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Structural Organization of the Optic Lobe of Grey Breasted Helmeted Guinea Fowl (*Numida Meleagris Galeata*) at Pre-Hatch Study

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Abstract

A total of one hundred and seventy-three fertilized eggs were used for morphometry, gross and histological studies. At day 4 of incubation, the mean body weight of the helmeted guinea fowl embryo was 0.6401 ± 0.0211 g. It was at day 10 of incubation that there was an increase in the whole body weight of the embryo to be 0.8650 ± 0.676 g. The whole brain weight indicated relative increased at day 4 as compared to that of the whole body weight. Graphically, there were steady increase in the body, brain and optic lobe weights. Histologically, cells and neurones that make up the optic lobe is probably as a result of the migration of immature cells from the ventricular neuroepithelium.

Keywords: Structural, Organization, Optic Lobe, Guinea Fowl

1. Introduction

The helmeted guinea fowl, (*Numidea meleagris galeata*) is widely distributed (Ayeni, 1980)

in the Guinea Savannah vegetation zone of Nigeria and estimated at 44 million in captivity. In Nigeria, two types of guinea fowl species are found; *Numida ptilorhycha* that is indigenous to the Southern part while *Numida meleagris* is domiciled in the Northern part, but it is spreading to other small-holder farming areas (Ayorinde, 1987). There exist eight known subspecies of helmeted Guinea fowl; *Numidea meleagris meleagris, Numida meleagris sabyi, Numida meleagris galeata, Numidea meleagris marungensis, Numidea meleagris damarensis, Numidea meleagris damarensis, Numidea meleagris coronate, Numidea meleagris mitrata and Numidea meleagris reichnowi* (Van Niekerk, 2010). Some people keep guinea fowl out of curiosity and as "watch animals" around homestead because they have excellent eye-sight, a harsh cry, and they shriek at the slightest provocation (Smith, 2000). They are also kept for income generation (Ligomela, 2000), and for the control of snakes, mice and ticks (Cactus, 2001) thus, encouraging its production. The increase in guinea fowl production has led to the development of informal traders who buy and sale the birds for breeding and consumption, especially during festive seasons (Fajemilehin, 2010).

Birds are subjected to a constant and potentially overwhelming barrage of information from the environment. The development of the brain is an important requirement for the survival of all birds because it controls the entire systems and most importantly, in matured birds, it plays a vital function on the skeletal movement properly, through continuous sensory feedback of information concerning the effect of its action (Snell, 2001).

Optic lobes are the visual roof of the mesencephalon, which is optic tectum in most avian and non-mammalian species (Northcutt. 2002). The avian optic lobe is well developed, thick and highly laminated. The optic tectum is responsible for the generation of orienting movements to stimuli of interest in the environment which may be a moving prey or predator. There are differences with respect to the relative sizes of the optic lobe compared to other vertebrates. It is quite large and the tectofugal pathway is generally regarded as the primary route of the visual information to the telencephalon, Butler and Hodos (2005).

Work done on the developmental anatomy of the avian include, Davey and Tickle (2010), Salami (2009) and Serdar and Emrah (2010). These three authors worked on the brain and cerebellar cortex development in chicken.

The basis of the study was to have a baseline structural organization of the optic lobe of the Grey breasted helmeted guinea fowl and relate it to its behavioural function in the wild and in domestication.

2. Materials and Methods

2.1 Experimental Eggs

One hundred and seventy three (173) fertilized guinea fowl eggs were purchased from National Veterinary Research Institution (NVRI) Vom, Jos, Plateau State, Nigeria. The eggs were transported to a hatchery, in Jos and incubated using their standard incubation guide. During incubation, the eggs were turned regularly (minimum of three times) each day for the first 24 days, Nwogu and Alawa (1995).

Seventy (70) fertilized eggs for pre-hatch study were collected daily from day 4 of incubation up to day twenty eight which is the final day of incubation. An opening was made on the large air space area and the entire egg dropped into a labeled container of 10 % buffered formalin for proper fixing.

2.2 Extraction of Embryo from Egg Shell

This was done at pre-hatch using a scalpel blade and clean transparent dish. The blunt side of the scalpel blade was used with the egg held on the palm, and a gentle tap was made on the egg until a crack was formed. Then, the crack was gently widened manually and the embryo collected in a transparent dish.

2.3 Extraction of Brain

At pre-hatch, because the entire skull is soft and pliable, scalpel blade and rat tooth forceps were used for extraction of the brain. At post-hatch, the keets were euthanized using Nembutal at 40 mg per body weight. Immediately, decapitation was made and the heads fixed in 10 % buffered formalin for 3-5 days. After ensuring proper fixation, a dissection was made at the angle of the beak up to the level of the occipital bone. The upper portion of the dissected area was pulled off gradually using the rat tooth forceps until the entire brain was exposed. The cranial nerves were severed to ease the lifting of the brain from the cranium. The extracted brains were fixed in Bouin's solution comprising 75 ml picric acid, 25 ml formaldehyde and 5 ml glacial acetic acid.

2.4 Gross and Morphometry

The weights of the whole bird, brain and optic lobe were taken using digital electronic balance; Model JJ1000, Max. 1000g, d=0.01g, e=10d, No. 21101101109, Analytical Weighing balance, Adventure QHAUS Corporation, Item No. AR3130, Max. Capacity= 310g Readability= 0.001g. Photographs of the dorsal and ventral aspects were taken using cannon digital camera (4x optical zoom lens 5.0 - 20.0 mm, 15.1 mega pixels Apple, Cannon) and Digital Handheld Microscope, Magnification 1000x, 5x Zoom, 3D stand high speed DSP, Made in China. Weights and lengths were all recorded in grams (g) and centimeter (cm) respectively.

2.5 Histological Techniques

One brain sample out of the seven samples was used. Fixed brain sample was washed using tap water and dehydrated through ascending grades of alcohol (70 %, 80 %, 90 %, 95 %, 100 %), within intervals of three (3) hours each, cleared in xylene for two hours and embedded in liquid paraffin at 50 0 C according to standard procedures as described by (Kiernan, 1990).

