Effects of Dietary Fats on Reproductive Performance, Egg Quality, Fatty Acid Composition of Tissue and Yolk and Prostaglandin Levels of Embryonic Tissues in Japanese Quail (*Coturnix coturnix japonica*).

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(ABSTRACT)

The effect of dietary fats on reproductive performance, egg quality, fatty acid composition of tissues and egg yolk, and prostaglandin levels in embryonic tissues in Japanese quail (*Coturnix, coturnix japonica*) were evaluated. The addition of 5.0% chicken fat (CHX), hydrogenated soybean oil (HSBO), menhaden fish oil (MENH) or soybean oil (SBO) to the maternal and paternal diet altered tissue and yolk composition of the hens and tissue composition of the males to reflect the dietary source. Comparisons were made to chicken fat which served as a control. Feeding MENH increased the omega three fatty acid concentrations in liver, heart, testicle, and yolk. Feeding HSBO and SBO increased the omega six fatty acid concentrations in yolk. Feeding MENH and SBO increased the total monosaturated fatty acids in yolk. The concentrations of polyunsaturated fatty acids were increased by feeding MENH and SBO. Feeding MENH decreased egg production. Feeding MENH and HSBO decreased hatchability. Feeding MENH decreased specific gravity of eggs at day 30, 60, and 90. Feeding CHX increased specific gravity at day 30, 60 and 120. In addition, feeding CHX increased chick weight. Feeding SBO decreased early embryonic death. There were no consistent differences noted in tissue prostaglandin levels of embryos from hens on the differing diets.

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INTRODUCTION

Lipids are necessary for normal growth and reproduction. The composition of lipids supplied in the diet can have a marked effect on the composition of the lipids within the animal. If the proper fatty acids are not supplied within the diet, or if the proper ratio of various fatty acids is not provided, then problems such as impairments in growth and/or reproduction can occur.

Birds are oviparous and lipids represent 7 to 8 % of whole eggs. The lipids are extremely important for embryonic development, serving as a source of energy as well as essential nutrients. The lipid composition of the egg is influenced by the dietary lipid composition of the hen.

The objectives of the present study were to evaluate the effect of dietary fats on reproductive performance, egg quality, fatty acid composition of tissues and egg yolk, and prostaglandin levels in embryonic tissues in Japanese quail (*Coturnix, coturnix japonica*). Japanese quail are a good model for research due to their small size, early sexual maturity (6-8 weeks), ability to produce three or four generations a year, and the relative ease of maintaining a colony (Wilson *et al.*, 1961; Vilchez *et al.*, 1990).

REVIEW OF THE LITERATURE

Digestive System.

The digestive system of the quail consists of: the mouth, including the beak, tongue and salivary glands, the pharynx, cranial esophagus (crop), caudal esophagus, proventriculus, ventriculus or gizzard, small intestine, large intestine and cloaca. The digestive tract is lined with a continuous mucous membrane from the mouth to the cloaca (Sturkie, 1986).

The accessory organs of digestion consist of: the liver, gall bladder and pancreas. The liver is situated in the cranioventral portion of the abdominal cavity, just caudal to the heart, and separated from the heart by the diaphragm. The gallbladder, a ventral dilation of the cystic duct of the liver, lies along the lateral surface of the pancreas. The pancreas is situated in the interduodenal space formed by the

ascending and descending limbs of the duodenum (Sturkie, 1986).

The small intestine is the principal site of chemical digestion involving enzymes of both intestinal and pancreatic origin. Most nutrient absorption occurs in the small intestine. Additionally, the small intestine secretes hormones that are involved in the regulation of gastric and intestinal actions (Sturkie, 1986).

The Female Reproductive System

The reproductive system of the female coturnix in general is similar to that described in the literature for avian species. The length of the oviduct in coturnix is longer on a total-bird weight basis than other avian species and the shell gland is pigmented. Longitudinal rugae are observed in each segment of the oviduct. (Fitzgerald, 1969).

The right ovary and oviduct are present during embryonic stages of all birds. The distribution of the primordial germ cells to the ovaries becomes asymmetrical and the right ovary regresses. The reproductive system of birds (galliformes) consists of a single left ovary and its oviduct. On rare occasions, a functional right ovary and oviduct may be present. The avian oviduct is derived from the left Muellerian duct and consists of five regions: the infundibulum, magnum, isthmus, shell gland and vagina (Johnson, 1986).

The infundibulum is 5 +/- 2 cm in length and engulfs the ovulated ovum. Infundibular activity is not controlled by ovulation, as objects placed near the ovary will be engulfed and passed into the reproductive tract. Fertilization of the ovum occurs in the infundibulum and the first layer of albumin is produced (Fitzgerald, 1969; Johnson, 1986).

The largest portion of the oviduct is the magnum, and this is where a majority of the albumen is formed. The magnum is 13 +/- 1 cm in length and is highly glandular and produces ovotransferrin and ovomucoid. The epithelial cells of the magnum synthesize avidin (Fitzgerald, 1969; Johnson, 1986).

The isthmus is 6 +/- 2 cm in length and is characterized by a layer of epithelial cells with underlying tubular gland cells. Both the inner and outer shell membranes are formed during passage through the isthmus (Fitzgerald, 1969; Johnson, 1986).

The shell gland or uterus is 2 +/- 1 cm in length and is characterized by a prominent muscle layer, and is lined with tubular and unicellular goblet cells. The shell gland in Coturnix is pigmented and dark brown in color. In the shell gland, the egg albumen absorbs salts and water. This process is termed "plumping" and occurs prior to calcification. Calcification of the eggshell also takes place in the shell gland. The initiation of calcification within the shell gland is associated with stimuli induced by ovulation. Neuroendocrine factors that control and coordinate both ovulation and calcium secretion may also be involved (Fitzgerald, 1969; Johnson, 1986; Kawashima *et al.*, 1999). Kawashima *et al*. (1999) have identified an androgen receptor within the uterus of the domestic fowl and suggest that androgen binding may be related to early stage of shell formation in the uterus of the laying hen.

The vagina is 1 cm in length, consists of mucosal folds lined with both ciliated and non-ciliated cells, and is devoid of secretory glands. The vagina is separated from the shell gland by the uterovaginal sphincter muscle, and terminates at the cloaca. The vagina has no role in the formation of the egg, but in coordination with the shell gland, participates in oviposition (Fitzgerald, 1969; Johnson, 1986).

In domestic fowl, spermatozoa are stored in specialized sperm-storage tubules located in the uterovaginal region and remain viable for a period of 7-14 days in the chicken, and for greater than 21 days in the turkey hen. Uterovaginal junction glands are apparently devoid of innervation and contractile tissue, but possess a well-developed vascular system. Evidence suggests that spermatozoa fill the uterovaginal glands in a sequential fashion without mixing so that with successive inseminations, sperm from the latest insemination is most likely to fertilize an ovum. Following oviposition of each egg, spermatozoa are released from these tubules by an unknown mechanism and migrate to the infundibulum for fertilization (Sturkie, 2000).

The ovarian follicle consists of concentric layers of tissue that surround the oocyte and yolk. These include: 1) the oocyte plasma membrane, 2) the perivitelline membrane, 3) granulosa cells, 4) basal lamina and 5) the theca interna and externa. The follicle is highly vascularized except at the stigma, which is the point of rupture during ovulation. The stigma contains a lesser number of underlying small veins and arteries (Johnson, 1986).

<u>The Female Reproductive Cycle</u>

Several steroids have been implicated in the induction of ovulation in the domestic hen. Luteinizing hormone (LH), required for ovulation, peaks 4-6 hours prior to ovulation. Progesterone secreted by the largest preovulatory follicle, peaks 4-6 hours prior to ovulation and coincides with the final LH peak. The increase in progesterone and LH is necessary for ovulation to occur (Johnson, 1986). Johnson and van Tienhoven, (1980) found that the highest plasma concentrations of estrone and estradiol 1-7 occurred 4-6 hours prior to ovulation. A preovulatory peak of plasma progesterone was accompanied by an increase in estrone and estradiol 1-7 . Estrogens, together with progesterone, are required for priming the hypothalamus and pituitary in order that progesterone can induce LH release (Johnson, 1986). Plasma corticosterone tended to be highest 2 hours prior to ovulation. The rise in corticosterone probably occurred because of the coincident oviposition, but, there is a possibility that corticosterone secretion via the adrenal gland regulates the timing of the preovulatory LH surge (Johnson and van Tienhoven, 1980; Johnson, 1986). Circulating prolactin has also been reported to increase at the onset of egg laying in Coturnix (Sturkie, 1986). The Japanese quail lays at roughly the same time every afternoon. Tanabe and Nakamura, (1980) noted that two peaks of plasma estradiol were observed at 22 hours and 6 hours prior to ovulation. The concentration of estradiol hormone reached minimal values at the time of ovulation. Increases in plasma LH and progesterone occurred shortly after the onset of light. A decrease in pituitary LH occurred coincidentally. Plasma progesterone increased shortly after the onset of light and reached a peak four hours before ovulation. The onset of daylight (or artificial light) is closely linked to the induction of plasma LH and progesterone increases and, is associated with the occurrence of ovulation in the Japanese quail (Tanabe and Nakamura, 1980).

The ovary of the hen secretes estrogen (estradiol and estrone), progestin (progesterone) and androgen (testosterone and dihydrotestosterone). A rise in follicle-stimulating hormone (FSH) has been observed 15 hours prior to ovulation in the domestic hen with additional peaks 11 hours before and immediately after ovulation (Johnson, 1986; Kawashima *et al.*, 1999). Additional study to determine the role of FSH in follicular maturation and the ovulatory process is necessary. Macrophages may be a potent regulator of ovarian function, follicular development and postovulatory follicular regression.

Ovarian macrophages have been reported as regulators of follicular development, steroidogenesis, ovulatory process and regression of corpus luteum in several mammalian species. Chicken macrophages have been reported to exert many functions similar to mammalian macrophages and therefor, may play important roles in the avian reproductive cycle. Cytokines such as tumor necrosis factor alpha and interleukins are produced by chicken macrophages and it is assumed that macrophages play a role in follicular development and postovulatory regression in the chicken (Barua *et al*., 1998). Barua *et al*. (1998) suggests that prostaglandins may be involved in the homing of macrophages to postovulatory follicular tissue.

The role of neurotransmitters and prostaglandins in the ovulatory process has been investigated (Johnson, 1986). There is experimental evidence that prostaglandins are involved in oviposition. Exogenous administration of $PGF_{2\ alpha}$ stimulates the shell-gland contractility, relaxes the vagina and induces premature oviposition (Shimada and Asai, 1979). Treatment with indomethacin or aspirin depresses the peak of prostaglandins in the plasma and in pre-and postovulatory follicles, suppresses uterine contractility and delays oviposition (Shimada and Asai, 1979). Injection of PGE_1 induces premature oviposition in chickens and , while passive immunization with PGE_1 antiserum delays oviposition (Sturkie, 1986). There is evidence to suggest that increased prostaglandin production occurs in the shell gland at the time of spontaneous oviposition (Sturkie,1986). Shimada *et al*. (1984), reported that there were significant increases of $PGF_{2\ alpha}$ and PGFM in the peripheral plasma when the maximum frequency of uterine contraction was reached about one hour prior to ovulation.

The process of oviposition in the hen involves the contraction of the uterus propelling the egg to the vagina and expelling the egg out through the cloaca. The neurohypophysial hormone arginine vasotocin, causes the initial contraction of the uterus. There is an increased binding of arginine vasotocin to its receptor just prior to oviposition. Further contractions of the uterus may be caused by increased release of arginine vasotocin and an increased production of prostaglandin in the uterine tissue. Alpha and beta-adrenergic receptors are present throughout the length of the oviduct and have been shown to affect oviduct motility (Sturkie, 1986, Shimada and Asai, 1979; Takahashi *et al*., 1998). Takahashi *et al*. (1998) have suggested that arginine vasotocin has an effect on the vagina at the time of oviposition.

The ovulation-oviposition cycle in Coturnix is 24.5 hours long with approximately 80 percent of coturnix eggs laid after 1600 hours (Wilson and Huang, 1962; Konishi, 1980). The number of eggs laid on successive days is called a sequence or clutch, and each sequence is separated by one or more days in which no egg is laid. There is a 1.5-2.0 hour delay in *Coturnix* between oviposition of successive eggs in a sequence, and Coturnix ovulate the first egg in a sequence 8-9 hours after onset of the photophase (Johnson, 1986).

Components of the Egg

Components of the egg include the yolk, albumen, organic matrix and the crystalline shell. There appears to be a remarkable uniformity among species of birds as to the density of yolk and albumen, as well as the proportion of egg yolk with respect to egg weight. Variations in the amino acid and carbohydrate content of the yolk and albumen do occur (Johnson, 1986).

The organic fraction of the eggshell consists of shell membranes, mammillary cores, shell matrix and the cuticle. The shell membranes are organized into an inner and outer membrane. These membranes are produced by the isthmus region of the oviduct and consist of a meshwork of protein fibers, cross-linked by disulfide and lysine derived bonds (Sturkie, 1986).

