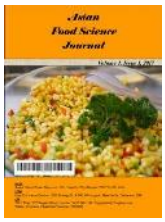

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Cholesterol and Fatty Acid Profiles of Some Bird Egg Varieties: Possible Health Implications

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Authors' contributions

This work was carried out in collaboration between all authors. Author EOO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author HNEO co-designed the study and managed the analyses of the study. Authors MII and IOW edited the draft and made some medical input in the study. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To determine the cholesterol and fatty acid profile of five bird egg varieties and the possible health implications of their consumption.
Study Design: Experimental design.
Place and Duration of Study: Department of Biochemistry, University of Calabar, Calabar, and Department of Pharmacology, University of Nigeria, Nsukka, February to July 2017.
Methodology: Five bird egg varieties studied in the raw and boiled forms were: Exotic chicken, local chicken, turkey, quail and guinea fowl eggs. Freshly-laid eggs were purchased from poultries, cleaned and divided into two- raw and boiled. After boiling, both groups of eggs were freeze-dried and milled before analyses. Standard AOAC method of Gas chromatography was used for cholesterol and fatty acid determination.
Results: The cholesterol content varied significantly (P = .05) among the raw egg varieties with

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values ranging from 435 mg/100 (quail egg) to 851 mg/100 (turkey egg). Similarly, among the boiled egg samples, quail egg had the lowest cholesterol content (455.4 ± mg/100) and turkey had the highest (1164 ± 1.33 mg/100). For the fatty acid composition, 3 saturated, 4 monounsaturated and 6 polyunsaturated fatty acids were reported in significant quantities. Palmitic acid was the most predominant saturated fatty acid with guinea fowl egg having significantly (P = .05) higher concentration and exotic chicken having significantly (P = .05) lower concentration. Oleic acid was the most predominant unsaturated fatty acid with boiled quail egg having significantly (P = .05) higher concentration (0.96 ± 0.001 g/100 g).

Conclusion: Quail egg was found to have relatively safer content of cholesterol and also had higher concentration of unsaturated fatty acids. Consequently, they are better recommended for consumption by individuals with dyslipidemia and diabetes. Consumption of turkey eggs should be moderate due to their exceptionally high cholesterol content.

Keywords: Fatty acid profile; eggs; cholesterol; health implications.

1. INTRODUCTION

According to WHO [1], non-communicable diseases (NCDs) are the leading cause of mortality in the world. It is estimated that 80% of premature heart disease, hypertension stroke and diabetes are preventable. Hypercholesterolaemia is a major risk factor for many NCDs including hypertension; WHO statistics [1] show that globally, 39% of males and 40% of females have elevated total cholesterol levels (> 5 mmol/L). This accounts for over 4.5% of deaths worldwide.

Research has shown that many animal-source foods contain cholesterol. Cholesterol, one of the most important and abundant steroids in the body, is found in the liver, bile salts and skin where it forms vitamin D. Cholesterol in the body is obtained from animal foods like eggs, milk and meat; it is also synthesised in the liver from fats, carbohydrates and proteins [2]. Dietary cholesterol is of relatively lesser amount compared with that synthesised in the liver, intestine, gonads, adrenals and skin [3]. On the other hand, approximately 40 - 50% of dietary cholesterol is absorbed. Cholesterol and saturated fat have been implicated in the etiology of some NCDs such as hypertension, stroke, obesity and diabetes [2]. It has also been discovered that eggs seem to have relatively higher content of cholesterol than some other animal foods. This is of concern considering the frequency of egg consumption by humans and the extensive use of eggs in the food and baking industry.

In the year 2000, the American Heart Association (AHA) revised its dietary guidelines and declared eggs to be nutritionally fit for healthy adults. The AHA guidelines now allow an egg a day for healthy adults while still advising a total daily cholesterol limit of 300 mg [4]. Eggs which have been consumed by humans for many centuries are a cheap and accessible source of protein. According to Pamplona-Roger [5], eggs provide the most complete protein of any food, together with fats, vitamins and minerals; hence they play an important role in human nutrition. An average egg weighs about 50 to 60 g and the egg white consists primarily of water (87%) and protein (13%) with no cholesterol and little if any fat [6]. Despite the good nutritional qualities of eggs, there has been a lot of controversy over the consumption of eggs especially with regards to some associated health risks such as hypercholesterolaemia. This is because of the relatively high content of cholesterol in eggs compared to some other foods: a large yolk contains more than two-thirds of the recommended daily intake (300 mg) of cholesterol [4].

