EFFECT OF MATERNAL DIETARY FATS AND ANTIOXIDANTS ON GROWTH RATE AND BONE DEVELOPMENT OF COMMERICAL BROILERS

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ABSTRACT

The effect of maternal dietary fats on growth rate and bone development of commercial broilers was examined. Three hundred fifty female chicks were winged banded, weighed and equally divided among six starter pens (1.52 X 3.66m) with litter floors. At 20 wk of age, each pen was fed a basal laying diet supplemented with either 3% chicken fat (CF), soybean oil (SBO) or menhaden oil (MO). Each diet was provided with or without the antioxidant ethoxyquin, producing a total of six dietary treatments. Addition of fats [soybean (SBO), menhaden oil (MO), chicken fat (CF), soybean + antioxidant (SA), menhaden + antioxidant (MA), and chicken + antioxidant (CA)] to the maternal diet altered the tissue and yolk composition of hens to reflect the dietary source. Response variables measured were body weight, tibia weight and length, and breaking strength (stress, force, energy, bone wall, and diameter). Chick tissue from hens fed a MO and MA diet exhibited greater ($P<0.01$) amounts of DPA (22:5n3), DHA (22:6n3) and total n-3 fatty acids than the remaining dietary treatments. Tissues from chicks fed a SBO and SA diet displayed larger levels of 18:2n6 and total n-6 fatty acids when compared to all other treatments. Male and female chicks from the menhaden type diets (MO and MA) were lighter ($P<0.01$) during grow out period than from soybean (SBO and SA) and chicken (CF and CA) type diets. Chicks tibiae diameter from CF maternal diet tended to be larger than the MO maternal diet, with significance being noted at d 14 ($P<0.01$) and 28 ($P<0.01$). Increases were observed in shear force and stress required to break chick tibia from SBO maternal diet compared to those from the CF and MO maternal diets. The SBO maternal diet stimulates growth rate and bone development and strength of the progeny.

(Key words: chickens, bone development, breaking strength, growth rate, fatty acids)

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TABLE OF CONTENTS

LIST OF TABLES

INTRODUCTION

Non-infectious skeletal diseases cost the commercial broiler industry millions of dollars annually. While the cause has not been elucidated, perhaps the most extensively studied area of skeletal abnormalities is the link between bone development and nutrition. For example, high levels of vitamin D in maternal and chick diets can cause a greater incidence of leg abnormalities, and elevated vitamin A can interfere with vitamin D absorption and utilization (Hargis, 1992).

The most practical method of increasing the metabolized energy (ME) of poultry diets is by the addition of fat. Fat supplies more metabolized energy per unit weight than any other ingredient in poultry diets. However, not all fats are equally utilized by poultry. The energy value of fat in diets is influenced by its chemical structure which affects fat digestion and absorption. Menhaden oils, rich in polyunsaturated fatty acids (PUFA), is highly digestible for chickens and represents a traditional fat sources in broiler diets (Engberg *et al.*,1996). However, PUFA's are highly susceptible to oxidation during storage, leading to an interest in determining fat quality for diet supplementation.

Recently, more fat has been added to commercial broiler diets to increase energy density. The addition of 5% poultry fat to broiler breeder diets has been reported to increase egg production (Brake *et al.*, 1989). Changes in the lipid fatty acid composition of the maternal diet are reflected in the fatty acid composition of the yolk. All the fatty acids found in the yolk are formed by the liver of the hen and deposited prior to oviposition. Since the egg is nutritionally isolated after oviposition, the hen has an important role in the nutrition of the developing embryo.

Denbow (1994) performed an experiment in which he used three different supplemental oils; menhaden (MO), soybean oil (SBO), and chicken fat (CF). While the focus of that study was on the effects of maternal fats on embryonic mortality, it was also observed that bones growth of chicks may be affected by maternal dietary lipid composition. At 4 weeks of age, chicks from hen fed MO and SBO diets exhibited larger body size, tibiae weight, and bone strength (as measured by breaking force, energy and stress) when compared to chicks fed CF diet. The present study was designed to focus on the relationship of dietary fat on bone growth, development, and tibia strength.

REVIEW OF LITERATURE

Yolk and Lipid Composition

There are two components of egg yolks: white and yellow. White yolk consist of approximately two-thirds protein and one-third fat, whereas yellow yolk consist of two-thirds fat and one-third protein and accounts for 98% of total yolk (Schjeide *et al.*, 1963). The white yolk lies beneath the germinal disc in alternating concentric rings with yellow yolk. Since the egg yolk contains most all the lipids found in the egg, lipid deposition must occur during yolk maturation and is not influenced by fertilization or transport through the oviduct (Noble and Cocchi,1990).

The yolk is initially bound by a four-layer vitelline membrane which is laid down during yolk maturation as it travels through the oviduct. The vitelline membrane provides mechanical strength but is permeable to water and mineral salts (King and McLelland, 1984).

The overall lipid to protein ratio in the egg is 2:1. The yolk of the average chicken egg contain 6g of lipid mainly in the form of triacylglyerols, phospholipids (such as phosphatidylcholine and phosphatidylethanolamine) and free cholesterol (Noble and Cocchi, 1989; 1990). Minor yolk components includes cholesteryl esters and free fatty acids. Each lipid component has a unique fatty acid profile. The phospholipid component exhibits characteristic levels of linoleic (18:2n6), arachidonic (20:4n6) and docoshexaenoic acid (DHA, 22:6n3) as well as other polyunsaturated fatty acids (Noble and Cocchi, 1990).

In the avian liver, extensive reprocessing of glycerides and fatty acid residues from the portomicrons remnants occurs. The modified lipid is then reformed and included in particles of very-low density lipoprotein (VLDL) which then pass to the Golgi complex where they acquire phospholipids and further glycosylation. Completed particles of VLDL are finally concentrated in secretory vesicles, and then discharge into the blood (Bensadoun and Rothfeld, 1972).

VLDL particles are then carried into the blood to the ovarian follicles where they diffuse through holes in the capillaries. The basal lamina offers some resistence and particles of VLDL accumulate in the layer. Evan *et al*. (1979) reported that basal lamina filters out all particles larger than the VLDL of laying birds. Once in the blood the particles enter the yolk by receptor-mediated endocytosis through the oolemma (Perry *et al.,* 1984).

Dietary Effects on Endogenous Fatty Acid Composition

Many studies have addressed changes in long chain polyunsaturated fatty acid (PUFA) metabolism in maternal-filial systems (Cherian and Sim, 1993). Once a fertilized egg is incubated, the constituents of the yolk are the sole supply of nutrients for the developing embryo. Therefore, it is possible to determine the net movement of n-3 or n-6 PUFA from the yolk to the developing embryo and quantify the changes in the lipid composition of the progeny. Studies indicate that the presence of n-3 or n-6 fatty acids in the laying hen diet can enrich the egg yolk lipids and the tissue of the chick (Cherian and Sim, 1992).

There have been attempts to increase the n-3 fatty acid content of poultry by supplementation of poultry diets with oils rich in n-3 fatty acids (Chanmugan *et al.*, 1992). Birds supplemented with linseed oil, rich in linolenic acid (C18:3n3), had significantly higher levels of n-3 fatty acids and higher n-3:n-6 ratios than those supplemented with the same level of menhaden oil, which is high in C18:3n3. Levels of eicosapentaenoic acid (C20:5n3) were increased in the group fed linseed oil or menhaden oil compared to those fed corn oil.

Although desaturase activities regulate tissue concentrations of fatty acids, especially for PUFA, dietary lipid can dictate fatty acid composition in poultry. Varying the type and amount of dietary unsaturated fat dramatically modifies the fatty acid composition of lipids in the hen yolk and in the tissues of growing chicks (Watkins, 1991). Feeding linseed oil, which is rich in α-linolenic acid, to chicks depresses the amount of arachidonic acid but concomitantly raises levels of eicosapentaenoic acid in organ lipids presumably by enhancing (n-3) PUFA formation. The long chain PUFA (especially eicosapentaenoic and docosahexaenoic acids) present in fish oils are extremely effective in lowering total (n-6) PUFA in chick liver and in depressing VLDL production rates in roosters (Phetteplace and Watkins, 1990).

Phetteplace and Watkins (1989) reported that high levels of 18:3n3 (linseed oil) in chicken formed 20:5n3 fatty acid, and that the conversion of 18:2n6 to 20:4n6 was decreased. High levels of 18:2n6 found in soybean oil increased the rate at which 20:4n6 were produced. Simopoulos (1988) found that liver tissue of chicks fed linseed oil displayed high levels of 18:3n3, 20:5n3, 225n3, and 22:6n3 fatty acids. Chickens fed menhaden oil had a decrease in the amount of 20:4n6 compared to the chicks fed soybean oil and chicken fat.

Maternal Dietary Influence on Egg Yolk Lipid Composition

Fatty acids destined from the yolk are synthesized in the hen's liver, permitting manipulation of fatty acid components through dietary measures (Cherian *et al.,* 1996). Cruickshank (1934) investigated the effects of degree of dietary fatty acid saturation on egg fatty acid composition. Eggs were collected and analyzed for fatty acid composition as determined by iodine value (degree of unsaturation). Hens fed hemp oil which is unsaturated produced eggs with iodine values of 124 and 126 compared with the control values of 84 to 88 found in eggs from birds fed a commercial mash diet. The iodine value of eggs from hens fed satrurated mutton fat was not different from that of the controls. Machlin *et al*. (1962) fed White Leghorns diets containing 15% safflower or hydrogenated coconut oil for a 12-wk period. Eggs from hens fed hydrogenated coconut oil contained significant quantities of lauric (12:0) and myristic (14:0) acids and significantly less 20:4n6 acid than hens fed safflower oil. Furthermore, egg yolks were enriched with n-6 or n-3 PUFA by incorporation of fats rich in these respective essential fatty acids (Cherian and Sim, 1993).

A maternal diet influence has also been reported for turkeys (Couch *et al*., 1974; Vilchez *et al.,* 1990). Couch *et al.* (1974) divided Beltsville White turkey breeder hens into five groups which received either a fat free, 3% SBO, 30% SBO, 3% neat's foot oil, or a control diet (2.26% total fats). Hens fed the fat-free diet laid eggs with the lowest stearic (18:0) acid level, whereas hens fed 30% SBO laid eggs with significantly higher levels. The opposite effect was seen with oleic (18:1n9) acid.

Vilchez *et al.* (1990) fed medium-sized turkey breeder hen diets containing no fat, 5% animal-vegetable (AV) fat, 5% corn oil (CO) or 5% olive oil (OO) for a 20 wk period. Hens fed the AV or CO diets had significantly higher plasma levels of 14:0 and 18:0 fatty acids than the controls. Hens fed the OO diet had significantly higher plasma 18:1n9 acid levels than the remaining treatments. Likewise, hens fed the CO diet had significantly higher 18:2n6 acid in plasma. Egg yolk fatty acid composition of birds fed the OO diet contained significantly higher 18:1n9 acid when compared with the remaining treatments. In contrast, yolk from hens fed the CO diet had higher levels of 18:0, 18:2n6 and 20:4n6 acids.

Fatty Acid Metabolism

Lipid metabolism is an important aspect of chick embryonic development because avian embryos derive over 90% of their caloric requirement from fatty acid oxidation (Donaldson, 1981). The embryo requires fatty acids to synthesize phospholipids for membrane formation, and for synthesis of triglycerides for energy storage (Donaldson, 1981). These properties make lipids a most efficient resevoir of energy.

Fat can represent 15-20% of the total body weight of broilers (Leveille *et al*., 1981). According to Evans (1977), greater than of 85% of the total body fat functions as an energy supply, and is stored in adipose tissue. Therefore, only 15% of the fat found in the body, or 2 to 3% of the total body weight, are used for functions other than energy storage.

Upon absorption from the intestinal lumen, hydrolyzed products of lipid digestion including long chain fatty acids and monoacylglycerols must be re-esterfied within the endoplasmic reticulum of the enterocytes prior to transport. The resultant triglycerides are packaged with cholesterol, phospholipids, and protein to form lipoproteins. In mammals, these lipoproteins are referred to as chylomicrons because they are transported within the lymphatic system (Bensadoun and Rothfeld, 1972). However, in poultry these lipoproteins are referred to as portomicrons, because they are transferred to the hepatic portal circulation (Bensadoun and Rothfeld, 1972). On the other hand, short chain fatty acids (<12 carbons) and free glycerol are transported directly to the liver via the portal system in both poultry and mammals.

Fatty acids are transported as triglycerides in very low density lipoproteins (VLDL) to adipose tissue storage sites (Leclercq *et al.*, 1974). Once the VLDL's released by the liver reach the target tissue, lipoprotein lipase hydrolyzes them for free fatty acid uptake by the cell. After hydrolyzation, most of the VLDL's are converted to low density lipoprotein (LDL). It is estimated that 50% of the LDL are eventually degraded by the liver and extrahepatic tissues (Leveille et al., 1979).

The parent compound of n-3 fatty acid is linolenic acid (18:3n3). This acid is converted in both mammalian and avian species by a delta 6 desaturase to 18:4n3. Desaturase enzymes remove a hydrogen thereby forming a carbon to carbon double bond in the backbone chain. The 20:5n3 serves as a precursor for series 3 prostanoids through the cyclooxygenase pathway and series 5 leukotrienes through the lipoxygenase pathway; it can also be desatuated (delta 4 - desaturase) to 22:6n3 (Simopoulos, 1988).

Metabolism of the n-6 fatty acids occurs via the same enzymes. The parent compound of the n-6 series, lenoleic acid (18:2n6), is desaturated to 18:3n6 by delta 6-desaturase. Elongation of γ-linolenic acid (18:3n6) produces 20:3n6; delta 5-desaturase converts the compound to arachidonic acid (20:4n6) (Ackerman, 1995). Arachidonic acid is further metabolized by either the cyclooxygenase or lipoxygenase pathway. The cyclooxygenase pathway converts arachidonic acid to prostaglandins (PGD_2, PGE_2, PGF_2, PGI_2) and thromboxanes (TxA_2). Lipoxygenase pathway converts arachidonic acid to leukotrienes (Smith, 1989).

There is competition between n-3 and n-6 fatty acids for all desaturase enzymes. It appears that n-3 fatty acid are preferred by delta 6-esaturase enzymes (Simopoulos, 1988). Inclusion of feed stuffs rich in n-3 fatty acids to the diet results in the replacement of n-6 with n-3 fatty acids in the cell membranes resulting in an increase of 20:5n3, PGI_3, TxA_3 and leukotriene B5 (Cahaner *et al.*, 1995). Conversely, diets high in 18:2n6 greatly increases the 20:4n6 content of tissue and therefore influences the production of prostaglandins and thromboxanes and decreases 20:5n3, PGI_3, TxA_3 and leukotriene B_5 (Smith, 1989). If diets contain appreciable amounts of both 18:2n6 and 18:3n3 acids, 18:3n3 is metabolized more readily than 18:2n6 (Simopoulos, 1988).

Effects of Menhaden Oil

In the poultry industry, menhaden oil has been an important ingredient of chicken and turkey rations for over 30 years. Menhaden oil (MO) is a rich dietary source of long chain n-3 PUFA, particularly 20:5n3, 22:5n3, and 22:6n3 acids. Marine oils have been shown to stimulate growth rates when used in conjunction with other fats, or when used alone (Dansky, 1961). Edward *et al* (1963), demonstrated that menhaden fish oil and safflower oil gave equal growth stimulation when added to a high protein diet (Dansky, 1961). The n-3 fatty acid content of broiler thigh muscle was increased by dietary supplementation with either linseed or menhaden oil (Chanmugam *et al.*, 1991).

Edwards and Marion (1963) studied the effect of MO on both growth rate and fatty acid composition of White Plymouth Rock cockerels. One group received a basal casein-gelatin diet supplemented with either 0% or 4% MO while the second group was fed a soybean protein diet supplemented with 0% or 4% MO. The addition of fat increased 4 wk BW regardless of diet. The fatty acid composition of the liver of birds supplemented with MO contained significantly elevated levels of 20:5n3, 22:6n3, and 22:5n3 acids. EPA and other n-3 PUFA levels in broilers

were enhanced as a result of feeding diets supplemented with menhaden oil (Marion and Woodroof, 1965; Edwards and May, 1995). Hulan *et al.* (1988) demonstrated that broiler chickens fed a diet containing 5.0% fish oil has substantial amounts of EPA, DHA, and other n-3 PUFA deposited in tissues. Fish oil added to diets of young turkeys consistently increased body weights. Potter (1980) reported there were factors responsible for the increase in growth rate, which were not present in water or ether extracts of fish meal but remained in the residues of these extractions.

Effects of Soybean Oil

Soybean oil contains large amounts of PUFA including 18:1n9, 18:2n6, 18:3n3, and 18:3n6 acids. These long chain PUFA have been shown to increase growth rate, lower feed intake and improve feed conversion (Atteh *et al.*, 1989; Scaife *et al*., 1994). Atteh *et al*. (1989) fed male broiler a basal diet with either 5% animal-vegetable blend (AV), SBO, canola oil (CAO) or canola soapstock (CS) from 0 to three wk. At three WOA, SBO-fed birds exhibited a higher BW (531.2g) than AV (527.2g), and CAO (502.8g) or CS (509.6g). Birds fed SBO also had lower feed intake and superior feed conversion when compared to other treatments. Scaife *et al.* (1994) fed 19-day of age broiler hens a basal diet supplemented with either 5% SBO or rapeseed oil (RSO) for a 5-wk period. At 54 d, SBO fed birds showed higher live weight gain when compared to the RSO fed birds.

Growth stimulation with fats has been obtained with practical broiler rations containing predominately corn meal and soybean meal. Including 7.5 % soybean oil in such a ration improved growth rate by approximately 8 percent over similar rations unsupplemented with fat (Skinner *et al.*, 1990). Watkins *et al.* (1996) reported that feeding soybean oil to chicks produced higher PGE_2 levels in bone and depressed bone formation rates when compared to feeding menhaden oil.

