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Improvement of biologic and nutritional value of eggs

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SUMMARY - Although egg consumption has experienced a continuously decreasing trend in Europe and North America, there have been tremendous efforts for increasing egg nutritional value. The health-conscious consumer is willing to pay more for a value-added product. A (partial) review is presented on some of the topics involved in the improvement of egg value: minerals, vitamins, carotenoids, ω -3 fatty acids, cholesterol and therapeutic antibodies; and some of their implications in human health.

Key words: Eggs, minerals, vitamins, carotenoids, ω -3 fatty acids, cholesterol, therapeutic antibodies.

RESUME - "Amélioration de la valeur biologique et nutritionnelle des oeufs". Malgré que la consommation d'oeufs a diminué d'une manière continue en Europe et Amérique du Nord, on a fait d'énormes efforts pour améliorer la valeur nutritionnelle de l'oeuf. Le consommateur conscient, est disposé à payer un supplément pour un produit avec une plus grande valeur. Une révision (partielle) est présentée, sur certains aspects impliqués dans l'amélioration de la valeur nutritionnelle de l'oeuf : minéraux, vitamines, caroténoïdes, acides gras ω -3, cholestérol, anticorps thérapeutiques, et certaines de ses implications dans la santé humaine.

Mots-clés : Oeufs, minéraux, vitamines, caroténoïdes, acides gras ω -3, cholestérol, anticorps thérapeutiques.

Introduction

Egg consumption has decreased in some parts of the world in the last decades. However, the value of eggs as human food has not. Moreover, there has been an increasing effort to improve its nutritional value lately.

The egg has not been conceived by the hen to be fed to human beings, but its objective of supporting the embryo development for three weeks, makes it a good supply of the three main nutritional requirements: energy, protein and essential accessory factors (vitamins, carotenoids, minerals and certain fatty acids and aminoacids).

According to the World Health Organization the protein in egg has the highest true digestibility of major foods, and its nutritive value is also high because it contains the essential aminoacids in the required proportions (Fig. 1).

The egg has also been described as a low energy source of equilibrated proteins and easily digested fats. It represents an important source of phosphorus (together with milk, it is the richest food in available phosphorus), iron and vitamins; however it is deficient in glucides, calcium and vitamin C. Although one third of the egg yolk are lipids, the egg as a whole is considered to be a low-calorie source of other nutrients. Moreover, from egg yolk lipids, just 1/3 are saturated fatty acids and eggs are rich in linolenic acid, essential in human nutrition. The lipids are present in the yolk in an emulsified form that favours their almost completely digestion by man (94-96%). They can also be enriched in ω -3 fatty acids, thus helping in preventing atherosclerosis, etc.

When the hen's diet is supplemented with magnesium, manganese, zinc, iodine, and selenium, the levels of these minerals raise in the egg; whereas the iron content is not as easily modified. A summary of the results from several experiments are presented in Table 1.

The egg content of vitamins can be modified by diet supplementation, the response varying depending on the vitamin: very high response can be obtained with the liposoluble vitamins A and D,

good response is obtained with riboflavin and vitamin B₁₂, and moderate response with other vitamins from B group (biotin; folic acid; pantothenic acid). Carotenoids can also be easily increased in the egg yolk with higher dosages in the diet; however, there is an interaction between carotenoids and several vitamins: if the carotenoid level in the diet is high, there is a reduction in the deposition of vitamin A and certain vitamins B in the yolk; the reverse is also true: high levels of vitamin A interfere with carotenoid absorption.

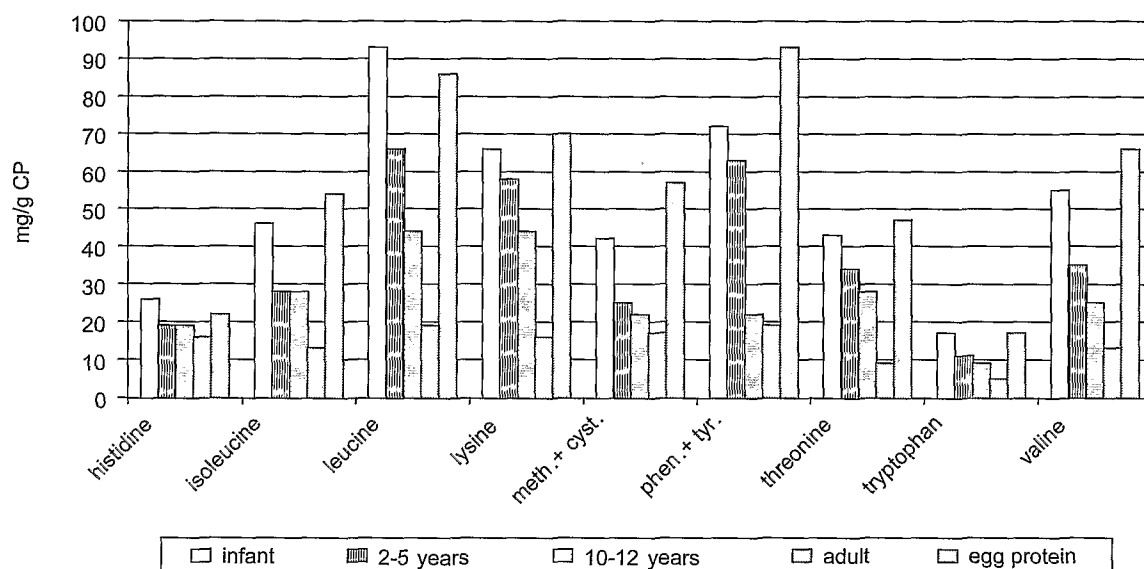


Fig. 1. Suggested essential amino acid Daily Requirements compared with the composition of hen's egg yolk protein (from Burley and Vadehra, 1989).

