

Effect of Whole Quail (*Coturnix japonica*) Egg Consumption on some Reproductive Parameters and Lipid Profile of Male Albino Rat (*Rattus norvegicus*)

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Abstract

The present study evaluated the effect of whole egg consumption on the liver, testes, cauda epididymal sperm reserve and lipid profile of male rats. These evaluations were carried out on adult twenty (20) male albino rats, which were randomly selected into four groups of 5 rats each, designated groups A, B, C and D. Group A was the control group and received only equivalent volume of distilled water, while groups B, C and D received 0.25 mg/kg, 0.5 mg/kg and 1.0 mg/kg body weight of the quail egg respectively. Standard procedures were carried out in the tissue processing, cauda epididymal sperm reserve and in lipid profile determinations. On days 35 and 49, the mean serum total cholesterol value of group D was significantly lower than that of the control group. On day 35, the mean serum low density lipoprotein and high density lipoprotein (LDL and HDL cholesterol) values of all the treatment groups were significantly lower and higher than that of the control group, respectively. However, on days 49 and 63, the mean serum very low density lipoprotein (VLDL cholesterol) and triglyceride values of all the treatment groups were significantly higher than that of the control group. A significant increase in caudal epididymal sperm count (CESR) was recorded on day 63 at the mid and high doses. No obvious pathological lesions were observed in the histomorphology of the testes and liver when compared to the control. Therefore, whole quail egg consumption caused an increase in serum triglyceride and very low density lipoprotein concentration, and also improved fertility. In other words, prolonged consumption of quail egg should be done with caution as it may predispose one to cardiovascular disease.

Keywords: cardiovascular disease, fertility, lipid profile, quail egg, *Rattus norvegicus*, testis, spermatozoa

Introduction

Japanese quail (*Coturnix japonica*) is a migratory pheasant bird that belongs to the family *Phasianidae* (Awad *et al.*, 2015). It was domesticated since the eleventh century (Campos *et al.*, 2013) and introduced in Nigeria in 1992 (NVRI, 1994). Japanese quail egg consumption is becoming increasingly popular in Nigeria (Onyewuchi *et al.*, 2013) and also in many Asian, European and American countries (Campos *et al.*, 2013; Tunsaringkarn *et al.*, 2013; Tolik *et al.*, 2014). This increasing popularity is due to its acclaimed nutritional (Campos *et al.*, 2013; Tolik *et al.*, 2014) and health benefits which may underscore its ethnomedical use

(Tunsaringkarn *et al.*, 2013). Quail egg is known to contain more nutrients, high protein value and lower fat content when compared to chicken eggs (Campos *et al.*, 2013; Tunsaringkarn *et al.*, 2013; Tolik *et al.*, 2014). Avidin found in egg albumen causes an increase in cholesterol level by producing a deficiency in bioavailability of biotin (Kovacs-Nolan *et al.*, 2005). Its cholesterol content is higher than that of chicken (Jalaludeen and Churchill 2006; Sinanoglou *et al.*, 2011; Aziz *et al.*, 2012) or about equal to that of chicken (Bragagnalo and Rodriguez-Amaya 2003; Tolik *et al.*, 2014).

Consumption of a large number of eggs is considered a risk factor for cardiovascular diseases due to high blood cholesterol levels (Bragagnalo and Rodriguez-Amaya 2003).

High blood cholesterol levels (hypercholesterolemia) could predispose to atherosclerosis, which could result in pathologies such as myocardial infarction, cerebral infarction, aortic aneurysm, chronic ischaemic heart disease and ischaemic encephalopathy (Ihedioha *et al.*, 2013). These cardiovascular pathologies could predispose to impotentia couendi due to erectile dysfunction consequent upon impaired cardiac supply and impaired arterial blood flow to the male reproductive organ resulting in infertility. The types of blood cholesterol associated with increased risk of atherosclerosis are the low density lipoprotein cholesterol (LDL-c) and very low density lipoprotein cholesterol (VLDL-c). LDL-c and VLDL-c carry cholesterol to tissues, unlike high density lipoprotein cholesterol (HDL-c) that clears cholesterol from developing and existing atheromas and carries it to the hepatic tissue for excretion in bile (Ihedioha *et al.*, 2013). Cholesterol is the precursor for sex hormones (Hu *et al.*, 2010), and Japanese quail egg is reported to contain sex hormones of which progesterone and testosterone are present in appreciable quantities (Tunsaringkarn *et al.*, 2013). These hormones do not only affect the reproductive system and fertility, but are pleiotropic and do possess anabolic properties (Tunsaringkarn *et al.*, 2013).

