Unhealthy Fats Can Be Declined in Enriched Eggs by Graded Levels of Polyunsaturated Oils and Selenium Sources

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ABSTRACT

Dietary saturated and trans fats are the primary culprit in today's society leading to worsening of the lipid profile and increasing atherosclerosis risk. This study was conducted to evaluate the effects of graded levels of oil sources and different types of selenium (organic and inorganic) on cholesterol (CH) and triglyceride (TG) concentrations and lipid oxidation content in fresh and stored table eggs. Two hundred eighty eight Hy-Line hens, 45-wk-old, were divided into six groups which received a basal diet supplemented with 1.5% of each of fish oil (FO), canola oil (CO) and sunflower oil (SO) and also 0.3 mg/kg from each organic and or inorganic selenium (selpex or sodium selenat) as well as the same doses of vitamins E and C. Amounts of cholesterol, triglyceride and lipid oxidation marker (2-thiobarbituric acid, TBA, -reactive substances) contents in fresh and stored eggs for one month at refrigerator temperature (+4°C) were measured. Fresh and stored eggs of hens fed on diet supplemented with 1.5%FO+1.5%CO+0.3 mg/kg organic Se had lowest CH, TG and also malondialdehyde (MDA) concentrates, followed by dietary 1.5%CO+1.5%SU+0.3 mg/kg organic Se group (P<0.01). TBARS value increased in eggs stored for a period of 1 month but differences were not significant in dietary fish and canola oils groups which also enriched by organic Se. The results related to oil × Se interactions showed that dietary enrichment with fish and canola oils (1.5 % from each) as well as selenium could decrease unhealthy fats and MDA concentrate in enriched eggs and supplementation with 0.3 mg/kg organic Se was enough to prevent lipid oxidation in enriched table eggs during storage period.

KEY WORDS: Enriched egg, fish oil, canola oil, sunflower oil, cholesterol, triglycerides, lipid oxidation

INTRODUCTION

All fats are not alike. Some types of fats are essential for good health. Other fats or dietary fats can raise blood cholesterol levels or have other negative effects on cardiovascular health. Unsaturated fats are found in plant foods or in fish that eat microscopic plants. One type of polyunsaturated fat - omega-3 fatty acids - has been found to have many positive effects. For example, omega-3 fatty acids may reduce the risk of sudden cardiac death, help keep blood vessels flexible and reduce excess blood clotting [1]. Other polyunsaturated and monounsaturated fats will lower low density lipoprotein (LDL) (“bad”) cholesterol and triglyceride when used in place of saturated fat (unhealthy fats). Foods rich in these “good” unsaturated fats are listed below:

A) Omega-3 Fatty Acids (a type of Polyunsaturated Fat): Fatty fish such as salmon, herring, sardines and trout; so that, the American Heart Association recommends eating at least two 3 oz. servings of fatty fish per week [2]. Flaxseed, walnuts and canola oil (all contain a less active form of omega-3)

B) Omega-6 Fatty Acids: Vegetable oils: corn oil, safflower oil, sesame oil, soybean oil, sunflower oil. Soft (liquid or tub) margarine, ideally one that is trans fat-free. Walnuts. Sunflower seeds, pumpkin seeds, sesame seeds. Soy “nuts” (roasted soy beans), soy nut butter and tofu.


All foods and oils contain a mixture of fats (fatty acids, to be more precise). Fats with negative health effects are saturated fats and trans fats. Saturated fats are found primarily in high-fat meats and dairy foods. Trans fatty acids (called “trans fats” for short) are present in foods that contain “partially hydrogenated” vegetable oils: fried foods, stick margarine, crackers, microwave popcorn, baked goods and other processed foods. Studies have shown that both saturated fats and trans fats can raise LDL (the “bad”) cholesterol. Saturated and trans fats may also make the lining of blood vessels (the endothelium) less flexible. In addition, trans fats may depress the “good” blood cholesterol (HDL cholesterol) when eaten in large quantities.

The foods contain “bad” unhealthy fats are listed below:


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B) Trans fatty acids (or “trans fats”): Stick margarine and some tub margarines. Vegetable shortening (e.g. original Crisco). Fried foods: doughnuts, French fries, other deep fried fast food items. Commercially prepared foods containing partially hydrogenated oils: crackers, cookies, cakes, pastries, microwave popcorn and other snack foods.