Nutrition deals with the effects of components of food on the human body, and it starts with the physiological and biochemical processes involved in nourishment – how substances in food provide energy or are converted into body tissues, and the diseases that result from insufficiency or excess of essential nutrients [7]. Consequently, nutrition is now seen as a primary modifiable determinant of chronic disease, with scientific evidence increasingly supporting the view that alterations in diet have strong effects, both positive and negative on health throughout life [8]. Strategies are now being employed to combat the problem of malnutrition. These strategies are either long term or short term and are usually designed to enhance the energy and nutrient density of meals and formulated diets, and also to increase the production and consumption of micronutrient-dense foods

(especially animal foods like eggs and milk) [9]. The concept of food biodiversity is now widely recognised to offer some sustainable solution for combating malnutrition and food insecurity [10]. The consumption of different breeds/varieties may have a significant impact on nutrition and health outcomes. Different varieties/breeds have been reported to vary significantly in their nutrient contents [11].

Numerous species of bird eggs exist which are nutritious but there seems to be inadequate information on the nutritional qualities of the various egg species except for the popular chicken egg. According to Kiple [12], the chicken egg is the most consumed by humans but other eggs including those of quail, guinea fowl, goose, turkey and duck are also important in human nutrition. This study, therefore, seeks to comparatively evaluate the cholesterol and fatty acid composition of five commonly consumed bird eggs namely exotic chicken, local chicken, turkey, quail and guinea fowl eggs.

2. MATERIALS AND METHODS

2.1 Egg Sample Collection and Preparation

Eggs used in this study were from poultry-bred birds fed with strict diets, sourced from poultries in Nsukka, Enugu State; only freshly laid eggs were purchased for the purpose of this research. The different egg varieties were cleaned and prepared separately for analyses.

2.1.1 Preparation of raw egg samples

Shells of fresh eggs were cleaned, broken and the contents emptied into clean glass beakers. The raw egg contents were then homogenised and frozen at -40°C. The homogenized raw egg samples were freeze-dried with liquid nitrogen at -47°C and 13 x 10^{-3} mbar for 48 hours, using a freeze-drier (Model-Virtis, Gardener, New York). The obtained freeze-dried samples were milled to give fine particles using a miller (Model-Breville kitchen Wizz BFP650). Milling was done under a very low temperature of 10°C.

2.1.2 Preparation of hard-boiled egg samples

The fresh eggs were submerged in boiled (100 °C) tap water and allowed to boil for 10 min. The boiled eggs were removed and allowed to cool in tap water at room temperature. The different varieties of eggs were boiled separately and then shelled after they had cooled. The boiled eggs were placed in clean, labelled beakers and sealed with parafilm prior to undergoing freeze-drying. The boiled, whole (shelled) eggs were mashed and then freeze-dried with liquid nitrogen at -47°C and 13 x 10^{-3} mbar for 48 hours. The freeze-dried samples were then milled using a miller at a very low temperature of 10°C.

After milling, each of the samples (raw and boiled freeze-dried samples) were stored in properly labelled, air-tight sample glass bottles until ready for analyses. All the reagents used in the laboratory analyses were of standard analytical grade (AR).

2.2 Determination of Cholesterol

This was carried out according to the method of AOAC [13]. The procedure involved fat saponification and extraction, derivatisation and then quantification by gas chromatography.

2.2.1 Principle and procedure

The method for the determination of cholesterol involves alcoholic potassium hydroxide (KOH) saponification to extract lipid from samples. The non-saponifiable fraction containing cholesterol and other sterols is extracted with hexane. Sterols are derivatized to form trimethylsilyl (TMS) ethers, which are determined quantitatively by chromatograph, using 5α-cholestane as internal standard.

2.2.1.1 Saponification and extraction

Sample (3 g) was accurately weighed into a screw-capped test tube, 50% aqueous KOH (1 mL) and 4 mL 95 % ethanol were added to the test tubes which were screwed, placed in a water bath (120°C) and refluxed for 1 hour. The water bath was turned off and 2.5 mL water was added after cooling, n-hexane (5 ml) was added and then shaken vigorously for 15 sec using vortex until the layers separated. The upper organic layer was removed with a Pasteur pipette and placed in an evaporating flask. This was repeated for a total of four extractions (4 x 5 mL n-hexane). The total extract was evaporated to dryness on a vacuum rotary evaporator at 40°C.

