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Evaluation of the effects of egg yolk on serum cholesterol level and liver function in rats

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ABSTRACT

Egg yolk (EY) is a major source of triglycerides, phospholipids, cholesterol, fatty acids and vitamins among food products. The aim of this study was to investigate the effect of egg yolk on lipid profile, liver function and atherosclerosis in rats. The experiment was carried out using forty male albino rats (Sprague-Dawley strain). The rats were divided into two main groups at random: the first main normal rats (20) were divided into: Subgroup (1) as a negative control group, rats were fed the basal diet (-Ve). Rats in subgroups 2–4 were fed a basal diet supplemented with (3 %, 5 % and 10 % of egg yolk from the basal diet respectively). The second atherosclerotic rats; groups (n=20) were induced with atherosclerosis using an atherogenic diet and fed orally with high levels of vitamin D for five days. Following this period, groups (n= 20 rats) were divided into four groups, each with five rats. Group (5) as a positive control group (+Ve), Rats were fed an atherogenic diet. Atherosclerotic rats (6, 7, and 8) were fed an atherogenic diet supplemented with (3 %, 5 % and 10 % of egg yolk from the atherogenic diet respectively). The experimental period was four weeks, Blood samples were collected. At the end of the experiment, the results showed that using (5% and 10%) levels of EY in atherosclerosis and normal rats increased the mean values of total cholesterol, total lipids and triglycerides, AST, ALT, and ALP but the level of 3% from EY doesn't increase that. This study recommends limiting egg yolk consumption to improve the lipid profile, liver function and atherosclerosis.

Keywords: Egg yolk, Atherosclerosis, Rats, Basal diet.

1. Introduction

An egg yolk is part of an egg which serves as the food source for the developing embryo inside. Prior to fertilization the yolk together with the germinal disc is a single cell. The egg yolk is suspended in the egg white (known more formally as albumen or ovalbumin) by one or two spiral bands of tissue called the chalazae (Nys and Sauveur, 2004).

The eggs used for culinary purposes generally come from birds (particularly poultry). Chickens in particular are extensively farmed for their eggs. Duck eggs are also sometimes used and are particularly suitable for use in baking cakes (Nys and Sauveur, 2004).

Because of the amount of cholesterol contained in eggs, a restricted consumption of eggs has been often recommended to reduce plasma cholesterol levels and the risk of cardiovascular disease. An egg contains more than 200 mg of cholesterol, which is the maximum recommended daily amount for patients with dyslipidemia and cardiovascular risk (National Institutes of Health, 2000). However, in addition to cholesterol, eggs contain nutrients such as essential amino acids, folate, riboflavin, selenium, choline, and vitamins B 12, A, D, E and K. Eggs also provide protein of high biological value and a small amount of total fat (Song and Kerver, 2000). Compared with other animal foods, eggs contain proportionally less saturated fat (Seuss-Baum *et al.,* 2011). It is conceivable, therefore, that the small adverse effects due to cholesterol could be counterbalanced by the potential beneficial effects of the other nutrients found in eggs (Hu *et al.,* 2001). Furthermore, evidence suggests that dietary cholesterol has a limited influence on serum cholesterol and cardiovascular risk (Song and Kerver, 2000). Previous studies indicate that the consumption of up to one egg a day is unlikely to have a substantial overall impact on the risk of coronary artery disease (CAD) or stroke among healthy men and women and that

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egg consumption was non-detrimental to endothelial function and serum lipids in hyperlipidemic adults (Njike *et al.,* 2010).

The increase in LDL cholesterol to HDL-C ratio and decreased levels of HDL-C, will lead to the formation of atherosclerosis is increasing cardiovascular risk. But increase in total cholesterol in plasma is still below the maximum threshold (200mg/dl). Similarly, the increase in LDL cholesterol was still in the normal range. There is surprisingly little evidence that egg consumption increases blood cholesterol levels, thereby increasing cardiovascular risk (Harman *et al.,* 2008).

Zhang *et al.* (2021) also showed that Habitual preserved egg consumption is associated with a modestly higher risk of NAFLD among the Chinese adult population. The mechanism underlying this association warrants further research.

The purpose of this study was to investigate into the impact of egg yolk on lipid profile, liver function, and atherosclerosis in rats.

2. Materials and Methods

2.1. Diet content

- Casein, all vitamins, minerals, cellulose and choline bitartrate were obtained from Elgomhoria Pharmaceutical Company, Cairo, Egypt.
- Egg yolks were purchased from the Egyptian local market.