The outermost surface of the egg is covered by a thin waxy cuticle composed of protein, polysaccharide and lipid. The function of the cuticle is to protect the egg and embryo from dehydration, microbial invasion and, to color the egg for protection from predators. The source of the cuticle has not been definitively identified. The cuticle of the eggshell is known to significantly influence the rate of water loss from eggs during incubation, and to have an effect of embryogenesis. The cuticle has a lipid component and dietary fat may influence its structure and composition (Peebles *et al*, 1998).

The calcified portion of the shell consists of the mammillary knob layer, the palisade layer and the outer surface crystal layer. Formation of the mammillary knobs occur in the shell gland. Crystallization of this layer is not even and pores remain. The palisade layer is a crystallized spongy layer that is composed primarily of crystalline calcium carbonate in the form of calcite and represents the largest proportion of the shell. Calcification of this layer also occurs in the shell gland. The surface crystal layer is a denser crystalline layer than the palisade layer. This layer lies perpendicular to the shell

surface (Board and Tullett, 1977; Sturkie, 1986).

Eggshell respiration

The pores of the shell are the result of areas of incomplete crystallization and are funnel-shaped openings that arise at the shell surface and persist through to the mammillary knob layer. In many species, the pores traverse the shell radially and branch longitudinally along the axis of the egg. The number of pores is related to egg weight, with the number of pores per unit decreasing with increasing egg weight. The function of the eggshell pores is to serve as a mechanism of chemical communication between the egg and the external environment (Board and Tullett, 1977; Peebles and Brake, 1985). The exchange of gasses occurs via passive diffusion. The total functional pore area and, the metabolic rate of the embryo determines the air-cell gas tensions (Paganelli, 1978). Vital gas diffusion occurs through the thousands of microscopic pores, which are the only means of communication between the external environment and the chorioallantoic membrane. The length of the pores and thus the diffusion pathway is equal to shell thickness (Soliman *et al.*, 1994).

The Male Reproductive System

The male reproductive system consists of paired testes, epididymis, vas deferens, ejaculatory groove, and phallus. The testes are white and ovoid to elliptical in shape and are located within the body cavity, craniomedial to the cranial pole of the kidneys. They are attached to the dorsal body wall by the mesorchium and are encapsulated by a fibrous inner layer the tunica albuginea and a thin outer layer, the tunica vaginalis. The size of the testes increases during the breeding season (Fitzgerald, 1969; Johnson, 1986).

The seminiferous tubules are lined with a simple cuboidal epithelium comparable to the Sertoli cells of the mammalian region. The majority of cells in the seminiferous tubules are spermatogenic cells. Spermatogenic cells undergo meiosis to form sperm. Between the tubules are modified interstitial cells that produce testosterone (Fitzgerald,1969; Johnson, 1986).

Blood flow to each testis is from the descending aorta, via a common trunk with the anterior renal artery to the testicular artery. Venous flow occurs via superficial veins that merge to form the testicular vein that empties into the posterior vena cava (Johnson, 1986).

The Japanese quail has a proctodeal gland, which is unique to this species. This gland is androgen-dependant and of uncertain function. It is well developed in the male and rudimentary in the female. In the mature male, there is a high correlation between size of this gland and weight of the testis (Johnson, 1986).

The phallus is a rudimentary copulatory structure located on the dorsal edge of the ventrum of the external cloacal orifice. The phallus consists of mucus membrane contiguous with the lining of the proctodeum and an outer surface of highly cornified stratified epithelium. During the breeding season, there is a pronounced hypertrophy of the musculature of the dorsal cloacal wall, which parallels the enlargement of the testes (Fitzgerald, 1969). The mature testis has a multilayered epithelium representing the various stages of spermatogenesis. Structurally, there are two types of avian spermatozoa. The spermatozoa of the passeriform birds are characterized as a complex type. Spermatozoa of the coturnix are classified as simple. The morphological appearance of avian spermatozoa is divided into the acrosome, head, neck, middle piece of the tail and principal piece of the tail. The composition and principal properties of avian semen differ from that of mammals. These differences are attributable to the lack of accessory glands in the reproductive tract (King and McLelland, 1984; Sturkie, 1986).

Embryogenesis

The first meiotic division occurs prior to ovulation of the follicle. Yoshimura *et al*. (1993) noted that the first polar body was in the perivitelline space one hour prior to ovulation. During the process of early oocyte maturation, the junctions between the oocyte and granulosa cells are disconnected. Factors that promote oocyte maturation may be activated in the germinal disc (Yoshimura *et al*., 1993). Fertilization occurs in the infundibulum, usually within 15 minutes of ovulation and initiates the second meiotic division. The egg proceeds to divide mitotically while still in the isthmus. In the chicken, the egg reaches the blastocyst stage in the shell gland. Cleavage, which is meroblastic, is at first confined to the blastodisc, and is referred to as the blastoderm. As the egg passes through the reproductive tract, the tertiary membranes are added (Eakin 1978; Johnson 1986; Speake *et al*., 1998).

Lipids.

Lipids include a variety of materials of differing chemical composition. Triglycerides are fatty acid esters of glycerol and serve as a source as well as a store of energy. Phospholipids, complex lipids found in all plant and animal tissues are especially abundant in nervous tissue. On hydrolysis, phospholipids yield glycerol, fatty acids, phosphoric acid and bases such as choline, ethanolamine or the amino acid serine. Other phospholipids yield glycerol, fatty acids, phosphoric acid and cyclic polyalcohol inositol. Sphingomyelins consist of a fatty acid, phosphoric acid, choline and the base sphingosine (Stryer, 1988; Ackerman, 1995).

Fatty acids are traditionally classified by the degree of saturation. The number of double bonds between the carbon atoms in the backbone of the fatty acid molecule determines the saturation. Saturated fats have carbon chains with no double bonds; the carbons are saturated with hydrogen atoms. Saturated fats are found in such hard fats as lard, meat and cocoa butter. Some vegetable oils such as coconut and palm also contain saturated fats. Double bonds are inserted into the fatty acid molecule by desaturase enzymes. Monosaturated fatty acids have one double bond and polyunsaturated fatty acids have more than one double bond. Fatty acids were originally named for their source instead of their chemical structure. Linoleic acid for example, derives its name from *linum*, the Latin word for flax, the original source of this acid (Brenner, 1981; Stryer, 1988; Ackerman, 1995).

The naming system in use currently names fatty acids based on their carbon length, number of double bonds and location of the first double bond as numbered from the methyl (omega) end of the molecule. This system gives rise to three different classes of fatty acids, the omega-3, omega-6 and, omega-9 fatty acids (Ackerman, 1995).

The omega-3 fatty acids have alpha-linolenic (18:3) acid as the parent compound. Alpha-linolenic acid can be converted by the action of desaturase and elongase enzymes to eicosapentaenoic (20:5) and docosahexanoic (22:6) acids. These compounds all remain omega-3 fatty acids despite the number of carbons and double bonds added (Drevon, 1992; Ackerman, 1995).

The omega-6 fatty acids are derived from linoleic acid (18:2). Delta-6-desaturase adds a double bond to form gamma-linolenic (18:3) acid. Gamma-linolenic acid (18:3, n-6) should not be

confused with alpha-linolenic acid (18:3, n-3). Due to elongase enzymes, dihomo-gamma-linolenic acid is formed from gamma-linolenic acid. Delta-5-desaturase enzyme adds a double bond to form arachidonic acid (20:4) (Drevon, 1992; Ackerman, 1995).

The omega-9 fatty acids are derived from oleic acid. Oleic acid has 18 carbons and only one double bond (18:1). Oleic acid is acted upon by the same desaturase and elongase enzymes as the omega-3 and omega-6 fatty acids. Therefore, when a deficiency exists, this non-essential fatty acid takes the place of the essential fatty acids (omega-3 and omega-6) in tissues and functional and clinical fatty acid deficiencies results (Drevon, 1992; Ackerman, 1995).

Most of the benefits attributable to the fatty acids are due to the effects of eicosanoid regulation (Drevon, 1992). Considerable evidence has accumulated that suggests that dietary fats may influence the immune response through their ability to modulate eicosanoid production (Fritsche *et al.*, 1991; Drevon, 1992). Fritsche *et al.* (1991) showed that feeding chicks fats rich in n-3 fatty acids decreased significantly the level of arachidonic acid. The eicosanoid products on the omega-3 fatty acids are less inflammatory than those of arachidonic acid (Ackerman, 1995). The principal steps in fatty acid metabolism have been described elsewhere (Brenner, 1981, Drevon, 1992; Ackerman, 1995).

A number of other compounds, extractable in such non-polar organic solvents as chloroform are included in the lipid group. Among these are medium and long carbon chain fatty acids, the glycolipids, long chain alcohols esterified with fatty acids, steroids and fat-soluble vitamins (Stryer, 1988).

Polyunsaturated fatty acids of both the n-3 and n-6 series play essential roles in embryonic and neonatal development. The primary function of these fatty acids relates to the development of neural tissue (Speake *et al*., 1998).

Dietary sources of n-3 fatty acids include linseed, canola and soybean oils. These sources contain significant amounts of alpha-linolenic acid, but they do not contain any very long-chain (more that 18 carbon atoms) fatty acids. Very long-chain fatty acids are made primarily by phytoplankton living in water and are transferred via the food chain to higher animals (Drevon, 1992).

Lipid Storage and Metabolism.

Body fat acts as an energy reserve, and it is the most variable among the major body constituents. While it varies with species, sex and age, it is strongly affected quantitatively and qualitatively by nutrition. It has been noted that the capability of birds for storing triglycerides as an energy reserve exceeds that of other classes of vertebrates. The fatty acids of these triglycerides are predominantly 16 and 18 carbon chains (Sturkie, 1986).

Following feeding, lipids are mostly stored in existing adipocyte vacuoles rather than in newly formed cells. Even following prolonged starvation, body fat is never completely depleted, and is not likely to drop below 4% of total body weight. This is due to the need to protect the integrity of tissues and organs, especially those containing phospholipids as a functional necessity (Sturkie, 1986).

Carbohydrates and proteins serve as a source of material for lipogenesis. A hen fed a commercial laying diet will absorb 3 grams of fat per day. The average egg yolk contains 6 grams of fat; an appreciable part of the yolk lipid must be synthesized from non-lipid constituents. Absorption of fats from the intestinal tract and the synthesis of fats from non-lipid compounds are then deposited as organ and egg lipids (Noble, 1987).

Lipase activity in the small intestine, and to a lesser extent in the stomach, hydrolyses triglycerides to diglycerides, monoglycerides, fatty acids and glycerol. In the laying hen, fatty acids may be absorbed by the portal system as portomicrons rather than the lymphatic system as chylomicrons as in mammals (Sturkie, 1986).

In the quail, three pancreatic bile ducts enter the ventromedial surface of the distal end of the duodenum. Lipids passing through the first part of the jejunum of the quail may therefore be hydrolyzed and emulsified for absorption at a more distal location than mammals. A large part of the fat adsorption takes place in the third portion of the small intestine. Furthermore, age may affect the site of absorption. The hen can absorb fat in more proximal sections than the chick (Whitehead, 1981).

The liver produces and secretes bile, which has several digestive functions. The principal function is emulsification of fats to aid in their absorption. Ninety percent of bile acids are absorbed with most of the absorption taking place in the proximal small intestine. Bile also serves as a vehicle for lipid excretion. Bile lipids of domestic fowl differ significantly from that of other animal species.

Mammalian bile lipids consist mostly of phospholipids and cholesterol, whereas fowl bile contains significant amounts of cholesterol esters and triglycerides (Noble and Connor, 1984).

The liver, as well as adipose tissue, contributes to the synthesis of fatty acids in the animal. In chickens and pigeons, the liver is the major site of fatty acid synthesis (Sturkie, 1986; Phetteplace and Watkins, 1990; Speake *et al*., 1998). In the egg producing female, the liver averages 4.8 percent of the body weight. The avian skeleton is also an important site of lipogenesis in the chick. Bone has one-tenth to one-third of the lipogenic activity of the liver, whereas bone marrow has approximately two-thirds of hepatic activity (Sturkie, 1986).

The two major enzyme systems involved in fatty acid synthesis are: 1) acetyl-Co-A carboxylase, a biotin dependent enzyme; and 2) fatty acid synthetase, a multi enzyme system. Acetyl-CoA may come from the oxidative decarboxylation of pyruvate, an end product of the oxidation of glucose via glycolysis. Acetyl-CoA can also result from the breakdown of ingested or previously synthesized fatty acids and from the catabolism of some amino acids (Ackerman, 1995). Donaldson and Fites (1970) have shown that *in vivo* lipogenesis can be affected by biotin status, dietary fat level, degree of unsaturation of dietary fat, starvation and dietary levels of mercury and selenium. Acetyl-CoA carboxylase is considered the rate-limiting enzyme, however, fatty acid synthetase may be subject to short-term control. Thus, limiting the rate of lipogenesis under conditions of fasting or continued feeding of high-fat diets.

Tissue lipids are derived not only from lipogenesis, but also from dietary lipids. The nature of the fat consumed will influence the composition of the lipids deposited in various tissues. The triglycerides in each tissue, as well as the compound lipids, have specific fatty acid compositions. When significant amounts of fats are consumed, the fatty acids of the ingested lipids will change the fatty acid composition of the tissue lipids to varying degrees. Because of the dynamic nature of adipose tissue and the lipid moieties of other tissues, changes occur continuously so that the fatty acid composition of a tissue may ultimately return to its constant composition (Adolf and Emken, 1986; Al-Athari and Watkins, 1988; Mantzioris *et al.*, 1995).

