THE USE OF THE AVIAN MODEL TO STUDY THE IMPACT OF MATERNAL DIETARY $\omega$-3 FATTY ACIDS COMPOSITION ON THE BRAIN, HEART AND SPLEEN C22:6$\omega$3 STATUS OF GROWING BROILER CHICKENS

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Summary

Broiler breeder hens were fed three diets that were low, moderate or high in $\omega$3 fatty acids. The diets were based on the addition of 50 g/kg sunflower oil (diet1), 25 g/kg each of sunflower oil and fish oil (diet2), and 50 g/kg fish oil (diet3) to a wheat-soybean meal basal diet. Samples of brain, heart and spleen were taken at hatch and at 2 weeks of age from broiler chicks hatched from eggs produced by these hens. The trend for C22:6$\omega$3 accretion were brain > heart > spleen at hatch and brain > spleen > heart at 2 weeks of age and diet 3 > diet 2 > diet 1 ($P<0.05$). In contrast, the trends for C20:5$\omega$3 were spleen > heart > brain at hatch, and heart > spleen > brain at 2 weeks of age, but again diet3 > diet2 > diet1 ($P<0.05$). The pattern of incorporation of C22:6$\omega$3 and C20:5$\omega$3 reflected essentiality (structural and energetic) and maternal dietary composition. The practical implications for these observations are related to the role of these fatty acids in brain development, cardiovascular disease and immune response.

I. INTRODUCTION

In recent years there has been an upsurge in the use of the avian and animal models to study metabolic and physiological events that could be of benefit to humans and commercial livestock and poultry producers. An example is the study of fatty acid (FA) synthesis, accretion and utilization during embryonic or fetal development and growth. Most of these studies have provided additional information on the nutritional essentiality and importance of $\omega$3-FA (Noble and Sand, 1985; Bowen and Clandinin, 2000). The $\omega$3-FA, in particular docosahexaenoic acid (DHA, C22:6$\omega$3) and the precursors of eicosapentaenoic acid (EPA, C20:5$\omega$3) have been implicated with the prevention of vascular diseases, platelet function, inflammation, immunology, visual process, mental acuity, reproductive function and tumor inhibition in humans and animals (Sim, 1998; Cunnane et al., 2000).

The usefulness of the avian model encompasses the following: (i) conducting experiments/ clinical procedures that cannot be performed using humans, (ii) short generation interval, (iii) multi-generation studies, (iv) self-contained nutritional environment of the egg, (v) low experimental cost, and (vi) anatomical and physiological similarities between the avian and mammalian species in the utilization and requirement for long chain polyunsaturated fatty acids (Cherian et al., 1997; Innis, 2000).

Therefore based on the avian model, this research was designed to investigate pre and post maternal dietary effects on the accretion of EPA and DHA into the brain, heart and spleen of growing broiler chickens.

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II. MATERIALS AND METHODS

This study was part of a larger study wherein fertile eggs were collected over a one-week period from artificially inseminated broiler breeder hens that were fed ad-libitum three experimental diets. The diets were: a wheat-soybean basal diet containing 50 g/kg sunflower oil, 25 g/kg sunflower oil plus 25 g/kg fish oil, and 50 g/kg fish oil as diets 1, 2 and 3 respectively. The eggs were incubated and one hundred newly hatched chicks from each treatment were distributed to floor pens and offered the same commercial broiler starter and finisher diets from hatch to 6 weeks of age (to delineate maternal dietary fatty acids residuum). Brain, heart and spleen tissues were collected at 0 (hatch), 2, 4 and 6 weeks of age from 5 broiler chicks per treatment which were killed by cervical decapitation, and all the samples were stored at −20°C for future chemical analysis. All the tissue, feed and egg yolk samples were analyzed for fatty acid composition, using the direct methylation method described by Wang et al. (2000). The data were all analyzed by ANOVA, using the general linear model procedure of SAS. The remaining chickens were used to study maternal effects on selected economic and physical traits, including mortality from sudden death syndrome (data not shown).