Table 1. Increment in the levels of several minerals in egg from several experiments

Mineral	Increment vs control		Source
Chromium	10	Fold	Kang <i>et al.</i> , 1996
	5	Fold	Meluzzi <i>et al.</i> , 1996
Iodine	10	Fold	Naber and Squires, 1991
Iron	1.4	Fold	ITPSA, 1997
Manganese	1.4	Fold	Jandrey, 1987 (cited by Naber and Squires, 1991)
Selenium	5	Fold	Latshaw and Osman, 1975
Zinc	1.9	Fold	Jandrey, 1987 (cited by Naber and Squires, 1991)

The most important developments in the chemistry of egg yolk in the recent years are the characterization of sugar chain structures, and the use of egg yolk antibodies as an alternative method to generate antibodies for a great range of applications (Baker & Halpin, 1991). The immunization of chickens and the extraction of antibodies from yolk (IgY) is a great advantage because the animal suffering is reduced, the yield of antibodies from eggs is much higher than from blood, and there are less cross-reactions with IgY in comparison to IgG from mammals.

Enriched eggs with some minerals, vitamins, carotens, fatty acids, and even specific antibodies or decreased cholesterol content, provide the possibility of new eggs and egg products on the present market and in the near future, yielding eggs with superior biologic and nutritional value for human consumption.

Minerals

Chromium

Mineral deficiencies are causing more health problems than the overconsumption of fats, cholesterol and refined carbohydrates that are the targets of almost all of public health measures (Brown, 1996). There are at least 256 perceived benefits of organic chromium. A portion of the need for effective fiber is, in fact, a need for organic chromium.

Anderson and Kozlovsky (Anderson & Kozlovsky, 1985) reported that the dietary chromium intake for normal subjects might not meet the suggested minimum daily intake. As chromium enhances the uptake of glucose, insufficient dietary chromium is associated with increased risk factors linked with noninsulin-dependent diabetes and cardiovascular disease. Variables in humans that have been shown to be improved following chromium supplementation include fasting blood glucose concentration and glucose tolerance, serum insulin and insulin binding, total cholesterol and HDL cholesterol (Anderson, 1992).

The chromium content in egg yolk and albumen was higher for chromium supplemented hens than for control ones, without differences from chromium source (inorganic or organic). The chromium content increased sharply the first two weeks (10 times) while decreased to two- three-fold the basal level thereafter (Kang et al., 1996).

Chromium content of eggs was also increased in other experiments (more than 5-fold) by supplementation of chromium to the hens diet; however, chromium content decreased with time of supplementation (30 to 75 d) (Meluzzi et al., 1996).

The amount of metal transferred to the edible parts of the egg was, however, irrelevant with respect to the amount the hens ingested to produce an egg. Moreover, egg production and weight were negatively affected by heavy metals at high dosages.

Those results indicate that although it is possible to accumulate chromium in egg yolk and albumen to supply it as a chromium source for humans, more studies should be performed before obtaining an economical chromium-enriched egg.

Iron

Iron plays an essential role in many metabolic processes including oxygen transport, oxidative metabolism, and cellular growth. Iron-deficient anemia is probably the most prevalent human nutritional disorder, leading to reduce work performance and increased morbidity. Individuals particularly vulnerable to iron deficiency are those with high iron requirements: children, pregnant women, persons with high iron loss due to hemorrhage or parasitic infections, and women with excessive and/or frequent menstrual losses. Therefore, iron deficiency occurs when the demand for iron is high (growth, high menstrual losses, pregnancy) and dietary intake is quantitatively inadequate or contains elements that render the iron unavailable for absorption.

The current Recommended Daily Allowances (National Research Council -NRC-1989) for adults are calculated to provide sufficient bioavailable iron to ensure the maintenance of an iron store. The Recommended Daily Allowance for women was set at 15 mg/d; for men, young girls and postmenopausal women 10 mg/d is considered to be enough. Data from the National Health and Nutrition Examination Survey III showed a lower than recommended iron intake for adolescent and menstruating women aged 12-50 years.

The presence of marginal vitamin A deficiency in a community may limit the effectiveness of an iron intervention program. It has also been shown that response to iron supplementation is greater when vitamin A is given in conjunction with iron. An immediate conclusion can be the simultaneous fortification of iron and vitamin A (although it seems to be not easy for any of them).

The Relative Biological Value of yolk iron, was not different from that of the reference salt, ferrous sulfate, without any interference of egg yolk in the absorption of the inorganic salt (Miller & McNeal,

1983). Eggs could contribute significantly to the dietary iron supply since one standard egg contains about 1 mg of iron. Furthermore, the ratio of iron to calories in eggs, 12.5 mg/1000 kcal, is about twice that in the average American diet. Eggs might be a food of choice for those who require a generous supply of dietary iron and a limited calorie intake, and iron fortified-eggs will have an increased value.

When hens were fed graded levels of iron from 15 to 65 ppm, a dose-response was observed of hematocrit and hatchability (Morck & Austic, 1981). Weight gain, hemoglobin, and hematocrit were markedly improved when increasing levels (0 to 80 mg/kg) of added iron from analytical grade ferrous sulfate were fed to chicks (Biehl et al., 1997). Organic sources of iron showed no clear advantage over the inorganic salt in several experiments (Biehl et al., 1997). The iron content of the egg yolk was increased up to 125% and 140% with inorganic and organic iron sources (ITPSA, 1997).

Organic acids have been characterized as enhancers of iron absorption in human (Lynch, 1997), then it might be possible that ascorbic, citric, malic, tartaric or lactic acids increase iron absorption by the hen. However, phytates, polyphenols and several vegetable proteins, all likely to be present in the hen's feed, are known as inhibitors of iron absorption. Also, there is an interaction between manganese and iron, in which excess manganese impairs iron utilization, but excess iron does not antagonize manganese (Baker & Halpin, 1991).