Quail egg is anecdotally used to enhance fertility yet there is a paucity of information in the available literature on the effect of quail egg consumption on the liver, fertility and reproduction. In the light of the increasing consumption of quail eggs and its ethnomedical use, especially to enhance fertility juxtaposed with the potential cardiovascular pathologies, this study evaluated the alterations in the serum lipid profile, cauda epididymal sperm reserves and histomorphology of the testis and liver of adult male albino rats following oral administration of varied doses of Japanese quail (*Coturnix japonica*) whole egg.

Materials and Methods

Experimental animals

A total of 20 adult male Sprague-Dawley albino rats were used for the study. The rats were procured from the Laboratory Animal House of the Department of Pathology and Microbiology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka. They were allowed two weeks for acclimatization. The rats were kept in groups in stainless steel cages in the Experimental Animal House of the Department of Veterinary Obstetrics and Reproductive Diseases, Faculty of Veterinary Medicine, University of Nigeria, Nsukka under standard conditions (ambient temperature: $\approx 28^\circ\text{C}$, approximately 12 hours natural day light, humidity 60-70%). Commercial pelleted feed (Vital, GCOML, Nigeria) containing approximately 14.5% crude protein and 2,500 Kcal/kg metabolizable energy, and potable water were provided *ad libitum*. Freshly laid quail eggs used for the study were procured from a commercial quail farm in Nsukka, Enugu State, Nigeria.

Experimental design

The rats were randomly assigned to four treatment groups designated A, B, C and D of 5 rats each. Group A was the control group and received only equivalent volume

of distilled water, while group B received 0.25 mg/kg, group C received 0.5 mg/kg and group D received 1.0 mg/kg body weight of the quail egg respectively for the entire duration of the study. A homogenised mixture of the egg white and yolk of freshly laid Japanese quail egg was prepared each day of dosing. The rats were dosed every other day for 9 weeks with the aid of an orogastric gavage between 09.00-10.00 hours.

Sample collection and analysis

Blood samples were collected on days 7, 21, 35, 49 and 63 of dosing from the retrobulbar plexus following the procedure of Stone (1954) between 10- 11 am. Serum was harvested from the blood samples one hour after clotting and centrifuged at 10,000 g for 10 minutes. The serum samples were used immediately for the serum lipid profile determination following standard procedures using Quimica Clinica Applicada test kits (Quimica Clinica Applicada, Spain) and digital colorimeter (Labtech, India). Determination of serum cholesterol was done by cholesterol oxidase-peroxidase method (Allain *et al.*, 1974), while serum high density lipoprotein-cholesterol (HDL-C) was done following the dextran sulphate-magnesium (II) method (Albers *et al.*, 1978). Serum triglyceride was determined by the glycerol phosphate oxidase enzymatic method (Bucolo and David, 1973), while determination of serum very low density lipoprotein cholesterol (VLDL-C) was obtained by dividing the serum triglyceride by 5 (Friedewald *et al.*, 1972). Determination of serum low density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald formula (Friedewald *et al.*, 1972; Warnick *et al.*, 1990).

At the end of the study the rats were humanely sacrificed, the testes and liver were carefully resected. Cauda epididymal sperm reserves were determined using the haemocytometric method described by Amman and Almquist (1961). The testicular and liver samples were promptly fixed in Bouin's fluid. Further histological preparations were carried out as described by Igwebuike and Eze (2010), Haschek *et al.* (2009), and stained with hematoxylin and eosin for light microscopy. Photomicrographs were captured using a Moticam[®] digital camera (Motic China Group Co., Ltd., Xiamen, China) including Motic Images Plus 2.0 software.