2.2. Induction of atherosclerosis

The first time succeeded in inducing atherosclerosis in rabbits by feeding cholesterol containing diet Development of atherosclerosis in rabbits usually takes at least 60 days of feeding an atherogenic diet. Rat is said to be resistant to such dietary manipulations for the development of atherosclerosis but will supplementation of very high doses of Vitamin-D, along with an atherogenic diet success has been achieved in developing atherosclerosis in rats in a short period of 5 days by fed on atherogenic diet and orally fed on vitamin D3 (3.20.000 IU) in 1.5 ml of olive oil for five days (Chandler *et al*., 1979). The atherogenic diet (AD) consisting 2 g of cholesterol and 8 of saturated fat and 100 mg calcium were added to 90 g of powdered standard basal diet and thoroughly mixed (Altman, 1973).

2.3. Experimental Design

Forty male rats were housed in well aerated cages under hygienic conditions and fed on basal diet for one week for adaptation in animal house in Agriculture Research Center, Cairo, Egypt. The basal diet were formulated according to Reeves *et al.* (1993). The basal diet consisted of protein (14%), fat (5%), salt mixture (3.5%), vitamin mixture (1%), Choline Chloride (0.2%), cellulose (5%), Sucrose (10%) and the remainder were Corn starch. These constituents were thoroughly mixed together. Rats were divided into two main groups, the first normal rats and second atherosclerotic rats. Each group (5 rats).

2.3.1. The first normal rats divided into

Group (1): Rats were fed on the basal diet during the experimental period as a negative control group (-Ve).
Group (2): Rats were fed on basal diet supplemented with 3% of egg yolk from basal diet.
Group (3): Rats were fed on basal diet supplemented with 5% of egg yolk from basal diet.
Group (4): Rats were fed on basal diet supplemented with 10 % of egg yolk from basal diet.

2.3.2. The second atherosclerotic rats

Rats (n=20) were inducted with atherosclerosis by using the method describe by Chandler *et al.* (1979) and Altman, (1973) by feeding rats on atherogenic diet and high doses of vitamin D according to the methods described mention before. After this period blood samples were taken from all rats for measuring total cholesterol level to be sure that all rats have been suffering from Atherosclerosis. Rats (n= 20 rats) were divided into 4 groups, 5 rats each as follows:

Group (5): Atherosclerotic Rats were fed on atherogenic diet as positive control group (+Ve).
Group (6): Atherosclerotic Rats were fed on atherogenic diet supplemented with (3 % of egg yolk from atherogenic diet).

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Group (7): Atherosclerotic Rats were fed on atherogenic diet supplemented with (5 % of egg yolk from atherogenic diet).

Group (8): Atherosclerotic Rats were fed on atherogenic diet supplemented with (10 % of egg yolk from atherogenic diet).

During the experimental period, water and diet will be introduced ad-Lib. under hygienic conditions. At the end of the feeding trial (Four weeks) rats were fasted over night before scarifying and blood was collected, then centrifuged to obtain serum for biochemical analysis.

2.4. Biological parameters

Feed intake (FI) was calculated daily. Body weight gain (BWG) and feed efficiency ratio (FER) were calculated according to Chapman *et al.* (1959).

2.5. Serum analyses

Serum cholesterol was determined according to Richmond, (1973), Triglyceride (Wahlefeld, 1974), Serum total lipids (Albers *et al.,* 1983 and Fridewald *et al.,* 1972), Alanine aminotransferase, Aspartate aminotransferase and Alkaline phosphatase according to Young, (2001).

2.6. Statistical analysis

The results were analyzed using Statistical Package for the Social Sciences (SPSS) for Windows, version 20 (SPSS Inc., Chicago, IL, USA). Collected data were presented as mean± standard deviation (SD). Analysis of Variance (ANOVA) test were used for determining the significances among different groups according to Armitage and Berry, (1987). All differences were considered at level of significant P-values < 0.05.

3. Results and Discussion

Data presented in Table (1) showed the effect of EY with three levels on feed intake (g/day), body weight gain% and feed efficiency ratio in rats. The results in this study revealed that, the atherosclerotic group (positive control group) showed an increase in feed intake (g/day), body weight gain% and feed efficiency ratio, as compared to rats fed on the basal diet (the negative control group). Data in this table revealed that, treating rats suffering from atherosclerosis with two levels (5% and 10%) from EY led to increase, as compared to the positive control group. Normal rats with tree levels (5% and 10%) from EY led to increase, as compared to the positive and negative control group. The group 3% EY is the best group in all treatment groups, because this group showed a non-significant difference, as compared to the negative control group.

