

**DIFFERENCES IN THE TISSUE CHOLESTEROL CONTENT OF WILD
AND DOMESTICATED BIRDS INHABITING VARIOUS MICRO-
ENVIRONMENTS IN NSUKKA, NIGERIA**

***¹Asogwa C. N. and ²Osibe D. A.**

¹Department of Zoology and Environmental Biology, Physiology Unit, University of Nigeria, 410001, Nsukka, Enugu State, Nigeria

²Department of Plant Science and Biotechnology, University of Nigeria, 410001, Nsukka, Enugu State, Nigeria, Nigeria.

Email: chinweike.asogwa@unn.edu.ng

DOI: <https://doi.org/10.5281/zenodo.17182646>

ABSTRACT: Information on the tissue cholesterol content of various avian species inhabiting contiguous microenvironments, with differences in diet and behavior, is limited. This study evaluated potential differences in tissue cholesterol concentrations of several avian species inhabiting varying, but sometimes overlapping, microhabitats in Nsukka, Nigeria. The birds included adult males of two domesticated and one wild species: turkey (*Meleagris gallopavo*, Galliformes), pigeon (*Columba livia*, Columbiformes), and wild Guinea fowl (*Numida meleagris*, Galliformes). Tissue samples were dissected from the brain, liver, breast muscle, gizzard, and heart (n = 5/species), and total lipids and total cholesterol levels in each tissue were extracted using standard methods. One-way analysis of variance was used to analyze potential differences in tissue cholesterol concentrations, while the mean differences in tissue cholesterol concentrations were considered significant at $p \leq 0.05$. The results show significant species differences in tissue cholesterol concentration ($p < 0.001$), with the brain and liver having the highest cholesterol concentrations. Significant intraspecific differences in cholesterol concentration were observed in each species ($p < 0.001$). Furthermore, the pairwise comparisons revealed significant differences in cholesterol levels for most pairs of tissues compared. These findings show that avian species inhabiting similar environments regulate cholesterol differently in different tissues within a species and in similar tissues across different species.

Keywords: Cholesterol, birds, variation, metabolism, tissue, adaptation.

Running title: Tissue cholesterol variation in Nsukka birds

INTRODUCTION

Animal species are endowed with adaptive features that optimize feeding, movement, defense, offense, resistance to parasites and diseases, and successful reproduction in a wide range of environments (Clauss et al., 2008; Naudi et al., 2013; Sejian et al., 2018; Olsen et al., 2021; Smith and Son, 2023; Chin, 2024). For example, avian species

in different habitats differ significantly in terms of morphological, behavioral, physiological, and biochemical features that aid in maximizing resource exploitation for survival (Davies, 1982; Prestrud et al., 1991; Russell and Tumlinson, 1996; Hedenstrom, 2008; Møller, 2015; Allam et al., 2019). Among the biochemical adaptations of birds, varying regulations of lipid metabolism have been observed at the tissue and species levels (Prestrud and Nilssen, 1992; Enriquez and Visser, 2023). Previous studies indicate that temperate birds generally have higher adipose deposits than tropical species (Corder et al., 2016; González-Medina et al., 2023), suggesting that genetics, environment, diet, or a combination of factors may influence species lipid regulation.

Cholesterol is a crucial lipid in animal tissues, essential for synthesizing sex hormones, steroids, and bile acids, and a major component of cellular and nuclear membranes (Bastiaanse et al., 1997; Zhang et al., 2018; Frangos et al., 2023). Additionally, cholesterol is vital for myelin sheaths around neurons in the nervous system (Mouritsen and Zuckermann, 2004; Poitelon et al., 2020; Barnes-Vélez et al., 2022). Like other vertebrates, birds produce endogenous cholesterol primarily in the brain and liver, with the liver contributing over 70% of tissue cholesterol, with the rest from diet (Frantz et al., 1954; Jeske and Dietschy, 1980). Tissue cholesterol content may vary based on genetics, physiological state, diet, or other factors (Al-ruwaili et al., 2014; Lorenz et al., 1938; Musacchia, 1953; Jeske and Dietschy, 1980; Al-ruwaili et al., 2014; Palmisano et al., 2018). While egg cholesterol content has received attention, tissue-specific cholesterol regulation is less explored (Kaźmierska et al., 2005; Oloyede, 2005; Faitarone et al., 2013; Akinwumi et al., 2019; Ossamulu et al., 2023). Investigating species differences in tissue cholesterol composition can enhance understanding of how species with similar habitats but different behaviors and diets regulate cholesterol for survival. This study aimed to assess potential variations in tissue cholesterol content in wild and domesticated birds in Nsukka, Nigeria, to determine whether birds in similar tropical habitats but with different behaviors and diets regulate cholesterol differently in various tissues. Currently, there is no information on tissue and species-specific differences in cholesterol in avian species in Nigeria.

2.0 MATERIALS AND METHODS

2.1 Bird procurement

Adult males of three bird species were used for this study: domesticated turkey (*Meleagris gallopavo*, Galliformes), pigeon (*Columba livia*, Columbiformes), and wild guinea fowl (*Numida meleagris*, Galliformes). Sex identification was primarily based on the presence of prominent testes upon dissection. Females were intentionally excluded to prevent the potential confounding effects of sex hormones on cholesterol regulation, which was not the focus of the study. A total of 15 birds ($n = 5/$ species) were included in the study, with domesticated species sourced from local breeders and wild guinea fowl obtained from local hunters. The birds were not fed or kept in captivity during the study.

