Chapter 20 Egg Enrichment in Omega-3 Fatty Acids

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1 Introduction

The role of food in human health is again in vogue, however, the link between what we eat and our health is a subject that was being written about long ago as 500 BC when Hippocrates wrote "let food be your medicine and medicine be your food." Eggs have been associated with creation, fertility, and new life since ancient times and are consumed by every generation from childhood to late life. Thus, compared with the hen's egg, no other single food of animal origin is eaten by so many people all over the world, and none is served in such a variety of ways. The egg is considered as nature's most complete food containing high quality proteins and a 2 to 1 ratio of unsaturated fats to saturated fats. It is an excellent source of iron, phosphorous, and other minerals, and contains all the vitamins with exception of vitamin C. The possibility of manipulating the nutrient composition of eggs was shown as long ago as 1934, and modification of the polyunsaturated fatty acid (PUFA) composition has been addressed since the early 1960s. Egg yolk composition depends on the dietary nutrient provision and information is accumulating suggesting that other nutrients in eggs can have their content manipulated.

Some egg producers today supply new-type or specialty eggs (organic eggs, free-range eggs, omega eggs, etc.). These eggs may be slightly different in nutrient value from regular eggs or they may come from hens housed or fed in a special way.

2 Fatty Acid Requirements in Humans

There are two families of PUFA, omega-6 (ω-6) and omega-3 (ω-3), defined by the position of the double bond close to the methyl end of the molecule (Simopoulos 2002).

The ω-3 fatty acids are long-chain PUFA (18–22 carbon atoms) with the first of many double bonds beginning from the third carbon atom when

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counting from the methyl end (Holub 2002). Especially, the ω-3 can be categorized according to their chain length. The 18-carbon n (ω) linolenic acid (ALA18:3n3) is precursor to the longer ω-3 polyunsaturated fatty acids eicosapentaenoic acid or EPA (20 carbon atoms, 5 double bonds, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3).

These ω-6 fatty acids are also long-chain PUFA with the first of many double bonds beginning at the sixth carbon atom from the methyl end. The parent acid of this family is linoleic acid (LA, 18:2, n-6) which is converted to the longer chain ω-6 fatty acid, arachidonic acid (AA, 20:4, n-6).

Of all fats found in food, ALA and LA cannot be synthesized in human body. These fats are called essential fatty acids because they are necessary for physiological functions.

2.1 Fatty Acid Metabolism in the Human Body

After ingestion, ALA and LA are converted into long chain PUFA. The liver hepatocyte is a main site for the biosynthesis of 20-and 22-carbon PUFA from ALA (e.g., readily to EPA and more slowly to DHA) and for the formulation of lipoproteins that transport fatty acids in the plasma (Pawlosky et al. 2001). The only known metabolic function of ALA is to be a dietary precursor of EPA and DHA. Conversely, supplementing the diet with ω-3 long chain PUFA (e.g., EPA and DHA) will not lead to increased tissue levels of ALA (Jones et al. 2001). Conversion of ALA to EPA and DHA occurs to a low degree (about 0–15 %) in the adult human body (Jiang and Sim 1993). This conversion is affected by several factors, including the concentration of ALA and ω-6 fatty acids, mainly LA, and it is not sufficient for optimal health (De Deckere et al. 1998). LA can also be elongated and desaturated into AA, but the conversion from LA to AA via γ-linolenic acid (20:3, n-6) appears to occur slowly in humans and there is a competition between ω-3 and ω-6 fatty acids for Δ-desaturase enzymes. Thus, a balance between ω-6 and ω-3 PUFA in the diet is important since both compete for the same enzymes and they have different biological functions. It appears that ω-3 fatty acids are preferred by 6-desaturase enzymes (Simopoulos 1988).

2.2 Effects of PUFA on Human Health

Researchers around the world have focused on the health effects of a dietary supply of ω-3 fatty acids, partly because those fatty acids have been reported to protect against cardiovascular and inflammatory diseases, as well as certain types of cancer, and partly because it has been shown that ω-3 fatty acids are essential nutrients for adults and children.