III. RESULTS

The values for total ω6-FA composition (%) of the maternal dietary fatty acids were 65, 42 and 19, and for total ω3-FA 2, 10 and 19 for diets 1, 2 and 3 respectively. The major ω3-FA were EPA and DHA for diets 2 and 3, and C18:3ω3 for diet 1. The commercial broiler starter and finisher diets contained similar proportions of total ω6-FA (~28%) mainly C18:2ω6, and total ω3-FA or C18:3ω3 (~7%) from canola oil. Total ω6-FA concentrations in the fertile eggs (mg/g yolk) were 73, 50 and 23, and total ω3-FA were 3, 18 and 26 for diets 1, 2 and 3 respectively. The most abundant ω6 and ω3-FA in all the egg yolks were C18:2ω6 and C22:6ω3, in addition to which, eggs from hens given diet 1 now contained other ω3-FA metabolites (C22:5ω3 and C22:6ω3). The DHA composition of the brain, spleen and heart at 0 and 2 weeks of age is shown in Figures 1 and 2. The trend for the organs were brain > heart > spleen at hatch, and brain > spleen > heart at 2 weeks of age. Differences between treatments were significant (P<0.05), with diet3 > diet2 > diet1 at both ages (P<0.05). Regarding EPA the trend was spleen > heart > brain at hatch and heart > spleen > brain at two weeks of age and the differences between treatments were significant (P<0.05), with diet3 > diet2 > diet1 at both ages (Figures 3 and 4).

![Figure 1. C22:6ω3 FA concentration of the brain, heart and spleen (mg/g) from newly hatched broiler chickens. **Columns with different superscripts are significant different at P<0.05. SO = Sunflower Oil FO = Fish Oil](Image)

![Figure 2. C22:6ω3 FA concentration of the brain, heart and spleen (mg/g) from two weeks old broiler chickens. **Columns with different superscripts are significantly different at P<0.05 SO = Sunflower Oil FO = Fish Oil](Image)
IV. DISCUSSION

The egg as the only source of nutrients for the developing embryo, excluded other extraneous nutrient sources from invalidating the use of the avian model in this study. However, there were parallels between maternal dietary fatty acids composition with that of the fertile eggs and all the selected organs from the newly hatched chicks. The enrichment of the hen’s egg with nutritionally desirable and function-specific fatty acids is a well-established concept, and this observation further confirms previous studies (Cherian et al., 1997). The high concentration of DHA in the brain at 0 and 2 weeks of age, and wide variations between the brain, heart and spleen indicate the major structural role of DHA as opposed to an energetic role in the heart and spleen. The wide variation observed between treatments for brain DHA concentration is contrary to the observation of Crawford et al. (1989), who observed similar DHA levels across mammalian species despite large dietary variations. The large variation in brain DHA observed from our study was directly related to the wide ω6:ω3-FA ratio of the maternal diets. Post hatch resumption of DHA in the chick’s brain up to 2 weeks of age underpins the importance of maternal dietary DHA status for brain growth and development. It has earlier been reported that brain accretion of DHA in humans increases dramatically during the last trimester of pregnancy and plateaus 2 years postpartum (Martinez, 1991), this is because 30% of the structural lipid of the cerebral gray matter is DHA. The heart and spleen seem to have greater requirements for EPA compared to the brain, which might indicate essentiality or requirements for ecosanoids production. The progeny diets were totally deficient in C20:4n6, C20:5n3 and C22:6n3 because vegetable oil was the major fat source, therefore the chicks would have relied on their precursors (C18:2n6 and C18:3n3) for their production. Results show that at two weeks of age, chicks from DHA deficient parents incorporated EPA into the heart and spleen tissues, which indicates the ability of the chick to synthesise DHA from C18:3n3. In contrast, no EPA was incorporated into the brain, which further supports the essentiality or specificity for DHA in the brain.

In summary, the brain, heart and spleen are major organs of the body that are involved primarily in mental acuity, blood circulation and immunity respectively. Their activity and functions are greatly influenced by membrane fatty acids composition viz a viz membrane permeability, which in turn is dependent on dietary fatty acid composition and concentration. The manipulation of dietary fatty acids, especially maternal fatty acids, will invariably affect membrane fatty acid composition of the brain, heart and spleen of the developing fetus or embryo and inevitably modulate certain physiological functions postpartum. Therefore, dietary intervention, in particular with ω3 FA has been proposed by some researchers as a strategy to reduce the incidence of certain diseases, improve mental acuity of infants and
modulate immune responses to inflammation (Sim, 2000). Currently, major sources of \( \omega 3 \) FA in human and animal nutrition include fish and fish products (C20:5\( \omega 3 \) and C22:6\( \omega 3 \)), plant products - flax and canola oils (C18:3\( \omega 3 \)), and modified poultry and animal products containing C20:5\( \omega 3 \) and C22:6\( \omega 3 \).

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REFERENCES