2.2 Collection of tissue samples

The birds were humanely euthanized with an overdose intramuscular injection of pentobarbital sodium. They were then pinned to a dissecting tray, defeathered around the chest and abdomen, and the liver, heart, gizzard, and breast muscle were swiftly dissected. The brain was extracted through deep incisions made in the midline of the skull and then around the base of the skull up to above the eye sockets. Dissected tissue samples were washed in 0.1 M PBS (pH 7.4), dried with filter paper, and either stored at $-20\text{ }^{\circ}\text{C}$ or used immediately for total lipid extraction and cholesterol quantification.

2.3 Total lipid extraction and cholesterol quantification in tissue samples

The total lipid content was extracted following the method described by Folch et al. (1957). Tissue samples were homogenized in a tissue blender with a chloroform/methanol solution (2:1, v/v). The mixture was then filtered,

and the lower lipid-containing layer was preserved for the quantification of total cholesterol. To quantify the total cholesterol concentration, we prepared Liebermann-Buchard reagent, which produces a blue-green product depending on the amount of cholesterol present. We also prepared a blank, a series of cholesterol standards, and added each to specific cuvettes. Next, the spectrophotometer was calibrated using the blank, and the optical absorbance of the standards and samples was measured at 550 nm. Finally, we determined the cholesterol level in each sample by comparing the optical absorbance of the samples with those of standards with known cholesterol concentrations.

2.4 Statistical analysis of the data

A one-way analysis of variance was used to assess differences in tissue cholesterol concentration within and across species. The Duncan post hoc test was used to identify differences in mean tissue cholesterol values within a species, whereas the least square difference test was used to separate the mean differences in cholesterol values between two tissues within a species or between different species. The results were considered statistically significant at $p \leq 0.05$ and presented as mean \pm standard error of the mean (SEM).

3.0 RESULTS

3.1 Intra-species differences in the cholesterol content of tissue

To test the possibility that different bird species regulate tissue cholesterol content, we compared cholesterol values among the brain, liver, breast muscle, heart, and gizzard of each species. The results show significant differences in tissue cholesterol contents (Figure 1, one-way ANOVA; post hoc, Duncan; $p = 2.76E-16$ for the pigeon; $p = 1.50E-18$ for Guinea fowl; and $p = 8.07E-23$ for the turkey). Further post hoc analysis revealed that pairs of tissues within a species also differed in terms of cholesterol levels. In the pigeon, the liver had a higher cholesterol concentration than the other tissues ($p < 0.001$ in all pairwise comparisons, except for the brain, where $p = 0.002$). In the guinea fowl, the liver had significantly higher cholesterol concentrations than the other tissues ($p < 0.001$), except for the brain, which did not attain statistical significance ($p = 0.318$). However, the comparison between the breast muscle and the gizzard was not significant ($p = 0.199$; Table 1). In the turkey, even though the liver contained a significantly higher level of cholesterol when paired with every other tissue ($p < 0.001$ in all cases), every other pairwise comparison was statistically significant except between the heart and the gizzard (Table 1, $p = 0.083$).

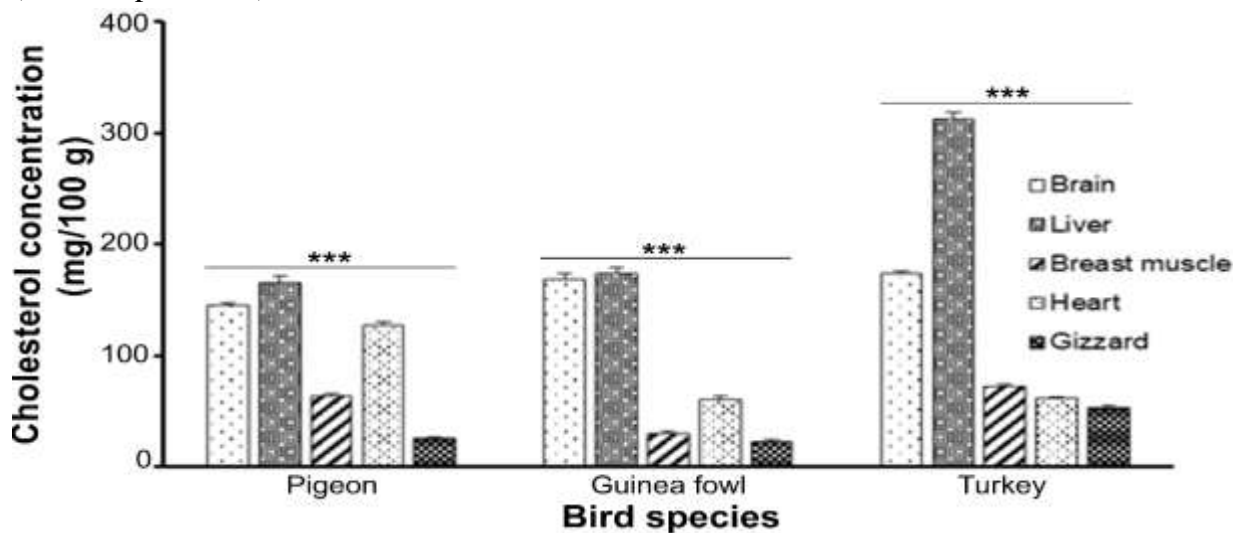


Figure 1: Interspecies variations in tissue cholesterol levels in male adult birds. Plotted values are mean \pm SEM. Error bars: SEM. Top right: Legends showing different tissues in a bird. *** indicates statistical significance ($n = 5$ birds/species, one-way ANOVA, $p \leq 0.001$ for all comparisons).