The potential health benefit of ω-3 fatty acids in the human diet has drawn attention since the original publication of Dyerberg and Bang (1974) who reported a link between dietary ω-3 fatty acid consumption and decreased incidence of cardiovascular disease in Eskimos. The results of more recent studies have suggested that ω-3 PUFA, as EPA and DHA, are important for brain development in children, prevent platelet aggregation, reduce serum triglycerides and very low density lipoproteins that promote the clogging of arteries, etc. An increased intake of ω-3 fatty acids is now known to protect against heart disease, some inflammatory diseases, and certain autoimmune disorders.

While ω-3 PUFA play a major role in infant growth and development and have been implicated in many other protective roles, ω-6 PUFA are metabolically and functionally distinct and in many cases have opposing physiological effects. The absolute amount of PUFA in the diet is important to human health. Health authorities recommend, for all adults of 19 years and over, an intake of LA of 11-17 g/day and for ALA of 1.1–1.6 g/day (Holub 1998; Howe 1998). The balance between the two major types, ω-6 and ω-3 fatty acids, may also be crucial. The proper ratio of ω-6 to ω-3 PUFA in the human diet appears to be in the range of 1:1 to 4:1.The current ratio in the human diet in the developed world is about 10:1 and thus individuals are advised to consume more foods containing ω-3 fatty acids (green leafy vegetables, legumes, fish, etc.).

3 Lipid Metabolism in Avians and Lipid Composition of Eggs

The yolk of the average chicken egg contains 6 g of lipids mainly in the form of triacylglycerols, phospholipids, and free cholesterol (Noble and Cocchi 1990). Minor yolk components include cholesterol esters and free fatty acids. The phospholipid component exhibits levels of LA, AA and DHA as well as other PUFA (Noble and Cocchi 1990). Fatty acids destined for the yolk are synthesized in the hen's liver, which permits the manipulation of fatty acid components through dietary measures (Cherian et al. 1996). As Sim (1993) reported, the yolk lipid composition is the result of a combination of de novo lipogenesis and incorporation of lipid components from the hen's diet. Thus, egg yolks can be enriched with ω-3 PUFA by the incorporation of fats rich in these essential fatty acids.

Upon absorption from the intestinal lumen, hydrolyzed products of lipid digestion, including long chain fatty acids and monoacylglycerols, must be re-esterified within the endoplasmic reticulum of enterocytes prior to transport (Taylor 1998). The resultant triglycerides are packaged with cholesterol, phospholipids, and proteins to form lipoproteins. In human, these lipoproteins (chylomicrons) are transported within the lymphatic system (Bensadoun and Rothfeld 1972). In poultry, those lipoproteins that include long-chain fatty acids are referred to as portomicrons, because they are transferred to the hepatic portal circulation (Bensadoun and Rothfeld 1972). On the other hand, short-chain fatty acids and free glycerol are transported as such in poultry (also to the liver via the portal system). In the avian liver, extensive reprocessing of glycerides and fatty acid residues from the portomicron remnants occurs. The lipids reform and are included in particles of very low density lipoprotein (VLDL), which then pass to the Golgi complex where they acquire phospholipids and further glycosylation. Finally, completed particles of VLDL concentrate in secretory vesicles and then discharge into the blood (Bensadoun and Rothfeld 1972).The VLDL are then carried by the blood to the ovarian follicles, where they diffuse through holes in the capillaries. The particles enter the yolk by receptor mediated endocytosis through the oolemma.

4 Omega-3 Enriched Eggs as a Means to Improve Human Health

Commercial table eggs contain a high proportion of ω-6 PUFA but they are a poor source of ω-3 fatty acids. There are two main approaches to increase the ω-3 content of eggs, either by the enrichment of the diet with ALA of a vegetable source (flaxseed, linseed, etc.) or by the addition of any source of EPA or DHA (fish meal, fish oil, algae, etc.).

Flaxseed is a rich source of unsaturated fats, it contains about 38% fat, and 50% of this fat is in the form of ALA. It appears that the concentration of ALA can be increased from a very low level of 21 mg/egg, when hens are fed diets without flaxseed, to a level of 580 mg/egg by the addition of flaxseed at levels of 20% (Table 1). ALA is the major ω-3 fatty acid that accumulates in the triglyceride fraction of yolk lipids due to the consumption of flaxseed, although research indicates that the concentration of EPA and DHA can also be increased to 8.5 and 87 mg/egg, respectively, for hens fed 10% flaxseed.

