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Full-Length Research Article

Species- and Tissue-Specific Variations in Cholesterol Content among Wild and Domesticated Birds in Nsukka, Nigeria

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Summary: Avian species exhibit variations in adaptive features, including differences in lipid metabolism, which are essential for successful survival and reproduction in unique habitats. However, there is a lack of information on species- and tissue-specific variations in cholesterol metabolism among avian species living in similar habitats. This study aimed to investigate potential variations in tissue cholesterol levels in adult males of the wild, white-breasted crow (*Corvus albus*, Passeriformes) and the local and exotic (broiler) breeds of domestic chicken (*Gallus gallus domesticus*, Galliformes) avian species found in Nsukka, Nigeria. These species have diverse diets and behaviours. Tissues from the brain, liver, gizzard, heart, and breast muscle were dissected and used for total lipid extraction and cholesterol quantification. The results showed significant species- and tissue-specific differences in cholesterol levels among the examined birds ($p < 0.001$). Moreover, the brain and liver had notably higher cholesterol content compared to other tissues ($p < 0.001$ in most cases). Lastly, multiple comparisons showed that domesticated species generally exhibited higher cholesterol levels than wild species. These findings support the hypothesis of species- and tissue-specific differences in cholesterol metabolism in avian species inhabiting similar environments but differing in diets and behaviours.

Keywords*:* *birds; cholesterol; diet; lipid metabolism; tissues*

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INTRODUCTION

Species survival and reproductive success require the development of unique adaptive characteristics to secure optimal shelter, food, defend territories, evade predators, find mating partners, and ultimately produce offspring for the next generation (Bevan *et al*., 1997; Broggi *et al*., 2011; Birn-Jeffery *et al*., 2012; Blix *et al*., 2016; Furness *et al*., 2022; Kerschbaumer *et al*., 2023; Pap *et al*., 2024). However, adaptive features vary depending on species, varieties, strains, diet, habitat, sex, and the presence, type or absence of predators. While morphological adaptations are often discussed, biochemical and physiological adaptations, including the incorporation of necessary lipids, play an even greater role in successful environmental adaptations.

Cholesterol is a crucial lipid found in living organisms, serving as a key component of cellular and nuclear membranes. It helps maintain the fluidity of the phospholipid bilayer, regulating the transport of nutrients, gases, ions, synthesized molecules, and water (Bastiaanse *et al*., 1997; Zhang *et al*., 2018; Frangos *et al*., 2023). Additionally, cholesterol plays a vital role in the myelin sheath and Schwann cells surrounding neuronal axons, aiding in the conduction of nerve signals (Mouritsen & Zuckermann, 2004; Poitelon *et al*., 2020; Barnes-Vélez *et al*., 2022). The vertebrate liver synthesizes and stores over 70% of the body's cholesterol, with the remaining 30% being produced in other cells or obtained from the diet (Jeske & Dietschy, 1980). Cholesterol synthesized in the liver is transported in the blood as low-density lipoprotein-cholesterol (LDL-C) to other organs for various functions, such as membrane incorporation, bile acid synthesis, and hormone production (Gustafsson *et al*., 1977; Rone *et al*., 2009; Miller & Bose, 2011; Ikonen & Zhou, 2021).

In contrast, the brain synthesizes its own cholesterol and there is no evidence to suggest that plasma LDL-borne cholesterol can cross the blood-brain barrier (Björkhem & Meaney, 2004). Tissue cholesterol content varies based on species, diet, tissue type, habitat, and sex (Lorenz *et al*., 1938; Musacchia, 1953; Mancinelli *et al*., 2022; Teekell *et al*., 1974; Al-ruwaili *et al*., 2014; Punam *et al*., 2024; Palmisano *et al*., 2018; Robinson *et al*., 2021; Conlon *et al*., 2023; Gould *et al*., 1953; Tomkins *et al*., 1953; Frantz *et al*., 1954; Jeske & Dietschy, 1980). For example, it has been shown that temperate mammals and birds have higher cholesterol levels compared to their tropical counterparts (Calhoon *et al*., 2014). Despite these findings, a comprehensive comparative study on tissue cholesterol composition among avian species with similar habitats but differing diets, behaviors, and breeding cycles is lacking. Such research could provide valuable insights into species- and tissue-specific cholesterol metabolism.

