

Effects of Four Dietary Oils on Cholesterol and Fatty Acid Composition of Egg Yolk in Layers

A. F. Agboola, B. R. O. Omidwura, A. Oyeyemi, E. A. Iyayi, A. S. Adelan

Abstract—Dietary cholesterol has elicited the most public interest as it relates with coronary heart disease. Thus, humans have been paying more attention to health, thereby reducing consumption of cholesterol enriched food. Egg is considered as one of the major sources of human dietary cholesterol. However, an alternative way to reduce the potential cholesterolemic effect of eggs is to modify the fatty acid composition of the yolk. The effect of palm oil (PO), soybean oil (SO), sesame seed oil (SSO) and fish oil (FO) supplementation in the diets of layers on egg yolk fatty acid, cholesterol, egg production and egg quality parameters were evaluated in a 42-day feeding trial. One hundred and five Isa Brown laying hens of 34 weeks of age were randomly distributed into seven groups of five replicates and three birds per replicate in a completely randomized design. Seven corn-soybean basal diets (BD) were formulated: BD+No oil (T₁), BD+1.5% PO (T₂), BD+1.5% SO (T₃), BD+1.5% SSO (T₄), BD+1.5% FO (T₅), BD+0.75% SO+0.75% FO (T₆) and BD+0.75% SSO+0.75% FO (T₇). Five eggs were randomly sampled at day 42 from each replicate to assay for the cholesterol, fatty acid profile of egg yolk and egg quality assessment. Results showed that there were no significant ($P>0.05$) differences observed in production performance, egg cholesterol and egg quality parameters except for yolk height, albumen height, yolk index, egg shape index, haugh unit, and yolk colour. There were no significant differences ($P>0.05$) observed in total cholesterol, high density lipoprotein and low density lipoprotein levels of egg yolk across the treatments. However, diets had effect ($P<0.05$) on TAG (triacylglycerol) and VLDL (very low density lipoprotein) of the egg yolk. The highest TAG (603.78 mg/dl) and VLDL values (120.76 mg/dl) were recorded in eggs of hens on T₄ (1.5% sesame seed oil) and was similar to those on T₃ (1.5% soybean oil), T₅ (1.5% fish oil) and T₆ (0.75% soybean oil + 0.75% fish oil). However, results revealed a significant ($P<0.05$) variations on eggs' summation of polyunsaturated fatty acid (PUFA). In conclusion, it is suggested that dietary oils could be included in layers' diets to produce designer eggs low in cholesterol and high in PUFA especially omega-3 fatty acids.

Keywords—Dietary oils, Egg cholesterol, Egg fatty acid profile, Egg quality parameters.

I. INTRODUCTION

EGG is considered as one of the major sources of human dietary cholesterol, the average content ranges from 195 to 230mg per egg. However, eggs are also very valuable source of proteins and contain many substances with biological functions beyond basic nutrition [1]-[3]. Egg lipids are confined to the yolk and account for about 30% of the fresh weight of the yolk and for 60% of the dry matter [4]. In chickens, breed or strain [5] or age of the hen [6] can influence

yolk cholesterol; nonetheless, information on the influence of strain and age on egg fatty acid composition throughout the laying cycle is controversial [7]. This is because the reduction of the cholesterol content of poultry products has met with little success; dietary fatty acid modification has been pursued as a viable method of adding value to poultry products. Due to the numerous proposed cardiovascular benefits associated with consumption of omega-3 (n-3) fatty acids, marketing of eggs enriched by n-3 fatty acid may benefit the producer [8]. An alternative way to reduce the potential cholesterolemic effect of eggs is to modify the fatty acid composition of the yolk [7]. The fatty acid composition of egg lipids in laying hens can be influenced by the fatty acid composition of their diet [9]. The eggs from hens provided with standard feed are poor in linolenic acid (LNA; C18:3n-3), and does not contain eicosapentaenoic (EPA; C20:5n-3) and docosahexaenoic (DHA; C22:6n-3) fatty acids [10]. The majority of the egg's fatty acids are monounsaturated (~44%) with saturated and polyunsaturated accounting for ~29 and 11%, respectively [11].

There is evidence that hens have a unique ability to deposit dietary lipid into the egg yolk, which makes the egg a potential source of polyunsaturated fatty acids (PUFAs) [12]. The inclusion of n-3 PUFA promotes a qualitative change in the yolk fatty acid profile and reducing the n-6/n-3 ratio to a more beneficial level concerning the human nutritional needs [13].

Several studies have been conducted to determine the effect of different n-3 fatty acids sources in diets on the cholesterol and fatty acid composition of egg yolk [3], [12], [14]-[17]. However, there is paucity of information on the comparative effects of palm oil, soybean oil, sesame seed oil, and fish oil on egg yolk cholesterol and fatty acid composition and performance characteristics of layers.

II. METHODOLOGY

A. Experimental Site

This study was conducted at the Egg Production Unit, Teaching and Research Farm, University of Ibadan, Ibadan, Oyo State, Nigeria.

B. Experimental Diets and Management of Birds

One hundred and five (105) Isa Brown layers of thirty-four weeks of age were used for this study. The layers were housed in a battery cage kept under semi-controlled environmental conditions with exposure to 16-hours photoperiod. Seven experimental diets were formulated according to [18] standard specifications for brown egg laying hens. Treatment 1 (T₁)

Adebisi Agboola is with the University of Ibadan, Nigeria (e-mail: adebisi.agboola@gmail.com).

was the basal diet with no dietary oil inclusion (positive control). Treatment 2 (T₂) contained basal diet + 1.5% palm oil (negative control); treatment 3 (T₃) basal diet + 1.5% soybean oil; treatment 4 (T₄) basal diet + 1.5% sesame seed oil; treatment 5 (T₅) basal diet + 1.5% fish oil; treatment 6 (T₆) basal diet + 0.75% soybean oil and 0.75% fish oil and treatment 7 (T₇) basal diet + 0.75% sesame seed oil and 0.75% fish oil. The study lasted for six weeks. Birds were weighed, tagged, and randomly assigned to receive one of the seven experimental diets consisted five replicates with three birds each. The layers were fed experimental diets and provided with clean water for the duration of the study.