On the other hand, menhaden and herring oils contain about 1% ALA but a total 12–14% of combined EPA and DHA. Three percent dietary menhaden oil increased yolk DHA to 252 mg/egg (Van Elswyk et al. 1995; Table 1). Although the use fish oils has the advantage of enriching the eggs mainly with long ω-3 fatty acids that are specially involved in human health, the maximum level of fish oils that can be used in the layer diet is limited due to production of eggs with a fishy flavor. Van Elswyk et al 1995 reported that treatments with less than 3% oil did not significantly lower overall flavor quality. Another approach for the enrichment of eggs with long chain ω-3 is to use microalgae in the laying hen diet. Different kinds of microalgae may contain up to 50% of their total fatty acids in the form of EPA and DHA.

In general the ω-3 fatty acid profile of the egg is dependent on which supplements hens are fed (Van Elswyk 1997). The levels of ω-3 in the yolk of eggs achieved by the inclusion of different sources of ω-3 fatty acids in the diet varies considerably (Jiang and Sim 1994; Surai 2003). For example

Table 1. Enrichment of the ω-3 fatty acid content in eggs using different ω-3 sources (mg/egg) and ω-3 content in different egg types

Diet	ALA	EPA	DHA	Source
20% flaxseed	580	8	74	Caston and Leeson 1990
2% linseed + 2% mackerel oils	214	32	214	Farrell et al. 1991
3% menhaden oil	21	24	252	Van Elswyk et al. 1995
12% herring meal	66	8	100	Nash et al. 1995
Egg type				
Supermarket egg	8.5	0	19	Simopoulos 2000
Greek egg	117	20	112	Simopoulos and Salem 1992
Fish meal egg	70	3.5	110	Simopoulos 2000
Flaxseed egg	362	8.5	87	Simopoulos 2000

(Table 1), the inclusion of flaxseed rich in ALA increased the level of DHA in the egg up to 74–87 mg. Greek free range eggs prior to 1960 provided 112 mg DHA (Simopoulos and Salem 1992), while a similar amount of DHA, 100 mg, was accumulated in the egg yolk as a result of using 12% herring meal (Nash et al.1995) or 1.5% of menhaden oil (106 mg DHA) (Marshall et al. 1994).

4.1 Enriched ω3 Eggs in Practice

In the case of the ω-3 enrichment, eggs are naturally enhanced through programmed feeding of laying hens with PUFA, antioxidants, and vitamins which are transferred to the egg. This natural program of enrichment of eggs may be economically and socially beneficial for the egg industry. When considering options of enrichment of eggs by certain nutrients, e.g., ω-3 fatty acids, it is necessary to take into account several factors (Sparks 2005; Seuss-Baum 2005):

1. Form of nutrient in the diet and efficiency of the transfer from feed into the egg
2. Possible toxicity or adverse effects or interactions of the increased dietary supplementation on chicken health and productive characteristics
3. Total amount of the nutrient delivered with a single egg in comparison with the daily requirement of this nutrient
4. Availability and cost of commercial sources of effective feeds
5. Stability during cooking and shelf life
6. Effect on appearance and organoleptic characteristics (flavor, odor)
7. Nutrition claims and health benefits

Table 2. Omega-3 fatty acid content (EPA and DHA) of selected fish and ALA in other feeds

Product	Concentration of sum EPA and DHA mg/100g
Mackerel	2,500
Herring	1,700
Salmon	1,200
Trout	500
Tuna	400
Shrimp	300
Cod	300
	Concentration of ALA mg/g net weight
Purslane	4.05
Spinach	0.89
Flaxseed	23.4 g /100g of edible portion

Since enrichment is based only on the natural transformation of the substances from feed to egg, such eggs are positively accepted by consumers. It needs to be stressed that PUFA, so important for human life and health, are present in oils, fish, and flaxseed in the amount of 300–2,500 mg/100g (Table 2).

However, a higher PUFA content leads to an increase in lipid unsaturation, and thus to a greater susceptibility to oxidation and free radical production. Lipid oxidation is of major importance because it can adversely affect the overall quality of foods, including flavor, taste, and nutritional value. Also, secondary reaction products of the lipid oxidation process have been related to the development of cardiovascular and other diseases. Moreover, several human diseases at different stages of their development have been associated with free radical production and their metabolism, since normally there is a balance between the amount of the free radicals generated in the body and the antioxidant capacity of the tissues. In order to prevent lipid peroxidation, antioxidants (vitamin E, herbal mix, selenium, etc.) have been widely used by the food industry.

