

Biological Evaluation of Normal and Low Cholesterol Eggs in Feeding Rats

Samah, M. Ismael¹, Ashoush, I. S.², Abdallah, M.A.² & Massri, M. M.³

¹ Home Economic Dept., Fac. of Specific Edu., Ain Shams Univ., Cairo, Egypt.

² Food Science Dept., Fac. of Agric., Ain Shams Univ., Cairo, Egypt.

³ Food Science Dept., Fac. of Agric., Al-Baath Univ., Homs, Syria.

ABSTRACT

The present investigation was performed by using albino male rats to study the effect of daily ingestion of normal and low cholesterol eggs at two levels (2 and 5%) for 28 successive days on some biological parameters to shed a light on what may happen in human nutrition. The effects of the investigated eggs on the lipid profile parameters as indicated from blood analysis of treated rats were examined throughout the scope of the study. The adverse effects of the dietary eggs on the internal organs and body weight gain were also considered. Moreover, fatty acid composition and total cholesterol in the tested Eggs were determined.

The results indicated that addition of normal eggs at the two levels under study (2 and 5%) caused significant increase in total lipid after 10 days while significant elevation was observed in triglycerides level after 30 days from feeding on normal eggs (2%), while significant decrease was occurred after 30 days from feeding on low cholesterol eggs (5%). In addition, there was significant increase in total cholesterol after 10 days from feeding on normal eggs (5%) and the same trend in LDL-cholesterol was found throughout the experimental period. Meanwhile, there was significant increase in HDL-cholesterol at the end of the experiment at 2% of low cholesterol diet. No significant difference in the relative weight of the internal organs such as (lung, heart and testes). On the other hand, a real increment was noticed in the body weight of the rats fed with the two types of egg at level 5% at the end of the experiment. Data also exhibited significant decrease in the activity of alanine aminotransferases (ALT) at the two levels (2 and 5%) of the normal and the low cholesterol (2%) eggs while, the activity of aspartate aminotransferase (AST) increased after 10, 20 days at 5% of normal eggs diet. In addition, after 10 days, the dietary eggs showed significant increase in liver and spleen weight in treatment 5% for the normal and the low cholesterol eggs as compared to the control group. Also, the chemical composition of the two types of egg showed significant decrease in fatty acid composition and total cholesterol in low cholesterol eggs as compared to normal egg.

Keywords: egg, cholesterol, biochemical analysis, bioassay.

INTRODUCTION

It is advised by some authors to restrict the egg consumption in efforts to lower blood cholesterol and mitigate risk of heart disease. On the contrary, there has been little evidence that egg consumption is directly related to cardiovascular risk. Epidemiological studies have indicated that consumption of one egg per day is unlikely to have any substantial effect on cardiovascular disease risk in healthy subjects. Moreover, there has been a lack of consistent literature to support the notion that regular or near-regular egg ingestion leads to substantial elevation in serum lipids and total cholesterol levels. Because there are many serum moieties used to gauge cardiac risk, a physiologic measure that captures their aggregate influence is desirable (Song & Kerver, 2000).

David *et al.* (2005) demonstrated that randomized controlled crossover trial was needed to determine the effect of egg ingestion on endothelial function, a reliable index of cardiovascular risk. In such a case, forty-nine healthy adults (mean age 56 years, 40% females) underwent a baseline brachial artery reactivity study (BARS), and were fed on two eggs or oats daily for 6 weeks in random sequence with a 4-week washout. The BARS was done at the end of each treatment phase, measuring flow-mediated vasodilation (FMD) in the brachial artery using a high-frequency ultrasound technique. The data indicate that FMD was stable in both egg and oat groups, and differences between-treatments were not significant (egg 0.96%, oatmeal 0.79%, *p* value > 0.05). Six weeks of egg ingestion had no effect on total cholesterol (baseline: 203.8, post-treatment: 205.3mg/dl) or LDL (baseline: 124.8,

post-treatment: 129.1 mg/dl). In contrast, 6 weeks of oats lowered total cholesterol to 194 mg/dl, ($p = 0.0017$) and LDL to 116.6 mg/dl, ($p = 0.012$). There were no differences in body mass index (BMI), triglyceride, HDL levels between egg and oat treatment assignments.

