Housing types and extract of oyster mushroom (*Pleurotus ostreatus*): Effects on egg qualities and egg yolk lipid profile of egg-type chickens

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This study, laid out in a 2 x 4 factorial arrangement compared egg lipid profile of egg-type chickens on two housing types (battery cage and litter-covered floor) and on extract of oyster mushroom (0, 10, 20 and 30 ml/L of water) at early and late lay phases. Each treatment consisted of 30 birds sub-divided into three replicates of ten birds each. Data obtained were subjected to analysis of variance. Results showed that birds in the battery cages recorded a higher ($P \leq 0.05$) deep yellowish yolk colour and higher ($P \leq 0.05$) triglycerides (1427 g/dl), and very low-density lipoprotein, VLDL, (285.40 g/dl) compared to those housed on litter-covered floor at early lay. In the extract of oyster mushroom (*Pleurotus ostreatus*), eggs from birds on 20 ml/L of water had the lowest ($P \leq 0.05$) triglyceride (1362 g/dl) and VLDL (272.40 g/dl) in early lay. At late lay, higher eggshell thickness of 0.39 mm was obtained in eggs from birds on the litter-covered floors. In the interaction between housing types and extract of oyster mushroom, significantly ($P \leq 0.05$) highest eggshell thickness (0.45 mm) was obtained in eggs from birds on litter-covered floors and on 10 ml/L extract of oyster mushroom. However, housing types and extract of oyster mushrooms did not significantly affect ($P > 0.05$) lipid profile of eggs at late lay. Housing types and extract of oyster mushroom neither increased nor decreased cholesterol contents of the eggs at both early and late lays.

Keywords: Egg yolk, egg cholesterol, oyster mushroom, egg consumers, litter-covered floor, battery cage

Hen eggs possess excellent nutritive value and constitute a traditional food used in many basic and formulated preparations. The quality of an egg is related to features affecting the acceptability of the egg to consumers (Ahmadi and Rahimi 2011; Ledvinka et al. 2012). The qualities of internal parts are a reflection of the freshness of the egg and are considered the best at the onset of lay and decrease with storage time (Philippe et al. 2020). Eggs have been reported to be successfully enriched with desired compounds through dietary interventions (Leeson and Caston 2003). For instance, selenium supplementation in layer diets increased egg levels of vitamin E while increased dietary vitamins E and C alongside supplemental omega n-3 found in fish oils for poultry helped prevent lipid peroxidation of eggs (Bou et al. 2005).

Housing types have been reported to influence meat (Olaniyi et al. 2012) and egg (Sogunle et al. 2014) qualities but there is dearth of information in literature on the use of oyster mushroom (*Pleurotus ostreatus*) in nutritional enrichment of eggs in spite of the fact that it is a good source of vitamins, especially vitamin D_2. It also contains B vitamins, and minerals including Fe, Mn, K, Ca, Mg, Cd, Cu, P and Zn (Li et al. 2017). Mushroom aggregates and production are increasing and they have a positive effect on broiler performance (Daneshmand et al. 2011).

Despite the nutritional value of egg, it is still confronted with marketing challenges and reduced quality over time (Sokołowicz et al. 2018; Selim and Hussein 2020). Consumption

