen diet can enrich the egg yolk lipids and further the tissues of the hatched chick with n-3 and n-6 fatty acids. The increased supply of dietary 18:3n-3 tended to increase the levels of the long-chain n-3 fatty acids such as EPA, DPA, DHA associated with a corresponding reduction in the level of 20:4n-6 in the egg yolk.

The major n-3 fatty acid in liver and brain was DHA. The low LNA and higher DHA levels in liver of n-3 fatty acid enriched FXC and CAC compared to SFC indicate the importance of liver in supplying DHA to the developing brain. Similar levels of DHA in the liver tissue of FXC and CAC chicks further support this observation

because the brain of the FXC and CAC chicks had similar DHA levels. Moreover, DHA was a major n-3 fatty acid in the chick plasma. Liver and plasma have been shown to provide DHA to the developing brain in rat pups when the maternal diet was enriched with 18:3n-3 (Scott and Bazan, 1989). Despite changes in the fatty acid composition of the brain tissue, the total PUFA content (n-6 plus n-3) remained constant in this tissue. Similar observations have been reported for other species such as in the rat (Yamamoto et al., 1987; Enslen et al., 1991) and pig (Hrboticky et al., 1989).

Chicks hatched from eggs enriched with n-3 fatty acids (flax group) showed reduced plasma and liver cholesterol and plasma total lipids. In contrast, chicks hatched from eggs enriched with n-6 fatty acids (sunflower group) showed a similar reduction in plasma cholesterol with an accumulation of cholesterol in the liver. However, this reduction was not evident in chicks hatched from hens fed diets with soy bean seeds and which might be due to the lower levels of n-3 fatty acids in the SBE eggs. Thus, the n-6 and n-3 fatty acids in egg yolk affected cholesterol metabolism in the developing progeny differently. The n-6 fatty acids caused a redistribution of cholesterol from the plasma to the liver pool.

Diets rich in n-6 fatty acids have been reported to increase cholesterol esterification. This increased esterification further increases the capacity of hepatic cells to take up more free cholesterol from circulating plasma (Garg et al., 1988; Reiber, 1978). The rate-limiting step in cholesterol synthesis is

3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) (Rodwell et al., 1976). Haave et al. (1990) reported an increased activity of this enzyme in the fetal liver of rat pups when the dam's diet was high in n-6 fatty acids. Differential effects of n-3 and n-6 fatty acids causing redistribution of cholesterol have been reported when rats were fed egg yolks enriched with n-3 or n-6 fatty acids (Jiang and Sim, 1992) or linseed and sunflower oil (Garg et al., 1988). However, the enrichment of n-3 and n-6 fatty acid in the egg and their effect on the developing chick have not been reported previously.

In summary, the type of fat fed to laying hens can affect the fatty acid composition of the yolk and also the fatty acid and cholesterol content of the developing progeny. Whether changes in fatty acid accretion during development due to alteration of yolk fatty acid composition may cause long term changes in cholesterol metabolism in the chick requires further study.

Table 6.1.

Composition of Laying Hen Diets

Ingredients	Experimental diets			
	Flax	Canola	Soy bean	Sunflower
	(%)			
Wheat	64.66	65.04	68.00	62.21
Soybean meal	10.28	11.05	13.16	12.01
Animal tallow	3.25	2.18	2.88	2.00
Flax seeds	10.00	---	---	---
Canola seeds	----	10.00	---	---
Sunflower seed	----	----	---	12.00
Soy bean seed	---	----	16.00	---
DiCal - phos	1.00	0.98	1.00	0.98
Limestone	8.33	8.29	8.32	8.34
Iodized salt	0.29	0.28	0.28	0.29
D/L-Methionine	0.07	0.07	0.10	0.09
Layer premix[a]	2.10	2.00	2.00	2.10
Calculated composition				
Crude protein (%)	16.50	16.80	16.80	16.50
ME (kcal/kg)	2700.00	2900.00	2900.00	2747.00

[a]Layer premix supplied per Kg of the diet the following : vitamin A, 8,000IU;vitamin D_3, 1,200 ICU; vitamin E, 5 IU; riboflavin, 4mg; calcium pantothenate, 6mg; niacin, 15 mg; vitamin B_{12},10 μg; choline chloride, 100 mg; biotin, 100 μg; DL-methionine, 500 mg; manganese sulfate, 146 mg; zinc oxide, 58 mg.

Table 6.2.

Total Lipid and Fatty Acid Composition of the Experimental Diets(%)

	Experimental Diets			
Fatty acid	Flax	Canola	Soy bean	Sunflower
18:2n-6	19.82	25.46	37.30	45.01
18:3n-3	27.03	10.98	5.46	3.31
Σ Saturated	18.80	14.55	21.51	19.06
Σ Monounsaturated	33.66	48.22	35.05	32.35
Σ n-6	19.82	25.46	37.30	45.01
Σ n-3	27.26	11.43	5.00	3.31
n-6/n-3	0.73	2.23	7.46	13.59
Σ Lipids(%)	5.20	5.85	4.06	4.25

Table 6.3

Fatty Acid Composition of the Egg Yolk Lipids

y acid	Experimental Diets			
	Flax	Canola	Soy bean	Sunflower
	---------- (% of total fatty acids) ----------			
18:2n-6	10.62 ± 0.18[b]	11.15 ± 0.12[b]	11.47 ± 0.16[b]	21.77 ± 0.22[a]
20:4n-6	0.82 ± 0.18[c]	1.37 ± 0.08[b]	1.98 ± 0.38[b]	2.18 ± 0.12[a]
18:3n-3	5.77 ± 0.09[a]	1.37 ± 0.10[b]	0.63 ± 0.07[c]	0.47 ± 0.09[c]
20:5n-3	0.18 ± 0.05[a]	0.08 ± 0.02[a]	ND	ND
22:5n-3	0.49 ± 0.02[a]	0.16 ± 0.01[a]	ND	ND
22:6n-3	1.85 ± 0.41[a]	1.22 ± 0.03[ab]	1.03 ± 0.26[b]	0.64 ± 0.26[c]
Σ n-6	11.44 ± 0.50[b]	12.52 ± 0.14[b]	13.45 ± 0.41[b]	23.95 ± 0.25[a]
Σ n-3	8.29 ± 0.61[a]	2.83 ± 0.17[b]	1.66 ± 0.35[c]	1.11 ± 0.34[c]
n-6/n-3	1.38 ± 0.49[d]	4.42 ± 0.19[c]	8.10 ± 0.26[b]	21.58 ± 0.39[a]
Lipids[e]	5.85 ± 0.32	5.41 ± 0.28	5.63 ± 0.23	5.88 ± 0.19

[a-d]Means with no common superscripts within the same row are significantly different ($P<0.05$). Data are presented as mean ± SE, n = 5.

ND, not detectable.

[e]Percent of total lipids.