For 3 weeks, 8 male Wistar rats of initial body weight 220 to 240 g were fed on stock diet with 16% crude protein or on cooked whole eggs. Final total lipid content of the liver was 3 times as high for rats given eggs as compared to control which were given stock diet. Incorporation of amino acids labelled with C14 into serum low-density lipoprotein apoproteins was 52% higher with egg than for controls. There was no effect on the incorporation into serum albumin or liver total protein. Turnover of low-density lipoprotein in rats fed on egg was very high (Nadkarni, 1984).

The total lipid, cholesterol, phospholipid, and fatty acid content of dried whole egg (DWE), a refrigerated liquid egg substitute, a powdered egg substitute, and a commercial egg yolk replacer (EYR) on rats were compared by Childs & Ostrander, (1976). All substitutes contained less total lipid, cholesterol, and phospholipid than DWE. The powdered substitute contained only half the total lipid content reported by the producers and only a fifth of the cholesterol of DWE, while the percentage distribution of fatty acids was similar to that of DWE. Rat growth and lipid response were compared for diets containing either DWE or a mixture of EYR and eggs white. Both diets were fed with and without vitamin and mineral supplements. Rat growth response was greater on the DWE diets, either with or without the supplements, was intermediate on the supplemented EYR diets, and was at the least on the unsupplemented EYR diets.

The consumption of DWE-containing diets, when compared with the EYR-containing diets, caused greater liver weight, greater liver total lipid and total cholesterol content, and slightly higher serum cholesterol.

MATERIALS AND METHODS

Materials

Low Cholesterol Egg were obtained from the Hashem Brothers farm (Kafr El-Sohby – Shibein El-Kanater – Kalyoubeya, Egypt). Eggs of normal level of cholesterol were purchased from local market, Cairo governorate, Egypt.

Methods

Preparation of samples:

The two types of egg were blanched for 10 min. in boiling water (100°C) and prepared to be dried at 40-50°C then grinded and kept in glass bottle for biological evaluation.

1- Animal Diets:

Twenty five albino rats (Wister strain), with an initial body weight of 130 g were housed in screen-bottomed aluminum cages in rooms maintained at $25 \pm 1^\circ\text{C}$ with alternating cycles of light and dark of 12hr duration. Rats were randomly allocated into five main groups each of which contains five animals with a mark on their tails as a mean of differentiation. The animals were fed experimental diets (Table 1) and the changes in body weight were recorded weekly. Blood samples were also obtained from the retro-orbital plexus of the eyes from all animals of each group on 10, 20 and 30 days, after which the rats were slaughtered at the end of the experiment for organs weight.

Table 1: Composition of the diets (g /kg⁻¹) fed to the investigated male albino rats

Ingredients	Control	Normal Eggs		Low Cholesterol Eggs	
		2%	5%	2%	5%
Corn starch	700	650	600	650	600
Casein	100	100	100	100	100
Corn Oil	100	100	100	100	100
Mineral mixture	40	40	40	40	40
Vitamin mixture	10	10	10	10	10
Cellulose	50	50	50	50	50
Egg	----	50	100	50	100

2- Animal experimentals

Blood samples were taken for the following biochemical analysis:

Liver function tests: The activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined colorimetrically according to the method adopted by Reitman & Frankel (1957), using special Biodiagnostic, (Egypt) Kits.

Lipid profiles analysis:

Total cholesterol: Enzymatic determination of cholesterol was carried out according to Allain, (1974) using kits obtained from Biodiagnostic, (Egypt).

Total triglycerides: Fully enzymatic determination of total triglycerides in plasma was measured colorimetrically at 546 nm. according to the method of Fossati & Principe (1982) using kits obtained from Biodiagnostic, (Egypt).

Total lipids: Plasma lipids were measured at 525 nm. according to the method of Schmidt (1964), using kits obtained from Biodiagnostic, (Egypt).

Determination of the low density lipoprotein (LDL): It was precipitated from the tested samples with polyvinyl sulphate. The concentration was calculated from the difference between serum total cholesterol and the cholesterol in the supernatant after centrifugation. The cholesterol was measured according to the method of Assmann, *et al.* (1984) using kits obtained from Biosystem, (Spain).

Determination of the very low density lipoprotein (VLDL): It was precipitated from the tested samples with phosphotungstate and magnesium ions. The residual supernatant was used for the analysis of high density lipoprotein (HDL). The HDL was measured according to the method of Burstein, *et al.* (1980) using kits obtained from Biosystem, (Spain).