Bone Development

Bone formation and bone resorption are regulated by systemic hormones and locally produced factors within the skeleton. Among these factors are cytokines and growth factors such as interleukin-1 (IL-1), IL-6, epidermal growth factor (EGF), fibroblast growth factor (FGF), insulin-like growth factor (IGF-I and II), transforming growth factor-β (TGF-β), and eicosanoids (Watkins *et al.*, 1996). Eicosanoids are local modifiers of bone metabolism and include prostaglandins which are derivatives of 20-carbon carboxylic acids formed by the cyclo-

oxygenase pathway. Prostaglandin E_2 (PGE_2), derived from n-6 PUFA, causes resorption of bone mineral and the release of calcium *in vitro* (Klein and Raisz, 1970). Considerable clinical and experimental evidence has revealed that PG is a potent stimulator of bone formation (Marks and Miller, 1993). Recent studies on bone formation in chicks demonstrated that diets enriched with saturated fats or vitamin E stimulated bone formation (Xu *et al*., 1995). In addition, diets enriched with n-6 PUFA elevated *in vivo* bone PGE^2 production and lowered the rate of trabecular bone formation (Watkins *et. al.,* 1996).

Prostaglandin E^2 was reported to increase IGF-I transcript and polypeptide levels in rats calvaria cells (McCarthy *et al.,* 1991, Schmid *et al.* 1992) and stimulate the expression of mRNA for IGF binding protein-3 (BP-3) to enhance the IGFBP-3 binding affinity to rat calvaria (Schmid *et al*., 1992).

Dietary manipulation of fat intake may effect bone cell function and play a role in the local regulation of bone formation. Recently it was reported that 21-day-old chicks consuming a soybean oil diet rich in n-6 PUFA had higher concentration of the PGE_2 precursor 20:4n6, but lower concentrations of IGF-I in epiphyseal cartilage, cortical bone, and liver compared with those fed menhaden oil rich in (n-3) PUFA (Watkins *et al.,* 1996).

Bone Breaking Strength

In the poultry industry, processing of spent hens often results in many broken and shattered bones, especially in cage-maintained birds. Bone breaking strength has received considerable attention during the past decade. Bone breaking strength and bone ash content are common methods used to evaluate dietary adequacy, bone mineralization and bone fragility (Rowland, *et al.,* 1967). Rowland *et al.* (1967) noted that the bone ashing process was more time consuming than bone breaking strength tests, but that both tests were equally reliable. Both procedures require defleshing of bone after excision and weighing (Orban *et al.,* 1993).

Frost and Rowland (1991) and Orban *et al.* (1993) reported a correlation between bone breaking strength and bone density (or bone mineral mass). Positive correlations were found between bone breaking strength and bone density (.81), bone density and weight (.82), tibia breaking strength and tibia weight (.70) and tibia weight and body weight (.62).

Lott *et al.* (1992) examined the effects of bone handling on bone breaking strength. Fresh, frozen, and oven dried bones were compared. Only minor differences in breaking strength were detected between fresh and frozen bone. However, drying the bones decreased

their strength approximately 50%. The breaking strength of fresh and frozen bones of male broilers was significantly higher than that of females (Lott *et al*., 1992). However, these differences disappeared after the bones were dried (Lott *et al*., 1992). Lott et al. (1992) also tested poultry bones in the fresh, frozen and thawed, and dried condition. They observed no differences between fresh and frozen but noted a 50% decrease in the strength of dried bones.

Dietary Fats in Broiler Breeder Diet

The addition of fat to the broiler breeder diets has been used to increase metabolizable energy, feed conversion, egg production, fertility and hatchability (Atteh and Lesson, 1983; Brake, 1990; Triyuwanta *et al.*, 1992). Fats are widely used as a source of energy in broiler diets, although its efficiency of utilization is dependent on the fatty acid composition. Atteh and Lesson (1983) found that saturated fat is less efficiently utilized than unsaturated fatty acids, confirming earlier studies by Renner and Hill (1961). Atteh and Lesson (1984) fed male broiler chicks a basal diet supplemented with either 8.8% 18:1n9 acid or 8.8% 16:0 acid. The 16:0 acid fed birds ate more feed (29.4 g/bird), gained less overall weight (365.7 g) and had lower feed conversion (1.72) than 18:1n9 acid fed birds (26.6 g/bird, 411.9 g and 1.59, respectively). Certain fat sources can also form insoluble soaps comprised of fatty acids and minerals during digestion, causing these components to become unavailable thus having a detrimental effect on mineral metabolism (Atteh and Lessom, 1983).

Materials and Methods

Broiler Breeder

Commercially sexed Abor Acre broiler breeder chicks arrived July, 1996. Three hundred fifty female chicks were winged banded, weighed and equally divided among six starter pens (1.52 x 3.66m) with litter floors. Water and a commerical broiler starter feed composition (Table 2) were provided for *ad libitum* consumption for 10d. From d 10 through 20, the females were fed specific daily amounts with unlimited water access. At 20 d, a skip-a-day feeding regime was implemented as specified in the Arbor Acre Broiler Breeder Growth Guidelines while water was provided *ad libitum*. Artificial lighting was provided continuously on d one and then gradually decreased as follows: 16h on d 3 and 4, 12h from d 5-7, and 8h daily starting d 8 onward as per breeder company specifications.

Seventy-five male chicks were also wing-banded, weighed and equally divided among two grower pens (1.52 x 1.33m). Water and a commercial broiler starter feed were provided for *ad libitum* consumption until the birds were 4 weeks of age. Thereafter, a skip-a-day feeding program was implemented with water provided *ad libitum*. Artificial lighting was provided as described above. Male and female BW was monitored weekly and compared to the Arbor Acre breeder guidelines. Feed was adjusted to maintain body weight guidelines.

At 8 wk of age, the birds were moved into growing pens (3.04 x 2.66m). The skip-a-day feeding program was maintained as per company specifications and water was provided *ad libitum*. Artificial lights were kept constant at 8 h/d.

At 20 wk of age, the number of hens was reduced to 300 by culling those farthest from the flock average. The hens were randomly divided among six treatment pens (3.04 x 2.66m). Seven roosters were also placed in each pen. In addition nine culled hens were sacrificed by cervical dislocation. The heart, uterus and liver were removed, placed on ice, then immediately frozen for subsequent fatty acid analysis.

Begining at 20 wk of age, hens was fed a basal laying diet (Table 1) supplement with either 3% chicken fat (CF), soybean oil (SBO) or menhaden oil (MO). In addition, each diet was provided with or without the antioxidant ethoxyquin (0.00025%), producing a total of six dietary treatment groups. The antioxidant was added to each fat prior to inclusion in the diet. Samples of each individual fat were taken and immediately frozen for subsequent fatty acid analysis.

The female diet was placed in feeders which had grills designed to deny male access. The grill had a 4.13 cm horizontal and 7.62 cm vertical opening. The males were fed a standard male breeder diet which was placed in feeders raised above the reach of the females. The skip-a-day feeding program was continued and water was provided for *ad libitum* comsumption. The skip-a-day feeding program was discontinued at 25 wk of age (WOA) and replaced with a daily restricted feeding program as specified by Arbor Acre breeder guidelines. Lighting was increased 1h weekly until 14h/d was reached and maintained. Both male and female BW was monitored biweekly and compared to the Arbor Acre broiler breeder guidelines. Feeding allocations were adjusted to maintain these body weight targets.

Egg production commenced at approximately 23 WOA. At 25 WOA, nine eggs were collected from each treatment group. The yolks were collected and immediately frozen at -20°C for subsequent fatty acid analysis. This procedure was repeated at 8 and 24 wk of production (33 and 49 WOA, respectively).

Beginning at 25 WOA, and every 3 weeks thereafter, eggs were collected for five consecutive d and set in the incubator. Percent fertility and hatchability were determined. All eggs that failed to hatch were opened and examined macroscopically. Embryos were classified as either early, mild, or late dead, or pipped embryos.

From 36 weeks of age, broiler breeder weights were monitored monthly and compared to Arbor Acre breeder weight guidelines. Water and artificial lighting were provided as described above until the end of the experiment (51 WOA). At 20 and 51 WOA the heart, uterus and liver were excised from nine hens per treatment group and immediately frozen at –20°C for subsequent fatty acid analysis.

Broiler Progeny

The progeny from those eggs collected at 25 WOA were hatched, wing-banded, weighed and placed in grower pens (1.52 x 3.66m) according to maternal diets. All chicks were fed an identical starter diet (Table 2) and raised under similar conditions. Feed and water were provided for *ad libitum* consumption, and lighting was continuous. The chicks were weighed weekly and sampled as described below. Broilers chicks were weighed weekly until the end of the experiment (4 wk).

For the first hatch, eggs were collected and set during the second wk of broiler breeder production (pre-peak). The total number of chicks placed in grower pens was as follows: MO

progeny, 86; SBO progeny, 101; and CF progeny, 107. At 2 and 4 WOA, 25 males from each treatment group (i.e. maternal diet) were sacrificed by cervical dislocation. The left or right tibiae were removed, weighed, the length and diameter measured, and then refrigerated for subsequent bone strength tests. At 4 WOA, the remaining birds were sacrificed by cervical dislocation. In addition to tibia measurements, the length of the shank and keel from each bird were measured.

During wk 8 of broiler breeder production (peak), eggs were collected, set, incubated and hatched. The total number of chicks placed into grower pens was as follows: MO progeny, 113; SBO progeny, 104; and CF progeny, 104. Measurements were made as described for hatch one. The total number of chicks from the third hatch included: MO progeny, 74; SBO progeny, 199; CF progeny, 214. Measurements were made as described for the first hatch.

Eggs from the last hatch was collected and set. Both female and males were used and the same procedures were followed as stated above. Upon hatching, nine chicks per treatment group were sacrificed at hatch by cervical dislocation and their heart, uterus, and liver immediately removed and placed on ice for subsequent fatty acid analysis. The total number of chicks from the third hatch included: MO progeny, 111; SBO progeny, 149; CF progeny, 178.

Fatty Acid Analysis

Fatty acid analysis was performed as described by Nelson (1975), Christi (1982) and Bear-roger (1985). The samples tested were as follows: 1) the individual dietary fats, 2) broiler breeder hen tissues including liver, heart, and uterus prior to feeding the experimental diets and at the end of the experiment, and 3) egg yolks from 2, 8, and 24 wk of production. The tissue required homogenization and filtration prior to fat extraction, methylation and analysis. A 0.5 g tissue sample was placed into a 50 ml glass screw-top tub and homogenized (Polytron homogenizer) in 2:1 chloroform:methanol. The solution was then filtered (Whatman #40 filter, 12.5 cm diameter) into a second test tube. KCL (0.88%) was added to filtrate; the test tubes were shaken (Eberbach Corp. horizontal shaker) and centrifuged (3 min at 3000 rpm, 1380 g) to separate the aqueous and organic layers. Upon completion, the aqueous layer was siphoned off, leaving the fatty acid dissolved in the lower, chloroform layer. The chloroform was then evaporated (Organomation Meyer N-evap analytical evaporator model # 112) under a N stream at 60 C. The resulting product was then transferred to a 15 ml glass screw-top tub to await methylation and analysis.

Lipid extraction of egg yolk and dietary fat followed a more simplified approach. All samples (0.5 g each) were dissolved in chloroform:methanol (2:1), shaken for 10 min (Eberbach corp. horizontal shaker) and vortexed. The samples were filtered (Whatman # 40 filter, 12.5 cm diameter). Once the samples were ready for methylation, the internal standard (40µg/ml 17:1 in chloroform:methanol (2:1, Nuchek Prep Inc.) and triglyceride standard (TG dissolved in chloroform:methanol (2:1) NuChek Prep Inc. were added, and the samples evaporated, under a stream of N at 60 C (organomation Meyer N-evap analytical evaporator model # 112) until approximately 2-3 drops remained. NaOH (400-µl, in methanol) was added, and the samples heated (5 min, 100 C) in a dry block heater to saponify the lipids. Once cooled, 0.4 ml BF3 was added, and the samples were again heated (5 min, 100 C) in a dry block heater to methylate the fatty acid.

The samples were cooled and iso-octane and distilled water was added; the test tubes were shaken (Eberbach Corp. horizontal shaker) and centrifuged (10 min, 2000 rpm, 650 g); the methylated fatty acids were dissolved in iso-octane once this step was completed. The iso-octane layer was then transferred to a crimp vial and ready for injection into a gas chromatographer (Hewlett Packard model # 5890, with automatic sampler (Hewlett Packard model # 7673), flame ionization detector and integrator {(Hewlett Packard model # 3393. The column used was fused silica capillary column (J & W Scientific model # DB225), 30 m long and 1.5 mm inner diameter}. Retention peaks for each fatty acid were compared to known standards. The 20:5n3 and 22:5n3 acids were calculated by comparison with the 17:1 (chloroforn:methanol, 2:1) internal standard.

Bone Breaking Strength

All mechanical testing was conducted on an Instron Universal Testing Machine (Model # 1011, Instron, Canton Mass.), which was set at a maximum load of 1000 and 2000 Newtons for bones from 2 and 4 week old chicks, respectively and cross head speed of 5 mm/min. Bones strength was measured by shear force and stress. Shear test was performed using a double shear block test fixture (Wilson *et al.,* 1984). The shear fixture was designed so that the shear force was exerted on a 12.7 mm section loaded at the center of the shaft. The bones were loaded at a rate of 5.00 mm/min. Test position of each bone was such that the smallest dimension of the cross-section was parallel to the direction of loading. These test resulted in the ultimate shear force, shear stress, and fracture energy being determined for each bone (Wilson *et al.,* 1984).

Shear stress was determined mathematically by dividing shear force by 2 times the cross sectional area of the bone, thus accounting for bone diameter and bone wall thickness.

Statistical Analysis

A one way ANOVA was conducted to analyze the effects of maternal dietary fat on growth rate and bone parameters (tibiae weight, length and diameter, shank and keel measurements, shear force and stress required to break individual tibiae) of broiler progeny, and yolk and tissue fatty acid composition of both broiler breeder hens and broiler progeny. Where significant differences were found among treatment (i.e. maternal diets), comparisons among multiple means were separated using a Duncan's Multiple Range test. Calculations were made using the General Linear Model of the SAS Institute Inc.

RESULTS

Maternal Tissue Fatty Acid Composition

Uterine tissue from hens fed diets containing chicken fat (CF) diet showed significantly ($p<0.01$) increased levels of 14:1n5 and trans-16:1n7 fatty acids when compared to those in the chicken-antioxidant (CA), soybean oil (SBO), soybean-antioxidant (SA), menhaden oil (MO), and menhaden-antioxidant (MA) treatments (Table 3). The levels of fatty acid 16:0 were not different in uterine tissue from the CF and SBO treatments, but were increased when compared to the MA and MO treatments. The levels in the CA treatment were intermediate. The levels of 18:0 were decreased in uterine tissues of hens fed SBO and SA compared to CF and CA, while the levels were intermediate in hens fed MA and MO treatments. Feeding CF significantly increased levels of 18:1(iso) compared to all other treatments.

Levels of 18:2n6 in uterine tissue of hens were highest in the SBO and SA treatments, compared to the remaining groups. The levels of 18:3n3 fatty acid were significantly decreased in the uterus of hens fed MO when compared to chicks fed CF diet. The level 20:0 was significantly elevated in the uterus of hens fed CF. Feeding chicken fat generally elevated the levels of 20:2n6 while decreasing 22:6n3 compared to diets containing menhaden oil. Levels of soybean fed hens were intermediate. Levels of 20:3n6 in uterine tissue were lowest in the MO and MA treatments compared to the remaining groups, whereas levels of n-3 fatty acids were significantly higher in uterus of hens fed MA and MO and lowest in other treatments. Total PUFA levels were highest in hens fed CF treatments, intermediate in SBO and lowest in the remaining groups.

Heart tissues from hens fed MA had significant levels of 15:0 fatty acid compared to hens fed CA, while values in the remaining treatments were intermediate. Tissues from hens fed CF diet contained significantly ($p<0.01$) higher levels of 16:0, trans-16:1n7, and 18:1(iso) fatty acids, and was also significantly different from all other treatments (Table 4). Levels of 16:1n7 was high in heart tissue from hens fed CF treatment, intermediate in MA and lowest in the SBO treatment. Feeding SBO and SA significantly increased levels of 18:2n6 fatty acid. Fatty acid 18:3n3 were present in the highest levels in hens fed a CF diet. Levels of 20:1n9 fatty acid was significantly ($p<0.01$) highest in hens fed CA compared to remaining treatments. The levels of n-3 and n-6 fatty acids followed the same trends seen in uterine tissue. Feeding chicken fat generally elevated the levels of total SUFA, MUFA, and PUFA.

Liver tissues from hens fed a MA treatment had significantly higher in levels of 15:0 fatty acid, while hens fed a CA treatment were significantly lower (Table 5). Fatty acid 17:0 increased significantly ($p<0.01$) in liver tissue of MA fed chicks. Levels of 18:2n6 fatty acids was highest in hens fed SBO treatment, intermediate in CF and lowest in the remaining groups. Levels of 22:4n6 in liver tissue of hens were highest in SBO treatment, intermediate in CF, and lowest in the remaining groups. Levels of 22:5n3 fatty acid were significantly elevated in hens fed a MO and MA treatment. Levels of total PUFA was highest in hens fed a SA treatment, intermediate in the CF, and lowest in all other groups.

Yolk Fatty Acid Composition

The yolk from hens fed diets containing fish oil had elevated amounts of 14:1n5 and 15:0 fatty acid compared to the remaining treatments (Table 6). The levels of 18:0 were highest in yolk from hens fed CA while there was no differences in the remaining groups. Levels of 18:2n6 fatty acid was high in yolk from the SBO and SA treatment, intermediate in CF, and lowest in all other groups. Yolk from SBO and SA treatment showed significantly high levels 18:3n3 fatty acid while yolk from MO treatment was intermediate and all other treatment were the lowest. Feeding SBO generally decreased levels of 22:6n3 fatty. Levels of total PUFA was highest in yolk from the MO and MA diet, intermediate in CF, and lowest in the remaining treatments.

Levels of 14:1n5 was highest in yolk fed a MO and MA treatment, while those fed a SA treatment was significantly lower (Table 7). Fatty acid 15:0 showed elevated levels in yolk from diets containing fish oil, but was significantly low in all other treatments. Levels of 17:0 fatty acid were highest in those yolk from hens fed a MO treatment, while all other treatments showed no significant difference. Levels of 18:0 fatty acid was highest in yolk from hens fed a SBO treatment, intermediate in those fed CF, and lowest in all other groups. Levels of 18:2n6 fatty acid was high in yolks from hens fed a SBO and SA treatment, intermediate in those fed diets containing chicken fat, and lowest in the remaining treatments. Levels of 18:3n3 were highest in yolk from hens fed a SBO diet and lowest in those fed a CF treatment.