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of eggs is affected by their being erroneously perceived to be a concentrated source of cholesterol (Lesnierowski and Stangierski 2018), and this often results in egg gluts. Dietary cholesterol is a fat-like substance existing in all animal cells. Cholesterol is found in the blood as particles called lipoproteins and it is a precursor of steroid hormones (Kasprzak and Hetmanski 2004). Schade et al. (2022) reported that for some individuals, dietary cholesterol has adverse effects and in others, a significant elevation in blood low-density lipoprotein may occur. Investigations have included alternative housing systems, genotype and age of hens (Sogunle et al. 2019; da Silva Pires et al. 2021), use of drugs, manipulation of dietary protein and energy levels of the dietary sources, supplementation with vitamins and the use of dietary fibre said to be hypocholesterolemic. However, the cholesterol content of an egg remained unchanged by diet regardless of the dietary fat content (Millet et al. 2006; Sokołowicz et al. 2018). In the phytochemical analysis of oyster mushroom carried out by Sogunle et al. (2019), the most abundant active ingredients were: 9-Octadecenoic acid (Z)-, methyl ester (oleic acid) which is a monounsaturated fatty acid associated with decreased LDL and possibly increased HDL; 9,12-Octadecadienoic acid (Z, Z)- known as linoleic acid that is associated with anti-inflammatory, hypocholesterolemic, cancer prevention, hepatoprotective, nematicide, insectifuge (Omega 6 fatty acid), antihistaminic, antieczemic, antiacne, 5-Alpha reductase inhibitor antiandrogenic, anti-arthritic, and anti-coronary activities; and methyl palmitate (an antioxidant and antimicrobial) which is the most common saturated fatty acid in animals, plants and micro-organisms. Bilal et al. (2010) stated that oyster mushroom has antibacterial, antifungal, antioxidant, antiviral and cholesterol-reducing properties. In a systematic review (da Silva Pires et al. 2021) on the relationship between egg quality and hen housing systems, it was reported that external and internal egg characteristics were affected by production systems. A reduction of cholesterol content of quality market eggs therefore becomes understandably a subject of interest to both egg producers and health-conscious consumers. However, a paucity of information on the usage of oyster mushroom, a phytogenic substance to maintain egg quality and as a cholesterol-reducing agent in eggs necessitated this study.

Materials and methods

Experimental site and management of birds

The experiment was carried out at the Poultry Unit of the Teaching and Research Farm, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria located at latitude 7° 15'N, longitude 3° 26'E and 76 m above sea level (Google Earth). A total of 240 birds of 21 weeks old were used for this study. There were two housing types, battery cage and litter-covered floor and four extracts of oyster mushroom: 0, 10, 20 and 30 ml/L of water. Each treatment combination in this 2 x 4 factorial arrangement had three replicates of ten birds. Birds on the control treatment (zero oyster mushroom) were administered an antibiotic monthly while the others were administered the extracts of oyster mushroom three times weekly in the course of the experiment.

Preparation of the extracts of oyster mushrooms

The extracts of oyster mushroom were prepared by heating 1000 g in 1 litre of water at 70°C for 20 minutes as described by Sogunle et al. (2019). The newly formed extracts were then cooled and strained off the mushrooms with the aid of a sieve. The extracts were kept in a dark-coloured recipient, to prevent photolysis, and then stored in the refrigerator until needed.

Data collection

Egg quality parameters

For each of the eight treatment combinations, four eggs were collected for the evaluation of egg quality characteristics at early (21 - 30 weeks) and late (41 - 50 weeks) laying periods.

External egg qualities

Egg weight: Each egg sample collected from the treatment was weighed using a weighing scale with 0.01 g sensitivity.

Egg length and width: The length and width of each egg were measured using a vernier calliper. The width was measured as the distance between two ends of the egg at the widest cross-sectional region. The length was measured as the distance between the broad and narrow ends of the eggs.

Egg shape index: This was measured as the percentage of the egg breadth (width) to the width:

Egg shape index = Width of egg/length of egg x 100

Shell weight: Eggshells were air-dried and weighed using a weighing scale with 0.01 g sensitivity.

Shell thickness: The thickness of the air-dried shells was measured to the nearest 0.01 mm using a micrometre screw gauge.

Internal egg qualities

Albumen height: The eggs were gently broken, and the maximum albumen height was measured with tripod micrometre (Dikmen et al. 2017).

Yolk colour: This was determined for individual yolks by comparing the yolk with colour chips of a Hoffman La Roche yolk colour fan. The yolk colour fan has different scores for different colours.

Haugh unit (HU): This was calculated using the values obtained for the egg weight and albumen height (Şekeroğlu and Altuntaş 2009).

$$HU: 100 \log (H+7.57 - 1.7W^{0.37})$$

Where,

H = albumen height (mm);

W = egg weight (g).

Collection of egg for the lipid profile

Egg samples were collected from the birds randomly from each replicate (three eggs per replicate) and were taken to the laboratory in order to determine total cholesterol, high density lipoprotein (HDL), low-density lipoprotein (LDL) and very low lipoprotein (VLDL). The egg samples were collected very early in the morning at the 30th and 50th weeks of the experiment.