Chemical analysis:

Fatty acids

Fatty acid methyl esters (FAME) of the egg samples under investigation were prepared after saponification using the method of the AOAC, (2000). The gas chromatography (G.C) Varian GC-14-B Shimatzo-Japan was applied with a column package of Diethylene Glycol Succinate DEGS) 2.1 m × 3.2mm. Program temperature for identification, starting with 80°C for 2 min. then was elevated to 180°C by rate of 5°C/min. then isothermal for 30 min. Fatty acids (%) were calculated in relative to others from the equation of Gehrke *et al.*, (1968).

Total Cholesterol

Total cholesterol was determined using Gas Chromatography (GC) Varian Sigma 3B Perkin-Elmer, at an oven temp., of 290 °C while 310 °C was used for each of detector and injection aspects.

Statistical analysis

All data were subjected to statistical analysis according to the procedure reported by Snedecor & Cochran, (1980) and the statistical analysis system program (SAS, 1996) using student's t-test and factorial analysis.

RESULTS AND DISCUSSION

Effect of treatment with the investigated eggs on the body weight and internal organs weights of male albino rats

The different investigated groups of rats were fed on the tested diets including two levels of the investigated eggs to look forward towards the generation efficiency of blood lipids. Subsequently, body weight gain, of the different groups of rats during the whole period of experiment was estimated as presented in Table (2). The obtained body weight

Table 2: Body weight gain of male albino rats as a result of treatment with different concentration of the eggs

Tested Diets	Level % out of the diet	Body weight gain (g) at the indicated post-treatment (weeks)		
		1	2	3
Control		3.000 ± 0.764	0.833 ± 0.167	0.567 ± 0.089
Normal Eggs	2	1.067 ± 0.296	3.833 ± 0.167**	1.733 ± 0.593
	5	1.967 ± 0.318	4.300 ± 0.079**	5.366 ± 0.095*
Low Cholesterol Eggs	2	1.033 ± 0.437	2.467 ± 0.328*	2.600 ± 0.071*
	5	2.000 ± 0.472	2.067 ± 0.677	5.666 ± 0.745***

* Statistical significant differences (P<0.05) ** Statistical significant differences (P<0.01) *** Statistical significant differences (P<0.001)

of rats fed on basal diet indicated a decrement pattern as compared to that of rats fed on the two type of eggs especially at the level 5% out of the diet and by the end of experimental period. Such results agree with those obtained by David, *et al.* (2005) who mentioned that, six weeks of eggs ingestion had no effect on body mass index (BMI).

Statistical analysis of the data in Table (3) did not reveal significant differences in the relative weight of the considered internal organs of rats with some exception in liver and spleen weight which showed significant increase in treatment 5% for the normal and the low cholesterol eggs as compared to the control group. Such results are in harmony with those obtained by Childs & Ostrander, (1976).

Biochemical analysis of the blood plasma of male albino rats:

Liver function parameters:

Data recorded in Table (4) indicated that the normal eggs added to the diet at level 5% caused significant increase in the activity of aspartate aminotransferase (AST) after 10 and 20 days of starting the experiment and returned to the normal range after 30 days. On the other hand, the normal eggs at levels 2, 5% and low cholesterol eggs at level 2% caused significant decrease in the activity of alanine aminotransferases (ALT) after 10 days of starting the experiment and returned to the normal range at the end of the experiment as compared to control. These findings are on the contrary with those obtained by Nadkarni (1984) and Aguila, *et al.* (2003) who found that, final total

Table 3: Weight of selected internal organs of male albino rats after treatment with different concentration of the eggs

Tested Organs	Control	Organs weight (g/100g body weight) after 30 days of feeding			
		Normal		Low Cholesterol	
		2%	5%	2%	5%
Liver	2.900±0.000	3.100±0.153	4.367±0.569*	2.933±0.233	4.033±0.033***
Kidney	0.700±0.000	0.833±0.088	0.933±0.033*	0.800±0.058	0.900±0.057
Lung	0.967±0.033	1.033±0.120	1.200±0.058	1.000±0.000	1.000±0.100
Spleen	0.333±0.033	0.400±0.000	0.666±0.033*	0.367±0.033	0.567±0.033*
Heart	0.367±0.033	0.400±0.00	0.367±0.567	0.433±0.033	0.533±0.033
Testes	1.967±0.067	2.167±0.233	2.500±0.305	2.000±0.058	2.000±0.208