C. Experimental Design

The experimental design was a completely randomised design (CRD).

D. Data and Sample Collection

Performance parameters (feed intake, egg production, egg weight, and feed conversion ratio) were calculated during the course of the trial. To determine the cholesterol and fatty acid profile of egg yolk, three eggs were randomly sampled at week six of the experiment from each treatment respectively. Egg quality parameters (yolk weight, yolk index, albumin weight, haugh unit, shell weight, and shell thickness) were measured at week six, using five eggs from each treatment.

E. Performance Evaluation

Daily egg production per replicate was recorded and number of eggs per hen per week was calculated. Eggs laid per replicate were weighed daily and average weight for that particular week was calculated. The data thus generated (Egg production and Egg weight) was used to calculate egg mass/bird/week (weekly egg no. in replicate x average egg weight). Weekly feed intake was determined (total feed offered during a week - Feed refused at the end of week). Data on feed intake and egg mass were used to calculate feed conversion (feed intake/egg mass; g/g).

F. Egg Quality Evaluation

1. External Qualities

Egg weight was measured using Mettler top-loading weigh balance. The length and width of each egg (in cm) was measured using Vernier calliper. The width was measured as the distance between two ends of the egg at the widest cross sectional region using Vernier calliper. The length was measured as the distance between the broad and narrow ends of the eggs.

2. Egg Shape Index (ESI)

This was calculated as the percentage of the egg breadth (width) to the egg length [19]. The formula that was used is as follows:

$$\text{Egg Shape Index} = \frac{\text{Width of egg (mm)}}{\text{Length of egg (mm)}} \times 100$$

The thickness of individual air-dried shells is measured to the nearest 0.01mm using micrometre screw gauge [20]. Eggshells were air-dried in the crates. The relative shell weight was calculated by relating the shell weight to the weight of the egg. Shell thickness was measured using a micrometre meter gauge (in mm).

G. Internal Qualities

Yolk height, yolk width and yolk diameter (in cm) were measured using a Vernier calliper. **Albumen height:** The egg was gently broken and the maximum albumen height was measured with tripod micrometre [21]. **Albumen weight:** is the difference between the egg weight and the sum of weight of yolk and dry eggshell expressed as a percentage of the whole egg. **Percentage Albumen weight:** was calculated as the percentage of the albumen weight to other egg weight. **Yolk weight:** was measured using Mettler top-loading weighing balance. **Percentage Yolk weight:** was calculated as the percentage of the yolk weight to the egg weight. **Yolk index:** was estimated from ratio of yolk height to yolk width. **Visual yolk colour:** was determined with a yolk colour fan (scale 1 to 15). **Haugh unit (HU):** is a relationship between egg weight and height of thick albumen surrounding yolk. This was calculated using the values obtained from the egg weight and albumen height as expressed by [22] in the formula:

$$HU = 100 \log [H + 7.57 - 1.7 W^{0.37}]$$

where, H = Albumen Height (mm) and W = Weight of the egg (g).

1. Fatty Acid Analysis of Egg Yolk and Experimental Diets

Fatty acid analysis of egg yolk, test diets, and dietary oils was done as described by [23]. 2g of sample was weighed into 100ml conical flask; 20ml of benzene was added, shaking thoroughly to extract all the fatty acids. The mixture was transferred into 250ml separating funnel to separate the benzene extract from the aqueous extract. 5ml aliquot of the benzene extract was pipetted into 15ml test tube and 2ml of 10% copper acetate was added to develop colour. Absorbance or optical density of sample extracts was read on a spectrophotometer at a wavelength of 630nm.

Standard solutions of each fatty acids were prepared in the range 0-10ppm from 100ppm stock solution of each fatty acids. Absorbance or optical density of standard solutions of different concentrations were read on a spectrophotometer at a wavelength defined for each fatty acids as listed thus: Capric acid (700nm), Lauric acid (640nm), Myristic acid (655nm), Palmitic acid (630nm), Palmitoleic acid (685nm), Stearic acid (650nm), Oleic acid (670nm), Linoleic acid (660nm), Linolenic acid (680nm) and Arachidonic acid (690nm).

The percentage of each fatty acid was obtained using:

$$\% \text{Fatty Acid} = \frac{\text{Absorbance of sample} \times \text{Gradient Factor of a specific fatty acid} \times \text{Dilution Factor}}{\text{Weight of Sample} \times 10000}$$

TABLE I
GROSS COMPOSITIONS OF EXPERIMENTAL DIETS (G/100G DM)

Ingredient	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇
Maize	58.05	53.55	53.55	53.55	53.55	53.55	53.55
Soybean meal	22.00	22.00	22.00	22.00	22.00	22.00	22.00
Wheat offal	9.20	12.20	12.20	12.20	12.20	12.20	12.20
Palm oil	0.00	1.50	0.00	0.00	0.00	0.00	0.00
Soya bean oil	0.00	0.00	1.50	0.00	0.00	0.75	0.00
Sesame oil	0.00	0.00	0.00	1.50	0.00	0.00	0.75
Fish oil	0.00	0.00	0.00	0.00	1.50	0.75	0.75
Limestone	8.50	8.50	8.50	8.50	8.50	8.50	8.50
Di-Calcium Phosphate	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Vit-Min Premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30
DL-Methionine	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Lysine	0.10	0.10	0.10	0.10	0.10	0.10	0.10
TOTAL	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated Nutrient							
Crude Protein	17.11	17.16	17.16	17.16	17.16	17.16	17.16
ME, kcal/kg	2699.80	2718.61	2723.75	2723.02	2720.54	2722.15	2721.78
Fat	2.73	2.70	2.70	2.70	2.70	2.70	2.69
Crude Fibre	3.22	3.46	3.46	3.46	3.46	3.46	3.46
Calcium	3.60	3.61	3.61	3.61	3.61	3.61	3.61
Total Phosphorus	0.49	0.50	0.50	0.50	0.50	0.50	0.50
Methionine	0.38	0.38	0.38	0.38	0.38	0.38	0.38
Lysine	0.98	0.99	0.99	0.99	0.99	0.99	0.99
Sodium	0.22	0.23	0.23	0.23	0.23	0.23	0.23