Table 1: Effect of egg yolk on body weight, BWG %, FI and FER in rats

Parameters	-ve control	+ve control	3% EY + Atherosclerosis	5% EY + Atherosclerosis	10%EY + Atherosclerosis	3% EY	5% EY	10% EY
BWG	16.66 $\pm 5.13^{d}$	32.96 $\pm 5.6^{b}$	33.69 $\pm 7.44^{b}$	41.89 $\pm 1.66^{a}$	44.19 $\pm 3^{a}$	19.12 $\pm 1.2^{d}$	24.32 $\pm 0.88^{c}$	26.88 $\pm 5.4^{c}$
FER	0.142 $\pm 0.41^{d}$	0.188 $\pm 0.88^{b}$	0.189 $\pm 1.078^{b}$	0.213 $\pm 0.22^{a}$	0.22 $\pm 0.36^{a}$	0.143 $\pm 0.084^{d}$	0.153 $\pm 0.056^{c}$	0.158 $\pm 0.3^{c}$
FI	9.5	12.4	12.8	13.89	14.20	9.74	10.83	11.36

Mean values are expressed as means ± SE.

Means with different superscript letters in the row are significantly different at P ≤ 0.05.

From this present study, it could be observed that, EY has a greater enhancement of weight gain and feed in rats. This increased FER of the diet presented for each group. Bogoriani *et al.* (2019) showed that there was increased in the body weight of rats in both treatments with a significant difference (P <0.05) compared to controls in weeks 3 and 4. According to Miranda *et al.* (2015) that egg-containing food full of nutrition. Eggs are easily digested and absorbed by the intestine so that the rapid body weight increased over the controls. Egg yolk is a major source of cholesterol with an average egg containing 200 mg - 750 mg (Sunwoo and Gujral, 2015 and Spence, 2010). Beside that Eggs are an excellent natural source of folate, riboflavin, selenium, choline, vitamin B12, and vitamins of A, D, E, and K of fat-soluble (Miranda *et al.,* 2015; Bogoriani *et al.,* 2019; Herron and Fernandez, 2004; Song and Kerver, 2000 and Valentine *et al.,* 2010).

As demonstrated in Table (2), the mean value ± SE of serum LDL, VLDL, cholesterol and triglycerides of the positive control group significantly increased p≤ 0.05, as compared to the negative control group. While there was a significant decreased in the serum of HDL when compared to the negative control group. In the normal groups feeding with three levels from EY showed that group 3% EY is the best group in all treatment groups, because this group showed a non-significant difference, as compared to the negative control group. non-significant difference in serum cholesterol was observed between the group treated with 3% EY in atherosclerosis rats and the positive group

In the present study, groups treated with 5% and 10%, EY led to a significant increase in lipid profile (serum LDL, VLDL, cholesterol and triglycerides) as compared to the basal diet group. A similar result was reported by Rouhani *et al.* (2018) published that egg consumption increases total cholesterol, LDL-C and HDL-C.

Table 2: Effect of EY on Total cholesterol, Lipoproteins and Triglycerides of rats

Parameters	-ve control	+ve control	3% EY + Atherosclerosis	5% EY + Athero-sclerosis	10% EY + Athero-sclerosis	3% EY	5% EY	10% EY
Cholesterol	60.64 ±2.59[e]	210.42 ±3.25[b]	213.9 ±3.9[b]	235.66 ±1.71[a]	238.96 ±1.82[a]	63.39 ±2.36[e]	99.13 ±1.87[d]	114.53 ±2.35[c]
Triglyceride	52.84 ±2.07[d]	90.08 ±1.97[b]	92.1 ±2.08[b]	95.78 ±3.12[ab]	100 ±3.29[a]	55.81 ±4.8[d]	75.2 ±1.85[c]	80.36 ±0.55[c]
HDL	46.46 ±1.34[a]	25.66 ±1.68[cd]	22.97 ±1.93[de]	19.84 ±1.35[ef]	18.03 ±1.79[f]	43.52 ±1.72[a]	33.11 ±1.4[b]	29.4 ±0.6[bc]
LDL	15.75 ±2.25[d]	101.2 ±2.82[b]	103.8 ±3.2[b]	107.84 ±2.84[ab]	113.13 ±2.68[a]	17.72 ±2.18[d]	59.55 ±2.34[c]	67.79 ±4.13[c]
VLDL	11.88 ±0.83[d]	33.97 ±1.8[b]	33.96 ±0.82[b]	38.44 ±1.35[a]	40.87 ±0.92[a]	14.15 ±0.79[d]	25.43 ±1.56[c]	27.76 ±1.072[c]

Mean values are expressed as means ± SE.
Means with different superscript letters in the row are significantly different at P ≤ 0.05.