* Statistical significant differences (P < 0.05)

*** Statistical significant differences (P < 0.001)

Table 4: Activity level of AST and ALT in male albino rats

Treatments	Level % out of the diet	Activity of AST (U/L) at the indicated post-treatment (days)		
		10	20	30
Control		13.997 ± 3.459	18.042 ± 0.879	23.997 ± 0.668
Normal eggs	2	21.116 ± 2.0511	19.984 ± 8.414	18.074 ± 1.975
	5	30.178 ± 1.100*	37.945 ± 1.044***	23.382 ± 2.049
Low Cholesterol eggs	2	22.896 ± 0.701	25.485 ± 2.546	25.942 ± 1.023
	5	24.353 ± 2.917	26.780 ± 2.087	23.867 ± 1.714
Activity of ALT (U/L) at the indicated post-treatment (days)				
Control		24.112 ± 0.410	14.975 ± 1.465	16.531 ± 1.735
Normal eggs	2	8.376 ± 2.637*	12.112 ± 1.034	19.053 ± 2.782
	5	14.721 ± 1.319*	16.497 ± 8.206	21.218 ± 2.931
Low Cholesterol eggs	2	14.636 ± 1.391*	25.381 ± 7.180	27.025 ± 2.879
	5	19.137 ± 0.732	8.376 ± 1.758	16.832 ± 1.465

* Statistical significant differences (P < 0.05)

*** Statistical significant differences (P < 0.005)

lipid content of the liver was 3 times as high, for rats given egg as for controls given stock diet.

Parameters of lipid profiles

Data recorded in Table (5) show significant increase after 10 days from feeding normal egg 2, 5% stability in the total lipid in both normal and low cholesterol egg groups at the end of thirty days of eggs feeding. The results of plasma triglyceride levels (mg/dl) are shown in the same Table. It could be no-

ticed that, the total serum triglycerides after 30 days was significantly ($P < 0.05$) the highest in animals fed on the normal egg 2% diet as compared to animals fed on the control diet. While, a significant decrease was observed in the triglyceride level after 30 days in low cholesterol eggs group 5%. These findings are on the contrary with those obtained by Jiang & Sim (1991) and David, *et al.* (2005).

From data recorded in Table (6), the treatment with the two types of egg do not affect the plasma

Table 5: Effect of different concentration of the investigated eggs on total lipid and triglycerides in the tested male albino rats

Treatments	Level % out of the diet	Total Lipid (g/L) level at the indicated (days)		
		10	20	30
Control		0.596 $\pm$ 0.303	2.474 $\pm$ 0.421	1.552 $\pm$ 0.684
Normal eggs	2	2.649 $\pm$ 0.238*	1.609 $\pm$ 0.150	1.965 $\pm$ 0.507
	5	2.868 $\pm$ 0.259*	1.693 $\pm$ 0.727	2.052 $\pm$ 0.638
Low Cholesterol eggs	2	1.974 $\pm$ 0.836	1.605 $\pm$ 0.053	2.052 $\pm$ 0.596
	5	1.228 $\pm$ 0.408	2.079 $\pm$ 0.615	1.719 $\pm$ 0.735
Triglycerides (mg/dl) level at the indicated (days)				
Control		123.768 $\pm$ 16.823	95.811 $\pm$ 22.578	108.551 $\pm$ 6.245
Normal eggs	2	118.551 $\pm$ 4.355	133.333 $\pm$ 3.92	137.10 $\pm$ 6.540*
	5	111.884 $\pm$ 30.386	103.043 $\pm$ 5.69	98.971 $\pm$ 6.76
Low Cholesterol eggs	2	103.188 $\pm$ 16.36	92.464 $\pm$ 2.188	95.913 $\pm$ 8.58
	5	101.594 $\pm$ 7.148	96.652 $\pm$ 3.525	80.391 $\pm$ 1.240*

Table 6: Change in the concentration of total cholesterol, LDL and HDL levels in rats