T₁- Basal diet; T₂- Basal diet + 1.5% Palm oil; T₃- Basal diet + 1.5% Soybean oil; T₄- Basal diet + 1.5% Sesame seed oil; T₅- Basal diet + 1.5% Fish oil; T₆- Basal diet + 0.75% Soya-bean oil + 0.75% Fish oil; T₇- Basal diet + 0.75% Sesame seed oil + 0.75% Fish oil, Vit-Min Premix- Vitamin-Mineral Premix, ME- Metabolisable Energy

*2.5kg Premix supplied contain Vitamin A-10,000,000Iu; Vit D3-2,000,000Iu; Vit. E-23,000m; Vit K3-2,000mg; Vit B1-3000mg; Vit B2-6,000mg; Nicotinic acid-50,000mg; Calcium Pantothenate-50,000mg; Vit B6-5,000mg; Vit B12-25mg folic acid-1000mg; Biotin-50mg; choline chloride-400,000mg; manganese-120,000mg; iron-100,000mg; zinc-80,000mg; copper-8,500mg; Iodine-1,500mg; cobalt-300mg; selenium-120mg and Anti-Oxidant-120,000mg.

H. Egg Yolk Cholesterol Quantification

The eggs used for this analysis were prepared according to the procedure described by [24]. The eggs were first hard-cooked, allowed to cool, after which the weight of the boiled eggs was noted. The eggshell was peeled off and weighed followed by careful removal of the egg albumen. The yolks were separated, weighed, and crumbled. 1g of the yolk was homogenized with 15ml of chloroform-methanol 2:1 (v/v), thoroughly mixed, and filtered. Egg homogenate filtrates were designated egg yolk samples. Total Cholesterol (TC), HDL, and Total Triglyceride (TG) concentration in the egg yolk were determined using the respective cholesterol assay kit. The kit contained cholesterol assay reagent and standard cholesterol solution, used for calibration curve. TC was determined using 10µl of egg yolk sample and 10µl of deionized water were pipetted into a test tube, followed by 1000 µl 6 recorded as mg/g of egg yolk was computed from various values obtained from the various cholesterol standard. This method was used to analyse the HDL, except that HDL reagent was used. TG were automated, gradients was collected in tubes and was analysed using an auto-analyser.

LDL was calculated as expressed by [25]:

$$\text{LDL} = \text{TC} - \text{HDL} - (\text{TG}/5)$$

where LDL is Low Density Lipoprotein, TC is Total Cholesterol, HDL is High Density Lipoprotein, and TG is Triglyceride. VLDL was calculated as expressed by [25]:

$$\text{VLDL} = \text{TG}/5$$

where VLDL is Very low Density Lipoprotein and TG is Triglyceride.

I. Proximate Analysis

Proximate analysis of the test diet samples was determined following the procedures of [26]. Moisture content was determined by drying 2g of feed samples in an oven at 100-105°C for 24 hours until a constant weight was reached. Moisture content value was obtained by subtracting the weight of oven-dried sample from the feed sample. Crude protein was determined by using Kjeldahl method, which comprised digestion, distillation, and titration of the distillate. Crude

protein value was obtained by multiplying percentage nitrogen content with 6.25 (crude protein content). Fat was determined with soxhlet extraction method using petroleum ether. Ash content was determined by igniting 2g of the feed sample in a Muffle furnace set between 550 and 600°C for 4 hours, the residue was allowed to cooled and weighed. The ash content value was obtained by subtracting the weight of ash residue from the feed sample.

J. Statistical Analysis

Data were analysed using descriptive statistics and Analysis of Variance (ANOVA) of GLM procedure [27]. Means were separated using Duncan Multiple Range Test [28].

III. RESULTS

The results on the effect of dietary oils inclusion on the fatty acid composition of egg yolk at day 42 are presented in Table II. There were no significant differences ($P>0.05$) observed in $C_{16:0}$ -Palmitic acid, $C_{18:0}$ -Stearic acid, $C_{18:1}$ -Oleic acid, $C_{18:2n-6}$ -Linoleic acid, $C_{18:3n-3}$ -Linolenic acid and $C_{20:0}$ -Arachidonic acid levels of egg yolk. However, $C_{10:0}$ -Lauric acid level of egg yolk was significant ($P<0.05$) across the treatments. The $C_{10:0}$ level (11.21%) recorded in egg yolks from birds on treatment 4 (1.5% sesame seed oil inclusion) was similar to those on treatments 3 (1.5% soybean oil), 5 (1.5% fish oil), 6 (0.75% soybean oil + 0.75% fish oil), and 1 (no dietary oil inclusion). These were significantly ($P<0.05$) higher than that of treatments 2 (1.5% palm oil) and 7 (0.75% sesame seed oil + 0.75% fish oil). The summation of the saturated fatty acids, monounsaturated fatty acids) [$C_{18:1}$ -Oleic acid] and n-6/n-3 ratio (omega-6 to omega-3 ratio) in this study were not significant ($P>0.05$). However, the summation of PUFA

(polyunsaturated fatty acids) [$C_{18:2n-6}$ -Linoleic acid and $C_{18:3n-3}$ -Linolenic acid] was significant ($P<0.05$) across the treatments. The PUFA level (31.26%) recorded in eggs of hens on treatments 5 (1.5% fish oil inclusion) and 6 (0.75% soybean oil + 0.75% fish oil inclusion) which was similar to those on other treatments except treatment 2 (30.77%) which contained 1.5% palm oil inclusion.