Determination of triglyceride

Serum triglyceride was determined by colorimetric method (Li-Hua et al. 2019). The serum (20 µL) was hydrolysed completely to free fatty acids and glycerol by lipoprotein lipase from *Pseudomonas fluorescens*. The released glycerol was oxidised with glycerol dehydrogenase from *Erwinia aroideae* in the presence of nicotinamide adenine dinucleotide (NAD+). The reduction of the enzyme-linked NAD+ is coupled to the reduction of nitro blue tetrazolium as a chromogenic indicator with phenazine methosulfate serving as an intermediate electron carrier of nicotinamide adenine dinucleotide plus hydrogen (NADH). The absorbance was read at a 570 nm after a 10 min rapid incubation.

Determination of total cholesterol

Cholesterol was determined spectrophotometrically as described by Li-Hua et al. (2019). The reagent was made up of three enzymes, cholesterol esterase (C, E) cholesterol oxidase

(CO) and peroxidase (POD), and two substrates; 4-aminoantipyrine (4-AA) and phenol. Three clean test tubes labelled blank, standard, and test were arranged in a test tube rack; 10 ml distilled water, standard cholesterol and eggs were added to each of the test tubes respectively. About 1 ml of reagent was added to all test tubes, the reaction mixtures were mixed and incubated for 10 minutes at room temperature, the absorbance of the samples (both standard and test) was read at 550 mm wavelength against the reagent blank.

$$\text{Cholesterol concentration in egg (mg/dL)} = \frac{\text{Absorbance of test x concentration of standard}}{\text{Absorbance of standard}}$$

Determination of HDL cholesterol

Three clean test tubes marked and labelled blank, standard, and test were arranged in a test tube rack. To each of these tubes was added 1 ml of working reagent with 0.05ml of distilled water, HDL standard and super nutrient were added to each test tube respectively. The reaction mixture was incubated at 37^{o}C for five minutes. The absorbance of standard and test samples against the blank were measured within 60 minutes using a spectrophotometer.

$$\text{HDL cholesterol (mg/dL)} = \frac{\text{Absorbance of test x concentration of standard}}{\text{Absorbance of standard}}$$

Determination of LDL cholesterol

Three clean test tubes marked and labelled blank, standard, and test were arranged in a test tube rack 1000 ml of cholesterol reagent was added to each test tube, 50 ml of distilled water, cholesterol stand, and super nutrient was added to each test tube respectively. The reaction mixture was incubated for 10 minutes at 25^{0}C and the absorbance of the standard and test samples against the blank were measured within 60 minutes using a spectrophotometer.

$$\text{LDL cholesterol (mg/dL)} = \frac{\text{Absorbance of test x concentration of standard}}{\text{Absorbance of standard}}$$

Determination of VLDL cholesterol

The concentration of very low-density lipoprotein (VLDL) cholesterol was calculated by modification of Freidewald's formula (Martin et al. 2013).

$$\text{VLDL cholesterol} = 0.2*\text{triglyceride}$$

Statistical Analysis

Data obtained were arranged in a 2 x 4 factorial experimental layout and subjected to one-way analysis of variance. Significant ($P \leq 0.05$) differences among variable means were separated using Tukey's test as contained in Minitab (2013) statistical package.

Results

Effects of housing types and extract of oyster mushroom on egg qualities of egg-type chickens at early lay (21-30 weeks of age)

Table 1 shows the effects of housing types and extract of oyster mushroom on egg qualities at early lay. In the housing types, significant ($P \leq 0.05$) difference was obtained in the yolk colour with a higher deep yellowish yolk colour of 9.50 in eggs from birds in battery cages while a lower value of 8.83 was obtained in eggs from birds on litter-covered floors. Extract of oyster mushroom, and the interaction between housing types and extract of oyster mushroom did not significantly ($P > 0.05$) influence the parameters obtained across the treatment groups.