Treatments	Level % out of the diet	T. Cholesterol (mg/dl) level at the indicated (days)		
		10	20	30
Control		60.000 $\pm$ 8.696	109.855 $\pm$ 24.615	92.319 $\pm$ 3.292
Normal eggs	2	59.710 $\pm$ 5.883	070.145 $\pm$ 0.618	80.290 $\pm$ 8.276
	5	91.884 $\pm$ 8.254*	108.406 $\pm$ 2.395	94.637 $\pm$ 3.053
Low Cholesterol eggs	2	48.116 $\pm$ 5.903	067.536 $\pm$ 2.188	77.971 $\pm$ 9.618
	5	76.521 $\pm$ 5.645	062.898 $\pm$ 9.298	65.217 $\pm$ 12.382
LDL (mg/dl) level at the indicated (days)				
Control		80.520 $\pm$ 1.416	70.187 $\pm$ 1.606	56.018 $\pm$ 2.560
Normal eggs	2	63.364 $\pm$ 4.166	72.277 $\pm$ 13.207	63.623 $\pm$ 6.455
	5	124.588 $\pm$ 1.956**	122.124 $\pm$ 7.727**	107.830 $\pm$ 3.087**
Low Cholesterol eggs	2	54.528 $\pm$ 11.609	52.839 $\pm$ 9.651	51.352 $\pm$ 2.616
	5	76.122 $\pm$ 5.741	71.271 $\pm$ 3.151	57.879 $\pm$ 7.667
HDL (mg/dl) level at the indicated (days)				
Control		14.512 $\pm$ 2.509	17.685 $\pm$ 3.561	17.156 $\pm$ 4.179
Normal eggs	2	16.097 $\pm$ 1.090	19.431 $\pm$ 1.991	20.738 $\pm$ 6.057
	5	17.656 $\pm$ 6.037	19.928 $\pm$ 7.026	17.718 $\pm$ 3.114
Low Cholesterol eggs	2	20.582 $\pm$ 0.561	22.083 $\pm$ 2.859	26.281 $\pm$ 4.807*
	5	19.991 $\pm$ 2.487	20.293 $\pm$ 2.507	21.237 $\pm$ 5.259

* Statistical significant differences ($P < 0.05$)

** Statistical significant differences ($P < 0.01$)

total cholesterol concentration except there was significant increase after 10 days from feeding on normal egg at level 5% then returned to the normal range at the end of the experiment. On the contrary, the animals fed on normal egg at level 5% exhibited significant increase in LDL-cholesterol levels throughout the experimental course. Meanwhile, it could be observed that HDL-cholesterol levels did not change during the experimental period except there was a significant elevation in HDL-cholesterol in animals fed on low cholesterol eggs 2% after 30 days. The obtained data agreed with Taneja & Rakha, (2005), they reported that low cholesterol eggs enriched with vitamin-E and omega-3 fatty acids have been developed and are marketed under different brands claiming them as heart friendly or smart eggs. 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.

Fatty acid composition in the normal and low cholesterol eggs

Saturated fatty acids having 14 or more carbon atoms are solid at room temperature and the most common saturated fatty acids in the foods are lauric, myristic, palmitic, and stearic with 12, 14, 16 and 18 carbons, respectively. Stearic acid (C18) is found almost in all naturally occurring animal, marine and vegetable oils (Montano *et al.*, 2001)

Fig (1) shows the identified fatty acids in normal and low cholesterol egg samples. The concentration of C18:1 acid in the normal egg sample came in the first order (47.845 %) followed by that of low cholesterol egg 43.937 %. The level of C16:0 fatty acid in the same sources was 26.638% in low cholesterol egg and 24.807% in normal egg. When a comparison was carried out between normal egg and low cholesterol egg it was found that the identified fatty acids were characterized by their higher contents of C16:0, C16:1, C18:0, C18:2, C18:3 and C14:0, in low cholesterol egg as seen in the same Fig, while the C18:1 was higher in the normal eggs.

Total Cholesterol

The egg industry continues to suffer from decreased consumption because of the cholesterol problem. Eggs are one of nature's most complete