4.2 Health Benefits of ω-3 Enriched Eggs

The effect of consuming ω-3 enriched eggs has been the subject of many studies in healthy adults, the very young, and the aged (Sparks 2005).The major advantage of the consumption of ω-3 eggs is an enrichment of plasma lipids with these fatty acids (Farrell 1998). Sindelar et al (2004) reported that consumption of one ω-3 enriched egg results in elevated serum levels of ALA and triglycerides. Blood pressure was decreased and DHA concentration in the plasma increased as well (Farrell 1998). Consumption of four ω-3 eggs a week by volunteers for 6 weeks caused a significant decrease in platelet aggregation (Van Elswyk et al. 1998). Therefore, depending on the amount of eggs consumed, ω-3 eggs are proved to have health-promoting properties by increasing ω-3 fatty acid levels in blood lipids and, in some cases, even reducing cholesterol and triglyceride levels in the plasma. Yannakopoulos et al. (1999a), reported that plasma total cholesterol was reduced ($P < 0.05$) in humans aged 41–50 years who consumed ω-3 eggs, while HDL cholesterol was raised. Narahari (2004) conducted an experiment with human volunteers to determine the effects of the consumption of designer eggs. He reported that the consumption of herbal enriched functional eggs not only reduced the serum triglycerides and LDL cholesterol levels, but also increased the HDL cholesterol level. The increasing awareness of these benefits may have helped the egg industry to rebound from a declining consumption over the past twenty years (Asselin 2005).

4.3 Organoleptic Characteristics

Eggs enriched with PUFA may be associated with off-odors and particular fishy taints. However, these can be minimized if the hens are fed 1.5% or less of a high quality fish oil, or 5% or less flaxseed, and if the PUFA are protected both in the diet and in the egg from oxidation (Surai and Sparks 2000).

The organoleptic quality of ω-3 eggs tends to be similar to regular table eggs although in some cases panelists are able to detect off flavors. A "fishy" or fish-product-related flavor was detected in eggs from hens fed on diets containing 15–20 % flaxseed.

Cooking characteristics of ω-3 eggs, including emulsification capacity, hardness, and springiness of sponge cakes prepared using these, were the same as in regular eggs.

Data on effects of ω-3 enrichment on egg quality during storage are limited and sometimes contradictory. Van Elswyk et al. (1992) reported that there is no alteration in the fatty acid profile of eggs enriched with ω-3 PUFA during cooking or during storage for 7 weeks at 25 °C (Oku et al. 1996).

4.4 The ω-3 Egg in the Market

Egg demand has been and continues to be affected by health information and nutrition concerns. The egg industry has been very responsive in seeking new technology to improve consumers' negative perception of the egg associated with cholesterol. Enriched eggs may constitute a valuable and safe supplement to the diet of children, smokers, people on unbalanced diets, people on slimming diets, vegetarians, and people engaged in intensive sports.

The early generation of modified eggs was enriched with ω-3 and vitamin E. For this purpose hens' diets were usually enriched with flaxseed, linseed, fish oil, algae, etc. Such eggs are now available in Europe, Asia, America, and

Australia, and constitute the most common type of enriched eggs. The predominance of these eggs in the marketplace is determined by two key characteristics. First, the general public is sensitized to the health benefits of consuming less saturated and more PUFA and, to a lesser extent, the need to adjust the fatty acid balance of the diet so that LA and ALA ratio falls between 5:1 and 10:1 (Anonymous 1998, Simopoulos 1998). Secondly, it is biologically possible to produce eggs with favorable fatty acid profiles. A new generation of ω-3 eggs has been enriched with ω-3 fatty acids and herbal mixtures or other antioxidants such as selenium, vitamin E, etc., simultaneously.

An additional requirement for enriched eggs should be a guaranteed and consistent product quality. The ω-3 egg occupies a niche market, but it is undoubtedly a niche that can be expanded, provided that the consumers remain confident in the product and in the claims made for it. In Greece, one of main reasons consumers buy ω-3 eggs is their "health value", since the 93.3% of the consumers believe that ω-3 eggs are healthier than the regular ones (Sosidou et al. 2005).