Maternal Diet and Growth Rate

At hatch (d 0), male chick (35.80 ± 0.43g) from diets containing menhaden oil weighed significantly ($p<0.01$) less than chicks coming from diets containing chicken fat and soybean oil (Table 8). Over the next 21d, chicks from SBO fed hens weighed significantly more than all other treatments. Chicks from the CF maternal diet were significantly larger than those from

MO fed hens. At d 28, chicks (1119.09 ± 14.30g) from the SBO fed hens and those from the SA fed hens (1115.36 ± 23.11g) were significantly larger than all other treatments. Feeding a CA treatment significantly increased shank and keel measurements (Table 18). Shank measurements were intermediate in the SA treatment and lowest in the MA treatment. Keel measurements were low in those chicks fed a CF treatment.

Upon hatch, chicks (41.78 ± 0.33g and 43.08 ± 0.39g) whose maternal diet was MO and MA were significantly lighter than chicks from CF and SBO maternal diets (44.15 ± 0.40g and 43.64 ± 0.37g, respectively)(Table 9). Chicks from the MO and MA treatment continued to be significantly lighter through the following 21 d, and were still significantly lighter (1138.28 ± 20.43g and 1036.92 ± 32.39g) at the end of 28 d (chicks from CF, CA, SBO, and SA fed hens were 1215.59, 1221.64, 1225.87, and 1221.96, respectively). Shank and keel measurements were significantly larger in those chicks fed a CF treatment (Table 19).

At hatch (d 0), chicks from MO fed hens weighed significantly less than chicks from all other treatments (Table 10). Over the next 21 d, the chicks from the CF fed hens weighed more than those from MO and SBO fed hens. Chicks from the SBO maternal diet were larger than the chicks from the MO fed hens. At d 28, chicks (1052.21 ± 18.86g) from the SA fed hens and the CF fed hens (1048.74 ± 14.27g) chicks were significantly larger than the MO treatment (952.92 ± 15.58g). Shank and keel measurements were larger in those chicks fed a CF diet (Table 20).

Tibia Development and Bone Strength

There was no significant difference observed in tibia weight or length, among chicks according to maternal diet (Table 12). At 14 DOA, the diameter of tibiae excised from male chicks whose maternal diet was SBO and SA was significantly lower and different from all the other diets. Also at 14 DOA, there was no significant difference in force and bone wall thickness. As shown in (Table 13) at 28 d, there was still no significant difference in weight, nor was there any difference in diameter of tibiae from SA and CF diets. Chicks fed SBO type diet showed significant differences in force and energy. There was no significant ($p<0.01$) difference in bone wall thickness between CF, CA, SA, and MO extracted tibiae, but CF and SA was different from MA.

At 14 DOA, there was no significant difference in tibia weight or length according to maternal diet (Table 14). Male tibiae from the diet CA showed significant differences in force

and stress when compared to all the other maternal diets. Those chicks fed a diet containing CF or SBO was significantly high in energy when compared to those chicks fed MO treatments. As shown in (Table15) at 28 DOA, tibia from chicks fed CA showed significant ($p<0.01$) differences in tibia length, but not weight when compared to all other treatments. The tibiae from a CF diet showed significantly higher bone wall thickness when compared to the remaining dietary treatments.

At 14 DOA, tibiae from female chicks fed a SA diet showed higher tibia weights, and SBO displayed significantly higher tibia length among all other maternal diets (Table 16). Force and energy were significantly ($p<0.01$) higher in tibia from CF fed chicks. Also at 14 DOA, stress was significantly ($p<0.01$) higher in tibia of MO fed chicks. At 28 d, tibia weight and diameter were significantly ($p<0.01$) greater in those chicks in which their dietary treatment was CF. The tibia from CF fed chicks showed significantly higher levels of force and stress when compared to al other maternal diets (Table 17).

DISCUSSION

Fatty acid composition of CF, MO, and SBO is well documented (Dansky, 1961; Atteh *et al*., 1989; Chanmugan *et al.*, 1994; Scaife *et al.*, 1994). Results of this study confirmed the observation of these authors. SBO was rich in 18:2n6, 18:3n3, total PUFA and n-6 fatty acids whereas MO was rich in 22:5n3, 22:6n3 and n-3 fatty acids. The only n-3 fatty acid not present in large amounts is 18:3n3. The fatty acid profile of the MO and SBO diets were in accordance with other researchers (Machlin *et al.*, 1962; Skinner *et al.*, 1990; Lin *et al.*, 1991).

Addition of chicken fat into broiler breeder diets caused an increase in total MUFA. The n-3 and n-6 fatty acids are highly dependent on the ratio of 18:2n6 and 18:3n3 in the diet (Elswyk *et al.*, 1994). Dietary lipid composition significantly altered the fatty acid composition of all the tissues examined. When rich in n-3 fatty acids were included in the diet of hens, there was a significant ($P<0.05$) incorporation of long chain EPA (22:5n3) and DHA (22:6N3) with a concomitant reduction in arachidonic acid (20:4n6) in the liver and eggs when compared to feeding CF or SBO. This agrees with the results of Cherian *et al*. (1996).

As reported by Hulan *et al*. (1988), Simopouls (1988) and Chanmugan *et al*. (1991), desaturase enzymes prefer n-3 acids as a substrate over n-6 fatty acids. Therefore, n-3 fatty acids are more readily metabolized by the liver than n-6 fatty acids resulting in a decrease of 20:4n6 acids and subsequent prostoglandin production (Cherian *et al*., 1996). Addition of soybean oil (SBO) elevated levels of 18:2n6 and decreased 20:2n6, particularly when SBO was the sole lipid supplement. The proportions of 20:5n3, 22:5n3, and 22:6n3 were significantly reduced in the liver tissue from hens fed SBO supplemented diets (Scaife *et al*., 1993). Incorporation of MO into the breeder hen diet significantly increased the concentration of long chain n-3 fatty acids and significantly decreased the amounts of 20:4n6 acids within 2 wk of production. These effects lasted throughout the entire production period.

The major changes in egg yolk fatty acids by dietary linolenic acid (LNA) can be summarized as an increase in polyunsaturated fatty acids (PUFA) and a decrease in mono-unsaturated fatty acids (MUFA). However, the increase in PUFA in the yolk lipid from LNA diets was mainly caused by the increase in n-3 fatty acids 18:3n3, 22:5n3, and 22:6n3 (Ahn *et al*., 1995). The maternal tissue fatty acid composition paralleled that of the dietary source. In this study, levels of 22:5n3 and 22:6n3 in all groups fed menhaden oil (MO) were significantly higher than the groups fed the same level of either CF or SBO. Similarly, levels of

docosapentaenoic acid (22:6n3) were higher in controls fed the same levels of corn oil than groups fed linseed oil (Chanmugam *et al.,* 1992). Lipids of all groups fed menhaden oil had significantly higher 22:5n3 to 20:4n6 ratios compared with linseed and corn oil groups fed the same levels of oil.

The present study indicates that the inclusion of n-3 or n-6 fatty acids in the laying hen diet can enrich the egg yolk lipids and tissue lipids of the hatched chicks with n-3 and n-6 fatty acids, respectively. The increased supply of dietary 18:3n3 tends 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 20:4n6 in the egg yolk (Cherian and Sim, 1992).

Antioxidants play an integral role in providing a defense mechanism against the damaging effects of reactive free radical and singlet oxygen. Diet is an important source of antioxidants that falls into two classes: water soluble and lipid-soluble (Bhagavan and Nair, 1996). Amino acids have been reported to be either antioxidants or prooxidants or, to have no effect on the oxidation of lipids. Alaiz *et al*. (1995) reported that addition of oxidized lipids/amino acids reaction products (OLAARP) efficiently reduced peroxidation in a soybean oil diet. In this study, chicks fed diets containing antioxidants showed no significant differences in parameters measured. Alaiz *et al*. (1995) was not able to find significant differences in growth rate as a result of employing either butylhydroxytoluene (BHT) or ethoxyquin (EQ) in broiler feed. Male tibia parameters at 14 d showed no significant differences. Female tibia followed the same result stated for the male tibia.

Reports on the influence of maternal fatty acids on yolk composition are numerous, but reports on the influence of those fatty acids on total yolk or egg weight are few. Vilchez *et al.* (1992) reported that hens fed MO diets laid eggs which weighed significantly ($P<0.01$) less than eggs from hens fed CF diets. Quails fed diets enriched with linoleic acid (18:2n6) produced eggs with yolk that weighed significantly ($P<0.05$) more than those from quail hens fed diet high in 18:0 or 14:0 acids. Unfortunately, total egg weight and quail chick weight was not reported (Vilchez *et al.,* 1992).

Some of the fatty acids of yolk lipid from different strain of hens varied significantly. Among the fatty acids, 18:1n9 and 18:2n6 had large variation. As n-3 fatty acids in eggs increased, the percentage incorporation of those fatty acids to the progeny tissue tended to

decrease. The higher level of LNA in the egg from n-3 PUFA diet did not cause any change in the percentage incorporation of LNA (Vilchez *et al.,* 1992).

Fatty acids present in the day-old chicks reflected those contained in the egg yolk, and, therefore reflected those of the maternal diet. Alterations in fatty acids composition of egg yolk can have a dramatic impact on embryonic development (Donaldson, 1981). This has been reported from current studies among embryos from menhaden oil (MO) fed hens. Dietary oils significantly ($P<0.05$) altered the fatty acid composition of all the tissues examined. When n-3 fatty acid rich in MO was included in the diet, a significant ($P<0.05$) incorporation of longer chain EPA and DHA with reduction in arachidonic acid (20:4n6) was observed in the liver and eggs (Cherian *et al.,* 1996). Long chain n-3 fatty acids (FA) were not detected in the adipose tissue of many of the birds except those fed diets containing menhaden oil.

Body weight had been reported to correlate with both tibia breaking strength and bone density. A significant relationship was also found between body weight (BW) and dry tibia weight ($r=.66$, $P<0.0001$) (Frost and Roland, 1990). Adding higher levels of 1, 25-$(OH)_2$ D_3 caused highly significant linear increases in tibia breaking strength, tibia weight, and bone density.

The growth promoting properties of SBO were reported in Scaife *et al*. (1994) and confirmed by the data from this experiment. The presence of increased levels of 18:2n6 acid, which is rapidly metabolized to 20:4n6 acid, then enters the cyclooxygenase pathway to produce prostaglandins which stimulate growth rate and bone development in animals (Lefkowth *et al*., 1986; Scaife *et al*., 1994). Atteh *et al*. (1984) did a study on the effect of fats in broilers chick diets and reported that soybean oil (SBO) fed chicks exhibited a significant ($P<0.01$) decrease in bone ash.

Arachidonic acid (20:4n6) is a precursor for prostaglandins (PGI_2 and PGE_2) which stimulate bone growth. Chicks from SBO and CF fed hens had ($P<0.01$) higher levels of 20:4n6 acid than the same chick tissue from MO fed hens. Prostaglandin E_2 and I_2 have been known to have a positive effect on growth and bone development (Friedman, 1981; Croft *et al.,* 1985; Ackerman, 1995). Male chick parameter exhibited very little significant differences at 14 day of age.

At 14 DOA, tibia diameter of hens fed CF and MO diet were larger than those fed a SBO treatment. At d 28, male chick parameters exhibited significant differences. Chicks from hens

fed a SBO and CF diet had heavier tibia weights than those from hens fed a MO diet. These data corroborated a study by (Scaife *et al.* 1994). Since SBO and CF contained higher levels of arachidonic (20:4n6) acid and hence more prostaglandins, the present results should be expected. Similarly, chick tibia from the SBO and CF diets were significantly thicker than MO maternal diet tibiae. The diameter continued to be significantly larger in those chicks fed a CF and SBO maternal diet. Shear force showed significant differences in those chick fed a diet containing soybean oil. Shank measurements were larger in those chicks fed a CA maternal diet when compared to all other treatments.

In this study, male chick tibia parameters exhibited significant differences. At 14 DOA, there were no significant differences in tibia weight and length. Both shear force and stress were significantly increased in those chicks fed a CA maternal diet. By 28 DOA, the SBO maternal diet produced a thicker tibia than both CF and MO maternal diet tibiae. Similarly, chick tibia from the SBO maternal diet were significantly ($P<0.01$) thicker than MO and CF maternal diet tibiae. Throughout the entire grow out period, there was no significant difference detected in tibia length according to the maternal diet.

In this study, tibiae of females chicks were weighed at 14 and 28 days of age (DOA) and the tibiae from the hens fed the SBO and CF diet were significantly ($P<0.01$) heavier than tibiae from MO maternal diets. The length and diameter of female chick tibiae were significantly ($P<0.01$) different at these same time periods confirming results by Frost and Roland (1991). At both 14 and 28d, shear force and energy was extremely high in those female tibia in which their maternal diet was CF. Shank and keel was significantly larger in those female chicks fed a CA maternal diet.

IMPLICATIONS

Non-infectious skeletal diseases cause the commercial broiler industry millions of dollars annually. Poultry nutritionists are being challenged to develop new diets that would maximize the broilers genetic potential and reduce related bone stress disorders. If a maternal dietary influence on bone growth and development can be found, poultry nutritionist could formulate a more precise ration that would meet the needs of the commercial broiler industry. Reducing the occurrences of these skeletal diseases by dietary mean would increase the overall health of the birds and decrease the amount of revenue lost yearly due to these abnormalities. This study shows that if dietary fats particularly soybean oil (SBO) and chicken fat (CF) into the broiler breeders diet it would increase egg and body weight and help supply those essential fatty acids need for growth and development.

Table 1. Broiler Breeder Hen Diets

	Diets					
Ingredient	CF	CA	SBO	SA	MO	MA
Corn Meal	58.70	58.70	58.70	58.70	58.70	58.70
Soybean Meal	20.50	20.50	20.50	20.50	20.50	20.50
Wheat Middlings	8.65	8.65	8.65	8.65	8.65	8.65
Limestone	6.53	6.53	6.53	6.53	6.53	6.53
Fat[γ]						
Chicken Fat	3.00					
Chicken_Antioxidant		3.00				
Soybean Oil			3.00			
Soybean + Antioxidant				3.00		
Menhaden Oil					3.00	
Menhaden + Antioxidant						3.00
Ethoxyquin		0.0025		0.0025		0.0025
Defluorinated P	1.86	1.86	1.86	1.86	1.86	1.86
Salt	0.25	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.18	0.18	0.18	0.18	0.18	0.18
Lysine HCL	0.03	0.03	0.03	0.03	0.03	0.03
Baciform 50[α]	0.05	0.05	0.05	0.05	0.05	0.05
Vitamin/Mineral Mix[β]	0.25	0.25	0.25	0.25	0.25	0.25

[1]CF = Chicken Fat; CA =Chicken + Antioxidant; SBO = Soybean oil; SA = Soybean + Antioxidant; MO = Menhaden Oil; MA = Menhaden + Antioxidant.

Ethoxyquin (0.0025%) was added directly to the fats in order to prevent oxidation prior to mixing.

[α]Baciform 50 is a coccidiostat.

[β] Supplied by Hoffman-LaRoche, formulated to supply the following to each kilogram of finished feed: vitamin A, 11,000 IU; cholecalciferol, 2,750 ICU; vitamin E, 22 IU; riboflavin, 7.7 mg; menadione sodium bisulfite, 4.96 mg; niacin, 38.6 mg; d-pantothenic acid, 13.2 mg; folic acid, 1.1 mg, vitamin B_{12} , 13µg; biotin, 110µg; choline chloride, 441 mg; thiamine, 1.8 mg; pyridoxine, 4.7 mg; ethoxyquin, 55 mg; manganese, 55mg; zinc, 50 mg; iron, 30 mg; copper, 5 mg; iodine, 0.5 mg; and selenium, 0.1 mg.

Table 2. Broiler Chick Commercial Starter Diet[1]

Ingredients	(%)
Corn Meal	60.45
Soybean Meal	23.45
Stabilized Animal Fat	4.00
Fish Meal	2.50
Corn Gluten Meal	4.00
Alfalfa Meal	2.00
Defluorinated Phosphate	1.50
Limestone	1.00
Salt	0.40
Trace Mineral Mix$^{\beta}$	0.10
Vitamin Premix$^{\delta}$	0.50
Baciform 50$^{\alpha}$	0.05

[1]All chicks were fed this diet regardless of maternal diet.

$^{\beta}$Provides per kilogram of diet: cobalt, 450 mg; copper, 5 g; iodine, 2 g; manganese, 120 g, zinc, 120 g; iron, 40 g, with calcium carbonate as a diluent.

$^{\delta}$Provides per kilogram of diet: vitamin A, 8.81×10^5 USP; vitamin D_3, 4.41×10^5; vitamin E, 220 IU; menadione sodium bisulfite complex, 350 g; menadione, 180 mg; riboflavin, 660 mg; d-calcium pantothenate, 1.3 g; d-pentothenic acid, 1.2 g; niacin, 6.6 g; choline choride, 50 g; choline, 43 g; vitamin B_{12}, 1 g; selenium, 40 g; methionine, 100 g; folic acid, 60 mg; ethoxyquin, 25 g.

$^{\alpha}$Baciform 50 is a coccidiostat.

