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(54) Titre : OEUFS COMPRENANTS UN MELANGE D'ANTIOXYDANTS ET D'ACIDES GRAS POLYINSATURES EN FAIBLE QUANTITE

(54) Title: EGGS WITH A MIXTURE OF ANTIOXIDANTS AND LOW AMOUNTS OF POLY-UNSATURATED FATTY ACIDS

(57) Abrégé/Abstract:
Chicken eggs are provided which contain synergistic composition of antioxidants and low PUFA (poly-unsaturated fatty acids). The eggs comprising no more than 15.5 % PUFA of the egg's fatty acids and controlled amount of Vit E, iodine, edible cartoneoids and additional edible antioxidants. The eggs provide an antioxidative environment which reduce the oxidizability of the consumer's LDL, which is accepted as a risk factor for cardiovascular disease. The eggs are produced by maintaining egg laying chickens on a regime wherein conventional feed ingredient and supplements are selected to provide about 0.7- 1.5 wt.% PUFA of the entire diet, controlled amount Vit E and iodine, edible carotenoids and additional edible antioxidants.

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Plasma vitamins E, A and Carotenoids following eggs consumption (mg/dl)

Time on Eggs Supplement.	0 Baseline	3 Weeks Control Eggs	6 Weeks Enriched	9 Weeks Enriched Low Pufa
Vitamin E	43±6	44±8	63±17*	54±11*
Vitamin A	0.55±0.11	0.58±0.13	0.77±0.18*	0.66±0.21*
Carotenoids	1.11±0.21	1.09±0.27	1.60±0.57*	1.69±0.45*

*p<0.01 (vs. 3 Weeks)

(57) Abstract

Chicken eggs are provided which contain synergistic composition of antioxidants and low PUFA (poly-unsaturated fatty acids). The eggs comprising no more than 15.5 % PUFA of the egg's fatty acids and controlled amount of Vit E, iodine, edible cartoneoids and additional edible antioxidants. The eggs provide an antioxidative environment which reduce the oxidizability of the consumer's LDL, which is accepted as a risk factor for cardiovascular disease. The eggs are produced by maintaining egg laying chickens on a regime wherein conventional feed ingredient and supplements are selected to provide about 0.7-1.5 wt.% PUFA of the entire diet, controlled amount Vit E and iodine, edible carotenoids and additional edible antioxidants.

EGGS WITH A MIXTURE OF ANTIOXYDANTS AND LOW AMOUNTS OF POLY-UNSATURATED FATTY ACIDS

The present invention relates to eggs comprising a synergistic composition of antioxidants and a low amount of poly-unsaturated fatty acids (PUFA) (hereinafter called "low PUFA") for reducing the oxidation stress of LDL-cholesterol (hereinafter called "LDL") and to a method for their production (The LDL is the high cholesterol and high risk fraction in the human plasma.)

Researches all over the world proved the link between heart disease and high blood cholesterol, especially high LDL and high LDL/HDL ratio which is accepted as "risk factor".

Early experiments in animals and humans showed that diets comprising large amounts of saturated fatty acids (SFA) and cholesterol increase the risk of high blood cholesterol and cardio vascular (CV) conditions. This leads to the "lipid hypothesis" suggesting that atherosclerosis is caused by hypercholesterolemia-induced deposition of lipids in the vessel wall and to the public recommendation to restrict the cholesterol consumption to 300 mg/day and to reduce the SFA consumption, especially from animal source. The arithmetic manifestation of this approach is defined by "The Cholesterol saturated fat index (CSI) for coronary prevention: background, use and a comprehensive table of foods", Connor et al, J.Am.Diet Assoc., 1989, June 89 (69), pg. 807-16.

According to this approach low CSI, i.e. food low in SFA and/or cholesterol content is considered to have a hypocholesterolemic and low atherogenic potential.

As egg yolk contains both 213-240 mg of cholesterol and

"animal fat" . Thus, egg was among the first food to be excluded from the diet of western countries consumers.

Modifying the egg quality by changing the chicken diet was challenged by many researches and patents, i.e. trying to invent an egg compatible with low CSI, namely having a low cholesterol and low SFA content in order to prevent the increase of blood cholesterol and SFA in the human consumer.

There have been performed many researches in order to produce an egg which would solve the above problem. The results of said researches are described, e.g. in the following Patent specifications:

U.S. Pat. No. 4,187,294, U.S. Pat. No. 4, 394, 376, Ca Pat. No. 1,115,983, U.S. Pat. No. 4,410,541, U.S. Pat. No. 4,197,293, U.S. Pat. No. 4,197,294 , U.S. Pat. No. 4,128,640, U.S. Pat. No. 5,012,761 , U.S. Pat. No. 4,738,853, U.S. Pat. No. 4,868,001 , U.S. Pat. No. 3,657,423 and U.S. Pat. No. 5,246,717.

Said specifications substantially describe and claim eggs compatible with a cholesterol-reducing diet and methods of producing the same. As can be seen said methods comprise increasing the iodine and the PUFA content of the food and of the eggs, etc.

In U.S. Patent Specification No. 5,246,717 it is indicated that for eggs to be compatitable with a cholesterol reducing diet they should contain not more than 34% of SFA. No differentiation was made between the amounts of the various unsaturated fatty acids, namely between (PUFA) and mono-unsaturated acids (MUFA) present.

All the above publications relate to the blood cholesterol concentration, which is compatitable with the "lipid hypothesis".

However, a paper of Brown et. al; J.Am. Diet Assoc. 46, pg. 189 - 192, 1965, mentioned in U.S. Specification 5,246,7-17 (column 5, lines 62 - 67) states ".....consumptions of modified eggs rich in polyunsaturated fat was ineffective in reducing serum cholesterol.." . This means that neither the inventors of the invention claimed in said specification nor the authors of said publication were aware of the fact that a large amount of PUFA in the egg has a bad effect on the blood cholesterol and in particular on the oxidiability of the LDL as will be shown hereinafter.

It has also recently been found that only those people responding to dietary cholesterol are "responders" and are sensitive to external/dietary cholesterol. In "non-responders" the external cholesterol consumed operates effectively the feed-back mechanism to keep cholesterol within a normal range. This individual variation could explain the contradictory results in studies on the dietary effect on the blood cholesterol concentration.

Another competing hypothesis of atherosclerosis is the "response to injury hypothesis". This explains the first phase, the initiation of the atherogenic process. Much evidence suggests that lipid oxidized products (LOPS) obtained either as a result of a diet or formed in vivo both initiate and promote the process, i.e. cholesterol oxidized products (COPS) and/or oxidized LDL are more atherogenic than

the non-oxidized forms. Thus, low rancidity, oxidized lipids and a large amount of antioxidants reduce the production of lipids and of LDL oxidation. Here PUFA due to its great reactivity to oxidation differs greatly from MUFA which is rather resistant.

As easily can be understood from the above, although there exists a wide agreement that the LDL oxidation in the blood is a major risk for arteriosclerosis. So far no research regarding the correlation between eating eggs and the sensitivity of LDL to oxidation has been performed.

