Influence of low cholesterol eggs enriched with vitamin–E and omega-3 fatty acid on blood lipid profile of wistar rats

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In the recent past, low cholesterol eggs enriched with vitamin-E and omega-3 fatty acid have been developed and are marketed under different brands claiming them as heart friendly. The influence of these eggs (smart eggs) on lipid profile of rats was evaluated in comparison to that of the standard eggs. Data of 4 week dietary treatment revealed that total plasma cholesterol, low density lipoprotein (LDL) and very low density lipoprotein (VLDL) cholesterol increased only 22% in rats fed on diet containing 4 smart eggs per kg of semi-synthetic diet in contrast to the increase of more than 100 % when fed on diet containing standard eggs. The results suggest that it is not the low cholesterol content alone but also vitamin E and omega-3 fatty acids present in smart eggs that act synergically to prevent a substantial change in blood lipid profile and impose no serious risk to the health of the consumers.

Keywords: Blood, Lipid profile, Low cholesterol eggs, Omega-3 fatty acids, Vitamin-E

Poultry egg is a highly nutritious food product as it contains high quality proteins containing all amino acids, polyunsaturated fatty acids, phospholipids, almost all vitamins (except vitamin –C) and all essential minerals. Inspite of its health benefits, there is an increasing reluctance to consume eggs all over the world due to high cholesterol content present in its yolk, as there are increasing evidences of linkage between cholesterol concentration and coronary heart diseases.

In recent past, low cholesterol eggs enriched with vitamin-E and omega-3 fatty acids have been developed and are marketed under different brand names [Diet eggs, Kool Kombestibles Pvt. Ltd., Bangalore, India; Organoeggs, Tegma Agrotech Ltd., Panchkula, India; Smart eggs, Jaya Health care, Panchkula, India] claiming them as heart friendly as the inclusion of vitamin –E and omega–3 fatty acids in the diet have been reported to increase high density lipoprotein (HDL) cholesterol and decrease low density lipoprotein (LDL) cholesterol. However no conclusive study has been undertaken under controlled conditions in support of this claim from vitamin E and omega-3 fatty acids enriched eggs.

Therefore, the present communication reports the influence of the low-cholesterol eggs enriched with vitamin-E and omega-3 fatty acids sold under brand name “smart eggs” on the lipid profile of rats.

Materials and Methods

Analysis of eggs— Smart eggs and standard eggs were procured locally. Eggs of identical weight (50g) were separated and analyzed for their cholesterol, triglycerides, total lipids, and vitamin E and omega-3 fatty acid (Table 1).

Lipids were extracted from homogenates of eggs by the method of Folch et al., using chloroform and methanol (2:1) as the solvent. Cholesterol, triglycerides, and total lipids were estimated from the lipid extract. Vitamin E was extracted using methanol, ascorbic acid in ethanol and petroleum ether as solvent and was estimated according to the method of Taylor et al. as detailed by Barker. Omega –3 fatty acid (linolenic acid) was estimated according to the method of Binjal and Wadhwa using gas liquid chromatography operated at sensitivity of 1000 with column temperature of 150°C for 15 min and 225°C for 12 min.

Preparation of diet for rats —Semi-synthetic basal diet for the rats was prepared following Orgebin crist et al. It contained (g/100 g of diet): casein, 30; agar,
2.00; corn oil, 5; cellulose, 8; sucrose, 51; vitamin mixture**, 0.5; mineral mixture*, 3.5.

The basal diet was further divided into 3 parts and modified as: control diet consisting of basal diet only (Group I), test-A diet containing 4 homogenized standard eggs (group II) and test-B diet containing 4 homogenized smart eggs per kg of basal diet (Group III). For each diet, the minerals and water-soluble vitamins were ground in sucrose and fat-soluble vitamins were dissolved in corn oil. Agar, which served as a binder was dissolved in 25 ml of triple distilled deionized warm water (60°C). On cooling to 40°C, the contents of each diet were thoroughly mixed in separate containers. The dough so formed was put in petridishes and solidified in refrigerator. The solidified diet was cut into small pieces of 2 x 2 x 2 cm size and stored in the container at the temperature > - 4°C.