Serial transverse sections of 5 µ were made using Jung rotary microtome (Model 42339, Berlin, Germany). Sections were mounted on glass slides and allowed to dry; were deparaffinized, stained, hydrated and coverslipped using diphynylpthalate propylene xylene (DPX) as mountant (Drury and Wellington, 1980).

Sections were stained with hematoxylin and eosin (H & E), and cresyl fast violet (CFV) for nuclei and photomicrographs of sections were taken using digital eyepiece (Scopetek DCM 500, Resolution: 15 Pixels) attached to a light microscope (OLYMPUS XSZ107BN, Hamburg, Germany).

2.6 Data Analysis

Mean standard error of mean (MEAN ± SEM) using statistical package for social sciences (SPSS) version 17 was used in finding values of weights and chats were plotted.

3. Results

3.1 Morphometric Results

The mean weight of the whole embryo body from day 4 to day 28 pre-hatch period indicated that, at day 4 when the embryo of the HGF was first observed, the body mean weight was 0.6401 ± 0.0211 g. From day 7, 16, 23 and 28, their mean weights were taken to be; 0.8180 ± 0.0192 g, 3.7537 ± 0.5553 g, 12.592 ± 1.3083 g and 18.6871 ± 1.6767 g respectively (Table 1). There was a steady increase in the body weights from day 10 of incubation (figure 1). The whole brain mean weight was taken from day 4 and was observed to be 0.509 ± 0.004 g. At day 10, 19, 25 and 28, the various brain weights were observed to be, 1.405 ± 0.065 g, 2.872 ± 0.164 g, 3.987 ± 0.176 and 4.402 ± 0.122 g respectively (Table 2). It was on days 16, 23 and 28 that there was an enormous increase in the mean brain weight (figure 2). The mean weights of the optic lobe from day 8 of incubation were seen to be 0.0109 ± 0.0049 g. The highest mean weight was that of day 28 which did not vary from that of day 26. On day 14 and 23 there mean weights was observed to increase greatly (Table3) and (figure 3).

Table 1. Mean weights of the embryo of guinea fowl at days 4-28 pre-hatch (in gram)

	Bird (n=7)		
Days	Min.	Max.	Mean ± SEM
4	0.0164	0.1671	0.6401 ± 0. 0211
7	0.0709	0.2044	0.8180 ± 0.0192
10	0.5034	0.9652	0.8650 ± 0.0676
13	0.7121	2.6244	1.8109 ± 0.2843
16	1.1306	5.5191	3.7537 ± 0.5553
19	3.0974	9.3138	7.0807 ± 0.8996
21	5.5941	11.5214	8.7716 ± 0.9481
23	6.0395	14.9034	12.592 ± 1.3083
25	7.2809	18.1108	15.1409 ± 1.4697
28	9.1085	21.5106	18.6871 ± 1.6767

n; Number of birds used per day, Min; Minimum, Max; Maximum, SEM; Standard Error of Mean

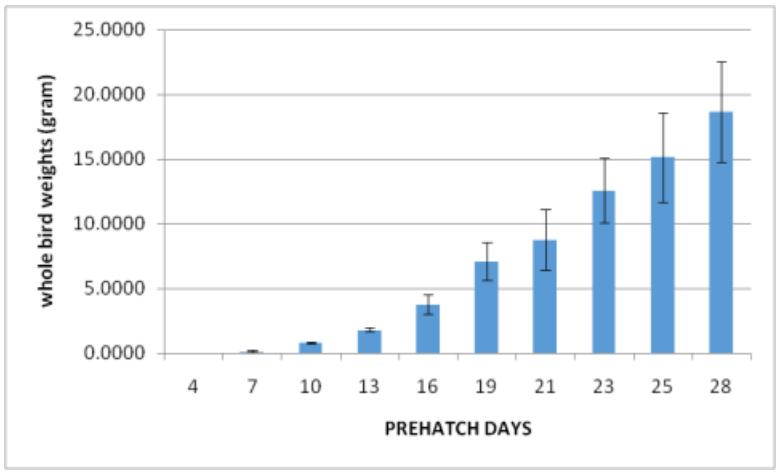

Figure 1. Mean weight of the guinea fowl embryo at days 4-28 pre-hatch (in gram).

Table 2. Mean weight of the guinea fowl whole brain at day 4 - 28 pre-hatch period (in gram).

	Bain (n=7)		
Days	Min.	Max	Mean ±SEM
4	0.004	0.016	0.509 ±0.004
7	0.021	0.062	0.913 ±0.015
10	0.071	0.246	1.405 ±0.065
13	0.190	0.499	1.951 ±0.108
16	0.212	0.570	2.386 ±0.115
19	0.222	0.694	2.872 ±0.164
22	0.509	0.828	3.382 ±0.112
25	0.750	1.225	3.987 ±0.176
28	0.815	1.131	4.402 ±0.122

n; Number of birds used per day, Min; Minimum, Max; Maximum, SEM; Standard Error of Mean

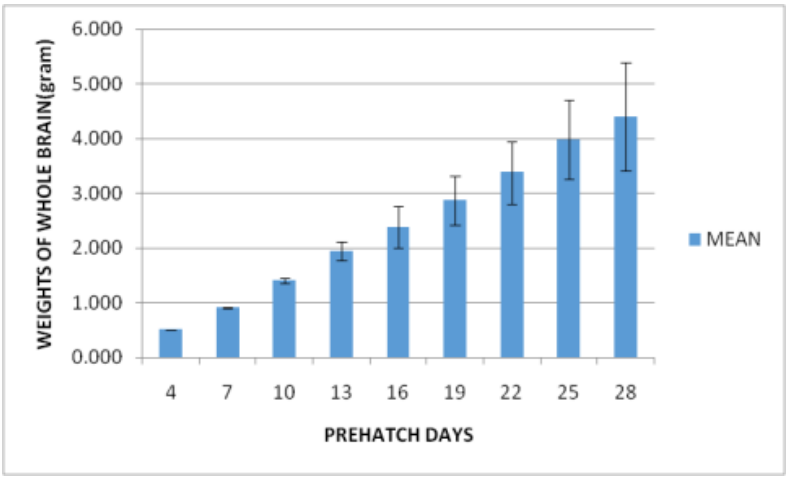

Figure 2. Mean weight of the guinea fowl whole brain at days 4 - 28 pre-hatch period (in gram)