The results on the effect of dietary oils inclusion on egg quality are presented in Table V. There were no significant differences ($P>0.05$) observed in egg weight, yolk weight, yolk ratio, albumen weight, albumen ratio, shell weight, shell ratio, and shell thickness. However, there were significant differences ($P<0.05$) recorded in yolk height, albumen height, yolk index, egg shape index, haugh unit, and yolk colour.

The results on the effect of dietary oils inclusion on the lipid profile of egg yolk at day 42 of the trial are presented in Table III. There were no significant differences ($P>0.05$) observed in total cholesterol, high density lipoprotein and low density lipoprotein levels of egg yolk across the treatments. However, there were significant differences ($P<0.05$) recorded in TAG (triacylglycerol) and VLDL (very low density lipoprotein) levels of egg yolk across treatment. The higher TAG level (603.78 mg/dl) and VLDL level (120.76 mg/dl) were recorded in eggs of hens on treatment 4 (1.5% sesame seed oil inclusion) and was similar to those on treatments 3 (1.5% soybean oil inclusion), 5 (1.5% fish oil inclusion) and 6 (0.75% soybean oil + 0.75% fish oil).

TABLE II
DIETARY OILS INCLUSION ON THE FATTY ACID COMPOSITIONS OF EGG YOLK AT DAY 42 OF THE EXPERIMENT

Fatty Acids	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	SEM	P value
$C_{10:0}$	11.20 ^a	11.07 ^c	11.18 ^{ab}	11.21 ^a	11.17 ^{ab}	11.19 ^a	11.11 ^{bc}	0.02	0.0078
$C_{16:0}$	14.33	14.17	14.36	14.24	14.11	14.15	14.29	0.09	0.3755
$C_{18:0}$	10.38	10.28	10.33	10.36	10.27	10.33	10.42	0.06	0.4716
$C_{18:1}$	15.71	15.78	15.66	15.63	15.72	15.63	15.86	0.08	0.4274
$C_{18:2n-6}$	15.58	15.44	15.53	15.63	15.72	15.63	15.66	0.11	0.6742
$C_{18:3n-3}$	15.58	15.33	15.53	15.51	15.54	15.63	15.42	0.10	0.4773
$C_{20:0}$	17.24	17.33	17.39	17.42	17.48	17.46	17.23	0.12	0.6463
Σ saturated	53.14	52.85	53.26	53.23	53.03	53.13	53.05	1.60	1.0000
Σ MUFA	15.71	15.78	15.66	15.63	15.72	15.63	15.86	0.08	0.4274
Σ PUFA	31.16 ^{ab}	30.77 ^b	31.06 ^{ab}	31.14 ^{ab}	31.26 ^a	31.26 ^a	31.08 ^{ab}	0.06	0.0257
n-6/n-3 ratio	1.00	1.01	1.00	1.01	1.01	1.00	1.02	1.10	1.4125

*Means on the same row with different superscripts are significantly ($P<0.05$) different. SEM-Standard Error of Mean; $C_{10:0}$ -Lauric, $C_{16:0}$ -Palmitic, $C_{18:0}$ -Stearic, $C_{18:1}$ -Oleic, $C_{18:2n-6}$ -Linoleic, $C_{18:3n-3}$ -Linolenic, $C_{20:0}$ -Arachidonic; Σ -Summation, MUFA-Monounsaturated Fatty Acid, PUFA-Polyunsaturated Fatty Acid, n-6 (omega-6), n-3 (omega-3), T₁- Basal diet; T₂- Basal diet + 1.5% Palm oil; T₃- Basal diet + 1.5% Soybean oil; T₄- Basal diet + 1.5% Sesame seed oil; T₅- Basal diet + 1.5% Fish oil; T₆- Basal diet + 0.75% Soybean oil + 0.75% Fish oil; T₇- Basal diet + 0.75% Sesame seed oil + 0.75% Fish oil

TABLE III
LIPID PROFILE (MG/DL) IN EGG YOLK OF HENS ON DIETARY OILS SUPPLEMENTED DIETS

Lipid Profile	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	SEM	P value
TC	82.36	72.22	71.91	84.37	56.30	74.13	62.22	10.05	0.4623
TAG	437.79 ^b	461.82 ^b	501.90 ^{ab}	603.78 ^a	489.27 ^{ab}	485.49 ^{ab}	439.80 ^b	41.34	0.0158
HDL	7.143	5.953	6.273	3.273	5.953	5.353	6.843	1.84	0.8110
LDL	0.933	4.833	4.293	0.000	0.000	2.643	0.000	2.66	0.7081
VLDL	87.56 ^b	92.36 ^b	100.38 ^{ab}	120.76 ^a	97.85 ^{ab}	97.10 ^{ab}	87.96 ^b	8.27	0.0158

*Means on the same row with different superscripts are significantly ($P < 0.05$) different. SEM-Standard Error of Mean; TC-Total Cholesterol, TAG-Triacylglycerol, HDL-High Density Lipoprotein, LDL-Low Density Lipoprotein, VLDL-Very Low Density Lipoprotein

T₁- Basal diet; T₂- Basal diet + 1.5% Palm oil; T₃- Basal diet + 1.5% Soybean oil; T₄- Basal diet + 1.5% Sesame seed oil; T₅- Basal diet + 1.5% Fish oil; T₆- Basal diet + 0.75% Soybean oil + 0.75% Fish oil; T₇- Basal diet + 0.75% Sesame seed oil + 0.75% Fish oil

