Original Research Article

Comparative morphometry of the brainstem of the male and female Helmeted Guinea Fowl *(Numida meleagris)*

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Abstract

The aim of this study was to obtain base-line morphometric data on the whole brain and brainstem in the adult male and female helmeted guinea fowl *(Numida meleagris)*, and compare the result in the two sexes. Brain samples of seventy adult helmeted guinea fowls, comprising 35 males and 35 females, intended for slaughter, were extracted for the study. The body weights for both male and female helmeted guinea fowls were 1247 ± 5.51 g and 1230 ± 4.85 g, respectively. The mean brain weights were 3.39 ± 0.02 g and 3.47 ± 0.18 g for male and female, respectively, and the difference in the values was not significant ($P > 0.05$). The lengths of the brain and medulla oblongata, as well as the lengths of the midbrain and mesencephalic tectum, did not differ between the two sexes. Neither the mean weights nor lengths of the pons differed between the two sexes. In conclusion, the results of this study showed that there was no sex dimorphism observed in the morphometry of the midbrain, medulla oblongata and pons in the helmeted guinea fowl.

Keywords: galliform birds; medulla oblongata; mesencephalic tectum; pons.

INTRODUCTION

The helmeted guinea fowl *(Numida meleagris)* is a semi-wild bird belonging to the family *Numididae,* order *Galliformes,* They are indigenous to Africa (Belshaw, 2010). There are two species of helmeted guinea fowl in Nigeria, *Numida ptilorhycha* and *Numida meleagris* (Gosomji et al., 2016). The guinea fowl brainstem consists of the midbrain, pons and medulla oblongata (Umosen et al., 2008).

This study was initiated by the increased demand for guinea fowl meat that has led to its breeding and domestication in Nigeria. Behavioural traits in birds are better understood by first having a good mastery of the neurobiology of the bird. There is limited scientific information on the brainstem morphometry in the male and female helmeted guinea fowl. The null hypothesis of the work is that there is no sexual dimorphism in the whole brain and brainstem structures in the helmeted guinea fowl. Therefore, the objective of this study was to investigate the morphometry of the whole brain and brainstem of the helmeted guinea fowl with respect to sex so as to offer a reference for future research.

MATERIALS AND METHODS

Seventy adult helmeted guinea fowls, comprising 35 males and 35 females, intended for slaughter, were used for this study. The birds were purchased from a local market and housed in the Department of Veterinary Anatomy Research Laboratory, Ahmadu Bello University, Zaria, Nigeria. Prior to the purchase, birds whose wattles inclined at an angle to the attachment in the lower jaw were selected as males, and those whose wattles hung vertical in the normal position were selected as females (Umosen et al., 2008). The birds were provided commercial poultry feed and drinking water *ad libitum*. The experimental protocol was approved by the Ethical Committee of Ahmadu Bello University, Zaria, Nigeria. Management of the experimental animals was as stipulated in the Guide for the Care and Use of Laboratory Animals, 8th Edition, National Research Council, USA (National Academic Press, Washington D.C.: www.nap.edu.).

Table 1. Body and whole brain weights of the helmeted guinea fowl

Sex	Body Weight (g)		Absolute Brain Weight (g)		Relative brain Weight (%)
	Min–Max	Mean ± SEM	Min–Max	Mean ± SEM	
Female (n = 35)	950–1350	1230 ± 4.85	3.02–4.88	3.47 ± 0.18[b]	0.28 ± 0.16
Male (n = 35)	1000–1480	1247 ± 5.51	3.33–3.52	3.39 ± 0.02[b]	0.27 ± 0.03

Table 2. Morphometric values (Mean ± SEM) of the midbrain in the helmeted guinea fowl

Sex	Weight (g)	Relative weight (%)	Length (mm)	Relative Length (%)	Width (mm)
Female (n=35)	0.58 ± 0.01	17.81 ± 0.29	8.59 ± 0.32	40.92 ± 0.44	9.16 ± 0.34
Male (n=35)	0.59 ± 0.02	17.86 ± 0.29	8.60 ± 0.40	40.92 ± 0.37	8.25 ± 0.37