Table 6.4
Total Lipids and Fatty Acid Composition of Tissues of Newly Hatched Chicks

Tissue	Fatty acid	Flax	Canola	Soybean	Sunflower
		---------- (% of total fatty acids) ----------			
Whole Body	18:2n-6	12.01 ± 0.18^{c}	12.96 ± 0.12^{c}	15.71 ± 0.16^{b}	22.17 ± 0.1
	20:4n-6	1.10 ± 0.21^{c}	1.22 ± 0.08^{c}	1.87 ± 0.02^{b}	2.22 ± 0.1
	22:4n-6	ND	0.14 ± 0.02^{b}	0.10 ± 0.01^{b}	0.45 ± 0.0
	22:5n-6	ND	ND	ND	0.40 ± 0.1
	18:3n-3	4.79 ± 0.20^{a}	1.55 ± 0.02^{b}	0.46 ± 0.01^{c}	0.38 ± 0.0
	20:5n-3	0.11 ± 0.05	0.13 ± 0.02	ND	ND
	22:5n-3	1.47 ± 0.02^{a}	0.19 ± 0.01^{b}	ND	ND
	22:6n-3	1.05 ± 0.41^{a}	0.84 ± 0.03^{b}	0.94 ± 0.26^{ab}	0.92 ± 0.2
	Σ n-6	13.11 ± 0.50^{c}	14.32 ± 0.14^{c}	17.68 ± 0.41^{b}	25.24 ± 0.2
	Σ n-3	7.87 ± 0.50^{a}	2.71 ± 0.17^{b}	1.40 ± 0.35^{c}	1.30 ± 0.1
	n-6/n-3	1.67 ± 0.09^{d}	5.28 ± 0.12^{c}	12.62 ± 0.21^{b}	19.42 ± 0.2
	Lipids(%)	5.66 ± 0.18	4.43 ± 0.28	4.07 ± 0.23	4.43 ± 0.2
Liver	18:2n-6	10.96 ± 0.32^{c}	11.33 ± 0.12^{c}	12.71 ± 0.22^{b}	14.45 ± 0.0
	20:4n-6	4.07 ± 0.12^{c}	6.25 ± 0.08^{b}	7.10 ± 0.38^{b}	9.35 ± 0.1
	22:4n-6	ND	ND	0.22 ± 0.02^{b}	0.55 ± 0.0
	22:5n-6	ND	ND	0.32 ± 0.01^{b}	1.04 ± 0.0
	18:3n-3	2.01 ± 0.09^{a}	0.73 ± 0.10^{b}	0.26 ± 0.07^{c}	ND
	20:5n-3	0.18 ± 0.05^{b}	0.30 ± 0.05^{a}	0.31 ± 0.02^{a}	ND
	22:5n-3	0.45 ± 0.02	0.47 ± 0.01	ND	ND
	22:6n-3	5.30 ± 0.41^{a}	4.92 ± 0.03^{a}	3.87 ± 0.26^{ab}	2.43 ± 0.2
	Σ n-6	15.03 ± 0.23^{d}	17.58 ± 0.56^{c}	20.35 ± 0.89^{b}	25.39 ± 0.5
	Σ n-3	7.94 ± 0.36^{a}	6.42 ± 0.11^{a}	4.44 ± 0.27^{b}	2.43 ± 0.1
	n-6/ n-3	1.89 ± 0.24^{d}	2.74 ± 0.17^{c}	4.58 ± 0.23^{b}	10.45 ± 0.2
	Lipids(%)	13.69 ± 0.34	12.91 ± 0.46	12.14 ± 0.28	12.81 ± 0.3
Plasma	18:2n-6	20.46 ± 0.22^{c}	21.08 ± 0.27^{c}	24.26 ± 0.19^{b}	27.81 ± 0.3
	20:4n-6	3.96 ± 0.03^{d}	5.96 ± 0.10^{c}	8.00 ± 0.07^{b}	10.41 ± 0.1
	22:4n-6	ND	0.25 ± 0.01	0.26 ± 0.03^{b}	0.47 ± 0.0
	22:5n-6	ND	ND	0.26 ± 0.01	0.29 ± 0.0
	18:3n-3	4.15 ± 0.16^{a}	1.12 ± 0.03^{b}	0.72 ± 0.01^{c}	0.30 ± 0.0
	20:5n-3	0.97 ± 0.05^{a}	0.61 ± 0.02^{b}	0.43 ± 0.02^{c}	ND
	22:5n-3	2.62 ± 0.02^{a}	0.39 ± 0.01^{b}	0.35 ± 0.02^{b}	ND
	22:6n-3	3.80 ± 0.09^{a}	2.43 ± 0.16^{b}	2.23 ± 0.06^{c}	1.55 ± 0.0
	Σ n-6	24.42 ± 0.25^{d}	27.29 ± 0.45^{c}	32.78 ± 0.61^{b}	38.98 ± 1.45
	Σ n-3	11.54 ± 0.23^{a}	4.55 ± 0.29^{b}	3.73 ± 0.32^{c}	1.85 ± 0.32
	n-6/n-3	2.12 ± 0.14^{d}	5.99 ± 0.23^{c}	8.79 ± 0.19^{b}	21.07 ± 0.29
	Lipids(%)^{e}	0.93 ± 0.02^{b}	0.88 ± 0.49^{b}	1.27 ± 0.07^{a}	1.18 ± 0.03

$^{a-d}$Means with no common superscripts within the same rows are significantly different ($P<0.05$). Data are presented as mean ± SEM, n = 5. ^{e}g/dL.

Table 6.5

Total Lipids and Fatty Acid Composition of Brain Tissue of Newly Hatched Chicks(%)

Fatty acid	Experimental Diets			
	Flax	Canola	Soy bean	Sunflower
	---------- (% of total fatty acids) ----------			
18:2n-6	2.34 ± 0.04[b]	2.62 ± 0.09[b]	2.25 ± 0.25[b]	3.47 ± 0.13[a]
20:4n-6	5.77 ± 0.04[c]	7.55 ± 0.45[b]	8.74 ± 0.26[a]	9.38 ± 0.26[a]
22:4n-6	1.00 ± 0.03[c]	1.2 ± 0.03[c]	1.70 ± 0.07[b]	2.40 ± 0.12[a]
22:5n-6	1.11 ± 0.11[b]	1.13 ± 0.11[b]	1.18 ± 0.12[b]	3.77 ± 0.33[a]
18:3n-3	0.26 ± 0.04	0.30 ± 0.04	ND	ND
20:5n-3	0.69 ± 0.05[a]	0.72 ± 0.05[a]	0.31 ± 0.11[b]	0.12 ± 0.03[c]
22:5n-3	2.04 ± 0.06[a]	2.19 ± 0.08[a]	0.31 ± 0.02[b]	ND
22:6n-3	18.67 ± 0.09[a]	17.39 ± 0.45[a]	13.21 ± 0.46[b]	10.12 ± 0.45[c]
Σ n-6	10.22 ± 0.06[c]	12.50 ± 0.56[b]	13.87 ± 0.66[b]	19.02 ± 1.01[a]
Σ n-3	21.66 ± 0.73[a]	20.60 ± 0.73[a]	13.83 ± 0.76[b]	10.24 ± 0.73[c]
n-6/n-3	0.47 ± 0.12[c]	0.61 ± 0.02[c]	1.00 ± 0.24[b]	1.86 ± 0.09[a]
Lipids[e]	4.41 ± 0.18	3.98 ± 0.08	4.36 ± 0.06	4.21 ± 0.16

[a-c]Means with no common superscripts within the same row are significantly different ($P<0.05$). Data are presented as mean ± SEM, n = 5.

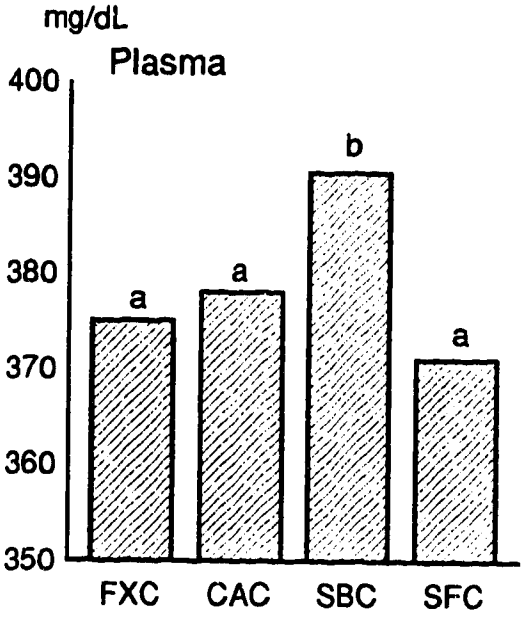
[e] Percent of total lipids.