TABLE IV
DIETARY OILS INCLUSION ON PRODUCTION PERFORMANCE OF LAYING BIRDS

Production Performance	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	SEM	P value
Egg production (%)	83.97	80.53	81.75	83.76	78.03	85.71	82.06	3.85	0.8526
Egg weight (g)	61.83	59.99	57.23	58.52	57.65	60.74	58.70	1.44	0.2648
Layers' weight (kg)	1.64	1.60	1.64	1.63	1.57	1.62	1.64	0.04	0.9381
Feed intake (g/hen/day)	103.89	98.43	99.29	106.22	101.20	106.58	107.10	2.96	0.2170
FCR (g feed/g egg)	0.85	1.08	1.08	0.97	1.17	0.85	1.10	0.15	0.6319

*Means on the same row with different superscripts are significantly ($P < 0.05$) different. SEM-Standard Error of Mean, FCR-Feed Conversion Ratio, T₁- Basal diet; T₂- Basal diet + 1.5% Palm oil; T₃- Basal diet + 1.5% Soybean oil; T₄- Basal diet + 1.5% Sesame seed oil; T₅- Basal diet + 1.5% Fish oil; T₆- Basal diet + 0.75% Soybean oil + 0.75% Fish oil; T₇- Basal diet + 0.75% Sesame seed oil + 0.75% Fish oil

The results on the effect of dietary oils inclusion on production performance are presented in Table IV. There were no significant differences ($P > 0.05$) observed in egg production, egg weight, layers' weight, feed intake, and FCR (feed conversion ratio) across the treatments.

The values of yolk height, albumen height, yolk index, and haught unit statistically followed a similar trend. Egg shape index of 79.97 recorded in eggs of hens on treatment 1 were identical to those on treatments 2, 5, 6 and 7. Yolk colour value of 7.20 was recorded in eggs laid by hens on treatments 5, 6 and 7 and was similar to those on treatments 2, 3 and 4 respectively.

IV. DISCUSSION

The total percentage compositions of saturated, monounsaturated and polyunsaturated fatty acids in the egg yolks of the different treatments varied in relation to the source of lipids present in the diets (Table II). The hens on treatments 3 (1.5% soybean oil inclusion) and 7 (0.75% sesame seed oil + 0.75% fish oil) laid eggs showing higher total saturated fatty acids level (53.26%) and MUFA (monounsaturated fatty acids) (15.86%) respectively than the egg yolks of hens on other treatments, although not significant. This is in consonance with the findings of [29] who discovered that saturated fatty acids of egg yolk are not influenced by dietary strategies, although, it contradicts the report that the amounts of saturated fatty acids and MUFA decreased with the use of diets containing vegetable oils [30], [1], [31]. However, total PUFA (polyunsaturated fatty acids) [C18:2n-6-Linoleic acid and C18:3n-3-Linolenic acid] was significant across the treatments. The hens on treatments 5 (1.5% fish oil inclusion) and 6 (0.75% soybean oil + 0.75% fish oil inclusion) laid eggs showing higher total PUFA level (31.26%), with treatments 2 (1.5% palm oil inclusion) recording the lesser percentage (30.77%). This can be

attributed to the fact that the fatty acid composition of palm oil is higher in the saturated fatty acids components especially palmitic acid than the PUFA components. These results are consistent with the findings of [1], who observed an increase in PUFA egg percentage when evaluating diets containing 3% sunflower oil or linseed oil. However, n-6/n-3 ratio (omega-6 to omega-3 ratio) was not significant across treatments.

No significant differences were observed in TC (total cholesterol), HDL (high density lipoprotein) and LDL (low density lipoprotein) levels of egg yolk across the treatments (Table III). These results are in accordance with the findings of [32] who established that different levels of dietary n-3 PUFA had no effect on the cholesterol contents of egg yolks in the laying hens when compared with the control. In contrast to our finding, [33] discovered that yolk cholesterol level was remarkably lower in the quails treated orally with fish oil capsules compared to the control group. It has been hypothesised that the inability to markedly reduce egg cholesterol levels is due to a physiological control mechanism that ultimately causes the cessation of egg production when yolk cholesterol deposition is inadequate for embryo survival [34]. However, there were significant differences recorded in TAG (triacylglycerol) and VLDL (very low density lipoprotein) levels of egg yolk across treatments. The higher TAG level (603.78 mg/dl) and VLDL level (120.76 mg/dl) was recorded in eggs laid by hens on treatment 4 (1.5% sesame seed oil), and the lower TAG level (437.79 mg/dl) and VLDL level (87.56 mg/dl) was recorded with treatment 1 (no dietary oil inclusion). These results contradict findings of [35] who were unable to find any reduction in egg yolk cholesterol and lipoprotein in the hens fed dietary oils. Similarly, [36] was unable to find any change in total TAG and cholesterol content of the eggs produced by the hens fed sources of n-3 PUFA.

Data on production performance of layers is presented in Table IV showing the effect of dietary oils inclusion on egg production, egg weight, layers' weight, feed intake and feed conversion. Egg production was more than 80 percent for all treatments except for treatment 5, which was lower (78.03%). However, results were not significant among the egg

production of birds across the treatments. According to previous studies, egg production was also not affected with the addition of different oils in the diets of layers [1], [12], [30], [32], [36], [37]. In addition, diets had no effect on feed intake of hens.