Zinc

In a survey of the incidence of cancer in children, it was found that serum concentrations of beta-carotene, alpha-tocopherol and zinc were significantly inversely associated with cancer, and no significant association with cancer was observed for serum values of selenium (Malvy et al., 1997).

From a therapeutic standpoint, the micronutrient and protein deficiencies at diagnosis in childhood malignancy may have pathogenic implications, and theoretically could be corrected by a nutritional rehabilitation comprising supplemental vitamins and trace elements (zinc) administration.

Zinc has been recognized for years as an important trace mineral for maintaining health in humans and other animals. Zinc is ubiquitous in cellular metabolism and functions as a catalyst and stabilizes the quaternary structure of metalloenzymes, RNA, DNA, and ribosomes (O'Dell, 1992). More than 200 proteins contain zinc, and several biological functions of zinc have been identified. The most notable hormones that require zinc include insulin, adrenal corticosteroids, and testosterone.

Kidd *et al.* (Kidd et al., 1992) did a study in which progeny from broiler breeders fed 72 mg Zn/kg diet had heavier embryonic bones and improved immune status, as a result of Zn accumulation in the egg. Similar results were found by Flinchum *et al.* (Flinchum et al., 1989). Eggs represent a good opportunity for supplying extra zinc to human diets. Zinc content of egg yolk is closely related to diet content (Jandrey, 1987, cited by (Naber & Squires, 1991): adding 80 mg/kg of zinc to a laying hen diet containing 65 mg/kg (NRC states for 50 mg/kg addition of Zn to laying hen diets) increased egg yolk zinc levels from 0.84 to 1.62 mg/egg.

Iodine, manganese and selenium

Other elements that vary according to the diet are iodine and manganese (Naber, 1979).

Feeding potassium iodide, kelp or iodized linseed meal to laying hens resulted in a high egg uptakes of this element that were 10 times the basal level (up to 400 µg/egg) when 16 mg/kg of iodine from these sources were fed (NRC 0.3 mg/kg) (Naber & Squires, 1991). Egg iodine can be increased markedly and it has recently been postulated that kelp-fed laying hens may produce eggs that reduce blood cholesterol in human subjects. Ternes and Leitsch (Ternes & Leitsch, 1997) refer to other authors that found that a lower enrichment of iodine in the diet (2.6 mg/kg) produced iodine enriched eggs (74 µg/egg compared to 26 µg/egg of generic eggs).

Manganese levels in egg yolk responded only fractionally to the increase in the dietary manganese supplement. Adding 60 mg/kg of manganese to a laying diet containing 37.5 mg/kg (NRC 30 mg/kg of

manganese addition) increased egg yolk manganese levels from 156 to 222 µg/egg (Jandrey, 1987; cited by (Naber & Squires, 1991). However, there is an impairment of iron absorption when high levels of manganese are fed (Baker & Halpin, 1991).

Egg selenium values reflect changes in dietary selenium in a short period of time. In addition, various selenium compounds give different patterns of distribution because egg white proteins are synthesized in the oviduct and yolk proteins are synthesized in the liver. Selenium-methionine was particularly effective in increasing egg albumen selenium concentration while sodium selenite and selenium-cysteine were more effective in increasing egg yolk concentration. Latshaw and Osman (Latshaw & Osman, 1975) found increments up to five-fold in selenium content of eggs (from 2 to 10 µg/egg). Appropriate selenium supplementation of rations for laying hens may help to provide for selenium needs of the human via the egg.

Vitamins

A study comprising 243 people aimed to determine the vitamin status of young British people (Benton et al., 1997). The status of young British adults of riboflavin and pyridoxine was either borderline or deficient. The thiamin and biotin status of a minority of both sexes, and retinol in the females, was marginal. The status of ascorbic acid, cyanocobalamin, α-tocopherol, folic acid, and in males retinol, was adequate in the majority of cases.

The recently published National Health and Nutrition Examination Survey (NHANES III) found that vitamin E intake was 10.00 mg in men and 7.57 mg in women; comparable values were found in European countries. Those values are close to the practical allowance stated by the current tenth edition of the Recommended Daily Allowances (10 mg for adult males and 8 mg for adult females). However, based upon studies of vitamin E kinetics and metabolism, a daily vitamin E intake of 135-150 IU is suggested by Weber *et al.* (Weber et al., 1997).

The main topic of vitamin research in eggs is the enrichment of vitamins. The enrichment of eggs with vitamin D and folic acid is a new development for fortified eggs. Vitamin E is also considered a target not just as a fortification per se, but also as a complement to improve the stability of other components. The increments achieved in egg content of several vitamins by dietary supplementation are presented next in Table 2.

Table 2. Increment in the levels of several vitamins in egg from several experiments

Compound	Increment vs control		Source
Vitamin A	2	Fold	Naber and Squires, 1991
Vitamin B ₁₂	5	Fold	Naber and Squires, 1991
Vitamin E	100	Fold	Surai <i>et al.</i> , 1997
Biotin	2.2	Fold	Robel, 1991
Riboflavin	3	Fold	Squires and Naber, 1993

Vitamin A

Vitamins in the egg would be expected to vary with the concentration in the feed. However, for some vitamins, as vitamin A, the liver acts as a reservoir so that the concentration in the yolk is buffered against large changes in the diet (Naber, 1979).

Naber and Squires (Naber & Squires, 1991) performed experiments with this vitamin and laying hens. At low dietary levels, liver storage was minimal but egg yolk increased sharply. At higher dietary levels liver storage increased markedly while egg yolk level continued to increase at a moderate rate. Over the range of 2600 to 22000 IU/kg diet liver storage of the vitamin approached a 200 fold increase while egg yolk vitamin content doubled (NRC 4000 IU/kg, 117 IU/egg). There is a delay of 8-

10 weeks on the supplementation of the diet with vitamin A and the increase of this vitamin in the egg, as liver acts as a regulatory-storage.