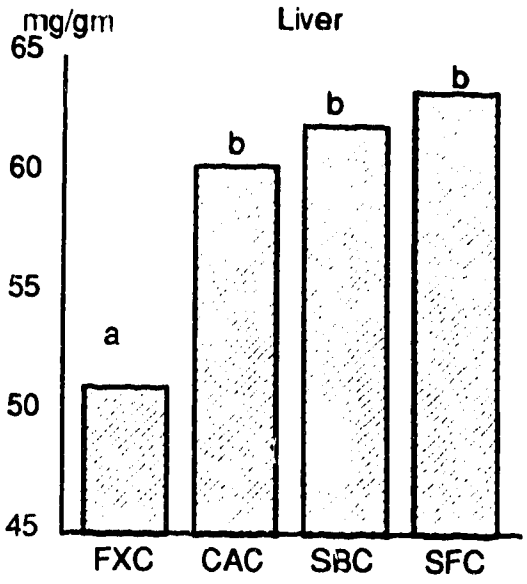
Figure 6.1.

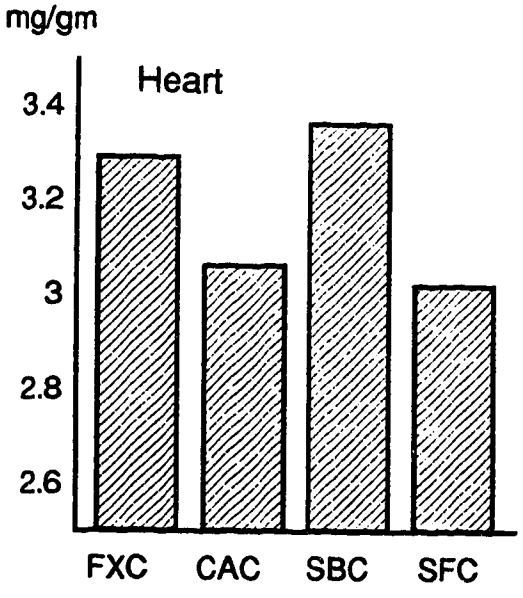
Cholesterol content of of newly hatched chick tissues from eggs enriched with n-3 or n-6 fatty acids

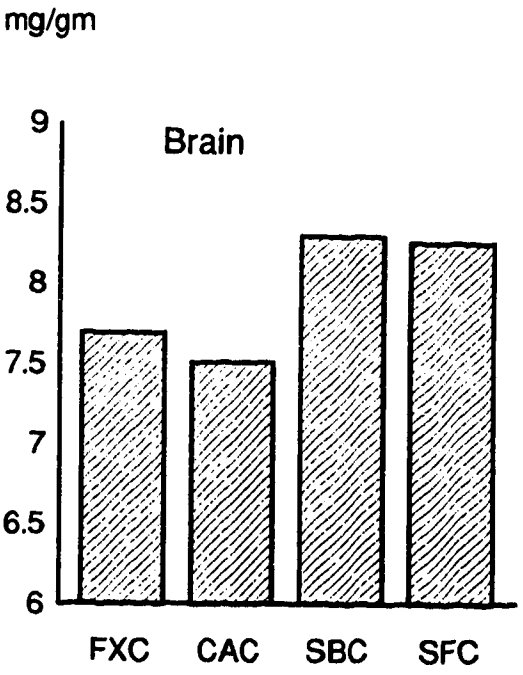
Values are presented in mg/gm of tissue or mg/dL. n=5.

FXC, CAC, SBC, and SFC represents chicks hatched from hens fed diets containing ground flax, canola, soy or sunflower seeds respectively.


mg/dL
Plasma
400
390
380
370
360
350
a
a
b
a
FXC
CAC
SBC
SFC


mg/gm
Liver
65
60
55
50
45
a
b
b
b
FXC
CAC
SBC
SFC


mg/gm
Heart
3.4
3.2
3
2.8
2.6
FXC
CAC
SBC
SFC


mg/gm
Brain
9
8.5
8
7.5
7
6.5
6
FXC
CAC
SBC
SFC

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Chapter 7. n-3 Fatty Acid Modified Egg as an Infant Food

7.1. Consumption of n-3 Fatty Acid Enriched Eggs by Nursing Mothers and Transfer of n-3 Long Chain Polyunsaturated Fatty Acids to Breast Milk Lipids

7.1. Introduction.

In the previous chapters the role of yolk n-3 fatty acids in modifying the n-3 PUFA composition of the chick brain tissue was addressed. Recent years have witnessed an increasing interest in the role of lipids in growth and development. Particular emphasis has been focussed on the role of docosahexaenoic acid (DHA, C22:6 n-3) in the developing central nervous system because of reports of impaired cognitive performance (Yamamoto et al., 1987; Bourre et al., 1989) and electroretinographic abnormalities in rats (Benolken et al., 1973; Bourre et al., 1989) and altered visual function in monkeys (Connor and Neuringer, 1988) and human infants (Carlson et al., 1989; Uauy et al., 1990) with low DHA levels. The imbalance of n-6:n-3 fatty acids has been reported to influence brain phospholipid fatty acid composition and phosphotidyl choline synthesis (Hagreaves and Clandinin, 1990). Therefore, it is logical to postulate that the ratio of n-6:n-3 fatty acids fed during periods of rapid brain growth are important determinants for structural and functional parameters of the brain tissue.

In human infants, C20 and C22 PUFA accumulate during the last intrauterine trimester and during early stages of life (Dobbing and Sands, 1979), suggesting that these fatty acids may be indispensible for neural development of the newborn (Neuringer and Connor, 1986). After birth,

infants consume n-6 and n-3 fatty acids both as C18:2 n-6 and C18:3 n-3 unless fed on breast milk which contains C20:4 n-6 and C22:6 n-6, and small amounts of other C20 and C22 PUFA. However, breast milk fatty acids reflect the mother's diet (Insull and Aherns, 1965) and because modern diets are low in n-3 PUFA and vary in their n-6 to n-3 ratio (Simopoulos, 1991, Lands, 1992), it may be assumed that infants at present are receiving less long chain n-3 or n-6 fatty acids from their mother's milk.

Due to convenience and life style, infant formulas remain the major source of nutrition for many infants during the first 12 months of life (Fomon, 1987). The fat in the infant formulas is a blend of one or more vegetable oils and thus they do not contain C20 and C22 PUFA, and differ markedly in their n-6 to n-3 ratios. Formulas with low levels of C18:3 n-3 and no C22:6 n-3 have been reported to limit accretion of n-3 PUFA to the developing piglet brain (Hrboticky et al., 1990). Because of the lack of long chain PUFA in formula diets, and the limited ability of the infant in synthesizing from precursor (Putnam et al., 1982; Carlson et al., 1986), there is a good reason to question and evaluate the adequacy of today's infant formula. Hopefully the information could be used to design an improved fatty acid balance to meet the infant's C20 and C22 PUFA requirements.

Aware of the importance of n-3 PUFA in the development of central nervous system, we investigate, in this chapter, the magnitude of changes in the fatty acid composition of total lipids in the breast milk of nursing women consuming eggs enriched with n-3 fatty acids. A survey of the fatty acid composition of commercially available infant formulas and the potential of using egg yolk lipids as an alternative lipid source for

infant formula preparation is discussed.

7.1.2. Subjects and Methods

Nursing women (n=8) were recruited from the University of Alberta. Signed consent was obtained from each participant. All protocols, survey forms, and questionaires were approved by the ethics committee on human research at the University of Alberta.

N-3 fatty Acid Enriched Eggs

Eggs enriched with n-3 fatty acids (n-3 Egg) were obtained from a local producer. These eggs were produced by feeding diets containing flax seeds to laying hens.

