# **Fats and Fatty Acids in Provide the State of Control For State of Con**

Edited by Gita Cherian Reza Poureslami



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#### FATS AND FATTY ACIDS IN LAYING HENS

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#### Introduction

The laying hen does not have a specific requirement for fat as a nutrient, but rather fats supply the laying hen energy (calories), essential fatty acids, and improved absorption of fat soluble vitamins and other feed supplements such as pigmentors (NRC, 1994). When formulating a laying hen ration, the nutritionist rarely sets a minimum level of fat in the ration. However, a minimum level of the essential fatty acid - linoleic acid (C18:2) will usually be set into the least cost formulation at a rate recommended by the primary breeder or the NRC Nutrient Requirements for Poultry (1994).

Fats can be provided to the laying hen diet from a multitude of ingredients including grains such as corn, oats, wheat; vegetable protein sources such as soybeans, canola, dried distillers grains, peas; animal protein sources such as meat and bone meal, poultry meal, blood meal, and finally from pure fat sources such as vegetable oils, tallow, lard, palm oil, and restaurant grease blends. These fats serve as concentrated sources of energy to the laying hen and add caloric and economic value to laying hen rations. When formulating a least cost laying hen ration, the program will balance for protein and energy, looking for concentrated sources of energy such as fats to meet the hen's high energy requirements for optimum egg production. It is not uncommon to see 2-4%added fat sources in laying hen rations during peak egg production. The rate of added fat will change depending on the base ingredient price differences when formulating the ration. For example, when corn prices are high and a low price restaurant grease is available, the least cost feed formulation program is likely

#### FATS AND FATTY ACIDS IN MEAT-TYPE BROILER BIRDS

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#### Introduction

Dietary fat composition affects the amount of fat and the fatty acid composition of the fat deposited in the body. On one hand, polyunsaturated fatty acids (PUFA) appear to result in lower fat deposition than fats rich in monounsaturated (MUFA) or saturated fatty acids (SFA). Conjugated linoleic acid (CLA) also appears to affect fat deposition, although results are controversial. Since chickens are normally in a positive energy balance, fatty acids are mostly deposited unaltered and the composition of the fat tends to reflect that of the diet, although there are some modifications which are discussed below.

#### Fat deposition

Fatty acids affect differently the amount of fat deposited in the body of chickens. Diets containing PUFA cause lower fat deposition than diets containing similar amounts of SFA or MUFA. A summary of results is presented in Table 1. Pan et al. (1979) replaced tallow (T) by soybean oil (SO) in broiler diets and observed lower abdominal fat pad (AFP) deposition. Pichasov and Nir (1992) found that high levels of a mixture of sunflower oil (SFO) and SO replacing T also resulted in lower AFP and lower body fat (BF). Vilà and Esteve-Garcia (1996) observed that saturated acid oils, resulted in higher AFP deposition than unsaturated acid oils although they had much lower metabolizable energy. A study was designed to test this effect, and diets differing in their fatty acid profile were compared to determine their effect on AFP deposition. Birds fed SFO -rich in linoleic acid (LA)- and linseed oil (LO) -rich in  $\alpha$ -linolenic acid (LNA)- presented better values of feed efficiency than those fed T, AFP and

#### LIPOPROTEINS AND THEIR METABOLISM IN POULTRY

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#### Introduction

Lipoproteins and their metabolism have been of intense research interest to poultry scientists for a very longtime. Lipoproteins are distinctive macromolecular structures assembled in a structurally specific manner that reflects their physiological purpose (Walzem, 1996). While poultry typically consume feed with a very low fat content, lipid transport and tissue lipid composition are of great interest to poultry biologists due to the roles such processes play in determining the nutritional value of poultry products and bird health. Past reviews have summarized the general biochemical features and physiological processes of avian lipoprotein metabolism (Chapman, 1980; Gruffat et al., 1996; Hermier, 1997; Walzem, 1996). However, now that the complete sequence of the avian genome is publically available new molecular detail can be developed to guide further improvement of our understanding of these biologically active nanoparticles. Thus the goal of this chapter is to update biochemical information and, where possible, to provide molecular context for the reader with regard to the processes through which diet and dietary fatty acids influence the multiple aspects of poultry biology covered in other chapters within this book.