Table 1: Effects of housing types and extract of oyster mushroom on egg qualities of egg-type chickens at early lay (21 – 30 weeks)

		Egg weight (g)	Egg length (mm)	Egg width (g)	Egg shape index (%)	Shell thickness (mm)	Shell weight (g)	Albumen height (mm)	Haugh unit	Yolk colour
Housing type	BC	48.67	51.56	40.96	79.45	0.33	5.16	7.02	86.08	9.50[a]
	LCF	48.67	52.50	40.75	77.68	0.31	5.25	6.29	80.67	8.83[b]
	SEM	0.99	0.43	0.34	0.86	0.02	0.18	0.51	3.26	0.20
	P-Value	1.00	0.14	0.67	0.17	0.56	0.72	0.33	0.26	0.04
Extract of oyster mushroom	0 ml/L	47.50	51.70	40.52	78.38	0.31	5.42	5.87	78.67	9.17
	10 ml/L	47.00	51.60	40.32	78.14	0.30	4.73	7.32	87.00	9.33
	20 ml/L	50.50	52.50	41.51	79.08	0.36	5.57	6.53	83.00	9.17
	30 ml/L	49.67	52.32	41.07	78.78	0.32	5.10	6.90	84.83	9.00
	SEM	1.40	0.61	0.48	1.22	0.03	0.25	0.72	4.62	0.29
	P-Value	0.26	0.67	0.33	0.96	0.58	0.14	0.55	0.63	0.88
Housing type * Extract of oyster mushroom	BC*0 ml/L	47.00	50.61	40.52	80.05	0.31	5.57	6.30	83.00	9.33
	BC*10 ml/L	47.67	51.60	40.41	78.32	0.34	4.77	7.27	85.67	9.67
	BC*20 ml/L	49.67	51.66	41.39	80.11	0.37	5.43	7.27	88.00	9.67
	BC*30 ml/L	50.33	52.38	41.51	79.30	0.31	4.87	7.23	87.67	9.33
	LCF* 0 ml/L	48.00	52.79	40.51	76.78	0.32	5.27	5.43	74.33	9.00
	LCF*10 ml/L	46.33	51.60	40.23	78.11	0.27	4.70	7.37	88.33	9.00
	LCF*20 ml/L	51.33	53.34	41.63	78.05	0.35	5.70	5.80	78.00	8.67
	LCF*30 ml/L	49.00	52.26	40.62	77.79	0.32	5.33	6.57	82.00	8.67
	SEM	1.98	0.87	0.69	1.72	0.05	0.36	1.01	6.53	0.41
	P-Value	0.82	0.46	0.86	0.84	0.81	0.72	0.89	0.77	0.88

[a, b:] Means in the same column by factor with different superscripts are significantly ($P \leq 0.05$) different
BC = Battery cage, LCF = Litter-covered floor, SEM = Standard error of means

Effects of housing types and extract of oyster mushroom on egg lipid profile of egg-type chickens at early lay (21-30 weeks of age)

Effect of housing types and extract of oyster mushroom on egg lipid profile of egg-type chickens at early lay is shown in Table 2. The triglycerides and very low-density lipoproteins (VLDL) were significantly ($P \leq 0.05$) influenced by the housing types and extract of oyster mushroom. Eggs from birds in battery cages had higher (1427 g/dl) triglyceride than eggs from birds on the litter-covered floors (1381 g/dl). This same trend was recorded in VLDL. Eggs from birds on 10 ml extract of oyster mushroom/litre of water had the highest (1456 g/dl) triglycerides and VLDL which were similar to the values recorded in eggs from birds on 0 and 30ml extract of oyster mushroom/litre of water. In the interaction between housing types and extract of oyster mushroom, eggs from birds on the battery cage and on 10 ml/L extract of oyster mushroom recorded the highest ($P \leq 0.05$) triglycerides (1523 g/dl) and VLDL (304.6 g/dl) while the lowest triglycerides (1370 and 1373 g/dl) and VLDL (274.0 and 274.5 g/dl) were recorded in eggs from birds on 30 ml extract of oyster mushroom/litre of water on litter covered floors and battery cages respectively.