The dietary fat source does not alter the amount of lipid deposited in the yolk, but the

unsaturated and polyunsaturated component of the yolk can be altered to reflect dietary lipids (Guenter *et al.*, 1971; Hargis *et al*., 1991; Speake *et al*., 1998). The ability to successfully alter yolk polyunsaturated fatty acid content in response to dietary manipulation is related to the unique aspects of lipid metabolism in the hen. Unlike mammals, portomicrons are absorbed into the portal blood for transport to the liver. This allows direct exposure of the liver to dietary fat. The yolk lipids are synthesized in the liver and transported via the bloodstream for yolk deposition (Phetteplace and Watkins, 1990; Van Elswyk *et al*., 1994).

The fatty acids that are necessary for normal physiological functions but cannot be synthesized by the organism are called essential fatty acids (EFA). Linoleic, linolenic and arachidonic are examples of EFA. Linoleic acid can be converted to arachidonic acid by α-linolenic acid. A dietary supply of linoleic acid can satisfy the need for arachidonic acid. Linoleic is the essential fatty acid in poultry. The question of essentiality of linolenic acid is controversial, and an absolute requirement for linolenic acid in poultry has not been determined (Budowski *et al.*, 1961; Sturkie, 1986).

Japanese quail on a linoleic acid-deficient diet gained only a few grams over a four week period (Sturkie, 1986). Balnave (1971) has shown that one-quarter of dietary linolenate was deposited rapidly as ovarian fatty acid. He also demonstrated an increase in liver size and lipid concentration, and in the specific activity of hepatic lipogenic and glutamate-metabolizing enzymes in response to an EFA-deficient diet. EFA's , especially linoleic acid and linolenate, appear in various tissues as constituent fatty acids of phospholipids and cholesterol esters. Their major biological function appears to be as precursors of prostaglandins and thromboxanes.

The lipids circulating in the blood are derived from synthesis, intestinal absorption, or are mobilized from body fat stores. Blood lipids of birds are qualitatively and quantitatively similar to those of other vertebrates, except that very high levels are observed in mature females during periods of egg formation. In part, this is caused by the lipoprotein complexes that transport the lipids synthesized in the liver to the ovary for deposition in the follicles (Sturkie, 1986).

Lipids; Reproduction and Growth.

Estrogen enhances lipid metabolism. The increased blood lipids of the avian female during the

reproductive stage appear necessary for the deposition of the relatively large amounts of fat needed for yolk formation. Most of the yolk lipid is accumulated during the main vitellogenic period of maximum ovum development with takes place during the last 7-11 days of ovum maturation in the chicken. Extensive alterations to the capillary and membrane structure of the follicle in conjunction with a receptor mediated process of endocytosis across the ovum basement membrane allows a massive transfer of the yolk lipid precursors from the plasma. In contrast to mammals, lipid synthesis in Aves is predominantly associated with the liver rather than adipose tissue (Noble and Cocchi, 1990; Speake *et al.*, 1998).

Lipids are necessary for normal growth and reproduction. The composition of the lipids supplied in the diet, can have a marked effect on the composition of the lipids within the animal. Studies have shown that the composition of the feed-grade fats can vary tremendously (Al-Athari and Watkins, 1988). If the proper fatty acids are not supplied within the diet, or if the proper ratio of various fatty acids is not provided, then problems such as impairments in growth and/or reproduction can occur. The addition of fat to broiler breeder diets has been reported to increase egg production. The addition of fat to diets has been reported to either decrease or increase fertility (Brake, 1990; Vilchez *et al.*, 1991). Menge *et al*. (1965) reported that in essential fatty acid deficient hens, with every incremental increase in dietary 18:2 fatty acid, there was a decrease in tissue 20:3 fatty acid and an increase in egg size, production, hatchability and a decrease in early embryonic mortality.

Lipids represent a major constituent of the egg, constituting 7-8 % of whole eggs, and lipids are extremely important for embryonic development (Noble and Connor, 1984 and Ding *et al.*, 1995). The yolk of the average chicken egg weighs 60 grams and contains 6 grams of lipid, mainly in the form of triglyceride and phospholipid (Noble, 1987; Ding *et al.*, 1995). Palmitic (16:0) and oleic (18:1n-9) acids are the major acyl components of all the lipid classes. The levels of linoleic (18:2n-6), alpha linolenic (18:3n-3), arachidonic (20:4n-6) and docosahexanoic (22:6n-3) depend largely on the diet of the hen (Speake *et al*., 1998). Lipids provide 90% of the embryonic energy requirements and embryonic membrane constituents. Virtually the entire lipid content of the yolk is mobilized and absorbed into embryonic tissues during the last 7 days of incubation in the chicken (Noble and Shand,

1985). Carbohydrate and protein metabolism predominate during the first stages of incubation. It has been observed that the yolk sack membrane is capable of making a considerable contribution to the accumulation of mono- and polyunsaturated fatty acids in the embryonic liver via desaturation. Yolk lipids do not satisfy the requirements for these mono- and polyunsaturated fatty acids (Noble and Moore, 1965; Noble and Shand, 1985). Following the emergence of the chick and withdrawal of the remnant yolk complex into the abdominal cavity, assimilation and metabolism of the yolk lipid continues and is sufficient for the adequate maintenance of the chick for several days post hatching. During the development of the chick embryo, it is estimated that 90% of the total energy requirement is derived from oxidation of yolk fatty acid (Noble, 1987). Yolk lipid performs a role both as an energy source and as a supply of nutritionally essential tissue components. The transfer of the yolk to the embryo is important for successful emergence of the chick (Speake *et al.*, 1998).

Lipid Transport in the Hen and Embryo

The hen's fertilized egg constitutes an isolated environment, which, except for respiration, remains free of nutrient uptake until hatching. Early development of the embryo is confined to the migration and rearrangement of the cells within the germinal disc. The germinal disc divides into ectodermal, mesodermal and endodermal regions. These regions correspond with the development of specific organs and tissues. In addition to the true embryonic tissues, there is an associated development of extra-embryonic structures including the yolk sac membrane. During the first few days of *in ovo* development, the growing embryo floats on the surface of the yolk. The yolk sac membrane grows outward from the body of the embryo to progressively cover and eventually surround the yolk completely. The inner endoderm of the yolk sac membrane consists of a single layer of columnar cells in contact with the yolk surface. The absorptive area of the yolk sac membrane is enhanced by the formation of folds and villi-like projections into the yolk. The apical surface of the endodermal cells is rich in microvilli and a network of capillaries adjacent to the basal surface of the endodermal layer feed into the vitelline veins. The vitelline veins enter the portal system of the embryo via the yolk stalk, which connects the yolk sac membrane to the body of the embryo. Yolk lipid uptake by the yolk sac occurs through non-specific phagocytosis (Budowski *et al.*, 1961; Noble and Cocchi, 1990; Speake *et al.*,

1998).

Lipids and Feedstuffs

Lipids are routinely added to breeder diets as a source of energy and, in some cases to affect egg size (Balnave, 1971; Scragg *et al.*, 1987; March and MacMillian, 1990). Consideration is usually not given to the compositions of the lipids, amount of lipid incorporated into the diet, or what affect the lipids may have on reproduction, embryonic development or hatchability. Brake, (1990) noted that 4% added fat is near optimum for broiler breeders. There is evidence that alterations in dietary fatty acid composition can influence the fatty acid profile of birds and the eggs produced by birds consuming these diets (Chen, 1965; Phetteplace and Watkins, 1990). Fatty acids play an integral role in embryonic development. Through desaturation and chain elongation reactions, there is extensive conversion of linoleic and alpha-linolenic acids to longer chain polyunsaturated fatty acids, which are necessary for the embryo (Bordoni *et al.*, 1986).

In order to stabilize fat to prevent oxidative rancidity, many producers hydrogenate (saturate) the fat. Hydrogenation of unsaturated fat is done in the presence of a catalyst, usually nickel, and this procedure hardens the fat. Such chemical modifications cause geometric and positional modifications resulting in the production of *trans* fatty acids. Geometric isomerism depends on the orientation of atoms or groups around the axis of double bond. Geometric isomers are defined as those isomers containing *trans* double bonds in addition to, or instead of, the *cis* double bonds (Adolf and Emken, 1986). Positional isomers are formed by movement of the double bond along the fatty acid chain (Al Athari and Watkins, 1988). For geometrical isomers, if the radicals that are being considered are on the same side of the bond, the compound is called *cis*; if on the opposite sides, *trans*. In acids with a greater degree of unsaturation there are more geometric isomers. Naturally occurring unsaturated long-chain fatty acids are usually of the *cis* configuration. The molecule is bent at the position of the double bond (Al-Athari and Watkins, 1988; Stryer 1988).

As reported by Al-Athari and Watkins (1988), *trans* fatty acids can represent up to 11.7% of available blended feed-grade fats. There has been increased attention placed on the role of dietary *trans* fatty acids and their interference with fatty acid metabolism and effect on growth (Kinsella *et al.*,

1981; Al-Athari and Watkins 1988). Eicosanoids, derived from eicosapolyenoic fatty acids comprise the prostaglandins, prostacyclins, leukotrienes and thromboxanes. Prostaglandins exist in every mammalian tissue and have important physiological and pharmacologic activities. *Trans* fatty acids can potentially interfere with many physiological systems dependent on eicosanoids, including reproduction and embryonic development (Kinsella *et al*., 1981).

Affect of Age of Breeder Stock on Hatchability.

The parental age of the breeder flock has a marked affect upon hatchability. Eggs from young flocks have lower hatchability than those from older flocks (McNaughton *et al*., 1978; Garwood and Lowe, 1982). While the cause for lower hatchability is not understood, Nobel *et al*. (1986) reported that the higher mortality of embryos from young parents was associated with a malfunction of lipid absorption and mobilization from the yolk during the last week of incubation. Yafei and Noble (1990) showed that lower plasma low-density lipoprotein levels were present in the blood of embryos from young parents compared to those from older parents. Latour *et al*. (1996) reported that serum lipids and yolk retention in chicks are influenced by breeder hen age and may be associated with alterations in other associated physiological and molecular processes. Therefore, absorption of lipids in embryos from young parents appears to be impaired, leading to increased embryonic mortality. The mechanism for this impairment is uncertain. However, this does attest to the importance of lipid metabolism on embryonic viability.

Dietary Lipids and Egg Weight, Hatchability and Eggshell Quality.

Given the high percentage of lipid in the egg, it is not surprising that dietary lipids can influence various egg characteristics. Egg weight is affected by the inclusion of fat in the breeder diet (March and MacMillan, 1990). This effect was seen most consistently with vegetable oils high in linoleic acid (Shutze *et al.*, 1962). It is generally believed that the increase in egg size is due to increasing dietary linoleic acid (March and MacMillan, 1990; Whitehead *et al.*, 1993), and not dietary energy (Balnave, 1971). While egg weight changed, egg production was not affected by diets differing markedly in linoleic acid content (Scragg *et al.*, 1987). Insko *et al*. (1971) reported that coturnix eggs weighing less than 7.1 grams should be avoided.

Heywang (1942) reported that varying the corn oil content of feed from 1% to 8% had no effect on hatchability or embryonic mortality. However, other studies have shown that feeding certain fats can influence hatchability. Ringrose *et al.* (1941) found that feeding 3.6% crude cottonseed oil reduced hatchability and increased embryonic mortality. Donaldson and Fites (1970) investigated the effect of feeding cyclopropene fatty acids on embryonic mortality in quail. While it had been believed that embryonic mortality was due to toxicity of these fatty acids, this study revealed that mortality actually resulted from an increased ratio of saturated to unsaturated fatty acids in the egg yolk, and not from a direct effect of cyclopropene fatty acids.

The influence of eggshell quality on hatchability of avian embryos has been reported by several researchers (McDaniel *et al*., 1979; Brake, 1990; Peebles and Marks, 1991). The eggshell is the major determinant of respiratory gas exchange of the developing embryo, and its porosity is essential for embryonic development (Peebles and Brake, 1985; Roque and Soares, 1994). An egg must exchange adequate amounts of CO_2 and O_2 and loose 12-15% of its weight as water vapor during incubation in order to hatch (Peebles and Marks, 1991). Gas diffusion occurs through thousands of microscopic pores in the shell, which act as the only means of communication between the external environment and the chorioallantois (Peebles and Brake, 1985; Soliman *et al.*, 1994). The greatest weight loss in the coturnix egg occurs from day 5-10 and day 15-18 during incubation (Soliman *et al.*, 1994).

Egg specific gravity (SG), which estimates eggshell thickness (Bennett, 1992; Roque and Soares, 1994), is one of the most widely used methods to assess shell quality. Reductions in SG have been associated with lowered hatchability. In eggs with SG higher than 1.080, there seems to be little relationship between SG and hatchability (McDaniel *et al.*, 1979). Optimum hatchability depends on proper eggshell region-pore concentration as well as a sufficiently high number of pores. Embryonic mortality may result from insufficient pore numbers, and a thick shell may negate the compensatory effects of high pore numbers (Peebles and Brake, 1985).