Feeding of rats — Male wistar rats (18), 2-3 months old and weighing 175-178 g were procured from central Animal House of Panjab University, Chandigarh.

They were maintained in plastic cages with stainless steel grill at room temperature (20°-30°C) with 14:10 hr L:D cycle and 70-80% RH. They were fed on standard pellet rat feed for one week to acclimatize. Thereafter, rats were equally divided into 3 groups in such a way that their mean body weight remained similar in each group.

Group-I, which served as control, was fed ad libitum on control diet while those of group II and group III were fed on test-A diet and test-B diets respectively for a period of 30 days. After the dietary treatment, rats were sacrificed using diethyl ether as anaesthesia and blood was collected by puncturing the libitum group III were fed on test-A diet and test-B diets respectively for a period of 30

Results and Discussion

The results revealed that the gain in body weight was displayed by all the 3 groups but it was maximum in group II followed by group III and group I (Table 2). The difference in gain in body weight was significantly more in group II than that of group I (P<0.001). The gain was though more in group III than the control group but was significantly less than those of group II rats. Higher gain in body weight in group II and group III than that of group I can be attributed to the addition of eggs in their diet which made their diet energy dense relative to group I and the lesser gain in group III than that of group II to the omega-3 fatty acids whose concentration in smart eggs have been estimated 100% more than that in standard eggs (Table 1). Omega-3 fatty acids are known to increase the rate of oxidative metabolism of fats. This has been supported by the triglycerides fraction in the blood which was estimated 75% less in blood plasma of group III than that of group II.

Further, the cholesterol level in plasma increased by 22% in rats of group III in contrast to its increase by 122% in group II than those of the control rats (Table 3). Certainly this observed difference was due to the addition of eggs in their diet which made their diet energy dense relative to group I and the lesser gain in group III than that of group II to the omega-3 fatty acids whose concentration in smart eggs have been estimated 100% more than that in standard eggs (Table 1). Omega-3 fatty acids are known to increase the rate of oxidative metabolism of fats. This has been supported by the triglycerides fraction in the blood which was estimated 75% less in blood plasma of group III than that of group II.

Table 1 — Cholesterol, triglycerides, total lipids, vitamin-E and omega-3 fatty acids in smart and standard eggs (50 g)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Standard eggs</th>
<th>Smart eggs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol *</td>
<td>211.080 ± 2.870</td>
<td>126.165 ± 2.78 &lt;0.001</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>4.078±0.040</td>
<td>3.026±0.040 &lt;0.001</td>
</tr>
<tr>
<td>Total lipids</td>
<td>7.006 ± 0.090</td>
<td>5.975 ± 0.060 &lt;0.001</td>
</tr>
<tr>
<td>Vitamin-E *</td>
<td>0.836 ± 0.029</td>
<td>3.156 ± 0.082 &lt;0.001</td>
</tr>
<tr>
<td>Omega-3 fatty acids*</td>
<td>36.021 ± 3.018</td>
<td>73.840 ± 2.570 &lt;0.001</td>
</tr>
</tbody>
</table>

Units: *mg/egg; **/g egg

P values: *<0.001; **<0.01
Table 2—Body weight of rats fed on basal diet [control (Group I)], basal diet mixed with standard eggs [Group II] and basal diet mixed with smart eggs [Group III]