2.2.1.2 Derivatization

Residue was dissolved in 3 mL dimethylformamide (DMF) and 1 mL of DMF sample solution was transferred into a silanized

test tube. In the fume cupboard, 0.2 mL hexamethyldisilazane (HMDS) and 0.1 mL trimethylsilyl chloride (TMCS) were added to each tube, and shaken vigorously for 1 min using vortex mixer. The solution stood undisturbed for 15 min after which 1 mL 5-α-cholestane (0.1 mg/mL) and 10 mL distilled water was added and the test tube shaken vigorously for 1 min.

2.2.1.3 GLC Measurement

One microlitre of upper heptane layer was injected into gas chromatograph followed by 1 µl each of standard solution.

2.2.1.4 Calculation

Cholesterol (mg/100 g) = [{Cholesterol from standard curve (mg/mL)/ Weight of sample (g)} x dilution x 100]

2.3 Determination of Fatty Acid Composition

This was carried out after saponification of fat and derivatisation to fatty acid methyl esters (FAMEs) using the method of Wang [14].

2.3.1 Principle and procedure

For fatty acid profile, the lipid fraction is obtained by cold extraction using a mixture of chloroform and methanol. Pyrogallic acid is added to minimise oxidative degradation of fatty acids during analysis. The lipid portion is then saponified, derivatised to FAMEs with boron trifluoride/methanol, and determined by gas chromatography using Flame Ionisation Detector (FID). This method determines the percentage area of the fatty acids and the absolute concentrations of specific fatty acids were estimated using fatty acids internal standards.

2.3.1.1 Fat extraction (cold extraction)

Sample (5 g) was accurately weighed into a 125 mL round bottom flask. Methanol (50 mL) was poured into the flask, then a magnetic bar was added, and stirred for 30 min. The solution was filtered through filter paper No.1 into a separating funnel. The flask was rinsed with 2 x 25 mL methanol and added into the same funnel then distilled water (20 mL) was poured into the separating funnel and swirled gently. This was kept standing until two layers were completely separated. The lower layer was collected into a round bottom flask, and then the solvent evaporated off by rotary evaporator until the solution was nearly dry. This was dissolved with 2 mL of each of iso-propanol and hexane; evaporation was continued to remove the solvents until nearly dry. The residue was blown with oxygen-free nitrogen (OFN) until dry. The residue obtained was the extracted lipid to be analysed for fatty acids.

2.3.1.2 Saponification and methylation

Extracted fat of 0.1 g was weighed into screw cap tube. A portion (2 mL) of 0.5 N NaOH was added to the methanol in the tube and shaken. The tube was placed in a water bath at 100°C for 8 min, cooled and then 3 mL BF_3 was added. This was shaken and heated at 90ºC for 15 min, then cooled. Iso-octane (3 mL) and 3 mL of saturated NaCl solution was added and the test tube shaken vigorously. This was centrifuged until the iso-octane (upper) layer separated from the aqueous phase. The upper layer was passed through a small amount of anhydrous Na_2SO_4 into a test tube using a Pasteur pipette. The aqueous phase was re-extracted twice with 2 mL iso-octane, then iso-octane layer was transferred through the Na_2SO_4 into the same test tube using the same Pasteur pipette. The solvent was evaporated off by rotary evaporator under a stream of OFN. The FAMEs were dissolved and diluted to an appropriate volume using isooctane in a volumetric flask. The sample was ready and then injected to the GC column.

2.3.1.3 Calculation

Quantification as absolute concentration of specific fatty acids is given by:

Concentration of fatty acids (mg/g of fat) = (Ax × WIS × CFx × 1000/ AIS × WS × Fx)

where:

Ax = area of each fatty acid methyl ester
AIS = area of fatty acid internal standard
WIS = weight of internal standard (mg)
WS = weight of sample (g)
CFx = correction factor of detector for certain fatty acid obtained from AOCS reference mixture
Fx = factor necessary to express result as mg fatty acid/g oil (rather than as methyl ester)
=MW (molecular weight) of ester / MW of fatty acid.

(Results were later multiplied by fat content of the samples (g/100 g) to convert the fatty acid content to mg/100 g sample).