TABLE V
EGG QUALITY PARAMETERS OF LAYERS ON DIETS CONTAINING VARYING LEVELS OF DIETARY OILS

Egg Quality Parameter	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	SEM	P value
Egg weight (g)	63.24	61.44	57.90	58.48	57.56	60.48	61.46	1.99	0.3554
Yolk weight (g)	18.22	17.68	17.26	18.10	16.62	17.64	18.20	0.71	0.6656
Yolk ratio	28.87	28.80	29.80	31.01	28.95	29.16	29.62	0.95	0.6642
Albumen weight (g)	38.34	37.32	34.86	34.04	34.62	36.28	36.84	1.43	0.3227
Albumen ratio	60.56	60.70	60.27	58.13	60.08	60.01	59.98	1.05	0.6789
Shell weight (g)	6.68	6.44	5.78	6.34	6.32	6.56	6.42	0.33	0.6218
Shell ratio	10.57	10.50	9.93	10.86	10.97	10.84	10.40	0.37	0.5045
Shell thickness (mm)	0.05	0.05	0.04	0.05	0.05	0.05	0.03	0.01	0.6267
Yolk height (mm)	1.27 ^a	1.21 ^{ab}	1.16 ^{ab}	1.17 ^{ab}	1.06 ^b	1.08 ^b	1.12 ^{ab}	0.06	0.0156
Albumen height (mm)	5.36 ^a	4.22 ^{ab}	4.82 ^{ab}	3.91 ^b	4.28 ^{ab}	4.30 ^{ab}	4.68 ^{ab}	0.36	0.0131
Yolk index	0.31 ^a	0.29 ^{ab}	0.28 ^{ab}	0.28 ^{ab}	0.25 ^b	0.26 ^{ab}	0.28 ^{ab}	0.02	0.0256
Egg shape index	79.97 ^a	76.14 ^{abc}	71.85 ^c	72.87 ^{bc}	77.21 ^{ab}	76.84 ^{abc}	77.18 ^{ab}	1.62	0.0234
Haugh unit	69.65 ^a	59.30 ^{ab}	65.29 ^{ab}	57.31 ^b	61.47 ^{ab}	59.93 ^{ab}	63.75 ^{ab}	3.38	0.0203
Yolk colour	5.40 ^b	6.60 ^{ab}	6.40 ^{ab}	6.40 ^{ab}	7.20 ^a	7.20 ^a	7.20 ^a	0.54	0.0225

*Means on the same row with different superscripts are significantly ($P < 0.05$) different; SEM-Standard Error of Mean.

T₁- Basal diet; T₂- Basal diet + 1.5% Palm oil; T₃- Basal diet + 1.5% Soybean oil; T₄- Basal diet + 1.5% Sesame seed oil; T₅- Basal diet + 1.5% Fish oil; T₆- Basal diet + 0.75% Soybean oil + 0.75% Fish oil; T₇- Basal diet + 0.75% Sesame seed oil + 0.75% Fish oil.

There was no difference in feed conversion due to persistent egg production across all the treatments. This finding is in agreement with previous researchers who found no change in feed intake nor feed efficiency of hens fed different levels of dietary omega-3 (n-3) polyunsaturated fatty acids (PUFA) [37] and [38]. Similarly, there were no differences in egg weight and layers' weight among different dietary treatments of the experiment. This result agrees with the findings of various studies that egg weight was not influenced by feeding different sources and levels of n-3 PUFA in the diet of laying hens [30], [36], [37], [39], [40].

The effect of dietary oils inclusion on egg quality parameters as presented in Table V had no observable influence on egg weight, yolk weight, yolk ratio, albumen weight, albumen ratio, shell weight, shell ratio, and shell thickness. The findings of this experiment are in agreement with those found by [1] who found consistency in the proportion of yolk or albumen to total egg weight. In addition, it was reported that feeding n-3 PUFA to hens did not change egg yolk weight [9], [41], [42]. Similarly, it was reported that the egg white weight was not affected by 5% canola oil supplementation to hens [43]. Non-significant effects of dietary oils on shell thickness are in line with previous findings [1]. It was also reported that flaxseed did not affect eggshell quality [44]. Albumen height and Haugh unit were significant across the treatments. Haugh unit based on the albumen height and egg weight is an acceptable measure of the quality and freshness of shell eggs. Eggs produced by hens on treatment 1 (no dietary oil inclusion) had higher albumen height (5.36mm) and Haugh unit (69.65) while treatment 4

(1.5% sesame seed oil inclusion) had lower albumen height (3.91mm) and Haugh unit (57.31). This is in line with the findings of [45] who found lower values of Haugh unit of eggs obtained by feeding of sunflower seed to laying hens. Contrary to this, [1] while investigating the effect of different fat sources on egg quality, found no difference in Haugh unit estimates of eggs by different treatments. Yolk height was dependent of dietary treatments, thus significant results for yolk index estimates of eggs. Effect of dietary oils inclusion was pronounced for yolk colour of eggs recorded with eggs produced by hens on treatments 5, 6 and 7. This supports the previous findings that proportion of feedstuffs, rich in xanthophylls, in the poultry rations can affect yolk colour [46], [47]. In contrast, [1] did not observe significant difference in yolk colour while comparing the nutritional value of various fat sources for layers. This disparity may be due to the type and level of inclusion of ingredients used to produce the designer eggs.

V. CONCLUSION AND RECOMMENDATION

The current study shows that supplementation of different oil sources into laying hen diets had no negative effect on production performance, egg cholesterol and egg quality parameters. The dietary oils added to the basal diet affected the PUFA (polyunsaturated fatty acid) content of the eggs positively but had no effect on the MUFA (monounsaturated fatty acid) and saturated fatty acids contents of eggs across the treatments. It can therefore be recommended that producers of designer eggs should adopt the use of fish oil in formulating layers' ration to produce eggs that is higher in PUFA. In

addition, higher inclusion levels of the dietary oils in layers' diet should be experimented in subsequent studies.