However, a large amount of resources have been spent on finding a correlation between the oxidation of LDL to arteriosclerosis and the reasons causing said oxidation and preventing same. This can be seen, e.g. from the following publications:

1. "Low density lipoprotein rich in oleic acid is protected against oxidative modification: Implications for dietary prevention of atherosclerosis" Sampath Parthasarthy et al. Proc. Nat Acad. Sci. USA; Vol. 87 pp. 3894-3898 May 1990 Medical Sciences.

2. "Inverse correlation between plasma vitamin E and morality from ischemic heart disease in cross-cultural epidemiology." K Fred Gey et al. Am J Clin Nutr 1991;53:626S-34S, 1991 USA.

3. "Antioxidant Vitamins and Low-density Lipoprotein Oxidation". Abbey, M., Nestel, P.J. et al; Am. J. Clin. Nutr. 58:525-532, 1993, USA.

4. "Comparative Study on the Effect of Low-Dose Vitamin E and Probucol on the Susceptibility of LDL to Oxidation and the Progression of Atherosclerosis in Watanabe Heritable Hyperlipidemic Rabbits". Kleinveld H.A., et al; Arterioscler. Thromb. 14:1386-1391, 1994.

5. "Increase in Oxidation Resistance of Atherogenic Serum Lipoproteins Following Antioxidant Supplementation:A Randomized Double-Blind Placebo-Controlled Clinical Trial" Nyyssonen, K., et al. Eur. J. Clin. Nutr. 48:633-642, 1994.

6. "The Effect of Alpha-tocopherol Supplementation on LDL Oxidation". Jialal, i., et al. Arterioscler. Thromb. Vasc. Biol. 15:190-198, 1995.

7. "Dietary Supplementation with Vitamins C and E inhibits in Vitro Oxidation of Lipoproteins". Rifici, V.A. and et al. J. AM. Coll. Nutr. 12:631-637, 1993.

8. "Vitamin E Consumption and the Risk Coronary Heart Disease in Men." Rimm, E.B., New Engl. J. Med. 328:1450-1456,1993.

9. "Antioxidant Vitamins and Coronary Heart Disease". The New England Journal of Medicine Vol 323, pg 1487-1489, 1993.

10. "Antioxidant-Mediated Inhibition of Macrophage Modification of low density Lipoprotein" .Life Chemistry Reports, 1994, Vol. 12, pp 69-78.

None of said publications has checked the effect of egg PUFA on the plasma LDL. The publication of Parthasarathy et. al. which was performed with a special sunflower oil (Trisun* 80) comprising a low amount of PUFA (8% of lineolic acid) shows that due to this fact inhibition of LDL oxidation is achieved. However, as is readily understood an experiment performed with oil in feeding rabbits cannot be conclusive for the production of a functional egg. Moreover, in said publications there is no indication given whatsoever that the addition of carotenoids and of other antioxidants would increase resistance of the LDL against oxidation.

As is known in the nutrition science in each food condition and diet each factor has to be considered on its own merits.

As cholesterol is very reactive to oxidation and as peroxidation is a chain reaction, a lower amount of antioxidative materials are required for protecting the cholesterol at the initial phases than later on. It could therefore be expected ingesting "Protected" cholesterol, namely cholesterol in an environment of low oxidative stress, i.e. comprising a large amount of high antioxidants and low PUFA, which may become "Pro-oxidants", could give a significant impact on the plasma LDL oxidizability. Thus, an egg enriched with antioxidants, known for the LDL protection potential and low PUFA can provide such "protecting" environment.

Indeed some research papers show that the reactivity of LDL to oxidation is determined not only by its antioxidant

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content but also by other compositional factors and more specifically by the ratio of oleic acid content to linoleic acid content. (See: "oxidation resistance, oxidation rate and extent of oxidation of human low density lipoprotein depend on the ratio of oleic acid content to linoleic acid content: studies in vit E deficient subjects; Kleinveld et al.; Free radic med 1993 SIP 15(3) 273-80.)

Although diet may greatly affect the fatty acid profile of the eggs the question is how much this can influence the LDL in the consumer. It was shown that when primates on high cholesterol diet received various amounts of FA, that the FA profile in LDL was indeed influenced by the diet but linoleic acid was the predominant PUFA in all of the LDLs. The rates of LDL oxidation were linearly dependent upon the concentration of PUFA.

The final proof that PUFA and mainly linoleic acid are involved in LDL oxidation was recently shown by the analysis of the lipid oxidation products in the oxidized LDL following immunological activation of the human monocytes: the major FA oxidation product was esterified hydroperoxyoctadecadienoic acid (HPODE) which is the oxidized product of the main PUFA in human LDL-linoleic acid.

It has thus been desirable to devise an egg comprising a synergistic composition of antioxidants preventing the oxidation of LDL. The production of said egg should use as many as possible ingredients of the standard mixtures. The method should thus be simple and not require many changes by the manufacturer of the mixture or by the farmer growing the

chickens.

It is well known that various egg components are affected by chicken feed e.g. Vitamins E, A and other vitamins. (See: modifying vitamin composition of eggs: A review by E C Naber.J.Applied poultry res 2:385-393, 1993). Recently a paper " α-tocopherol, ß-carotene and retinol enrichment of chicken eggs" Jiang YH et al., Poult SCI, 1994 JUL:73(7):1137-43) showed that it is possible to enrich these components in the egg significantly. However, supplemental ß-carotene may markedly decrease the yolk deposition of vitamin E. Moreover, ß-carotene is most intensively transformed in the chicken to vitamin A and only traces of it attain the yolk compared to the rapid and effective deposition of dietary oxycarotenoids. The kind and concentration of oxycarotenoids in the yolk is strongly influenced by the diet, i.e. the carotenoid concentration, protective antioxidants, destructive factors, e.g. proxidants such as PUFA, storage and processing conditions, etc. Oxycarotenoids are considered in relation to their pigmentation attributes, since they contribute most of the yolk pigments. ß-carotene is thus not the selected antioxidant for chicken feed. Moreover, it is known that oxycarotenoids which are readily deposited in the yolk perform an antioxidative function.

In the present invention those principles were applied to obtain an egg comprising more iodine, carotenoids and vitamin E.

A recent research on antioxidants showed that adding vitamin E which increased LDL content 2.5 times the baseline

amount, reduced the reactivity of LDL to oxidation by 50% . (See: Effect of dietary antioxidant combinations in humans, protection of LDL by vitamin E but not by ß-carotene, Reaven et al. ,1933, Arterioscler - Thromb Apr. 13(4) 590-600).

As in the process of oxidation-protection, vitamin E itself is consumed, and thus its concentration should be increased in proportion to PUFA. The generally accepted ratio being between 0.4-0.6 mg Vitamin E/1 g PUFA.

Moreover, recent research has raised the question regarding the potential of oxidized vitamins (antioxidants) becoming a proxidant and then facilitating oxidation. Thus, it might not be enough to increase the amount of vitamin E but rather provide further protection, i.e. by carotenoids, vitamin C, flavonoids and/or other antioxidants to create a synergistic effect in antioxidative processes.