Ethical approval

The housing, handling and welfare of the rats used for the study were duly observed in accordance with the Ethics and Regulation Guiding the use of animals as approved by the University of Nigeria Senate committee on Medical and Research ethics, with approval number 09/57172.

Data analysis

Data generated were subjected to one-way analysis of variance (ANOVA) using SPSS 16. Data having different time points were subjected to repeated measures ANOVA. Variant means were separated by the least significant difference (LSD) method. Significance was accepted at a probability level of less than 0.05. Results were presented as means \pm standard error (SE).

Results

There was no significant ($P > 0.05$) variation in the mean serum cholesterol concentration of all the treatment groups given graded doses of quail egg orally when compared to the control group on days 7 and 21 (Table 1). However, on days 35 and 49 the mean serum cholesterol concentration of only group D was significantly lower than that of the control group, but by the end of dosing on day 63, there was no significant variation in the mean serum cholesterol concentration of all the treatment groups when compared to the control group (Table 1).

The mean serum LDL cholesterol concentration of only group D was significantly lower than that of the control group on day 7 of dosing; but by day 21 the mean serum LDL cholesterol concentration of groups A and B were significantly ($P < 0.05$) lower, whilst that of group D was significantly higher than that of the control group (Table 2). By day 35 of dosing, the mean serum LDL cholesterol concentration of all the treatment groups was significantly lower than that of the control group (Table 2). However, by day 49 of dosing there was no significant variation in the mean serum LDL cholesterol concentration of all the groups; but by the end of dosing on day 63, only that of group C was lower than that of the control group (Table 2).

On day 7, the mean serum HDL cholesterol concentration of all the treatment groups were lower than

that of the control group, but by day 21, the mean serum HDL cholesterol concentration of only group D was significantly lower than that of the control (Table 3). However, by day 35 the mean serum HDL cholesterol concentration of all the treatment groups was significantly higher than that of the control, but by day 49 of dosing, the mean serum HDL cholesterol concentration of only group D was lower than that of the control group (Table 3). By the end of dosing on day 63, there was no significant variation in the mean serum HDL cholesterol concentration of all the treatment groups when compared to that of the control (Table 3).

There was no significant variation in the mean serum vLDL cholesterol concentration of all the treatment groups when compared to that of the control group on day 7 of dosing; but on days 21 and 35, the mean serum vLDL cholesterol concentration of group C was higher, whilst that of group D was lower when compared to that of the control group (Table 4). However, on days 49 and 63 the mean serum vLDL cholesterol concentration of all the treatment groups was significantly higher than that of the control group (Table 4).

The mean serum triglyceride concentration of only group D was significantly lower than that of the control group on day 7 of dosing (Table 5). On days 21 and 35, the mean serum triglyceride concentration of group C was higher whilst that of group D was significantly lower than

Table 1. Mean serum total cholesterol concentration (mg/dl) of albino rats given graded doses of whole quail egg per kilogram body weight (\pm SEM)

Days	Means \pm SEM			
	Group A (Control)	Group B (0.25 mg/kg)	Group C (0.5 mg/kg)	Group D (1.0 mg/kg)
7	97.44 \pm 13.57 ^a	92.31 \pm 14.04 ^a	92.31 \pm 8.88 ^a	103.85 \pm 3.85 ^a
21	96.43 \pm 3.57 ^a	100.00 \pm 0.00 ^a	95.24 \pm 12.60 ^a	103.57 \pm 3.58 ^a
35	104.76 \pm 4.76 ^a	96.43 \pm 4.57 ^a	92.24 \pm 9.53 ^{ab}	85.71 \pm 5.83 ^b
49	90.48 \pm 9.52 ^{ab}	78.57 \pm 4.12 ^{bc}	90.47 \pm 4.76 ^a	71.43 \pm 5.83 ^c
63	71.43 \pm 14.29 ^a	75.00 \pm 8.99 ^a	71.43 \pm 5.83 ^a	92.86 \pm 22.21 ^a

^{abc} superscripts across a row indicate significant difference between the means of the different groups ($p \leq 0.05$).