Among the factors influencing eggshell quality, dietary components are the most investigated. Brake (1990) reported that inclusion of 5% poultry fat to a broiler breeder diet significantly increased egg weight, a result consistent with aforementioned reports. Fertility and hatchability of fertile eggs

significantly decreased with the inclusion of fat to the diet. Vilchez *et al*. (1992) showed that feeding oleic acid to coturnix hens resulted in eggs with SG values higher than those fed either palmitic or linoleic acids. But, the differences in hatchability noted in this study were not explained by the differences SG.

Trans Fatty Acids and Egg Production, Fertility and Embryonic Development.

In mammals, the presence of *trans* fatty acids in the diet has been correlated with decreased growth and enhanced fatty acid deficiency syndromes (Mahfouz *et al.*, 1980). Positional and geometrical isomers of 18:1 appear to impair essential fatty acid metabolism in the chick (Al-Athari and Watkins, 1988). *Trans* and *cis* isomers of 18:2 are equally incorporated into egg yolk neutral lipids and phospholipids (Al-Athari and Watkins, 1988).

Only recently have scientists become aware of the potential physiological effects of dietary *trans* fatty acids. *Trans* fatty acids can constitute a considerable portion of the blended fats that are commonly added to commercial breeder diets. Little information is available on the role of these fatty acids in reproduction and embryonic development (Al-Athari and Watkins, 1988).

The relatively high content of polyunsaturated fatty acids (PUFA) found in soybean oil (SBO) and other vegetable oils causes chemical instability and contributes to oxidative rancidity (Kinsella *et al*., 1981). In order to increase consumption of SBO, the chemical stability of SBO is improved by hydrogenating SBO to some degree. An important side reaction of catalytic hydrogenation is isomerization, which forms more than 20 positional and geometrical fatty acid isomers (Emken, 1984; Lawson *et al*., 1985). *Trans* and *cis* 18:1 positional isomers found in hydrogenated soybean oil (HSBO) normally have the double bonds distributed between the 7 through 14 positions (Emken *et al.*, 1986). Mahfouz *et al*. (1981) and Emken (1984) indicated that hydrogenated fats and specific fatty acid isomers can influence the activity of desaturases, elongases, acyltransferases, oxygenases and prostaglandin synthetases. The amount of dietary fatty acids, which are incorporated into tissue lipids, depends on their concentration in the diet. *Trans* fatty acids are oxidized at rates equivalent to the corresponding *cis* isomers. Complete oxidation of trans isomers may not occur in all instances. *Trans* fatty acids in the diet tend to increase the need for essential fatty acids. However, long-term multi-generation feeding of processed fats with up to 50% *trans* fatty acids in the presence of adequate

linoleic acid to rats showed no evidence of any overall deleterious effects (Kinsella *et al.*, 1981). Mahfouz *et al.* (1984) found that trans acids in partially hydrogenated soybean oil affected the metabolism of essential fatty acids. Dietary *trans* fatty acids can inhibit the desaturase enzyme (Kinsella *et al.*, 1981; Mahfouz *et al.*, 1984). In addition, there may be an inhibition of the conversion of *trans* linoleic acid to arachidonic acid (Kinsella *et al.*, 1981; Mahfouz *et al.*, 1981; Lawson *et al.*, 1985) . The incorporation of *trans* fatty acids into membrane phospholipids may alter the packing of the phospholipids and possibly influence the physical properties of the membrane as well as the activities of the membrane (Mahfouz *et al.*, 1981; Kinsella *et al.*, 1981; Lawson *et al.*, 1985).

Prostaglandins:

Prostaglandins are a group of naturally occurring, chemically related, long-chain hydroxy fatty acids that are endogenous mediators to biological responses. Von Euler initially introduced the term prostaglandin in 1935 (see Schror and Smith, 1990) to characterize an acidic lipid from seminal fluid that reduced blood pressure and exerted contractile and relaxing effects on nonvascular smooth muscle. Originally, he suggested that the compound was formed by the prostate gland. Later investigations confirmed the biosynthesis of prostaglandins in the prostate were of the F-type, but also showed that the seminal vesicle rather than the prostate was the predominant source of prostaglandins in the seminal fluid. There are nine types of prostaglandins: A, B, C, D, E, F, G, H and I. The first prostaglandins that were isolated were PGE and PGF. These terms were chosen because of the distribution of the prostaglandin containing lipid fractions between ether (E) and phosphate (F) (fosfat in Swedish) phases. PGA and PGB were products of PGE in acidic (A) and alkaline (B) (for basic) medium. Conversion of PGA to PGB for a hypertensive intermediate PGC whose formation was stimulated by cat (C) plasma. PGD is 11-dehydro (D) -PGF. There are three prostaglandin endoperoxides: PGG_1, PGG_2 and PGG_3. The most important metabolite is PGG_2, the first cyclooxygenase metabolite of arachidonic acid. PGH_2 is generated from PGG_2 by reduction. PGH_2 is a direct precursor of thromboxane and prostacyclin. PGI_2, also know as prostacyclin, is formed enzymatically from PGH_2. PGI_2 is the dominating cyclooxygenase metabolite of arachidonic acid in vascular epithelium, stomach mucosa, uterus and renal cortex. A suffix number (1-3) denotes the number of double bonds in the molecule. Prostaglandins of

arachidonic acid carry two double bonds, from dihomo-gamma-linolenic acid have one double bond and those from eicosapentaenoic acid have three double bonds. The Greek characters alpha and beta denote the stearic position of the hydroxyl group at C-9 of the characteristic cyclopentaenoic ring structure. Alpha denotes above and beta denotes below the ring structure. All natural prostaglandins have an alpha-configuration at C-9. "Prostaglandins" is incorrectly used to refer to all 20 carbon arachidonic acid metabolites of the cyclooxygenase pathway, but the correct terminology for oxygenated metabolites from polyunsaturated 20 carbon fatty acids is eicosanoids (Schror and Smith, 1990).

Eicosanoids are a family of compounds derived from arachidonic acid and include the prostaglandins, prostacyclins and thromboxanes. Eicosanoids represent a class of lipid mediators derived from peroxidation of C-20 carbon polyunsaturated fatty acids. The major natural source is arachidonic acid, a constituent of complex lipids. In addition to arachidonic acid, eicosanoids can also be formed from other 20-carbon polyunsaturated fatty acids. Eicosanoids represents a major group of biological mediators. The predominant role of these autocoids in physiological and pathophysiological processes is best underlined by their widespread distribution and innumerable actions on all cells and tissues of the body (Schror and Smith, 1990).

Ingestion of diets enriched with omega-3 fatty acids reduces overall eicosanoid production. Incorporation of omega-3 fatty acids into cell membranes reduces the quantity of arachidonic acid available for eicosanoid synthesis. Eicosapentaenoic (EPA) acid, derived from omega-3 fatty acids competitively inhibits the activity of the cyclooxygenase enzyme, which is necessary for eicosanoid production. The eicosanoids produced from EPA are less pro-inflammatory or antiinflammatory than those produced from arachidonic acid, which is derived from the omega-6 fatty acids (McCann and Carrik, 1998).

PGE_2, PGF_2 and PGFM:

Due to the lack of data on prostaglandin analysis in the avian species, the known data in mammalian system will be reviewed. There are three physiological E-type prostaglandins: PGE_{1-3}. All compounds have similar pharmacokinetics but differ considerable in their biological activities. PGE_2, the

natural metabolite of arachidonic acid, is the most important PGE compound. PGE_1 is present in significant amounts in human seminal fluid. PGE_3 appears also to be a natural PGE-type compound, but less is formed of this compound under physiological conditions (Schror and Smith, 1990).

PGE_2 is formed from the prostaglandin endoperoxide PGH_2 via the prostaglandin endoperoxide PGE-isomerase (9-keto-PG-isomerase). This enzyme is membrane-bound and requires reduced glutathione as a cofactor. Particularly high enzymatic activity is present in the seminal vesicle. The half-life of PGE_2 in plasma is less than one minute. Circulating PGE_2 is rapidly metabolized by passage through the lung producing a substrate for subsequent formation of biologically inactive metabolites. Further metabolism occurs primarily in the liver by beta- and omega- oxidation. In addition to the gastrointestinal system, uterus and kidney, vascular smooth muscle cells and the endothelium in the microvascular are significant sites for PGE_2 formation. PGE_2 induces relaxation of arterial vessels and contraction of venous vessels. PGE_2 sensitizes pain receptors during inflammation. PGE_2 inhibits acid and pepsin secretions in the stomach and exhibits cytoprotective effects on stomach mucosa. PGE_2, in addition to $PGF_{2\text{-alpha}}$, is an endogenous mediator of labor and therefore essential for physiological delivery (Schror and Smith, 1990).

There are two stereoisomeric PGF compounds, but only the alpha-derivatives are biologically active. $PGF_{1\text{-alpha}}$ and its metabolites are present in significant amounts only in human seminal fluid. Biosynthesis of $PGF_{2\text{-alpha}}$ from the prostaglandin endoperoxide PGH_2 occurs via two different pathways: direct reduction of PGH_2 and isomerization on PGE_2 with subsequent reduction of the 9-keto group by the prostaglandin 9-keto reductase. In addition, $PGF_{2\text{-alpha}}$ can also be formed non-enzymatically by spontaneous hydrolysis of prostaglandin endoperoxides at low conversion rates in the central nervous system or in presence of thromboxane synthase inhibitors. The metabolism of $PGF_{2\text{-alpha}}$ occurs predominately in the lungs. The primarily metabolite is 15-keto-$PGF_{2\text{-alpha}}$ which is further metabolized to 13,14-dihydro-15-keto$PGF_{2\text{-alpha}}$(PGFM). This metabolism is associated with a loss of biological activity. Further metabolism occurs predominately in the liver by beta- and omega- oxidation (Schror and Smith, 1990).

Prostaglandins (PG) PG_1 and PG_2 are synthesized from linoleic acid whereas PG_3 is synthesized

from eicosapentaenoic (20:5n3) which is derived from linolenic acid. It has become increasingly obvious that prostaglandins play an integral role in organismic homeostasis. Prostaglandins have been found in most organ systems of the body, including the kidney, digestive system, skeletal system and reproductive tract (Shimada and Asai, 1979; Hester *et al.*, 1991). Because of their localized synthesis and extremely labile nature, it is accepted that prostaglandins are autocrine/paracrine factors acting as localized hormones. Since prostaglandins are ultimately synthesized from essential fatty acids, and prostaglandins are important regulators of biological functions, the lipid composition of the diet can potentially have marked effects on various physiological systems.

MATERIALS AND METHOS

Experiment 1.

Two hundred and eighty Japanese quail breeder hens and two hundred Japanese quail breeder males were raised from eggs obtained from the University of Georgia. The eggs were incubated and hatched and the chicks were raised in a Petersime battery brooder with a modified crinoline floor. They were fed a commercial quail starter diet and maintained on 24 hours of light until they were 4 weeks of age.

At 4 weeks of age 320 females and 250 males were weighed wing banded, and moved to individual wire cages (152 X 229 X 175 mm). One hundred and sixty females and eighty males were randomly assigned to each of four treatment groups of forty females and twenty males. The remaining birds were randomly assigned to similar treatment groups, housed and fed identically, and used as substitutes when death occurred in the breeding flock and for tissue analysis. Each group was fed a diet containing 5.0% of either chicken fat (CHX), hydrogenated soybean oil (HSBO), menhaden fish oil (MENH) or soybean oil (SBO), (Table 1). All birds had access to feed and water *ad libitum*. The birds were maintained on 12:12 light schedule. Diets were made by mixing all ingredients required for 400 pounds of diet except the fat in a bulk mixer. The diets minus the fats were partitioned into 100 pound lots and the differing fats were blended using a standing mixer that was cleaned between each fat. To prevent oxidative rancidity the bulk of the diets were held at 8.0 °C. Weekly rations were removed and kept at room temperature.

The birds were allowed to acclimate for one week prior to mating. The males were then rotated between two females on an every other day basis. One male was switched between the same two females prior to 0830 every Monday, Wednesday, and Friday. Woodward and Abplanalp (1967) reported that the highest fertility was obtained with a ratio of 1 male mated to 2 females. Aggressive pecking by males causing significant head injuries has been reported (Vilchez *et al.*, 1991; Wechsler and Schmid, 1998). For welfare reasons, males were housed singly and were only left with the females for thirty minutes. Satisfactory fertility has been observed in quail with a male to female ratio of 1:2 to 1:12 (Narahari *et al.*, 1988; Gebhardt-Henrich and Marks, 1991; Wechsler and Schmid, 1998).

Maximum duration of fertility for females mated 1 to 4 days occurred from 1 to 3 days following removal of the males. In all groups fertility of eggs began to drop sharply after 3 or 4 days following removal of males. Gebhardt-Henrich and Marks (1991) reported that females that were only mated every 4th day laid fewer eggs than permanently paired females. Woodward and Abplanalp, (1967) reported that in all mating groups, the first fertile egg was not laid until the second day after introduction of the males. Wechsler and Schmid (1998) reported that fertility was as high as 92% with a male to female ratio of 1:8. The presence of eggs in the shell gland at the time of mating may interfere with fertilization of the succeeding eggs and thus increase the average delay of fertility. For practical purposes and high fertility the males remained with one female for 30 minutes.

Hen Weight: Beginning at four weeks of age, hens were weighed once a month to the nearest gram. If there was not an egg present in the cage, the average egg weight for that hen during that week period was subtracted from the body weight of the hen.