Table 1: Multiple comparisons of intra-tissue variations in tissue cholesterol levels in different birds.

	(i)Tissues	J(Tissues)	Mean difference(i-j)	SEM	Sig.	Lower bound	Upper bound
Pigeon	Brain	Liver	-19.52*	5.51	0.002	-31.01	-8.03
		Breast muscle	81.80*	5.51	<0.001	70.31	93.29
		Heart	18.12*	5.51	0.004	6.63	29.61
		Gizzard	120.24*	5.51	0.002	108.75	131.73
	Liver	Brain	19.52*	5.51	0.002	8.03	31.01
		Breast muscle	101.32*	5.51	<0.001	89.83	112.81
		Heart	37.64*	5.51	<0.001	26.15	49.13
		Gizzard	139.76*	5.51	<0.001	128.27	151.25
	Breast muscle	Brain	-81.80*	5.51	<0.001	-93.29	-70.31
		Liver	-101.32*	5.51	<0.001	-112.81	-89.83
		Heart	-63.68*	5.51	<0.001	-75.17	-52.19
		Gizzard	38.44*	5.51	<0.001	26.95	49.93
	Heart	Brain	-81.12*	5.51	0.004	29.61	-6.63
		Liver	-37.64*	5.51	<0.001	-49.13	-26.15
		Breast muscle	63.68*	5.51	<0.001	52.19	75.17
		Gizzard	102.12*	5.51	<0.001	90.63	113.61
Gizzard	Brain	-120.24*	5.51	<0.001	-131.73	-108.75	
	Liver	-139.76*	5.51	<0.001	-151.25	-128.27	
	Breast muscle	-38.44*	5.51	<0.001	-49.93	-26.95	
	Heart	-5.54*	5.51	<0.001	-113.61	-90.63	
Guinea fowl	Brain	Liver	-5.54 ^{ns}	5.41	0.318	-16.81	5.74
		Breast muscle	138.51*	5.41	<0.001	127.23	149.78
		Heart	107.56*	5.41	<0.001	96.29	118.84
		Gizzard	145.69*	5.41	<0.001	134.42	156.97

Turkey	Liver	Brain	5.54 ^{ns}	5.41	0.318	-5.74	16.81
		Breast muscle	144.04*	5.41	<0.001	132.77	155.34
	Breast muscle	Heart	113.10*	5.41	<0.001	101.83	124.38
		Gizzard	151.23*	5.41	<0.001	139.95	162.51
		Brain	-138.51*	5.41	<0.001	-149.78	-127.23
	Heart	Liver	-144.04*	5.41	<0.001	-155.32	-132.77
		Heart	-30.94*	5.41	<0.001	-42.22	-19.66
		Gizzard	7.19*	5.41	0.199	-4.09	18.46
	Gizzard	Brain	-107.56*	5.41	<0.001	-118.84	-96.29
		Liver	-13.10*	5.41	<0.001	-124.38	-101.83
		Breast muscle	30.93*	5.41	<0.001	19.66	4.22
	Brain	Gizzard	38.13*	5.41	<0.001	26.85	49.40
		Brain	-145.69*	5.41	<0.001	156.97	134.42
		Liver	-151.23*	5.41	<0.001	-162.51	-139.96
	Liver	Breast muscle	-7.19 ^{ns}	5.41	0.199	-18.46	4.09
		Heart	-38.13*	5.41	<0.001	-49.40	-26.85
		Liver	-138.97*	4.90	<0.001	-149.18	-128.75
	Breast muscle	Breast muscle	101.47*	4.90	<0.001	91.25	111.68
		Heart	111.83*	4.90	<0.001	101.62	122.05
		Gizzard	120.77*	4.90	<0.001	110.56	130.99
	Liver	Brain	138.97*	4.90	<0.001	128.75	149.18
		Breast muscle	240.43*	4.90	<0.001	230.22	250.65
		Heart	250.80*	4.90	<0.001	240.59	261.01
	Breast muscle	Gizzard	259.74*	4.90	<0.001	249.53	269.96
		Brain	-101.47*	4.90	<0.001	-111.68	-91.25
		Liver	-240.43*	4.90	<0.001	-250.65	-230.22
	Heart	Heart	10.37*	4.90	0.047	0.15	20.58
		Gizzard	19.31*	4.90	<0.001	9.09	29.52
Brain		-111.83*	4.90	<0.001	-118.84	-96.29	

	Liver	-250.80*	4.90	<0.001	-124.38	-101.83
	Breast muscle	-10.37*	4.90	0.047	19.66	42.22
	Gizzard	8.94*	4.90	0.08	26.85	49.40
Gizzard	Brain	-120.77*	4.90	<0.001	130.99	-110.56
	Liver	-259.74*	4.90	<0.001	-269.96	-249.53
	Breast muscle	19.31*	4.90	<0.001	-29.52	-9.09
	Heart	-8.94 ^{ns}	4.90	0.083	-19.16	1.27

Notes: Asterisks indicate significant differences; ns: not significant. SEM: standard error of the mean; n = 5/species