Methods

The mothers were encouraged to continue their usual diets supplemented with 2 n-3 eggs per day and to limit the intake of sea foods during the six week study. Breast milk samples were collected once every week and quickly frozen, then kept at -30 C until analysis.

Infant Formulas

Five different brands of infant formulae (n=5) were selected randomly from local market. These formulas all reported the source of fat as being of vegetable oil origin, and none of them reported the percentage of n-3 fatty acids on the labels. These five different brands represented the usual food supply available to the infant.

Analysis

The frozen milk samples were thawed and 3 mL of the sample was taken from the breast milk or formulae (n=5). Total lipids were extracted from breast milk and formulas according to the method of Folch et al., 1957. Briefly, 20 ml chloroform:methanol (2:1) was added to 3 ml of breast milk or

formule and homogenised using a polytron for 30 seconds. To this homogenate 7 ml of 0.7% NaCl was added and mixed and centrifuged at 1000 rpm for 5 minutes. The top layer was removed by suction and an aliquot of the bottom chloroform layer was taken for fatty acid analyses.

The total lipids were dried under nitrogen and methylated using a mixture of boron trifluoride methanol (Metcalfe et al., 1961). Fatty acid methyl esters were separated and quantified by an automated gas choramotography equipped with autosampler and flame ionisation detector[4], using a 30 m x .25 mm inside diameter fused silica capillary column[5]. The initial column temperature was set at 50 C for 1 min, increased to 170 C at 30 C/min, and held for 8 min. Next the column temperature was elevated to 230 C at temperature of 20 C/min and held at the final temperature for 5 min. Helium was used as the carrier gas at a flow rate of 3.0 mL/min. The flame ionization detector was set at 240 C. A Shimadzu EZChrom[6] laboratory automation system was used to integrate peak areas.

The data for the effect of n-3 egg consumption on the changes in breast milk fatty acids were analysed by a paired t-test. The fatty acid composition of the infant formulas were analysed by one-way analyses of variance, using the General Linear Model (GLM) procedure (SAS, 1985). Significant differences among means were tested using Student-Neuman-Keuls

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[2] Sigma Chemical Co., St. Louis, MO 63178-9916

[3] Fisher Scientific Ltd., Ottawa, ON, K1G 4A9, Canada.

[4] Model 3400, Varian Assosciates, Inc., Sunnyvale, CA 94089.

[5] Supelco Canada Ltd., Oakville, ON, L6K 3V1, Canada.

[6] Shimadzu Scientific Instruments, Inc, Columbia, MD, 21046

multiple range test at the 5% probablity level (Steel and torrie, 1980).

7.1.3. Results

The n3-eggs contained 13.5% n-3 and 14.4% n-6 fatty acids. The total longer chain n-3 and n-6 fatty acids constituted 3.2 and 1.4% respectively. Thus, an average chicken egg could provide up to 690 mg of n-3 fatty acids with 165 mg being longer chain C20 or C22 n-3 fatty acids (Figure 7.1). The analysis of commercially available infant formulas showed a wide variation in fatty acid composition (Table 7.1). Saturated fatty acids appeared to be the major fatty acid varying from 47 to 61% of the total lipids and monounsaturated fatty acids from 14 to 36% ($P<.05$). Significant differences ($P<.05$) were also observed in the C18:2 n-6 (13.9 to 31%) and C18:3 (1.9 to 4.4%). No longer chain C20 or C22 fatty acids were observed. The total n-6 to n-3 ratio of the formulas were in the range of 6.9 to 7.6% respectively.

Figure 7.2. shows the fatty acid composition of breast milk total lipids. Addition of n-3 eggs did not result in any changes in the saturated or monounsaturated fatty acid content of breast milk. C16:0 was the major saturated fatty acid in the breast milk followed by C18:0. The total n-3 fatty acids at the end of the trial was 3.8% compared with 1.9% for the pre-test milk. The major n-3 fatty acid in the breast milk was C18:3 which comprised 2.5% compared with 1.6% for the pre-test milk. The proportions of C20 and C22 n-3 fatty acids constituted 1.3% with DHA being 0.6% compared with .3% for pre-test milk samples. Addition of n-eggs in the diet did not cause any reduction in the C20:4n-6 content. However, the total n-6 to n-3 ratio was significantly lower ($P<.05$) in the n3-egg consuming group (3.0 vs 6.7).

7.1.4. Discussion

The dietary requirement of n-6 and n-3 PUFA for normal biochemical and functional development of the CNS is an important, unresolved issue in infant nutrition. Western diets are high in total fat and saturated fats with a ratio of n-6 to n-3 PUFA of 10:1 to 25:1 (Simopoulos, 1991). Therefore, it could be assumed that infants fed breast milk are also receiving less n-3 PUFA. In the present study, we employed the n-3 fatty acid enriched chicken egg as an alternate vehicle for providing the much needed n-3 PUFA and for lowering the n-6 to n-3 ratio in the breast milk. Addition of two n3-eggs could provide up to 1.5 g of n-3 fatty acids with 325 mg being long chain n-3 fatty acids such as 20:5 n-3, 22:5 n-3 and 22:6 n-3. Furthermore, the modification of yolk fatty acid composition in the n-3 eggs led to reduction of n-6 to n-3 ratio to 1.07, when compared with those of supermarket eggs which are in the range of 15 to 20 (Sim et al., 1991; Simopoulos, 1992).

The present study demonstrates the dependency of milk n-3 fatty acids on maternal dietary fat composition and also the nutritional supremacy of human milk over infant formulas with respect to its C0 and C22 PUFA content. Although there is considerable information on the major fatty acids of human milk, information on the fatty acid composition of lipid class or the content of long chain PUFA is more limited. Human milk contains a variety of C20 and C22 PUFA, the levels of these fatty acids are influenced by dietary pattern. The content of n-3 fatty PUFA has been reported to be higher in Inuit women who consume relatively large amounts of marine mammal flesh compared with those eating western diet (Innis and Kuhnlein, 1988) and also in women consuming an omnivorous rather than a

vegetarian diet (Sanders and Dickerson, 1978).

The n-6 and n-3 fatty acid composition of the pre-test breast milk was similar to previous reports for women consuming mixed diets (Jensen et al., 1992). In comparison to the pre-test milk, the higher levels of n-3 fatty acids in the post-test milk lipids reflects the increase dietary intake of n-3 fatty acids. The dependency of milk fatty acids on dietary fats have been well documented (Harris et al., 1984; Innis and Kuhnlein, 1988; Sanders and Reddy, 1992). Henderson et al. (1992) reported that addition of Bio-EFA capsules containing 180 mg of 20:5 n-3 and 120 mg of C22:6 n-3 produced an increase in breast milk n-3 fatty to 2.3% with longer chain n-3 fatty acids such as C20:5 n-3, C22:5 n-3 and C22:6 n-3 fatty acids comprising 1.54% and an n-6 to n-3 ratio in the breast milk lipids of 6:1. The n3-egg used in the present study could provide 127 mg of C22:6 n-3 and 10 mg of C20:5 n-3 per medium sized egg and resulted in an enrichment of total n-3 fatty to 3.8% with longer chain n-3 fatty acids comprising 1.3% and an n-6 to n-3 ratio of 2.9. The addition of n-3 eggs did not affect the C20:4 n-6 fatty acids cor[illegible] of breast milk. Long term supplementation of marine oils in premature i[illegible]ants (Carlson et al., 1992) resulted in reduced blood phospholipid C20:4 n-6 concentrations and poorer growth in the infants given marine oils. Studies of piglets fed with formulas supplemented with fish oil rich in C20:5 n-3 were made by Arbuckle et al., (1992a) who reported a decrease in C20:4 n-6 in the liver and plasma phospholipids. Arachidonic acid (C20:4 n-6) is an important eicosanoid precursor and (Carlson et al., 1992) indicated a need for caution in using formula supplemented with fish oil. Similar decrease levels of C20:4 n-6 were also observed in rat pups when the dam's diet was

enriched with menhaden oil (Yeh et al., 1990).