Table 3. Mean weight of the guinea fowl embryo optic lobe at days 8-28 pre-hatch period (in gram)

	Bird (n=7)		
	Min	Max	Mean ± SEM
8	0.0051	0.0210	0.0109 ± 0.0049
11	0.0114	0.0304	0.0154 ± 0.0067
14	0.0214	0.0586	0.0332 ± 0.0127
17	0.0291	0.0670	0.0422 ± 0.0130
20	0.0329	0.0729	0.0485 ± 0.0151
23	0.0381	0.0805	0.0547 ± 0.015
26	0.0299	0.0829	0.0639 ± 0.0182
28	0.0388	0.0851	0.0682 ± 0.0166

n; Number of birds used per day, Min; Minimum, Max; Maximum, SEM; Standard Error of Mean

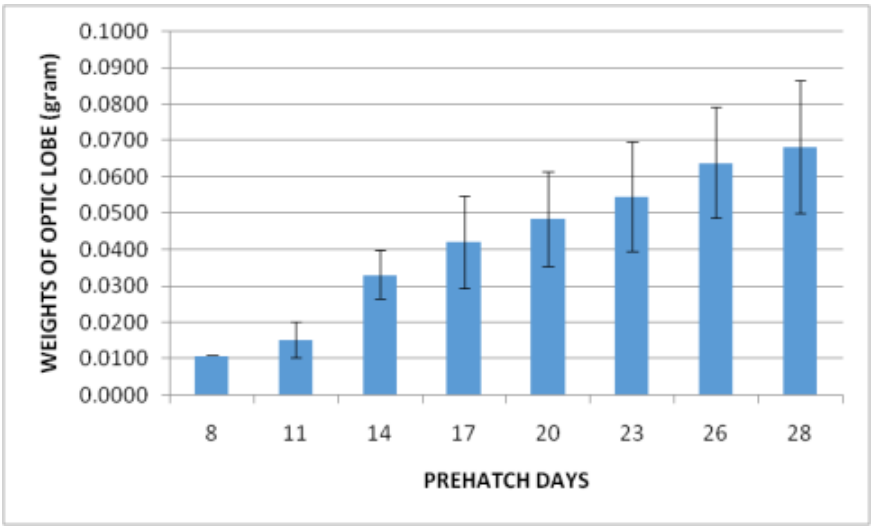

Figure 3. Mean weight of the guinea fowl embryo optic lobe at days 8-28 pre-hatch (in gram)

3.2 Gross Anatomy

The optic lobe of the HGF was first observed to appear on day 8 of incubation with the cerebral hemispheres located rostral to it. The optic lobe was observed to be paired, separated from each other by a longitudinal fissure which ran from the rostral end of the cerebral hemispheres to terminate caudally. Dorsolateraly, it appeared convex and tend to be flattened at the caudal borders (Plate I). The optic lobe subsequently, appeared somewhat oval, caudally separated from the cerebellum by a transverse process and ventrally, it was attached to the midbrain (Plate II). The shape of the optic lobe was observed to be kidney or bean shaped. Subsequently, there was a simultaneous increased in the shapes of cerebrum and cerebellum causing the dorsomedial face of the optic lobe to appear like the head of a maggot on day 18 of incubation (Plate III). At day 27, the optic lobe was found to be firmly attached at the lateral sides of the midbrain. The dorsal, lateral and ventral aspects were seen to be

convex. The cerebrum almost overlaps the dorsal surface of the optic lobe. Ventrally, the optic lobe is attached to the midbrain and cerebrum (Plate IV).

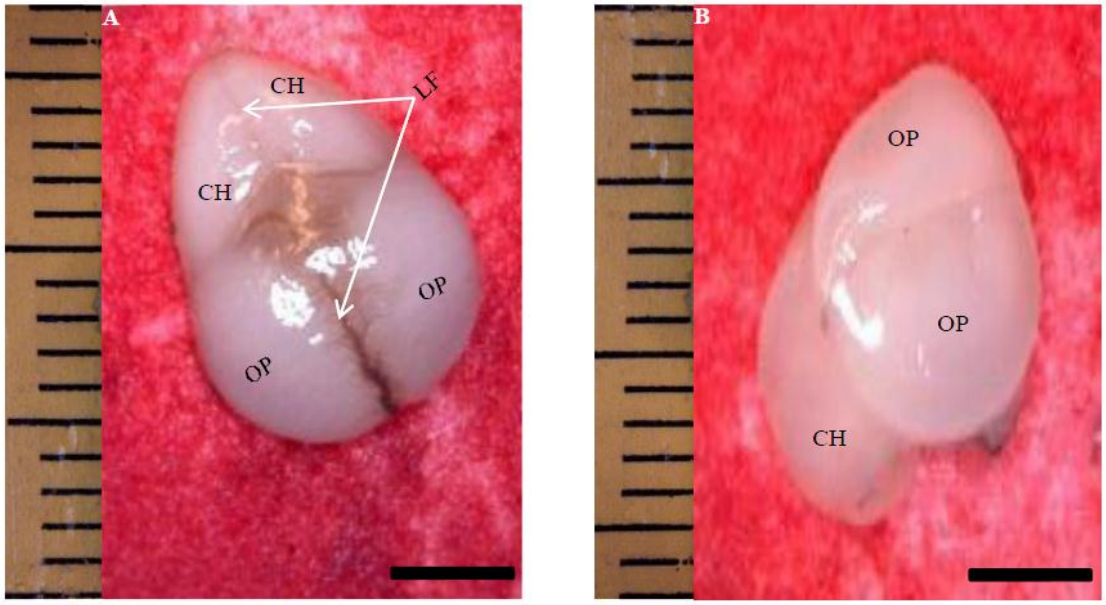

PLATE I. Dorsal and lateral views of the brain of the embryo: (A): CH; Cerebral hemispheres, OP; Optic lobe, LF; Longitudinal fissure, (B): CH; Cerebral hemispheres, OP; Optic lobe, (Day 8 pre-hatch), X500

