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THE EFFECT OF INTAKE DUCK EGG YOLK ON BODY WEIGHT, LIPIDS PROFILE AND ATHEROSCLEROSIS DISEASES IN MALE WISTAR RATS

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Keywords:

Duck egg yolk, Triglycerides, Total cholesterol, HDL cholesterol, LDL cholesterol

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ABSTRACT: Duck egg yolks contained fat and higher cholesterol than chicken eggs. A high-fat diet has caused obesity to impact for the development of diseases such as type II diabetes, stroke, atherosclerosis, hypertension, cardiovascular disease and certain forms of cancer. The purpose of using laboratory animals determined the effects of duck egg yolk to body weight, lipids profile, and atherosclerosis diseases. The study design is randomized post-test only control group design with Wistar rats. Twenty-seven rats were divided into three groups (nine rats per group): control, group I and II. The control group was given standard diet, and other groups received their respective agents (pellet duck egg yolk and raw duck egg yolk). After rats were treated for 30 days, blood samples were taken for examination of blood plasma lipids profile. These results have indicated that an increase in body weight of rats in both treatment as well as increase total cholesterol, LDL cholesterol, triglycerides, ratio of total cholesterol to HDL cholesterol, atherogenic index (AI), VLDL, and decreased in blood plasma HDL cholesterol were significantly ($p<0.05$) in both treatment well with duck egg yolk pellets or raw duck egg yolk. Based on these study results can be concluded that the duck egg yolk intake caused an increase in body weight of rats and total cholesterol, LDL cholesterol, the ratio of total cholesterol to HDL cholesterol, AI, VLDL, and decreased HDL cholesterol and thus potentially lead to obesity, atherosclerosis, and coronary heart disease.

INTRODUCTION: Eggs are foods that contain lots of protein, fat, cholesterol, B_6 vitamins, B_{12}, A, folic acid, half of the riboflavin, thiamine, and minerals most of the calcium, phosphorus, iron, zinc [1, 2, 3, 4, 5]. Eggs as a source of animal protein, easily digested and absorbed by the intestine. According to Miranda *et al*., (2015) eggs are sources of protein, fats and micronutrients and content of the egg white is almost non-existent. Sudaryani (2003) reported that almost all of the fat contained in the yolk, which reached 32%, an observation fat and cholesterol are more effectively done in egg yolk.

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Duck egg yolks contain fat and higher cholesterol than chicken eggs. Egg yolk composition depending on several factors such as the type of food the hen diet, age, strain and environmental [5, 6]. Nutrient composition of duck eggs per 100 grams, namely energy (kcal) 185; protein (g) 12.81; total fat (g) 3.77; carbohydrate (g) 1.45; and cholesterol (mg) 884. [8] Fats are generally contained in egg yolk are triglycerides (neutral fats) and phospholipids (lecithin).

Effect of dietary fat and cholesterol can cause hyperlipidemia and obesity which is a major risk factor of atherosclerosis, stroke, type II diabetes and occurrence of cardiovascular disease [9, 30, 34, 35, 36, 37]. Cardiovascular diseases become the leading cause of death in developed countries like Indonesia [29, 30]. The duck egg is a dietary that is easily obtained, the most favored and has high religious value in Bali, so the price is higher than

chicken eggs. The content of cholesterol and fat of eggs often makes people anxious, because it was thought that foods containing high fat and cholesterol could cause atherosclerosis, obesity and coronary heart disease [2, 3, 12, 34, 35, 36, 37]. To know more about the effects of fat and cholesterol in the body, that has researched the effects of duck egg yolk of body weight, lipids profile and atherosclerosis disease of the Wistar rats.

MATERIALS AND METHODS:

List of Chemical Materials Used:

- ✓ Duck eggs were obtained from duck breeder.
- ✓ Lipid profile kit for cholesterol, triglyceride (TG), and high-density lipoprotein (HDL) (AGAPE Diagnostic Ltd.)
- ✓ All reagents in his study were of analytical grade.
- ✓ Standard diet cp 551 (from PT. Tohpati Poultry).

Diet of Animal: Diet control as standard diet for rats is CP 551 has a composition of 13% water content; 18.50 to 20.50% protein; fat, 4%; fibre 6%; ash 8%; 0.90% calcium and 0.70% phosphorus. Diet of duck egg yolk (pellet) and row is the standard diet (80%) and a duck egg yolk (20%).