Vitamin E also protects other antioxidants, e.g. carotenoids. Thus, it enhances pigmentation in the yolk (See: "Oxycarotenoids in Poultry Feeds, Carotenoids as Colorants and Vitamins as Precursors" Marusich and Bauernfeind, Academic Press 1981, pages 319 - 444). This results in a considerable increase of the xanthophyll concentration in blood plasma (See: Carotens and other Vitamin A Precursors in Animal Feed, Bauernfeind et al., Carotenoids as Colorants and Vitamin A Precursors, Academic Press 1981.) Other antioxidants were also effective in the same manner, i.e. ethoxyquin (EMQ) and butylated hydroxytoluene (BHT) are also known to reduce the oxidative destruction of unsaturated

molecules such as PUFA and carotenoids, and thus improve pigmentation (Carotenoids-their Nature and Significance in Animal Feeds by T Latsch, Dept. of Animal Nutrition and Health, HOFFMANNN LA ROCHE LTD BASEL SWITZERLAND, 1990). The above antioxidants are synthetic compounds and do not have any nutritional value, but they can contribute to reduce the LDL oxidation.

As indicated above the LDL is usually rich with PUFA which is mainly C-18:2 (linoleic acid) which is very reactive in the oxidation of LDL. On the other hand the presence of a large amount of MUFA, e.g. C-18:1, oleic acid contributes to the increase of the LDL stability as far as oxidation of LDL is concerned. Also SFA are rather resistant to oxidation.

As can be understood from the above the PUFA are very sensitive to oxidation and therefore increase the risk to the LDL oxidation. Thus, one major aspect of the present invention is to reduce the amount of the PUFA present in the egg.

The present invention thus consists of an egg comprising:

(a) not more than 15.5 % poly unsaturated fatty acids of the egg's fatty acids concentration;

(b) 2-11 mg vitamin E per 59 grams of whole shell egg; and

(c) 10-60 (g of edible carotenoids per gram of egg yolk.

More specifically the present invention also consists in an egg comprising a synergistic composition of antioxidants and of low PUFA (as herein defined) comprising:

not more than 15.5% (PUFA) of the egg's fatty acid concentrate and/or not more than 1.5 wt% of same in the eggs;

2- 11 mg vitamin E per 59g of whole shell egg; per 50g of liquid egg; or per 16.6g of yolk;

40 - 112 (g iodine per 59g of whole shell egg; per 50g of liquid weight; or per 16.6g of egg yolk; and

10 - 60 µg of edible carotenoids which are readily deposited in the yolk per g of yolk.

"Liquid egg" according to the present invention indicates

the liquid content of an egg, that is the whole shell egg minus the shell.

The amount of edible carotenoids present in the egg is preferably 20-45µg/g yolk.

Said edible carotenoids may be selected, e.g. among:

Carotenoids such as oxycarotenoids (xanthophylls) : i.e. lutein, zeaxanthin, cryptoxanthin, violaxanthin, neoxanthin, antheraxanthin, polyoxyxanthophylls, etc.

The sources of the xanthophylls may be: yellow corn, corn gluten meal, lucerne (alfalfa) meal, grass meal, dehydrated alfalfa, alfalfa concentrate, grass meal,paprika meal, alga meal, seaweed, kelp, marigold meal/concentrate, tagetes meal and/or other suitable synthetic carotenoids such as yellow or red carophyll, etc.

The egg according to the present invention comprises advantageously additional edible antioxidants.

These antioxidants may be selected, e.g. among:

synthetic antioxidants which have been found to protect vitamin E, Carotenoids, PUFA, etc. such as BHT, EMQ, N,N'-diphenyl-p-phenylenediamine (DPPD), Ionol, Diludin, Digisan, Tanan, Kurasan, etc.;

Phenols and Flavonoids from herbs and plants, e.g.sage, Rosemarine, green and black tea, etc.; or pure forms like Hydroxyflavone, Galanin, Quercetine, Catechines,

Ubiquinol, etc;

Selenium; Vitamin C (asAscorbic Acid or Ascorbyl Esters); mixtures of the above; etc.

All said antioxidants should be within the recommended dietary allowance (RDA) and not exceed twice the amount allowed by RDA in one egg. The amount of the antioxidant differs according to the kind of antioxidant combinations thereof utilized.

Said antioxidants are fed to the chicken as part of the standard mixtures or of water, advantageously as part of a premix.

The fatty acids fed to the chickens usually comprise both PUFA and MUFA. The amount of PUFA in the egg should not exceed the amount indicated above preferably not 13%. The amount of MUFA is suitably 38% - 57%, advantageously 47 - 53% of the egg's total amount of fatty acids. The remainder are SFA. Animal fats comprise a large amount of SFA which is a disadvantage to the cholesterol metabolism. Thus, as indicated above, animal fat is not the first fat/oil selection of the diet. However, it can be used to be part of the composition and texture of the feed mixture.

The source of the fatty acids is advantageously raw canola oil which is low in PUFA and very rich in antioxidants. However, any other suitable oil may be used, e.g. Trisun 80; olive oil; avocado oil; peanut oil; corn oil; soy oil; combinations of all said oils; etc.

A preferred egg comprises 2 to 9 mg, advantageously 4 to 9 mg, of vitamin E per 59 g of whole shell egg, per 50 g of

liquid egg; or per 16.6 g of egg yolk.

The source of vitamin E is advantageously alfalfa meal/concentrate or pure vitamin E or salts thereof or mixtures of them.

A preferred egg comprises 50 to about 100 µg, preferably 50 - 85 µg, advantageously 65 µg of iodine, per 59 g of whole shell egg; per 50 g of liquid egg or 16.6 g of egg yolk.

The source of the iodine may be, for example, seaweed e.g. kelp; calcium iodide; potassium iodide; sodium iodide; cuprous iodide; thymol iodide; ethylene dihydroiodide; or combinations thereof.

The present invention consists also in a method for producing chicken eggs (as defined above) which consists in feeding chickens with a standard feed mixture comprising low PUFA supplemented with vitamin E, iodine and carotenoids in amounts ascertaining that the eggs so produced contain the target amounts of said ingredients.

Said ingredients may be part of the standard food mixture; be added as part of a premix, in water or separately.

Should other antioxidants besides carotenoids and vitamin E have to be present they are fed to the chicken in adequate amounts.

The standard grain based food mixture is advantageously a milo, and/or barley, rye oat, wheat, rice, corn, etc. based food mixture.

The method according to the present invention preferably

comprises feeding chickens with standard ingredients and fat, supplemented with from about 0.2 to about 3.0 wt.% edible oil to attain at least 65 wt. % unsaturated fatty acids, the amount of PUFA being about 0.7 - 1.5 wt% of the entire diet, at the utmost 20-45% of the total fatty acids (preferably 35%) further supplemented with iodine and vitamin E thus that the iodine content of the feed is from 2.5 to about 7.5 mg per kg of feed, and the vitamin E content of the feed is from about 100 to about 300 mg per kg of feed, (preferably 100 mg/kg) further supplemented with edible carotenoids such that the carotenoid content of the feed is from 15 to about 45 mg per kg of the feed, and optionally further supplemented with edible antioxidants; the antioxidant content of the egg not exceeding 2 times the amount allowed by RDA/egg.