TABLE 3. FATTY ACID COMPOSITION OF BROILER BREEDER HEN UTERUS 50 WOA[1]

Diets

(μg/mg tissue)

FATTY ACID	CF			CA			MO			MA			SBO			SA		
14:1n5	12.82^{A}	±	3.09	0.0^{B}	±	0.0	1.37^{B}	±	0.90	0.48^{B}	±	0.48	0.63^{B}	±	0.63	0.00^{B}	±	0.00
15:0	9.46	±	1.79	4.90	±	1.19	5.92	±	0.22	7.80	±	0.90	6.54	±	1.64	6.07	±	0.73
16:0	2505.22^{A}	±	320.70	1476.86^{BC}	±	183.23	857.05^{C}	±	70.08	896.99^{C}	±	115.34	1977.76^{AB}	±	369.56	1103.44^{C}	±	194.57
t16:1n7	126.47^{A}	±	27.67	58.71^{B}	±	11.18	19.24^{B}	±	7.88	62.12^{B}	±	13.32	57.70^{B}	±	18.17	34.96^{B}	±	9.42
16:1n7	55.95	±	10.26	25.19	±	6.35	74.45	±	29.47	13.57	±	1.66	22.01	±	2.68	126.35	±	107.53
17:0	75.80	±	13.43	41.19	±	9.36	45.41	±	6.93	48.29	±	7.03	34.21	±	7.76	175.57	±	114.05
18:0	2587.95^{ab}	±	193.64	2859.23^{a}	±	776.84	1963.7^{abc}	±	208.45	2103.64^{abc}	±	255.75	1498.82^{bc}	±	267.54	1187.20^{c}	±	277.82
18:1	382.50^{a}	±	56.43	241.60^{b}	±	35.53	235.03^{b}	±	39.34	197.06^{b}	±	27.80	196.88^{b}	±	31.30	194.86^{b}	±	33.47
18:2n6	2478.48^{AB}	±	628.38	2017.74^{AB}	±	420.10	1502.75^{B}	±	150.99	1635.21^{B}	±	246.70	3701.90^{A}	±	558.79	3242.03^{AB}	±	983.43
18:3n6	7.08	±	5.15	0.00	±	0.00	5.03	±	2.89	1.37	±	0.90	1.56	±	1.56	0.00	±	0.00
18:3n3	82.28^{A}	±	21.80	25.98^{BC}	±	14.97	6.98^{C}	±	2.59	33.76^{BC}	±	13.13	29.13^{BC}	±	7.40	46.31^{AB}	±	11.95
20:0	289.39^{A}	±	86.25	95.16^{B}	±	24.75	53.37^{B}	±	12.82	51.97^{B}	±	10.81	78.36^{B}	±	17.71	94.29^{B}	±	23.44
20:1n9	164.64	±	59.00	99.69	±	66.05	98.40	±	40.47	174.05	±	53.80	99.88	±	58.56	34.79	±	25.48
20:2n6	230.67^{a}	±	108.72	133.92^{ab}	±	40.07	34.90^{b}	±	14.54	31.92^{b}	±	11.03	91.15^{b}	±	44.86	47.78^{b}	±	11.62
20:3n6	4274.50^{A}	±	544.02	4324.94^{A}	±	482.39	1972.49^{B}	±	306.24	2183.43^{B}	±	240.23	5026.57^{A}	±	545.04	5715.09^{A}	±	847.12
20:4n6	2182.40^{AB}	±	740.34	1808.97^{AB}	±	54.14	771.33B	±	293.50	1714.72^{AB}	±	128.24	2990.23^{A}	±	929.14	1195.86^{AB}	±	485.71
22:5n3	266.34^{B}	±	131.73	162.04^{B}	±	56.7	947.52^{A}	±	105.78	1238.17^{A}	±	186.67	202.84^{B}	±	62.07	361.63^{B}	±	141.34
22:6n3	3.01^{B}	±	3.01	2.82^{B}	±	2.01	10.53^{B}	±	2.24	26.74^{A}	±	5.32	1.93^{B}	±	0.99	4.77^{B}	±	3.10
TOTS	5467.83^{A}	±	456.15	4477.37^{AB}	±	936.59	2925.52^{BC}	±	262.69	3108.72^{BC}	±	375.64	3595.74^{BC}	±	359.28	2566.58^{C}	±	368.40
TOTM	742.40	±	92.45	425.21	±	78.44	428.52	±	42.09	447.29	±	81.66	377.12	±	89.05	390.98	±	165.47
TOTP	10967.2^{A}	±	2140.22	8476.4^{ABC}	±	628.01	5250.6^{C}	±	632.45	6872.5^{BC}	±	576.60	10127.8^{AB}	±	1288.52	9187.9^{AB}	±	1326.46

[1]Values represent mean ± standard error; N=7 for CF; 9 for CA; 9 for MA; 8 for MO; 9 for SA; 9 for SBO.

[2]Dietary treatments represent the maternal diet; CF = Chicken Fat; CA = Chicken + Antioxidant; SBO = Soybean Oil; SA = Soybean + Antioxidant; MO = Menhaden Oil; MA = Menhaden + Antioxidant; TOTS = Total Saturated Fatty Acid; TOTM = Total Monounsaturated Fatty Acid; TOTP = Total Polyunsaturated Fatty Acid.

$^{A-C}$ Means within a row lacking the same superscript differ significantly (P<0.01).

$^{a-c}$ Means within a row lacking the same superscript differ significant (P<0.05).

TABLE 4. FATTY ACID COMPOSITION OF BROILER BREEDER HEN HEART 50 WOA[1]

	Diets (μg/mg tissue)																							
FATTY ACID	**CF**			**CA**			**MO**			**MA**			**SBO**			**SA**								
14:1n5	27.82	±	4.64	14.68	±	4.34	20.58	±	4.03	22.44	±	9.81	12.68	±	2.86	10.30	±	3.61						
15:0	13.08^{abc}	±	1.52	5.43^{c}	±	1.54	16.90^{ab}	±	2.56	18.43^{a}	±	2.81	8.60^{bc}	±	3.53	12.94^{abc}	±	4.56						
16:0	5428.79^{A}	±	511.55	3568.23^{B}	±	383.56	3913.56^{B}	±	206.38	3848.11^{B}	±	380.46	3295.42^{B}	±	200.48	4144.00^{B}	±	265.08						
t16:1n7	117.67^{a}	±	19.21	73.87^{b}	±	14.61	65.54^{b}	±	8.15	74.07^{b}	±	20.29	48.17^{b}	±	8.70	70.14^{b}	±	11.06						
16:1n7	617.54^{A}	±	103.81	314.90^{B}	±	70.14	356.27^{B}	±	40.62	419.76^{AB}	±	122.10	201.35^{B}	±	19.80	261.47^{B}	±	67.46						
17:0	38.89	±	4.21	32.06	±	2.68	55.84	±	2.56	52.10	±	3.52	33.82	±	1.56	44.51	±	2.44						
18:0	2830.91	±	155.05	2539.35	±	28.14	2607.08	±	81.32	2580.92	±	239.54	2684.71	±	111.45	2813.95	±	111.54						
18:1	4912.56^{A}	±	597.40	2551.79^{B}	±	354.26	2443.19^{B}	±	385.89	1920.88^{B}	±	431.79	1981.64^{B}	±	169.74	2919.62^{B}	±	397.12						
18:2n6	4221.71^{A}	±	330.73	3189.15^{B}	±	252.79	3258.40^{B}	±	149.75	2708.49^{B}	±	156.39	4027.30^{A}	±	264.83	4721.18^{A}	±	256.43						
18:3n3	48.17^{A}	±	10.01	16.53^{B}	±	6.71	9.21^{B}	±	3.09	5.64^{B}	±	2.22	5.53^{B}	±	2.43	16.95^{B}	±	8.95						
20:0	100.36	±	14.09	37.64	±	13.18	54.84	±	9.54	38.70	±	15.09	121.16	±	21.26	164.74	±	27.54						
20:1n9	10.35^{b}	±	5.26	25.84^{a}	±	5.55	4.45^{b}	±	2.99	6.28^{b}	±	4.94	7.42^{b}	±	5.22	5.63^{b}	±	3.78						
20:2n6	339.92^{A}	±	49.87	218.09^{AB}	±	49.50	168.21^{B}	±	55.21	100.79^{B}	±	48.00	75.68^{B}	±	23.59	177.22^{B}	±	44.22						
20:4n6	183.14^{B}	±	42.14	269.62^{B}	±	36.51	414.96^{A}	±	56.77	269.29^{B}	±	47.07	140.34^{B}	±	24.23	234.22^{B}	±	54.80						
22:4n6	4188.52^{A}	±	183.60	4233.24^{A}	±	168.82	3176.25^{B}	±	188.82	2954.98^{B}	±	224.55	4179.94^{A}	±	175.36	4108.29^{A}	±	195.01						
22:5n3	27.51^{B}	±	14.23	12.45^{B}	±	6.78	84.08^{A}	±	17.62	81.42^{A}	±	16.82	3.67^{B}	±	1.95	12.86^{B}	±	4.35						
22:6n3	77.99^{B}	±	18.18	72.92^{B}	±	28.75	1574.51^{A}	±	235.77	1453.81^{A}	±	216.05	109.2^{B}	±	43.15	156.58^{B}	±	59.82						
TOTS	8412.05^{a}	±	658.09	6182.72^{b}	±	513.55	6648.23^{b}	±	328.79	6538.28^{b}	±	532.63	6143.73^{b}	±	302.76	7180.16^{ab}	±	380.01						
TOTM	5685.95^{A}	±	708.50	2981.10^{B}	±	444.08	2890.05^{B}	±	403.14	2443.45^{B}	±	556.52	2251.28^{B}	±	176.86	3267.18^{B}	±	465.96						
TOTP	12535.30^{A}	±	674.33	11574.92^{AB}	±	773.48	7365.95^{C}	±	371.17	6245.16^{C}	±	388.81	10312.67^{B}	±	533.29	12220.03^{AB}	±	995.34						

[1]Values represent mean ± standard error; N=9 for CF; 9 for CA; 8 for MA; 9 for MO; 9 for SA; 9 for SBO.

[2]Dietary treatments represent the maternal diet; CF = Chicken Fat; CA = Chicken + Antioxidant; SBO = Soybean Oil; SA = Soybean + Antioxidant; MO = Menhaden Oil; MA = Menhaden + Antioxidant; TOTS = Total Saturated Fatty Acid; TOTM = Total Monounsaturated Fatty Acid; TOTP = Total Polyunsaturated Fatty Acid.

$^{A-C}$ Means within a row lacking the same superscript differ significantly (P<0.01).

$^{a-c}$ Means within a row lacking the same superscript differ significant (P<0.05).

TABLE 5. FATTYACID COMPOSITION OF BROILER BREEDER HEN LIVER 50 WOA[1]

	Diets											
	(µg/mg tissue)											
FATTY ACID	**CF**		**CA**		**MO**		**MA**		**SBO**		**SA**	
14:1n5	21.22	± 8.52	11.69	± 3.68	28.58	± 8.80	40.26	± 12.14	11.25	± 2.73	20.08	± 4.69
15:0	19.58^{C}	± 3.53	11.54^{C}	± 0.99	34.79^{B}	± 5.32	49.42^{A}	± 8.34	14.19^{C}	± 3.06	18.89^{C}	± 3.33
16:0	11359.71	± 2095.09	7329.56	± 564.30	9617.99	± 1553.72	13275.21	± 2196.46	8806.03	± 1220.67	11524.72	± 1847.73
t16:1n7	385.27	± 141.86	141.06	± 19.11	191.84	± 41.50	385.21	± 96.07	178.03	± 54.15	215.48	± 48.80
16:1n7	793.57	± 278.23	303.51	± 24.47	594.48	± 130.04	1135.67	± 366.00	308.86	± 74.81	1236.64	± 627.12
17:0	114.32^{B}	± 21.59	66.02^{B}	± 6.13	131.40^{B}	± 24.02	206.27^{A}	± 42.29	90.39^{B}	± 12.95	114.43^{B}	± 16.32
18:0	5441.12	± 734.37	3358.84	± 256.63	3518.24	± 473.80	6147.5	± 1420.77	4750.58	± 690.22	4478.25	± 738.56
18:1	13829.66	± 4205.41	4921.52	± 660.36	9403.09	± 2104.42	17382.58	± 5257.24	6846.88	± 2035.17	9193.12	± 2601.62
18:2n6	3836.4^{B}	± 637.51	2608.8^{B}	± 408.18	2699.50^{B}	± 386.20	3760.50^{B}	± 658.01	4321.1^{AB}	± 799.68	5770.9^{A}	± 599.40
18:3n3	54.06^{B}	± 14.21	54.78^{B}	± 28.06	64.35^{B}	± 14.05	92.39^{B}	± 31.99	149.56^{B}	± 32.56	278.55^{A}	± 50.68
20:2n6	21.39	± 14.72	0.0	± 0.0	64.35	± 47.47	17.25	± 12.90	5.26	± 3.68	24.65	± 16.31
20:3n6	220.24	± 67.49	87.66	± 24.06	77.69	± 28.77	178.38	± 60.43	74.71	± 34.22	137.65	± 50.76
20:4n6	93.29	± 46.96	56.70	± 13.09	42.66	± 39.08	24.39	± 12.25	97.69	± 20.56	80.44	± 15.90
22:4n6	3794.50^{B}	± 1582.34	2180.19^{B}	± 190.51	775.36^{B}	± 154.00	2535.91^{B}	± 1293.77	9088.20^{A}	± 3128.92	2270.14^{B}	± 105.19
22:5n3	267.60^{B}	± 203.27	77.70^{B}	± 29.74	2005.1^{A}	± 1283.71	915.60^{AB}	± 186.85	305.90^{B}	± 82.39	196.20^{B}	± 163.13
22:6n3	2942.61	± 725.03	1818.52	± 228.75	93.86	± 19.28	204.21	± 58.70	2440.14	± 293.91	4050.03	± 1311.39
TOTS	16934.75	± 2750.56	10765.97	± 807.95	13302.43	± 2043.49	19678.50	± 3568.54	13661.20	± 1621.65	16136.30	± 2512.17
TOTM	15064.62	± 734.71	5452.59	± 703.68	10282.35	± 2290.46	19036.13	± 5738.54	7494.60	± 2178.51	10943.89	± 2947.49
TOTP	11822.29^{B}	± 6244.06	7520.8^{BC}	± 429.78	5720.50^{C}	± 1330.46	7052.1^{BC}	± 1689.06	10037.15^{BC}	± 728.91	19446.98^{A}	± 3031.81

[1]Values represent mean± standard error; N=9 for CF; 9 for CA; 9 for MA; 8 for MO; 9 for SA; 9 for SBO.

[2]Dietary treatments represent the maternal diet; CF = Chicken Fat; CA = Chicken + Antioxidant; SBO = Soybean Oil; SA = Soybean + Antioxidant; MO = Menhaden Oil; MA = Menhaden + Antioxidant; TOTS = Total Saturated Fatty Acid; TOTM = Total Monounsaturated Fatty Acid; TOTP = Total Polyunsaturated Fatty Acid.

[A-C] Means within a row lacking the same superscript differ significantly ($P<0.01$).

TABLE 6. FATTY ACID COMPOSITION OF BROILER BREEDER HEN YOLK 2 WK OF LAY (HATCH 1) [1]

Diets

(μg/mg tissue)

FATTY ACID	CF	CA	MO	MA	SBO	SA
14:1n5	13.61^{BC} ± 2.56	8.87^{C} ± 1.42	22.59^{A} ± 3.07	21.18^{AB} ± 4.89	10.30^{C} ± 1.76	10.61^{C} ± 1.38
15:0	7.24^{B} ± 1.32	5.30^{B} ± 0.69	15.70^{A} ± 1.72	11.82^{A} ± 2.69	4.95^{B} ± 1.07	5.44^{B} ± 0.64
16:0	4264.52 ± 556.84	3786.77 ± 257.72	4518.01 ± 426.23	4087.24 ± 407.79	4355.31 ± 453.08	3897.89 ± 265.41
t16:1n7	81.82 ± 11.07	641.67 ± 461.17	63.75 ± 15.78	131.80 ± 70.06	93.79 ± 25.48	82.02 ± 14.48
16:1n7	450.33 ± 69.02	203.54 ± 62.28	468.39 ± 41.88	330.56 ± 84.06	266.66 ± 41.43	258.12 ± 43.33
17:0	29.38^{b} ± 3.68	857.99^{a} ± 388.49	46.90^{b} ± 4..38	254.36^{b} ± 156.52	44.66^{b} ± 17.17	289.60^{ab} ± 260.46
18:0	1542.88^{AB} ± 310.30	2461.92^{A} ± 626.09	1007.14^{B} ± 108.37	751.56^{B} ± 116.65	1039.70^{B} ± 169.21	1236.90^{B} ± 334.15
18:1	3820.16 ± 912.28	1178.54 ± 711.22	3552.37 ± 720.10	1443.72 ± 667.73	3035.02 ± 614.90	2012.32 ± 664.71
18:2n6	1323.3^{BC} ± 212.78	637.2^{D} ± 207.86	1154.3^{BCD} ± 121.42	774.0^{CD} ± 189.20	1960.0^{A} ± 186.44	1682.70^{AB} ± 250.22
18:3n6	1323.28 ± 212.78	637.19 ± 207.86	1154.3 ± 121.42	774.04 ± 189.20	1689.70 ± 275.03	1682.68 ± 250.22
18:3n3	34.97^{BC} ± 6.42	11.94^{C} ± 3.92	50.51^{B} ± 15.71	31.09^{BC} ± 10.55	86.26^{A} ± 13.33	86.97^{A} ± 13.89
20:1n9	34.97 ± 6.42	11.94 ± 4.87	50.81 ± 5.19	31.09 ± 9.05	86.26 ± 13.33	86.97 ± 13.98
20:2n6	24.73 ± 14.93	35.67 ± 24.24	15.71 ± 15.71	6.68 ± 6.68	10.53 ± 10.53	4.28 ± 4.28
20:3n6	38.15 ± 13.22	23.25 ± 10.29	32.41 ± 18.43	44.02 ± 17.86	39.82 ± 21.37	36.22 ± 16.95
20:4n6	1.76 ± 1.16	10.89 ± 8.70	3.67 ± 2.05	2.33 ± 1.61	13.23 ± 5.69	12.87 ± 5.46
22:4n6	1622.17 ± 1138.83	4086.92 ± 1617.96	2488.38 ± 1649.22	2493.80 ± 1643.41	23.87 ± 13.26	111.64 ± 99.15
22:6n3	366.69 ± 154.31	166.71 ± 111.56	1.12 ± 0.82	25.79 ± 24.24	167.16 ± 39.59	129.06 ± 52.37
TOTS	5844.02 ± 666.69	7111.99 ± 723.90	5587.72 ± 536.30	5105.10 ± 506.23	5444.62 ± 537.67	5429.84 ± 434.89
TOTM	4400.91 ± 982.33	2044.58 ± 754.69	4157.93 ± 759.88	1949.32 ± 694.49	3492.05 ± 631.96	2450.06 ± 683.72
TOTP	9421.71^{ab} ± 1065.69	7881.98^{ab} ± 1252.41	11529.32^{a} ± 1017.43	10230.86^{a} ± 1135.87	5761.09^{b} ± 1248.01	9160.49^{ab} ± 1495.98

[1]Values represent mean ± standard error; N=9 for CF; 9 for CA; 9 for MA; 8 for MO; 9 for SA; 9 for SBO.