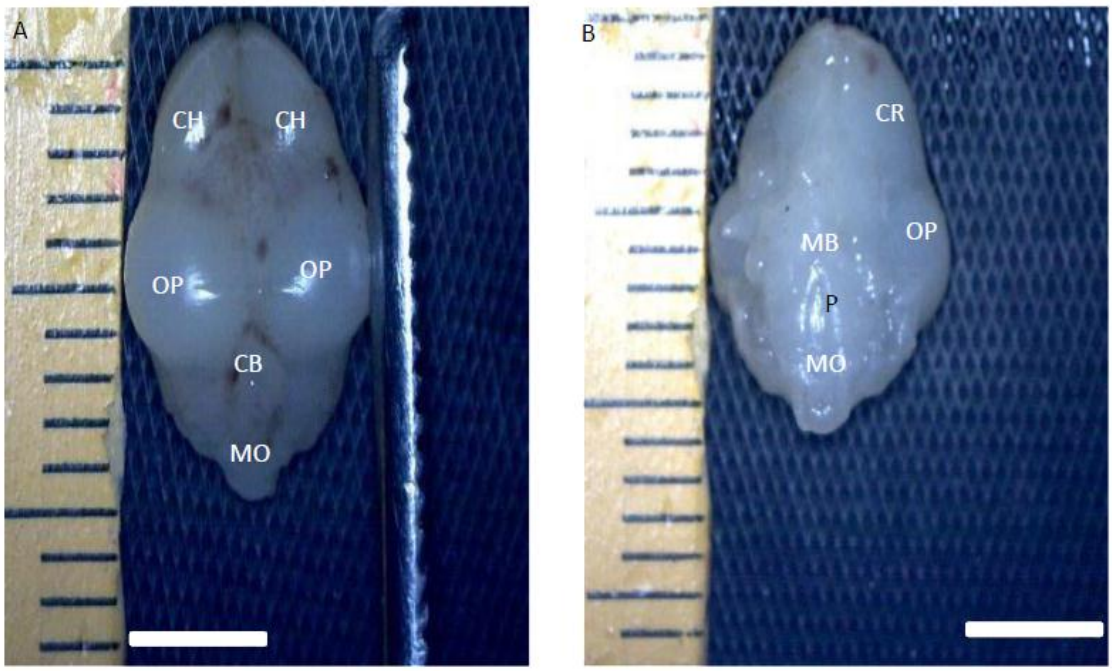

PLATE II. Developing brain of the helmeted guinea fowl, (A): Dorsal aspects, CH; Cerebral hemisphere, OP; Optic lobe, CB; Cerebellum, MO; Medulla oblongata, (B): Ventral aspect, CR; Cerebrum, MB; Midbrain, P; Pons, MO; Medulla oblongata, (Day 11 pre-hatch,) X500.

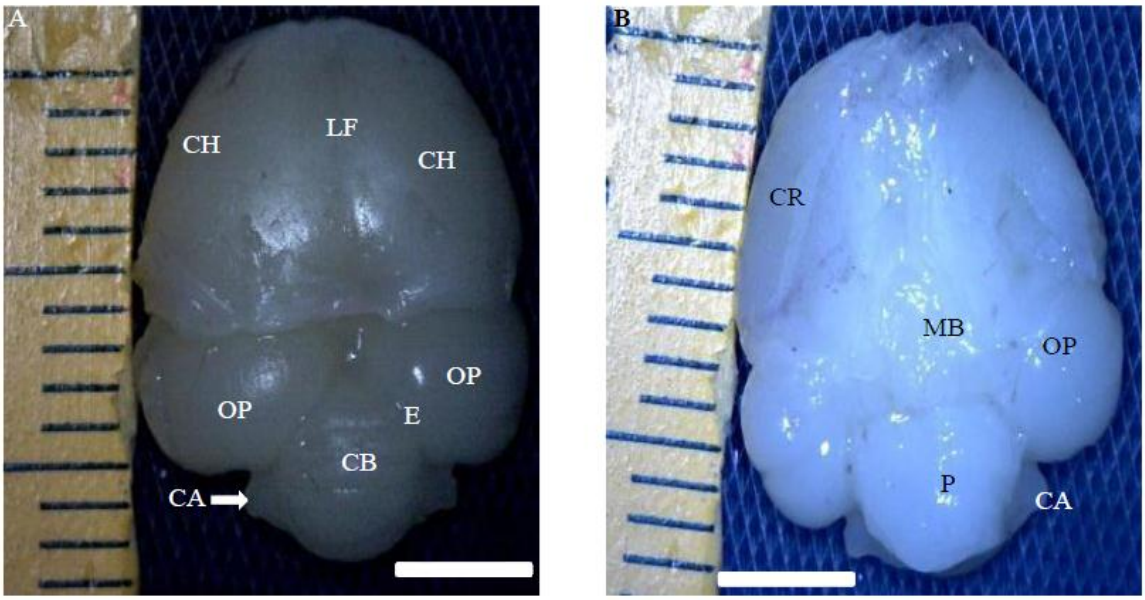

PLATE III. Dorsal and ventral aspects of the developing brain: (A): CH; Cerebral hemisphere, LF; Longitudinal fissure, E; Transverse fissures, OP; Optic lobe, CB; Cerebellum, CA; Cerebellar auricle, (B): CR; Cerebrum, MB; Midbrain, OP; Optic lobe, P; Pons, CA; Cerebellar auricle, (Day 18 pre-hatch) X500