Table 2: Effects of housing types and extract of oyster mushroom on egg lipids profile of egg type-chicken at early lay phase (21-30 weeks)

		Total cholesterol (g/dl)	Triglycerides (g/dl)	High density lipoprotein (g/dl)	Low-density lipoprotein (g/dl)	Very low-density lipoprotein (g/dl)
Housing type	Battery cage	1105	1427[a]	236.2	538.8	285.4[a]
	LCF	1105	1381[ab]	234.0	594.5	276.34[b]
	SEM	15.5	12.3	13.2	21.0	2.54
	P-Value	0.97	0.04	0.92	0.75	0.05
Extract of oyster mushroom	0 ml/L	1103	1426[ab]	211.9	605.6	285.2[ab]
	10 ml/L	1096	1456[a]	246.3	558.5	291.7[a]
	20 ml/L	1116	1362[b]	245.1	598.5	272.4[b]
	30 ml/L	1105	1371[ab]	237.0	594.0	274.3[ab]
	SEM	18.9	21.3	18.7	29.7	3.59
	P-Value	0.89	0.02	0.57	0.69	0.02
Housing type * Extract of oyster mushroom	BC*0 ml/L	1103	1430[ab]	190.6	625.8	286.1[ab]
	BC*10 ml/L	1087	1523[a]	245.7	536.2	304.6[a]
	BC*20 ml/L	1149	1382[ab]	251.0	621.6	276.4[ab]
	BC*30 ml/L	1084	1373[b]	257.4	551.6	274.5[b]
	LCF* 0 ml/L	1103	1422[ab]	233.3	585.4	284.3[ab]
	LCF*10 ml/L	1105	1389[ab]	247.0	580.7	278.8[ab]
	LCF*20 ml/L	1083	1342[b]	239.3	575.3	268.4[b]
	LCF*30 ml/L	1127	1370[ab]	216.6	636.4	274.0[ab]
	SEM	26.8	24.6	26.5	42.1	5.08
	P-Value	0.33	0.12	0.57	0.45	0.04

[a, b:] Means in the same column by factor with different superscripts are significantly ($P \leq 0.05$) different
BC = Battery cage, LCF = Litter-covered floor, SEM = Standard error of means

Effects of housing types and extract of oyster mushroom on egg qualities of egg-type chickens at late lay (41-50 weeks of age)

Table 3 shows the effects of housing types and extract of oyster mushroom on egg qualities of egg-type chickens at late lay. Housing types significantly ($P \leq 0.05$) influenced eggshell thickness. A higher eggshell thickness of 0.39 mm was obtained in eggs from birds on litter-covered floors. The interaction between housing types and extract of oyster mushroom significantly ($P \leq 0.05$) influenced the eggshell thickness. The highest value (0.45 mm) was obtained in eggs from birds on litter-covered floors and 10 ml/L extract of oyster mushroom: the lowest (0.30 mm) was obtained in eggs from birds in battery cages and on 0 ml/L extract of oyster mushroom. This value (0.30 mm) was however statistically ($P > 0.05$) similar to the other values obtained.

Effects of housing types and extract of oyster mushroom on egg lipid profile of egg-type chickens at late lay (41-50 weeks of age).

The effects of housing types and extract of oyster mushroom on egg lipid profile of egg-type chickens at late lay showed no significant ($P > 0.05$) differences in all the parameters measured across the treatment groups (Table 4).

Table 3: Effects of housing types and extract of oyster mushroom on egg qualities of egg-type chickens at late lay (41-50 weeks)