The mean daily intake of breast milk by a one month old infant has been reported to be 794 mL (Boersma et al., 1991). Assuming the intake to be 794 mL, then infants nursed from women consuming n-3 eggs could have up to 280 mg of long chain n-3 fatty acids such as 20:5 n-3, 22:5 n-3 and 22:6 n-3. Thus it is clear from the present results that a diet supplemented with n-3 eggs should provide the necessary amount of DHA to the developing infant in a natural way through breast milk. These observed differences in the n-3 PUFA content of the breast milk could have implications for the development of the lipid structures of the central nervous system and possibly neurological development of the infant. Much more work in this direction is necessary.

Infant formulae do not contain C20 and C22 PUFA and they show wide variation in saturated and monounsaturated fatty acid content. Studies in piglets reported an impact of milk saturated fat composition on liver tissue C16:0 (Arbuckle, 1992b). The proportion of saturated fatty acids in the membrane lipids tends to be inversely related to membrane fluidity (Stubbs and Smith, 1984). If so, the effect of the wide variation in formula saturated fatty acids (47%-61%) compared with the breast milk saturated fatty acids (38%-39%) on the formula fed infant needs to be considered. Thus provision of a formula with fatty acid composition that ensures optimal structural-lipid accretion is of major importance. This optimal structural lipid accretion could be achieved by properly designing the formula with added longer chain PUFA in a manner and composition that would reflect the fatty acid composition of breast milk. Comparison of the fatty acid composition of n-3 fatty acid enriched egg yolk lipids with the

breast milk lipids shows similarities in saturated, monounsaturated as well as PUFA. Thus egg yolk lipids extracted from n-3 fatty acid enriched egg would seem ideal as a natural lipid alternative to be incorporated in formula preparations to achieve an improved fatty acid balance. Furthermore, n-3 eggs containing an abudance of amino acids, vitamins and minerals would be a useful supplemental or weaning food for infants.

7.2. Positional Distribution of Fatty Acids in the Breast Milk Phospholipids

7.2.1. Introduction

Although phospholipids of bovine milk have been studied extensively (Moore et al., 1968; Morrison et al., 1965) information on human milk phospholipids are limited. Phospholipids are a rich source of essential fatty acids. After hydrolysis by phospholipase A_2, phospholipids are absorbed by the enterocyte as 1-lysocompound. The position of essential fatty acids in phospholipid may be important because non-esterified essential fatty acids are the substrate for the synthesis of prostaglandins and hence phospholipase may be responsible for the controlled release of prostaglandin precursors. In the present study, we hypothesized that the increase in the n-3 content of breast milk observed (Chapter 7.1) will alter the positional distribution of essential fatty acids in the milk phospholipids. In addition to obtaining information on the positional distribution of essential fatty acids in breast milk phospholipids, the present study may also provide insights into ways of properly designing infant formulas.

7.2.2. Materials and methods

Lipid class separation

The lipid extract (Chapter 7.1) was concentrated under nitrogen at 40 C and was applied on to precoated silica gel G plates[2] (20 x 20 cm) that had been previously activated by heating at 70 C for 1 h. Lipid standards were mixed and applied aside the lipid extract. The plates were developed in chloroform/methanol/water/acetic acid (65:43:3:1). The plates were then air-dried, and sprayed with .1% (wt/vol) 2',7'-dichlorofluorescein in ethanol. The spots corresponding to phospholipids (PL) and triglycerides were identified under ultraviolet light, and were scraped off into screw-capped tubes. Triglycerides were methylated with boron trifluoride methanol. Phospholipids were extracted with 25 ml of chloroform/methanol (2:1, vol/vol), silica gel was removed by filtration and was washed by 10 ml of chloroform/methanol (2:1, vol/vol). The organic phases were pooled and the solvent was removed by drying under nitrogen.

Phospholipase A2 degradation of phospholipids

Phospholipids (5-7 mg) were dissolved in diethylether (5 ml) containing hexadecyltrimethylammonium bromide (20 mg); 10 mM 4-morpholinoethene-sulfonic acid (MES) buffer, pH 7.0, containing 1mM ethylenediamine-tetraacetic acid (EDTA) (1mL) and 100 units of phospholipase A2 (porcine pancreas; Sigma) and the mixture was shaken vigorously for 3 h at 37 C (Kendrick and Ratledge, 1992). The top layer was removed and washed with 15 ml of diethylether and dried under nitrogen. The residue (free fatty acids) was methylated with boron trifluoride methanol. The aqueous layer was mixed with ethanol (20 ml), the solvent was removed by drying under nitrogen and the residue of lysophospholipids were methylated with boron

trifluoride methanol (Metcalfe et al., 1961). The fatty acid methyl esters of sn-1 and sn-2 position phospholipids and the milk triglycerides were identified by comparison with authentic standards. The conditions of the gas chromatograph were as described earlier (Chap 7.1).

The data for the fatty acids of sn-1 and sn-2 position were compared by t-test. Comparison between sn-1, sn-2 and triglycerides fatty acids were by ANOVA (. Significant differences among means were dertermined using Student-Newman-Keul's Multiple Range test (Steel and Torrie, 1980).

7.2.3. Results

The results from the present study indicated significant differences between the distribution of fatty acids in the sn-1 and sn-2 position of milk phospholipids (Table 7.3). In sn-1 position, saturated fatty acids comprised 54.38%. Among the saturated fatty acids 16:0 was the major fatty acid followed by 18:0. The levels of shorter chain fatty acids (<12:0) were higher in the sn-2 position. Compared with sn-2 position, monounsaturated fatty acids were significantly ($P < .05$)lower in the sn-1 position. The levels of total polyunsaturates (n-6+n-3) as well as total longer (>20C) n-3 and n-6 fatty acids were significantly ($P < .05$) higher in the sn-1 position. The levels of DHA was 4.59 in the sn-1 position compared with 1.45 ($P < .05$) in the sn-2 position. Of interest was the significant increase in 20:4 n-6 and 20:5 n-3 in the sn-2 position. These fatty acids (20:4 n-6 and 20:5 n-3) are the precursor for prostaglandins. Thus the increased prevelance of these fatty acids may be of nutritional value to the infant.

Palmitic acid was the major saturated fatty acid and oleic acid was the major monounsaturated fatty acid in the milk TG. Linolenic acid was the

major n-3 fatty acid in the breast milk at 2.1%. Compared with the phospholipids, the TG fraction contained lesser amounts of longer (<C20) PUFA (Fig. 7.2.1).

7.2.4. Discussion

Milk lipids in addition to being the major energy source, supply fatty acids crucial to normal growth and development. About 98% of milk lipids is triglycerides, which are present in the core of the milk fat globule, and are held in solution in the aqueous milk environment by surface layer of phospholipids and constitutes 1 to 1.4 % of milk total lipids (Jensen, 1978; Morrisson and Smith, 1965). The phospholipid fatty acid composition has not been reported recently and those composition reported earlier indicated large variations probably due to analytical variations (Morrisson and Smith, 1965).

Under resting conditions, the phospholipids of membranes are thought to be stable (Gurr and James, 1986). However, physiological conditions such as rapid growth initiates 'phospholipid turnover.' Thus in the new born, the turnovers of fatty acids may increase dramatically in nervous tissue during early stages of life due to cellular proliferation. Therefore, during these critical periods of neural tissue development the fatty acid pattern of diet or breast milk could be 'tailored' to meet the requirements of the cell.