The weight of each live adult bird was obtained using digital electronic balance (Citizen Electronic Scale, PVT Ltd., 0.01g). Thereafter, each bird was sacrificed and decapitated. The sex of each bird was confirmed when dissected. The presence of testes confirmed males while the presence of ovaries confirmed females. The lower jaw was then separated from its point of articulation with the quadrate bone using a pair of scissors. The samples were then fixed in Bouin's solution for 24 hours. Brain samples were extracted and weighed using a sensitive electronic balance (Mettler balance P 1210, Mettler instruments AG. Switzerland; sensitivity: 0.01 g). Brain lengths were obtained using a digital Vernier caliper (MG6001DC, General Tools and Instruments Company, New York; sensitivity: 0.01 mm).

To separate the brainstem from the rest of the brain, the cerebrum was removed from the rest of the brain by gentle pulling of the cerebral hemispheres apart at the occipital pole and severing it at the midline. The midbrain was severed at the level of 3rd ventricle. This completely separated the forebrain from the brainstem and cerebellum. In order to separate the cerebellum from brainstem, the floculli of the cerebellum were manually raised to expose the cerebellar peduncles. These peduncles were then severed on both sides. Finally, the brainstem was freed from the arachnoidea and cranial nerves by simple trimming. Using a scalpel blade, the midbrain was separated from the pons, and an incision at the pontomedullary junction separated the medulla oblongata from the pons. The dimensions of brainstem structures were obtained using the same instruments mentioned above.

Transverse extent of the caudal surface of the midbrain, rostral to the pons was measured as width of midbrain. Width of Pons was obtained as the extent of the pons along the width of the brain, while the length of the Pons was the extent of the pons along the rostrocaudal axis of the brain. The rostrocaudal extent from the pontomedullary junction to the point of pyramidal decussation was measured as the length of medulla oblongata.

The relative lengths of the brainstem structures were obtained as a percentage of the whole brainstem length occupied by the particular brainstem structure. Similarly, the relative weight of each brainstem structure was obtained as a percentage of the weight of the brainstem occupied by the particular brainstem structure.

The data obtained were analysed using Graph Pad Prism version 4.0. The result of whole brain and brainstem and relative weights and lengths of the brainstem structures were expressed as Mean ± SEM and subjected to Student t-test to determine the level of differences in the values obtained in both sexes. Values of $P > 0.05$ were considered significant.

RESULTS

Body and Brain weight

The mean body and brain weights of the male and female helmeted guinea fowls are presented in Table 1. There was no difference in body or brain weight between male and female guinea fowls ($P > 0.05$).

Weight and morphometry of brainstem structures

The absolute and relative weights of the midbrain in the male and female helmeted guinea fowls are presented in Table 2. The difference in the relative mean weights of the midbrain for the male and female guinea fowls was not significant ($P > 0.05$). The absolute weights of the pons for the male and female helmeted guinea fowls are shown in Table 3, and neither this difference was significant. The absolute weights of the medulla oblongata for the male and female helmeted guinea fowls are presented in Table 4 with differences in the values not statistically significant ($P > 0.05$).

Dimensions of brainstem structures

The absolute and relative lengths and widths of the midbrain in the male and female helmeted guinea fowls are presented in Table 2. The difference in

Table 3. Morphometric values (Mean ± SEM) of the pons in the helmeted guinea fowl

Sex	Weight (g)	Relative weight (%)	Length (mm)	Relative length (%)	Width (mm)
Female (n=35)	1.16 ± 0.02	4.96 ± 0.01	9.15 ± 0.02	35.37 ± 0.42	8.14 ± 0.02
Male (n=35)	1.16 ± 0.01	4.97 ± 0.01	9.14 ± 0.03	35.27 ± 0.28	8.38 ± 0.03

Table 4. Morphometric values (Mean ± SEM) of the medulla oblongata in the helmeted guinea fowl

Sex	Weight (g)	Relative weight (%)	Length (mm)	Relative length (%)	Width (mm)
Female (n=35)	1.53 ± 0.03	6.65± 0.03	6.33 ± 0.01	24.44± 0.03	7.45± 0.02
Male (n=35)	1.57 ± 0.05	6.72± 0.02	6.31± 0.03	24.36± 0.03	7.11± 0.05

the relative lengths of the midbrain between the sexes was not statistically significant ($P > 0.05$). The absolute lengths of the pons for the male and female helmeted guinea fowls are presented in Table 3. Neither this difference was significant. For the absolute lengths of the medulla oblongata see Table 4. Again, the differences in the values were not statistically significant ($P > 0.05$).