3.2 Interspecies variations in the cholesterol concentration in tissue

To assess potential interspecies variations in cholesterol regulation, the cholesterol levels in a particular tissue were compared among the three species. The results show significant interspecies differences in cholesterol concentration in the tissues examined (Figure 2, one-way ANOVA, N = 5 birds/species, $p = 3.4E-4$ for the brain, $p = 1.55E-9$ for the liver, $p = 3.86E-8$ for the breast muscle, $p = 4.58E-10$ for the heart, and $p = 2.54E-7$ for the gizzard). Of all the species, the turkey had the highest cholesterol concentrations in all tissues, except in the heart where the pigeon had a higher cholesterol concentration than the other species (127.11 ± 3.64 mg/100 g in the pigeon vs. 60.76 ± 2.32 mg/100 g and 61.52 ± 1.44 mg/100 g in the guinea fowl and turkey, respectively). Compared with other tissues, the gizzard had the lowest tissue cholesterol levels. Further multiple comparisons (LSD) revealed significant differences in tissue cholesterol concentrations when specific tissues were compared between the two species (Table 2). For example, the brain cholesterol level of the pigeon significantly differed from those of the guinea fowl and turkey (pigeon vs. guinea fowl: $p = 7.94E-4$; pigeon vs. turkey: $1.55E-4$), whereas the guinea fowl did not differ from the turkey ($p = 0.352$). In the liver, the cholesterol levels of the pigeon differed significantly from those of the turkey, but not the guinea fowl ($p = 1.33E-9$ and $p = 0.329$, respectively), whereas the guinea fowl differed significantly from the turkey ($2.76E-9$). In the breast muscle, the cholesterol concentrations of the pigeon significantly differed from those of the guinea fowl and turkey ($p = 2.05E-7$ and $p = 0.021$, respectively), and the guinea fowl also differed from the turkey ($p = 1.69E-8$). In the heart, the pigeon had a significantly higher cholesterol value than the guinea fowl and turkey ($p = 5.22E-10$ and $5.97E-10$, respectively), whereas the differences in cholesterol content between the guinea fowl and turkey were not significant ($p = 0.841$). The cholesterol levels in the pigeon gizzard were not significantly different from those in the guinea fowl but significantly different from those in the turkey ($p = 0.422$ and $p = 4.65E-7$, respectively), whereas the comparison between the guinea fowl and the turkey was significantly different ($p = 1.91E-7$).

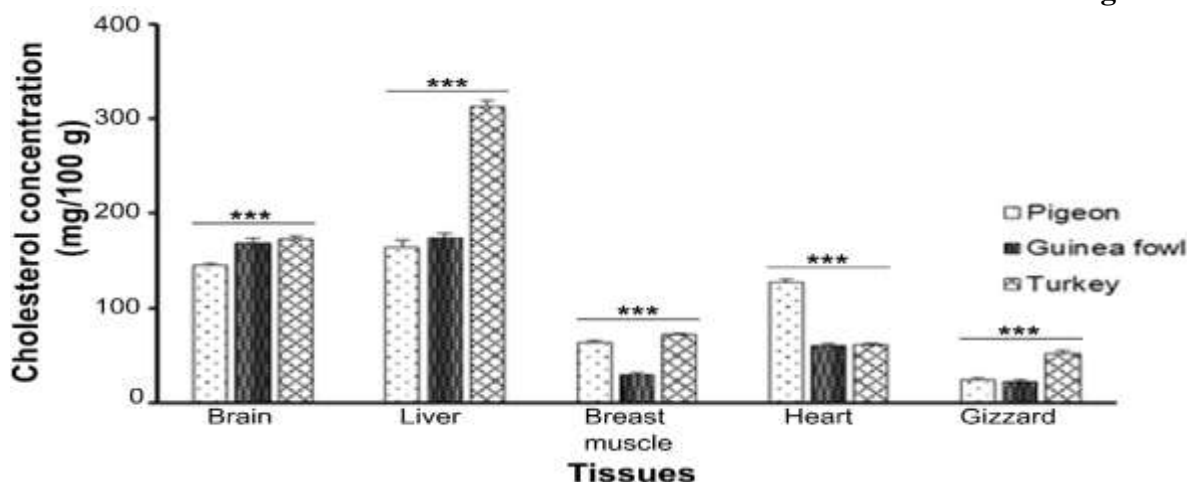


Figure 2: Inter-species differences in tissue cholesterol levels among adult male Nsukka birds. Values plotted are mean \pm SEM. Error bars: SEM. Top right: Legends showing different tissues in a bird. *** indicates statistical significance ($n = 5$ birds/species, one-way ANOVA, $p \leq 0.001$ for all comparisons).

Table 2: Multiple comparisons of interspecies variations in tissue cholesterol levels in different birds.

Dependent variable	(i)Tissues	(j)Tissues	Mean difference (i-j)	SEM	Sig.	Lower bound	Upper bound
Brain	Pigeon	Guinea fowl	-23.10*	5.19	<0.001	-34.41	-11.79
		Turkey	-28.13*	5.19	<0.001	-39.44	-16.81
	Guinea fowl	Pigeon	23.10*	5.19	<0.001	11.79	34.41
		Turkey	-5.03 ^{ns}	5.19	0.352	-16.34	6.29
	Turkey	Pigeon	28.13*	5.19	<0.001	16.81	39.44
		Guinea fowl	5.03 ^{ns}	5.19	0.352	-6.29	16.34
Liver	Pigeon	Guinea fowl	-9.12 ^{ns}	8.96	0.329	-28.64	10.40
		Turkey	-147.58*	8.96	<0.001	-167.10	-128.06
	Guinea fowl	Pigeon	9.12 ^{ns}	8.96	0.329	-10.40	28.64
		Turkey	-138.46*	8.96	<0.001	-157.98	-118.94
	Turkey	Pigeon	147.58*	8.96	<0.001	128.06	167.10
		Guinea fowl	138.46*	8.96	<0.001	118.94	157.98
Breast muscle	Pigeon	Guinea fowl	33.61*	3.19	<0.001	26.65	40.56
		Turkey	-8.46*	3.19	0.021	-15.42	-1.51
	Guinea fowl	Pigeon	-33.61*	3.19	<0.001	-40.56	-26.65