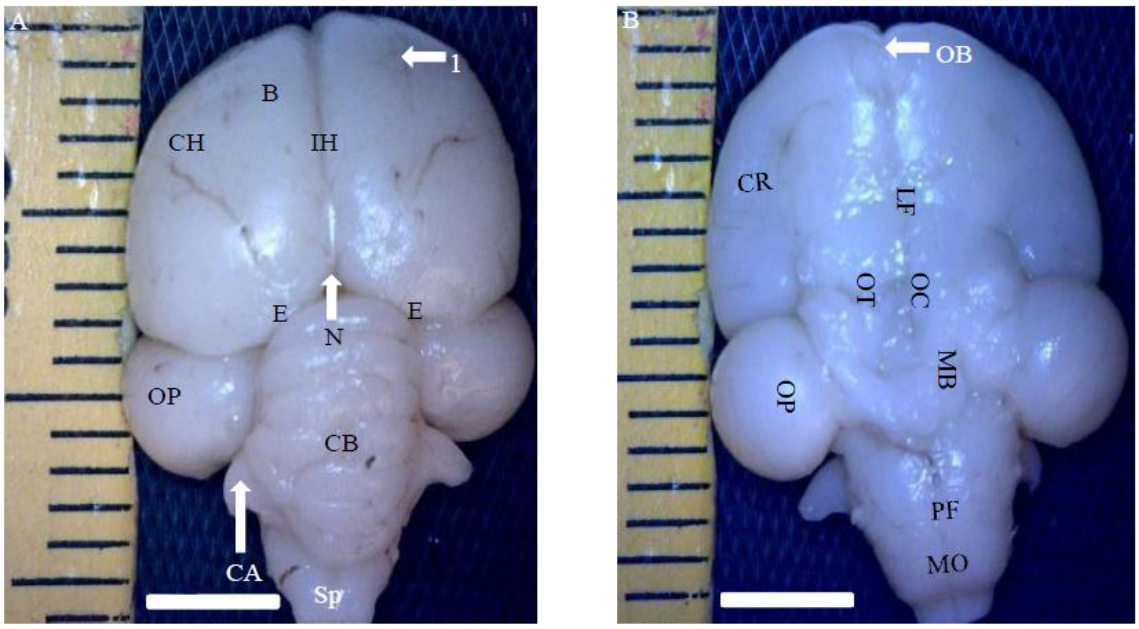

PLATE IV. Brain of guinea fowl, (A): B; Wulst, IH; Interhemispheric fissure, E; Transverse fissure, N; Notch, CH; Cerebral hemisphere, OP; Optic lobe, CB; Cerebellum, CA; Cerebellar auricle, 1; Vallecular, SP; Spinal cord, (B): LF; Longitudinal fissures, MO; Medulla oblongata, OB; Olfactory bulb, OC; Optic chiasm, OT; Optic tract, MB; Midbrain, PF; Pontine flexure, X500 (Day 27 pre-hatch).

3.3 Histological Features

Histologically, the optic lobe, midbrain, neuroepithelial cells, the ventricle, neuroglial cells and granule cells developed from day 7 of incubation (Plate V). At day 8 the optic lobes were first observed grossly, the superficial layer, deep layer, ventricular neuroepithelium and stratification of the superficial layer into sublaminae were formed (Plate VI). Cells found in the deep layer were mostly granular in nature migrating from the ventricular neuroepithelium toward the surface there by decreasing the wide space initially occupied by the superficial layer (Plate VII).

The uppermost strata, the stratum opticum, neuropil and stratum griseum periventriculare were observed. Large cells that form the fourth strata were first seen at day 16 of incubation. Numerous granule cells that occupied the deep layer became reduce and tend to organize itself into various stratum at day 16 of incubation (Plate VIII). At day 18, large cells which were likely to become future pyramidal and fusiform neurones were observed among numerous granule ells. These large cells were seen within the layers; stratum griseum periventriculare (SGP), stratum griseum centrale (SGP) and Stratum album centrale (SAC) (Plate IX). The large irregular shaped neurones were seen to be concentrated within the stratum album centrale with neuroglia cells. The stratum griseum centrale has few large immature neurones with scanty granular cells. The optic lobe at day 22 was organized and the pyramidal cell, fusiform neurones, neuroglia and dense granules were all visible. Stratum album centrale was observed to be composed of large neuronal cells with varied position of nucleoli, interposed by granule cells which were seen to have a dark stained nuclei (Plate XI).