Table 2. Mean serum low density lipoprotein cholesterol concentration (mg/dl) of albino rats given graded doses of whole quail egg per kilogram body weight (\pm SEM)

Days	Means \pm SEM			
	Group A (Control)	Group B (0.25 mg/kg)	Group C (0.5 mg/kg)	Group D (1.0 mg/kg)
7	27.28 \pm 14.34 ^a	30.98 \pm 11.38 ^a	45.96 \pm 11.45 ^{ab}	60.83 \pm 4.96 ^b
21	51.67 \pm 0.00 ^a	33.10 \pm 8.43 ^b	25.24 \pm 14.56 ^b	56.08 \pm 0.60 ^a
35	65.79 \pm 6.29 ^a	30.43 \pm 5.93 ^b	25.29 \pm 13.59 ^b	39.25 \pm 4.53 ^b
49	34.97 \pm 12.55 ^a	20.07 \pm 3.50 ^a	20.47 \pm 6.17 ^a	19.10 \pm 5.92 ^a
63	55.95 \pm 7.14 ^a	45.71 \pm 9.91 ^{ab}	38.81 \pm 8.06 ^b	57.81 \pm 24.72 ^{ab}

^{abc} superscripts across a row indicate significant difference between the means of the different groups ($p \leq 0.05$).

Table 3. Mean serum high density lipoprotein cholesterol concentration (mg/dl) of albino rats given graded doses of whole quail egg per kilogram body weight (\pm SEM)

Days	Means \pm SE			
	Group A (Control)	Group B (0.25 mg/kg)	Group C (0.5 mg/kg)	Group D (1.0 mg/kg)
7	57.14 \pm 0.00 ^a	46.43 \pm 3.58 ^b	28.57 \pm 0.00 ^c	28.57 \pm 0.00 ^c
21	42.86 \pm 0.00 ^a	42.86 \pm 0.00 ^a	38.10 \pm 4.76 ^{ab}	35.72 \pm 4.13 ^b
35	25.64 \pm 5.13 ^a	50.00 \pm 3.85 ^b	51.28 \pm 5.13 ^b	38.46 \pm 4.44 ^c
49	44.44 \pm 5.56 ^a	37.50 \pm 4.17 ^{ab}	50.00 \pm 9.62 ^a	33.33 \pm 0.00 ^b
63	18.18 \pm 0.00 ^a	18.18 \pm 0.00 ^a	22.73 \pm 4.44 ^b	27.27 \pm 9.09 ^a

^{abc} superscripts across a row indicate significant difference between the means of the different groups ($p \leq 0.05$).

Table 4. Mean serum very low density lipoprotein cholesterol concentration (mg/dl) of albino rats given graded doses of whole quail egg per kilogram body weight (\pm SEM)

Days	Means \pm SEM			
	Group A (Control)	Group B (0.25 mg/kg)	Group C (0.5 mg/kg)	Group D (1.0 mg/kg)
7	17.78 \pm 6.79 ^a	21.11 \pm 3.33 ^a	17.78 \pm 2.57 ^a	14.44 \pm 1.11 ^a
21	15.00 \pm 0.00 ^a	17.50 \pm 2.50 ^{ab}	20.00 \pm 2.89 ^b	10.00 \pm 0.00 ^c
35	13.33 \pm 1.33 ^a	16.00 \pm 2.83 ^{ab}	18.67 \pm 2.67 ^b	8.00 \pm 1.63 ^c
49	10.67 \pm 2.67 ^a	21.00 \pm 1.92 ^b	20.00 \pm 2.31 ^b	19.00 \pm 1.00 ^b
63	4.44 \pm 0.00 ^a	11.11 \pm 1.28 ^b	10.00 \pm 2.13 ^{bc}	7.78 \pm 1.11 ^c

^{abc} superscripts across a row indicate significant difference between the means of the different groups ($p \leq 0.05$).