Heart	Turkey	Turkey	-42.07*	3.19	<0.001	-49.02	-35.11	
		Pigeon	8.46*	3.19	0.021	1.51	15.42	
		Guinea fowl	42.07*	3.19	<0.001	35.11	49.02	
	Pigeon	Guinea fowl	66.35*	3.72	<0.001	58.25	74.44	
		Turkey	65.59*	3.72	<0.001	57.49	73.68	
		Guinea fowl	-66.35*	3.72	<0.001	-74.44	-58.25	
	Gizzard	Turkey	Turkey	-0.76 ^{ns}	3.72	0.841	-8.86	7.33
			Pigeon	-65.59*	3.72	<0.001	-73.64	-57.49
			Guinea fowl	0.76 ^{ns}	3.72	0.841	-7.33	8.86
Pigeon		Guinea fowl	2.35 ^{ns}	2.83	0.422	-3.81	8.51	
		Turkey	-27.59*	2.83	<0.001	-33.75	-21.43	
		Guinea fowl	-2.35 ^{ns}	2.83	0.422	-8.51	3.81	
Turkey	Turkey	-29.95*	2.83	<0.001	-36.12	-23.79		
	Pigeon	27.59*	2.83	<0.001	21.43	33.75		
	Guinea fowl	29.95*	2.83	<0.001	23.79	36.12		

Notes: Significant differences are indicated by asterisks; ns: not significant; SEM: standard error of the mean; n = 5/species.

4.0 DISCUSSION

This study investigated potential tissue- and species-specific differences in the regulation of cholesterol in wild and domesticated adult birds in Nsukka, Nigeria. The findings show significant variations in the cholesterol composition of tissues and species. Notably, the liver and brain maintained significantly higher cholesterol levels than other tissues in all birds examined. Furthermore, the domesticated turkey had higher cholesterol content in all tissues compared to other species, except for the pigeon, which had higher cholesterol in the heart.

Like other vertebrates, birds synthesize endogenous cholesterol in the liver and brain; over 70% of cholesterol in other tissues is produced by the liver, with the remainder sourced from diets (Jeske and Dietschy, 1980). Unlike other tissues, the brain does not absorb or secrete cholesterol (Björkhem and Meaney, 2004), which shows that its cholesterol level is independent of plasma cholesterol fluctuations. The findings of this study align with the concept that the liver and brain maintain the highest cholesterol levels compared with other tissues. The brain contains more than 20% of the total body cholesterol (Björkhem and Meaney, 2004), which is crucial for the rapid conduction of nerve impulses essential for survival (Mouritsen and Zuckermann, 2004; Orth and Bellosta, 2012; Hussain et al., 2019; Poitelon et al., 2020; Barnes-Vélez et al., 2022). In contrast, the regulation of cholesterol in other tissues strongly depends on plasma cholesterol levels (Lin and Connor, 1980; Hopkins, 1992). Liver-synthesized cholesterol is transported in plasma as low-density lipoproteins, whereas excess cholesterol in

other tissues is transported back to the liver as high-density lipoprotein via the reverse cholesterol transport pathway (Ouimet et al., 2019; Ohkawa et al., 2020).

Limited information on the lipid composition of bird heart and gizzard comes from gible analysis. For example, Jokanović et al. (2014) reported a higher total lipid content in the heart, followed by the liver, and the lowest in the gizzard of chickens. Another study in Brazil also found a higher total lipid content in the heart than in the liver and gizzard of chickens, with a higher cholesterol level in roasted heart followed by fried liver, and lowest in cooked gizzard (Pereira et al., 2002). Antunes et al. (2018) found the highest total cholesterol concentration in the heart, followed by the gizzard and the liver, while the lowest was found in thigh and leg meats of domesticated ostriches (*Struthio camelus var. domesticus*). These findings, like our results showing higher cholesterol content in pigeon hearts, contrast with the belief that the liver and brain have the highest cholesterol content among the major internal organs. However, the lower cholesterol concentration in ostrich muscles aligns with the lower breast muscle cholesterol levels found in this study. Although total cholesterol was not measured in the study, these findings agree with the lower cholesterol content in the gizzard in this study. Whether the higher total lipids in the heart also reflect a higher cholesterol level remains unclear. Notably, this study also found a higher cholesterol content in the heart than in the gizzard of pigeons. It remains to be understood if there are other avian species that maintain a higher cholesterol level in the heart than in the liver.

Tissue lipid levels largely reflect the current physiological/metabolic state of animals. For example, Musacchia (1953) found higher total cholesterol levels in the liver than in the kidney of four migratory birds. No correlation was found between sex and body weight and total cholesterol levels although wide individual differences existed. Several decades ago, studies showed a causal relationship between physiological states and tissue lipids (Bloor, 1943; Wilber and Musacchia, 1950). Tissue lipids are correlated with egg-laying in chickens and turtles (Lorenz et al., 1938; Chaikoff and Entenman, 1946). These findings mean that changes in lipid levels in tissues largely represent internal regulatory states.