Vitamin B₁₂

It is also possible to modify the content of the egg with vitamin B₁₂, as described by Naber and Squires (Naber & Squires, 1991). When increasing the feed dosage of vitamin B₁₂ from 24 to 200 µg/kg (NRC 4 µg/kg), there was a 5 fold increase in yolk vitamin level (normal level 0.6 µg/egg). Therefore, a considerable vitamin B₁₂ enrichment of egg yolk is possible.

Vitamin D

In contrast to the situation with vitamin A, there is a linear relationship of dietary vitamin D to vitamin D in egg yolk. Excess dietary vitamin D is readily absorbed, transferred to the ovary and secreted in egg yolk during its formation (Naber & Squires, 1991). In other experiments, the concentration of vitamin D₂ in the egg yolk was 14.5 IU/g when the concentration in the diet was 20 IU/g (Kawazoe et al., 1996). Hence egg yolk can be fortified with vitamin D in order to increase its nutritional value.

Vitamin E

The primary physiologic role of vitamin E is as a biological antioxidant, and its potential role in human health includes enhancement in the immune response, decreased risk of cancer and cardiovascular and coronary heart diseases.

A number of studies in animals and in humans report that the amount of vitamin E needed to protect polyunsaturated fatty acids against oxidative damage ranges from 0.4-0.8 mg vitamin E per gram polyunsaturated fatty acid intake. The current tenth edition of the Recommended Daily Allowances acknowledges that a ratio of 0.4 mg of RRR- α -tocopherol per gram polyunsaturated fatty acids has been suggested by scientists.

When vitamin E was supplemented at 200 µg/g of feed, the concentration of vitamin E in the egg was 188 µg/g. Eggs from laying hens fed diets with 10 (basal level), 200, 2000 and 20000 mg/kg of α -tocopheryl acetate contained up to 2, 15, 100 and more than 200 mg α -tocopherol per egg respectively. The increase in vitamin E content of the egg was in a dose-dependent manner, and after a maximal at 3 weeks after starting diet supplementation it decreased to approximately half values from 7-8 weeks on (Surai et al., 1997). This drop in vitamin E content of the yolk might be related to the mechanisms of absorption and transport of vitamin E to the egg yolk.

From these vitamin E content it becomes clear that those enriched-eggs may be effectively used as a good source of vitamin E for human consumption, where increased vitamin E doses are recommended.

Another possibility is a storage of egg powder without accumulation of peroxidation products having harmful effects on human health. Also, simultaneous enrichment of eggs with ω -3 polyunsaturated fatty acids and vitamin E will be seen more desirable, with higher consumer acceptability.

Experiments with rats (Yoshizawa *et al.*, 1991; cited by (Surai et al., 1997) showed that egg yolk tocopherol ingested as part of the diet prevented lipid peroxidation, haemolysis and testicular atrophy.

Comparing the vitamin content of different meat cuts from several species and eggs, from animals fed diets supplemented with vitamin E, (Leonhardt et al., 1997) concluded that egg was the best source of vitamin E for human nutrition by dietary modification.

All together, these results indicate that vitamin E-enriched eggs may have a very good prospect to use in the processing industry and consumer preferences.

Biotin

Biotin has several important functions in metabolism. As a coenzyme, the biotin moiety functions as a carboxyl carrier. The biotin-dependent enzymes are involved in carboxylation in such reactions as gluconeogenesis, lipogenesis, and fatty acid biosynthesis (Chen et al., 1994).

Concentrations of biotin in egg albumen increased with incremental dietary biotin levels, but egg yolk concentrations were stable. There is a positive relationship between dietary biotin and the amount of biotin in the egg (Robel, 1991).

Folic acid

(Sherwood T.A. et al., 1993) studied the supplementation of food with folic acid; they found that folates in egg yolk were 43 times more concentrated than in hen plasma; and the folate concentration in egg yolk was 100% above that in egg white. As folic acid deficiency is sometimes observed in pregnant women, eggs can be a good source for folic acid supplementation if its concentration is raised.

Riboflavin

Deposition of riboflavin in egg yolk and albumen is dependent on dietary riboflavin and reaches half-maximal values at about 2 mg of supplemental riboflavin/kg (White et al., 1986). The maximal amount of riboflavin deposited in the yolk is limited stoichiometrically by the amount of riboflavin-binding protein, whereas the maximum amount of riboflavin deposited in albumen is limited by other factors before saturation occurs. The amount of riboflavin-binding protein in yolk and albumen is independent of dietary riboflavin.

The results observed for riboflavin and riboflavin binding protein here are in contrast with those for biotin and its two binding proteins in laying hens. With biotin only saturated proteins are found in plasma and yolk, and the production of the proteins is in some way controlled by the bioavailability of riboflavin.

There is a nearly linear relationship of diet riboflavin to egg riboflavin in the range of 1.5 to 5.0 µg/g of feed (NRC 2.2 µg/g) (Naber & Squires, 1991). At 2 to 4 times the dietary requirement of the hen, riboflavin deposition in egg yolk and albumen is limited by the transfer of riboflavin into the ovum, and increases up to 3 fold (Squires and Naber, 1993).

Carotenoids

Singlet molecular oxygen has been shown to be generated in biological systems and is capable of damaging proteins, lipids and DNA. When the ability of some biological antioxidants to quench singlet oxygen was studied, the quenching ability of carotenoids was higher than that of tocopherols. Both carotenoids and tocopherols may contribute to the protection of tissues against the deleterious effects of singlet oxygen. Moreover, carotenoids and tocopherols have been reported to exert a protective action against some types of cancer (DiMascio et al., 1990).