Cholesterol is not a fat. It is a waxy substance found only in foods of animal origin: meat, poultry, seafood, egg yolks and dairy products. Humans do not need to consume any cholesterol because our cells can produce all the cholesterol our bodies need for use in cell membranes and hormones [3]. High intakes of dietary cholesterol can raise LDL cholesterol and can increase heart disease risk in other ways. However, this effect is generally not as strong as that of saturated fats and trans fats. People who have high blood cholesterol levels, heart disease or diabetes should limit their intake of dietary cholesterol such as egg yolks or whole eggs (limit to 2 per week), organ meats (liver, brains, kidney and sweetbreads) and shrimp and squid/calamari (one serving a week is okay).

Due to the health benefits associated with the consumption of omega (ω) 3-fatty acids (FAs), much research has been done in recent years to enrich important animal products, such as eggs or broiler meat [4, 5, 6]. Eggs, one of the cheapest sources of animal protein are easily available all over the world [7]. Furthermore, eggs are nutritious favorites providing complete nutritional proteins, lipids, vitamins, minerals and some key nutrients such as omega -3 fatty acid, amino-acids, vitamins A, D, B1, B2 and E, selenium and iron. These are considered particularly important in human nutrition and body health [8, 9]. Since most egg lipids are located in the yolk, they are susceptible to oxidation and thus require quality control [4, 10, 11].

Yolk lipids generally include triglycerides, phospholipids, cholesterol and other trace compounds. These and other different components of yolk lipids may result in adverse effects on human health named as unhealthy fats. The LC n-3 PUFAs present in FO, mainly EPA and DHA, reduce the very low-density lipoprotein (VLDL) levels in blood [12]. This effect, is because of acting to lower the circulating free low density lipoprotein (LDL) concentration which is normally delivered to tissues for fat storage or deposited directly in the arteries, and thus reduces the rate of TG synthesis in the liver [3]. Omega-3 (ω3) and omega-6 (ω6) fatty acids gotten from natural sources such as fish, canola and sunflower oils are highly unsaturated and are susceptible to peroxidation when there is excessive level of its consumption without sufficient amount of antioxidants [13, 14].

Supplementation with selenium has been demonstrated to beneficially affect enhancement of lipid stability in foods from animal origin, such as avian eggs and meat. However, it is well known that oxidative deterioration, quality characteristics, nutritional value of eggs, consumer acceptability and deleterious biological effects are important nutritional factors that researchers do not test for without antioxidant substances [15].

Analyzing recent publications that relate human health to useful poultry products, it is evident that Se-enriched eggs can be used as an important delivery system of this trace mineral for humans. In particular, developments and commercialization of organic forms of selenium has initiated a new era in the availability of selenium-enriched products. It has been shown that egg selenium content can be easily manipulated to give increased levels, especially when organic selenium is included in hen’s diet at levels that provide 0.3-0.5 ppm Se in feed [16, 17, 18]. Results derived from various research studies conducted over the last few years have indicated that the Se-enrichment of animal-derived foods (mainly meat, milk and eggs) with selenium via supplementation of animal feeds can be an effective way of increasing human selenium status [16, 19].

In order to decrease unhealthy cholesterol and triglyceride contents and also prevent the oxidation (formation of thiobarbituric acid reactive substances; TBARS) of yolk lipids and improve the oxidative stability of enriched products such as omega-3 fatty acids in eggs, the current experiment was conducted to study of the effects of dietary fat sources (fish, canola or sunflower oils) and dietary types of selenium (organic and inorganic) on cholesterol (CH) and triglyceride (TG) concentrations and lipid oxidation content in fresh and stored table eggs.

MATERIALS AND METHODS

Two hundred eighty eight Hy-Line hens at 45 weeks of age were randomly housed into six groups in laying cages (4 replicates, 3 cages per replicate and 4 birds per cage) in a windowed poultry house with a light regimen of 16 h light to 8 h dark periods.

A basal diet (Table 1) without oil was supplemented with 0.3 mg/kg from each of organic or inorganic selenium (selsplex or sodium selenat) as well as same doses of vitamins E and C in all experimental diets. Both organic and inorganic Se-supplemented diets were mixed with 3% of two oil source (1.5% of each of FO, CO or SO). Diets (Table 1) were formulated to meet the requirements for nutrient and energy for laying hens on the base of nutrients recommended by (NRC, 1994).