Table 5. Mean serum triglyceride concentration (mg/dl) of albino rats given graded doses of whole quail egg per kilogram body weight (\pm SEM)

Days	Means \pm SE			
	Group A (Control)	Group B (0.25 mg/kg)	Group C (0.5 mg/kg)	Group D (1.0 mg/kg)
7	88.89 \pm 33.94 ^{ab}	105.56 \pm 16.67 ^a	88.89 \pm 12.83 ^{ab}	72.23 \pm 5.56 ^b
21	75.00 \pm 0.00 ^a	87.50 \pm 12.50 ^{ab}	100.00 \pm 14.43 ^b	50.00 \pm 0.00 ^c
35	66.67 \pm 6.67 ^a	80.00 \pm 14.14 ^{ab}	93.33 \pm 13.33 ^b	40.00 \pm 8.17 ^c
49	53.33 \pm 13.33 ^a	105.00 \pm 9.57 ^b	100.00 \pm 11.55 ^b	95.00 \pm 5.00 ^b
63	22.22 \pm 0.00 ^a	55.56 \pm 6.42 ^b	50.00 \pm 10.64 ^{bc}	38.89 \pm 5.56 ^c

^{abc} superscripts across a row indicate significant difference between the means of the different groups ($p \leq 0.05$).

Table 6. Mean cauda epididymal sperm reserves of albino rats given graded doses of whole quail egg per kilogram body weight (\pm SEM)

Days	Means \pm SE			
	Group A (Control)	Group B (0.25 mg/kg)	Group C (0.5 mg/kg)	Group D (0.1 mg/kg)
63	314000000 ^a \pm 8185350	332000000 ^{ab} \pm 29143320	395000000 ^b \pm 53436360	625000000 ^c \pm 43247350

^{abc} superscripts across a row indicate significant difference between the means of the different groups ($p \leq 0.05$).

that of the control group (Table 5). However, on days 49 and 63 of dosing the mean serum triglyceride concentration of all the treatment groups was higher than that of the control group (Table 5). At the end of dosing on day 63, the cauda epididymal sperm reserve of groups C and D was significantly higher than that of the control group (Table 6).

Photomicrographs of sections of the testes and liver from rats (Fig. 1-5) in the treatment groups did not show any obvious histopathologic lesions and also did not differ from those of the control group. Thus, they did not show any deviation from the normal testicular and hepatic histomorphology. The photomicrographs of the testes in all the groups showed normal seminiferous tubules lined by stratified epithelium of spermatogenic cells and Sertoli cells (S). Between the basement membranes of the seminiferous tubules are thin vacular loose connective tissue which holds the Leydig cells in all the groups. Late spermatids (Ls), early spermatids (Es), primary spermatids (Ps), spermatogonium (Sp), the lumen of a seminiferous tubule (L), interstitial cells were also shown. The liver showed hepatic lobules with normal hepatocytes arranged in chords (arrow) around the central veins (V), radiating towards the portal areas which showed the hepatic artery (HA), hepatic vein (HV) and bile duct (B).

Discussion

The results of the hereby study revealed that oral administration of raw whole Japanese quail eggs to rats produced a hypocholesterolemic effect by the end of days 35 and 49 at the highest dose. However, the cholesterol level recorded for the different treatment groups fell within the



Fig. 1. Histological appearance of testis of rat given 0.25 mg/kg body weight of quail egg orally for 63 days, showing no obvious lesions. Note active seminiferous tubules (S) bearing spermatogonium (Sp), primary spermatids (Ps), late spermatids (Ls) and interstitium (T); Hematoxylin & Eosin (H&E) \times 400

minimum and maximum cholesterol reference values reported by Ihedioha *et al.* (2013) for rats in the same geographic location. Oral administration of raw whole Japanese quail eggs to rats at the doses of 0.25, 0.5 and 1.0 ml/kg body weight had varied effects on the different density fractions of serum lipoprotein cholesterol. At the dose of 1.0 ml/kg body weight there was an initial reducing