Experiment Animal: Male Wistar white rats (150-200 g) of either sex approximately 11-12 weeks, procured from the Research Laboratory Center Study of Animal Disease (CSAD), Faculty of Veterinary Medicine of Udayana University. They were housed in polypropylene cage and fed with standard rodent pellet diet (CP 551) and water *ad libitum*. The animals from each group individually housed at room temperature, with cycles of light: dark 12:12 h. All the experimental works with the animal were carried out after obtaining approval from the organization of Animal Ethics Committee (ethical clearance) no: 0142/KE-PH/VIII/2013.

Design of Experimental: Twenty-seven Wistar rats were divided into 3 groups (nine rats per group). Group I were given a standard diet (CP 551, 25 g/d) as control, group I and II received their respective agents (pellet duct egg yolk 25 g/day) was paced in the cage carefully, and row 22.5 g/d of CP 551 and 2.5 g/d of row duct egg yolk), respectively. After treatment 30 days, rats fasted for 14 h. Blood was withdrawn using micro hematocrit capillaries from retro-orbital sinus, accommodated in the tubes of blood containing EDTA solution. The plasma was obtained after centrifuging the blood, which was used to estimate the concentration of biochemical parameters using the semi autoanalyzer and relevant lipid profile kits.

Biochemical Test for Lipids: Total cholesterol was determined by CHOD-PAP method by E. Merck. Total cholesterol was calculated by absorbance of the sample divided by the absorbance of the standard cholesterol (0.240) multiplied by constant standard cholesterol (200 mg/dl). High-density lipoprotein-cholesterol (HDL) cholesterol analysis principle according to [9], namely the provision of phosphotungstic acid and magnesium ions into the sample so that chylomicrons, very low-density lipoprotein-cholesterol (VLDL) and LDL will settle. HDL levels were calculated with the absorbance of the sample multiplied by 318 (mg/dl). Low-density lipoprotein-cholesterol (LDL) cholesterol checks were done by reducing the total cholesterol in VLDL and HDL, while VLDL calculation was done by using triglycerides, VLDL which is equal to one-fifth (1/5) of triglycerides.

Triglycerides examination conducted by GPO-PAP method. Triglyceride was determined after enzymatic hydrolysis with lipase: quinoneimin indicator formed from hydrogen peroxide, 4-aminoantipirin and 4-chlorophenol under the influence of peroxide catalysis. Triglyceride levels calculated by absorbance of the sample divided by the standard absorbance triglycerides (0.145) multiplied by constant triglycerides (200 mg/dl).

Measurement of Coronary Diseases Risk Factor: Atherogenic index (AI) was calculated using the following formula, and the results were tabulated:

$$AI = \text{LDL-cholesterol} / \text{HDL-cholesterol}$$

$$\% \text{ Protection} = \frac{\text{AI of control-AI of the treated group}}{\text{AI of control}} \times 100$$

Histopathological Studies: A portion of liver tissue of normal control, duct egg yolk of pellet, and raw egg yolk were stored in containers for 24 h in 10% formalin solution and subjected to

histopathological studies [34]. Observed micro-scopically for histopathological changes, *i.e*., normal liver, damaged and recovered liver was studied and compared.

Statistical Analysis: The analysis of result data was conducted by statistical. The difference between the average levels of the test groups was tested with One Way ANOVA followed by Post Hoc LSD, where statistical tests indicated significant if the values of $p<0.05$.

RESULTS AND DISCUSSION:

Results:

Body Weight of Wistar Rats: Observations of body weight development in each group of test rats, every week during the 30 day study period was also carried out and presented in **Table 1**.