DISCUSSION

The present study has generated a base-line data on the morphometry of the brainstem structure in the male and female helmeted guinea fowls. Although the male helmeted guinea fowl had a slightly higher numerical mean body weight values than the female, the difference was not significant. This may imply that there is little or no interaction or correlation between sex and body weight in the helmeted guinea fowl, at least in indigenous birds. This finding is in agreement with Nahashon et al. (2006a, b). On the other hand, Baéza et al. (2001) and Mroz et al. (2016) found that in selected strains, the body weight was affected by sex, with females being heavier than males (Mroz et al. 2016).

The utility of brain weight rests in the ability to calculate organ to brain weight ratios, otherwise, known as relative brain weight. Some authors consider evaluation of organ to brain weight ratio helpful when terminal body weights are affected by the test article or to normalize organ weight data when there is large inter-animal variability. Therefore it is vital to document brain weights so that organ to brain weight ratio (relative brain weights) may be calculated if needed. The study of McDaniel (2005) and Narr et al. (2007) opined that there is a correlation between brain weight and cognition. Although there are more sensitive brain markers for cognitive ability, such as cortical gray matter thickness, encephalisation quotient and cortical neurone number (Roth and Dicke, 2005; Herculano-Houzel, 2007), absolute and relative brain weights are still considered as markers for cognition. Results of the present study indicated no significant difference in the absolute and relative brain weight of the male and female helmeted guinea fowl. This may imply the non-existence of any variation in the cognitive ability of the two sexes. This can also be buttressed by the report of Owen and Hartley (1998) in which they inferred that sexual dimorphism in birds may or may not be significant, depending on the phylogenetic trend and social mating. The absolute brain weight of either the male or the female bird from the present study was lower than 4.78 g reported as the absolute brain weight of the wild African parrot by Wanmi et al. (2018). Unfortunately, there is dearth of information on the sexual differences in brain parameters of birds in available literature.

The brainstem consists of the midbrain, pons and medulla oblongata. The midbrain is the most cranial structure of the brainstem (Ibe et al., 2017). It narrows rostrally into the third ventricle, and is connected to the pons caudally. The dorsal surface, called the mesencephalic tectum, has a dual function: it is the centre for auditory and visual reflexes, and a nerve pathway of the cerebral cortex (Walton et al., 2007; Loftus et al., 2008; Ibe et al., 2017). The medulla oblongata is the most caudal part of the brainstem; it is continuous with the spinal cord caudally and the pons rostrally; it lies in the cranial vault, superior to the foramen magnum (Suckow et al., 2006). The medulla oblongata is the control centre for autonomic functions and a relay centre for impulses between the spinal cord and higher brain centres. It coordinates autonomic functions such as respiration, blood pressure and heart rate (Nolte, 2002; Gourine et al., 2005).

The brainstem morphometry in the helmeted guinea fowl observed in the present study did not show considerable sexual dimorphism. The absence of any significant sexual difference in these brainstem structures in the helmeted guinea fowl implies that their respective functions may not be different in terms of efficiency in either of the sexes. Several studies have shown that different demands on the visual system in birds are correlated with variation in the relative size of visual areas in the brain (Iwaniuk et al. 2010).

One of the main visual pathways in birds is the tectofugal pathway, comprising three main

structures: optic tectum, nucleus rotundus and entopallium (Nguyen et al. 2004). Therefore, in respect to the functions of the optic tectum in the visual pathway, it can be inferred that both the male and female helmeted guinea fowl have equally good visual sense.

CONCLUSIONS

The results of this study showed that there was no sex dimorphism in the morphometry of the midbrain, medulla oblongata and pons of the helmeted guinea fowl. This in turn may not show any difference in functional performance in both sexes of the fowl. Further, no sex dimorphism was observed in the mean body and brain weights of the helmeted guinea fowls. Finally, this result can also be useful in comparative avian neuroanatomy.

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