The antioxidant activity in biological systems of carotenoids appear to be involved in both the protection against singlet oxygen and triplet oxygen (as radical chain breaking antioxidants). Epidemiological investigations reveal an inverse relationship between serum carotenoid level and the incidence of several types of cancer (Krinsky, 1988).

Studies so far demonstrated that the quenching ability of carotenoids depends on the length of the conjugated polyene chain and for efficient energy transfer at least nine conjugated double bonds are

necessary. It was also found that the quenching ability can be influenced by introducing oxo or hydroxy substituents in the β -ionone group (Mathis & Schenck, 1982).

However, (Crabtree & Adler, 1997) suggest that β -carotene's primary *in vivo* function is to serve as a reservoir for retinol, retinaldehyde and retinoic acid. Although β -carotene may have other functions, regardless of its concentration, or the partial pressure oxygen, its intended function is not that of a free-radical scavenger because it does not meet the requirements: regenerability and tolerable toxicity. They even say that although the function of lutein and zeaxanthin in the macula *lutea* is still open to speculation, for the same reasons as for β -carotene, it is doubtful that their intended function is that of free-radical scavengers.

Moreover, (Haila et al., 1996) showed that lutein, lycopene, and β -carotene increased the hydroperoxide formation of autoxidized triglycerides. However, their results also show that minor concentrations of γ -tocopherol may inhibit the prooxidant effect of lutein and lycopene. A combination of lutein and γ -tocopherol was more efficient than α -tocopherol in inhibiting the hydroperoxide formation of triglycerides.

On the other hand, (Chen et al., 1997) found that capsanthin, and to a lesser extent lutein and β -carotene, exhibited the ability to quench singlet oxygen to reduce the photosensitized oxidation of both soybean oil and selected flavour compounds. For them, these results suggest that capsanthin as well as other carotenoids may be applied to food systems which contain food lipids or flavour compounds to minimize food photodeterioration.

The presence of the oxidation products of lutein and zeaxanthin in the human retina supports the hypothesis that dietary lutein and zeaxanthin may act as antioxidants to protect the retina against short-wavelength visible light (Khachik et al., 1997). Lutein and zeaxanthin were also recently identified in the human lenses and may also play a role in prevention of age-related macular degeneration and cataracts. Therefore, the presence of these compounds in the retina and lens at reasonably high concentrations may be essential to prevent macular degeneration and cataracts.

A leading cause of blindness in Western countries is age-related macular degeneration. Because there are currently no effective treatment strategies for most patients, attention has focused on efforts to prevent the damage leading to this condition (Hammond et al., 1997). Of particular interest is the possibility that nutritional counseling or intervention might reduce its incidence or retard its progression. One possible approach is to increase the dietary intake of the carotenoids lutein and zeaxanthin. There was an age-dependent, inverse relationship between macular pigment density and lens density (Hammond et al., 1997). Thus, an inverse relationship between these two variables suggests that lutein and zeaxanthin may retard age-related increases in lens density.

Dietary lutein increased tumor latency, suppressed mammary tumor growth and enhanced lymphocyte proliferation in a dose-dependent manner in mice (Chew et al., 1996). For them, it is unlikely that this antitumor activity is mediated through lutein's antioxidant activity; it seems more plausible to be mediated through the stimulation of certain immune function.

Higher breast adipose concentrations of retinoids and some carotenoids (β -carotene, lutein and zeaxanthin) may be associated with decreased risk of breast cancer. However, no significant correlation was found between adipose concentration of these compounds and dietary intake (Zhang et al., 1997).

Inhibitory effect of four carotenoids prevalent in human blood and tissues against the formation of colonic aberrant crypt foci (an intermediate biomarker for colon cancer) was examined in Sprague-Dawley rats by (Narisawa et al., 1996). Lycopene, lutein, α -carotene and palm carotenes (α - and β -carotene, and lycopene) inhibited the development of the aberrant crypt foci, but β -carotene did not. The results suggested that lycopene and lutein in small doses may potentially prevent colon carcinogenesis.

A tendency to a reduction in risk of lung cancer with increased intake was observed for all vegetables, but these associations did not reach statistical significance (Agudo et al., 1997). Given the results of recent intervention trials, β -carotene seems not to be associated with lung cancer risk, but the role of other carotenoids cannot be discarded.

Supplementation of broiler breeder diets with 400 ppm of β -carotene, canthaxanthin or lutein, increased their respective concentrations in the yolk up to 14, 426 and 701 ppm respectively, being lutein the most effectively deposited (Haq et al., 1996).

When Single Comb White Leghorn laying hens were fed diets supplemented with β -carotene, DL- α -tocopheryl acetate, or their combination. Yolk concentration of β -carotene increased from 0.14 to 5.19 ppm at 200 ppm diet inclusion. The increment in yolk retinol levels, although significant, was not as important (20% maximal increase) (Jiang et al., 1994). Alpha-tocopherol in egg yolk was raised up to three fold, although a negative interaction with diet supplementation together with β -carotene was found. When yellow carotenoids (lutein and zeaxanthin) were fed up to 500 ppm in the diet, a marked increase in yolk carotenoids was observed from 0 to 100 ppm dietary supplementation, reaching a plateau level from 200 ppm on (ITPSA, 1997). Increments in egg content of β -carotene, lutein and zeaxanthin are summarized below in Table 3.

Table 3. Increment in the levels of several carotenoids in egg from several experiments

Compound	Increment vs control		Source
β -carotene	2	Fold	Jiang et al., 1994
Lutein	25	Fold	ITPSA, 1997
Zeaxanthin	15	Fold	ITPSA, 1997

Therefore, it is possible to significantly increase the concentration of β -carotene and retinol in chicken eggs; however, because of the relative expense involved (eggs price will be at least 160% that of normal eggs for β -carotene) it may not be very attractive from the commercial standpoint to increase egg yolk concentrations of β -carotene and retinol by supplementing β -carotene in the diet. Increments in the lutein content of egg yolk are much more feasible and economical.