After ten weeks of feeding experimental diets four egg per replicate were collected and yolks of two eggs were pooled to compose one sample. The fresh samples were examined for cholesterol (CH), triglyceride (TG) lipid peroxidation (MDA) contents. Cholesterol and triglyceride of yolk were extracted with chloroform: methanol (2:1 vol/vol) according to the procedure of Folch et al. [19] method. Four eggs for each replicates stored at +4°C for a period of month then were analyzed for CH, TG and MDA contents. All the samples sliced with a homogenized blade cutter during 4 minute. Total lipids of all samples were extracted by Folch reagent. After extraction process, 4-milliliters aliquots from ready samples were transported to commercial kits [21], and the cholesterol and triglyceride were analyzed by means of Autoanalyzer [22].
Table 1. Composition of the basal diet

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>%</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>40.50</td>
<td>Metabolizable energy, (Kcal/kg) 2900</td>
</tr>
<tr>
<td>Soybean meal (44%CP)</td>
<td>21.00</td>
<td>Crude protein (%) 15.02</td>
</tr>
<tr>
<td>Wheat</td>
<td>25.25</td>
<td>Crude fiber (%) 3.05</td>
</tr>
<tr>
<td>Added oil1</td>
<td>3.00</td>
<td>Calcium1 (%) 3.48</td>
</tr>
<tr>
<td>Oyster shell</td>
<td>8.00</td>
<td>Available Phosphorus4(%) 0.34</td>
</tr>
<tr>
<td>Bone meal</td>
<td>1.25</td>
<td>Lysine2 (%) 0.68</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.30</td>
<td>Methionine+cystine4 (%) 0.57</td>
</tr>
<tr>
<td>Vitamin premix2</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>Trace-Mineral premix3</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>Salt</td>
<td>0.20</td>
<td></td>
</tr>
</tbody>
</table>

1 Fish oil or sunflower oil. 2 Supplied per kilogram of diet: 3520000 IU vit.A, 1000000 IU vit.D, 4400 IU vit.E, 880 mg vit.K, 738.5 mg vit.B6, 1600 mg vit.B12, 3136 mg vit.B6, 13860 mg vit.B12, 984.8 mg vit.B6, 192 mg vit.B12, 4 mg vit.B12, 60 mg biotin, 80000 mg choline chloride, 400 mg anti oxidant, 3 Supplied per kg of diet: 25870 mg Zn, 30000 mg Fe, 29760 mg Mn, 2400 mg Cu, 346.8 mg I, 80 mg Se. 4 Calculated value.

Thiobarbituric acid (TBA) determination

The TBA values were determined for the Malonaldehyde (MDA) formed in fresh eggs and those that were refrigerated. This secondary oxidation product (MDA) was measured according to the TBA method described by Botsoglou et al. [23] using third derivative spectrophotometry with some modifications. Yolk samples were homogenized (Polytron homogenizer, PCU, Switzerland) in the presence of 8 ml 5% aqueous trichloroacetic acid (TCA). 5 ml 0.8% butyrate hydroxytoluene in hexane was immediately added and the mixture centrifuged. The top layer was discarded and a 2.5 ml aliquot from the bottom layer was mixed with 1.5 ml of 0.8% aqueous 2-thiobarbituric acid, and was further incubated at 70°C for 30 min. Following incubation, the mixture was cooled under tap water and submitted to conventional spectrophotometry [24] in the range of 400 - 650 nm. Third order derivative spectra were produced by digital differentiation of the normal spectra using a derivative wavelength difference setting of 21 nm. The concentration of MDA in analyzed samples (ng/g yolk) was calculated on the basis of the height of the third-order derivative peak at 521.5 nm by referring to slope and intercept data of the computed least-squares fit of a standard calibration curve prepared using 1, 3, 3, 3 tetraethoxypropane, the precursor of MDA.

Statistical analysis

Data were subjected to a one-way analysis of variance using the General Linear Models (GLM), procedure of SAS User’s guide [25]. When significant difference among means was found, means were separated using Duncan’s multiple range tests.

RESULTS AND DISCUSSION

Fresh and stored eggs of hens fed on diet supplemented with 1.5%FO+1.5%CO+0.3 mg/kg organic Se had lowest CH, TG and also malondialdehyde (MDA) concentrates, followed by dietary 1.5%CO+1.5%SO+0.3 organic Se group (P<0.01). TBARS value increased in eggs stored for a period of 1 month but differences were not significant in dietary fish and canola oils groups which also enriched by organic Se.