The present invention will now be illustrated with reference to the following examples, tables and Figs. without being limited by same:

The term "Enriched" used herein refers to diet 2 and/or eggs enriched with vitamin E, Iodine, and Carotenoids.

The term "Enriched, low PUFA" used herein refers to "Enriched" as defined above and comprises low PUFA (diet 3).

Example 1

Producing the eggs

Materials and Methods

One hundred laying pullets (Yarkon, PBU) 4.5 weeks of age, were located in individual battery cages in an open shaded poultry house and divided into two groups.

Diets were formulated (Table 1) and prepared monthly

with fresh premixes. Control chickens got the layers Vitamins and Minerals premix of Kofolk Ltd. (Israel). The enriched diet comprised The Biotene Total PX* (England's Best) premix having the following composition comprising suitable amounts of :

Rice Hulls, Dehydrated Alfalfa Meal, Rice Bran, Dehydrated Kelp, Vitamin A Supplement, Vitamin D3 Supplement, Vitamin E Supplement, Menadione Sodium Bisulfite Complex, Riboflavin Supplement, d-Calcium Pantothenate, Niacin Supplement, Vitamin B12 Supplement, Pyridoxine Hydrochloride, Thiamine Mononitrate, Folic Acid, Biotin, Manganese Sulfate, Manganous Oxide, Zinc Sulfate, Zinc Oxide, Iron Sulfate, Copper Sulfate, Sodium Selenite, Sodium Bicarbonate, Propionic Acid, Acetic Acid, Sorbic Acid, Benzoic Acid, Mono and Diesters of 1,2, Propanediol, Hydrated Ammonium Phosphate, Calcium Silicate, Ethoxyquin, Butylated Hydroxyanisole, Disodium EDTA, Phosphoric Acid, Citric Acid, Mono- and Diglycerides.

The control, diet 1 (6% wt fat) comprised soy oil, diet 2 (6% wt fat) comprised extracted canola oil and diet 3 (3% wt fat) comprised raw canola oil.

Feed intake, egg production, egg weight, egg composition, and shell quality were determined throughout the experiment. Chemical analyses were performed by Aminolab, Spectrolab, Siap.

The eggs enriched with vitamin E, iodine and carotenoids; and control eggs were daily collected in an air-conditioned room or refrigerator and were weekly transported to

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the pool and stored in a refrigerator before they were distributed to volunteers for the clinical study.

During the last feeding month were added 0.5 ml of Selen Vitamin of Koffolk Ltd to 4 L of the drinking water.

Chemical Analyses

The eggs were weighed monthly. The shell was removed with water, dried (100°C for 1 h) and weighed. Egg freshness was estimated by Hugh units.

Vitamin E, iodine and fatty acids were determined on pooled samples of three eggs per group. Egg vitamin E was determined in HPLC according to J. Food Sci. 1993 (p. 669). Egg iodine was determined according to the Food Chemical Codex method. Egg cholesterol was determined according to the Liebermann-Burchard Reaction. Fatty acid profile in yolks and feeds was determined by gas chromatography after lipid extraction, saponification and methylation.

Statistical analysis were carried out by the statistical software of Excel (Microsoft^{R}).

Differences between treatment groups were analysed by t-test.

Results

Feed intake and egg production (Table 2)

No significant difference between the 3 diets was observed in feed intake, which varied between 113 to 120 g/hen/day, and the layers performance indicating that the feed was optimal and balanced. Egg production rate increased with age to 31 weeks of age and stabilized thereafter.

Egg weight and its component (Table 3)

Egg weight and its components did not differ between the various diets, but the significantly increased with age. Egg weight increased with age, from 58 g at 27 weeks of age to 65 g at 39 weeks. Shell weight increased with age from 9 to about 9.45 g (per 100 g), with no significant differences between the various treatment groups. The weight of the yolk increased with age mainly after 31 weeks of age. Similar tendency was observed of Hough units.

Eggs iodine and vitamin E levels were determined several times during the experiment (Table 4).

When measured at 31 weeks of age, dietary vitamin E (tocopheryl acetate) was 5-fold higher in the enriched than in the control groups (158 and 28.6 mg/kg diet, respectively).

The levels in the control eggs ranged between 17 to 23 mg/kg egg, and that of the enriched eggs was about 5-9 fold higher. The highest value 41 weeks were attained under reduced PUFA.

The iodine level increased in the enriched eggs by 2-2.5-fold in comparison with the control eggs (Table 4).

Fatty acid prof egg lipids (triglycerides and phospholipides) show (Table 5) that palmitic acid (C18:1) and oleic acids (C16:0) are the major fatty acids in eggs, and linoleic acid (C18:2) is the third component.

Due to the dietary fat reduction (wt %) from 6% (diet 1 and 2) to 3% (diet 3) and in particular the reduction of linoleic acid from 3.05% (diet 1) to 1.44 % (diet 3) (see

Table 1) the lineolic acid in the egg was reduced from 21 and 21.6% to 12.4% in diets 1, 2 and 3, respectively. The oleic acid increase corresponded to the decrease of linoleic acid (Table 5).

Example 2

The clinical evaluation of the eggs

The purpose of this experiment was to examine the influence of eating eggs comprising a low amount of linoleic acid and a large amount of antioxidants on the LDL-oxidation.

The research was performed on 17 healthy volunteers,14 women and 3 men, aged 30 to 50, non smokers taking no medication and consuming a standard Israeli diet (26% fat, 53% carbohydrates and 21% protein). Before the beginning of the study the volunteers ate eggs only occasionally. The volunteers were asked to maintain their habitual diet and lifestyle from 2 weeks before and during the 9 weeks of the experiment. Every volunteer went through the following three stages of the experiment:

1) Three weeks with 2 control eggs daily.
2) Three weeks with 2 eggs daily enriched with iodine, vitamin E and carotenoids
3) Three weeks with 2 eggs daily enriched as in 2 comprising a reduced amount of PUFA.

Blood samples were taken after a fast of 14 hours (of Baseline), thereafter 3, 6, and 9 weeks on the 2 eggs feeding regime and tested for blood chemistry, lipids , vitamin E, cartenoids, cholesterol, and LDL oxidation.

The LDL was separated from the plasma by the density

gradient and was oxidized by incubation with copper ions. The oxidation rate of the LDL was determined by the kinetic differences in the formation of coupled diens (at 234 nm) as well as on the basis of the Malon Diadehyde levels in LDL. (By the TBARS method).