ω -3 fatty acids

In the 1950s it was discovered that polyunsaturated vegetable oils had a pronounced plasma cholesterol-lowering effect (Goodnight et al., 1982), and it was also noted that fish oil had a similar effect. But it was not until the observations of (Bang & Dyerberg, 1973) in Greenland Eskimos that special attention was focused on eicosapentaenoic and docosahexaenoic acid.

In the early 1980s the concept that ω -3 fatty acids could be beneficial to heart health gained nutritional attention. Docosahexaenoic acid decreases blood platelet function and is highly concentrated in the brain where its depletion leads to functional impairments (Brossard et al., 1997).

Current research in nutritional medicine indicates that the ω -3 fatty acids are becoming more and more prominent as essential components of the human diet. As a summary, the physiological effects of ω -3 fatty acids are (Mishra et al., 1993): lowering of plasma triglycerides; increased aggregation time for platelets; decreased viscosity of blood; decreased blood pressure; reduction in atherosclerosis; reduction of inflammation; and reduction in tumors.

The consumption of ω -3-enriched eggs has beneficial effects in decreasing serum triglycerides and increasing the HDL-2 subfraction without adversely affecting serum cholesterol. The beneficial effect of docosahexaenoic acid- and eicosapentaenoic acid-enriched eggs on serum lipids was studied by (Oh et al., 1991), they found that those eggs reduced serum triglycerides and blood pressure, and kept serum cholesterol from increasing (although the consumption was of 4 enriched-eggs per day). Related research with linolenic acid-enriched eggs showed no such beneficial effects, but did improve the ratio of ω -3/ ω -6 acids. As from the 1997 World Health Report, an estimated 691 million people have high blood pressure, the importance of ω -3-rich products (i.e., ω -3 enriched eggs) becomes clear.

There are two main approaches in order to increase the ω -3 content of the eggs. Either the enrichment of the diet with linolenic fatty acid or a vegetable source of it (flax seed, rapeseed, linseed) or the addition of any source of eicosapentaenoic and/or docosahexaenoic fatty acids.

Flax seed from 0 to 30% dietary level produced a large increase of ω -3 fatty acids (mainly linolenic), although 20% appeared most effective (egg content: 580 mg linolenic acid, 8 mg eicosapentaenoic acid, 14 mg docosahexaenoic acid); dietary treatment had no effect on egg cholesterol (200 mg/egg in all treatments) (Caston & Leeson, 1990). However, flaxseed decreased feed consumption, weight gain and egg weights in other experiments (Scheideler & Froning, 1996).

Increasing levels of linseed oil (0 to 6%) had no effect on total-, neutral- and polar-lipid in egg yolk, but significantly increased the contents of α -linolenic, eicosapentaenoic acid and docosahexaenoic acid; consequently, the ratios of ω -3/ ω -6 fatty acids in the total egg yolk lipid raised from 0.11 to 0.92 (ω -6/ ω -3 decreased from 9 to 1). The concentrations of α -tocopherol in egg yolk decreased at linseed oil supplementation of 2% or higher (Suzuki et al., 1994).

Dietary linolenic acid increased the amount of ω -3 fatty acids; over 70% was linolenic acid; 20-25% docosahexaenoic acid and 5-10% eicosapentaenoic acid. No consistent trend was observed for tocopherol content by treatment and during storage. The flavour scores of eggs from control diet were more favourable than those of eggs from linolenic acid diet, but their differences were only minor; some differences were also found among laying hen strains on araquidonic and docosahexaenoic fatty acids, as well as for the flavour scores of hard-boiled eggs with linolenic acid diets (Ahn et al., 1995). In some experiments, vitamin E significantly improved egg production (Scheideler & Froning, 1996). Levels of 10 to 15% flaxseed yield eggs with 4 to 7% yolk ω -3 fatty acids, respectively, making these eggs rich sources of ω -3 fatty acids.

Ten percent fish meal resulted in undesirable egg flavour except when Peruvian anchovy meal was fed early in the laying cycle. Comparisons of the effects of 5% levels of some fish meals, and their oils at the levels found in the meals, indicated that the cause of objectionable flavour is not contained specifically in the fish oil. A higher level (1.5%) of oil, however, resulted in off-flavoured eggs (Koehler et al., 1975). However, when 2% refined fish oil was added to hens' diet, no differences were found for egg taste nor flavour (ITPSA, 1998).

Egg production, feed efficiency, 490-d body weight and Haugh Units were not different among diets (0 to 12% herring meal). Dietary herring meal levels did not influence the total lipid content of the egg yolk, but significant incorporation of ω -3 fatty acids in the egg was obtained by feeding herring meal at 4-12% to laying hens, with a linear relationship. As the hens aged, the effects of dietary herring meal levels on yolk eicosapentaenoic acid decreased linearly. Eicosapentaenoic acid and docosahexaenoic acid in the yolk were 7.8 and 100.5 mg/yolk compared with 0.8 and 39.6 mg/yolk with the control diet (Nash et al., 1995).

Three percent dietary menhaden oil did not alter egg production, egg weight, total yolk fat, or yolk cholesterol. Three percent of dietary menhaden oil increased yolk- ω -3 fatty acids: linolenic from 14 to 21-25 mg/egg; eicosapentaenoic acid from <1 to 15-28 mg/egg; docosahexaenoic acid from 19-39 to 123-178 mg/egg; while the ratio of ω -6/ ω -3 fatty acids decreased from 15 to 3 (Van Elswyk et al., 1992). Similar values were found by (Hargis et al., 1991): the ω -6/ ω -3 ratio decreased from 18 to 3. The increase of ω -3 fatty acids was similar for 1.5 and 3% menhaden oil, and close to the levels described above but for docosahexaenoic acid, in other experiment: linolenic 21 mg/yolk; eicosapentaenoic acid 14 and 24 mg/yolk; docosahexaenoic acid 228 and 252 mg/yolk (Van Elswyk et al., 1995).