It would be very interesting to create a regulation for the EU-wide uniform addition of ω-3 to eggs. At present, such a lack has led to an obstruction in the free movement of these eggs because of the member states' separate regulations. Over the world, only Health Canada has made amendments to the regulation on ω-3 eggs in order to improve nutritional information. Regarding such regulations and labeling, since ω-3 eggs have a different nutrient profile, the fatty acid composition (e.g., content of EPA and DHA) should be declared on the package so that consumers are informed about the nutritional value of the food.

In general terms, labels on ω-3 eggs should bear the following information:

- National identification, name, and address of the organization that has packed the eggs
- Number, quality grade, and size of eggs
- "Best before" date
- Consumer advice, such as "keep refrigerated"
- Farming system for the eggs (battery, free range or barn, organic)

Of note is that the Joint Australia/New Zealand Food Standards Code now includes a nutrition claim for the ω-3 content of foods contain the following points:

- A food may be labeled as a "source" of ω-3 if it contains at least 200 mg ALA or 30 mg of combined EPA and DHA per serving.
- A food may be labeled as a "good source" of ω-3 if it contains at least 60 mg of combined EPA and DHA per serving.

Nutrient-enhanced eggs in the EU market comprise between 1and 5% and show an increasing trend yearly. The production of enhanced eggs involves more cost and they are usually sold at higher prices than regular eggs (2–3 times more).The prices are about to become similar to prices currently paid for free range and organically produced eggs. It is about 0.40 euro/egg.

5 Combined Enrichment with Omega-3 and Natural Antioxidants

Plants and their extracts have formed part of animal diets as preservatives, flavors, digestive enhancers, and remedies for millennia. Farmers in several countries use medicinal plants in the maintenance and conservation of the healthcare of the livestock. Medicinal plants are an integral component of ethnoveterinary medicine. Specific botanical components have been shown to be active against damaging compounds, such as mycotoxins, which they strongly bind and inhibit. A correct formulation of botanical supplements and ingredient activities is key for a product that delivers consistent benefits in vivo and optimizes the balance between cost of inclusion and overall efficacy (Botsoglou et al. 1997, Yannakopoulos et al. 2001). The herbs contain ingredients active in three main areas: gut microflora and environment, antioxidation, and liver function (Lange 1998). In Greece, Yannakopoulos et al. (1999b) have developed herbal enriched ω-3 eggs which are not only rich in ω-3 fatty acids and vitamin E, but also in active herbal principles such as natural antioxidants (thymol, carvacrol, and others). Since 1997, research has been undertaken to produce these herbal enriched ω-3 eggs (named Vi-ωmega-3; Table 3) (Yannakopoulos et al. 2004).

The Vi-ωmega-3 eggs were found to contain less saturated lipids and more PUFA compared to regular eggs. These eggs were also found to contain higher levels of ω-3 fatty acids, particularly of the DHA type, compared to regular eggs (120 mg vs 0 mg). This may be due to the diet supplementation with the herbal additive, which might have helped in a more efficient conversion of ALA to DHA. The Vi-ωmega-3 eggs have also been found to contain cholesterol at markedly lower levels than the control eggs, possibly due to the combined action of the herbal additive constituents. The higher levels of vitamin E compared to the control eggs, as well as the possible transfer of natural antioxidant compounds from the herbal additive, could justify the

Table 3. Comparison of Vi-ωmega 3 eggs with regular eggs of net weight of 53.6 g

Nutrients	Regular eggs	Vi-omega-3 eggs
Lipids per egg (g)	4.74	4.15
Saturated (g)	1.5	1.16
Monounsaturated (g)	2.1	1.62
Polyunsaturated (g)	0.9	1.19
N-3 fatty acids (mg)	40	350
DHA fatty acid (mg)	0.0	120
Cholesterol (mg per egg)	220	175
Vitamin E (mg per egg)	0.7	3.5

higher oxidative stability exhibited by the Vi-ωmega-3 eggs when all eggs were submitted to experimentally induced lipid oxidation (Botsoglou et al. 1997, Yannakopoulos et al.2004). Results from the taste panel showed that the organoleptic acceptability was higher in the ω-3 herbal enriched eggs compared to the control eggs (Tserveni-Gousi 2001). This could be due to the combined action of the herbs, vitamins, and minerals occurring in the herbal additive, since it is known that the ingredients used in layer diets to enrich eggs with polyunsaturated fatty acids often influence the sensory characteristics of the eggs (Van Elswyk 1997).

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