In **Table 1** showed the average of body weight into three groups of rats at initial almost the same ($p> 0.05$) with an average body weight of control group was 156.54 ± 9.0 g, the group treated with DEYP was 165.74 ± 8:03 g, with the treatment group DEYR, was 166.00 ± 9:08 g, body weight increased during the 30 days is almost the same for all groups of experimental rats were measured weekly. Occurs significantly increase in body weight at week 2; 3 and 4 of the study ($p<0.05$). The average body weight increased of rats week 3 of each group of the rat: DEYP group was 239.08 ± 12.09 g *vs.* control group 199.90 ± 17.00 g, DEYR group 247.13 ± 3.81 g *vs.* control (199.90 ± 17.00 g), $p<0.05$, was significantly different compared to control. The effect of DEYP body weight at week 4 (282.02 ± 3.41) *vs.* control (220.58 ± 15.76 g), $p<0.05$ and DEYR group (282.41 ± 2.68 g) *vs.* control (220.58 ± 15.76 g), was a difference of significant compared to control. So there is a treatment effect on the increase of body weight of rats.

Plasma Lipids Profile: Wistar rats were given treatment for 30 days and on the last day of the study of the rats were fasted for 14 h to pull all food and drink from his cage, and then have blood drawn and evaluated according to the study protocol. An average ratio of total cholesterol to HDL-C, LDL-C, HDL-C, triglycerides, VLDL, and the ratio of LDL-C to HDL cholesterol (AI) can be seen in **Table 2**.

TABLE 1: THE AVERAGE BODY WEIGHT DEVELOPMENT WISTAR RATS

Groups	Control	DEYP	*p*	DEYR	*p*
Initial BW (g)	156.50 ± 9.10	165.74 ± 11.59	0.06	166.00 ± 9.08	0.06
Week 1 BW (g)	172.59 ± 13.17	181.34 ± 8.02	0.07	179.96 ± 15.67	0.07
Week 2 BW (g)	191.56 ± 17.86	206.67 ± 12.61[a]	0.00	212.31 ± 3.91[a]	0.00
Week 3 BW (g)	199.90 ± 17.00	239.08 ± 12.09[a]	0.00	247.13 ± 3.81[a]	0.00
Week 4 BW (g)	220.58 ± 15.76	282.02 ± 3.41[a]	0.00	282.41 ± 2.68[a]	0.00

Mean ± SD (n =9) followed by different letters (superscript) in the same row indicate significant differences; Duck Egg Yolk Pellet (DEYP), Duck Egg Yolk Row (DEYR); Body Weight (BW); gram (g). [a] Represents significant difference from control $p<0.05$.

TABLE 2: AN AVERAGE PLASMA LIPIDS PROFILE BLOOD RATS

Group	Control	DEYP	*p*	DEYR	*p*
Chol-tot	63.11 ± 4.68[b,c]	143.33 ± 1.87[a,c]	0.000	108.78 ± 1.09[a,b]	0.000
(mg/dl)			0.000		0.000
LDL-C	11.97 ± 6.38[b,c]	96.43 ± 1.56[a]	0.000	97.07 ± 1.17[a]	0.000
(mg/dl)			0.730		0.730
HDL-C	43.44 ± 4.76[b,c]	27.88 ± 0.73[a]	0.000	26.51 ± 2.23[a]	0.000
(mg/dl)			0.353		0.353
TG	52.78 ± 1.56[b,c]	86.22 ± 2.54[a,c]	0.000	138.56 ± 4.33[a,b]	0.000
(mg/dl)			0.000		0.000
Chol-tot /	1.49 ± 0.11[b,c]	5.15 ± 0.12[a,c]	0.000	4.13 ± 0.34[a,b]	0.000
HDL-C			0.000		0.000
AI (LDL-C/	0.27 ± 0.13[b,c]	3.23 ± 0.26[a,c]	0.000	3.58 ± 0.40[a,b]	0.000
HDL-C)			0.015		0.015
VLDL	10.56 ± 0.31[b,c]	33.69 ± 0.91[a,c]	0.000	32.80[a,b]	0.000
(mg/dl)			0.020		0.020

Mean ± SD (n =9) followed by different letters (superscript) in the same row indicate significant differences; Duck Egg Yolk Pellet (DEYP), Duck Egg Yolk Row (DEYR); Chol-tot (Total cholesterol); TG (triglyceride); LDL-C (low Density lipoprotein-cholesterol); HDL-C (high density lipoprotein-cholesterol);VLDL (very low density lipoprotein) mg (milligram); dl (deciliter). [a] Represents significant difference from control $p<0.05$. [b] Represents significant difference from DEYP $p<0.05$. [c] Represents significant difference from DEYR $p<0.05$

Based of the research of initial data (control) and after feeding with DEYP 20% and DEYR 20% of plasma chol-tot, LDL-C, TG, HDL-C, LDL-C/HDL-C ratio and Chol-tot/HDL-C ratio can be seen in **Table 2** and **Fig. 1** indicated that effect of levels DEYP plasma chol-tot (143.33 ± 1.87 mg/dl) *vs.* control 63.11 ± 4.68 mg/dl, p<0.05 and *vs.* DEYR plasma (108.78 ± 1.09 mg/dl), p<0.05, were significantly compared to control and DEYR group.