3. RESULTS AND DISCUSSION

3.1 Cholesterol Content

The cholesterol content (Fig. 1) varied significantly (P = .05) between all the raw egg varieties with values ranging from 435 to 851 mg/100 g however, boiled turkey egg had the highest cholesterol content of 1164 mg/100 g. The cholesterol content of the boiled egg samples were higher than those of the raw egg samples. Raw quail egg had the lowest value and raw turkey egg had the highest value and a similar trend was observed among the boiled samples with boiled quail egg having the lowest content and boiled turkey egg having the highest content. Only the cholesterol content of boiled exotic chicken egg and local chicken egg were statistically similar (P = .05). The other boiled eggs varied significantly (P = .05) in their cholesterol concentrations.

The cholesterol content of turkey egg was almost double that of the other eggs both among the raw and boiled samples. This calls for concern because an average turkey egg weighs about 70 g with some weighing as high as 110 g depending on the age of the bird or breed. This means that one turkey egg can supply over 3 times the RDA for cholesterol. This could be quite unsafe for individuals with health conditions such as diabetes, obesity and hypercholesterolaemia. The cholesterol content of quail egg (both raw and boiled) was significantly lower (P = .05) than those of the other four varieties. This makes quail eggs safe to some extent, even for people with dyslipidemias. In addition, an average quail egg is small in size and weighs about 15 g, hence consumption of one or two quail eggs in a day, will not yield up to one-third the RDA for cholesterol. These results confirm that different varieties/species vary significantly in their nutrient contents, and some varieties/breeds are healthier in nutrient content than others. Only the cholesterol content of exotic chicken and local chicken eggs were slightly similar in both the raw and boiled forms. The values obtained in this

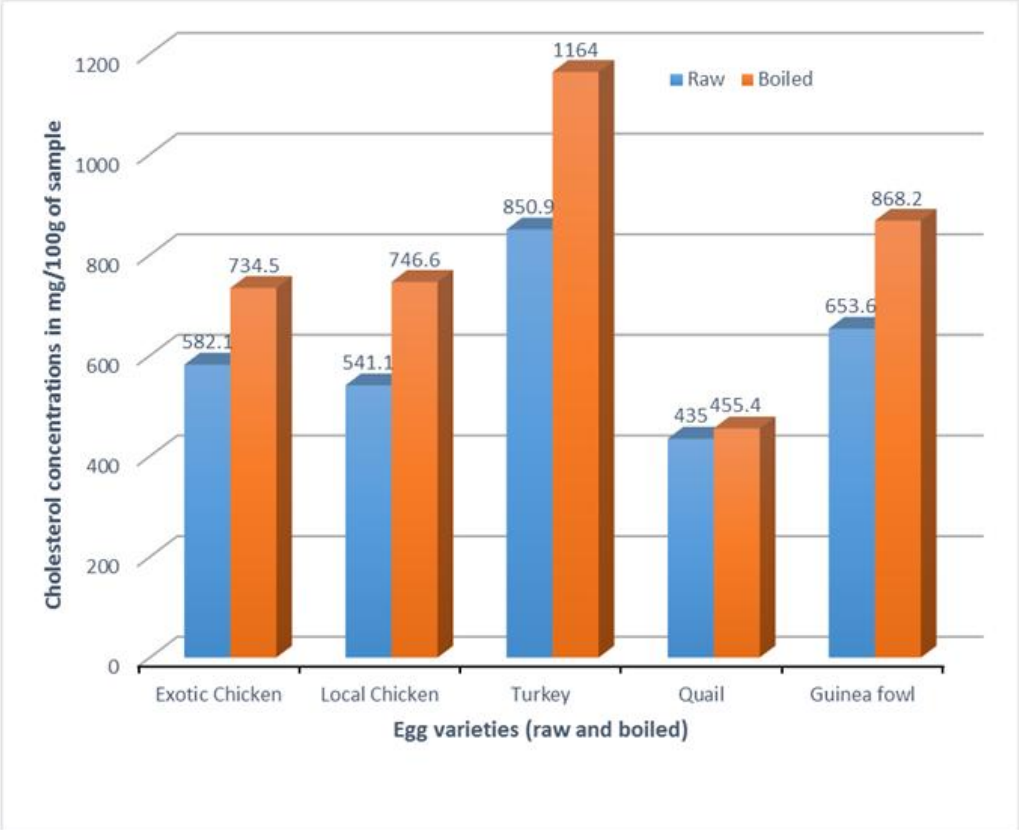

Fig. 1. Cholesterol content of five bird eggs in their raw and boiled forms
Mean ± S.E.M = Mean values ± Standard error of means of three experiments

study were similar to those reported by Jaludeen and Churchill [15] except for that of quail eggs which had higher cholesterol values in their research. This may be due to the breed of quail birds, diets fed to the birds and the analytical methods used in their study. On the other hand, the increase in cholesterol values in the boiled samples may be as a result of coagulation of the egg proteins (particularly albumin); during protein coagulation, moisture is said to be absorbed by protein binding with the water hydroden molecules thereby causing a reduction in moisture content and an increase in dry matter of the boiled samples [16]. This may explain the increased cholesterol concentrations of the boiled eggs.