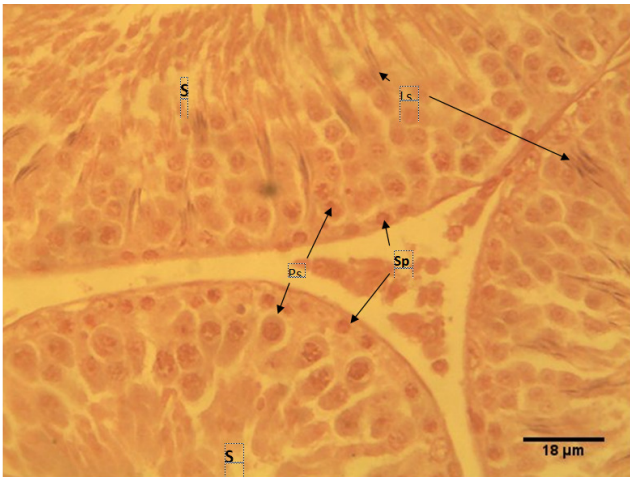


Fig. 2. Histological appearance of testis of rat given 0.5 mg/kg body weight of quail egg orally for 63 days, showing no obvious lesions. Note active seminiferous tubules (S) bearing spermatogonium (Sp), primary spermatids (Ps), late spermatids (Ls); Hematoxylin & Eosin (H&E) \times 400

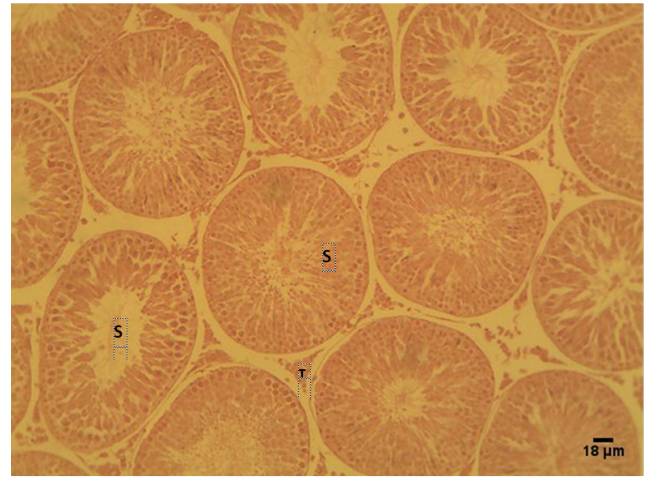


Fig. 3. Histological appearance of testis of rat given 0.5 mg/kg body weight of quail egg orally for 63 days, showing no obvious lesions. Note active seminiferous tubules (S) and interstitium (T); Hematoxylin & Eosin (H&E) \times 100

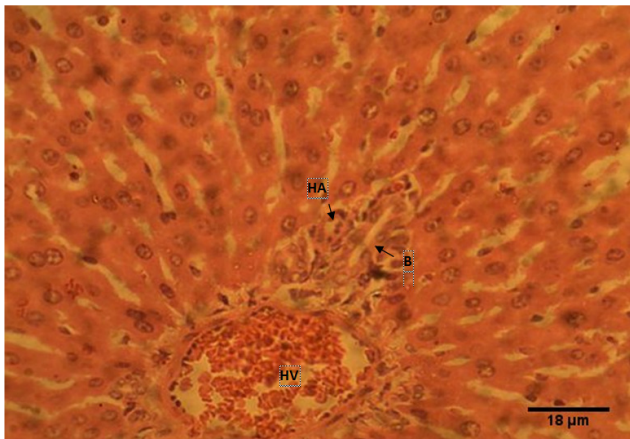


Fig. 4. Histological appearance of liver of rat given 0.25 mg/kg body weight of quail egg orally for 63 days, showing no obvious lesions. Note the hepatic lobules with normal hepatocytes arranged in chords radiating towards the portal areas which show the hepatic artery (HA), hepatic vein (HV) and bile duct (B); Hematoxylin & Eosin (H&E) \times 400