The levels of membrane longer chain n-3 and n-6 PUFA have been reported to be lower in infants fed on formulas compared with those fed breast milk (Innis, 1992; Carlson et al., 1992; Clandinin, 1989). The changes in the fatty acid composition of the milk phospholipids observed in the present study might have implications in the integrity of cellular

membranes of the central nervous system. Much work in this direction is necessary.

Considering the importance of n-3 and n-6 PUFA in the development of the central nervous system, the present study may have implications on the feeding of the new born. Human breast milk contains phopholipids rich in long chain n-3 and n-6 fatty acids. However, currently available infant formulas do not contain fatty acids above 18 carbons (Clandinin et al., 1989). The results presented here may have implications for the design and appropriate lipid composition of infant formulas.

Table 7.1.1

Fatty acid composition of infant formulas

Fatty Acid (%)	INFANT FORMULA 1	2	3	4	5	SEM
8:0	2.2^{c}	4.5^{a}	3.4^{b}	3.2^{b}	3.2^{b}	0.06
10:0	1.9^{c}	3.5^{a}	2.7^{b}	2.5^{b}	2.5^{b}	0.04
12:0	13.8^{d}	27.0^{a}	26.11^{a}	21.5^{b}	19.5^{c}	0.29
14:0	6.8^{c}	10.5^{a}	8.1^{b}	4.0^{d}	7.5^{bc}	0.07
16:0	15.0^{a}	11.2^{bc}	11.7^{b}	11.6^{b}	10.9^{c}	0.14
17:0	0.6^{a}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.01
18:0	7.6^{a}	4.1^{b}	4.5^{b}	4.5^{b}	4.2^{b}	0.07
16:1	1.3^{a}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.00
18:1	34.5^{a}	14.3^{c}	14.5^{c}	17.1^{b}	17.0^{b}	0.13
18:2 n-6	13.9^{c}	22.08^{b}	23.6^{b}	31.2^{a}	31.1^{a}	0.34
18:3 n-3	1.9^{d}	3.1^{c}	3.5^{c}	4.4^{a}	4.1^{b}	0.04
SAT	47.9^{b}	60.8^{a}	56.5^{a}	47.3^{b}	47.8^{b}	0.25
MUFA	35.8^{a}	14.3^{c}	14.5^{c}	17.1^{b}	17.0^{b}	0.13
Total n-6	13.9^{c}	22.1^{b}	23.6^{b}	31.2^{a}	31.1^{a}	0.34
Total n-3	1.9^{d}	3.1^{c}	3.5^{c}	4.4^{a}	4.1^{b}	0.04
n-6/n-3	7.3^{ab}	7.1^{b}	6.7^{c}	7.1^{b}	7.6^{a}	0.10

SAT = total saturated fatty acid, MUFA = total monounsaturated fatty acid.

$^{a-d}$ Means within the same row with no common superscripts are significantly different ($P<0.05$)

SEM = standard error of the mean

Figure 7.1. Total n-3 and total long chain n-3 fatty acid content (mg/gm) of yolk in n-3 polyunsaturated fatty acid enriched eggs versus regular supermarket eggs

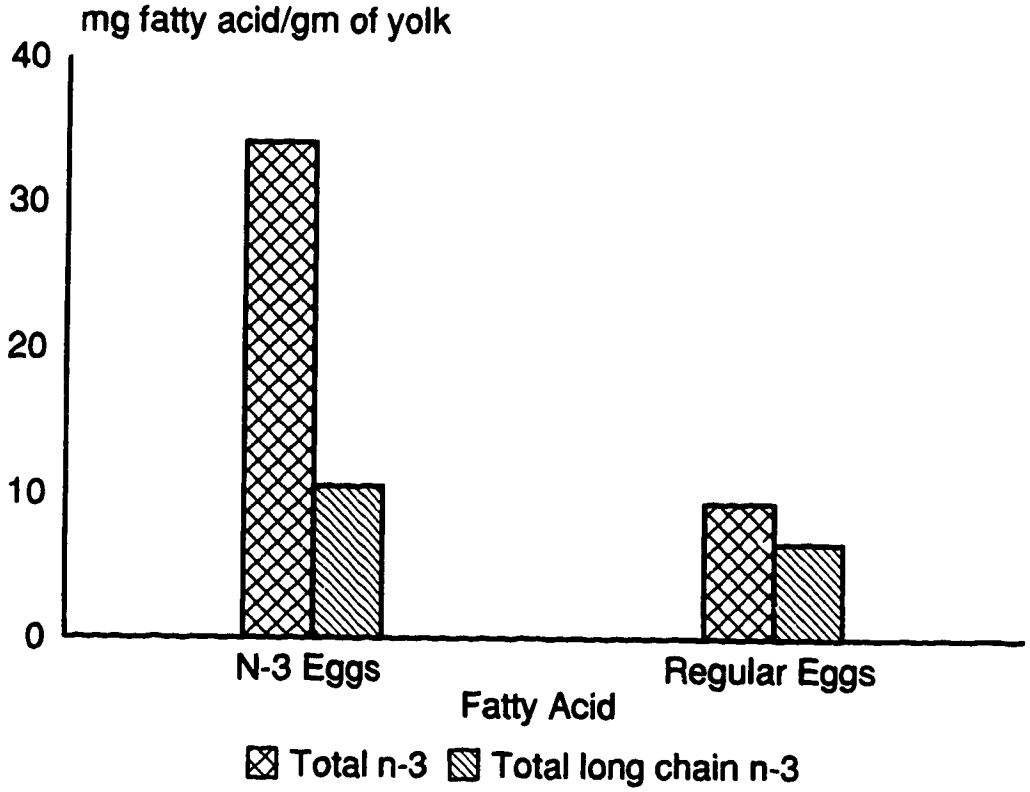

mg fatty acid/gm of yolk
40
30
20
10
0
N-3 Eggs
Regular Eggs
Fatty Acid
Total n-3
Total long chain n-3

Figure 7.2. Changes in the n-6 and n-3 fatty acid content of breast milk total lipids in women consuming n-3 fatty acid enriched eggs (2 per day) before and after six weeks. * indicates significantly different ($P<.05$). n=8