As a result of the rising prevalence of NCDs such as hypertension and obesity, a lot of research is ongoing in the area of risk factors causing these diseases. The risk factors include hyperglycaemia, hypercholesterolaemia and unhealthy lifestyles. The results of researches carried out in different parts of the world such as Natoli, et al. [17] and Hu, et al. [18] showed that increase in dietary cholesterol intake did not cause a corresponding (unhealthy) increase in blood cholesterol levels. This means that increased dietary cholesterol intake may only lead to hypercholesterolaemia in individuals with genetic or pre-existing problems of dyslipidaemia and diabetes. In order to maintain a healthy blood lipid profile, such individuals have to restrict their dietary intake of both cholesterol and fats (especially saturated and trans fats); not only from eggs but also from other dietary sources. On the other hand, McNamara [19] reported that increase in dietary cholesterol also increased HDL-c thereby reducing the LDL/HDL ratio and risk of coronary heart disease (CHD). This should go a long way to dispel the myth that 'eggs are bad for your blood cholesterol' thereby allowing a lot of people (not children only), to benefit from the exceptional nutritional value of different varieties of eggs.

3.2 Fatty Acid Composition

The results of the fatty acid compositions are presented in Table 1. Thirteen fatty acids were found present in significant quantities in the five egg species. Out of these thirteen fatty acids, three were saturated fatty acids (SFAs), four were monounsaturated fatty acids (MUFAs) and the other six were polyunsaturated fatty acids (PUFAs). Among the saturated fatty acids, palmitic acid had the highest concentrations, with values ranging from 0.45 to 0.90 g/100 g. In both raw and boiled samples, guinea fowl egg had significantly (P = .05) higher palmitic acid content than the other egg varieties. Among the ten unsaturated fatty acids present, oleic acid was the most abundant with raw and boiled quail eggs having significantly (P = .05) higher values than the other varieties. Erucic acid was also present in significant quantities in both raw and boiled eggs with the boiled samples having significantly (P = .05) greater values; clupanodonic acid and docosahexaenoic acid (DHA) were only found in trace amounts in turkey and quail eggs (in both their raw and boiled forms). Local chicken had the highest concentration of DHA in both raw and boiled forms. Calculating percentage compositions of the raw eggs, the MUFAs had the highest percentage composition ranging from 87.6% (in quail eggs) to 72.2% (in turkey eggs). Turkey egg had the highest content of SFA (14%) while that of quail egg was the least (8.1%). For the PUFAs, turkey eggs had the highest percentage composition (12.1%) while quail eggs had the least (3.2%).

The unsaturated fatty acids (both MUFAs and PUFAs) which are considered to be healthier than the saturated fatty acids (SFAs), made up between 78 to 88% of the various eggs' fatty acid profiles. The SFAs constituted between 8.1% in quail eggs to 12.8% in guinea fowl eggs. Quail egg also had a high percentage content of the MUFAs. Combined with its low SFA content, quail eggs may boost HDL-c and have a cardio-protective effect. Epidemiological and clinical studies have established that among the PUFAs, the n-6 fatty acid linoleic acid (LA), and the n-3 fatty acids, linolenic acid (LNA), DHA and EPA collectively protect against coronary heart disease, when they are in adequate balance [17]. LA is the major dietary fatty acid regulating LDL-c metabolism by downregulating LDL-c production and enhancing its clearance; LA further determines the hyperlipidemic effects of other dietary fat components such as SFAs and trans fatty acids [20]. DHA and EPA also improve vascular endothelial function and help lower blood pressure and serum TG level. Most of the egg varieties had similar concentrations of these PUFAs. Guinea fowl, exotic and local chicken eggs had significant quantities of arachidonic acid (ARA) – an important n-6 fatty acid. DHA and ARA are found in the grey matter of the brain and play an important function in both fetal and infant brain development [21]; hence consumption of these three egg varieties by