[2]Dietary treatments represent the maternal diet; CF = Chicken Fat; CA = Chicken + Antioxidant; SBO = Soybean Oil; SA = Soybean + Antioxidant; MO = Menhaden Oil; MA = Menhaden + Antioxidant; TOTS = Total Saturated Fatty Acid; TOTM = Total Monounsaturated Fatty Acid; TOTP = Total Polyunsaturated Fatty Acid.

$^{A-D}$ Means within a row lacking the same superscript differ significantly ($P<0.01$).

$^{a-c}$ Means within a row lacking the same superscript differ significant ($P<0.05$).

TABLE 7. FATTY ACID COMPOSITION OF BROILER BREEDER HEN YOLK 24 WK OF LAY (HATCH 2) [1]

Diets

(µg/mg tissue)

FATTY ACID	CF	CA	MO	MA	SBO	SA
14:1n5	4.04^{C} ± 1.12	5.45^{BC} ± 0.91	13.81^{A} ± 2.83	10.09^{AB} ± 1.97	4.10^{C} ± 1.34	2.62^{C} ± 0.76
15:0	4.96^{B} ± 0.67	5.27^{B} ± 0.84	12.77^{A} ± 1.33	10.67^{A} ± 1.40	5.39^{B} ± 0.84	4.96^{B} ± 0.52
16:0	3978.62 ± 225.67	4240.24 ± 260.62	4356.98 ± 405.00	3972.00 ± 400.25	4681.12 ± 273.10	3788.31 ± 312.63
t16:1n7	78.34 ± 6.89	80.26 ± 10.48	87.57 ± 11.78	57.14 ± 10.55	81.57 ± 11.71	58.48 ± 7.45
16:1n7	310.33 ± 21.65	364.26 ± 29.99	383.47 ± 61.96	315.72 ± 44.00	266.56 ± 25.37	197.29 ± 16.29
17:0	29.76^{B} ± 1.46	30.37^{B} ± 2.12	50.31^{A} ± 5.72	39.98^{B} ± 2.47	29.50^{B} ± 4.99	30.20^{B} ± 2.20
18:0	1171.84^{ab} ± 79.52	1145.39^{ab} ± 107.12	1031.71^{b} ± 109.29	911.84^{b} ± 96.43	1356.30^{a} ± 67.52	1114.11^{ab} ± 92.39
18:1	4067.58 ± 774.92	4631.07 ± 641.46	4364.35 ± 679.31	4106.02 ± 429.16	4153.29 ± 812.80	3299.60 ± 675.01
18:2n6	1317.47^{B} ± 83.98	1486.33^{B} ± 103.15	1191.60^{B} ± 120.71	1174.14^{B} ± 123.57	2118.20^{A} ± 111.49	1998.97^{A} ± 182.23
18:3n3	31.33^{B} ± 3.75	35.31^{B} ± 4.49	40.08^{B} ± 7.16	33.54^{B} ± 8.04	108.22^{A} ± 6.02	102.51^{A} ± 12.93
20:2n6	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
20:3n6	28.27 ± 11.23	37.79 ± 19.60	11.03 ± 7.42	0.00 ± 0.00	3.28 ± 3.28	17.30 ± 8.88
20:4n6	0.00 ± 0.00	3.90 ± 1.56	0.00 ± 0.00	1.53 ± 1.53	1.99 ± 1.36	2.49 ± 1.88
22:4n6	7.46 ± 5.50	8.69 ± 6.17	2.66 ± 2.66	7.17 ± 5.10	0.00 ± 0.00	11.38 ± 5.73
TOTS	5185.20 ± 303.27	5421.28 ± 671.19	5451.79 ± 515.48	4934.50 ± 496.10	6073.45 ± 336.86	4937.58 ± 398.55
TOTM	4491.63 ± 798.37	5116.37 ± 671.19	4889.30 ± 745.13	4522.53 ± 478.89	4613.76 ± 832.62	3660.53 ± 690.79
TOTP	10510.60 ± 1573.68	8575.09 ± 1368.90	13557.49 ± 632.14	10431.47 ± 1857.14	12202.48 ± 1833.51	9208.38 ± 1623.91

[1]Values represent µg/ mg tissue ± SEM; N=9 for CF; 9 for CA; 9 for MA; 8 for MO; 9 for SA; 9 for SBO.

[2]Dietary treatments represent the maternal diet; CF = Chicken Fat; CA = Chicken + Antioxidant; SBO = Soybean Oil; SA = Soybean + Antioxidant; MO = Menhaden Oil; MA = Menhaden + Antioxidant; TOTS = Total Saturated Fatty Acid; TOTM = Total Monounsaturated Fatty Acid; TOTP = Total Polyunsaturated Fatty Acid.

[A-C] Means within a row lacking the same superscript differ significantly ($P<0.01$).

[a-b] Means within a row lacking the same superscript differ significant ($P<0.05$).

TABLE 8. MALE BODY WEIGHTS ACCORDING TO MATERNAL DIET (TRIAL 1)[1]

Age	CF	CA	MO	MA	SBO	SA
	(g)					
1 d	$37.16^{AB} \pm 0.42$	$36.80^{BC} \pm 0.32$	$35.80^{C} \pm 0.43$	$36.03^{BC} \pm 0.33$	$36.04^{BC} \pm 0.41$	$38.23^{A} \pm 0.38$
7 d	$107.83^{B} \pm 1.32$	$107.18^{B} \pm 0.18$	$108.03^{B} \pm 2.13$	$110.22^{B} \pm 1.74$	$112.45^{B} \pm 1.57$	$119.35^{A} \pm 1.66$
14 d	$318.66^{A} \pm 4.11$	$321.75^{A} \pm 4.20$	$314.13^{AB} \pm 5.44$	$298.87^{C} \pm 4.66$	$323.76^{A} \pm 3.74$	$303.85^{BC} \pm 4.12$
21 d	$646.63^{ab} \pm 10.26$	$628.80^{b} \pm 10.75$	$625.67^{b} \pm 14.03$	$616.56^{b} \pm 13.28$	$661.64^{a} \pm 9.40$	$638.80^{ab} \pm 12.59$
28 d	$1112.45^{A} \pm 19.15$	$1108.08^{A} \pm 15.40$	$1065.12^{B} \pm 22.12$	$963.72^{B} \pm 27.26$	$1119.08^{A} \pm 14.30$	$1115.36^{A} \pm 23.11$

[1]Dietary treatments represent the maternal diet; CF = Chicken Fat; CA = Chicken + Antioxidant; SBO = Soybean Oil; SA = Soybean + Antioxidant; MO = Menhaden Oil; MA = Menhaden + Antioxidant

$^{A-C}$ Means within a row lacking the same superscript differ significantly ($P<0.01$).

$^{a-c}$ Means within a row lacking the same superscript differ significant ($P<0.05$).

TABLE 9. MALE BODY WEIGHTS ACCORDING TO MATERNAL DIET (HATCH 2)[1]

Age	CF	CA	MO	MA	SBO	SA
	(g)					
1 d	44.15^{AB} ± 0.40	43.45^{B} ± 0.33	41.78^{B} ± 0.46	43.08^{B} ± 0.39	43.64^{AB} ± 0.37	44.84^{A} ± 0.36
7 d	129.33^{AB} ± 1.61	123.68^{C} ± 1.87	116.21^{C} ± 1.57	123.83^{BC} ± 1.82	128.73^{AB} ± 1.68	133.43^{A} ± 1.52
14 d	364.54^{A} ± 5.13	359.24^{A} ± 4.21	335.17^{B} ± 4.12	332.93^{B} ± 4.92	358.08^{A} ± 3.80	357.27^{A} ± 4.63
21 d	741.27^{A} ± 10.42	737.19^{AB} ± 9.45	671.93^{C} ± 14.07	660.50^{C} ± 15.55	734.00^{AB} ± 7.85	717.07^{B} ± 11.80
28 d	1215.59^{B} ± 19.78	1221.64^{AB} ± 18.18	1138.28^{B} ± 20.43	1036.92^{C} ± 32.39	1225.87^{A} ± 18.46	1221.96^{AB} ± 20.43

[1]Dietary treatments represent the maternal diet; CF = Chicken Fat; CA = Chicken + Antioxidant; SBO = Soybean Oil; SA = Soybean + Antioxidant; MO = Menhaden Oil; MA = Menhaden + Antioxidant

[A-C] Means within a row lacking the same superscript differ significantly ($P<0.01$).

TABLE 10. FEMALE BODY WEIGHTS ACCORDING TO MATERNAL DIET (HATCH 3)[1]

Age	CF	CA	MO	MA	SBO	SA
	(g)					
1 d	48.08^{A} ± 0.47	46.76^{B} ± 0.44	45.09^{C} ± 0.32	46.10^{BC} ± 0.39	46.11^{BC} ± 0.55	47.44^{AB} ± 0.38
7 d	125.89 ± 1.48	128.95 ± 1.58	125.78 ± 1.58	123.09 ± 2.08	126.37 ± 1.55	123.50 ± 1.48
14 d	288.79 ± 2.98	282.46 ± 3.57	290.72 ± 4.13	286.55 ± 4.63	295.58 ± 3.31	289.66 ± 3.74
21 d	657.05^{a} ± 7.60	617.63^{b} ± 9.67	624.43^{b} ± 10.94	628.73^{ab} ± 11.21	645.41^{ab} ± 9.00	631.34^{ab} ± 11.55
28 d	1048.74^{A} ± 14.27	993.48^{B} ± 12.48	952.92^{B} ± 15.58	1008.78^{B} ± 17.32	1024.54^{A} ± 12.51	1052.21^{A} ± 18.86

[1]Dietary treatments represent the maternal diet; CF = Chicken Fat; CA = Chicken + Antioxidant; SBO = Soybean Oil; SA = Soybean + Antioxidant; MO = Menhaden Oil; MA = Menhaden + Antioxidant

$^{A-C}$ Means within a row lacking the same superscript differ significantly ($P<0.01$).

$^{a-b}$ Means within a row lacking the same superscript differ significant ($P<0.05$).

TABLE 11. EGG WEIGHTS ACCORDING TO MATERNAL DIETS[1]

Wk of Lay	CF	CA	MO	MA	SBO	SA
	(g)					
2	$51.25^{a} \pm 0.87$	$48.17^{bc} \pm 1.08$	$48.81^{abc} \pm 0.95$	$47.96^{bc} \pm 0.81$	$47.20^{c} \pm 1.70$	$50.74^{ab} \pm 0.71$
6	$60.86^{A} \pm 0.35$	$59.96^{AB} \pm 0.42$	$57.51^{C} \pm 0.47$	$56.68^{C} \pm 0.41$	$58.99^{B} \pm 0.42$	$60.49^{A} \pm 0.34$
10	$63.81^{A} \pm 0.71$	$63.61^{A} \pm 0.43$	$60.87^{B} \pm 0.35$	$61.49^{B} \pm 0.46$	$62.02^{B} \pm 0.50$	$64.44^{A} \pm 0.44$
14	$66.63^{A} \pm 0.55$	$65.77^{A} \pm 0.48$	$62.92^{B} \pm 0.51$	$63.94^{B} \pm 0.38$	$65.62^{A} \pm 0.47$	$66.18^{A} \pm 0.42$
18	$68.25^{A} \pm 0.56$	$67.84^{A} \pm 0.57$	$65.49^{B} \pm 0.40$	$65.50^{B} \pm 0.53$	$68.21^{A} \pm 0.50$	$68.03^{A} \pm 0.40$
22	$70.09^{a} \pm 0.65$	$67.08^{ab} \pm 0.56$	$67.39^{b} \pm 0.47$	$67.99^{b} \pm 0.62$	$68.94^{ab} \pm 0.55$	$68.95^{ab} \pm 0.42$

[1]Dietary treatments represent the maternal diet; CF = Chicken Fat; CA = Chicken + Antioxidant; SBO = Soybean Oil; SA = Soybean + Antioxidant; MO = Menhaden Oil; MA = Menhaden + Antioxidant

$^{A-C}$ Means within a row lacking the same superscript differ significantly ($P<0.01$).

$^{a-c}$ Means within a row lacking the same superscript differ significant ($P<0.05$).

TABLE 12. MALE TIBIAE MEASUREMENTS ACCORDING TO MATERNAL DIET AT 14 DOA (TRIAL 1)[1]

	CF	CA	MO	MA	SBO	SA
Tibia Wt.(g)	3.45 ± 0.08	3.69 ± 0.10	3.39 ± 0.08	3.38 ± 0.11	3.51 ± 0.07	3.50 ± 0.09
Tibia Len. (mm)	56.08 ± 0.36	57.05 ± 0.49	56.72 ± 0.51	55.69 ± 0.35	56.62 ± 0.29	56.44 ± 0.36
Diameter (mm)	4.51^{AB} ± 0.08	4.73^{A} ± 0.09	4.49^{AB} ± 0.09	4.72^{A} ± 0.04	4.38^{B} ± 0.08	4.32^{B} ± 0.09
Force (N)	293.96 ± 17.56	308.13 ± 12.80	283.64 ± 14.43	296.75 ± 10.52	268.08 ± 10.31	291.96 ± 13.14
Energy (N-mm)	207.80 ± 31.86	252.04 ± 30.12	203.80 ± 22.11	235.20 ± 32.66	223.00 ± 20.81	199.96 ± 19.25
Bone Wall (mm)	1.41 ± 0.04	1.39 ± 0.06	1.45 ± 0.05	1.29 ± 0.05	1.38 ± 0.03	1.29 ± 0.06
Stress (MPa)	14.36 ± 0.93	14.32 ± 0.91	13.89 ± 0.70	14.29 ± 0.60	13.75 ± 0.62	16.05 ± 0.68

[1]Dietary treatments represent the maternal diet; CF = Chicken Fat; CA = Chicken + Antioxidant; SBO = Soybean Oil; SA = Soybean + Antioxidant; MO = Menhaden Oil; MA = Menhaden + Antioxidant

[A-B] Means within a row lacking the same superscript differ significantly ($P<0.01$).

TABLE 13. MALE TIBIAE WEIGHTS ACCORDING TO MATERNAL DIET AT 28 DOA (TRIAL1)[1]

	CF	CA	MO	MA	SBO	SA
Tibia Wt. (g)	12.00^{a} ± 0.32	11.86^{ab} ± 0.28	11.61^{ab} ± 0.28	10.98^{b} ± 0.35	12.45^{a} ± 0.28	12.45^{a} ± 0.34
Tibia Len. (mm)	85.16 ± 0.52	85.16 ± 0.67	84.71 ± 0.55	83.5 ± 0.71	85.97 ± 0.57	84.34 ± 0.61
Diameter (mm)	7.85^{A} ± 0.17	7.32^{BC} ± 0.13	7.12^{CD} ± 0.16	6.77^{D} ± 0.11	7.62^{AB} ± 0.14	7.50^{B} ± 0.14
Force (N)	494.65^{B} ± 31.04	491.52^{B} ± 34.57	497.68^{B} ± 41.19	441.83^{B} ± 36.74	634.32^{A} ± 58.63	628.50^{A} ± 49.31
Energy (N-mm)	456.26^{b} ± 62.14	519.04^{ab} ± 62.55	463.88^{b} ± 81.27	461.70^{b} ± 68.55	757.25^{a} ± 110.33	616.04^{ab} ± 93.49
Bone Wall (mm)	2.01^{A} ± 0.04	1.88^{AB} ± 0.03	1.90^{AB} ± 0.04	1.65^{C} ± 0.04	1.79^{B} ± 0.05	2.00^{A} ± 0.03
Stress (MPa)	8.75^{b} ± 0.43	10.03^{b} ± 0.60	10.24^{ab} ± 0.78	10.70^{ab} ± 0.86	12.70^{a} ± 1.05	10.04^{ab} ± 0.94

[1]Dietary treatments represent the maternal diet; CF = Chicken Fat; CA = Chicken + Antioxidant; SBO = Soybean Oil; SA = Soybean + Antioxidant; MO = Menhaden Oil; MA = Menhaden + Antioxidant

$^{A-D}$ Means within a row lacking the same superscript differ significantly ($P<0.01$).

$^{a-b}$ Means within a row lacking the same superscript differ significant ($P<0.05$).

TABLE 14. MALE TIBIAE MEASUREMENTS ACCORDING TO MATERNAL DIET AT 14 DOA (TRIAL 2)[1]

	CF	CA	MO	MA	SBO	SA
Tibia Wt. (g)	3.59 ± 0.09	3.88 ± 0.10	3.52 ± 0.80	3.52 ± 0.76	3.84 ± 0.10	3.86 ± 0.06
Tibia Len. (mm)	57.48 ± 0.39	58.41 ± 0.41	56.23 ± 0.89	54.18 ± 2.13	57.06 ± 0.60	57.93 ± 0.34
Diameter (mm)	4.68 ± 0.39	4.37 ± 0.09	4.49 ± 0.08	4.54 ± 1.07	4.32 ± 0.08	4.71 ± 0.08
Force (N)	340.04^{b} ± 17.10	391.16^{a} ± 18.40	337.72^{b} ± 12.00	312.01^{b} ± 19.77	354.80^{b} ± 15.09	333.04^{b} ± 11.80
Energy (N-mm)	284.32^{AB} ± 33.45	340.76^{A} ± 32.69	195.20^{C} ± 22.74	231.08^{BC} ± 25.74	293.58^{AB} ± 24.82	297.28^{AB} ± 30.83
Bone Wall (mm)	1.51 ± 0.05	1.72 ± 0.05	1.56 ± 0.03	1.75 ± 0.26	1.58 ± 0.04	1.52 ± 0.04
Stress (MPa)	14.98^{B} ± 0.70	18.09^{A} ± 0.90	15.60^{B} ± 0.60	14.77^{B} ± 0.85	16.79^{AB} ± 0.61	14.72^{B} ± 0.77

[1]Dietary treatments represent the maternal diet; CF = Chicken Fat; CA = Chicken + Antioxidant; SBO = Soybean Oil; SA = Soybean + Antioxidant; MO = Menhaden Oil; MA = Menhaden + Antioxidant

[A-B] Means within a row lacking the same superscript differ significantly ($P<0.01$).