To address this gap, we conducted a study, quantifying cholesterol levels in the brain, liver, breast muscle, heart, and gizzard of adult males from three avian species: local and exotic breeds of domestic chicken (*Gallus gallus domesticus*) and the white-breasted crows (*Corvus albus*). These birds were chosen for their genetic, dietary, and microhabitat differences. We hypothesized that birds inhabiting different microhabitats and with varied diets would have different intra- and inter-tissue cholesterol levels.

MATERIALS AND METHODS

Birds: A total of twenty (20) adult male birds (n=5/species/breed) were used for this study. The local and exotic (broiler) breeds of domestic chicken (*Gallus gallus domesticus*) were purchased from breeders at a local market, while the white-breasted crow (*Corvus albus*) was trapped by experienced local hunters using baited traps that inflicted no physical harm on the birds. The presence of external morphological features such as the comb in domestic chickens and the presence of testes upon dissection were used to confirm sexual maturity in male birds. Female birds were excluded from this study to prevent potential confounding effects of sex hormones on the data generated. Birds were sampled during their respective breeding seasons when mature males were more likely to have pronounced testis and other external sexual characteristics, which enhanced sex identification.

Tissue Extraction: Each bird was humanely euthanized with an overdose intramuscular injection of pentobarbital sodium. Once under permanent anesthesia (confirmed when there were no signs of breathing and the bird became unresponsive to a strong toe pinch), birds were pinned to a dissecting board, defeathered around the chest and abdomen, and samples of the liver, brain, heart, gizzard, and breast muscle were quickly dissected. To extract the brain, an incision was made in the skin to expose the skull, followed by incisions in the midline of the skull and then sideways through the top of the eye sockets to the base of the beak. Each half of the skull bone was gently pulled apart to expose the brain; the optic chiasma was cut to release the brain. All brain samples were washed thoroughly in 0.1 M PBS (pH 7.4), dried with filter paper.

Extraction of total lipids: Total lipids were extracted following the method by Folch *et al*. (1957). Each tissue was first dissected into a tissue blender and blended until a uniform homogenate was achieved, and for every 10 g of tissues, 10 mL of a chloroform-methanol solution (2:1, v/v) was added to the blender, mixed thoroughly, and transferred to a test tube. The blender was rinsed with an additional 10 mL of chloroform-methanol solution, and the rinse was transferred to the same test tube. The test tube was tightly sealed, shaken for 10 minutes, and left at room temperature for 45 minutes. The contents of the tube were filtered through filter paper into a test tube. Then, 0.2% CaCl2 (0.2 times the volume of the filtrate) was added to the filtrate, homogenized, and allowed to stand until two layers formed. The upper layer was aspirated and discarded, while the lower lipid-containing layer was preserved for quantification of total cholesterol.

Quantification of total cholesterol: Before quantifying total cholesterol in each sample, the lipid sample was dissolved in 2 mL of chloroform. The analytical method, which employed the Liebermann-Burchard reagent as described by Kim and Goldberg (1969), was used to quantify total cholesterol concentrations from extracted lipid samples. To quantify total cholesterol, 3 mL of Liebermann-Burchard reagent was added to 0.2 mL of each sample, gently mixed, and left in the dark until a blue-green coloration developed. For the blank, 3 mL of the Liebermann-Buchard reagent was added to 0.2 mL of chloroform. A standard solution containing 0.4 mg of cholesterol was also prepared. The blank was used to calibrate the spectrophotometer, and the optical density measured at 550 nm.

Statistical analysis: To assess if different avian species found in Nsukka regulate tissue cholesterol differently, One-way analysis of variance was used to compare intra- and inter-tissue total cholesterol among birds (SPSS, version 18.0 for Windows, IBM Statistics). Post hoc tests, Duncan and LSD, were used to identify significant differences in intra-species differences and inter-species differences in tissue cholesterol levels. Statistical significance was considered as $p \leq 0.05$ and the results were presented as mean ± standard error of the mean.