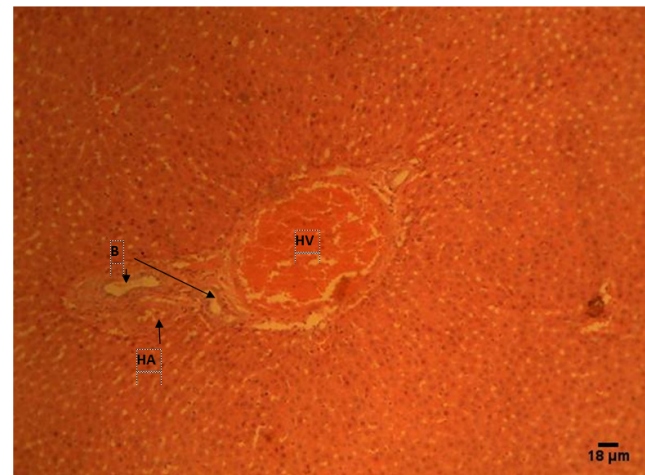


Fig. 5. Histological appearance of liver of rat given 0.5 mg/kg body weight of quail egg orally for 63 days, showing no obvious lesions. Note the hepatic artery (HA), hepatic vein (HV) and bile duct (B); Hematoxylin & Eosin (H&E) \times 100

effect on the vLDL cholesterol, but continued administration produced a net increase. There was a net reducing effect on the serum LDL cholesterol unlike the serum HDL cholesterol that was not consistent. The values of the serum vLDL, LDL, HDL cholesterol and triglyceride concentrations observed in this study were all below the upper limit for the reference values reported by Ihedioha *et al.* (2013) for rats in the same geographic location. The increase in serum vLDL cholesterol and triglyceride concentration observed in the study may suggest that consumption of quail eggs may predispose to cardiovascular pathologies, a finding supported by the report of Bragagnalo and Rodriguez-Amaya (2003).

The absence of any obvious pathological lesions in the histomorphology of the testes of rats post administration with 0.25, 0.5 and 1.0 ml/kg body weight of whole Japanese quail egg for 63 days suggests that whole quail egg may not

adversely affect the histomorphology of the testes. However, the finding of significantly increased CESR at the dosing regimen of 0.5 and 1.0 ml/kg at the end of 63 days shows that whole quail egg may potentiate the spermatogenic activities of the testes. Increase in sperm number and a normal testicular histology are positive indices of fertility (Al-Sa'aidi *et al.*, 2009; Etuk and Muhammad, 2009). It is possible that the dose-dependent increase in the CESR could be due to enhanced steroidogenesis (Pakarainen *et al.*, 2005) through the additional progesterone and testosterone supplied by the whole quail eggs (Tunsaringkarn *et al.*, 2013) through the delta-5 pathway (Jockenovel and Swerdloff, 2000) since there was no disruption of the histomorphology of the testes. This is further collaborating by the observed hypocholesterolemic effect of the whole quail egg, especially at the high dose regimen on days 35 and 49. Since cholesterol is a precursor of sex steroids (Hu *et al.*,

2010), higher CESR values should not be expected of this group that had lower cholesterol level.

The role of the liver in biotransformation of xenobiotics is of great importance (Haschek *et al.*, 2009). In addition, the histomorphologic evaluation of the liver could reveal alterations in this organ and the overall metabolic activities of the individual which may not be obvious following only clinical biochemical evaluation (Haschek *et al.*, 2009; Smith *et al.*, 2013). The absence of obvious histopathological lesions in liver of rat dosed with raw whole quail eggs in this study suggests that raw quail egg consumption may not directly affect the histomorphology of the liver.

Conclusions

Consumption of whole quail egg may enhance fertility of males attributed to the increase in cauda epididymal sperm reserve. In addition prolonged consumption may predispose to cardiovascular diseases due to the increases in serum vLDL cholesterol and triglyceride.

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