REFERENCES

- [1] M. R. Mazalli, D. E. Faria, D. Salvador, and D. T. Ito, A comparison of the feeding value of different sources of fats for laying hens: 1. Performance characteristics, *J. Appl. Poult. Res.*, 13: 274-279, 2004.
- [2] A. Laca, B. Pardes, and M. Diaz, A method of egg yolk fractionation characterisation of fractions, *Food Hydrocolloid*, 24: 434-443, 2010.
- [3] X. Liu, H. L. Zhao, S. Thiessen, J. D. House, and P. J. H. Jones, Effect of plant sterol-enriched diets on plasma and egg yolk cholesterol concentrations and cholesterol metabolism in laying hens. *Poult. Sci.*, 89: 270-175, 2010.
- [4] C. O. Leskanish, and R. C. Noble, Manipulation of the n-3 polyunsaturated fatty acid composition of avian meat. *World's Poult. Sci. J.*, 53: 156-182, 1997.
- [5] S.E. Chowdhury, S. D. Chowdhury, and T. K. Smith, Effects of dietary garlic on cholesterol metabolism in laying hens. *Poult. Sci.*, 81: 1856-1862, 2002.
- [6] L. Vorlava, E. Sieglava, R. Karpiskova, and V. Kopriva, Cholesterol content in eggs during the laying period. *Acta Veterinaria Brno*. 70: 387-390, 2001.
- [7] R. G. Elkin, Reducing shell egg cholesterol content. I. Overview, genetic approaches, and nutritional strategies. *World's Poult. Sci. J.*, 62: 665-687, 2006.
- [8] I. K. Paik, and R. Blair, Atherosclerosis, Cholesterol and Eggs. *AJAS*. 9 (1): 1-25, 1995.
- [9] A. C. Beynen, Fatty acid composition of eggs produced by hens fed diets containing groundnut, soybean or linseed. *N. J. Anim. Sci.*, 52, 3- 10, 2004.
- [10] J. G. Souza, F. G. P. Costa, R. C. R. E. Queiroga, S. H. V. Silva, A. R. P. Schwler, and C. C. Goulart, Fatty acid profile of eggs of semi-heavy layers fed feeds containing linseed oil. *Braz. J. Poultry Sci.*, 10: 37-44, 2008.
- [11] Food Standards Agency (FSA). McCance and Widdowson's: The composition of Foods (Sixt Summary Edition). *Royal Society of Chemistry*, Cambridge. 2002.
- [12] R. S. Filardi, O. M. Junqueira, A. C. DE Laurentiz, E. M. Casartelli, E. A. Rodrigues, and L. F. Araujo, Influence of different fat sources on the performance, egg quality, and lipid profile of egg yolks of commercial layers in the second laying cycle. *J. Appl. Poultry Res.*, 14: 258-264, 2005.
- [13] A. P. Simopoulos, A. P. The omega-6/omega-3 ratio: The scientific evidence and the need to return omega-3 fatty acids into eggs and other food. In: (ed) Sim, J. and Sinwoo, H. H. The Amazing Egg: Nature's Perfect Functional Food for Health Promotion. University of Atwerta Edmonton, Alberta, Canada, 2006.
- [14] H. Basmacioglu, M. Cabuk, K. Unal, K., Ozkan, S. Akkan, and H. Yalcin, Effects of dietary fish oil and flax seed on cholesterol and fatty acid composition of egg yolk and blood parameters of laying hens. *S. Afr. J. Anim. Sci.* 33: 4, 2003.
- [15] M. J. Gonzalez-Munoz, S. Bastida, O. Jimenez, Lorenzo de C., G. Vergara, and F. J. Sacher-Muniz, The effect of dietary fat on the fatty acid composition and cholesterol content of eggs from Hy-line and Warren hens *Grassas Y Aceites*. 60(4): 350-359, 2009.
- [16] J. B. Lawlow, N. Gaudette, T. Dickson, and J. D. House, Fatty acid profile and sensory characteristics of table eggs from laying hens fed diets containing microencapsulated fish oil. *J. Anim. Feed Sci. and Tech.*, 156: 97-103, 2010.
- [17] N. Ceylan, I. Ciftci, C. Mizrak, Z. Kahraman, and H. Efil. (2011). Influence of different dietary oil sources on performance and fatty acid profile of egg yolk in laying hens. *J. Anim. Feed Sci.*, 20: 71-83, 2011.
- [18] NRC (National Research Council). Nutrient Requirements of Poultry. National Academy Press: Washington. DC. 9th ed. Pp. 35-39, 1994.
- [19] P. C. Panda, Shape and Texture. In: Textbook on egg and poultry technology (First Edition). New Delhi, India, 57, 1996.
- [20] S. D. Chowdhury, Effects of feeding steolathrogens to laying fowl (*Gallus domesticus*) with particular reference to eggshell and membrane quality. Ph.D. Thesis, University of London, UK, 1987.
- [21] G. Doyon, M. Bernier-Cardou, R. M. G. Hamilton, F. Eastaings, C. T. and Ramdald, Egg quality: Albumen quality of egg from five commercial strains of White Leghorn hens during one year of lay. *Poult. Sci.*, 65: 63-66. 1986.
- [22] R. R. Haugh, The Haugh unit for measuring egg quality. *US Egg Poult. Mag.*, 43: 522-555, 1937.
- [23] AOAC, Official and Tentative Methods of the American Oil Chemist' Society, 3rd edition, champaign, Illinois, 1978.
- [24] R. G. Elkin, Z. Yan, Y. Zhong, S. S. Donkin, K. K. Burham, J. A. Story, J. J. Turek, R. E. Porter, M. Anderson, R. Homan, and R. S. Newton, Select 3-Hydroxyl-3-Methylglutaryl-coenzyme: a reductase inhibitors vary in their ability to reduce egg yolk cholesterol levels in varying alternation of hepatic cholesterol synthesis and plasma VLDL composition. *J. Nutr.*, 129: 1010-1019, 1999.