Results

Eating 2 control eggs daily for three weeks did not affect significantly blood chemistry but slightly increased glucose and urea (BUN), within the normal range. Enriched eggs significantly reduced glucose (Table 6) back to the Baseline level. Eating 2 control eggs/day had a negative effect on blood cholesterol (Table 7); It increased the total cholesterol (TC) and LDL and reduced the HDL-Cholesterol (11%). Thus it increased significantly the risk factor (LDL/HDL) for atherosclerosis. Enriched eggs significantly increased the HDL back to the baseline level. Thus, reducing the risk factor, although it did not reduce the LDL level.

Table 8 shows a highly significant effect of the enriched eggs on the plasma antioxidants: compared to the baseline and after 3 weeks levels, the vitamin E, vitamin A and carotenoids increased (average of results after 6 and 9 weeks) by 34%, 26.5% and 49%, respectively. These results indicate that enriching the eggs did reduce the oxidative stress which is expected to protect against the harmful effect of LDL oxidation. The analysis of plasma FA shown in Table 9 does not reveal any significant trend. As the volunteers are under "free living" conditions, the eggs does not seem to be a major source of FA in the diet and thus it

is not expected that they affect significantly the blood levels.

The main purpose of the research to evaluate the potential of eggs to reduce the oxidizability of plasma LDL, was achieved by the regime maintained between 6 - 9 weeks (Figs. 1 and 2). As can be seen, eating 2 control eggs significantly increased the oxidizability of plasma-LDL. Enriching with iodine, vitamin E and carotenoids was not enough to restore the protection on LDL, unless the PUFA percentage was reduced in the feed and the eggs.On this regime the plasma levels of antioxidants (Table 8) i.e vitamins E ,A and carotenoids were increased by 23%, 14% and 55%, respectively. This emphasizes the potential contribution of each of the antioxidants and the synergistic effect attained by the eggs in diet 3.

The following examples represent low PUFA mixtures. The enrichment with iodine, vitamin E and carotenoids should preferably be formulated and based on the calculations of the ingredients in the premix. Special attention should be given to milo based low corn mixtures which comprise low PUFA but may be low in the amount of carotenoids present, e.g. Example 4, which comprises a low amount of carotenoids in the feed ingredients (compared with Example 3 which comprises at least 30 mg of carotenoids in the ingredients of 1 kg of feed). The mixture of Example 4 should thus advantageously be further supplemented with other sources of carotenoids, e.g. corn gluten meal, alfalfa, grass, algae, tagetes meals or extracts, and/or with carotenoid premixes, e.g. oro-glo

layer, pig well egg of Sun Gold and/or other synthetic carotenoids, etc.

Example 3

Ingredients (KG) in 1000 KG

Corn 400

Raw Canola oil 5

Dl Methionine 89 g

Limesstone 99

Di Calcium Phosphate 6.7

Salt 2.7

$NaSO_4$ 1

Soya 48% Protein 82

Corn Gluten (60%) 49

Barley Meal 48

Chopped Corn 182

Sun Flower Meal 100

Canola Meal 21

Layer Mix 3

Fat Pecentage 3%

Example 4

Ingredients (KG) in 1000 KG

Milo* 613

Raw Canola/Chicken Fat 8.2

Dl Methionine 0.3

Lime Stone 92

DCP 6.2

Salt 3

Na_2SO_4 1

* trademark

Soya 48% 121

Corn Gluten 60% 19

Barley 16

Chop corn 10

Sunflower 100

Canola Meal 8.5

Layer Mix 3

Fat content 2.9

2700 Kcal/17% Protein

Example 5

Ingredients (KG) in 1000 KG

Milo 510.5

Corn 100

Soya 44% 174.9

Corn Gluten 70

Remoulage 26.6

M.H.A 0.5

D.C.P 12

Lime stone 91

Lysine 0.05

Canola oil 7

Salt 3

Layer Mix 5

Metabolic energy 2760 Kcal

Fat 2.87%

Linoleic acid 1.14%

Protein 17.5%

Example 6

Ingredients (KG) in 1000 KG

Milo 621

Soya 48% 160

Bran 27

Corn Gluten 70

M.H.A 0.5

D.C.P 11

Lime stone 92

Lysine 0.2

Canola oil 10.000

Salt 3

Layer Mix 5

Metabolic energy 2779 Kcal

Fat 3.14 %

Linoleic 1.13 %

Protein 17.7 %

Example 7

Ingredients (KG) in 1000 KG

Milo 633.8

Soya 44% 174.6

Corn Gluten 70

M.H.A 0.5

D.C.P 12.3

Lime stone 90.7

Lysine 3.2

Canola oil 7

Salt 3

Layer Mix 5

Metabolic energy 2780 Kcal

Fat 2.79%

Linoleic 1.03%

Protein 17.5%

Example 8

Ingredients (KG) in 1000 KG

Milo 533

Barley 100

Soya 44% 143

Corn Gluten 70

M.H.A 0.8

D.C.P 12.1

Lime stone 90.9

Lysine 15.9

Canola oil 7

Salt 3

Layer Mix 5

Metabolic energy 2760 Kcal

Fat 2.7 %

Linoleic 1 %

Protein 17.5 %

WHAT IS CLAIMED IS:

1. An egg comprising:

(a) not more than 15.5 % poly unsaturated fatty acids of the egg's fatty acids concentration;

(b) 2-11 mg vitamin E per 59 grams of whole shell egg; and

(c) 10-60 μg of edible carotenoids per gram of egg yolk.

2. An egg according to claim 1, further comprising 38-57 % mono unsaturated fatty acids of the egg's fatty acid concentration.

3. An egg according to claim 1, further comprising edible antioxidants.

4. An egg according to claim 1, comprising not more than 13 % of poly unsaturated fatty acids.

5. An egg according to claim 1, comprising 2 to 9 mg of vitamin E per 59 grams of whole shell egg.

6. An egg according to claim 5, comprising 4 to 9 mg of vitamin E per 59 grams of whole shell egg.

7. An egg according to claim 1, wherein said carotenoids are oxycarotenoids (xanthophylls) selected from the group consisting of lutein, zeaxanthin, cryptoxanthin, violaxanthin, neoxanthin, antheraxanthin and polyoxyxanthophylls.

8. An egg according to claim 7, wherein the source of the xanthophylls is selected from the group consisting of yellow corn, corn gluten meal, lucerne (alfalfa) meal, dehydrated alfalfa meal, seaweed, kelp, marigold meal/concentrate, tagetes meal, and synthetic carotenoids.

9. An egg according to claim 3, comprising not more than twice the amount of antioxidants allowed by the RDA.

10. An egg according to claim 3, wherein said antioxidants are selected from the group consisting of BHT, EMQ, N,N-diphenyl-p-phenylenediamine (DPPD), Ionol, Diludin, Digisan, Tanan, Kurasan, Phenols, Flavonoids, Hydroxyflavone, Galanin, Quercetine, Catechines, Ubiquinol, Selenium, Vitamin C and mixtures of the above.

11. An egg according to claim 1, wherein the source of the fatty acids is selected from the group consisting of raw canola oil, Trisun-80, olive oil, avocado oil, peanut oil, corn oil, soy oil, and combinations thereof.