[a-b] Means within a row lacking the same superscript differ significant ($P<0.05$).

TABLE 15. MALE TIBIAE MEASUREMENTS ACCORDING TO MATERNAL DIET AT 28 DOA (TRIAL 2)[1]

	CF	CA	MO	MA	SBO	SA
Tibia Wt. (g)	12.77^{B} ± 0.25	13.52^{B} ± 0.29	12.43^{B} ± 0.29	11.01^{B} ± 0.42	20.76^{A} ± 8.03	14.68^{B} ± 0.32
Tibia Len. (mm)	86.65^{B} ± 0.49	88.58^{A} ± 0.47	85.82^{BC} ± 0.63	84.64^{C} ± 0.74	86.66^{B} ± 0.50	85.04^{BC} ± 0.52
Diameter (mm)	7.77^{A} ± 0.13	7.76^{A} ± 0.16	7.72^{A} ± 0.18	7.08^{B} ± 0.13	7.51^{AB} ± 0.16	7.40^{AB} ± 0.16
Force (N)	807.12^{A} ± 60.58	735.84^{AB} ± 51.90	614.80^{B} ± 34.62	616.83^{B} ± 45.13	693.16^{AB} ± 48.96	594.41^{B} ± 39.79
Bone Wall (mm)	2.07^{A} ± 0.03	1.94^{B} ± 0.04	1.75^{C} ± 0.03	1.74^{C} ± 0.03	1.85^{BC} ± 0.03	1.86^{BC} ± 0.03
Stress (MPa)	14.30 ± 1.08	13.30 ± 0.73	12.37 ± 0.71	13.56 ± 0.86	13.68 ± 0.82	12.04 ± 0.74

[1]Dietary treatments represent the maternal diet; CF = Chicken Fat; CA = Chicken + Antioxidant; SBO = Soybean Oil; SA = Soybean + Antioxidant; MO = Menhaden Oil; MA = Menhaden + Antioxidant

A-C Means within a row lacking the same superscript differ significantly ($P<0.01$).

TABLE 16. FEMALE TIBIAE MEASUREMENTS ACCORDING TO MATERNAL DIET AT 14 DOA (TRIAL 3)[1]

	CF	CA	MO	MA	SBO	SA
Tibia Wt. (g)	3.79^{A} ± 0.10	3.36^{C} ± 0.06	3.41^{BC} ± 0.07	3.33^{C} ± 0.11	3.66^{AB} ± 0.11	3.93^{A} ± 0.11
Tibia Len. (mm)	55.86^{ab} ± 0.45	55.16^{b} ± 0.34	56.28^{ab} ± 0.30	55.29^{b} ± 0.44	56.93^{a} ± 0.49	56.02^{ab} ± 0.34
Diameter (mm)	5.01^{A} ± 0.10	4.71^{B} ± 0.07	4.60^{B} ± 0.07	4.65^{B} ± 0.08	4.63^{B} ± 0.07	4.68^{B} ± 0.07
Force (N)	340.56^{A} ± 10.67	281.72^{C} ± 12.32	330.48^{AB} ± 14.68	293.95^{BC} ± 13.05	331.84^{AB} ± 11.01	326.58^{AB} ± 12.88
Energy (N-mm)	269.80^{A} ± 27.66	170.36^{B} ± 17.50	188.44^{B} ± 23.01	178.83^{B} ± 26.43	260.72^{A} ± 23.46	258.41^{A} ± 27.13
Bone Wall (mm)	1.37 ± 0.02	1.35 ± 0.03	1.26 ± 0.04	1.39 ± 0.05	1.31 ± 0.03	1.44 ± 0.03
Stress (MPa)	14.50^{BC} ± 0.63	12.91^{C} ± 0.48	16.44^{A} ± 0.59	14.64^{BC} ± 0.76	16.13^{AB} ± 0.56	14.69^{BC} ± 0.43

[1]Dietary treatments represent the maternal diet; CF = Chicken Fat; CA = Chicken + Antioxidant; SBO = Soybean Oil; SA = Soybean + Antioxidant; MO = Menhaden Oil; MA = Menhaden + Antioxidant

A-C Means within a row lacking the same superscript differ significantly ($P<0.01$).

TABLE 17. FEMALE TIBIAE MEASUREMENTS ACCORDING TO MATERNAL DIET AT 28 DOA[1]

	CF	CA	MO	MA	SBO	SA
Tibia Wt. (g)	11.91^{A} ± 0.25	9.88^{C} ± 0.24	10.05^{C} ± 0.23	10.35^{B} ± 0.21	10.41^{B} ± 0.07	10.77^{B} ± 0.25
Tibia Len. (mm)	84.39 ± 0.45	82.41 ± 0.49	83.72 ± 0.42	84.22 ± 0.59	84.04 ± 0.64	84.30 ± 0.50
Diameter (mm)	7.90^{A} ± 0.15	7.24^{BC} ± 0.13	7.46^{B} ± 0.12	7.26^{B} ± 0.13	6.85^{C} ± 0.14	7.50^{B} ± 0.14
Force (N)	496.24^{A} ± 46.54	344.88^{BC} ± 20.78	431.35^{AB} ± 29.91	394.78^{BC} ± 35.04	314.56^{C} ± 10.31	366.88^{BC} ± 21.20
Energy (N-mm)	349.37 ± 67.34	210.24 ± 28.38	260.04 ± 43.91	234.66 ± 50.45	181.88 ± 17.14	255.12 ± 48.84
Bone Wall (mm)	1.51 ± 0.05	1.60 ± 0.05	1.62 ± 0.05	1.59 ± 0.04	1.76 ± 0.05	1.63 ± 0.05
Stress (MPa)	10.88^{A} ± 1.07	8.10^{B} ± 0.45	9.44^{AB} ± 0.57	9.25^{AB} ± 0.79	7.50^{B} ± 0.38	8.21^{B} ± 0.51

[1]Dietary treatments represent the maternal diet; CF = Chicken Fat; CA = Chicken + Antioxidant; SBO = Soybean Oil; SA = Soybean + Antioxidant; MO = Menhaden Oil; MA = Menhaden + Antioxidant

A-C Means within a row lacking the same superscript differ significantly ($P<0.01$).

TABLE 18. SHANK AND KEEL MEASUREMENTS OF 4 WOA MALE CHICKS[1]

	cm					
	CF	**CA**	**MO**	**MA**	**SBO**	**SA**
Shank	$7.986^{B} \pm 0.0978$	$8.354^{A} \pm 0.0605$	$8.113^{B} \pm 0.0822$	$8.020^{B} \pm 0.0668$	$8.054^{B} \pm 0.0816$	$7.876^{B} \pm 0.1165$
Shank/BW	$0.007^{BC} \pm 0.0001$	$0.007^{C} \pm 0.0001$	$0.007^{B} \pm 0.0001$	$0.007^{BC} \pm 0.0001$	$0.007^{B} \pm 0.0001$	$0.008^{A} \pm 0.0002$
Keel	$9.208^{D} \pm 0.0083$	$10.573^{A} \pm 0.0983$	$10.013^{B} \pm 0.1348$	$9.652^{BC} \pm 0.1340$	$10.487^{A} \pm 0.0776$	$9.528^{CD} \pm 0.1273$
Keel/BW	$0.008^{C} \pm 0.0002$	$0.009^{A} \pm 0.0001$	$0.009^{B} \pm 0.0001$	$0.008^{BC} \pm 0.0001$	$0.009^{A} \pm 0.0001$	$0.009^{A} \pm 0.0002$

[1]Values represent mean ± standard error; N=23 for CF; 26 for CA; 25 for MA; 24 for MO; 23 for SA; 25 for SBO.
[2]Dietary treatments represent the maternal diet; CF = Chicken Fat; CA = Chicken + Antioxidant; SBO = Soybean Oil; SA = Soybean + Antioxidant; MO = Menhaden Oil; MA = Menhaden + Antioxidant.
[A-D] Means within a row lacking the same superscript differ significantly ($P<0.01$)

TABLE 19. SHANK AND KEEL MEASUREMENT OF MALES CHICKS 4-WOA TRIAL 2[1]

	cm					
	CF	**CA**	**MO**	**MA**	**SBO**	**SA**
SHANK	$8.551^{AB} \pm 0.055$	$8.368^{BC} \pm 0.055$	$8.680^{A} \pm 0.131$	$8.192^{BC} \pm 0.091$	$8.560^{AB} \pm 0.064$	$8.428^{ABC} \pm 0.076$
SHANK/BW	$0.006^{C} \pm 0.0001$	$0.006^{C} \pm 0.0001$	$0.007^{B} \pm 0.0001$	$0.008^{A} \pm 0.0001$	$0.007^{C} \pm 0.0001$	$0.007^{B} \pm 0.0001$
KEEL	$10.829^{A} \pm 0.115$	$10.340^{B} \pm 0.142$	$10.952^{A} \pm 0.111$	$10.31^{B} \pm 0.133$	$10.691^{AB} \pm 0.091$	$10.568^{AB} \pm 0.152$
KEEL/BW	$0.008^{C} \pm 0.0001$	$0.008^{C} \pm 0.0001$	$0.029^{AB} \pm 0.0001$	$0.010^{A} \pm 0.0002$	$0.008^{C} \pm 0.0001$	$0.009^{B} \pm 0.0001$

[1]Values represent mean ± standard error; N=22 for CF; 27 for CA; 25 for MO; 25 for MA; 25 for SBO; 23 for SA.

[2]Dietary treatments represent the maternal diet; CF = Chicken Fat; CA = Chicken + Antioxidant; SBO = Soybean Oil; SA = Soybean + Antioxidant; MO = Menhaden Oil; MA = Menhaden + Antioxidant.

[A-C] Means within a row lacking the same superscript differ significantly ($P<0.01$).

TABLE 20. FEMALE SHANK AND KEEL MEASUREMENTS 4 WOA TRIAL 3[1]

	cm					
	CF	**CA**	**MO**	**MA**	**SBO**	**SA**
Shank	$7.616^{B} \pm 0.053$	$8.030^{A} \pm 0.066$	$7.725^{B} \pm 0.045$	$7.769^{B} \pm 0.072$	$7.725^{B} \pm 0.069$	$7.792^{B} \pm 0.045$
Shank/BW	0.007 ± 0.0001	0.007 ± 0.0001	0.007 ± 0.0001	0.007 ± 0.0001	0.007 ± 0.0001	0.007 ± 0.0001
Keel	$9.408^{C} \pm 0.102$	$9.917^{A} \pm 0.087$	$9.612^{BC} \pm 0.087$	$9.643^{BC} \pm 0.088$	$9.820^{AB} \pm 0.092$	$9.800^{AB} \pm 0.059$
Keel/BW	$0.009^{AB} \pm 0.0001$	$0.008^{C} \pm 0.001$	$0.009^{A} \pm 0.0001$	$0.009^{B} \pm 0.0001$	$0.009^{AB} \pm 0.0001$	$0.009^{A} \pm 0.0001$

[1]Values represent mean ± standard error; N=23 for CF; 25 for CA; 23 for MA; 24 for MO; 24 for SA; 25 for SBO.
[2]Dietary treatments represent the maternal diet; CF = Chicken Fat; CA = Chicken + Antioxidant; SBO = Soybean Oil; SA = Soybean + Antioxidant; MO = Menhaden Oil; MA = Menhaden + Antioxidant.
[A-C] Means within a row lacking the same superscript differ significantly ($P<0.01$).

TABLE 21. MALE SHANK AND KEEL MEASUREMENTS OF 4 WOA MALE CHICKS TRIAL 3[1]

	cm					
	CF	**CA**	**MO**	**MA**	**SBO**	**SA**
Shank	7.758 ± 0.141	7.900 ± 0.069	8.017 ± 0.166	7.680 ± 0.070	7.932 ± 0.052	7.744 ± 0.081
Shank/BW	0.007 ± 0.0001	0.007 ± 0.0001	0.007 ± 0.0001	0.007 ± 0.0001	0.007 ± 0.0001	0.007 ± 0.0001
Keel	9.179 ± 0.097	9.354 ± 0.101	9.473 ± 0.156	9.376 ± 0.138	9.704 ± 0.102	9.304 ± 0.090
Keel/BW	0.009 ± 0.0001	0.008 ± 0.0002	0.008 ± 0.0001	0.009 ± 0.0002	0.009 ± 0.0001	0.008 ± 0.0001

No significant differences (P>0.01) or (P<0.05)

[1]Values represent mean ± standard error; N=22 for CF; 27 for CA; 25 for MA; 25 for MO; 23 for SA; 25 for SBO.

[2]Dietary treatments represent the maternal diet; CF = Chicken Fat; CA = Chicken + Antioxidant; SBO = Soybean Oil; SA = Soybean + Antioxidant; MO = Menhaden Oil; MA = Menhaden + Antioxidant.

[A-C] Means within a row lacking the same superscript differ significantly (P<0.01).

TABLE 22. SHEAR FORCE REQUIRED TO BREAK MALE TIBIAE ACCORDING TO MATERNAL DIET[1]

	(N ± SEM)	
DIETS	**14 DOA**	**28 DOA**
CF	293.96 ± 17.56	494.65^{B} ± 31.04
CA	308.13 ± 12.80	491.52^{B} ± 34.57
MO	283.64 ± 14.43	497.68^{B} ± 41.19
MA	296.75 ± 10.52	441.83^{B} ± 36.74
SBO	268.03 ± 10.13	634.32^{A} ± 58.63
SA	291.96 ± 13.14	628.50^{A} ± 49.31

[1]Values represent mean ± standard error; N=22 for CF; 27 for CA; 25 for MA; 25 for MO; 23 for SA; 25 for SBO.
[2]Dietary treatments represent the maternal diet; CF = Chicken Fat; CA = Chicken + Antioxidant; SBO = Soybean Oil; SA = Soybean + Antioxidant; MO = Menhaden Oil; MA = Menhaden + Antioxidant.
[A-B] Means within a column lacking the same superscript differ significantly ($P<0.01$).

TABLE 23. SHEAR STRESS REQUIRED TO BREAK MALE CHICK TIBIAE ACCORDING TO MATERNAL DIET.
TRIAL 2[1]

	(N/mm^2 ± SEM)	
DIETS	**14DOA**	**28DOA**
CF	8.75^{b} ± 0.434	14.36 ± 0.939
CA	10.03^{b} ± 0.602	14.32 ± 0.911
MO	10.24^{ab} ± 0.786	13.89 ± 0.700
MA	10.70^{ab} ± 0.869	14.29 ± 0.602
SBO	12.70^{a} ± 1.051	13.75 ± 0.620
SA	11.04^{ab} ± 0.944	16.05 ± 0.680

[1]Values represent mean ± standard error; N=22 for CF; 27 for CA; 25 for MA; 25 for MO; 23 for SA; 25 for SBO.
[2]Dietary treatments represent the maternal diet; CF = Chicken Fat; CA = Chicken + Antioxidant; SBO = Soybean Oil; SA = Soybean + Antioxidant; MO = Menhaden Oil; MA = Menhaden + Antioxidant.
[A-B] Means within a row lacking the same superscript differ significantly ($P<0.01$).

TABLE 24. ANALYSIS OF VARIANCE FOR PARAMETER OF MALE CHICK TIBIAE ACCORDING TO MATERNAL DIETAT 14 DOA. (TRIAL 1)

Fatty Acid	Source of Variation	df	Mean Square	F	P
Tibia wt.	Maternal diet	5	1.657		
	Error	143	31.581	1.50	0.1932
Total		148			
Tibia wt./BW	Maternal diet	5	1.1×10^{-5}		
	Error	143	1.7×10^{-4}	1.95	0.0899
Total		148			
Tibia Length	Maternal diet	5	29.536		
	Error	143	5.8×10^{2}	1.43	0.2156
Total		148			
Tibia Length/BW	Maternal diet	5	5.6×10^{-3}		
	Error	143	2.9×10^{2}	5.54	0.0001
Total		148			
Diameter	Maternal diet	5	3.525		
	Error	143	26.044	3.87	0.0025
Total		148			
Force	Maternal diet	5	2.1×10^{4}		
	Error	140	6.1×10^{5}	0.99	0.4280
Total		145			
Stress	Maternal diet	5	84.894		
	Error	140	1.9×10^{3}	1.23	0.2989
Total		145			
Bone Wall	Maternal diet	5	5.3×10^{-6}		
	Error	140	1.2×10^{-4}	1.20	0.3115
Total		145			

TABLE 25. ANALYSIS OF VARIANCE FOR PARAMETER OF MALE CHICK TIBIAE ACCORDING TO MATERNAL DIET AT 14 DOA . (TRIAL 1)

Fatty Acid	Source of Variation	df	Mean Square	F	P
Tibia wt.	Maternal diet	5	38.345		
	Error	144	3.6×10^{2}	3.02	0.0126
Total		149			
Tibia wt./BW	Maternal diet	5	1.8×10^{-5}		
	Error	144	2.4×10^{-4}	2.27	0.506
Total		149			
Tibia Length	Maternal diet	5	83.201		
	Error	144	1.3×10^{3}	1.76	0.1245
Total		149			
Tibia Length/BW	Maternal diet	5	1.9×10^{-3}		
	Error	144	1.4×10^{2}	3.90	0.0024
Total		144			
Diameter	Maternal diet	5	25.803		
	Error	144	81.125	9.16	0.0001
Total		149			
Force	Maternal diet	5	7.8×10^{5}		
	Error	140	6.3×10^{6}	3.34	0.0058
Total		145			
Stress	Maternal diet	5	2.0×10^{2}		
	Error	141	2.2×10^{3}	2.57	0.0293
Total		146			
Bone Wall	Maternal diet	5	2.235		
	Error	141	7.474	8.43	0.0001
Total		146			

TABLE 26. ANALYSIS OF VARIANCE FOR PARAMETER OF MALE CHICK TIBIAE ACCORDING TO MATERNAL DIET AT14 DOA. (TRIAL 2)