The levels of DEYP plasma LDL-C (96.43 ± 1.56 mg/dl) *vs.* control (11.97 ± 6.38 mg/dl), p<0.05 and *vs.* DEYR plasma (97.07 ±1.17 mg/dl), p>0.05, was higher significantly compared to control and not significantly to DEYR group. The effect of DEYP plasma TG (86.22 ± 2.54 mg/dl) *vs.* 52.78 ± 1.56 mg/dl), p<0.05 and *vs.* DEYR 138.58 ± 4.33 mg/dl, p<0.05, were significantly difference to compare control and DEYR group. The levels of DEYP plasma Chol-tot/HDL-C (5.15 ± 0.12 mg/dl) *vs.* control 1.49 ± 0.11 mg/dl, p<0.05 and *vs.* DEYR group 4.13 ± 0.34, p<0.05, were difference significantly. The levels of DEYP plasma LDL-C/HDL-C ratio and VLDL respectively (3.23± 0.26; 33.69 ± 0.91, p<0.05, where a difference of significantly compared to control and DEYR group. Effect of DEYP plasma HDL-C 27.88 ±0.73 *vs.* 43.44 ± 4.76 mg/dl, p<0.05 and *vs.* DEYR group 26.51 ± 2.23 mg/dl, p>0.05, was significant compared to control and not significantly compared to DEYR group. The average increased in total cholesterol **Fig. 1**, LDL cholesterol, triglycerides, the ratio of total cholesterol to HDL cholesterol, ratio LDL-C to HDL-C, VLDL were significantly (p<0.05), compared with control, on treatment with duck egg yolk of pellets and raw. The average decreased in HDL cholesterol was significantly (p<0.05), compared to control and DEYR group.

Histopathology:

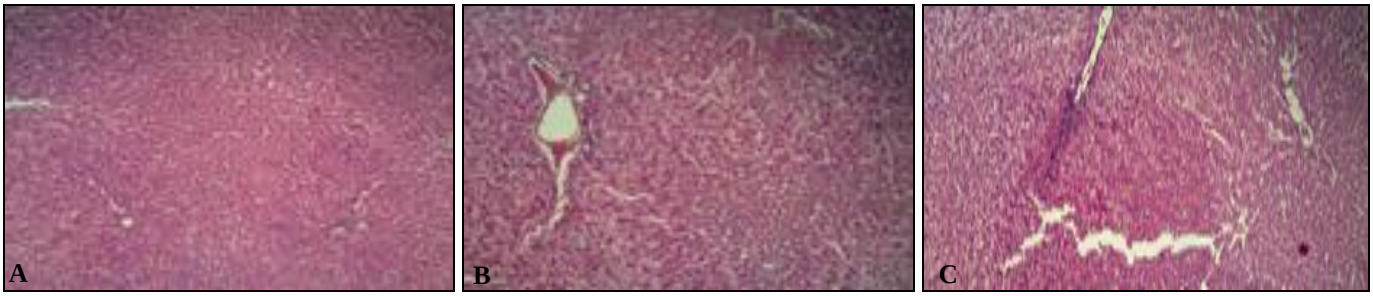

FIG. 1: HISTOPATHOLOGY (LIVER): A) NORMAL; B) DEYP (DUCK EGG YOLK PELLET); C) DEYR (DUCK EGG YOLK ROW)

DISCUSSION: According to **Table 1** showed that there was increased in 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 [4, 14]. 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 [6, 8, 15, 16, 17].