- [25] W. T. Friedewald, R. I. Levy, and D. S. Fredrickson, Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin. Chem.*, 18 (6): 499-502, 1972.
- [26] AOAC, Association of official analytical chemists. Official methods of analysis 16th Ed., Published by Association of Official Analytical Chemists. Inc., Washington, D. C., USA, 2000.
- [27] SAS, Statistical Analysis Systems User's guide (12.1 Ed.). SAS Institute Inc., Raleigh North Carolina, 2012.
- [28] D. B. Duncan, Multiple range and multiple F tests. *Biometrics*, 11: 1-42, 1955.
- [29] A. Meluzzi, F. Sirri, G. Manfreda, N. Tallarico, and A. Franchini, Effects of dietary vitamin E on the quality of table eggs enriched with n-3 long chain fatty acids. *Poult. Sci.*, 79: 539- 545, 2000.
- [30] M. D. Baucells, N. Crespo, A. C. Barroeta, S. Lopez-Ferrer, and M. A. Grashorn, Incorporation of different polyunsaturated fatty acids into eggs. *Poult. Sci.*, 79: 51- 59, 2000.
- [31] M. C. G. Pita, E. Piber Neto, P. R. Carvalho, and C. X. Mendonça Junior, Efeito da suplementação de linhaça, óleo de canola e vitamina E na dieta sobre as concentrações de ácidos graxos poliinsaturados em ovos de galinha. *Arq. Bras. Méd. Vet. Zootec.* 58: 925-931, 2006.
- [32] T. Ebeid, Y. Eid, A. Saleh, and H. Abd El-Hamid, Ovarian follicular development, lipid peroxidation, antioxidative status, and immune response in laying hens fed fish oil-supplemented diets to produce n-3-enriched eggs. *Animal*, 2: 84- 91, 2008.
- [33] E. Atakisi, O. Atakisi, H. Yaman, and I. Arslan, I. Omega-3 fatty acid application reduces yolk and plasma cholesterol levels in Japanese quails. *Food Chem. Toxicol.* 47: 2590-2593, 2009.
- [34] M. C. Milinsk, A. E. Murakami, S. T. M. Gomes, M. Matsushita, and N. E. De Souza, Fatty acid profile of egg yolk lipids from hens fed diets rich in n-3 fatty acids. *Food Chem.* 83: 287-292, 2003.
- [35] N. A. Botsoglou, L. A. Yannakopoulos, D. J. Fletouris, A. S. Tserveni-Goussi, and I. E. Psomas, Yolk fatty acid composition and cholesterol contents in response to level and form of dietary linseed. *J. Agri. Food Chem.*, 46: 4652- 4656, 1998.
- [36] S. Carrillo-Dominguez, I. M. E. Carranco-Jauregui, R. M. Castillo-Domínguez, and M. I. Castro-Gonzalez, Cholesterol and n-3 and n-6 fatty acid content in eggs from laying hens fed with red crab meal (*Pleuroncodes planipes*). *Poult. Sci.*, 84: 167- 172, 2005.
- [37] M. Schreiner, H. W. Hulan, E. Razzazi-Fazeli, J. Bohm, and C. Iben, Feeding laying hens seal blubber oil: Effects on egg yolk incorporation, stereospecific distribution of omega-3 fatty acids, and sensory aspects. *Poult. Sci.*, 83: 462- 473, 2004.
- [38] G. J. M. Coetzee, and L. C. Hoffman, Effects of various dietary n-3/n-6 fatty acid ratios on the performance and body composition of broilers. *S. Afr. J. Anim. Sci.*, 32(3): 175- 183, 2002.
- [39] P. S. Hargis, M. E. Van Elswyk, and B. M. Hargis, Dietary Modification of Yolk Lipid with Menhaden Oil. *Poult. Sci.*, 70 (4): 874-883, 1991.
- [40] I. D. Bean, and S. Leeson, Long-term effect of feeding linseed on performance and egg fatty acid composition of brown and white hens. *Poult. Sci.*, 82: 388- 394, 2003.
- [41] R. Augustyn, J. Barteczko, and S. Smulikowska, The effect of feeding regular or low α -linolenic acid linseed on laying performance and total cholesterol content in eggs. *J. Feed Sci.*, 15: 103-106 (Suppl. 1), 2006.
- [42] E. Sosin, F. Borowiec, J. Strzetelski, and S. mulikowska, The effect of feeding regular or low α -linolenic acid linseed on the fatty acid composition of egg yolks. *J. Anim. Feed Sci.*, 15: 641- 650, 2006.
- [43] E. Rowghani, M. Arab, S. Nazifi, and Z. Bakhtiari, Effect of canola oil on cholesterol and fatty acid composition of egg-yolk of laying hens. *Int. J. Poult. Sci.*, 6(2): 111-114, 2007.
- [44] C. Novak, and S. E. Scheideler, Long-term effects of feeding linseed-based diets. 1. Egg production parameters, components, and eggshell quality in two strains of laying hens. *Poult. Sci.*, 80: 1480- 1489, 2001.

- [45] Karunajeewa, H., Than, S. H. and Abu-Serewa, S. (1989). Sunflower seed meal, sunflower oil and full-fat sunflower seeds, hulls and kernels for laying hens. *Anim. Feed Sci. Technol.*, 26: 45-54.
- [46] Y. H. Jiang, R. B. McGeachin, and C. A. Bailey, α -tocopherol, β -carotene, and retinol enrichment of chicken eggs. *Poult. Sci.*, 73:1137–1143, 1994.
- [47] Scheideler, S. E., Jaroni, D. and Froning, G. (1998). Strain and age effects on egg composition from hens fed diets rich in n-3 fatty acids. *Poult. Sci.*, 77: 192–196.