		Egg weight (g)	Egg length (mm)	Egg width (g)	Egg shape index (%)	Shell thickness (mm)	Shell weight (g)	Albumen Height (mm)	Haugh unit	Yolk colour
Housing types	Battery cage	53.99	50.63	40.00	71.92	0.32[b]	5.23	5.08	64.42	6.83
	LCF	59.49	55.67	43.65	77.83	0.39[a]	5.40	4.84	66.39	7.86
	SEM	2.18	1.90	1.50	2.71	0.02	0.21	0.32	3.47	0.41
	P-Value	0.08	0.07	0.09	0.13	<0.0001	0.56	0.63	0.69	0.08
Extract of oyster mushroom	0 ml/L	57.26	52.56	41.41	74.00	0.34	5.16	4.86	63.67	7.67
	10 ml/L	57.01	52.72	41.56	73.89	0.38	5.14	4.76	62.94	7.72
	20 ml/L	54.96	52.08	41.28	74.11	0.35	5.37	5.18	68.28	6.67
	30 ml/L	57.76	55.25	43.04	77.50	0.36	5.58	5.06	66.72	7.33
	SEM	3.08	2.69	2.13	3.83	0.03	0.30	0.46	4.91	0.58
	P-Value	0.92	0.84	0.93	0.89	0.56	0.69	0.92	0.85	0.56
Housing type * Extract of oyster mushroom	BC*0 ml/L	54.68	49.85	39.03	69.22	0.30[b]	4.73	5.02	63.00	7.22
	BC*10 ml/L	52.23	49.06	38.74	69.89	0.31[ab]	4.98	5.14	64.78	7.44
	BC*20 ml/L	52.37	48.18	38.78	70.56	0.32[ab]	5.09	4.91	64.56	6.22
	BC*30 ml/L	58.68	55.42	43.44	78.00	0.33[ab]	6.10	5.26	65.33	6.44
	LCF* 0 ml/L	59.83	55.26	43.79	78.78	0.38[ab]	5.58	4.70	64.33	8.11
	LCF*10 ml/L	61.79	56.37	44.38	77.89	0.45[a]	5.30	4.38	61.11	8.00
	LCF*20 ml/L	59.56	55.98	43.78	77.67	0.38[ab]	5.64	5.44	68.00	7.11
	LCF*30 ml/L	56.79	55.08	42.63	77.00	0.37[ab]	5.07	4.86	68.11	8.22
	SEM	4.36	3.81	3.01	5.41	0.25	0.42	0.32	6.94	0.82
	P-Value	0.53	0.69	0.69	0.78	0.03	0.13	0.78	0.88	0.89

[a, b:] Means in the same column by factor with different superscripts are significantly ($P \leq 0.05$) different
BC = Battery cage, LCF = Litter-covered floor, SEM = Standard error of means

Table 4: Effects of housing types and extract of oyster mushroom on egg lipids profile of egg type-chicken at late laying phase (41-50 weeks)

		Total cholesterol (g/dl)	Triglycerides (g/dl)	High density lipoprotein (g/dl)	Low-density lipoprotein (g/dl)	Very low-density lipoprotein (g/dl)
Housing types	Battery cage	1063	1234	274.2	532.7	247.0
	LCF	1030	1237	276.4	505.7	247.5
	SEM	17.0	14.4	10.9	14.4	2.87
	P-Value	0.21	0.91	0.89	0.22	0.91
Extract of oyster mushroom	0 ml/L	1038	1245	273.1	515.6	249.1
	10 ml/L	1043	1218	279.7	519.8	243.5
	20 ml/L	1045	1269	259.0	532.0	253.8
	30 ml/L	1059	1213	289.3	509.4	242.5
	SEM	24.0	20.3	15.4	28.7	4.06
	P-Value	0.93	0.25	0.59	0.88	0.25
Housing type * Extract of oyster mushroom	BC*0 ml/L	1057	1228	290.0	521.3	245.6
	BC*10 ml/L	1025	1192	270.6	515.5	238.4
	BC*20 ml/L	1070	1290	242.6	568.9	258.0
	BC*30 ml/L	1101	1229	293.6	525.0	245.8
	LCF* 0 ml/L	1019	1262	256.2	509.8	252.5
	LCF*10 ml/L	1062	1243	288.9	524.0	248.6
	LCF*20 ml/L	1021	1248	275.4	495.0	249.5
	LCF*30 ml/L	1018	1196	284.9	493.8	239.2
	SEM	34.0	28.7	21.8	28.7	5.74
	P-Value	0.40	0.33	0.47	0.55	0.33

[a, b:] Means in the same column by factor with different superscripts are significantly ($P \leq 0.05$) different
BC = Battery cage, LCF = Litter-covered floor, SEM = Standard error of means