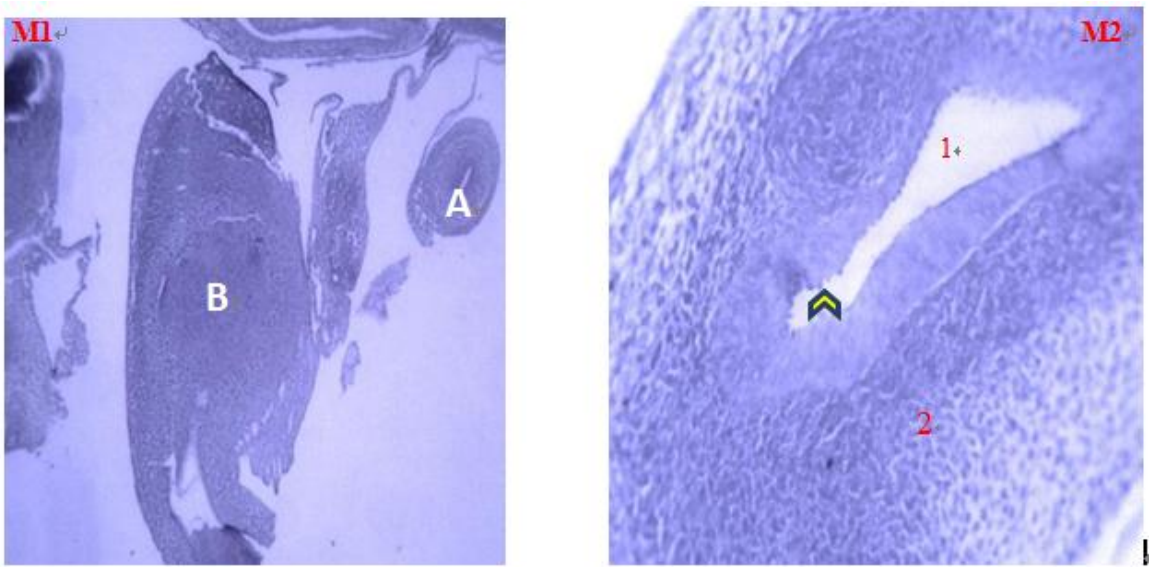

PLATE V. Pre-hatch development of the optic lobe of HGF indicating, (M1) A; Optic lobe, B; Midbrain, CFV. X40, (M2): Arrow; Neuroglial, 1; Ventricle, 2; Granule cells. X100. (Day 7 pre-hatch)

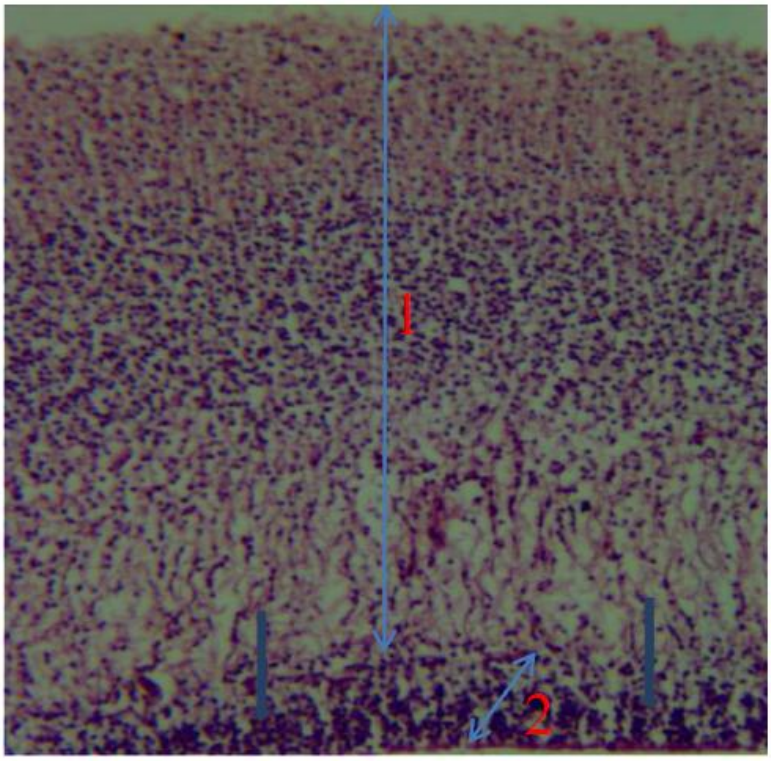

PLATE VI. Optic lobe showing, 1; Superficial layer, 2; Deep layer, Arrows; Ventricular neuroepithelium. Magnification X140. (Day 8 pre-hatch).

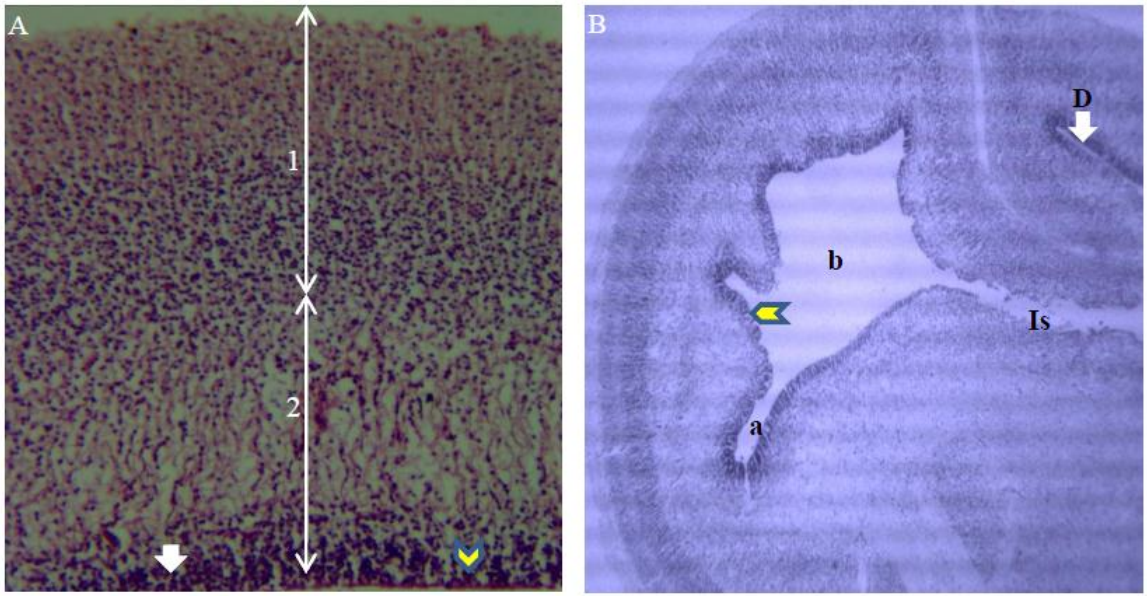

PLATE VII. Optic lobe of the HGF showing, (A): 1; Superficial layer, 2; Deep layer, Arrow; Granular cells, H & E. X140. (B): a; Cornus armornus, b; Ventricle, D; Mesencephalic aqueduct CFV. X140. (Day 14 pre-hatch).