Egg Production: Eggs were collected three times a week and the total number of eggs produced per week determined. Eggs that had soft shells were not counted as eggs produced. Eggs were collected on Tuesday, Thursday and Sunday by 0830 and stored at 13.3 °C until set. The eggs were allowed to come to room temperature (22.0 °C) for 10 hours prior to setting. The eggs were incubated to determine hatchability, chick weight, number of pips and embryonic death. All eggs were placed randomly into the same incubator at 30 °C wet bulb and 37.5 °C dry bulb. At twelve days post incubation, the eggs were transferred to the hatcher. All eggs were placed randomly into the same hatcher using settings previously found to be optimal for our machine. The hatcher was checked twice daily, and eggs were allowed to hatch over days 15-18. Hatched chicks were removed from the incubator and weighed on days 15-18. All eggs that failed to hatch by day eighteen were opened. We determined if fertilization had taken place and, if so, the time of embryonic mortality was determined. Embryonic mortality was classified as early embryonic death (EED), mid-embryonic death (MID) and late embryonic death (LED). The criteria used for these classifications were as follows: EED: exhibiting early embryonic development signs characterized by eye development but a lack of limb buds. MID: exhibiting mid-embryonic development signs characterized by developed limbs. LED: exhibiting late

embryonic development signs characterized by the presence of feathers. PIP: exhibiting signs of breakage by internal forces by day 18 at 8:00 am regardless of whether the chick was alive or dead were noted.

Chicks were weighed in grams as they hatched on days 15-18. Hatchability was calculated as all eggs that hatched and resulted in live chicks on days 15-18. All remaining non-hatched, or non-pipped eggs were opened and examined macroscopically. Fertility was calculated as all eggs that hatched and resulted in live chicks on days 15-18 and, eggs that were not hatched but were macroscopically determined to be fertile due to the presence of a zona pellucida and zone opaca. Non-fertile eggs lacked a zona pellucida and zona opaca. Chicks, pips and non-hatched but live chicks were euthanatized by cervical dislocation and CO_2 exposure.

Ten eggs per treatment were collected monthly and evaluated for quality and composition. Egg weight, specific gravity, egg shell weight, yolk weight and albumen weight were determined. All eggs were weighed at the same time in grams. Egg specific gravity was determined by dipping eggs in a series of solutions ranging from 1.020 to 1.080 specific gravity units at 0.005 intervals (Foster and Weatherup, 1979). Eggs that floated in a specific gravity solution were designated as having that specific gravity.

Egg Components: Eggs were collected and stored in a covered container. For analysis, the eggs were boiled for one minute. The wet weight of the shell, including the shell membranes, albumen and yolk were determined by weighing the separated egg components. The dry weight of the egg components was determined by weighing the egg components after they had been placed into a drying oven at 37.8 °C and allowed to dehydrate completely.

Ten eggs per treatment were collected at day 40, day 80 and day 120 for yolk lipid analysis. Lipids were extracted with chloroform and methanol (2:1 vol/vol) by the method of Folch *et al.* (1957). Fatty acid methyl esters were prepared following the procedure described by Metcalfe *et al.* (1966; see also Marcheselli and Bazan, 1990). The fatty acids were quantified by gas chromatography using a capillary column as described by Phetteplace and Watkins (1990).

Ten female and ten male birds per diet from the substitution groups were killed by cervical

dislocation on day 60 and day 120. The heart and liver from the females and males, and testes from the males were removed. The tissues were frozen in liquid nitrogen and stored at minus 70 °C. The fatty acids of the tissues were analyzed as described previously. The dietary fats and samples of each diet were analyzed as described above. The data were analyzed with one-way analysis of variance. When significant treatment effects were observed, the means were separated using Duncan's multiple range test. Angular transformations (arcsine of the square root of the proportion affected) were performed on all percentage data prior to separation by Duncan's multiple range test. The calculation used to obtain the pooled standard error (PSE) is the Root Mean Square Error divided by the square root of the number of observations per treatment mean. When treatment groups were not equal, a mean of the number of observations was used for the calculation. Values reported in the tables with different letters are significantly different.

Experiment 2.

An additional study was undertaken to quantify PG levels, specifically PGE_2, $PGF_{2\ alpha}$ and the metabolite of $PGF_{2\ alpha}$, PGFM, by enzyme linked immunosorbent assay. Additional eggs were obtained from the University of Georgia, hatched and the birds reared as described above. At 4 weeks of age females and males were weighed wing banded, placed in individual cages and divided into dietary treatment groups and eggs collected as described in Experiment 1.

The embryos were removed from the egg and immediately placed in liquid nitrogen. The frozen embryos were placed in plastic bags and buried in dry ice for transport. The embryos were kept frozen at -70 °C until prostaglandin analysis. Frozen brain, heart and femurs were removed from the embryos and placed in bags and buried in dry ice. For analysis, organs were sectioned into 1.0 gram samples and diluted 1:10 with cold acidified saline. The cold saline and frozen organ samples were homogenized, and every attempt was taken to keep the homogenate near freezing. The prostaglandin assay was performed using a plasma prostaglandin kit (Cayman Chemical, Ann Arbor MI) modified for tissue (Appendix 1). The plates were read using a Biotek Plate Reader at 415 nm. The data were analyzed as previously described.

RESULTS

The diets were designated by fat type, chicken fat (CHX), hydrogenated soybean oil (HSBO), menhaden fish oil (MENH) and soybean oil (SBO). CHX was the control diet. The type of fat in the diet did not affect the body weights of the adult birds. There was no significant difference in adult body weight throughout the study, however, the birds fed MENH tended to have lower body weights throughout the study compared to birds fed CHX (Table 2).

Feeding MENH negatively affected egg production, fertility and hatchability. Feeding HSBO also negatively affected hatchability when compared to CHX (Table 2). There was a decrease ($p = 0.0001$) in egg production (eggs per day per hen) by hatch, by month and total overall by those birds fed MENH when compared to CHX (Table 2). There was a decrease ($p = .0001$) in fertility in those birds fed MENH when compared to CHX (Table 2). There was a decrease ($p = .0001$) in hatchability of eggs from those birds fed both HSBO and MENH when compared to CHX (Table 2).

Feeding SBO increased ($p=.0001$) the incidence of pips when compared to CHX (Table 2). There was a decrease ($p = 0.0001$) in EED of eggs from those hens fed SBO when compared to CHX (Table 2). There were no differences noted in MED of eggs hens fed any of the diets (Table 2). Feeding CHX increased ($p=.0001$) the weight of chicks at hatch when compared to the other three diets (Table 2).

There was a decrease ($p = 0.0001$) in specific gravity in eggs from hens fed MENH at times 4, 8 and 16 weeks of production as compared to CHX (Table 3). There were no statistical differences noted in the weight of eggs from hens fed any of the diets.

There were differences ($p= 0.0001$) in egg composition throughout the study when the data was transposed to the arcsine for moisture content of the shell, albumin and yolk. These differences were only noted at the first observation. There were no clear trends due to dietary influences (Tables 4-8).

The fatty acid composition of the fats used in the diet was reflected in the fatty acid composition of the diets (Tables 9 and 10). The fatty acid composition of the tissue varied, and was reflective of the diet fed. There were differences ($p = 0.0001$) noted; MENH increased the omega three fatty acid content in liver, heart, testes and yolk when compared to CHX (Tables 11-14. The ratio of saturated

to polyunsaturated fatty acid was increased in the heart and liver tissues of birds fed MENH as compared to SBO (Tables 11 and 12).

There were increases (p= 0.0001) in omega three fatty acids in yolks from hens fed SBO as compared to hens fed CHX, MENH and HSBO. Hens fed HSBO and MENH had yolks with increased (p = 0.0001) concentrations of omega six fatty acids when compared to CHX and SBO. Yolks from hens fed MENH and SBO had decreased (p = 0.0001) total monosaturated (M) fatty acids when compared to CHX and HSBO. Yolks from hens fed MENH and SBO had an increased (p=0.0001) concentration of total polyunsaturated (P) fatty acids when compared to CHX and HSBO (Table 13).

There were no differences in $PGF_{2\ alpha}$ in embryonic bone as affected by diet (Table 18). There were differences (p = 0.0001) noted in $PGF_{2\ alpha}$ at day 14 in embryonic brain with levels being lower in embryos from hens fed MENH compared to SBO (Table 19). There were dietary differences (p = 0.0001) in PGFM and PGE_2 throughout the study, however, the differences were not consistent (Tables 15- 17 and 21-23).

Dietary Influences:

There was an increase in weight of chicks from hens fed CHX as compared to those hens fed HSBO, MENH and SBO. There was a decrease in specific gravity of eggs from hens fed CHX at time 90 compared to eggs from hens fed SBO.

There was a decrease in hatchability of eggs from hens fed HSBO compared to eggs from hens fed CHX and SBO. There was a decrease in embryonic death at the mid-embryonic time period in embryos from hens fed HSBO as compared to embryos from hens fed MENH. There also was a decrease in chick weight in chicks from hens fed HSBO as compared to those chicks from hens fed CHX. Lastly, there was a decrease in specific gravity of eggs at days 30 and 90 from hens fed HSBO compared to hens fed CHX at day 30, and SBO at day 90. There was a decrease in pips from hens fed HSBO compared to hens fed SBO.

Fewer eggs were produced per hen per day by hens fed MENH compared to hens fed CHX, HSBO or SBO. Hatchability of eggs decreased in hens fed MENH compared to hens fed CHX or

HSBO. The decrease in hatchability was not limited to one embryonic time period. The chicks that hatched weighed less than those from birds fed CHX. There also was a decrease in specific gravity of eggs from hens fed MENH compared to hens fed CHX at day 30, CHX, HSBO and SBO at day 60, SBO at day 90 and CHX and SBO at day 120. There was a decrease in the weight of eggs from hens fed MENH at day 30 compared to eggs from hens fed CHX. Decreased body weight in hens fed MENH was noted at day 30 compared to hens fed CHX, HSBO and SBO. There was a decrease in pips from hens fed MENH compared to hens fed SBO.

There was an increase in hatchability of eggs from those hens fed SBO compared to hens fed HSBO or MENH. The chicks that hatched weighed less than those from birds fed CHX. There was a decrease in embryonic death at the early-embryonic time period in those embryos from hens fed SBO as compared to embryos from hens fed CHX, HSBO and MENH. There was an increase in pips from those hens fed SBO as compared to CHX, HSBO and MENH. There was a decrease in egg weights from hens fed SBO at day 60 as compared to CHX, HSBO and MENH.

TABLE 1. Composition of diet

Ingredients	(% of diet)
Ground yellow corn	53.4
Dehulled soy bean meal	32.0
Fat[1]	5.0
Limestone	6.5
D-L Methionine	0.15
Dicalcium phosphate	1.50
Trace mineral mix[2]	0.10
Vitamin pre-mix[3]	1.0
Salt	0.35

[1] Fat was either chicken fat, hydrogenated soybean oil, menhaden fish oil or soybean oil.

[2]Provides the following per gram of diet: 0.45 mg cobalt, 5 mg copper, 2 mg iodine, 120 mg manganese, 120 mg zinc, and 40 mg iron, with calcium carbonate as a diluent.

[3] Provides the following per gram of diet: 2200 USP units retinol, 661 ICU cholecalciferol, 6.6 IU alpha tocopherol, 1.3 mg riboflavin, 3.0 mg d-pantothenic acid, 8.8 mg niacin, 75 mg choline chloride, 0.003 mg cyanocobalamin, 0.04 mg selenium, 0.22 mg folic acid, 1.07 mg menadione sodium bisulfite complex, 0.01 mg biotin, and 25 mg ethoxyquin (as a preservative).

TABLE 2. Mean egg production, fertility and hatchability of Japanese quail as affected by dietary fat.

	Chicken	Hydrogenated soybean oil	Menhaden fish oil	Soybean oil	PSE[1]
Egg/hen/day	0.76^{A}	0.76^{A}	0.67^{B}	0.76^{A}	.01
Fertility, %	86.0^{A}	87.79^{A}	74.41^{B}	88.44^{A}	NA
Hatchability, %	71.07^{A}	69.62^{B}	57.20^{B}	72.66^{A}	NA
EED[2]	6.23^{A}	7.80^{A}	7.29^{A}	4.42^{B}	NA
MID[3]	0.56^{AB}	0.33^{B}	0.89^{A}	0.29^{B}	NA
LED[4]	5.45	6.34	5.71	6.80	NA
PIP[5]	2.67^{B}	3.69^{AB}	3.29^{B}	4.26^{A}	NA
Chick weight, g	7.02^{A}	6.83^{B}	6.80^{B}	6.81^{B}	.03
Adult weight, g	134.43	134.50	130.80	131.02	2.41

^{A,B}Means within a row with different superscripts are significantly different (P≤.05).

[1]Pooled standard error.

[2]Early dead embryos were those that did not yet have limb bud development.

[3]Mid dead embryos were embryos which had a large, black eye, but prior to the appearance of feathers.

[4]Late dead embryos were those embryos that had feathers.

[5]Embryos that began to break through the shell, but did not completely hatch.

TABLE 3. Mean egg shell percent dry matter and moisture as affected by dietary fat at 9 and 18 weeks of production.