Fatty Acid	Source of Variation	df	Mean Square	F	P
Tibia wt.	Maternal diet	5	3.0×10^{3}		
	Error	144	9.4×10^{4}	0.92	0.4668
Total		149			
Tibia wt./BW	Maternal diet	5	3.7×10^{-2}		
	Error	144	1.127	0.96	0.4415
Total		149			
Tibia Length	Maternal diet	5	2.8×10^{2}		
	Error	144	3.6×10^{3}	2.25	0.0528
Total		149			
Tibia Length/BW	Maternal diet	5	3.3×10^{-4}		
	Error	144	6.6×10^{-2}	0.15	0.9806
Total		149			
Diameter	Maternal diet	5	25.067		
	Error	144	713.781	1.01	0.4132
Total		149			
Force	Maternal diet	5	8.5×10^{4}		
	Error	144	9.2×10^{5}	2.67	0.0243
Total		149			
Stress	Maternal diet	5	2.2×10^{2}		
	Error	144	2.0×10^{3}	3.23	0.0085
Total		149			
Bone Wall	Maternal diet	5	1.7×10^{3}		
	Error	144	5.1×10^{4}	0.98	0.4316
Total		149			

TABLE 27. ANALYSIS OF VARIANCE FOR PARAMETER OF MALE CHICK TIBIAE ACCORDING TO MATERNAL DIETAT 28 DOA. (TRIAL 2)

Fatty Acid	Source of Variation	df	Mean Square	F	P
Tibia wt.	Maternal diet	5	1.5×10^{3}		
	Error	144	3.9×10^{4}	1.17	0.3250
Total		149			
Tibia wt./BW	Maternal diet	5	9.7×10^{-4}		
	Error	144	2.5×10^{-2}	1.10	0.3628
Total		149			
Tibia Length	Maternal diet	5	2.4×10^{2}		
	Error	144	1.1×10^{-3}	6.11	0.0001
Total		149			
Tibia Length/BW	Maternal diet	5	3.7×10^{-3}		
	Error	144	6.7×10^{-3}	16.67	0.0001
Total		149			
Diameter	Maternal diet	5	9.173		
	Error	144	90.8840	2.91	0.0156
Total		149			
Force	Maternal diet	5	8.6×10^{5}		
	Error	144	7.9×10^{6}	3.07	0.0115
Total		149			
Stress	Maternal diet	5	89.045		
	Error	144	2.4×10^{3}	1.03	0.4034
Total		149			
Bone Wall	Maternal diet	5	1.935		
	Error	144	6.031	9.24	0.0001
Total		149			

TABLE 28. ANALYSIS OF VARIANCE FOR PARAMETER OF FEMALE CHICK TIBIAE ACCORDING TO MATERNAL DIET AT 14 DOA. (TRIAL 3)

Fatty Acid	Source of Variation	df	Mean Square	F	P
Tibia wt.	Maternal diet	5	7.867		
	Error	143	35.206	6.39	0.0001
Total		148			
Tibia wt./BW	Maternal diet	5	3.6×10^{-5}		
	Error	143	2.0×10^{-4}	5.03	0.0003
Total		148			
Tibia Length	Maternal diet	5	52.646		
	Error	143	574.638	2.62	0.0267
Total		148			
Tibia Length/BW	Maternal diet	5	3.2×10^{-3}		
	Error	143	2.8×10^{-2}	3.31	0.0074
Total		148			
Diameter	Maternal diet	5	3.236		
	Error	143	25.004	3.70	0.0035
Total		148			
Force	Maternal diet	5	6.9×10^{4}		
	Error	142	5.4×10^{5}	3.63	0.0040
Total		147			
Stress	Maternal diet	5	2.0×10^{2}		
	Error	142	1.2×10^{3}	4.75	0.0005
Total		147			
Bone Wall	Maternal diet	5	23.190		
	Error	142	6.8×10^{2}	0.96	0.4423
Total		147			

TABLE 29. ANALYSIS OF VARIANCE FOR PARAMETER OF FEMALE CHICK TIBIAE ACCORDING TO MATERNAL DIET AT 28 DOA. (TRIAL 3)

Fatty Acid	Source of Variation	df	Mean Square	F	P
Tibia wt.	Maternal diet	5	63.887		
	Error	143	199.846	9.14	0.0001
Total		148			
Tibia wt./BW	Maternal diet	5	1.2×10^{-5}		
	Error	143	1.5×10^{-4}	2.27	0.0508
Total		148			
Tibia Length	Maternal diet	5	68.982		
	Error	143	1.0×10^{3}	1.97	0.863
Total		148			
Tibia Length/BW	Maternal diet	5	2.1×10^{-3}		
	Error	143	8.1×10^{-2}	7.68	0.0001
Total		148			
Diameter	Maternal diet	5	15.307		
	Error	143	70.559	6.20	0.0001
Total		148			
Force	Maternal diet	5	5.2×10^{5}		
	Error	141	2.7×10^{6}	4.92	0.0003
Total		146			
Stress	Maternal diet	5	1.8×10^{2}		
	Error	141	1.5×10^{3}	3.32	0.0073
Total		146			
Bone Wall	Maternal diet	5	0.799		
	Error	142	10.398	2.18	0.0593
Total		147			

TABLE 30. ANALYSIS OF VARIANCE OF MALE BW ACCORDINGTO MATERNAL DIET. (TRIAL 1)

Age	Source of Variation	df	Mean Square	F	P
1d	Maternal diet	5	2.6×10^2		
	Error	378	3.6×10^3	5.52	0.0001
Total		383			
7d	Maternal diet	5	1.0×10^4		
	Error	373	6.5×10^4	11.52	0.0001
Total		378			
14d	Maternal diet	5	3.2×10^4		
	Error	373	4.5×10^5	5.30	0.0001
Total		378			
21d	Maternal diet	5	5.7×10^4		
	Error	216	1.1×10^6	2.27	0.0487
Total		221			
28d	Maternal diet	5	6.7×10^5		
	Error	216	3.3×10^6	8.65	0.0001
Total		221			

TABLE 31. ANALYSIS OF VARIANCE OF MALE BW ACCORDING TO MATERNAL DIET. (TRIAL 2)

Age	Source of Variation	df	Mean Square	F	P
1d	Maternal diet	5	4.3×10^3		
	Error	410	4.0×10^2	7.61	0.0001
Total		415			
7d	Maternal diet	5	1.1×10^4		
	Error	405	7.9×10^4	11.56	0.0001
Total		410			
14d	Maternal diet	5	5.5×10^4		
	Error	368	4.7×10^5	8.63	0.0001
Total		373			
21d	Maternal diet	5	2.8×10^5		
	Error	219	1.0×10^6	11.51	0.0001
Total		224			
28d	Maternal diet	5	8.5×10^5		
	Error	141	1.7×10^6	13.99	0.0001
Total		146			

TABLE 32. ANALYSIS OF VARIANCE OF MALE BW ACCORDING TO MATERNAL DIET. (TRIAL 3)

Age	Source of Variation	df	Mean Square	F	P
1d	Maternal diet	5	2.3×10^2		
	Error	395	5.5×10^3	3.39	0.0051
Total		400			
7d	Maternal diet	5	1.4×10^4		
	Error	391	7.4×10^4	15.65	0.0001
Total		396			
14d	Maternal diet	5	2.9×10^4		
	Error	361	3.9×10^5	5.33	0.0001
Total		366			
21d	Maternal diet	5	4.8×10^4		
	Error	208	1.0×10^6	2.01	0.0788
Total		213			
28d	Maternal diet	5	1.8×10^5		
	Error	199	2.9×10^6	2.59	0.0271
Total		204			

TABLE 33. ANALYSIS OF VARIANCE OF FEMALE BW ACCORDING TO MATERNAL DIET. (TRIAL 3)

Age	Source of Variation	df	Mean Square	F	P
1d	Maternal diet	5	3.7×10^2		
	Error	387	4.9×10^3	5.86	0.001
Total		392			
7d	Maternal diet	5	1.4×10^3		
	Error	386	6.6×10^4	1.66	0.1435
Total		391			
14d	Maternal diet	5	6.1×10^3		
	Error	356	2.9×10^5	1.47	0.2000
Total		361			
21d	Maternal diet	5	4.1×10^4		
	Error	210	7.5×10^5	2.29	0.0469
Total		215			
28d	Maternal diet	5	5.3×10^5		
	Error	199	1.5×10^6	13.87	0.0001
Total		204			

TABLE 34. ANALYSIS OF VARIANCE FOR FATTY ACID COMPOSITION OF 4 WOA CHICK YOLK TISSUE ACCORDING TO MATERNAL DIET. (TRIAL 1)

Fatty Acid	Source of Variation	df	Mean Square	F	P
14:1N5	Maternal diet	5	1.6×10^{3}		
	Error	48	3.3×10^{3}	4.47	0.0021
Total		53			
15:0	Maternal diet	5	8.7×10^{2}		
	Error	48	1.0×10^{3}	8.25	0.0001
Total		53			
16:0	Maternal diet	5	3.5×10^{6}		
	Error	48	7.2×10^{7}	0.47	0.7981
Total		53			
t16:1N7	Maternal diet	5	4.5×10^{3}		
	Error	48	5.2×10^{4}	1.40	0.2406
Total		53			
16:1N7	Maternal diet	5	1.6×10^{6}		
	Error	48	1.5×10^{7}	3.35	0.0113
Total		53			
17:0	Maternal diet	5	3.0×10^{3}		
	Error	48	5.2×10^{3}	2.48	0.0448
Total		53			
18:0	Maternal diet	5	9.8×10^{6}		
	Error	48	2.6×10^{7}	3.41	0.0103
Total		53			
18:1	Maternal diet	5	3.7×10^{7}		
	Error	48	2.3×10^{8}	2.40	0.0504
Total		53			

TABLE 35. ANALYSIS OF VARIANCE FOR FATTY ACID COMPOSITION OF 4 WOA CHICK YOLK TISSUE ACCORDING TO MATERNAL DIET. (TRIAL 1)

Fatty Acid	Source of Variation	df	Mean Square	F	P
18:2N6	Maternal diet	5	4.4×10^{2}		
	Error	48	2.1×10^{3}	2.28	0.0616
Total		53			
18:3N6	Maternal diet	5	6.8×10^{6}		
	Error	48	9.7×10^{6}	4.27	0.0027
Total		53			
18:3N3	Maternal diet	5	3.9×10^{2}		
	Error	48	3.4×10^{3}	1.00	0.4282
Total		53			
20:1N9	Maternal diet	5	4.5×10^{4}		
	Error	48	2.3×10^{4}	9.81	0.0001
Total		53			
20:2N6	Maternal diet	5	4.0×10^{3}		
	Error	48	3.7×10^{4}	0.70	0.6246
Total		53			
20:3N6	Maternal diet	5	3.5×10^{3}		
	Error	48	1.4×10^{5}	0.18	0.9673
Total		53			
20:4N6	Maternal diet	5	1.4×10^{3}		
	Error	48	9.6×10^{3}	1.21	0.3185
Total		53			
22:4N6	Maternal diet	5	4.2×10^{7}		
	Error	48	4.1×10^{8}	1.57	0.1867
Total		53			

TABLE 36. ANALYSIS OF VARIANCE FOR FATTY ACID COMPOSITION OF 4 WOA CHICK YOLK TISSUE ACCORDING TO MATERNAL DIET. (TRIAL 1)

Fatty Acid	Source of Variation	df	Mean Square	F	P
22:5N3	Maternal diet	5	2.1×10^8		
	Error	48	9.1×10^8	2.48	0.0442
Total		53			
22:6N3	Maternal diet	5	1.0×10^6		
	Error	48	3.2×10^6	1.96	0.1021
Total		53			
TOTS	Maternal diet	5	2.3×10^9		
	Error	48	4.4×10^8	1.74	0.1436
Total		53			
TOTM	Maternal diet	5	3.8×10^7		
	Error	48	2.9×10^8	1.99	0.0978
Total		53			
TOTP	Maternal diet	5	1.8×10^8		
	Error	48	6.3×10^8	2.70	0.0314
Total		53			

TABLE 37. ANALYSIS OF VARIANCE FOR FATTY ACID COMPOSITION OF 4 WOA CHICK YOLK TISSUE ACCORDING TO MATERNAL DIET. (TRIAL 2)

Fatty Acid	Source of Variation	df	Mean Square	F	P
14:1N5	Maternal diet	5	1.1×10^3		
	Error	48	8.4×10^2	6.87	0.0001
Total		53			
15:0	Maternal diet	5	540.20		
	Error	48	426.06	12.17	0.0001
Total		53			
16:0	Maternal diet	5	8.1×10^6		
	Error	48	7.2×10^7	1.07	0.3868
Total		53			
t16:1N7	Maternal diet	5	7.4×10^3		
	Error	48	4.3×10^4	1.65	0.1655
Total		53			
16:1N7	Maternal diet	5	5.8×10^5		
	Error	48	2.0×10^5	3.41	0.0103
Total		53			
17:0	Maternal diet	5	3.2×10^3		
	Error	48	5.4×10^3	5.76	0.0003
Total		53			
18:0	Maternal diet	5	9.9×10^5		
	Error	48	3.7×10^6	2.54	0.0406
Total		53			
18:1	Maternal diet	5	8.9×10^6		
	Error	48	1.9×10^8	0.43	0.8247
Total		53			

TABLE 38. ANALYSIS OF VARIANCE FOR FATTY ACID COMPOSITION OF 4 WOA CHICK YOLK TISSUE ACCORDING TO MATERNAL DIET. (TRIAL 2)

Fatty Acid	Source of Variation	df	Mean Square	F	P
18:3N6	Maternal diet	5	7.6×10^6		
	Error	48	6.7×10^6	10.97	0.0001
Total		53			
18:3N3	Maternal diet	5	1.2×10^3		
	Error	48	1.3×10^4	0.83	0.5332
Total		53			
20:1N9	Maternal diet	5	5.9×10^4		
	Error	48	2.5×10^4	22.54	0.001
Total		53			
20:3N6	Maternal diet	5	9.6×10^3		
	Error	48	4.7×10^4	1.96	0.1022
Total		53			
20:4N6	Maternal diet	5	102.53		
	Error	48	734.74	1.34	0.2638
Total		53			
22:4N6	Maternal diet	5	7.7×10^2		
	Error	48	9.6×10^3	0.77	0.5740
Total		53			
22:5N3	Maternal diet	5	7.5×10^5		
	Error	48	7.3×10^5	9.92	0.0001
Total		53			

TABLE 39. ANALYSIS OF VARIANCE FOR FATTY ACID COMPOSITION OF 4 WOA CHICK YOLK TISSUE ACCORDING TO MATERNAL DIET. (TRIAL 2)

Fatty Acid	Source of Variation	df	Mean Square	F	P
22:6N3	Maternal diet	5	1.8×10^8		
	Error	48	1.0×10^9	1.64	0.1667
Total		53			
TOTS	Maternal diet	5	2.2×10^7		
	Error	48	1.4×10^8	1.15	0.2050
Total		53			
TOTM	Maternal diet	5	1.1×10^7		
	Error	48	2.1×10^8	0.49	0.7825
Total		53			
TOTP	Maternal diet	5	1.5×10^8		
	Error	48	1.0×10^9	1.46	0.2209
Total		53			

TABLE 40. ANALYSIS OF VARIANCE FOR FATTY ACID COMPOSITION OF 4 WOA CHICK HEART TISSUE ACCORDING TO MATERNAL DIET.

Fatty Acid	Source of Variation	df	Mean Square	F	P
14:1N5	Maternal diet	5	1.9×10^3		
	Error	47	1.1×10^4	1.68	0.1539
Total		52			
15:0	Maternal diet	5	1.0×10^3		
	Error	47	3.6×10^3	2.69	0.0323
Total		52			
16:0	Maternal diet	5	2.4×10^7		
	Error	47	5.1×10^7	4.55	0.0018
Total		52			
t16:1N7	Maternal diet	5	2.3×10^4		
	Error	47	8.4×10^4	2.67	0.0332
Total		52			
16:1N7	Maternal diet	5	9.5×10^5		
	Error	47	2.4×10^6	3.69	0.0067
Total		52			
17:0	Maternal diet	5	4.1×10^3		
	Error	47	3.5×10^3	10.91	0.0001
Total		52			
18:0	Maternal diet	5	6.7×10^5		
	Error	47	8.3×10^6	0.75	0.5893
Total		52			
18:1	Maternal diet	5	5.4×10^7		
	Error	47	6.9×10^7	7.35	0.0001
Total		52			

TABLE 41. ANALYSIS OF VARIANCE FOR FATTY ACID COMPOSITION OF 4 WOA CHICK HEART TISSUE ACCORDING TO MATERNAL DIET.

Fatty Acid	Source of Variation	df	Mean Square	F	P
18:2N6	Maternal diet	5	4.3×10^{2}		
	Error	47	4.4×10^{3}	0.92	0.4734
Total		52			
18:3N6	Maternal diet	5	2.4×10^{7}		
	Error	47	2.5×10^{7}	9.22	0.0001
Total		52			
18:3N3	Maternal diet	5	1.1×10^{4}		
	Error	47	1.7×10^{4}	6.13	0.0002
Total		52			
20:0	Maternal diet	5	1.1×10^{3}		
	Error	47	1.3×10^{5}	8.21	0.0001
Total		52			
20:1N9	Maternal diet	5	2.8×10^{3}		
	Error	47	9.2×10^{3}	2.93	0.0219
Total		52			
20:2N6	Maternal diet	5	3.9×10^{5}		
	Error	47	8.8×10^{5}	4.17	0.0032
Total		52			
20:3N6	Maternal diet	5	9.6×10^{4}		
	Error	47	2.7×10^{5}	3.30	0.0124
Total		52			
2:4N6	Maternal diet	5	4.0×10^{5}		
	Error	47	8.3×10^{5}	4.51	0.0020
Total		52			

TABLE 42. ANALYSIS OF VARIANCE FOR FATTY ACID COMPOSITION OF 4 WOA CHICK HEART TISSUE ACCORDING TO MATERNAL DIET.

Fatty Acid	Source of Variation	df	Mean Square	F	P
22:4N6	Maternal diet	5	1.4×10^{7}		
	Error	47	4.4×10^{3}	9.12	0.0001
Total		52			
22:5N3	Maternal diet	5	5.7×10^{4}		
	Error	47	5.7×10^{4}	9.30	0.0001
Total		52			
22:6N3	Maternal diet	5	1.0×10^{8}		
	Error	47	7.4×10^{7}	13.16	0.0001
Total		52			
TOTS	Maternal diet	5	3.2×10^{7}		
	Error	47	9.0×10^{7}	3.37	0.0110
Total		52			
TOTM	Maternal diet	5	6.9×10^{7}		
	Error	47	9.7×10^{7}	6.71	0.0001
Total		52			
TOTP	Maternal diet	5	2.9×10^{8}		
	Error	47	1.8×10^{8}	15.16	0.0001
Total		52			

TABLE 43. ANALYSIS OF VARIANCE FOR FATTY ACID COMPOSITION OF 4 WOA CHICK LIVER TISSUE ACCORDING TO MATERNAL DIET.