#### Availability of genome data expands discovery potential

Our current understanding of fats and fatty acids in poultry nutrition and health is largely empirical, hard earned through a multitude of feeding trials. However, the availability of the genomes of chickens and species such as humans and

#### **MODIFYING EGG LIPIDS FOR ENHANCING HUMAN HEALTH**

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#### **Introduction**

During the past two decades, several ways of enhancing the nutritional value of food products, especially chicken eggs, have been attempted. These include genetic, pharmacological, dietary, biotechnological and processing methods. Among the different methods, dietary manipulation has been the most successful and most widely adopted. The current chapter will emphasize the role of diet in modifying egg lipid composition to meet the human requirement of essential nutrients and thereby contribute to human health in a holistic and sustainable manner. Research related to modifying egg lipid composition has been well documented, only some of the most recent research and/or reviews are included in the current chapter. Y0,''

#### Egg lipid composition

Eggs are store-houses of several essential nutrients and their contribution to the diet is based on size. Table eggs are categorized into small, medium, large or extra large, based on their weights. The nutrient contribution to the diet also varies depending on size. For example, a small egg weighing 38g could provide 50 calories while an extra large egg could contribute over 80 calories. Table 1 shows the lipid, fatty acid and cholesterol content in small, medium, large or extra large table eggs. Among several nutrients in eggs, fats or lipids have attracted the most attention among scientists, media and consumers due to the link between high dietary fat consumption and coronary heart disease.

#### MODIFYING MEAT LIPIDS FOR HUMAN HEALTH

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#### Fatty acids in human diet

Besides carbohydrates and proteins, lipids are one of the main components of food. Lipids have a crucial role in early development, pregnancy, lactation and provide an energy reserve for the body (Crawford, 1983). Lipids contain essential fatty acids which are needed for growth and development. Essential fatty acids act as precursor for long chain derivatives which are used in cell membrane and for synthesis of prostaglandins and hormone-like substrates involved in cell regulation, reproduction, immunity and blood flow. Fatty acids are the common structural units of lipids. Fatty acids consist of three elements; carbon (C), hydrogen (H) and oxygen (O) arranged as a carbon chain skeleton with a carboxyl group at the end (-COOH). Fatty acids are classified according to their chain length and number, position and configuration of double bonds. The impacts of quantity and quality of the dietary fat on the development of several diseases, including cardiovascular disease (CVD), some cancers and arthritis have been well-established (Simopoulos, 1999).

Dietary fats and oils represent a significant proportion of the caloric intake (>33%) in the United States (Bialostosky et al., 2002). Saturated fatty acids (SFA) are found in every type of food. Long chain SFA (>C12) are most common in animal fats and vegetable oils. Short chain SFA (C4-C10) are mainly found in milk fat. Milk and milk products (e.g., cheese and butter), ruminant meat and meat products are major sources of SFA in food (Givens et al., 2006). Dietary SFA are associated with an increase in LDL and a decrease in HDL, which is a risk factor for coronary heart diseases, risk of obesity and associated disorders (Lesna et al., 2008; Bergouignan et al., 2009). When contrasted to SFA, a high monounsaturated fatty acid (MUFA) content in the diet favors

#### **OXIDATIVE STABILITY OF FATTY** ACIDS

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#### Introduction

The unsaturated fatty acids docosahexaenoic acid (DHA) and alpha linolenic acid (ALA) have shown numerous benefits including improved nervous system development and cardiovascular health (Harris, 1989; Leaf, 1990; Martinez, 1995). This has generated interest in their use as feed additives (Rising et al., 1990; Kim et al., 1993; Watanabe, 1993; Enjalbert et al., 1997). However, DHA and ALA are particularly susceptible to oxidation due to the high degree of unsaturation. The oxidation of such unsaturated lipids generates malodorous compounds and leads to off flavors and the loss of bioactivity of the original lipid structures.

Fish oils are a rich source of polyunsaturated fatty acids and especially of the more highly unsaturated fatty acids such as DHA. However, when fish oils are used in the feed formulations of laying hens to improve the nutritional value of the eggs there is some decrease in sensory quality reported as fishy off-flavors (Van Elswyk et al., 1995; Van Elswyk, 1997; Gonzalez-Esgerra and Leeson, 2000). This can be alleviated by using marine algae as the primary source of the polyunsaturated fatty acids rather than the fish oil (Herber-McNeill and Van Elswyk, 1998). Similar studies with flaxseed as a source of linolenic acid (ALA) have shown that the polyunsaturated fatty acids do accumulate in the eggs, however, there are anti-nutritional compounds in the flaxseed that have negative effects on production (Bean and Leeson, 2003). When isolated ALA was fed to Shaver hens a reduction in hatchability was observed that could be off-set by supplementing the diet with soybean oil (Muma et al., 2006). These

#### FATTY ACIDS IN IMMUNE HEALTH

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## \$00 Introduction

Use of dietary lipids in poultry feed serve as immediate sources of energy, precursors for the synthesis of key metabolites in secondary metabolism, storage for future caloric needs, membrane composition and molecules critical in a variety of signal transduction pathways. With regards to immunity and inflammation in the fowl, lipids in cell membrane structures and their role as signaling molecules are likely the most direct mechanism by which lipids affect immune function. Literature and mechanism by which lipids influence immune and inflammatory responses is both vast and complex. In this chapter, key mechanisms by which fatty acids affect key pathways that direct immune defense and inflammatory response will be briefly introduced, then evidence of these mechanisms acting in avian species will be presented, and finally experimental nutritional trials involving dietary fatty acids and complex fats and oils on the immune response of the bird will be reviewed:where possible, specific fatty acids that are direct contributors to immune responsiveness will be described.