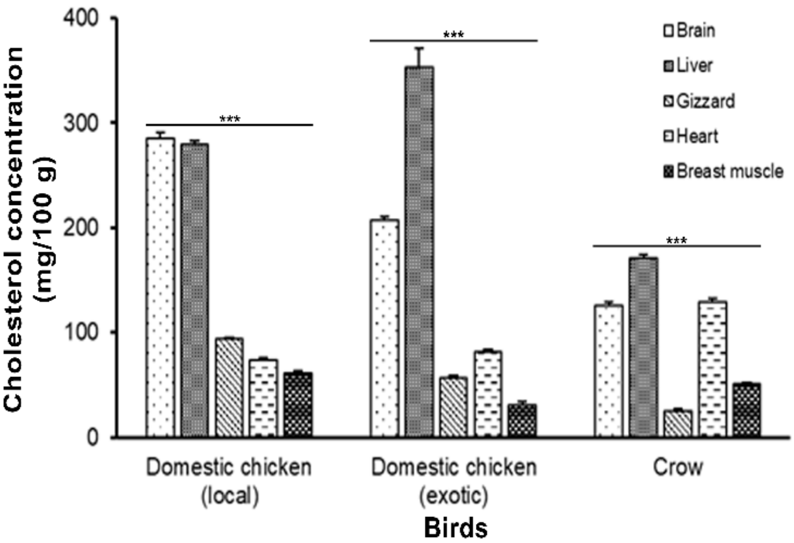

Figure 1:
Intra-species variations in tissue cholesterol content among adult in male avian species found in Nsukka. Plotted values are Mean ± SEM. Error bars: SEM. Top right: Legends. Bars with distinct patterns represent cholesterols in different tissues in a bird. Asterisks represent significant level (n = 5 tissues/species, One-way ANOVA, $p \leq 0.001$).

RESULTS

Intra-specific variations in tissue cholesterol contents: To assess potential intra-specific variations in tissue cholesterol metabolism, we analysed the mean differences in tissue cholesterol levels in three avian species found in Nsukka, Nigeria. The results show that there were significant differences in tissue cholesterol levels in all birds examined (Figure 1).

Table 1:
Multiple comparisons of intra-species differences in tissue cholesterol levels.