There is a risk of forming atherogenic risk factors, which factors are driving the increase in the formation of atherosclerosis. The increase in total cholesterol, LDL-C, triglycerides, and the ratio of total cholesterol / HDL-C and a decrease in HDL-C in treatment with egg yolk duck pellets and raw. Efforts to prevent hypercholesterolemia, should be limited to consume the yolk duck containing high cholesterol is below the quail egg yolk and above chicken (Mehdipour and Hamishehkar, 2018).

In another study, Li *et al.* (2020) concluded that medium and high egg intake per day may influence cardiovascular disease risks by increasing LDL-c and the LDL-c/HDL-c ratio, but low egg-consumption may lead to Lower LDL-c/HDL-c ratio and LDL-cin agreement with results, Wang *et al.* (2019).

To avoid elevations in blood cholesterol and reduce CHD risk, the public has been advised to consume no more than 300 mg/d of cholesterol and limit consumption of eggs, which contain about 213 mg of cholesterol per egg (National Cholesterol Education Program, 1991 and Krauss *et al.,* 1996). However, eggs contain many other nutrients besides cholesterol, including unsaturated fats, essential amino acids, folate, and other B vitamins. In addition, the consumption of eggs instead of carbohydrate-rich foods may raise high-density lipoprotein (HDL) cholesterol levels (McGee *et al.,* 1984 and Packard *et al.,* 1983) and decrease blood glycemic and insulinemic responses (Pelletier *et al.,* 1996).

As shown in Table (3), the mean value ± SE of serum AIT, ALP and AST of the positive control group increased significantly p≤ 0.05, as compared to the negative control group. In the normal groups feeding with three levels from EY showed that group 3% EY is the best group in all treatment groups, because this group showed a non-significant difference, as compared to the negative control group. Non-significant differences in serum AST, ALT and ALP were observed between the groups treated with 3% EY in atherosclerosis rats and the positive group.

In the present study, groups treated with two levels (5% and 10%) EY led to a significant increase in Liver function (AST, ALT and ALP) as compared to the basal diet group. A similar result was reported by Mazidi *et al.* (2020) found that subjects with higher egg intake have a greater chance of NAFLD and less favorable liver test results. These associations seemed to be partly attributable to cardiometabolic risk factors including triglycerides, HTN and DM. Mokhtari *et al.* (2017) and

Baumgartner *et al.* (2013) have evaluated the link of egg consumption with liver tests and chance of Non-alcoholic fatty liver disease.

Table 3: Effect of EY on serum ALT, AST and ALP in rats

Parameters	-ve control	-ve control	3% EY + Atherosclerosis	5% EY + Atherosclerosis	10% EY + Atherosclerosis	3% EY	5% EY	10% EY
ALT	29.423 $\pm0.575^{d}$	42.7 $\pm3.57^{b}$	45.15 $\pm2.94^{b}$	55.15 $\pm2.94^{a}$	57.7 $\pm2.6^{a}$	29.72 $\pm0.8^{d}$	34.56 $\pm0.4^{c}$	36.53 $\pm0.75^{c}$
AST	117.4 $\pm2.09^{e}$	160.2 $\pm1.85^{b}$	163.5 $\pm2.25^{b}$	175.3 $\pm2.25^{a}$	180.1 $\pm0.76^{a}$	120.5 $\pm2.6^{e}$	141.26 $\pm2.3^{d}$	151.16 $\pm1.73^{c}$
ALP	114.6 $\pm2.5^{d}$	150.58 $\pm0.99^{b}$	152.5 $\pm1.33^{b}$	158.5 $\pm1.33^{a}$	163.45 $\pm2.13^{a}$	118.38 $\pm2.3^{d}$	134.6 $\pm1.65^{c}$	139 $\pm1.77^{c}$

Mean values are expressed as means ± SE.
Means with different superscript letters in the row are significantly different at $P \leq 0$.

Eggs are loaded with nutrients that are beneficial (monounsaturated fatty acids, polyunsaturated fatty acids, arginine, a precursor to nitric oxide, vitamins, minerals and carotenoids); on the other hand, their high content of cholesterol and saturated fat and may influence metabolic disorders (Preli *et al.,* 2002 and Wallin *et al.,* 2016). It has been reported that the positive link between egg consumption and the chance of NAFLD, could be explained by the high cholesterol content of eggs (Mokhtari *et al.,* 2017). Previous studies have shown that higher consumption of cholesterol is associated with NAFLD and its exacerbation (including non-alcoholic steatohepatitis [NASH]) (Musso *et al.,* 2003; Yasutake *et al.,* 2009 and Baumgartner *et al.,* 2013). In addition, the presence of the high amount of cholesterol in the diet is necessary for NAFLD development (Emamat *et al.,* 2016). Moreover, it has been reported in many available trials that daily egg consumption significantly increased serum TC and especially, LDL-C levels (Baumgartner *et al.,* 2013).

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