	Egg weight				Specific gravity			
	4 wks	8 wks	12 wks	16 wks	4 wks	8 wks	12 wks	16 wks
Chicken	10.02	10.46	10.31	10.44	1.059[a]	1.066[a]	1.061[b]	1.059[a]
Soybean oil	9.72	9.92	10.22	10.07	1.060[a]	1.068[a]	1.064[a]	1.060[a]
Hydrogenated soybean oil	9.66	10.20	10.32	10.22	1.057[b]	1.066[a]	1.060[b]	1.059[ab]
Menhaden fish oil	9.62	10.16	10.19	10.18	1.056[b]	1.063[b]	1.060[b]	1.057[b]
Pooled Standard Error	.09	.11	.12	.10	.000	.000	.000	.000

[a-b]Means within a column with different superscripts are significantly different ($P \leq .05$).

TABLE 4. Mean egg shell percent dry matter and moisture as affected by dietary fat at 9 and 18 weeks of production.

	% Dry matter		% Moisture	
Diet	9 wk	18 wk	9 wk	18 wk
Chicken	7.51[a]	7.62[a]	24.2[ab]	18.8[a]
Soybean oil	7.56[a]	7.87	21.1[b]	18.1[a]
Hydrogenated soybean oil	7.23[ab]	7.61[a]	27.9[a]	17.0[a]
Menhaden fish oil	7.04[b]	7.47[a]	26.1[a]	19.3[a]
Pooled Standard Error	.001	.002	.016	.014

[a-b]Means within a column with different superscripts are significantly different (P≤.05).

TABLE 5. Mean weight (g) of eggs, wet shell weight, wet albumen and wet yolk of eggs from Japanese quail as affected by dietary fat at time at 9 weeks of production.[1]

	Chicken fat	Hydrogenated soybean oil	Menhaden fish oil	Soybean oil	PSE[2]
Egg	10.5[a]	10.08[a]	10.23[a]	10.25[a]	.11
Shell	1.04[a]	1.02[a]	0.98[a]	0.98[a]	.01
Albumen	5.95[a]	5.61[a]	5.89[a]	5.85[a]	.06
Yolk	3.21[a]	3.20[a]	3.10[a]	3.11[a]	.04

[1]There are no significant treatment effects (P > .05).
[2]Pooled standard error.

TABLE 6. Mean weight (g) of eggs, wet shell weight, wet albumen and wet yolk of eggs from Japanese quail as affected by dietary fat at 18 weeks at production.[1]

	Chicken fat	Hydrogenated soybean oil	Menhaden fish oil	Soybean oil	PSE[2]
Egg	10.50	10.52	10.24	10.31	.10
Shell	0.98	0.96	0.95	0.99	.01
Albumen	5.89	5.86	5.80	5.81	.05
Yolk	3.36	3.42	3.19	3.27	.04

[1]No significant treatment effects (P > .05).
[2]Pooled standard error.

TABLE 7. Mean percent albumen of eggs from Japanese quail as affected by dietary fat at 9 and 18 weeks of production.

	% Albumen dry matter		% Albumen moisture	
Diet	9 wk	18 wk	9 wk	18 wk
Chicken fat	7.19[a]	7.42	87.3[ab]	86.7
Soybean oil	7.33[a]	7.63	87.1[b]	86.4
Hydrogenated soybean oil	6.73[b]	7.44	87.9[a]	86.6
Menhaden fish oil	7.16[a]	7.47	87.5[ab]	86.8
Pooled Standard Error	.001	.001	.002	.002

[a-b]Means within a column with different superscripts are significantly different (P≤.05).

TABLE 8. Mean percent yolk of eggs from Japanese quail as affected by dietary fat at time 9 and 18 weeks of production.

	% Yolk dry matter		% Yolk moisture	
Diet	9 wk	18 wk	9 wk	18 wk
Chicken	15.3[ab]	16.4[ab]	49.9	48.7
Soybean oil	15.2[ab]	16.1[ab]	50.0	49.0
Hydrogenated soybean oil	15.8[a]	16.5[a]	50.1	49.2
Menhaden fish oil	14.8[b]	15.7[b]	50.9	49.2
Pooled Standard Error	.002	.003	.002	.002

[a-b]Means within a column with different superscripts are significantly different (P≤.05).

TABLE 9. Fatty acid composition of fats used in diets.1

Fatty acid	Chicken fat	Hydrogenated Soybean oil	Menhaden fish oil	Soybean oil
	ug/g			
18:2n6	7154	1141	241	18135
T18:2n6	0	1206	0	0
18:3n6	0	0	115	0
20:4n6	0	0	74	0
18:3n3	0	0	227	0
20:5n3	0	0	730	0
22:5n3	0	0	130	0
22:6n3	0	0	775	0
Total n-6	7154	2347	430	18315
Total n-3	0	0	18620	2132
16:0	8468	4314	4504	3314
17:0	0	0	313	33
18:0	0	0	61	120
22:0	0	0	0	144
16:1n7	3432	0	2705	28
18:1n9	16086	23715	2305	8827
20:1n9	0	0	361	98
Total SFA	10403	9325	5775	4917
Total MUFA	19518	23715	5371	8953
Total PUFA	7154	2347	2292	20447
SFA:MUFA	.533	.393	1.075	.549
SFA:PUFA	1.454	3.973	2.520	.240
18:3n3:182n6	0	0	.942	0

TABLE 10. Fatty acid composition of diets1.

Fatty acid	CHX	HSBO	MENH	SBO
	ug/g			
	8324	5371	3668	19867
T18:2n6	0	581	0	0
18:3n6	52	0	0	0
20:3n6	36	0	0	0
20:4n6	90	0	89	0
22:4n6	0	0	44	0
18:3n3	359	0	305	1834
20:5n3	0	0	3870	0
22:5n3	0	0	559	0
Total n-6	8502	5952	3801	19867
Total n-3	0	0	5856	1834
16:0	5398	3952	4049	4136
17:0	29	34	342	34
18:0	1178	3019	644	1182
20:0	46	110	52	120
22:0	24	97	32	117
16:1n7	1760	0	1330	61
18:1n9	9922	16688	2525	9073
20:1n9	105	74	152	106
Total SFA	6675	7212	5119	5589
Total MUFA	11787	16762	4007	9240
Total PUFA	850	5952	9657	21701
SFA:MUFA	.566	.430	1.278	.605
SFA:PUFA	.785	1.212	.530	.258
18:3n3:18:2n6	.043	0	.083	.092

[1]CHX = chicken fat; HSBO = hydrogenated soybean oil; MENH = Menhaden fish oil; SBO = soybean oil; SFA = saturated fatty acids; MUFA = monsaturated fatty acids; PUFA = polyunsaturated fatty acids.

TABLE 11. Fatty acid contents of liver (μg/g) of male and female Japanese quail as affected by dietary fat.[1]

	CHX		HSBO		MENH		SBO		PSE[2]	
	F	M	F	M	F	M	F	M	F	M
Total n-3[3]	887^{C}	1184^{c}	782^{C}	825^{c}	4764^{A}	6662^{a}	1784^{B}	2067^{b}	152	202
Total n-6[4]	3958^{B}	5077^{a}	5791^{A}	4178^{a}	1514^{C}	1281^{b}	3572^{B}	5596^{a}	609	492
Total sat.[5]	27737^{A}	1883^{a}	31896^{A}	12121^{b}	26428^{A}	19311^{a}	19380^{A}	15385^{ab}	1846	2015
Total mono[6]	32982^{Ab}	13720^{a}	40714^{A}	9258^{ab}	20925^{AB}	6693^{ab}	13593^{B}	5706^{b}	8143	2402
Total poly[7]	13210^{A}	13540^{AB}	15171^{A}	11028^{B}	12272^{A}	12265^{AB}	14426^{A}	14405^{A}	1846	1028
STOM[8]	.99^{B}	1.86^{b}	.90^{B}	1.39^{b}	1.62^{AB}	3.60^{a}	2.19^{A}	3.24^{a}	0.18	0.14
STOP[9]	1.95^{A}	1.40^{ab}	1.97^{A}	1.10^{b}	2.12^{A}	1.54^{a}	1.32^{B}	1.07^{b}	0.10	0.06

$^{a-c}$Means within a cow with different superscripts show significant differences among females (P≤.05).

$^{A-C}$Means within a row with different superscripts show significant differences among males (P≤.05).

[1]CHX = chicken fat; HSBO = hydrogenated soybean oil; MENH = Menhaden fish oil; SBO = soybean oil

[2]Pooled standard error.

[3]Total three chain: C18:3, C22:6, C20:5, C22:5.

[4]Total six chain: T18:2, C18:2, C18:3, C20:2, C20:3, C20:4, C22:4

[5]Total saturated: C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C22:0

[6]Total monosaturated: C12:1, C13:1, C14:1, C15:1, TC16:1, C16:1, TC18:1, C20:1, C22:1

[7]Total polyunsaturated: C18:2n6, C18:2n6C18:3n6,C18:3n6,C18:3n3, C20:2n6, C20:3n6, C20:4n6, C:20:5n3, C:22:4n6, C:22:6n3, C22:5n3.

[8]STOM Ratio: saturated to monosaturated.

[9] STOP Ratio: saturated to polyunsaturated.

TABLE 12 Fatty acid contents of heart (μg/g) of male and female Japanese quail as affected by diet.[1]

	CHX		HSBO		MENH		SBO		PSE[2]	
	F	M	F	M	F	M	F	M	F	M
Total n-3[3]	343[c]	249[B]	250[c]	251[B]	4298[a]	4065[A]	1784[b]	759[B]	156	169
Total n-6[4]	4798[a]	3964[A]	4174[a]	4626[A]	2206[b]	2193	4329[a]	4175[A]	294	224
Total sat.[5]	7825[ab]	6379[A]	6211[b]	6894[A]	7872AB	6797A	8461[a]	6379[A]	721	522
Total mono. [6]	4757[a]	2895[AB]	4796[a]	4475[AB]	2922A	2216B	4680[a]	4602[A]	785	755
Total poly.[7]	1.0x104B	7893[B]	8730[b]	9535[B]	9935[b]	9344[B]	1.5x10[4a]	1.1x10[4A]	1175	743
STOM[8]	2.06[ab]	2.28[B]	1.40[b]	1.63[C]	2.92[a]	3.40[A]	2.51[a]	2.31[B]	0.15	0.11
STOP[9]	.73[a]	.81[A]	.71[a]	.72[B]	.78[a]	.73[B]	.57[b]	.66[C]	0.01	0.01

[a-c]Means within a row with different superscripts show significant differences among females (P≤.05).
[A-C]Means within a row with different superscripts show significant differences among males (P≤.05).
[1]CHX = chicken fat; HSBO = hydrogenated soybean oil; MENH = Menhaden fish oil; SBO = soybean oil
[2]Pooled standard error.
[3]Total three chain: C18:3, C22:6, C20:5, C22:5.
[4]Total six chain: T18:2, C18:2, C18:3, C20:2, C20:3, C20:4, C22:4
[5]Total saturated: C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C22:0
[6]Total monosaturated: C12:1, C13:1, C14:1, C15:1, TC16:1, C16:1, TC18:1, C20:1, C22:1
[7]Total polyunsaturated: C18:2n6, C18:2n6, C18:3n6,C18:3n6,C18:3n3, C20:2n6, C20:3n6, C20:4n6, C:20:5n3, C:22:4n6, C:22:6n3, C22:5n3.
[8]Ratio STOM: saturated to monosaturated.
[9]Ratio STOP: saturated to polyunsaturated.

TABLE 13. Fatty acid contents ((μg/g) of egg yolk from Japanese quail at 9 and 18 weeks of production as affected by dietary fat.

	Chicken		Hydrogenated Soybean Oil		Menhaden fish oil		Soybean Oil		PSE[1]	
	9	18	9	18	9	18	9	18	9	18
Total n-3[2]	67[c]	0[C]	1273[c]	913[C]	4626[b]	4743 [B]	20924[a]	12516[A]	823	608
Total n-6[3]	5898[b]	4319[B]	8586[a]	6591[A]	7637[a]	6011 [A]	2119[c]	1629 [C]	416	351
Total sat.[4]	8.9X10^{4b}	8.1X10^{4B}	9.8X10^{4ab}	8.2X10^{4B}	9.6X10^{4ab}	8.8X10^{4AB}	1.0X10^{5a}	9.7 X10^{4A}	1429	4248
Total mono[5]	1.3X10^{5a}	1.1X10^{5A}	1.4X10^{5a}	1.1X10^{5A}	1.1X10^{5b}	9,4X10 ^{4B}	1.1X10^{5b}	1.0X10^{5AB}	6115	4981
Total poly.[6]	3.1X10^{4d}	2.5X10^{4C}	4.0X10^{4c}	3.3X10^{4B}	6.2X10^{4a}	5.4X10^{4A}	4.8X10^{4b}	3.3X10^{4B}	2623	2168
STOM[7]	.68[c]	.69 [B]	.70[c]	.69[B]	.86[b]	.93[A]	.95[a]	.93[A]	0.01	0.01
STOP[8]	2.85[a]	3.24[A]	2.44[b]	2.44[B]	1.55[d]	1.65[C]	2.18[c]	3.00[A]	0.04	0.06

[a-d]Means within a row with different superscripts show significant differences at 9 weeks (P≤.05).

[A-C]Means within a row with different superscripts show significant differences at 18 weeks (P≤.05).