Dependent variable	(i) Tissues	(j)Tissues	Mean difference (i-j)	SEM	Sig.	Lower bound	Upper bound
Crow	Brain	Gizzard	100.74*	3.96	<0.001	92.48	109.00
		Heart	-4.15ns	3.96	0.307	-12.41	4.11
		Liver	-45.24*	3.96	0.001	-53.50	-36.98
		Breast muscle	74.54*	3.96	0.001	66.28	82.80
	Gizzard	Brain	-100.74*	3.96	0.001	-109.00	-92.48
		Heart	-104.89*	3.96	0.001	-113.15	-96.63
		Liver	-145.99*	3.96	0.001	-154.25	-137.73
		Breast muscle	-26.20*	3.96	0.001	-34.46	-17.94
	Heart	Brain	4.15 ns	3.96	0.307	-4.11	12.41
		Gizzard	104.89*	3.96	0.001	96.63	113.15
		Liver	-41.10*	3.96	0.001	-49.36	-32.84
		Breast muscle	78.69*	3.96	0.001	70.43	86.95
	Liver	Brain	45.24*	3.96	0.001	36.98	53.50
		Gizzard	145.99*	3.96	0.001	137.73	154.25
		Heart	41.10*	3.96	0.001	32.84	49.36
		Breast muscle	119.78*	3.96	0.001	111.52	128.04
	Breast muscle	Brain	-74.54*	3.96	0.001	-82.80	-66.28
		Gizzard	26.20*	3.96	0.001	17.94	34.46
		Heart	-78.69*	3.96	0.001	-86.95	-70.43
		Liver	-119.03*	3.96	0.001	-128.04	-111.52
Domestic chicken (local)	Brain	Gizzard	191.03*	5.15	0.001	180.30	201.77
		Heart	211.25*	5.15	0.001	200.51	221.99
		Liver	4.9 ns	5.15	0.353	-5.84	15.64
		Breast muscle	223.24*	5.15	0.001	212.50	233.98
	Gizzard	Brain	-191.03*	5.15	0.001	-201.77	-180.29
		Heart	20.21*	5.15	0.001	9.47	30.95
		Liver	-186.13*	5.15	0.001	-196.87	-175.39
		Breast muscle	32.20*	5.15	0.001	21.46	42.94
	Heart	Brain	-211.25*	5.15	0.001	-221.99	-200.51
		Gizzard	-20.21*	5.15	0.001	-30.95	-9.47
		Liver	-206.35*	5.15	0.001	-217.09	-195.61
		Breast muscle	11.99*	5.15	0.030	1.25	22.73
	Liver	Brain	-4.90 ns	5.15	0.353	-15.64	5.84
		Gizzard	186.13*	5.15	0.001	175.35	196.87
		Heart	206.35*	5.15	0.001	195.61	217.09
		Breast muscle	218.34*	5.15	0.001	207.6	229.08
	Breast muscle	Brain	-223.24*	5.15	0.001	-233.98	-212.50
		Gizzard	-32.20*	5.15	0.001	-42.94	-21.46
		Heart	-11.99*	5.15	0.030	-22.73	-1.25
		Liver	-218.34*	5.15	0.001	-229.08	-207.60
Domestic chicken (Broiler)	Brain	Gizzard	151.09*	11.93	0.001	126.21	175.96
		Heart	126.28*	11.93	0.001	101.41	151.16
		Liver	-145.44*	11.93	0.001	-170.32	-120.56
		Breast muscle	176.60*	11.93	0.001	151.72	201.47
	Gizzard	Brain	-151.09*	11.93	0.001	-175.96	-126.21
		Heart	-24.80 ns	11.93	0.051	-49.68	0.07
		Liver	-296.53*	11.93	0.001	-321.41	-271.65
		Breast muscle	25.51*	11.93	0.045	0.64	50.39
	Heart	Brain	-126.28*	11.93	0.001	-151.16	-101.41
		Gizzard	24.80 ns	11.93	0.051	-0.72	49.68
		Liver	-271.73*	11.93	0.001	-296.60	-246.85
		Breast muscle	50.32*	11.93	0.001	25.44	75.19
	Liver	Brain	145.44*	11.93	0.001	120.57	170.32
		Gizzard	296.53*	11.93	0.001	271.65	321.41
		Heart	271.73*	11.93	0.001	246.85	296.60
		Breast muscle	322.04*	11.93	0.001	297.17	346.92
	Breast muscle	Brain	-176.60*	11.93	0.001	-201.47	-151.72
		Gizzard	-25.51*	11.93	0.045	-50.39	-0.64
		Heart	-50.32*	11.93	0.001	-75.19	-25.44
		Liver	-322.04*	11.93	0.001	-346.92	-297.17

Note: Values are expressed as mean ± SEM (n = 5 birds/species, One-way ANOVA, followed by LSD separation). *: significant, ns: Not significant.

Additionally, the brain and liver maintained significantly higher cholesterol levels compared to other tissues in all species studied, except for the crow that had heart cholesterol levels similar to the brain (129.52±3.32 mg/100 g in the heart vs 125.37±3.40 mg/100 g in the brain). For instance, there was a cholesterol concentration of 125.37±3.4 mg/100 g in the crow brain; 207.53±3.33 mg/100 g in the broiler; and 284.66±6.60 mg/100 g in the local chicken On the other hand, the gizzard (24.63±2.18 mg/100 g in the crow; 56.44±2.63 mg/100 g in the broiler; and 93.63±2.04 mg/100 g in the local chicken) and breast muscle (30.93±3.02 mg/100 g in the broiler; 50.83±1.37 mg/100 g in the crow; and 41.43±2.04 mg/100 g in the local domestic chicken) had the least amount of cholesterol compared to other tissues, except for the domestic chicken gizzard that had a higher cholesterol content (93.63±2.04 mg/100 g) compared to the heart (73.42±2.53 mg/100 g) and breast muscles (61.43±2.04 mg/100 g). The multiple species-specific comparisons of cholesterol levels revealed significant differences between most tissues, except for the brain vs heart in the crow (Table 1, p = 0.307), brain vs liver in the domestic chicken (p = 0.353), and heart vs gizzard in the exotic breed (broiler) (p = 0.051), which were not significantly different.