<table>
<thead>
<tr>
<th>Duration of Treatment</th>
<th>Group I (control)</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight</td>
<td>177.33 ± 0.398</td>
<td>176.455 ± 0.240</td>
<td>178.628 ± 0.508</td>
</tr>
<tr>
<td>Weight on day 8</td>
<td>182.333 ± 0.50</td>
<td>188.333 ± 0.667</td>
<td>185.667 ± 0.578</td>
</tr>
<tr>
<td>Weight on day 15</td>
<td>193.33 ± 1.00</td>
<td>205.167 ± 1.966</td>
<td>198.50 ± 1.571</td>
</tr>
<tr>
<td>Weight on day 22</td>
<td>205.833 ± 1.183</td>
<td>224.167 ± 1.166</td>
<td>213.33 ± 1.00</td>
</tr>
<tr>
<td>Weight on day 30</td>
<td>215.833 ± 0.555</td>
<td>241.833 ± 0.667</td>
<td>227.667 ± 0.800</td>
</tr>
</tbody>
</table>

(21.7) (37.02) (27.4)

Units: g

P values: * > 0.05; ** < 0.01; *** < 0.001 (values of Group-II and Group-III were compared with Group-I)

Table 3—Lipid profile in blood plasma of rats of Group I (control), Group II (fed with standard eggs in diet) and Group III (fed with smart eggs in diet)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I (Control)</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Lipids</td>
<td>189.102 ± 3.205</td>
<td>282.05 ± 4.054</td>
<td>208.332 ± 3.206</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>43.333 ± 2.108</td>
<td>96.660 ± 2.108</td>
<td>55.000 ± 2.236</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>48.887 ± 2.810</td>
<td>108.886 ± 5.352</td>
<td>59.995 ± 2.909</td>
</tr>
<tr>
<td>HDL</td>
<td>11.905 ± 1.984</td>
<td>8.332 ± 0.533</td>
<td>14.286 ± 1.065</td>
</tr>
<tr>
<td>LDL</td>
<td>28.315 ± 2.624</td>
<td>81.613 ± 5.425</td>
<td>34.705 ± 3.598</td>
</tr>
<tr>
<td>VLDL</td>
<td>8.667 ± 0.422</td>
<td>19.333 ± 0.422</td>
<td>11.000 ± 0.447</td>
</tr>
</tbody>
</table>

Units: mg/dl of blood plasma.

P values: * > 0.05; ** < 0.01; *** < 0.001 (values of Group-II and Group-III were compared with Group-I)

The analysis of eggs showed that there was approximately 40% difference in cholesterol level in two types of eggs but its difference in plasma of rats was almost 100%. This implies that it was not the difference in amount of cholesterol in diet alone but also the additional dietary factors that didn’t permit cholesterol level to rise proportionately to its level in diet. In recent past, vitamin -E and omega-3 fatty acids or their derivatives have been reported to lower the cholesterol content in serum along with rise in HDL cholesterol and fall in LDL cholesterol in hypercholesterolemic patients 23-28. Their presence in smart eggs in present study support observation of previous investigators since reduction of cholesterol, LDL and VLDL cholesterol concentrations between...
smart eggs and standard eggs were approximately 45% or above and almost equal increase in plasma HDL cholesterol (41%) in the former than the latter group of rats.

In spite of lesser amount of vitamin-E in smart eggs than that of vitamin-E enriched in diet of rats in such studies made previously, it could result in significant reduction in cholesterol, LDL and VLDL cholesterol and increase in HDL cholesterol. This may be possible due to two reasons. Firstly vitamin-E in smart eggs was in conjugate form that has greater rate of oxidation and more effective than the synthetic form of vitamin-E. Secondly, the role of omega-3 fatty acids in the smart eggs increases the rate of oxidation of fatty acids, which further supplement the role of Vitamin-E in lowering the lipid fraction per se.

Therefore, results of this study suggest that smart eggs are health friendly because they possess low cholesterol enriched with vitamin-E and omega-3 fatty acids. Since these eggs are easily available, their regular inclusion in diet is unlikely to impose a serious risk to the increase of serum cholesterol, LDL, VLDL cholesterol fractions in consumers.

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