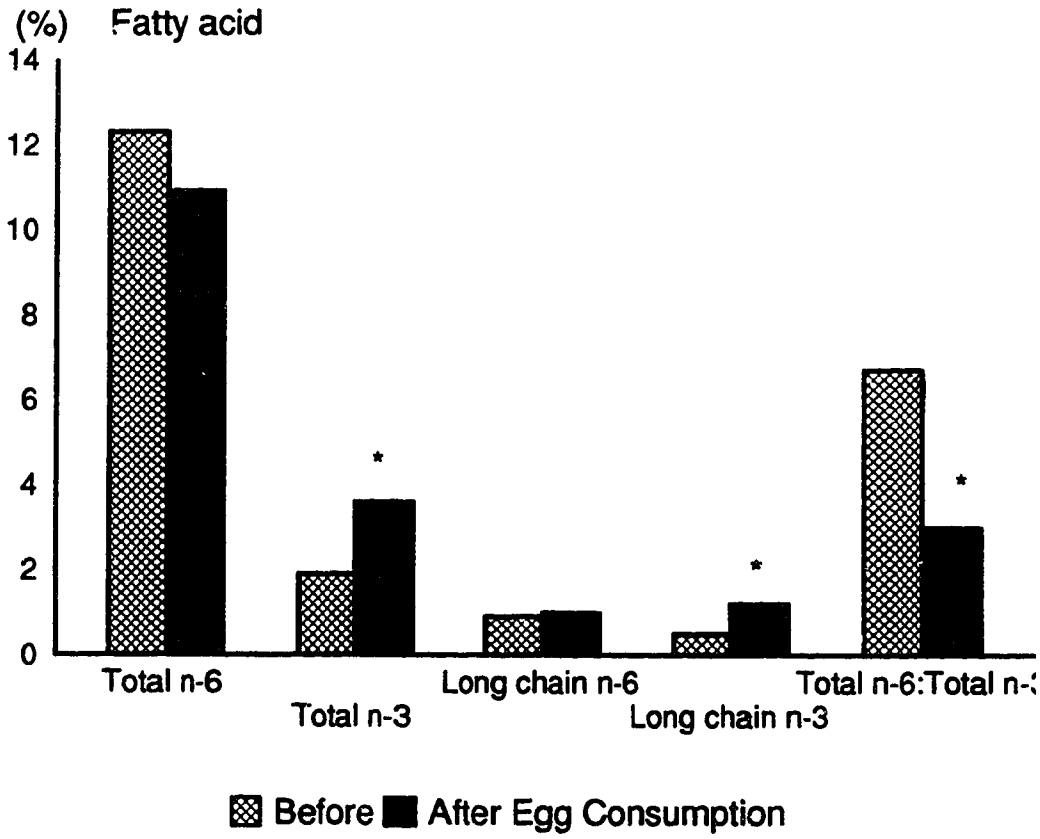
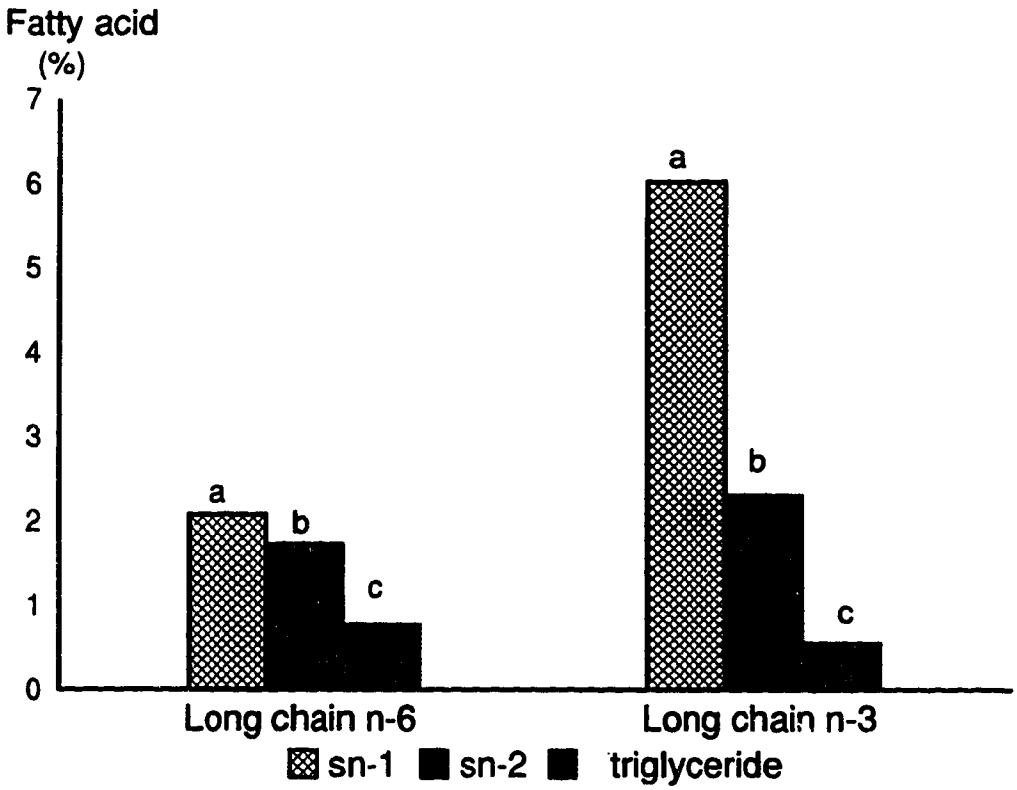

(%)
Fatty acid
14
12
10
8
6
4
2
0
*
*
*
Total n-6
Total n-3
Long chain n-6
Long chain n-3
Total n-6:Total n-3
Before
After Egg Consumption

Table 7.2 Positional distribution of fatty acids in the human breast milk phospholipids.

Fatty Acid[1]	sn-1 Position	sn-2 Position
4:0	0.00 ± 0.0	0.25 ± 0.1*
6:0	0.23 ± 0.7	0.24 ± 0.1
8:0	0.00 ± 0.0	0.22 ± 0.1*
10:0	0.00 ± 0.0	1.04 ± 0.1*
12:0	0.36 ± 0.4	3.91 ± 0.2*
14:0	1.33 ± 0.6	5.13 ± 0.3*
16:0	26.52 ± 2.3	23.16 ± 1.0
18:0	19.51 ± 1.1	10.73 ± 0.5*
20:0	2.56 ± 0.5	0.83 ± 0.1*
22:0	3.87 ± 0.7	0.67 ± 0.01*
14:1	0.00 ± 0.0	0.27 ± 0.1*
16:1	0.34 ± 0.3	1.83 ± 0.1*
18:1 n-9	15.04 ± 3.1	31.58 ± 1.9*
18:1 n-7	5.12 ± 2.4	2.25 ± 0.2
20:1 n-9	0.00 ± 0.0	0.85 ± 0.1*
20:1 n-12	2.56 ± 0.5	0.86 ± 0.1*
18:2 n-6	15.22 ± 1.1	9.22 ± 0.7*
20:3 n-6	0.13 ± 0.1	0.33 ± 0.1
20:4 n-6	.15 ± 0.1	1.15 ± 0.1*
22:2 n-6	0.00 ± 0.0	0.02 ± 0.0
22:4 n-6	3.87 ± 0.7	0.67 ± 0.1*
18:3 n-3	1.19 ± 0.5	2.02 ± 0.3
20:4 n-3	0.45 ± 0.5	0.35 ± 0.2
20:5 n-3	0.81 ± 0.4	0.78 ± 0.2
22:2 n-3	0.60 ± 0.4	0.39 ± 0.1
22:5 n-3	0.67 ± 0.7	0.19 ± 0.1
22:6 n-3	4.59 ± 0.4	1.45 ± 0.4*
Total SAT	54.38 ± 2.3	46.22 ± 1.8*
Total MUFA	21.11 ± 2.0	38.52 ± 1.6*
Total n-6	19.37 ± 1.3	11.39 ± 0.6*
Total n-3	7.21 ± 0.9	4.31 ± 0.6*
Total LC n-6	2.07 ± 0.8	1.72 ± 0.3
Total LC n-3	6.01 ± 0.8	2.29 ± 0.5*
Total PUFA	24.50 ± 1.7	15.26 ± 0.7*

SAT = saturated fatty acid: MUFA = monounsaturated fatty acid: LCn-6, longer chain n-6; LC n-3= longer chain n-3; PUFA = polyunsaturated fatty acid.
Data presented as Means ± S.E
*indicates significantly different (P<.05)


Fatty acid
(%)
7
6
5
4
3
2
1
0
a
b
c
a
b
c
Long chain n-6
Long chain n-3
sn-1
sn-2
triglyceride

7.5. References

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Chapter 8. General Discussion and Conclusion

Maternal\fetal\neonatal nutrition with respect to long chain n-3 polyunsaturated fatty acid (PUFA) has been studied extensively in mammals such as the rat or primates (Walker, 1967; Samulski and Walker, 1982; Connor et al., 1990). Nevertheless, the origin of long chain PUFA in the developing embryo is not clearly understood. In mammals, the developing fetus is continously nourished by the lipids of maternal circulation through the placenta. Therefore, the placenta may selectively transfer, or convert fatty acids of maternal origin making it difficult to understand the role of maternal diet in the n-3 PUFA nutrition of the progeny.