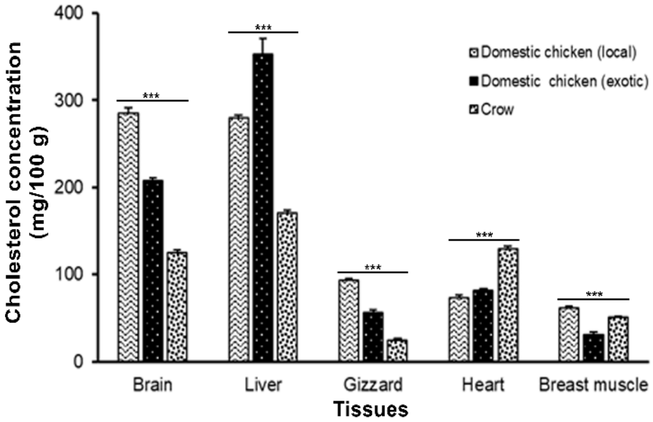

Figure 2:
Inter-species differences in tissue cholesterol levels in a specific tissue in Nsukka.
Plotted values are Mean ± SEM. **Error bars**: SEM. **Top right**: Legends. Bars with different patterns represent different species for a particular tissue. Asterisks represent significant level (n = 5 birds/species, One-way ANOVA, p ≤ 0.001).

Table 2:
Multiple comparisons of inter-species variations in tissue cholesterol contents.

Dependent variable	(I) Species	(J) Species	Mean difference (I-J)	SEM	Sig.	Lower bound	Upper bound
Brain	Crow	Domestic chicken (local)	-159.29*	6.64	<0.001	-173.77	-144.81
		Domestic chicken (Broiler)	-82.16*	6.64	<0.001	-96.64	-67.67
	Domestic chicken (local)	Crow	159.29*	6.64	<0.001	144.81	173.77
		Domestic chicken (Broiler)	77.13*	6.64	<0.001	62.65	91.62
	Domestic chicken (Broiler)	Crow	82.16*	6.64	<0.001	67.67	-62.65
		Domestic chicken (local)	-77.13*	6.64	<0.001	-91.62	-61.92
		Domestic chicken (Broiler)	-69.00*	6.64	<0.001	-76.08	-24.73
Liver	Crow	Domestic chicken (local)	-109.15*	15.08	<0.001	-142.01	-76.28
		Domestic chicken (Broiler)	182.36*	15.08	<0.001	-215.22	-149.49
	Domestic chicken (local)	Crow	109.15*	15.08	<0.001	76.28	142.01
		Domestic chicken (Broiler)	-73.21*	15.08	<0.001	-106.08	-40.34
	Domestic chicken (Broiler)	Crow	182.36*	15.08	<0.001	149.49	215.22
		Domestic chicken (local)	73.21*	15.08	<0.001	40.34	106.08
Gizzard	Crow	Domestic chicken (local)	-69.00*	3.25	<0.001	-76.03	-61.92
		Domestic chicken (Broiler)	-31.81*	3.25	<0.001	-38.90	-24.73
	Domestic chicken (local)	Crow	69.00*	3.25	<0.001	61.92	76.08
		Domestic chicken (Broiler)	37.19*	3.25	<0.001	30.11	44.27
	Domestic chicken (Broiler)	Crow	31.81*	3.25	<0.001	24.73	38.90
		Domestic chicken (local)	-37.19*	3.25	<0.001	-44.27	-30.11
Heart	Crow	Domestic chicken (local)	56.10*	3.87	<0.001	47.67	64.54
		Domestic chicken (Broiler)	48.27*	3.87	<0.001	39.84	56.71
	Domestic chicken (local)	Crow	-56.10*	3.87	<0.001	-64.54	-47.67
		Domestic chicken (Broiler)	-7.83	3.87	0.066	-16.26	0.60
	Domestic chicken (Broiler)	Crow	-48.27*	3.87	<0.001	-56.71	-39.84
		Domestic chicken (local)	7.83	3.87	0.066	-0.60	16.26
Breast muscle	Crow	Domestic chicken (local)	-10.59*	3.17	0.006	-17.51	-3.68
		Domestic chicken (Broiler)	19.90*	3.17	<0.001	12.99	26.82
	Domestic chicken (local)	Crow	10.59*	3.17	0.006	3.68	17.51
		Domestic chicken (Broiler)	30.50*	3.17	<0.001	23.58	37.41
	Domestic chicken (Broiler)	Crow	-19.90*	3.17	<0.001	-26.82	-12.99
		Domestic chicken (local)	-30.50*	3.17	<0.001	-37.41	-23.58

Note: Values are expressed as mean ± SEM (n = 5 birds/species, One-way ANOVA, followed by LSD separation). *: significant, ns: Not significant.