Based on **Table 2** can be seen that after 30 days, the effect of egg yolk on both treatments have started to appear. There is a tendency that during the study an increase in total cholesterol (127.11% DEYP; 28% DEYR) was significantly compared to control and DEYR group, triglycerides (63.36% DEYP; 163.52% DEYR) was significantly different compared to control and DEYR group p<0.05, LDL-C (705.59% DEYP; 710.94% DEYR) was significantly compared with control and not significantly to DEYR group, p>0.05; the ratio of total cholesterol / HDL-C (245.64% DEYP; 177.18% DEYR) was significantly compared to control and DEYR group respectively (Sitepoe, 1992); the ratio LDL-C to HDL-C (1096.30% DEYP; 1225.93% DEYR) and VLDL (219.03% DEYP; 210.61% DEYR) but decreased HDL cholesterol (35.82% DEYP; 38.97% DEYR) were significant of control and not significantly of DEYR group.

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 increased 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 [18]. Total cholesterol levels in the blood plasma of rats fed duck egg yolk with the form of pellets and raw egg yolk after 30 days increased significantly ($p<0.05$) compared with controls. HDL cholesterol levels decreased significantly ($p<0.05$) in blood plasma of rats in all treatments which were fed duck egg yolk. LDL cholesterol levels, difference significantly ($p<0.05$) compared with controls. The ratio of total cholesterol / HDL, VLDL, and AI showed a significant increase ($p<0.05$) in all treatments compared to controls. Triglyceride levels increased significantly in all treatments ($p<0.05$) compared to control, but not significantly difference of DEYP group compared to DEYR group ($p>0.05$). For years, the media and health-governing bodies issued warnings to avoid saturated fat at all costs because it was thought to be a major player in increasing one's risk for cardiovascular disease. Eggs, which happen to contain saturated fat in the yolk, were a primary target [19, 34, 35, 36, 37].

The reason saturated fat got such a bad rap was that of its supposed effect on cholesterol. Chronically elevated cholesterol, in combination with other cardiovascular disease risks, such as a sedentary lifestyle, diabetes, poor dietary choices, and high blood pressure, has been linked to various forms of heart disease [4, 19, 34, 35, 36, 37]. The evidence that regular consumption of egg yolks of diabetic patients increased the high risk of cardiovascular [20, 21]. A single egg yolk contains approximately 215 mg to 275 mg of cholesterol (depending on the size) [20, 21, 30]. The study showed that consumption of one egg a day in diabetic patients caused doubled their risk compared with less than one egg week (Spence *et al*., 2010) whereas some study has indicated positive associations between dietary and serum cholesterol [22, 23]. The others did not find any effect [23, 25]. There is effect variability of large in the response of an individual to dietary cholesterol [23, 24]. Also, the effect of dietary cholesterol on LDL-C observed in positive studies is modest compared with the LDL-raising effects of saturated and trans fatty acids [27, 28].

If the metabolism of cholesterol in the liver, indicating excessive cholesterol levels, it can interfere with its metabolism so that the cholesterol accumulate in the liver. If this situation is left for a long time, then the excess cholesterol gets stuck to the walls of blood vessels and lead to an atherosclerotic plaque that could increase the risk of CAD [1, 28, 29]. Based on the results of the analysis are presented in **Table 2** above, note that if every day were fed egg yolk, then there is an increase in total cholesterol levels in the blood. At 30 days, the total cholesterol level in blood plasma of rats is still below the maximum threshold (<200 mg/dl), but should be aware that if the yolk is given every day, total cholesterol levels in the blood of rat will continue to rise, although still below the threshold (<200 mg/dl).

Thus there is a risk of forming atherogenic risk factors [28], 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 [1, 2].

The recommended intake of daily dietary cholesterol continues to be 300 mg/day or less for healthy adults and less than 200 mg/day for persons with elevated cholesterol or heart disease [28, 30]. Given the widespread nature of this recommendation, there is surprisingly little evidence that egg consumption increases blood cholesterol levels, thereby increasing cardio-vascular risk [2, 18, 28, 32].

CONCLUSION: Based on these results it can be concluded that the duck egg yolk intake causes an increased in body weight of rats and increased in total cholesterol, LDL cholesterol, the ratio of total cholesterol to HDL cholesterol, AI, VLDL and decreased HDL cholesterol and potentially lead to atherosclerosis and coronary heart disease.

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CONFLICT OF INTEREST: Nil

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