Of the many fatty acids in the bird's diet, there are few that play a significant role in immune function. Critical fatty acids that serve as precursors for other fatty acids that effect immune events include linoleic acid C18:2, cis 9, cis 12, the conjugated dienes of linoleic acid (namely the most studied C18:2,cis (c) 9 trans (t) 11 and t10c12), and  $\alpha$ -linolenic acid (C18:3, c9c12c15). While the conjugated linoleic fatty acids have their biological effects directly, both linoleic acid and  $\alpha$ -linoleic acid are desaturated and elongated into fatty acids (linoleic acid to C20:4, c5c8c11c12 or arachidonic acid and alpha linoleic acid to C20:5, c5c8c11c14c17 or eicosapentaenoic acid or C22:6, c4c7c10c13c16c19 or docosahexaenoic acid) that directly influence signal transduction pathways.

#### DIETARY FAT AND NUTRIGENOMICS FOR POULTRY PRODUCTION

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Historically, nutritional research in the animal production industry has focused on identifying macronutrients and micronutrients that are essential for normal growth and development of animals. The most important, if not only, goal of nutrition research in the animal production industry has been to achieve maximal production through "least cost formulation". Least cost formulation ensures that a bird, in the case of poultry, consumes the right balance of nutrients to support increased production performance but at the same time costs the least to the poultry producers. This research approach has resulted in huge advances in reducing feed cost, thus, improving production performance of chickens with increased profit margin for poultry producers. Over the years, the advances owing to such empirical nutrition-based research have reached a plateau and, hence, researchers have started to identify unique interactions between nutrition, biochemistry, environment, gene expression, and immunology that will further advance profit margins achieved through empirical nutrition research. One such area of study is nutrigenomics, which is the study of interactions between dietary nutrients and gene expression.

#### Nutrigenomics

Dietary nutrients alter gene expression either directly or indirectly (Raqib and Cravioto, 2009). Nutrigenomics is the study of food and food-derived products on gene expression and how gene expression affects response to nutrients. From a nutrigenomics viewpoint, nutritional components act as signaling molecules that can activate several sensor systems in a cell, which ultimately alter gene and protein expression and control homeostasis (Muller and Kersten, 2003). Nutrients activate transcription factor sensor systems in the cell, which in

#### DIETARY FATTY ACIDS AND MALE FERTILITY IN POULTRY

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# Introduction

Spermatozoa are highly specialised cells with a unique shape and a singular function: the fertilisation of the egg. A typical spermatozoon consists of three main parts: the head, the midpiece, and the tail. Much of the head is occupied by the haploid nucleus, containing the paternal contribution to the genome of the potential embryo. Overlying the nucleus, at the tip of the head, is the acrosome, a Golgi-derived vesicle containing a range of enzymes whose action enables the spermatozoon to penetrate the outer layers of the egg and to fuse with the egg's plasma membrane. Mobility is provided by rhythmic, whiplash movements of the long, narrow tail, enabling the spermatozoa to move towards the egg and supplying the propulsive force required for fertilisation. These movements are powered by the energy generated by large numbers of mitochondria that are packaged within the midpiece of the sperm cells.

The characteristic long, narrow, extended shape of spermatozoa imparts these cells with a very high surface area in relation to their volume, and therefore with a relatively large amount of plasma membrane. This predominance of cell membrane is accomplished during spermatogenesis in the seminiferous tubules of the testis, where much of the cytoplasm of the sperm precursor cells (spermatogonia) is extruded. Other membranous structures within the cell, including the nucleus, acrosome and mitochondria, make further contributions to the total amount of spermatozoan biomembrane. Since the lipid bilayer of cell membranes consists of phospholipid (PL) plus some free cholesterol (chol), these amphiphilic lipids are seen as major structural components of spermatozoa. In fact, PL forms 70% (w/w) of the total lipid of spermatozoa