Using mackerel oil as a source of ω -3 fatty acids, maximum enrichment with total n-3 polyunsaturated fatty acids was achieved on diets with either mackerel oil at 6% (377 mg docosahexaenoic acid/egg) or a combination of linseed at 2% plus mackerel oil at 2% (214 mg docosahexaenoic acid/egg and 487 mg ω -3 FA/egg) (Farrell et al., 1991). A relevant incorporation in the yolk of ω -3 fatty acids, particularly eicosapentaenoic acid and docosahexaenoic acid, was achieved by supplementation of diet with 2 to 4% docosahexaenoic acid or eicosapentaenoic acid refined fish oils. No differences in docosahexaenoic acid content of eggs were found between both oils (149 and 147 mg/egg when 3% docosahexaenoic acid or eicosapentaenoic acid oils were used);

while significant differences were found with eicosapentaenoic acid (12.3 and 22.4 mg/egg) (Meluzzi et al., 1997).

A summary of the results obtained by several authors in the enrichment of eggs in ω -3 fatty acids is presented next in Table 4.

Table 4. Enrichment of ω -3 fatty acid content of eggs using several ω -3 fatty acids feed sources (expressed as mg of each fatty acid per egg)

Diet	LNA [†]	EPA ^{††}	DHA ^{†††}	Source
20% flaxseed	580	8	14	Caston and Leeson, 1990
2% linseed + 2% mackerel oils	214	32	214	Farrell et al., 1991
6% mackerel oil	20	71	377	Farrell et al., 1991
3% menhaden oil	25	28	178	van Elswyk et al., 1992
3% menhaden oil	21	24	252	van Elswyk et al., 1995
12% herring meal	66	8	100	Nash et al., 1995
3% refined EPA* fish oil	17	22	147	Meluzzi et al., 1997a
3% refined DHA* fish oil	20	12	149	Meluzzi et al., 1997a

[†]LNA: linolenic acid

^{††}EPA: eicosapentaenoic acid

^{†††}DHA: docosahexaenoic acid

Cooking did not significantly alter levels of ω -3 or ω -6 long-chain polyunsaturated fatty acids in the egg (Van Elswyk et al., 1992). Alterations in fatty acid composition did not significantly affect characteristic functional properties of eggs (emulsification capacity, sponge cake volumes). However, panelists differentiated between ω -3-enriched egg and control scrambled eggs (fish-like flavour), but not between hard-cooked eggs; heating during preparation and presentation may have enhanced availability of volatile compounds characteristic of marine products (Van Elswyk et al., 1992). Fishy taste, fishy aroma, and fishy aftertaste increased, generally in a dose-response fashion, as level of dietary fish oil increased. However, treatments with less than 3% oil were not significantly lower in overall flavour quality (Van Elswyk et al., 1995).

When docosahexaenoic acid or eicosapentaenoic acid refined fish oils were added to hens diet, substantial differences emerged between the two dietary oils tested for the sensory quality: with eicosapentaenoic acid-oil the fishy flavour was easily identified, particularly in boiled eggs, when supplementation level exceeded 2%. Docosahexaenoic acid-oil did not have any negative consequence on sensory egg quality at all the experimental doses. The different behaviour of the two oils might be linked to the different refining procedures to which they had been submitted and to the 13% unidentified fatty acids of eicosapentaenoic acid-oil which could have modified the organoleptic characteristics of the eggs (Meluzzi et al., 1997).

Some negative effect on performance and physiology of hens have also been described in several experiments. Reduction in hen body weight, egg weight, yolk weight, egg production, shell quality; all are negative consequences not consistently found while feeding high levels of flaxseed in the diets. Also, decreasing levels of circulating lipids as altered liver lipid metabolism have been described with ω -3 polyunsaturated fatty acids rich diets. Up to 30% reductions in circulating oestradiol in laying hens has also been reported. After 18 weeks of feeding fish-oil diets, no change in gross scoring of hepatic lipidosis was observed, although histologically, significantly greater hepatocellular lipid infiltration was observed, that may have significance for flocks predisposed to fatty liver syndrome (Hargis et al., 1991). At 462 d egg weights were inversely influenced by the dietary herring meal level of the diet, similar to the results of (Van Elswyk et al., 1994) for hens fed 3% menhaden oil.

Recent studies suggest that eggs rich in ω -3 polyunsaturated fatty acids may not be accepted for the production of spray dried yolks (Guardiola et al., 1995a,b). They reported that the n-3 polyunsaturated fatty acids content of typical eggs, albeit low, deteriorated unless powders were stored under vacuum and not exposed to light. These authors also reported the enhanced formation of cholesterol oxidation products, mainly oxysterol, following high temperature egg powder production. Simultaneous enrichment of eggs with ω -3 fatty acids and tocopherols could avoid this deterioration.

The concentration of docosahexaenoic acid in enriched eggs (at least 5 mg/g yolk) compares favourably to fish (2-3 mg/g of meat). In order to provide in one yolk the docosahexaenoic acid content of fish, the yolk docosahexaenoic acid content should be about 9.0 mg/g (achieved by feeding 15% ground flax seed, but not consistently and with some negative effects on production). Using more practical values, consumers may be advised that a serving of two enriched eggs would easily provide the docosahexaenoic acid content of a fish meal. Consumption of two ω -3 enriched eggs (hens fed diets with 2% linseed oil) per day for 3 weeks decreased plasma cholesterol, triacylglycerol and arachidonic fatty acid in young women (with low fish intake), whereas plasma docosahexaenoic acid, eicosapentaenoic acid and linolenic fatty acids increased (significantly for docosahexaenoic acid). Consequently, the ratio of ω -6/ ω -3 fatty acids in plasma was significantly decreased from 14.29 to 6.25 (Suzuki et al., 1995).