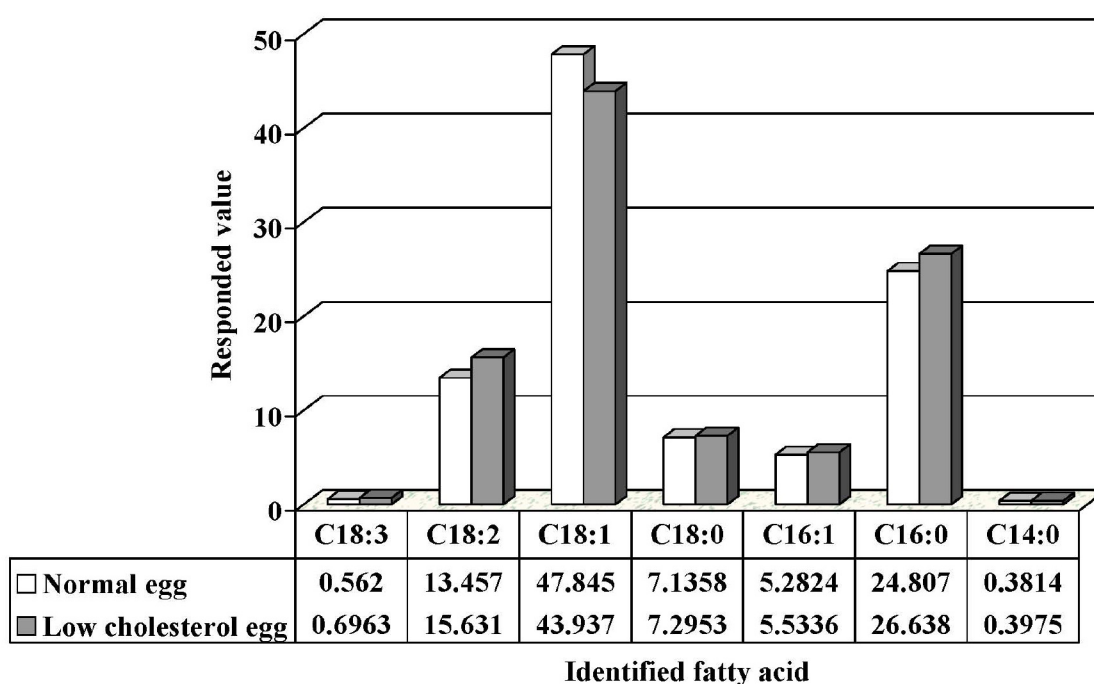


Fig. 1: Fatty acid composition in the tested egg

foods, containing high quality protein, vitamins and minerals. Nevertheless, health groups continue to emphasize the high cholesterol content of eggs.

If egg consumption trends are to be reversed, the egg industry should continue to pursue ways of lowering cholesterol in egg products. Numerous attempts have been made to lower yolk cholesterol through the hen's diet or genetic selection (Froning *et al.*, (1990).

Total cholesterol of the normal and low cholesterol egg samples determined by GC in the lipid extract indicated that, the total cholesterol of the normal cholesterol eggs was separated at distinguish retention time of 11.23 minutes (4394.82 mg chol/100 g lipid), were higher than in the low cholesterol eggs which appeared after 11.04 min on the chromatogram (4187.72 mg chol/ 100 g lipid) as seen in the Fig (2).

The relatively small differences could be attributed to the fact that cholesterol is naturally found in the eggs and in all the living animal cells and plays a vital role in the living cell.

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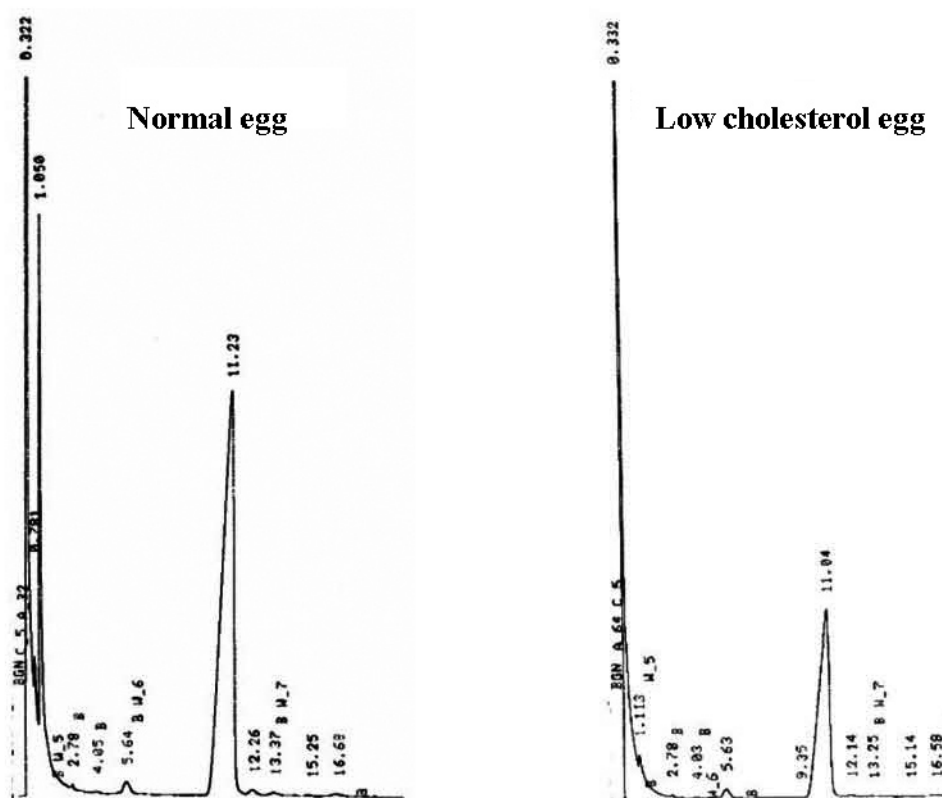