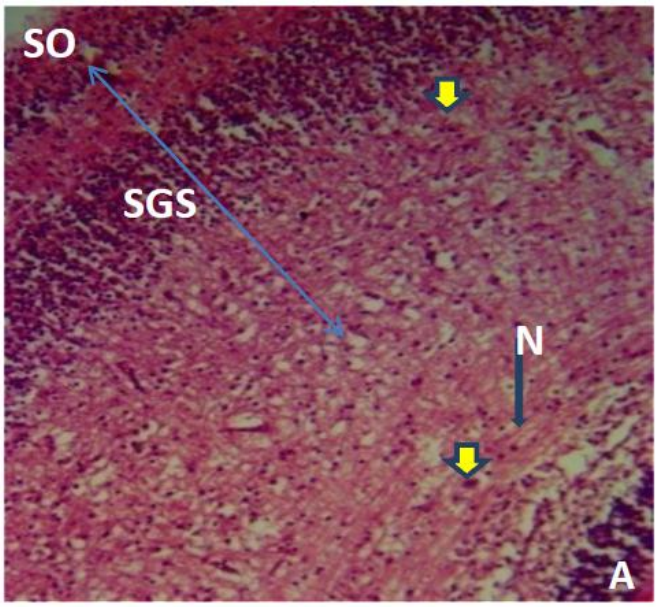

PLATE VIII. Optic lobe of the HGF showing, SO; Stratum opticum, SGS; Stratum griseum superficialis, N; Neuropil, Arrow; Cells that form future large neurones, A; Stratum fibrosum periventriculare. H & E. Magnification X140. (Day 16 pre-hatch).

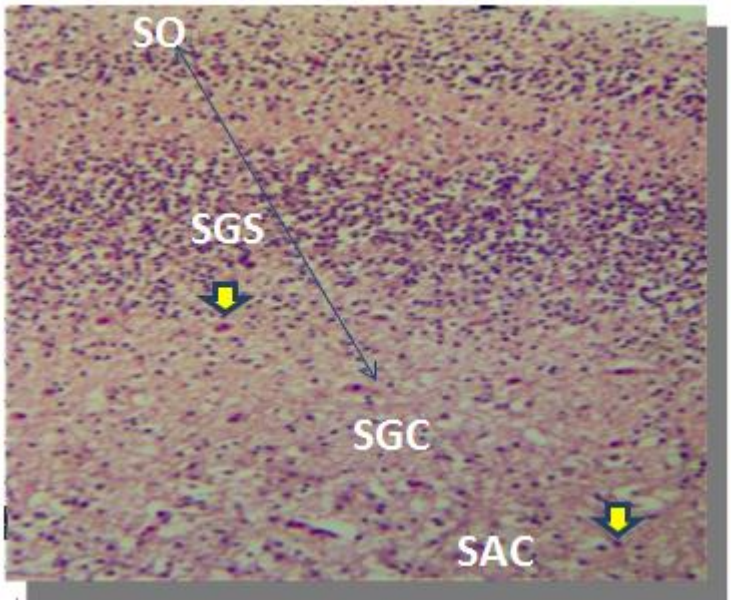

PLATE IX. Optic lobe from the guinea fowl brain showing, SO; Stratum opticum, SGP; Stratum griseum superficialis, SGC; Stratum griseum centrale, SAC; Stratum album centrale, Arrow; Cells that form future large and small pyramidal neurones. H&E. Magnification X140. (Day 18 pre-hatch).

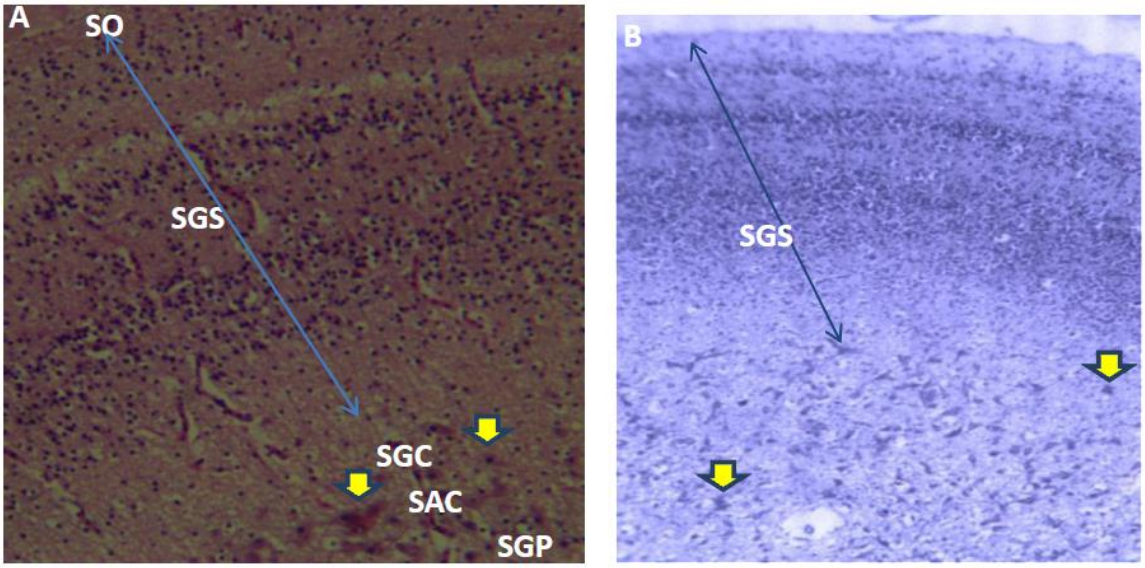

PLATE X. Optic lobe from the guinea fowl brain showing, (A): SO; Stratum opticum, SGS; Stratum griseum superficiale, SGC; Stratum album centrale, SGP; Stratum griseum periventriculare, Arrows; large neurones that form the layer Stratum album centrale, (B): SO; Stratum griseum superficiale, Arrow; Large neurones, A; H&E, B; CFV. Magnification X200. (Day 20 pre-hatch)