Cholesterol

Although cholesterol is a very small part of an egg, it has received as much attention as the major yolk components because of its continuing interest for human nutrition. Attempts at controlling the cholesterol content of eggs by variations in normal diets have not been completely successful.

It has been hypothesized that the inability to achieve reduced levels of cholesterol by selection is due to a physiological control mechanism which ultimately causes cessation of egg production when yolk cholesterol deposition is inadequate for embryo survival.

The average cholesterol content of commercially produced eggs is substantially lower than the estimate content of 274 mg/egg of the Consumer and Food Economics Institute (1976): in Georgia cholesterol content was 195 mg/egg (Beyer & Jensen, 1989); in Italy ranged from 173 to 257 mg, the mean value for all eggs being 222 mg (Meluzzi et al., 1993); while mean egg cholesterol levels were 207 mg/egg for HyLine white eggs and 213 mg/egg for Warren red eggs in commercial eggs in central Spain (Cores et al.). The total cholesterol content correlated positively with egg and yolk weight and negatively with egg production and dietary protein level, but these correlations were not significant when cholesterol was expressed as milligrams per gram of yolk.

Several nutritional approaches to decrease the cholesterol content of the egg have been reported, without achieving drastic results.

Ascorbic acid supplementation (1.3 g/kg) did not alter yolk weight or cholesterol content relative to controls; addition of sodium-potassium sulfate (1%) significantly increased cholesterol egg yolk content (Nockels, 1973).

Feeding of diets with 8% safflower oil did not increase the levels of egg yolk cholesterol; however, 8% hydrogenated coconut oil did increase significantly those levels; a significant reduction in cholesterol levels of both serum and egg yolk was obtained when 2% soysterols were added to diets containing either oil (Sim & Bragg, 1977). Functional site of plant sterols in regulating cholesterol metabolism in the fowl is not restricted to the absorptive site, in fact, these sterols may exert their effect via an enhanced rate of cholesterol turnover and excretion via bile rather than influencing cholesterol absorption (Hargis, 1988).

With diets including barley or a filtrate of *Trichoderma viride* culture, egg yolk cholesterol concentrations were lowered; however total egg yolk cholesterol was higher than control for the filtrate fed hens (as a result of a higher egg and yolk weight) and lower than control for the barley fed hens (lower concentration as well as lower egg and yolk weight) (Qureshi et al., 1984).

Addition of *Lactobacillus acidophilus* at 4.0×10^6 CFU/g of diet improved egg production, feed conversion efficiency and reduced the egg yolk cholesterol concentration by 11, 18 and 17% respectively (Abdulrahim et al., 1996).

Therapeutic antibodies

Passive immunization, as opposed to vaccination, consists of oral administration of antibodies for disease prevention, and has emerged as an alternative to the use of antibiotics, without any possible resistance from the microorganisms. Passive immunization requires large amounts of antibodies. Avian IgY can be produced in larger amounts and lesser cost than mammalian IgG, and it has additional advantages: welfare of the antibody-producing animals, the collection of the antibody source is much easier, there are less cross-reactions when administered.

Moreover, antigen binding and bacterial cell agglutinating capacity of the IgY remains after heating up to 70°C for 15 min, after digestion with alimentary tract proteases, freezing and freeze-drying repeated several times. Considering its availability to obtain in the technical scale, it seems a good idea to select yolk gammaglobulin, as a food additive to fortify the products ability to last longer and be safer for the consumer. (Stefaniak & Kopec, 1997) obtained egg yolk IgYs against several species of bacteria-human alimentary tract pathogens (*Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella enteritidis*, *S. typhimurium*), with no evidence of loss of activity of the IgY from eggs refrigerated up to 21 days.

The specific antibody enriched eggs can be directed either to human or livestock disease prevention, an several reports have been published recently confirming its efficacy in both cases.

Post-weaning *Escherichia coli* diarrhoea and *E. coli* enterotoxaemia continue to cause considerable economic loss. In both diseases the clinical signs and death of the pigs are caused by one or several toxins released by bacteria colonizing the small intestine. The situation is in many regards comparable to human cholera and enterotoxigenic *E. coli* infections. Passive immunization may therefore be an attractive alternative. Antibodies extracted from chicken eggs or even non-purified whole egg powder are therapeutically and prophylactically effective in neonatal pigs infected with enterotoxigenic *E. coli* (Yokoyama et al., 1992; Yokoyama et al., 1993; Imberechts et al., 1995; Imberechts et al., 1997; Zúñiga et al., 1997). The repercussions are not only for livestock, if we consider that diarrhoeal diseases killed two and a half million people in 1996 according to the 1997 World Health Report.

Antibodies derived from egg yolks obtained from hens immunized to *Streptococcus mutans* inhibited its adherence compared to controls. Successful experiment with animal models have been reported (Hamada et al., 1991; Otake et al., 1991). The results in human beings have also been encouraging: using a mouth rinse containing immune IgY against *S. mutans* prevented the colonization of this pathogen (Hatta et al., 1997).

Interferon- γ is an important regulator of immune responses and inflammation. Studies in animal models of inflammation, autoimmunity, cancer, transplant rejection and delayed-type hypersensitivity have indicated that administration of antibodies against interferon- γ can prevent the occurrence of diseases or alleviate disease manifestations. Therefore, it is speculated that such antibodies may have therapeutical efficacy in human diseases (Froyen & Billiau, 1997). Since mammal-derived antibodies are immunogenic in patients, egg yolk-IgY obtained from immunized laying hens could be the most cost-effective treatment, rather than recombinant DNA technology.

Therefore, eggs can be considered a convenient source for the production of polyclonal antibody specific to a variety of antigens such as bacteria, virus, enzyme, and hormone.

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