Fig. 2: Auto scaled chromatogram of total cholesterol of the normal and low cholesterol eggs

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التقييم البيولوجي للبيض العادي ومنخفض الكوليستيرول في فئران التجارب

سماح محمد إسماعيل^١، إيهاب صلاح عشوش^٢، محمد أمين عبد الله^٣، محمد محمود مصرى^٣

^١ قسم الإقتصاد المنزلي، كلية التربية النوعية، جامعة عين شمس، القاهرة، مصر.

^٢ قسم علوم الأغذية، كلية الزراعة، جامعة عين شمس، شبرا الخيمة، القاهرة، مصر.

^٣ قسم علوم الأغذية، كلية الزراعة، جامعة البعث، حمص، سوريا.

استهدف هذا البحث دراسة تأثير المتناول اليومي من البيض العادي ومنخفض الكوليستيرول بمستويين ٢، ٥٪ يوميا لمدة ٢٨ يوماً على ذكور الفئران البيضاء البالغة، تم وزن الفئران أسبوعياً وأخذ عينات الدم من الحيوانات بعد ١٠، ٢٠، ٣٠ يوم من المعاملة. وقد تم دراسة جوانب ومعايير الأيض متمثلة في صورة لبيدات الدم، وظائف الكبد. كذلك تم دراسة التغيرات في زيادة وزن الجسم الحي وبعض الأعضاء الداخلية. وبالإضافة إلى ذلك فقد تم دراسة تركيب البيض المختبر من أحماض دهنية وعدد الروابط الزوجية والكوليستيرول الكلي.

ولقد أظهرت النتائج المتحصل عليها أن المعاملة بالبيض بنوعيه بالمستويين موضع الدراسة أحدثت زيادة معنوية في اللبيدات الكلية بعد عشرة أيام بينما لوحظ زيادة في جلسريدات البلازما عند مستوى ٢٪ من التغذية على البيض العادي بعد ثلاثين يوماً وعلى العكس لوحظ إنخفاضها بعد نفس المدة من التغذية على البيض منخفض الكوليستيرول عند مستوى ٥٪. أيضاً فقد حدثت زيادة في مستوى الكوليستيرول الكلي عند التغذية على ٥٪ من البيض العادي. بالإضافة إلى ذلك حدثت زيادة معنوية في الكوليستيرول منخفض الكثافة أثناء فترة التجربة عند التغذية على ٥٪ بيض عادي بينما أظهرت النتائج حدوث زيادة معنوية في الكوليستيرول عالي الكثافة بنهاية التجربة عند مستوى ٢٪ من التغذية على البيض منخفض الكوليستيرول في حين لم يظهر أى تأثير معنوي في وزن أعضاء الرئة والقلب والخصية. ومن ناحية أخرى أحدثت المعاملة بالبيض العادي والمنخفض في الكوليستيرول عند مستوى ٥٪ من المعاملة زيادة معنوية في وزن الجسم بنهاية التجربة مقارنة بالمجموعة الضابطة. كما وجدت زيادة معنوية في وزن كل من الكبد والطحال عند مستوى ٥٪ من المعاملة كما حدث إنخفاض معنوي في نشاط الإنزيم الناقل للألمين (ALT) بينما حدثت زيادة في نشاط الإنزيم الناقل للألمين (AST). وأظهرت نتائج التحليل الكيماوي لنوعي البيض تحت الدراسة أن البيض منخفض الكوليستيرول أظهر إنخفاضاً معنوياً في محتوى الكوليستيرول الكلي والأحماض الدهنية مقارنة بالبيض العادي.