Diet is another key variable that strongly influences the regulation of tissue lipids in birds. For instance, Donaldson et al. (2017) found that high-fat diets composed of different animal and vegetable fat sources significantly influenced the serum and liver cholesterol and total lipid levels of Japanese quail (*Coturnix coturnix japonica*) compared with those of quails fed a standard diet. Chen et al. (2023) found that a high-fat diet significantly increased total fat content, lipid droplet area, and polyunsaturated fatty acid content in the liver, as well as abdominal fat weight, subcutaneous fat weight, and total fat in Perkin ducks. In a developmental study, Adamson et al. (1961) found that chicks (New Hampshires) fed a cholesterol-rich diet had liver cholesterol levels that were over five times higher than those fed a cholesterol-free diet.

Among the three bird species examined in this study, turkeys are bred mostly in captivity for enhanced maturity and marketability using standard commercial diets. In addition to other nutrients, such commercial diets are rich in fat and cholesterol, which are intended to boost meat production and increase fat content. Although the precise diet of the pigeon and the Guinea fowl remains unknown, the pigeon feeds mainly on dried seeds and grains, whereas the Guinea fowl feeds mainly on dried seeds, insects, and tubers. Regardless, the higher cholesterol levels found in most turkey tissues were likely due to its enriched diet. However, the similar levels of cholesterol in the brain and liver of the examined birds which suggests that irrespective of diet and behavior, cholesterol regulatory mechanisms are conserved in domesticated and wild birds.

Conclusion

This study revealed wide variations in tissue cholesterol metabolism among domesticated and wild species of Nsukka birds inhabiting varying habitats and with different diets. Periodic evaluations of variation in lipid

metabolism in these and other birds will be beneficial in assessing possible changes in abiotic factors, including food supply, in Nsukka.

Acknowledgements

We would like to thank the head of the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka, for providing a conducive environment for conducting the study.

REFERENCES

- Adamson, L. F., Leeper, G. K. and Ross, E. (1961). Influence of dietary fats and cholesterol on chicken tissue lipids. *Journal of Nutrition*, 73(3): 247-258. <https://doi.org/10.1093/jn/73.3.247>.
- Akinwumi, A.O., Atandah, R.A., Olawuyi, B.S., Olagoke, O.C., Ojebiyi, O.O. and Odunsi, A.A. (2019). Comparative evaluation on preference and composition of different avian egg types. *Journal of Research in Agricultural Sciences*, 6(5): 2348-3997.
- Allam, A. A., Abo-Eleneen, R. E. and Othman, S. I. (2019). Scale microstructures in selected lizard species. *Saudi Journal of Biological Sciences*, 26: 129-136. <https://doi.org/10.1016/j.sjbs.2017.03.012>.
- Al-Ruwaili, M., Herzallah, S., Al-Dmoor, H. and Al-Atiyat, R. (2014). Effect of commercial broiler strains on total and free cholesterol levels in chicken muscle tissues. *Global Veterinarian*, 12(3): 381-383. <https://doi.org/10.5829/idosi.gv.2014.12.03.82362>.
- Antunes, C., Ribeiro, M.F., Pimentel, F.B., Alves, S.P., Oliveira, M.B.P.P., Bessa, R.J.B. and Quaresma, M.A.G. (2018). Lipid profile and quality indices of ostrich meat and giblets. *Poultry Science*, 97: 1073-1081. <https://doi.org/10.3382/ps/pex379>.
- Barnes-Vélez, J. A., Yasar, F. B. A. and Hu, J. (2022). Myelin lipid metabolism and its role in myelination and maintenance. *The Innovation*, 4(1): 100360. <https://doi.org/10.1016/j.xinn.2022.100360>.
- Bastiaanse, E. M. L., Ho, K. M. and Van der Laarse, A. (1997). Effect of membrane cholesterol content on plasma membrane ion transport processes. *Cardiovascular Research*, 33: 272-283. [https://doi.org/10.1016/s0008-6363\(96\)00193-9](https://doi.org/10.1016/s0008-6363(96)00193-9).
- Björkhem, I. and Meaney, S. (2004). Brain cholesterol: a long secret life behind a barrier. *Arteriosclerosis Thrombosis Vascular Biology*, 24: 806-815. <https://doi.org/10.1161/01.ATV.0000120374.59826.1b>.
- Bloor, W. R. (1943). *Biochemistry of the fatty acids and their compounds, the lipids*. New York: Reinhold.
- Chaikoff, I.L. and Entenman, C. (1946). Lipids of the blood, liver, and egg yolk of the turtle. *Journal of Biological Chemistry*, 166: 683-689.
- Chen, G. H., Zhang, K. Y., Tian, G., Bai, S. P., Ding, X. M., Wang, J. P., Lv, L., Xuan, Y. and Zeng, Q. F. (2023). Effects of a high-fat diet on growth performance, lipid metabolism, and liver and skin fat fatty acid composition in Pekin ducks aged 10–40 days. *Poultry Science*, 102: 102429. <https://doi.org/10.1016/j.psj.2022.102429>.