Fatty Acid	Source of Variation	df	Mean Square	F	P
14:1N5.	Maternal diet	5	5.3×10^{3}		
	Error	47	2.3×10^{4}	2.17	0.0732
Total		52			
15:0	Maternal diet	5	9.4×10^{3}		
	Error	47	9.0×10^{3}	9.77	0.0001
Total		52			
16:0	Maternal diet	5	2.0×10^{8}		
	Error	47	1.1×10^{9}	1.65	0.1650
Total		52			
t16:1N7	Maternal diet	5	4.7×10^{5}		
	Error	47	2.7×10^{6}	1.65	0.1655
Total		52			
CC16:1N7	Maternal diet	5	1.4×10^{6}		
	Error	47	9.5×10^{5}	1.51	0.2054
Total		52			
17:0	Maternal diet	5	1.0×10^{5}		
	Error	47	2.2×10^{5}	4.22	0.0030
Total		52			
18:0	Maternal diet	5	5.1×10^{7}		
	Error	47	2.7×10^{8}	1.76	0.1402
Total		52			
18:1	Maternal diet	5	9.4×10^{8}		
	Error	47	4.3×10^{9}	2.06	0.1402
Total		52			

TABLE 44. ANALYSIS OF VARIANCE FOR FATTY ACID COMPOSITION OF 4 WOA CHICK LIVER TISSUE ACCORDING TO MATERNAL DIET.

Fatty Acid	Source of Variation	df	Mean Square	F	P
18:2N6	Maternal diet	5	6.1×10^5		
	Error	47	6.2×10^6	0.92	0.4736
Total		52			
18:3N3	Maternal diet	5	8.9×10^5		
	Error	47	7.9×10^6	1.06	0.3946
Total		52			
20:1N9	Maternal diet	5	2.1×10^4		
	Error	47	1.7×10^5	1.16	0.3426
Total		52			
20.2N6	Maternal diet	5	2.1×10^4		
	Error	47	1.7×10^5	1.16	0.3426
Total		52			
20:3N6	Maternal diet	5	1.6×10^5		
	Error	47	9.4×10^5	1.59	0.1816
Total		52			
20:4N6	Maternal diet	5	3.3×10^4		
	Error	47	3.1×10^5	1.00	0.4309
Total		52			
22:4N6	Maternal diet	5	3.8×10^8		
	Error	47	1.0×10^9	3.54	0.0085
Total		52			
22:5N3	Maternal diet	5	1.7×10^7		
	Error	47	1.1×10^8	1.40	0.2418
Total		52			

TABLE 45. ANALYSIS OF VARIANCE FOR FATTY ACID COMPOSITION OF 4 WOA CHICK LIVER TISSUE ACCORDING TO MATERNAL DIET.

Fatty Acid	Source of Variation	df	Mean Square	F	P
22:6N3	Maternal diet	5	1.0×10^8		
	Error	47	1.7×10^8	5.79	0.0003
Total		52			
TOTS	Maternal diet	5	4.4×10^8		
	Error	47	2.3×10^9	1.74	0.1436
Total		52			
TOTM	Maternal diet	5	1.1×10^9		
	Error	47	5.1×10^9	2.02	0.0935
Total		52			
TOTP	Maternal diet	5	1.1×10^9		
	Error	47	1.2×10^9	8.32	0.0001
Total		52			

TABLE 46. ANALYSIS OF VARIANCE FOR FATTY ACID COMPOSITION OF 4 WOA CHICK UTERUS TISSUE ACCORDING TO MATERNAL DIET.

Fatty Acid	Source of Variation	df	Mean Square	F	P
14:1N5.	Maternal diet	5	9.3×10^{2}		
	Error	45	4.9×10^{2}	16.98	0.0001
Total		50			
15:0	Maternal diet	5	1.0×10^{2}		
	Error	45	5.3×10^{2}	1.73	0.1476
Total		50			
16:0	Maternal diet	5	1.6×10^{7}		
	Error	45	2.0×10^{7}	7.43	0.0001
Total		50			
Ct16:1N7	Maternal diet	5	4.9 x 10		
	Error	45	8.7 x 10	5.11	0.0009
Total		50			
CC16:1N7	Maternal diet	5	8.7×10^{4}		
	Error	45	8.8×10^{5}	0.83	0.5370
Total		50			
17:0	Maternal diet	5	1.2×10^{5}		
	Error	45	9.6×10^{5}	1.20	0.3225
Total		50			
18:0	Maternal diet	5	1.7×10^{7}		
	Error	45	6.2×10^{7}	2.49	0.0452
Total		50			
18:1	Maternal diet	5	1.9×10^{5}		
	Error	45	5.1×10^{5}	3.35	0.0117
Total		50			

TABLE 47. ANALYSIS OF VARIANCE FOR FATTY ACID COMPOSITION OF 4 WOA CHICK UTERUS TISSUE ACCORDING TO MATERNAL DIET.

Fatty Acid	Source of Variation	df	Mean Square	F	P
t18:2N6	Maternal diet	5	1.8×10^{7}		
	Error	45	1.1×10^{8}	1.48	0.2162
Total		50			
18:2N6	Maternal diet	5	4.6×10^{2}		
	Error	45	1.5×10^{3}	2.61	0.0369
Total		50			
18:3N6	Maternal diet	5	3.9×10^{2}		
	Error	45	1.8×10^{3}	1.87	0.1193
Total		50			
18:3N3	Maternal diet	5	2.4×10^{4}		
	Error	45	6.1×10^{4}	3.65	0.0074
Total		50			
20:0	Maternal diet	5	2.9×10^{5}		
	Error	45	4.3×10^{5}	6.04	0.0002
Total		50			
20:1N9	Maternal diet	5	1.1×10^{5}		
	Error	45	1.0×10^{6}	0.95	0.4563
Total		50			
20:2N6	Maternal diet	5	2.2×10^{5}		
	Error	45	7.8×10^{5}	2.59	0.0385
Total		50			
20:3N6	Maternal diet	5	9.3×10^{7}		
	Error	45	9.9×10^{7}	8.51	0.0001
Total		50			

TABLE 48. ANALYSIS OF VARIANCE FOR FATTY ACID COMPOSITION OF 4 WOA CHICK UTERUS TISSUE ACCORDING TO MATERNAL DIET.

Fatty Acid	Source of Variation	df	Mean Square	F	P
22:4N6	Maternal diet	5	2.5×10^{7}		
	Error	45	1.3×10^{8}	1.77	0.1392
Total		50			
22:5N3	Maternal diet	5	4.0×10^{3}		
	Error	45	3.7×10^{3}	9.68	0.0001
Total		50			
22:6N3	Maternal diet	5	8.8×10^{6}		
	Error	45	5.8×10^{6}	13.68	0.0001
Total		50			
TOTS	Maternal diet	5	4.6×10^{7}		
	Error	45	1.0×10^{8}	1.57	0.1888
Total		50			
TOTM	Maternal diet	5	6.8×10^{5}		
	Error	45	3.9×10^{6}	1.57	0.1888
Total		50			
TOTP	Maternal diet	5	1.7×10^{8}		
	Error	45	5.1×10^{8}	3.14	0.0164
Total		50			

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APPENDICES

Appendix A

Tissue Homogenization and Extraction of Lipids

Equipment:

Polytron homogenizer

N-Evap (Organomation Meyer N-Evap analytical evaporator model #112)

Aspiration apparatus (large Erlenmeyer flask, tubing and Pasteur pipette)

Horizontal shaker (Eberbach Corp.)

Centrifuge

Reagent:

Methanol (Chromatography grade) – Baxter Catalogue #230-4

Chloroform (Chromatographic grade) – Baxter Catalogue # 049-4

Chloroform : methanol (2:1, v/v)

0.88% KCl (8.8 g KCl per liter distilled water)

Supplies:

General:

Teflon policeman spatula (Baxter Catalogue #R5115-2)

3 – 50 ml glass test tubes (for rinse solutions)

Pasteur pipettes

Per tissue sample:

2 - 50 ml glass screw – top tubes with Teflon lined caps

1 - 15 ml glass screw – top test tube with Teflon lined cap

1 – short stem glass funnel with #40 Whatman filter paper (12.5 cm diameter)

Procedure:]

1. Weigh out 0.5 g tissue and place it in a 50 ml glass screw – top test tube.
2. Add 7 ml of methanol.
3. Homogenize for 20 seconds with Polytron homogenizer.
4. Add 14 ml of chloroform and homogenize again for 20 seconds.

5. Filter homogenate through #40 Whatman filter paper into another 50 ml glass screw-top test tube.
6. Clean homogenizer. Fill 3 - 50 ml glass tubes: 2 with chloroform: methanol (2:1) and one with distilled water. Run, for several seconds, the first tube of chloroform: methanol (2:1), followed by the distilled water and the second tube of chloroform: methanol (2:1). The same rinse solution can be used for 4 samples; then discard the solution into the waste collection jar and replace the solution.
7. Allow samples to filter completely, but do not let it dry.
8. Using the police-man spatula, scrape the homogenate off the filter paper and place it back into the same 50 ml glass tube used for the previous homogenization.
9. Add 12 ml chloroform: methanol (2:1), homogenize for 20 seconds, and refilter through the same filter paper used previously; thus, collecting all of the solvent in the same tube.
10. Add another 12 ml of chloroform: methanol (2:1) to the 50 ml glass tube and homogenize for a short time (to recover all of the tissue homogenate) and pour the solution over the residue from the previus filteration. Clean homogenizer as outlined above in step 6.
11. When filteration is complete, add 8.5 ml (0.88%) KCl to each of the 50 ml tubes containing the filtered liquid.
12. Cap the 50 ml glass tubes tightly and place in a horizontal shaker on low for 5 minutes.
13. Centrifuge the tubes for approximately 3 minutes a 1000 rpm to separate the aqueous and organic phases. The lipids will remain in the lower (chloroform) layter.
14. Remove the upper, aqueous layer via vacuum aspiration.
15. Evaporate the chloroform layter which remains under a steady stream of N at 60°C using N- Evap until approximately 3 ml remain.
16. Using a Pasteur pipette, transfer the remaining solution to a 15 ml screw - top glass test tube. Rinse the 50 ml tube with 8 ml chloroform: methanol (2:1) and transfer the rinsings to the same 15 ml tube.
17. This preparation will be methylated to determine total fatty acid content, following the methylation procedures found in Appendix C.

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Appendix B

Yolk and Diet Lipid Extraction

Equipment:

N-Evap (Organomation Meyer N-Evap analyical evaporator model #112).

Horizontal shaker (Eberbach Corp.)

Multi tube vortex (American Scientific Product)

Reagents:

Methanol (Chromatographic grade) – Baxter Catalogue # 230-4

Chloroform (Chromatographic grade) – Baxter Catalogue #049-4

Chloroform : methanol (2:1, v/v)

Supplies:

General:

Teflon policeman spatula (Baxter Calalogue #R5115-2

3 - 50 ml glass test tubes (for rinse solutions)

Pasteur pipettes

Per yolk or fat sample:

2 - 50 ml glass screw – top test tubes with Teflon lined caps

1 - 15 ml glass screw – top test tube with a Teflon lined caps

1 – short stem glass funnel with #40 Whatman filter paper (12.5 cm diameter)

Procedure:

1. Weigh out 0.5 yolk or fat and place it in a 50 ml glass screw – top test tube.
2. Add 9.5 ml chloroform: methanol (2:1) tightly cap the tubes and place in a test tube rack.
3. Place the rack in a horizontal shaker on low for 5-10 minutes.
4. Move the shaken test tube rack to a rack vortexer and vortex for 3-5 minutes.
5. Filter the homogenate through #40 Whatman filter paper into another 50ml glass screw – top test tube.
6. Allow sample to filter completely into a second 50 ml glass screw – top test tube.
7. Using a Pasteur pipette, transfere 400 ? into a 15 ml glass screw –top tube.

8. This preparation will be methylated to determine total fatty acid content, following the methylation procedures found in Appendi.

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Appendix C

Methylation of Lipids

Equipment:

N-Evap (Organomation Meyer N-Evap analytical evaporator model #112).

100-1000 ? Eppendorf pippettor

Dry block heater

Centrifuge

Reagents:

Iso-octane (Chromatographic grade) – Baxter Catalogue #362-4

Boron Trifluoride (BF3, 12% in methanol) – Supelco Catalogue #3-3021

0.5 N NaOH (in methanol)

Triglyceride standard solution in chloroform: methanol (2:1)- NuChek Prep Inc.

Fatty Acid	**Catalogue**	**Amount (mg)**
16:1	T-150	850.0
16:1n7	T-215	150.0
17:0	T-155	50.0
18:0	T-160	900.0
18:1n9	T-235	1050.0
t18:2n6	T-255	50.0
18:2n6	T-250	1000.0
18:3n6	T-265	50.0
18:3n3	T-260	75.0
20:0	T-170	50.0
20:1n9	T-270	50.0
20:2n6	T-280	50.0
20:3n6	T-285	50.0
20:4n6	T-295	400.0
22:4n6	U-83-M	75.0
22:6n3	T-310	100.0

Reagents: (cont'd)

Internal standard solution (40µg/µl 17:1 in chloroform: methanol (2:1)

NuChek Prep Inc. – C 17: 1 10-heptadecaenoic methyl-code U-42-M

Supplies: (per sample being methylated)

15 ml glass screw-top test tube

Eppendorf pipette tips

Pasteur pipettes

Crimp vials (Hewlett Packard Catalogue #5181-3375) and caps (Hewlett Packard Catalogue #5181-1210)

Procedure:

1. Lipid samples are already dissolved in chloroform: methanol (2:1) and ready for methylation in 15 ml glass screw – top test tubes.
2. Add appropriate amount of troglyceride standard (TG STD) and internal standard (INT STD) to each 15 ml glass screw- top test tube:

Sample	**TG STD**	**INT STD**
Yolk or Pure Fat	10µl	10µl
Heart or Liver:		
Day old	10µl	10µl
4 WOA	20µl	20µl
Hen	10µl	20µl
Uterus	10µl	10µl

3. Evaporate the solution down to 2-3 drops, under a steady stream of N at 60°C, using N-Evap.
4. Add 400µl 0.5 N NaOH to the 15 ml glass screw-top test tubes and cap tightly.
5. Place tubes in a 100°C dry block heater for 5 munutes to saponify the lipids.
6. Remove tubes from the dry block heater and place into a test tube rack. Cool the test tubes by running cold tap water over the rack.
7. Uncap tubes (keeping the caps with the corresponding tubes) and add 0.4 ml BF3, recap all tubes tightly.
8. Place tubes in a 100°C dry block heater for 5 minutes to methylate the fatty acids.
9. Remove tubes from the dry block heater and place into a test tube rack. Cool tubes by running cold tap water over the rack.
10. Uncap tubes (keeping each cap with the corresponding tube), add 1 ml iso-octane and 8.5 ml distilled water. Recap the tubes tightly and place in a test tube rack.
11. Place the test tube rack in a horizontal shaker on a low speed for 5 minutes.

12. Centrifuge samples for 10 minutes at 2000 rpm to separate the solvent phases (While this is occurring, label 1.5 ml crimp vials with both a diamond tipped pen and a marker; place sodium sulfate, a dessicant, in each vial to a depth of 1 mm.).
13. Remove the test tubes from the centrifuge. Using a Pasteur pipette, the upper iso-octane layer (containing the methylated fatty acids) to a crimp vial and cap.
14. The sample is now ready for injection into the gas chromatograph. Store at -20°C until all samples are ready for fatty acid analysis by the GC.

References:

Christopherson, S. W., and R. L. Glass, 1969. Preparation of milk fat methyl esters by alcoholysis in an essentially nonalcoholic solution. J. Dairy Sci. 52:1289-1290.

Metacalfe, L. D., and A. A. Schmitz, 1961. The rapid preparation of fatty acids esters for gas chromatographic analysis. Anal. Chem. 33:514-515.

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

Nelson, G. J., S. Darshan, and J. E. Hunt, 1986. Effect of nutritional status on fatty acid Composition of rat liver and cultured hepatocytes. Lipids 21:454-459.

Appendix D

Fatty Acid Analysis by Gas Chromatography

Equipment:

Crimp vials (Hewlett Packard Catalogue #5181-3375) with sealed caps (Hewlett Packard Catalogue #518101210) containing prepared samples for fatty acid analysis.

Gas chromatograph (Hewlett Packard model #5890) with automatic sampler (Hewlett Packard model #7673) and integrator (Hewlett Packard model #3393)

Fused silica capillary column (J & W Scientific model #DB225), 30 m long, 0.15 mm inner diameter and 0.15μm wide.

Procedure:

1. Load prepared standard vials into the automatic samplers first and begin analysis. Use these results as standards to compare all results to.
2. Load all other prepared vials into the automatic sampler and run.
3. Multiply yolk and dietary fatty acids by 20 (only $1/20^{th}$ of the sample was used.

References:

Hewlett Packard GC model #5890 with automatic sampler (Hewlett Packard model #7673) and integrator (Hewlett Packard model #3393) instruction guide.

Nelson, G. J., S. Darshan, and J. E. Hunt, 1986. Effect of nutritional status on fatty acid composition of rat liver and cultured hepatocytes. Lipids 21:454-459.

VITA

Douglas Lumont Taylor was born on April 11, 1974 in Garysburg, North Carolina to Omessia and Haywood L. Arrington. After attending public school in Garysburg, North Carolina, he graduated from Northampton County High School-West in June, 1992. He pursued his studies at North Carolina Agricultural and Technical State University and graduated with an B. S. degree in Agricultural Science (Animal Science) in May, 1996. In August, 1996 he entered the graduate program at Virginia Polytechnic Institute and State University, Blacksburg, Virginia, and received a M. S. in Animal and Poultry Science in May, 1998 under the supervision of Dr. D. M. Denbow

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Douglas L. Taylor