12. An egg according to claim 1, wherein the source of Vitamin E is selected from the group consisting of alfalfa meal/concentrate, pure vitamin E, salts thereof, and mixtures of the foregoing.

13. An egg according to claim 1, further comprising:

(d) 40-112 μg iodine per 59 grams of whole shell egg.

14. An egg according to claim 13, comprising 50 to 100 μg of iodine per 59 grams of whole shell egg.

15. An egg according to claim 14, comprising 50-85 μg of iodine per 59 grams of whole shell egg.

16. An egg according to claim 14, comprising 65 μg of iodine per 59 grams of whole shell egg.

17. An egg according to claim 14, wherein the source of iodine is selected from the group consisting of seaweed, kelp, calcium iodide, potassium

iodide, sodium iodide, cuprous iodide, thymol iodide, ethylene dihydroiodide and combinations thereof.

18. An egg according to claim 1, comprising 20-45 μg of carotenoids per gram of egg yolk.

19. A method for producing eggs, the method comprising the step of feeding chickens with standard ingredients and fat, supplemented with from about 0.2 to about 3.0 wt.% edible oil to attain at least 65 wt.% unsaturated fatty acids, the amount of poly-unsaturated fatty acids being about 0.7-1.5 wt.% of a diet of the chickens, further supplemented vitamin E, such that the vitamin E content of the feed is from about 100 to about 300 mg per kg of feed, and still further supplemented with edible carotenoids such that the carotenoid content of the feed is from 15 to about 45 mg per kg of the feed.

20. A method according to claim 19, wherein the feed is further supplemented with iodine, the iodine content of the feed is from 2.5 to about 7.5 mg per kg of feed.

21. A method according to claim 19, wherein the feed is further supplemented with edible antioxidants so that the antioxidant content of the feed should not exceed the amount of 2 times allowed by the RDA/egg.

22. A method according to claim 19, wherein the standard grain based food is selected from the group consisting of milo, barley, rye oat, wheat, rice and corn based food mixture.

23. A use of eggs in a human diet designed to reduce the responsive increase of LDL oxidation associated with consuming eggs which are higher in polyunsaturated fatty acids, each egg comprising:

(a) not more than 15.5 % poly unsaturated fatty acids of the egg's fatty acids concentration;

(b) 2-11 mg vitamin E per 59 grams of whole shell egg; and

(c) 10-60 μg of edible carotenoids per gram of egg yolk.

24. The use of eggs of claim 23, wherein each egg further comprises 38-57 % mono unsaturated fatty acids of the egg's fatty acid concentration.

25. The use of eggs of claim 23, wherein each egg further comprises edible antioxidants.

26. The use of eggs of claim 23, wherein each egg comprises not more than 13 % of poly unsaturated fatty acids of the egg's fatty acid concentration.

27. The use of eggs of claim 23, wherein each egg comprises 2 to 9 mg of vitamin E per 59 grams of whole shell egg.

28. The use of eggs of claim 27, wherein each egg comprises 4 to 9 mg of vitamin E per 59 grams of whole shell egg.

29. The use of eggs of claim 23, wherein said carotenoids are oxycarotenoids (xanthophylls) selected from the group consisting of lutein, zeaxanthin, cryptoxanthin, violaxanthin, neoxanthin, antheraxanthin and polyoxyxanthophylls.

30. The use of eggs of claim 29, wherein the source of the xanthophylls is selected from the group consisting of yellow corn, corn gluten meal, lucerne (alfalfa) meal, dehydrated alfalfa meal, seaweed, kelp, marigold meal/concentrate, tagetes meal, and synthetic carotenoids.

31. The use of eggs of claim 25, wherein each egg comprises not more than twice the amount of antioxidants allowed by the RDA.

32. The use of eggs of claim 25, wherein said antioxidants are selected from the group consisting of BHT, EMQ, N,N-diphenyl-p-phenylenediamine (DPPD), Ionol, Diludin, Digisan, Tanan, Kurasan, Phenols, Flavonoids, Hydroxyflavone, Galanin, Quercetine, Catechines, Ubiquinol, Selenium, Vitamin C and mixtures of the above.

33. The use of eggs of claim 23, wherein the source of the fatty acids is selected from the group consisting of raw canola oil, Trisun-80, olive oil, avocado oil, peanut oil, corn oil, soy oil, and combinations thereof.

34. The use of eggs of claim 23, wherein the source of Vitamin E is selected from the group consisting of alfalfa meal/concentrate, pure vitamin E, salts thereof, and mixtures of the foregoing.

35. The use of eggs of claim 23, wherein each egg further comprises:

(c) 40-112 μg iodine per 59 grams of whole shell egg.

36. The use of eggs of claim 35, wherein each egg further comprises 50 to 100 μg of iodine per 59 grams of whole shell egg.

37. The use of eggs of claim 36, wherein each egg comprises 50-85 μg of iodine per 59 grams of whole shell egg.

38. The use of eggs of claim 36, wherein each egg comprises 65 μg of iodine per 59 grams of whole shell egg.

39. The use of eggs of claim 36, wherein the source of iodine is selected from the group consisting of seaweed, kelp, calcium iodide, potassium iodide, sodium iodide, cuprous iodide, thymol iodide, ethylene dihydroiodide and combinations thereof.

40. The use of eggs of claim 23, wherein each egg comprises 20-45 μg of carotenoids per gram of egg yolk.

Fig 1. Composition of diets

Components(g/kg)	Diet 1	Diet2**	Diet3
Metabolizable energy(kcal/kg)	2800	2800	2800
Protein	183	183	180
Arg	12.4	12.4	13
Lys	10.6	10.6	10.5
Met	3.9	3.9	4
Met+Cys	6.7	6.7	7.1
Linoleic acid	30.5	24	14
Calcium	37	37	38
Available Phosphor	4	4	3.1
Fat(%)	6	6	3
Ingredients (kg/100 kg)			
Milo			621
Corn	500	500	
Soybean meal 44	310	310	
Soybean meal 48			160
Corn gluten meal			70
Barley	30	30	
Wheat bran			27
Soapstock oil	40	40	
Canola oil		40	10
Limestone	86	86	92
DCP	16	16	11
Salt	3.3	3.3	3
DL-Methionine	0.4	0.4	0.514
Synthetic lysine			0.187
Vitamin and mineral premix***	10	10	10

** Same composition as diet 1 but soapstock oil was replaced by canola oil.

*** In diets 2 and 3 the Biotene Total PX (England's Best) premix was enriched with I and vitamin E. Diet 1 (control) contained a commercial premix of Koffolk Ltd.