Inter-specific variations in tissue cholesterol levels: To evaluate the possibility that avian species inhabiting similar environments, differing in diets and behaviours regulated cholesterol metabolism in the same tissue differently, this compared cholesterol levels in each of the brain, liver, heart gizzard, and breast muscle among the crow, domestic local and exotic chickens. The results show significant difference in tissue-specific cholesterol levels among the species examined (Figure 2, n = 5 birds/species, One-way ANOVA, $p < 0.001$ for all comparisons), with the local and exotic chickens having higher cholesterol levels in all tissues except in the heart where the crow had significantly higher cholesterol levels (129.52±3.32 mg/100 g in the crow heart, one-way ANOVA, $p < 0.001$). For instance, the brain cholesterol concentration was 284.66±6.6 mg/100 g in the local chicken; 207.53±3.33 mg/100 gin the broiler; and 125.37±3.40 mg/100 g in the crow, whereas the liver cholesterol concentration was 352.97±17.98 mg/100 g in the broiler; 279.76±2.82 mg/100 g in the local chicken; and 170.62±3.14 mg/100 g in the crow. On the other hand, the lowest cholesterol concentrations were found in the breast muscles (30.93±3.02 mg/100 g in the broiler; 50.83±1.37 mg/100 g in the crow, and 61.43±2.04 mg/100 g in the local domestic chicken). Additionally, except for the higher gizzard cholesterol level in the local domestic chicken, the birds examined had comparable gizzard cholesterol concentration to levels found in the breast muscle. The multiple comparisons of inter-specific variations in tissue cholesterol contents revealed significant differences for all pairs of tissues compared except for the broiler vs domestic comparison in the heart (Table 2, $p = 0.066$).

DISCUSSION

This study investigated the possibility that tissue cholesterol content varied within and among avian species that differ in diet, behaviour and micro-habitat preferences. Analysis of the tissue cholesterol content in three avian species revealed both significant intra- and inter-species differences. Liver and brain tissues recorded relatively higher cholesterol levels compared to the heart, gizzard, and breast muscle in all the species examined. Furthermore, we reported that the two breeds of domestic chickens exhibited higher cholesterol levels in all examined tissues relative to other species. Lastly, the exotic breed had higher cholesterol levels in all tissues compared with the local breed of the domestic chicken.

Although we recorded intra- and inter-species differences in brain and liver cholesterol levels like previous studies, the observed cholesterol levels appeared to be higher than previous reports in various chicken strains. For instance, whereas we recorded liver cholesterol levels of 174.21±4.22, 170.62 ± 3.15, 279.76±2.82, and 352.97±17.97 for the quail, crow, local domestic chicken, and the exotic chicken respectively, Konjufca *et al*. (1997) reported liver and muscle cholesterol levels of 143 mg/100g and 46 mg/100g in males of the fast-growing Ross 208 broiler strain raised on a corn and soybean-based diet. On the other hand, Al-ruwaili *et al*. (2014) found strain-specific differences in thigh, breast, and liver cholesterol contents of four broiler strains including Ross. However, the liver cholesterol level recorded by Al-ruwaili and colleagues was greater than that recorded by Konjufca *et al*. (1997) in the same strain (160.37 vs 143 mg/100g), suggesting that tissue cholesterol levels may vary even within the same strain (Ross). The breast muscle and liver cholesterol levels recorded in our current study were higher compared to values in Al-ruwaili *et al*. (2014) for the Ross, Lohman, Hubbard, and the local (Baladi) broiler strains. However, the Baladi strain had higher breast muscle cholesterol compared to the local and exotic chickens in our study (92.55 mg/100g vs 61.42±2.04 mg/100g and 30.93±3.02 mg/100g). In male cross-bred chicks, a protein-rich diet increased cholesterol synthesis in the liver and intestine compared to other organs (Yeh and Leveille 1971). Although we do not have precise information about the diets of our bird samples, it is likely that genetic, habitat, seasonal, and dietary differences could explain the discrepancies between our findings and previous studies. For instance, diet supplementation with 2% cholesterol increased the liver free cholesterol levels in White Leghorns (Teekell *et al*., 1974). In dogs fed a diet supplemented with 1 g of cholesterol for seven days, liver cholesterol levels strongly increased in cholesterol-fed dogs compared to controls (315 vs 246 mg/100g) (Gould *et al*., 1953). These findings support our thinking that diet could be one of the major factors that influenced tissue cholesterol levels recorded in the present study and that irrespective of genetics, diet has a strong impact on the modulation of cholesterol homeostasis in animals. The crow and quail in this study subsist on unique diets. Whereas the crow feeds mainly on waste food scraps and occasionally, dead rodents and chicken eggs the quail feeds on dry seed, insects, and fresh and dry tubers (cassava, potato, and yam). An examination of variations in cholesterol content in other wild and domesticated birds may help resolve the idea that domesticated species may have relatively higher levels of tissue cholesterol compared to their wild counterparts.