Table 1. Fatty acid composition of raw and boiled egg varieties in g/100 g

Egg species	Saturated fatty acids			Monounsaturated fatty acids				Polyunsaturated fatty acids					
	Myristic	Palmitic	Stearic	P/oleic	Oleic	Gadoleic	Erucic	Linoleic	α-Linolenic	Parinaric	Arach	Clupano	DHA
Raw													
Exotic chicken	0.05^{a}	0.52^{b}	0.18^{c}	0.18^{c}	2.66^{c}	0.02^{b}	1.89^{c}	0.05^{b}	0.04^{b}	0.19^{b}	0.09^{a}	0.06^{a}	0.05^{a}
Local chicken	0.04^{a}	0.45^{c}	0.15^{d}	0.16^{d}	2.31^{d}	0.02^{b}	1.64^{e}	0.04^{c}	0.04^{b}	0.17^{c}	0.08^{a}	0.05^{b}	0.06^{a}
Turkey	0.04^{a}	0.58^{b}	0.29^{a}	0.19^{c}	2.17^{e}	0.01^{c}	1.82^{d}	0.38^{a}	0.05^{b}	0.20^{b}	0.07^{b}	Tr	Tr
Quail	0.05^{a}	0.58^{b}	0.26^{b}	0.74^{a}	5.79^{a}	0.01^{c}	1.96^{b}	0.04^{c}	0.03^{b}	0.19^{b}	0.05^{b}	Tr	Tr
Guinea fowl	0.05^{a}	0.69^{a}	0.16^{d}	0.21^{b}	2.75^{b}	0.04^{a}	2.48^{a}	0.04^{c}	0.14^{a}	0.29^{a}	0.08^{a}	0.05^{b}	0.05^{a}
Boiled													
Exotic chicken	0.05^{a}	0.48^{e}	0.37^{d}	0.48^{c}	5.30^{c}	0.09^{a}	3.07^{c}	0.05^{b}	0.06^{c}	0.25^{b}	0.12^{a}	0.08^{a}	0.07^{a}
Local chicken	0.04^{b}	0.71^{c}	0.34^{e}	0.40^{e}	5.18^{d}	0.07^{b}	2.83^{e}	0.04^{b}	0.05^{c}	0.24^{b}	0.13^{a}	0.08^{a}	0.08^{a}
Turkey	0.05^{a}	0.89^{b}	0.57^{a}	0.64^{b}	5.08^{e}	0.06^{b}	3.45^{b}	0.52^{a}	0.11^{a}	0.25^{b}	0.11^{b}	Tr	Tr
Quail	0.05^{a}	0.64^{d}	0.40^{c}	0.96^{a}	7.27^{a}	0.04^{c}	2.85^{d}	0.04^{b}	0.04^{d}	0.15^{c}	0.06^{c}	Tr	Tr
Guinea fowl	0.06^{a}	0.90^{a}	0.46^{b}	0.46^{d}	6.91^{b}	0.10^{a}	4.65^{a}	0.05^{b}	0.26^{b}	0.37^{a}	0.13^{a}	0.07^{a}	0.07^{a}

Values are expressed as mean ± SEM, n = 3 at p = .05; Note: All the SEMs were less than 0.0005 and hence rounded off. Values with different superscripts on the same column are significantly different (P = .05) from each other

pregnant mothers and growing infants, should be encouraged. As a result of the increasing awareness and demand for functional foods, several PUFA-enriched eggs are now produced and sold; these are commonly called 'specialty eggs' [22]. Simopoulos and Salem [23] reported higher levels of n-3 PUFAs in eggs from free range birds consuming a variety of diets. This may explain why the turkey and quail eggs contained trace amounts of DHA as the eggs used in this study were from poultry-bred birds, fed strict diets (formulated feeds). In another study by Beynen [24], it was discovered that feeding hens with linseed and soya beans significantly raised the concentrations of DHA in the hen eggs thus making these eggs contribute significantly to the daily DHA requirement of humans.

4. CONCLUSION

Assessment of the cholesterol and fatty acid composition of some bird eggs has shown that quail eggs have relatively safer content of cholesterol and healthier fatty acid profile than others. In order to reduce the risk of NCDs, consumption of varieties such as quail egg should be more recommended especially for adults with dyslipidaemias and diabetes; while other varieties like turkey and guinea fowl eggs should be consumed in moderation due to the far higher cholesterol and fatty acid content they possess. The AHA [4] guidelines may not have covered the eggs with exceptionally high cholesterol and fat content, hence caution should be applied when recommending the daily intake for adults especially those with pre-existing health conditions.

ETHICAL APPROVAL

As per international standard or university standard written ethical permission has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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