Considerable similarity exists in mammalian and avian species in the overall relationship between PUFA supplied by the mother and those accumulated by the developing embryos (Noble and Cocchi, 1989). In oviparous species the developing embryo is absolutely dependent on nutrients stored in the egg yolk. Once a fertilized egg is incubated, lipid rich yolk is the only supply of fatty acids for energy and structural membranes of the developing embryo. Thus this biologically self 'contained' model allows a close correlation between nutritive substances and their physiological utilization.

Poultry are monogastrics and as such, tissue fatty acid composition is determined by dietary fatty acid composition. The possibility that chicken egg yolk lipids could be altered by dietary fatty acid prompted the usage of the avian model (hen, egg and the hatched chick) as a research tool in studying the n-3 PUFA metabolism in the maternal\fetal\ neonatal system.

As an n-3 fatty acid enriched egg is incubated, the developing embryo is in a self 'contained' n-3 fatty acid rich environment. Therefore, this model could be used to study the net transfer of n-3 fatty acids, and accumulation or conversion of longer chain n-3 fatty acids by the developing chick embryo.

In Canada, flax (Linum usitatissium) and canola (Brassica napus) cultivars are major export oil crops. The mature seeds are high in fat (41-43%) and protein (21-23%) and α-linolenic acid (flax oil, 48-58%; canola oil, 10-12%) (Sim, 1990). When incorporated into poultry diets, they serve as a source of energy, protein and omega-3 fatty acids.

The first chapter of the thesis presented the evaluation of the potential of using full fat flax or canola seeds rich in LNA to enrich the egg yolk lipids with n-3 fatty acids and investigating the role of yolk n-3 fatty acids (maternal sources) on the fatty acid composition of the developing chick. The efficacy of LNA in raising the level of long chain metabolites of LNA such as EPA, DPA, and DHA has been questioned in both human (Dyerberg et al., 1980) and animal studies (Anderson et al., 1989). In studies reported in chickens and pigs, the long chain n-3 PUFA in tissues and egg yolk increase when LNA is fed (Phetteplace and Watkins, 1989; Farrell and Gibson, 1990; Ajuyah et al., 1991). Results from Chapter 2 demonstrated that 18:3 n-3 in the laying hen diet (maternal diet) enriched the egg lipids with n-3 fatty acids. The major n-3 fatty acid in the egg is LNA, however, the longer chain metabolites such as EPA, DPA and DHA are higher in the eggs from hens fed flax or canola seed indicating the laying hen can convert LNA to its elongated metabolites and deposit these in the egg yolk. Chicks hatched from n-3 PUFA enriched eggs

incorporated higher DHA in to brain tissue with a concomitant reduction in arachidonic acid compared with chicks from hens fed wheat-soy based control diet. The increase in brain DHA observed could be a selective transfer from yolk DHA, or conversion of yolk LNA.

In view of the importance of n-3 fatty acids during brain development, it was important to assess the net synthesis or transfer of these fatty acids or their precursor for optimum accretion. Therefore, in the experiment described in Chapter 3, we investigated the net transfer of yolk n-3 fatty acids to the embryo during development. We observed an adaptive mechanism of the developing chick embryo for maximum utilization of n-6 and n-3 fatty acids. The presence of C22:4 n-6, C22:5 n-6, C20:5 n-3, and C22:5 n-3 in the progeny were higher than their original amount in the yolk lipids. This indicates that the requirement for these fatty acids are not satisfied by yolk lipids. These fatty acids are produced by chain elongation and desaturation of 18:2 n-6 and 18:3 n-3 (Dyerberg, 1986). This indicates that the developing embryos are not only sequestering the preformed long chain metabolites, but are converting yolk LA or LNA to these long chain PUFA to meet the higher requirement of C20 and C22 PUFA during cell division and growth. Upon examining the lipid class composition of the brain and liver tissue, we observed a preferential incorporation of long chain n-3 fatty acids (20:5 n-3, 22:5 n-3 and 22:6 n-3) in the phosphotidyl ethanolamine fraction of the brain tissue. In contrast, no preferential incorporation of DHA in the PE fraction of liver tissue was observed. The liver n-3 fatty acids were evenly distributed in the PC and PE (Chapter 4). After 21 days of incubation, of the various fatty acids present in the yolk sac, C20:4 n-6

and C22:6 n-3 concentrations decreased. This suggests that the developing embryos preferentially utilized these fatty acids during development. Further, the increased presence of C20 and C22 PUFA (Chapter 3) prompted us to examine the Δ-6 desaturation activity in the liver tissue of hatched chicks. Results demonstrated lower Δ-6 desaturase activity and conversion of 18:2 n-6 to 18:3 n-6 in the liver tissue of chicks hatched from n-3 PUFA enriched eggs. This reduction in the enzyme activity was reflected in the reduced arachidonic acid content in the liver and brain tissue of n-3 PUFA chicks. The results from this experiment suggests the role of chick liver in providing DHA to the developing chick brain. Results from chapters (2-5) demonstrated the role of maternal fat (yolk fatty acids) in modulating the n-3 PUFA metabolism in the brain tissue of the developing chick.

Further, using the avian model we investigated the role of maternal dietary fat in modulating the cholesterol metabolism of the chick. Results from this study (Chapter 6) demonstrated that chicks hatched from n-3 PUFA enriched eggs reduced plasma and liver cholesterol compared with n-6 fatty acid enriched eggs which reduced the plasma cholesterol with an accumulation of cholesterol in the liver. Therefore, the type of fat fed to laying hens can affect the fatty acid composition of the yolk and also the fatty acid composition and the cholesterol content of the progeny (Cherian and Sim, 1992).

Pioneering studies by Lamptey and Walker (1976), Clandinin et al., (1980) and Crawford et al., (1981) have reported the important roles of C20 and C22 PUFA in the development of the central nervous system of the human infant. Research by Jensen et al., (1978), Innis (1992) and Carlson

et al., (1986, 1992) has added considerably to the knowledge of infant formula fatty acids as well as vegetable and marine oils in modulating the plasma fatty acids of new born.

We evaluated the n-3 polyunsaturated fatty acid content of infant formulas in the commercial market and our results showed an absence of C20 and C22 PUFA and a wide variation in formula saturated fatty acids (47%-61%). As our results from Chapters 2,3,4, and 5 indicated the role of yolk lipids in modifying the n-3 PUFA composition of the chick brain tissue, we then proceeded to use the n-3 fatty acid enriched egg as a vehicle for delivering these essential nutrients to the newborn. Consumption of two medium sized eggs for a period of six weeks resulted in an enrichment of total n-3 fatty to 3.8% with longer chain n-3 fatty acids comprising to 1.3% and an n-6 to n-3 ratio of 2.9. Consuming n-3 eggs did not change the C20:4 n-6 fatty acids content of breast milk. It thus demonstrated that a diet supplemented with n-3 fatty acid enriched egg would be an alternate way of providing longer chain n-3 fatty acids to the developing infant in a natural way through breast milk. Further, we investigated the distribution of fatty acid in the breast milk fatty acids and the study revealed a predominance of saturated fatty acids and DHA in sn-1 position and arachidonic acid in sn-2 position. The results presented here may have implications for the proper design and appropriate lipid composition of infant formulas.

Therefore, the chicken egg yolk with proper manipulation of the hen's diet could be an excellent dietary source of n-3 essential fatty acids. Further, the lipids extracted from n-3 fatty acid enriched egg would seem ideal as a natural lipid alternative to be incorporated in formula

preparations to achieve an improved fatty acid composition that would reflect the fatty acid composition of breast milk. However, further research is needed to investigate the optimum levels of n-3 fatty acid enriched egg yolk lipids which could ensure an optimal structural-lipid accretion in the formula fed infant.

8.1. References

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