Discussion

The significant difference in the egg yolk colour due to the effects of housing types in the present study could not be attributable to any factor from literature. However, the ability of the birds to exhibit their natural behaviour better on the litter-covered floor could influence better nutrient utilisation and pigmentation of the yolk. The non-significant differences observed in the effects of extract of oyster mushroom, and effects of interaction between housing types and extract of oyster mushroom on egg qualities were in concordance with Paguia et al. (2012) where no differences were recorded in egg weight from layers in battery cage fed extracts of *Moringa oleifera* leaf and Twig. Mužić et al. (2005) also reported that feed supplementation with phytobiotics did not affect egg quality. Opinions differ on egg weights; Leyendecker et al. (2001) observed higher egg weights from hens that were housed in cages, whereas others (Tumova and Ebeid, 2005; Pistekova et al. 2006) reported heavier eggs from deep litter systems or litter-covered floors. In the late lay, significantly higher eggshell thickness was observed in the eggs from birds on litter-covered floors than in eggs from birds reared in battery cages. The values obtained were generally thicker than 0.31 mm reported by Chineke (2001), and 0.32 mm reported by Abutu et al. (2008). This could be due to adequate utilisation of calcium and phosphorus needed for shell formation. The contrasting results found in literature do not support a clear influence of the housing types on eggshell thickness; a major part of shell strength as reported by Sokołowicz et al. (2018). Sokołowicz et al. (2018) further stated that direct or indirect shell strength measures (such as shell thickness and weight, shell percentage and density, shell deformation, or resistance to breakage) are more dependent upon other factors, such as genetics, age, feed composition, or even egg size, than on the housing types.

The non-significance differences in albumen height and Haugh unit in this study corroborate the findings of Uuganbayar et al. (2006) and Deng et al. (2011) that no changes in albumen index and Haugh unit were observed when alfalfa extract and green tea were fed layer hens. Cho et al. (2010) observed no significant difference in Haugh unit, but a deep egg yolk colour was achieved in birds on the fermented spent mushroom substrate contrary to the findings of this study.

In line with the findings of Millet et al. (2006), the cholesterol content of the eggs was not influenced by the inclusion of extract of oyster mushroom in the present study. However, the triglycerides and very low-density lipoprotein of the eggs were influenced by the housing types (battery cage and litter-covered floor) and extract of oyster mushroom in the main effects, and the effects of interaction between the housing types and extract of oyster mushroom. The non-significant differences, either due to housing types or extract of oyster mushroom inclusion recorded in the cholesterol, low-density lipoprotein and high-density lipoprotein of the eggs corroborate Salama et al. (2015), where no significant effects on serum biochemical parameters were observed due to housing model. Also, Salama et al. (2015) did not record significant variations in lipid contents of yolks from birds housed in cages, barn or organic system in a whole cycle of lay. It was revealed in this study that triglycerides and VLDL reduced at 20 ml extract of oyster mushroom per litre of water though not significantly from the values obtained in the control and 30 ml extract of oyster mushroom per litre of water. This could probably be due to the hypocholesterolemic effects of the fruiting bodies of edible mushroom as reported by Guillamón et al. (2010), but possibly to the fact that dietary cholesterol contributes one fourth of the absorbed cholesterol (Schade et al. 2022). The values obtained were with the range for healthy birds. The results on egg lipid profile in the interaction between housing types and extract of oyster mushroom (*Pleurotus ostreatus*) confirmed the fact that cholesterol content of an egg

remained unchanged regardless of the dietary manipulations (Millet et al. 2006).

Conclusion

The study concluded that housing types and extract of oyster mushroom did not increase or reduce the cholesterol contents of the eggs at both early and late lays.

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Conflict of interest

The authors hereby declare that there is no conflict of interest in the conception, design and execution of the study.

Authors contributions

M.A. Ogundele was the research student involved in the field study while O.M. Sogunle conceptualised the study and supervised the student together with A.O. Oso and O.S. Akinola. O.J. Odutayo and O.M. Sogunle did the statistical analysis, and the interpretation was done by K.K. Safiyu and M.A. Ogundele. O.M. Sogunle wrote the manuscript; it was reviewed by I.O. Opowoye and K.K. Safiyu.

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