Fig 2. Egg production and feed intake of layers on "Enriched" vs control premix[1]

Age(wks)	22	27	31	35	39	44
	Egg production(egg/b/d)					
Control	0.60	0.87	0.89	0.91	0.91	0.91
Enriched	0.76*	0.87	0.91	0.88	0.93	0.90
	Feed intake(kg/b/d)					
Control		0.124	0.114	0.115	0.115	0.113
Enriched		0.126	0.120	0.116	0.113	0.115

1 An average of monthly data of 30 to 40 replicates per group.

* Statistically significant at $P<0.05$

Fig 3. Egg weight and components from layers on Enriched and Control regime

Age(wks)	22	27	31	35	39
			Egg wt.(g)		
Control		58.0	61.2	64.57	63.78
Enriched		58.5	61.5	64.65	64.77
			Shell wt.(%)		
Control	9.01		9.11		9.45
Enriched	8.76		9.03		9.35
			Yolk wt.(g)		
control		14.2	15.3		16.92
Enriched		13.8	15.2		17.24
			Hough units		
Control		5.8	6.6		7.40
Enriched		5.7	6.8		7.56

Fig 4. Egg vitamin E and iodine of layers on enriched and control regime

Age(wks)	27	29	31	41
	Dietary tocopheryl acetate (mg/kg feed)			
Control			28.6	
Enriched			158.2	
	Egg tocopherol (mg/kg egg)			
Control		22.9	17.4	
Enriched		112.7	82.5	158.3
	Egg iodine (ug/g)			
Control	2.9	3.8		
Enriched	3.6	7.9		

Fig 5. Fatty acid composition in diets and eggs

In Eggs (%)

FA		Control	Enriched	Enriched II	Enriched Low Pufa
Lauric	12:0				
Myristic	14:0	0.3	0.3	0.5	0.4
Palmitic	16:0	30.1	29.9	25	27.2
Palmitoleic	16:1	0.8	0.8	4.7	4.5
Stearic	18:0	10.5	10.7	4.2	
Oleic	18:1	37.4	34.1	37.3	47.3
Linoleic	18:2	17.9	21.5	21	12.4
Linolenic	18:3	0.4	0.4	2.3	0.6
Arachidic	20:0	0.3	0.3	0.8	0.3
Lingoceric	24:0	0.2	0.2		0.8

Table 5(Cont.)

Fatty acid composition (%)

In Diets

	Control	Enriched	Enriched II	Enriched Low Pufa
12:0	0.3	0.3		
14:0	0.5	0.3	0.2	0.7
16:0	12.2	8.5	11.2	26.1
16:1	0.4	0.1		4.5
18:0	5.9	3	1.5	4.4
18:1	30.7	37.6	36.9	37
18:2	41.1	41.1	40.8	21.6
18:3	2.9	6.7	4.8	1.52
20:0	1	0.2	0.3	0.2
20:3	2.2	0.4	0.9	
20:4	1.3	1	0.2	
22:0	0.1	0.2		
24:0	0.5	0.1		1.14

Fig 6. Blood chemistry, following eggs consumption (mg/dl)

Time on Eggs Supplementation	0 Baseline	3 Weeks Control Eggs	6 Weeks Enriched	9 Weeks Enriched, Low Pufa
CK	110±30	118±33	111±45	107±32
Amylase(IU/ml)	53±11	50±10	50±12	55±12
AST(IU/ml)	19±6	22±3	18±4	18±3
ALT(IU/ml)	20±10	19±9	19±11	19±10
T.Bilirubin	0.7±0.2	.6±0.3	0.5±0.3	0.5±0.2
D.Bilirubin	0.06±0.02	0.05±0.02	0.07±0.07	0.06±0.1
Calcium	10±0.4	9±0.3	10±0.3	9.8±0.3
Glucose	84±8	98±8*	85±7+	85±7+
BUN	12±4	16±2*	15±4	14±4
Createnine	0.9±0.1	0.8±0.2	0.9±0.1	0.7±0.1
Alkaline phosphatase	50±11	54±13	52±15	54±15

*p<0.01 (vs. Baseline). +p<0.05 (vs. 3 Weeks)

Fig 7. Plasma lipids and lipoproteins following eggs consumption (mg/dl)

Time on Eggs Supplementation	0 Baseline	3 Weeks Control Eggs	6 Weeks Enriched	9 Weeks Enriched Low Pufa
Cholesterol	185±24	210±40*	220±45	202±41
HDL-C	54± 7	48± 7*	55±15+	54± 7+
LDL-C	111±20	132±23*	144±20	130±21
VLDL-C	22±10	20±10	23±10	24± 9
Apo-A-I	143±20	148±14	146±25	142±19
Apo-B-100	90±19	91±20	98±12	107±27
Triglycerids	110±44	101±40	116±38	115±39

*p<0.01 (vs. Baseline). +p<0.05 (vs. 3 Weeks)

Fig 8. Plasma vitamins E, A and Carotenoids following eggs consumption (mg/dl)

Time on Eggs Supplement.	0 Baseline	3 Weeks Control Eggs	6 Weeks Enriched	9 Weeks Enriched Low Pufa
Vitamin E	43±6	44±8	63±17*	54±11*
Vitamin A	0.55±0.11	0.58±0.13	0.77±0.18*	0.66±0.21*
Carotenoids	1.11±0.21	1.09±0.27	1.60±0.57*	1.69±0.45*

*p<0.01 (vs. 3 Weeks)

Fig 9. Plasma fatty acids following eggs consumption (%)