The current study also reports a higher brain cholesterol level compared to the heart, gizzard, and breast muscle. An animal's body contains, on average, about 2100 mg/kg of body weight of cholesterol and in humans and mice, 15% and 23% of cholesterol are found in the central nervous system (Dietschy, 2009). The brain synthesizes its cholesterol from astrocytes and oligodendrocytes and there is no evidence of uptake of the plasma-borne LDL-C across the blood-brain-barrier even in newborn lambs (Cavender *et al*., 1995). In humans, the average amount of free cholesterol in the central nervous system (CNS) is around 23 mg/g, the highest of all tissues (Dietschy & Turley, 2004). As we do not have extant data on the brain cholesterol content of birds, these studies suggest that at baseline physiological conditions, the brain may have cholesterol levels like the liver, but greater than other internal organs. Put together these studies and our current findings support unique but conserved tissue-specific mechanisms for cholesterol homeostasis across different avian species inhabiting the same or different environments. The range of gizzard cholesterol levels recorded in our study does not compare well with previous studies. For instance, cooked gizzard and roasted heart giblets had cholesterol contents of 72.68 and 213.18 mg/100g respectively (Pereira *et al*., 2002), while Antunes *et al*. (2018) recorded a value of 176.8 mg/100g for the ostrich gizzard. On the other hand, we recorded gizzard cholesterol values ranging from 24.63 mg/100g in the crow to 93.63 mg/100g in the local domestic chicken, whereas cholesterol values in the heart ranged from

51.98±3.61 mg/100g in the quail to 129.52±3.32 mg/100g in the crow. These discrepancies could be due to genetic, nutritional, environmental differences, or an interplay of factors. A recent study found sex differences in liver cholesterol levels of males and females of the Ongole cross breed cattle and the Kacang goat, respectively, with males of both species having higher liver cholesterol levels compared to females (Susanto *et al*., 2022). Since all subjects in our study were males, we do not know the extent to which sex differences impacted our findings. However, Rule *et al*. (2002) reported a higher cholesterol content in chicken breast muscle compared to the longissimus dorsi and semitendinosus muscles in the bison.

In conclusion, we present evidence supporting intra- and inter-species differences in tissue cholesterol metabolism among various avian species inhabiting similar environments, but unique in genetics, diet, and behaviour. More extensive studies are recommended for other wild and domesticated species to further reveal the extent to which differences in genetics, habitat, diet, sex, and behaviour impact on tissue-specific cholesterol metabolism.

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Author contributions
CN Asogwa: Conceptualization, ran wet experiments, design, statistical analysis, and drafting the manuscript, and correspondence. CA Ezema: Statistical analysis, investigation, writing. DA Osibe: Sample collection, design, writing the manuscript.CI Ugwu: Sample collection, collation, supervision.

Ethics statement
Permission to conduct this investigation was obtained from the Faculty of Biological Sciences Ethics Committee on Animal Experiments. This Committee's guidelines are based on the regulations for animal welfare in Nigeria which ensures humane treatment and management of experimental animals.

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