- Chin, W. (2024). Role of morphological adaptations in environmental resilience: a comparative study across species. *Journal of Morphology and Anatomy*, 8(4): 341-343.
- Clauss, M., Kaiser, T. and Hummel, J. (2008). Morphophysiological adaptations of browsing and grazing mammals In: Gordon, I.J. Prins, H.H.T. (Eds.). *Ecology of browsing and grazing*. Ecological Studies, 195: 47–88.
- Corder, R. R., DeMoranville, K. J., Russell, D. E., Huss, J. M., & Schaeffer, P. J. (2016). Regulation of lipid metabolism and storage and association with PPARs in a migrant species: the gray catbird (*Dumetella carolinensis*). *Journal of Experimental Biology*, 219: 3391-3398. <https://doi.org/10.1242/jeb.141408>.
- Davies, S. J. F. (1982). Behavioral adaptations of birds to environments with high evaporation and short water supply. *Comparative Biochemistry Physiology*, 71A (4): 557-566. [https://doi.org/10.1016/0300-9629\(82\)90204-3](https://doi.org/10.1016/0300-9629(82)90204-3).
- Donaldson, J., Madziva, M. T. and Erlwanger, K. H. (2017). Effects of high-fat diets composed of different animal and vegetable fat sources on the health status and tissue lipid profiles of male Japanese quail (*Coturnix coturnix japonica*). *Asian-Australasia Journal Animal Sciences*, 30(5): 700-711. <https://doi.org/10.5713/ajas.16.0486>.
- Enriquez, T. and Visser, B. (2023). Importance of fat accumulation and reserves for insect overwintering *Current Opinion in Insect Sciences*, 60: 101118. <https://doi.org/10.1016/j.cois.2023.101118>.
- Faitarone, A. B. G., Garcia, E. A., Roça, R., Ricardo, H., Andrade, E.N., Pelícia, K. and Vercese, F. (2013). Cholesterol levels and nutritional composition of commercial layers of eggs fed with different vegetable oils. *Brazilian Journal of Poultry Sciences*, 15(1): 31-38. <https://doi.org/10.1590/S1516-635X2013000100006>.
- Folch, J., Lees, M., & Stanley, G. H. S. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, 226(1): 497-509. [https://doi.org/10.1016/S0021-9258\(18\)64849-5](https://doi.org/10.1016/S0021-9258(18)64849-5).
- Frangos, Z. J., Wilson, K. A., Aitken, H. M., Chater, R. C., Vandenberg, R. J. and O'Mara, M. L. (2023). Membrane cholesterol regulates inhibition and substrate transport by GlyT2. *Life Sciences Alliance*, 6(4): e202201708. <https://doi.org/10.26508/lsa.202201708>.
- Frantz, I. D., Schneider, H. S. and Hinkelman, B. T. (1954). Suppression of hepatic cholesterol synthesis by cholesterol feeding in rats. *Journal of Biological Chemistry*, 206(1): 465-469.
- González-Medina, E., Playà-Montmany, N., Cabello-Vergel, J., Parejo, M., Abad-Gómez, J.M., Sánchez-Guzmán, J.M., Villegas, A., Gutiérrez, J.S. and Masero, J.A. (2023). Mediterranean songbirds show pronounced seasonal variations in their thermoregulatory traits. *Comparative Biochemistry and Physiology A*, 280: 111408. <https://doi.org/10.1016/j.cbpa.2023.111408>.

- Hedenstrom, A. (2008). Adaptations to migration in birds: behavioral strategies, morphology, and scaling effects. *Philosophical Transactions of the Royal Society B*, 363: 287-299. <https://doi/10.1098/rstb.2007.2140>.
- Hopkins, P. N. (1992). Effects of dietary cholesterol on serum cholesterol: a meta-analysis and review *American Journal of Clinical Nutrition*, 55: 1060-1070. <https://doi.org/10.1093/ajcn/55.6.1060>.
- Hussain, G., Wang, J., Rasul, A., Anwar, A., Imran, A., Qasim, M., Zafar, M., Kamran, S. K. S., Razzaq, A. Aziz, N., Ahmad, W., Shabbir, A., Iqbal, J., Baig, S. M. and Sun, T. (2019). Role of Cholesterol and Sphingolipids in Brain Development and Neurological Diseases. *Lipids in Health and Disease*, 18(26): 1-12. <https://doi.org/10.1186/s12944-019-0965-z>.
- Jeske, D. J. and Dietschy, J. M. (1980). Regulation of cholesterol synthesis rates in vivo in the liver and carcass of rats measured using [3H] water. *Journal of Lipid Research*, 21: 364376. [https://doi.org/10.1016/S0022-2275\(20\)39816-3](https://doi.org/10.1016/S0022-2275(20)39816-3).
- Jokanović, M., Tomović, V.M., Jović, M.T., Škaljac, S.B., Šojić, B.V., Ikonić, P.M. and Tasić, T.A., 2014. Proximate and mineral composition of chicken giblets from Vojvodina. *Serbia International Journal of Biological Veterinary Agricultural Food Engineering*, 8(9): 911-914.
- Kaźmierska, M., Jarosz, B., Korzeniowska, M., Trziszka, T. and Dobrzański, Z. (2005). Comparative analysis of the fatty acid profile and cholesterol content of egg yolks from different bird species. *Polish Journal of Food and Nutrition Sciences*, 14/55(1): 69-73.
- Lin, D. S. and Connor, W. E. (1980). Long-term effects of dietary cholesterol on plasma lipids, lipoproteins, cholesterol absorption, and sterol balance in humans: feedback inhibition of cholesterol biosynthesis and increased bile acid excretion *Journal of Lipid Research*, 21: 1042-1052. [https://doi.org/10.1016/S0022-2275\(20\)34764-7](https://doi.org/10.1016/S0022-2275(20)34764-7).
- Lorenz, F. W., Chaikoff, I. L. and Entenman, C. (1938). Liver lipids of laying and non-laying birds *Journal of Biological Chemistry*, 123(2): 577-585. [https://doi.org/10.1016/S0021-9258\(18\)74144-6](https://doi.org/10.1016/S0021-9258(18)74144-6).
- Møller, A. P. (2015). Allometry of the number of feathers in birds changes seasonally. *Avian Research*, 6(2): 2-5. <https://doi.org/10.1186/s40657-015-0012-3>.
- Mouritsen, O. G. and Zuckermann, M. J. (2004). What is very special about cholesterol? *Lipids*, 39: 1101-1113. <https://doi.org/10.1007/s11745-004-1336-x>.
- Musacchia, X. J. (1953). Lipids in Arctic migratory birds. *The Condor*, 55: 305-312.
- Naudí, A., Jové, M., Ayala, V., Portero-Otín, M., Barja, G. and Pamplona, R. (2013). Membrane lipid unsaturation as a physiological adaptation to animal longevity. *Frontiers in Physiology*, 4(432): 1-13. <https://doi.org/10.3389/fphys.2013.00372>.