[1]Pooled standard error.

[2]Total three chain: C18:3, C22:6, C20:5, C22:5.

[3]Total six chain: T:18:2, C18:2, C18:3, C20:2, C20:3, C20:4, C22:4

[4]Total saturated: C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C22:0

[5]Total monosaturated: C12:1, C13:1, C14:1, C15:1, TC16:1, C16:1, TC18:1, C20:1, C22:1

[6]Total polyunsaturated: C18:2n6, C18:2n6, C18:3n6,C18:3n6,C18:3n3, C20:2n6, C20:3n6, C20:4n6, C:20:5n3, C:22:4n6, C:22:6n3, C22:5n3.

[7]STOM Ratio: saturated to monosaturated.

[8]STOP Ratio: saturated to polyunsaturated.

TABLE 14. Fatty acid contents of testes (μg/g) from Japanese quail as affected by diet.[1]

	CHX	HSBO	MENH	SBO	PSE[2]
Total n-3[3]	119[B]	178[B]	1061 [A]	185 [B]	54.87
Total n-6[4]	3090[AB]	2696[B]	1961 [C]	3326 [A]	198.9
Total sat.[5]	3772	3534	3666	4062	301.3
Total mono[6]	1891	1730	2074	1802	240.0
Total poly.[7]	3427	3094	3248[A]	3812	249.6
STOM[8]	2.03 [B]	2.08[AB]	1.90[B]	2.42[A]	0.06
STOP[9]	1.09	1.21	1.31	1.05	0.03

[A-C]Means within a row with different superscripts are significantly different (P≤.05).

[1]CHX = chicken fat; HSBO = hydrogenated soybean oil; MENH = Menhaden fish oil; SBO = soybean oil

[2]Pooled standard error.

[3]Total three chain: C18:3, C22:6, C20:5, C22:5.

[4]Total six chain: T18:2, C18:2, C18:3, C20:2, C20:3, C20:4, C22:4.

[5]Total saturated: C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C22:0.

[6]Total monosaturated: C12:1, C13:1, C14:1, C15:1, TC16:1, C16:1, TC18:1, C20:1, C22:1

[7]Total polyunsaturated: C18:2N6, C18:2N6, C18:3N6,C18:3N3, C20:2N6, C20:3N6, C20:4N6, C:20:5N3, C:22:4N6, C:22:6N3, C22:5N3.

[8]STOM Ratio: saturated to monosaturated.

[9]STOP Ratio: saturated to polyunsaturated.

[8] STOP Ratio: saturated to polyunsaturated.

TABLE 15. Prostaglandin E_2 levels ((μg/g) embryonic bone tissue, of Japanese quail as affected by maternal dietary fat.[1,2]

DAY	CHX	HSBO	MENH	SBO	PSE[3]
12	8.7	5.8	5.5	11.1	1.1
14	4.9	7.7	3.3	5.1	8.7
16	10.8	12.8	5.7	22.8	3.4

[1]No significant diet effects (P > .05).
[2]CHX = chicken fat; HSBO = hydrogenated soybean oil; MENH = Menhaden fish oil; SBO = soybean oil
[3]Pooled standard error.

TABLE 16. Prostaglandin E_2 levels ((μg/g) of embryonic brain tissue of Japanese quail as affected by maternal dietary fat.[1]

DAY	CHX	HSBO	MENH	SBO	PSE[2]
12	0.49^{A}	0.68^{A}	0.34^{A}	0.50^{A}	0.06
14	0.49^{BC}	1.4^{A}	0.36^{C}	1.3^{AB}	.016
16	2.5^{A}	1.7^{AB}	1.0^{B}	1.3^{B}	.017

$^{A-C}$ Means within a row with superscripts are significantly different (P≤.05).
[1]CHX = chicken fat; HSBO = hydrogenated soybean oil; MENH = Menhaden fish oil; SBO = soybean oil
[2]Pooled standard error.

TABLE 17. Prostaglandin E_2 levels ((μg/g) of embryonic heart tissue of Japanese quail as affected by maternal dietary fat.[1,2]

DAY	CHX	HSBO	MENH	SBO	PSE[3]
12	8.1	6.9	9.0	6.3	1.4
14	8.0	6.8	12.6	10.4	2.2
16	7.9	10.4	9.6	10.3	0.8

[1]No significant diet effects (P > .05).
[2]CHX = chicken fat; HSBO = hydrogenated soybean oil; MENH = Menhaden fish oil; SBO = soybean oil
[3] Pooled standard error.

TABLE 18. Prostaglandin $F_{2\text{-alpha}}$ levels (μg/g) of embryonic bone tissue of Japanese quail as affected by diet.[1,2]

DAY	CHX	HSBO	MENH	SBO	PSE[3]
12	4.3	7.4	12.8	11.8	3.6
14	.67	0.47	0.18	0.52	.07
16	24.5	13.0	11.8	10.2	4.3

[1]No significant diet effects (P > .05).
[2]CHX = chicken fat; HSBO = hydrogenated soybean oil; MENH = Menhaden fish oil; SBO = soybean oil
[3]Pooled standard error.

TABLE 19. Prostaglandin $PGF_{2\text{-alpha}}$ levels (μg/g) of embryonic brain tissue of Japanese quail as affected by diet.[1]

DAY	CHX	HSBO	MENH	SBO	PSE[2]
12	0.14	0.25	0.17	0.23	.04
14	0.18^{AB}	0.19^{AB}	0.10^{B}	0.30^{A}	.02
16	2.3	2.0	2.0	1.8	.14

[A,B]Means within a row with different superscripts are significantly different (P≤.05).
[1]CHX = chicken fat; HSBO = hydrogenated soybean oil; MENH = Menhaden fish oil; SBO = soybean oil
[2]Pooled standard error.

TABLE 20. Prostaglandin $PGF_{2\text{-alpha}}$ levels (μg/g) analysis of embryonic heart tissue of Japanese quail as affected by diet.[1,2]

DAY	CHX	HSBO	MENH	SBO	PSE[3]
12	25.9	17.1	5.5	7.5	3.7
14	1.4	1.7	1.1	1.5	0.1
16	17.2	10.2	13.4	7.2	2.8

[1]No significant diet effects (P > .05).
[2]CHX = chicken fat; HSBO = hydrogenated soybean oil; MENH = Menhaden fish oil; SBO = soybean oil
[3]Pooled standard error.

TABLE 21. Prostaglandin FM levels (μg/g) of embryonic bone tissue of Japanese quail as affected by diet.[1]

DAY	CHX	HSBO	MENH	SBO	PSE[2]
12	0.06	0.18	0.15	0.42	0.10
14	0.24[A]	0.21[A]	0.12[B]	0.24[A]	0.01
16	37.1[A]	14.0[B]	8.8[B]	9.4[B]	3.0

[A,B]Means within a row with different superscripts are significantly different (P≤.05).
[1]CHX = chicken fat; HSBO = hydrogenated soybean oil; MENH = Menhaden fish oil; SBO = soybean oil
[2]Pooled standard error.

TABLE 22. Prostaglandin FM levels (μg/g) of embryonic brain tissue of Japanese quail as affected by diet.[1,2]

DAY	CHX	HSBO	MENH	SBO	PSE[3]
12	0.001	0.004	0.004	0.004	.008
14	0.06	0.11	0.06	0.09	.01
16	184.2	200.0	90.0	69.0	39.0

[1]No significant diet effects (P > .05).
[2]CHX = chicken fat; HSBO = hydrogenated soybean oil; MENH = Menhaden fish oil; SBO = soybean oil
[3]Pooled standard error.

Table 23. Prostaglandin FM levels (μg/g) of embryonic heart tissue of Japanese quail as affected by diet.[1]

DAY	CHX	HSBO	MENH	SBO	PSE[2]
12	0.04[B]	0.97[A]	0.04[B]	0.42[B]	.004
14	0.47	0.49	0.05	0.24	.04
16	50.7	42.0	90.0	9.0	9.38

[A,B]Means within a row with different superscripts are significantly different (P≤.05).
[1]CHX = chicken fat; HSBO = hydrogenated soybean oil; MENH = Menhaden fish oil; SBO = soybean oil
[2]Pooled standard error.

DISCUSSION

The favorable effects of supplemental fats on growth and feed efficiency in domestic fowl have been known for more than four decades (Jin *et al*., 1998). Besides increasing energy density, certain fats and oils may be used as precursors of hormones, intracellular messengers, enzyme co-factors and structural components of cells (Peebles *et al*., 1998). The lipids of the yolk represent the primary nutrient source for the chick embryo, providing over 90% of the energy required for development, as well as supplying a range of structural components for biogenesis (Speake *et al*., 1998). Little work has been done on the fatty acid analysis of diets and subsequent affect of tissue and yolk lipids in the Japanese quail. The results noted in this study were similar for what has been reported in the chicken (Phetteplace and Watkins, 1990). Hormones and dietary factors have been shown to influence desaturation of polyunsaturated fatty acids in mammals (Brenner, 1998), but this has not been shown in poultry; the research has yet to be done. Much of the early research regarding the influence of fat on production in poultry has used fat deficient models as controls. All hens in the present experiments were fed 5.0% fat which exceeds Brake's (1990) recommendation of 4.0% fat as adequate.

Varying the amount and type of fat in the hen diet modifies that fatty acid composition of lipid in the hen yolk (Guenter *et al.*, 197; Hargis *et al.*, 1991), tissues (Menge, 1971; Watkins, 1988; Phetteplace and Watkins, 1989). There was modification in fatty acid composition of yolk and tissue in the current experiment that reflected the dietary fatty acid composition of the hens. Nevertheless, the modifications do not explain the differences noted in production, fertility and hatchability. Egg quality was not altered by the differing fatty acid profiles.

The effects of dietary omega three long-chain polyunsaturated fatty acids of marine origin have gained increasing attention. It is known that the omega three fatty acids 1) inhibit the synthesis of arachidonic acid and, 2) compete with arachidonic acid for incorporation into the sn-2 position of glycerophospholipids as a substrate for the cyclooxygenase, lipoxygenase and epoxygenase enzymes. The competition reduces the level of arachidonic acid in phospholipids and leads to formation of eicosanoids with lower biological activity (Baguma-Nibasheka *et al*, 1999; Turek *et al*., 1998; Kemp,

1998). The effect of fatty acids on the immune system is an area of intense research in mammalian species. Turek *et al*. (1998) reports that PGE_2 suppresses T-lymphocyte proliferation and cytotoxic activity of natural killer cells and inhibits the production of tumor necrosis factor in rats. Lokesh *et al*. (1986) demonstrated that diets high in menhaden fish oil depressed the synthesis of prostaglandins in the mouse.

The lipid composition of monocytes, macrophages and lymphocytes reflects the fatty acid composition of dietary lipids, and these cells require essential fatty acids for production of arachidonic, eicosapentaenoic and docosahexanoic acid from circulating blood plasma lipids. These cells lack delta 6 desaturase and rely on hepatic delta 6 desaturase for production of arachidonic acid. Factors that affect the activity of the enzyme influence the arachidonic acid content of immune cells. The fluidity of the membranes in which the immune cells are embedded is also affected by the types of fats fed. Alterations in the composition or physical properties of the membranes may affect the efficacy of the immune system by altering receptors and therefore intracellular communications (Lands, 1986; Kinsella and Lokesh, 1990). Lokesh *et al*. (1986) demonstrated that mouse macrophage lipids were altered by menhaden-rich diets and this alteration depressed the synthesis of prostaglandins without affecting the normal physiological functions of the macrophages. Chicken macrophages have been reported to exert many functions similar to mammalian macrophages. Barua *et al*. (1998) reports that macrophages in the chicken ovary may play an important role in the development and regression of postovulatory follicles. Effects of altered prostaglandin synthesis could affect the reproductive cycle and thus the viability of the ovum. Macrophages in the postovulatory follicles may play a role in the removal of apoptotic cellular elements and that there is a secretion that is responsible for the infiltration of macrophages into the postovulatory follicle. In the chicken, the largest postovulatory follicle is reported to contain $PGF_{2\ alpha}$. It is theorized that prostaglandins may be involved in the homing of macrophages to the postovulatory tissues (Barua *et al.*, 1998).

The interest in the effect of fatty acids reproduction at the biochemical and histological level has been examined in many mammalian species. In human pregnancy, a positive correlation has been

shown between prolonged dietary intake of n-3 fatty acids in late pregnancy and gestational length and birth weight. This may be due to the influence of fatty acids on the stimulatory and inhibitory prostaglandins. A delay in onset of labor and delivery was noted in ewes administered an omega-3 fatty acid emulsion. Maternal plasma estradiol and maternal and fetal PGE_2 increased in control ewes but not in those infused with omega-3 fatty acids (Baguma-Nibasheka *et al*, 1999). Baguma-Nibasheka *et al*. (1999) speculate that the observed hormonal changes may be due to a decrease in activity of placental 17 alpha-hydroxylase, with a resultant decrease in conversion of progesterone to estradiol. Decreased estrogen production would result in decreased PGE_2 synthesis and this would delay or prevent the change of the myometrium to a labor-type pattern. Kemp *et al*. (1999) showed that in the bovine, increased placental expulsion times were correlated to decreased PGFM levels at parturition. This suggests that low PGFM production can be a risk factor for retained placenta. Alterations in PGFM in the uterus of aves could have similar detrimental effects. The transit time of the egg in the avian uterus is fixed due to the complex alterations that occur around the ovum to provide a cledonic environment. This is unique in aves and alterations to this transit could have deleterious effects on the embryo. It is reasonable to assume that fatty acids have the ability to alter reproductive function at many levels.