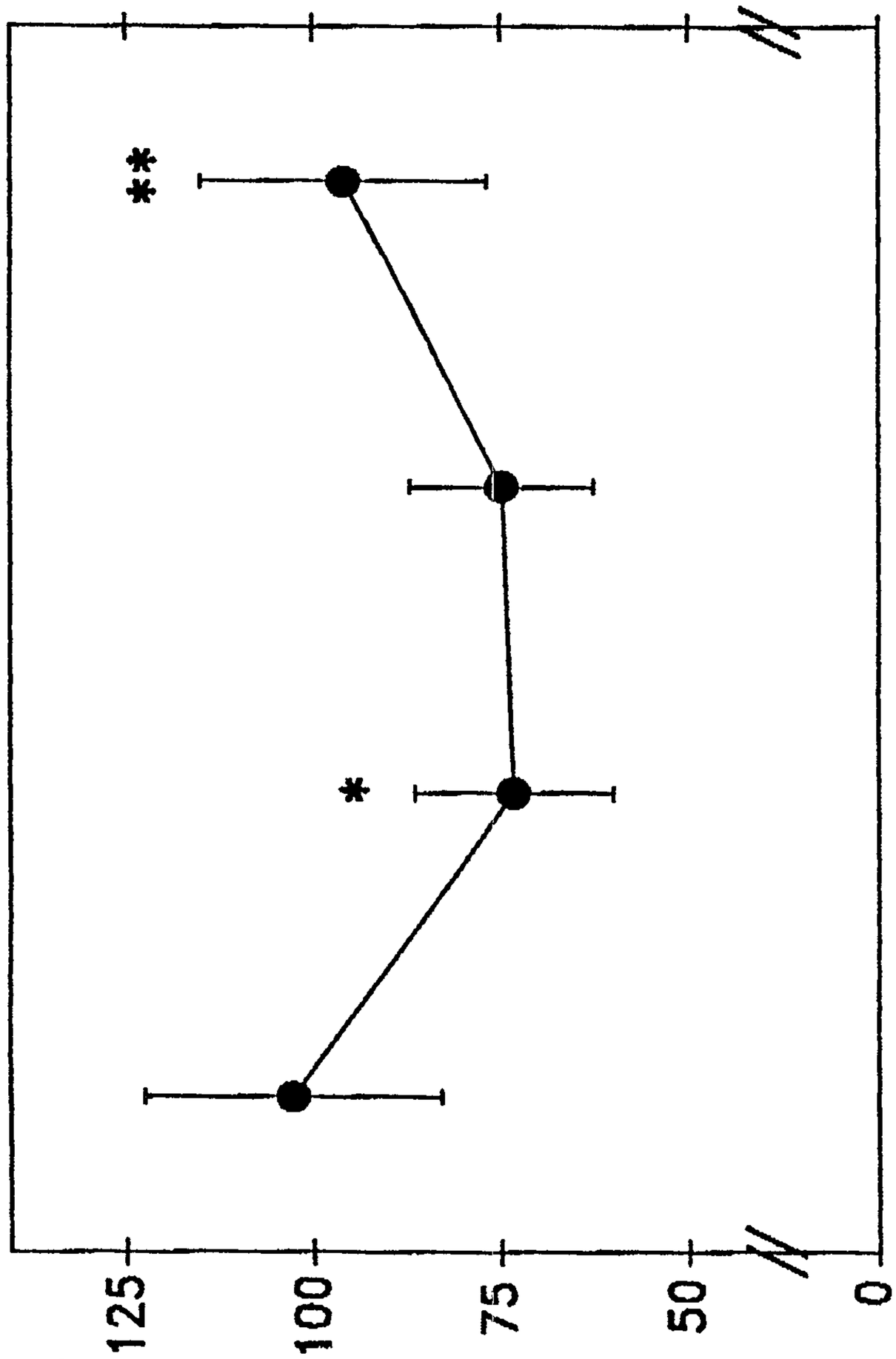
Fatty Acid

Time on Eggs Supplementation	0 Baseline	3 Weeks Control Eggs	6 Weeks Enriched	9 Weeks Enriched Low Pufa
16:0(Palmitic)	19±2	21±3	20±2	19±1
18:0(Stearic)	8±1	10±1	10±2	8±2
18:1(Oleic)	11±2	12±2	9±2	16±3++
18:2(Linoleic)	29±4	20±5*	25±5+	24±5
20:4(Archidonix)	10±2	10±3	11±2	9±2
Other fatty acids	23	28	25	24

*p<0.01 (vs. Baseline). +p<0.05 (vs. 3 Weeks)

++p<0.01(vs. 6 Weeks)

Fig 10. EFFECT OF CONTROL, ENRICHED LOW PUFA, 2 DAILY EGGS ON LDL OXIDIZABILITY



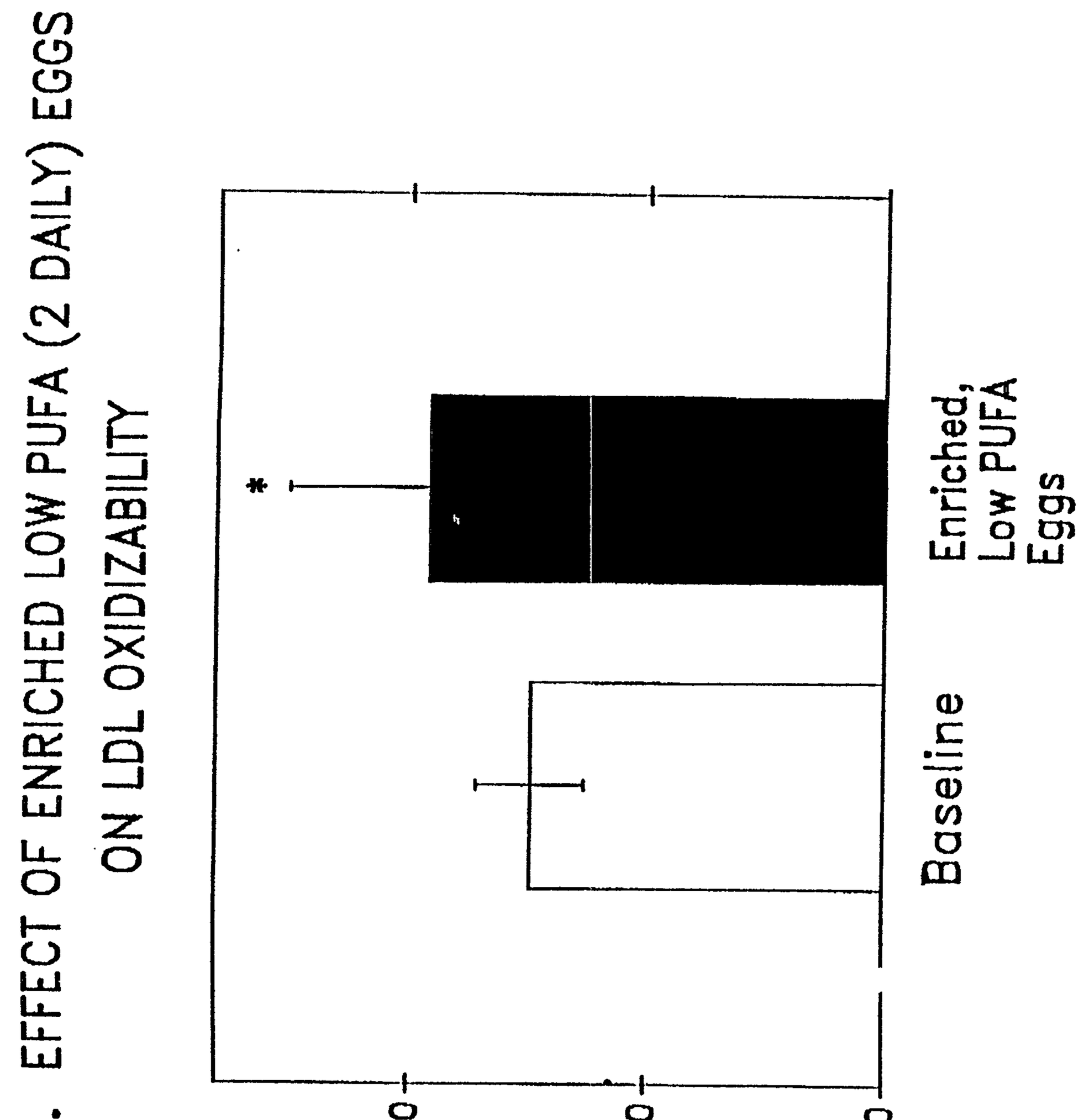

EFFECT OF ENRICHED EGGS
ON LDL OXIDIZABILITY
LOW PUFA (2 DAILY)
Enriched,
Low PUFA
Eggs
Baseline
*
100
50
0
LDL Oxidation
[Lag time (min)]

Fig 11.