#### USE OF FATTY ACIDS TO CONTROL ENTERIC PATHOGENS IN POULTRY

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#### Introduction

Research over the last two decades worldwide has yielded substantial information on the effect of supplemented unsaturated long chain fatty acids on the meat quality of food animals, including poultry (Wood et al., 2008; Qi et al., 2010). However, short-chain and medium chain fatty acids on the other hand have been primarily investigated for improving animal gut health and reducing pathogen colonization. Besides their use as such (Van Immerseel et al., 2002; Kollanoor Johny et al., 2009a; Solis de los Santos et al., 2009, 2010), these fatty acids are also used in animal diet as sodium, potassium or calcium salts, due to their higher solubility in water, odorless nature, low volatility, less corrosive property and easiness to handle in solid form (Huyghebaert et al., 2010; Metcalf et al., 2011). Although a variety of pathogenic organisms, including Salmonella spp., Campylobacter spp., Listeria spp., Clostridium perfringens, Helicobacter pullorum, Arcobacter butzleri and Eimeria spp. colonize the chicken gastrointestinal tract, most of the research on the antimicrobial properties of fatty acids in chickens has focused on the former two pathogens. This is chiefly because Salmonella Enteritidis and Campylobacter jejuni represent the two most common food-borne pathogens transmitted to humans through poultry products, together accounting for more than two-third of the total laboratoryconfirmed cases of bacterial gastroenteritis (Anonymous, 2005). Therefore, the microbiological safety of poultry-derived food products are of great concern for the government, regulatory agencies and consumers, thus triggering research on controlling S. Enteritidis and C. jejuni carriage in chickens.

Among different classes of fatty acids, short- and medium- chain fatty acids have been investigated for their potential roles as antimicrobial agents to

#### FATTY ACIDS OF EGGS OF WILD AND DOMESTICATED BIRDS AND THEIR ROLES IN EMBRYONIC DEVELOPMENT

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#### Introduction

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When attempting to ascertain the "ideal" mix of maternal dietary fatty acids for supporting egg formation, successful embryonic development and post hatch health, it is worth considering the diversity of yolk fatty acid compositions that are achieved naturally by different species of birds in the wild. Avian yolk is a rich blend of lipids and proteins in aqueous suspension or solution, capable of supporting the growth and development of the embryo to the point of hatch. In fact the nutritive role of the yolk is often sustained for a few days after hatching, when the residual yolk that has not been utilised during the embryonic period is retracted into the body cavity of the chick where it continues to act as a food source. The lipids of the yolk are almost entirely located in sub-microscopic lipoprotein particles, millions of which are packaged within the vitelline membrane. The fatty acids that comprise the yolk lipids are not present in the free form but are esterified to glycerol or cholesterol to give the complex lipids, triacylglycerol (TAG), phospholipid (PL) and cholesterol ester (CE).

Abbreviations: CE, cholesterol ester; chol, free (i.e. non-esterified) cholesterol; FFA, free (i.e. non-esterified) fatty acid; LPL, lipoprotein lipase; MG, monoacylglycerol; PL, phospholipid; PUFA, polyunsaturated fatty acid; TAG, triacylglycerol; VLDL, very-low density lipoprotein; YSM, yolk sac membrane.

#### APPROPRIATE EXTRACTION AND METHYLATION TECHNIQUES FOR LIPID ANALYSIS

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#### Introduction

A common question often asked when conducting lipid research using animals is what needs to be analyzed to support the hypothesis under consideration. Studies based on feeding specific lipid enriched diets or feeding components that affect lipid metabolism in animals may involve investigating digestion, absorption, tissue accretion, fatty acid (FA) metabolism, or their effects on other

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Abbreviations: Alk-1-enyl methyl ether, AME; American Oil Chemists' Society, AOCS; Association of Analytical Communities, AOAC; attenuated total reflection, ATR; cholesterol ester, CE; conjugated linoleic acid, CLA; diazomethane, DAM; dimethyl acetal, DMA; dimethyloxazoline, DMOX; empirical correction factor, ECF; fatty acid, FA; fatty acid methyl ester, FAME; flame ionization detector, FID; Fourier transform infrared, FTIR; Fourier transform near infrared, FT-NIR; free fatty acids, FFA; gas chromatography, GC; gas liquid chromatography, GLC; high performance liquid chromatography, HPLC; internal standard, IS; international organization for standards, ISO; Japanese Oil Chemists' Society, JOCS; partially hydrogenated vegetable oil, PHVO; mass spectrometry, MS; phospholipid, PL; polyethylene glycol, PEG; solid phase extraction, SPE; triacylglycerol, TAG; thin-layer chromatography, TLC; polyunsaturated fatty acid, PUFA; trimethylsilyl-diazomethane, TMS-DAM.

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