Table 2. Effect of dietary oil source and selenium on cholesterol, triglyceride and MDA in fresh and stored eggs

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Fresh egg</th>
<th>Stored egg</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CH mg/g</td>
<td>TG mg/g</td>
<td>MDA ng/g</td>
</tr>
<tr>
<td>Oil source (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.5%FO+1.5%CO</td>
<td>175.0a</td>
<td>753b</td>
<td>2.04a</td>
</tr>
<tr>
<td>1.5%FO+1.5%SO</td>
<td>194.4b</td>
<td>859a</td>
<td>2.70a</td>
</tr>
<tr>
<td>1.5%CO+1.5%SO</td>
<td>222.2a</td>
<td>883a</td>
<td>2.65a</td>
</tr>
<tr>
<td>Selenium (mg/kg)</td>
<td>ns</td>
<td>ns</td>
<td>**</td>
</tr>
<tr>
<td>Organic Se</td>
<td>194.0a</td>
<td>833a</td>
<td>2.15a</td>
</tr>
<tr>
<td>Inorganic Se</td>
<td>219.5a</td>
<td>853a</td>
<td>2.70a</td>
</tr>
</tbody>
</table>

Oil × Se interaction

*= P<0.05; **= P<0.01; ns= not significant or P>0.05; MDA= malondialdehyde; CH= cholesterol; TG= triglyceride; FO= fish oil; CO= canola oil; SO= sunflower oil.

As shown in Table 2, egg yolk cholesterol (TC) and triglycerides (TG) contents in fresh and stored eggs and also their lipid peroxidation (MDA) were affected by dietary treatments, significantly between oils groups and different types of supplemented selenium. Farhoomand and Chekanizer [12] stated that the long chained n-3 PUFAs present in FO, mainly eicosapentanoic acid (EPA) and docosahexaenoic acid (DHA), could reduce the very low-density lipoprotein (VLDL) levels in blood [12] and then in tissue. This effect, is because of acting to lower the circulating free low density lipoprotein (LDL)
concentration which is normally delivered to tissues for fat storage or deposited directly in the arteries, and thus reduces the rate of TG synthesis in the liver [3].

However, egg yolk total cholesterol content has shown no differences among laying hens fed diets containing soybean or coconut oil, lard or tallow [26], as well as laying hens fed with the mixture of sunflower and palm oil, in comparison to the three dietary regimens-tallow, crude and refined sunflower phospholipids [27]. Also, in the investigations performed by Hodzic et al. [28] statistically significant differences were found in the concentration and content of yolk total cholesterol in experimental groups. As a consequence, the physiological mechanisms of egg yolk formation are flexible enough to overcome the dietary intervention and keep the cholesterol homeostasis in the egg [29].

The lowest TBA value in fish and canola oil groups were observed. Also lowest MDA level was found in organic Se-enriched eggs in 0.3 mg/kg. Surai et al. [8] reported that Se-enriched eggs are a good provider of selenium for human and also selenium acted as a prooxidant at the egg yolk. Eggs stored for a period of 1 month had higher values of the yolk CH, TG and MDA in compared to fresh eggs by different oil sources (Table 2). Cholesterol and triglycerides contents was significant in stored eggs (P<0.01) and highest level in dietary sunflower oil groups which was enriched with 0.3 mg/kg inorganic Se eggs were found. Results related to cholesterol and triglyceride are more differented in recent study; so that, Liu et al. [30] reported that egg yolk oil with rich very low cholesterol (0.9%) was obtained by firstly extracting the egg yolk with alcohol, and followed by extracting the alcohol extract with acetone to remove the cholesterol therein. Selenium could prevent oxidative processes in enriched eggs. However, it is documented that when ordinary table eggs were stored in refrigerated conditions, the development of oxidative products was negligible [31].

CONCLUSION

In conclusion, the oils rich in polyunsaturated fatty acids plus selenium which are used in the current experiment seem to be appropriate to decrease the cholesterol and triglyceride of enriched egg. The n-3 PUFA content is considered mainly responsible for the susceptibility of egg yolk to lipid peroxidation during storage, while selenium could be a strong protector of these fatty acids, in this regard. Dietary supplementation of 0.3 mg/kg organic Se could lead to lower yolk MDA content, but its effectiveness may be influenced by the source of dietary oil.

The results related to oil × Se interaction showed that dietary enrichment with fish and canola oils (1.5 % from each) as well as selenium could decrease unhealthy fats and MDA concentrate in enriched eggs and supplementation with 0.3 mg/kg organic Se was enough to prevent lipid oxidation in enriched table eggs during storage period.

It is concluded that selenium especially in organic type enhances the oxidative stability of eggs rich in n-3 PUFA; however, it is effective at the levels used in this trial in reducing TBARS values of table eggs.

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REFERENCES