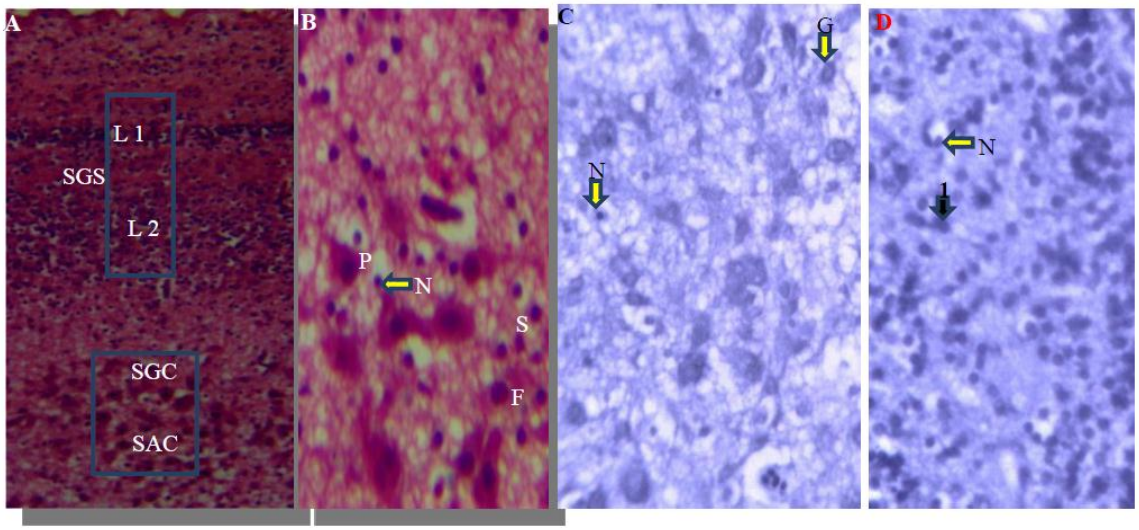

PLATE XI. Layers and sub laminae structrues of the optic lobe at day 22 pre-hatch, (A): Some layers of the optic lobe, SGS; Stratum griseum superficiale, SGC, Stratum griseum centrale, SAC; Stratum album Centrale, L1and L2; Sub laminae of SGP. H&E, X140 (B): Cellular features of the SGC and SAC, F; Fusiform neurons, P; Pyramidal neurone, N; Neuroglia cell, S; Small neurone. H&E, X200 (C); Sublamina L2 of SGS, N; Neuroglia cell, G; Small neurone. CVF, X400. (D); Sublamina L1 of SGP, N; Nerve cell, 1; Small neurone. CVF, X400.

4. Discussion

In this present study, the mean weight of the embryo body at day 4 of incubation was 0.6401

± 0.0211 g. The increased in weights was progressive graphically. The mean weights of the whole brain at day 4 and the optic lobe at day 8 of incubation were 0.509 ± 0.004 g and 0.0109 ± 0.0049 g respectively. This indicates that the optic lobe at day 8 of incubation constitute about 24.28 % of the total brain weight. The brain weight was observed to increase as the body weight increases but less than those of the body weights. This is in agreement with (Portmann and Stingelin, 1961) who stated that brain weight always increases less than those of the body weight and that galliformes had the lowest values which were not constant.

The optic lobe was first seen to appear at day 8 of pre-hatch period with the cerebral hemisphere located rostrally which was observed to have appeared earlier. Different parts of the brain in the avian and other animal species are seen to appear at different days and in chicken the cerebellum was observed to appear on day 7 of incubation (Serdar and Emrah, 2010).

At day 27, the optic lobes were somewhat oval, attached firmly at the lateral sides of the midbrain, caudolateral to the cerebrum and craniolateral to the cerebellum. This is in agreement with (Kent, 1987) that most bird has large optic lobe and (Iwaniuk, 2003) also observed this in the parrots. Some birds tend to have a relatively small optic lobe such as kiwi and humming birds (Martin, *et al*. 2007). In the pre-hatch period, the optic lobe of the HGF was seen to be located on the lateral side and overlapping dorsally by the cerebrum. Different bird posse's different orientation of the position of optic lobe, it is ventral in the parrots and corvids (Iwaniuk, 2003), while it is lateral below the cerebral hemisphere in most raptor birds which agrees with the present study.

The optic lobes at day 8 were seen to be stratified and laminated with the formation of superficial and deep layers and ventricular neuroepithelium. This is in agreement with finding of (Dong, *et al*. 2002) in the domestic chicken. The stratum opticum, neuropil and the stratum periventriculare were seen to possess large cells that form fourth strata which was observed at day 16 of incubation. At day 18, large cells that were to form large neurones were observed within the following layers; SGP, SGC and SAC. In mature birds according to (Cook, 2002) the SGC is made up of different sizes of neurones which renders most bird to have large receptive fields. The SAC was seen to have diverse types of cell possessing varied position of nucleoli, granule cells having dark stained nucleoli. There are differences with respect to the sizes of visual nuclei that tends to increase with the increase in species typical visual behaviour. Humming birds shows massive hypertrophy of the pretectal nucleus of the optokinetic response and analysis of optic flow (Iwaniuk and Wylie, 2007).

5. Conclusion

This study was aimed at having a baseline data for the development of the optic lobe of the grey breasted HGF which was seen to appear first at day 8 of incubation period. Structurally, before hatch, the optic lobes were observed to be large, developed and laminated. This is a typical characteristic ascribed to precusian birds which tends to relatively survive on its own in the ecosystem and as such are used in the control of insects, snakes and rodents because of its excellent vision.

6. Acknowledgement

This piece of work has come thus far with the aid of Mr. Tobias P.P Choji of NVRI Vom, JOS and Mr. Peters of the Department of Human Anatomy, A.B.U. Zaria. They take their precious time to work with me in the gross and histology Laboratories even some times into the nights to see that this work come to this phase.

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