Two isoforms of cyclooxygenase enzyme also known as prostaglandin endoperoxide H synthase have been identified: cyclooxygenase 1 and 2 (COX-1, COX-2). The two isoforms generate the same prostanoids but are encoded by different genes and are regulated differently. The COX-1 isoform of the enzyme is presumed to be ubiquitously present and functions as a "housekeeping" enzyme that is constitutively expressed. The COX-2 isoform is induced by specific ligands such as mitogens, cytokines and tumor promoters. Except in the brain and kidney which appears to express COX-2 constitutively, the concentration of COX-2 gene transcripts is normally below detectable limits and production of prostanoids by this isoform usually occurs with a certain delay (Fuchs *et al*., 1999; Johnston and Budsberg, 1997).

Bovine uterine tissue expresses predominantly the COX-2 isoform during pregnancy. In the bovine, there was a significant labor-associated increase in COX-2 mRNA in reproductive tissues.

Injection of oxytocin induced a significant accumulation of COX-2 transcripts in the endometrium and also induced significant release of PGE_2 from the cervical mucosa *in vivo.* Transcription induced in chicken embryo fibroblasts occurs rapidly and is followed by COX-2 mRNA (Fuchs *et al*, 1999). Staples *et al*. (1998) suggest that in the bovine, fatty acids in fish meal can influence $PGF_{2\ alpha}$, and that fatty acids may decrease the synthesis of $PGF_{2\ alpha}$ from the uterus. If the COX-2 isoform is induced by pregnancy, it may also be induced by ovum transit in aves. Alterations in induction of this form of cyclooxygenase by fatty acids could have deleterious effects on reproduction.

It has been documented that fatty acid alterations can lead to ammeriolation of clinical signs due pathological processes. Mooney *et al*. (1998) found that omega-3 fatty acid-enriched diets could be used to control inflammation associated with dermatologic conditions in the dog. As the ration of n-6 to n-3 fatty acids decreased in the diet, the anti-inflammatory fatty acids and eicosanoids decreased in the skin wound. There may be a degree of inflammation that exists in the reproductive tract due to the presence of the egg. This inflammation may serve a physiological process such as signaling cuticle production and alterations in this process may be detrimental.

Wiseman (1990) suggests that energy deficiencies can exist in diets differing in fats due to low absorbability or digestibility of certain fats. Vilchez *et al*. (1990) reported that in rats, a diet supplemented with linolenic acid resulted in lower feed consumption. Vilchez *et al*. (1991) reported that the inclusion of 3% of either palmitic or oleic acid in the diets of quail breeder hens resulted in an improved reproductive performance when compared with a diet supplemented with 3% linoleic acid. Feed intake was not measured in this experiment. All diets in the present study were consumed equally as noted by consistent levels of feed in the troughs and similar feeding schedules required among the birds on the four diets.

Van Elswyk *et al*. (1994) reports that a 3% menhaden fish oil diet contributes to hepatic lipidosis in laying hens. Serum triglycerides and cholesterol concentrations were reduced in hens fed menhaden fish oil compared to birds fed animal-vegetable oil. Yolk and total egg weight of hens fed menhaden fish oil were decreased as compared to those hens fed animal-vegetable oil. Less lipid may

have been available from the liver for yolk formation, thereby decreasing yolk size. Hepatic lipidosis was not reported in males fed the same diet. In the case of Aves, the decrease in hepatic triglyceride synthesis may not be coupled with the decrease rate of triglyceride secretion from the liver as it is reported by Van Elswyk (1994) in the rat. There were no differences in yolk and total egg weight in the present study. The liver is involved in digestive functions and metabolism of proteins, fats and carbohydrates. It also functions to detoxify metabolites. Alteration in the structure of the liver as with hepatic lipidosis can lead to altered function. Alterations of function can lead to decreased metabolism of fats and decreased clearance of metabolites. Histological examination of the liver was not undertaken in this study.

Highly unsaturated omega three fatty acids contained in menhaden fish oil can produce a state of oxidative stress in the chicken. Oxidative stress is generated by peroxidation of the omega three fatty acids incorporated in the tissues. The immunomodulating effects from feeding diets rich in n-3 fatty acids center around the capacity of the omega three fatty acids to reduce PGE production through competition with arachidonic acid as a substrate for cyclooxygenase. In chronic inflammation, reduction in PGE by n-3 fatty acids has an anti-inflammatory effect. In infections, reduction of PGE by n-3 fatty acids stimulates immunity by increasing tumor necrosis factor (Allen and Danforth, 1998).

Supplemental fat has been shown to increase the transit time of ingesta in chickens. By increasing transit time, supplemental fats may improve digestibility of other dietary constituents and therefore increasing utilization of dietary energy (Mateos *et al.*, 1982). Differing fats may have different transit times and thus influence the utilization of other nutrients. Transit times of the digesta were not measured however, a gross increase of fecal material was not noted in any of the hens on any of the diets. Jones (1989) suggests that the degree of unsaturation of a fatty acid consumed influences its rate of conversion to carbon dioxide. Partitioning of dietary fat for energy production versus retention within storage pools is dependent on fatty acid composition. Jones, 1989, found that feeding dietary fish oil compared to corn oil in the hamster favors energy substrate oxidation reducing the fraction of metabolizable energy partitioned for storage, and thus a reduced efficiency of energy utilization. Jones

(1989) also suggests that levels of prostaglandins essential for physiological functions are influenced by the type of dietary fat, and therefore, fatty acid composition may potentially alter whole body energy homeostasis. Kasting and Martin (1984) and Lands (1986) have shown that endogenous prostaglandins are important in physiological growth hormone and thyrotropin release in the rat. These interactions are proximal to the adrenergic synapse on growth hormone-releasing factor and postsynaptically to the adregenergic synapse on the thyrotropin releasing hormone neurons.

The successful development of the chick is dependent on the provision of sufficient lipid of the appropriate composition from the yolk to the embryo and on the metabolic activity of the embryonic tissues to utilize the lipid components for growth and differentiation. The composition of the yolk lipid largely reflects that of the precursor lipoproteins. The yolk sac membrane transfers lipid from the yolk to the embryo by non-specific phagocytosis at the apical surface. The carbohydrate content of the egg is very low and its contribution to energy production in the embryo is limited to the first few days of development. It is suggested that beta-oxidation of fatty acids is the predominant pathway of energy provision. It has been estimated that 94% of the total energy needs of the embryo during development are derived from the oxidation of fatty acids (Noble and Cocci, 1990; Speake *et al*., 1998). The potential for improving hatchability and promoting viability and health in the chick by providing an optimal profile of polyunsaturated fatty acids to the embryo remains to be evaluated. It is noted that ostriches, pheasants and geese grazing in the wild, incorporate very high levels of omega three polyunsaturated fatty acids into their yolk lipid in comparison with the same genera in captivity maintained on omega 6 polyunsaturated grain based diets (Speake *et al.*, 1998). In the current study, hens fed SBO had the highest level of omega three fatty acids in their yolks.

The energy utilization of the embryo is divided between the requirements for growth and maintenance. The requirements for maintenance are dependent on embryo mass and therefore will be greatest at the end of the developmental period. Energy required for growth will be greatest during the period of most rapid tissue synthesis, but will be low by the end of incubation (Speake *et al.*, 1998).

The cuticle of the eggshell is known to significantly influence the rate of water loss from eggs

during incubation and to have subsequent effects on embryogenesis. Because the cuticle has a lipid component, dietary fat may influence the structure and function of the cuticle (Peebles *et al.*, 1998). As stated previously, the transit time of the egg is fixed and alterations of this in addition to changes in inflammation may result in eggs in which the cuticle is not optimal.

CONCLUSION

In conclusion, feeding 5% menhaden fish oil to Japanese quail resulted in a reduction in egg production, egg quality, fertility and hatchability. Feeding 5% hydrogenated soybean oil to Japanese quail resulted in reduced hatchability. Feeding 5% soybean oil to Japanese quail resulted in decreased early embryonic death and increased pips. Feeding 5% chicken fat to Japanese quail increased chick weight. Much information regarding the reproductive system and cycle in aves has yet to be discovered. It needs to be determined in aves what physiological events require eicosanoids to be maintained. Whether the reduction in fertility and hatchability was due metabolic or extrametabolic effects in this study was not elucidated. It is possible that an alteration of homeostasis occurred at the level of prostaglandin synthesis. Analysis of maternal and paternal prostaglandin levels is warranted as is the effect of the diet on the male reproductive system. Histological analysis of the maternal liver is also warranted.

ADDENDUM 1

Modification of plasma prostaglandin assay for tissue samples:

Reagents:

1. Acidified saline: (2.5:1.0)
2. Saline: 1 N HCL
   to make 1 N HCL, use 83 ml concentrated HCL/L of H_2O.
3. Potassium phosphate buffer:
   106.772 g/L K_2HPO_4 (dibasic)
   52.667 g/L K_2HPO_4 (monobasic)
   combine monobasic solution to the dibasic solution until the pH is 7.4.
4. EIA buffer:
   100.0 ml KPO_4 buffer (pH = 7.4)
   100.0 mg sodium azide
   23.4 sodium chloride
   370.0 mg tetrasodium EDTA
   1.0 g BSA (add slowly to reduce foaming)
   q.s. to 1.0 L with megaPure H_2O.
5. Saturation Buffer:
   1.0 l EIA buffer
   200.0 mg sodium azide
   2.0 g BSA
   pH = 7.4
6. Mouse Monoclonal anti-Rabbit IgG:
   (Cayman Chemical cat.# 400003-2500).
7. Deionized water:
   filtered with activated carbon filters or other organic scavengers, free of trace organic contaminants.
8. Standards: PGE_2, PGF_{2a} and PGFM
(Cayman Chemical cat. # 414014, 416014, and 416674)
9. Antiserum: PGE_2, PGF_{2a} and PGFM
   Cayman Chemical cat. # 414012, 416012, and 416672)
10. Tracer: PGE_2, PGF_{2a} and PGFM
    (Cayman Chemical cat. # 414010, 416010, and 416670).
11. Wash Buffer:
    10 ml Potassium Phosphate buffer (pH = 7.4)
    0.5 ml Tween 20
    q.s. to 1.0 L with megaPure water.
12. Ellman's Reagent:
    (Cayman Chemical cat.# 400050)

Extraction Procedure:

1. Pipette 200 µl of sample into 13x100 Borosilicate glass tubes.
2. add 25 µl of 1N HCl.

3. add 2 ml Ethyl Acetate.
4. Vortex covered tube for 2 minutes.
5. Centrifuge at 1700 g for 10 minutes.
6. Pour aqueous phase into new 13x100 Borosilicate glass tube and evaporate:
   with air for PGFM
   with nitrogen at 6L/min for PGF_{2a}.
7. To the solid phase add 2 ml Ethyl Acetate and vortex for 2 minutes.
8. Centrifuge, pour aqueous phase into the 13x100 Borosilicate tubes used in step 6 and evaporate as described above.
9. Rinse with 500 µl of HPLC grade Methanol and evaporate as described above.
10. Add 200 µl EIA Buffer.
11. Vortex for 30 sec.
12. Cover and let sit:
    PGFM overnight at room temperature.
    PGF_{2a} for 30 minutes at room temperature.

Prostaglandin Assay Procedure:

1. Solution 1. 25 ml of Potassium Phosphate buffer and 475 ml of MegaPure water.
2. Reconstitute mouse monoclonal Anti-Rabbit IgG with 500 ml of solution 1. Let stand at room temperature for 45 minutes.
3. Add 200 µl of Mouse Monoclonal IgG into each well and cover plate with cover sheet. Let stand at room temperature for 18 hours.
4. Add 100 µl of saturation buffer to the well containing 200 µl of Mouse Monoclonal IgG. Cover at refrigerate at 4 C for at least 18 hours.
5. Reconstitute and store all standards, antiserum and tracer as per literature provided by Cayman Chemicals.
6. Dump and wash wells four times with wash buffer. Invert well on paper towel and tap to remove fluid.
7. Add 100 µl EIA buffer into NSB wells, pipet 50 µl EIA buffer into TB wells.
9. Pipette 50 µl of lowest standard into first standard wells, continue with remaining standards and wells.
10. Pipet 50 µl of sample into well.
11. To each 3.33 ml of tracer, add 1.67 ml of EIA buffer. Vortex. Add 50 µl of tracer to all wells except TA wells.
12. Add 50 µl of antiserum to all wells except TA and NSB wells.
13. Cover and incubate at room temperature for 18 hours.

Prostaglandin Assay Procedure:

14. Dump and rinse plates 4 times, invert and remove remaining fluid.
15. Reconstitute Ellman's reagent with 50 ml MegaPure water and add 200 µl to all wells.
16. Add 5 µl tracer to TA wells.
17. Cover and incubate at room temperature on a shaker. Cover to plates to allow development without light. PGF_{2a} and PGFM assay develop in 4 hours, PGE_2 may take up to 6 hours.
18. Read with Biotek Plate Reader.

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