- Ohkawa, R., Low, H., Mukhamedova, N., Fu, Y., Lai, S.-J., Sasaoka, M., Hara, A., Yamazaki, A., Kameda, T., Horiuchi, Y., Meikle, P. J., Pernes, G., Lancaster, G., Ditiatkovski, M., Nestel, P., Vaisman, B., Sviridov, D., Murphy, A., Remaley, A. T., Sviridov, D. and Tozuka, M. (2020). Cholesterol transport between erythrocytes and lipoproteins contributes to blood cholesterol metabolism. *Journal of Lipid Research*, 61(12): 1577-1588. <https://doi.org/10.1194/jlr.RA120000635>.
- Oloyede, O. I. (2005). A Comparative Study on the Cholesterol Content of Fractionated Egg Yolk Products. *Pakistan Journal of Nutrition*, 4(5): 310-312. <https://doi.org/10.3923/pjn.2005.310.312>.
- Olsen, L., Thum, E. and Rohner, N. (2021). Lipid metabolism in adaptation to extreme nutritional challenges. *Developmental Cell*, 56: 1417-1429. <https://doi.org/10.1016/j.devcel.2021.02.024>.
- Orth, M. and Bellosta, S. (2012). Cholesterol: its regulation and role in central nervous system disorders. *Cholesterol*, 2012: 1-19. <https://doi.org/10.1155/2012/292598>.
- Ossamulu, I. F., Ogunsanya, M. U., Odu, N. M., Salubuyi, S., Musa, M. and Ariyeloye, S. D. (2023). Nutrient evaluation of eggs of different avian species from Minna, Nigeria. *Agricultura, Tropica, et Subtropica*, 56: 41-49. <https://doi.org/10.2478/ats-2023-0005>.
- Ouimet, M., Barrett, T. J. and Fisher, E. A. (2019). HDL and reverse cholesterol transport basic mechanisms and their roles in vascular health and disease. *Circulation Research*, 124: 1505-1518. <https://doi.org/10.1161/CIRCRESAHA.119.312617>.
- Palmisano, B. T., Zhu, L., Eckel, R. H. and Stafford, J. M. (2018). Sex differences in lipid and lipoprotein metabolism. *Molecular Metabolism*, 15: 45e55. <https://doi.org/10.1016/j.molmet.2018.05.008>.
- Pereira, N. R., Muniz, E. C., Matsushita, M. and De Souza, N. E. (2002). Cholesterol and fatty acid profile of commercial Brazilian chicken giblets. *Archivos Latinoamericanos de Nutrición*, 52(2): 1-5.
- Poitelon, Y., Kopec, A. M. and Belin, M. (2020). Myelin fat facts: an overview of lipids and fatty acid metabolism. *Cells*, 9: 812. <https://doi.org/10.3390/cells9040812>.
- Prestrud, P. and Nilssen, K. (1992). Fat Deposition and Seasonal Variation in Arctic Fox Body Composition in Svalbard. *Journal of Wildlife Management*, 56(2): 221-233. <https://doi.org/10.2307/3808816>.
- Prestrud, P. (1991). Adaptations by the Arctic fox (*Alopex lagopus*) to the polar. *Arctic*, 44(2): 132-138. <https://www.jstor.org/stable/40511073>.
- Russell, J. E. and Tumilson, R. (1996). Comparison of the microstructures of the white winter and brown summer furs of some Arctic mammals. *Acta Zoologica*, (Stockholm) 77(4): 279-282. <https://doi.org/10.1111/j.1463-6395.1996.tb01272.x>.
- Sejian, V., Bhatta, R., Gaughan, J. B., Dunshea, F. R. and Lacetera, N. (2018). Review: adaptation of animals to heat stress. *Animal*, 12(2): s431-s444. <https://doi.org/10.1017/S1751731118001945>.

- Smith, E. and Son, E. (2023). Role of animal morphology in adaptation and survival *Journal of Zoological Research*, 5(2): 1-7.
- Wilber, C. G. and Musacchia, X. J. (1950). Fat metabolism in Arctic ground squirrels *Journal of Mammalogy*, 31: 304-309. <https://doi.org/10.2307/1375299>.
- Zhanga, X., Barrazaa, K. M. and Beauchampa, J. L. (2018). Cholesterol provides non-sacrificial protection of membrane lipids from chemical damage at the air–water interface. *Proceedings of the National Academy of Sciences USA*, 115(13): 3255-3260. <https://doi.org/10.1073/pnas.1722323115>.