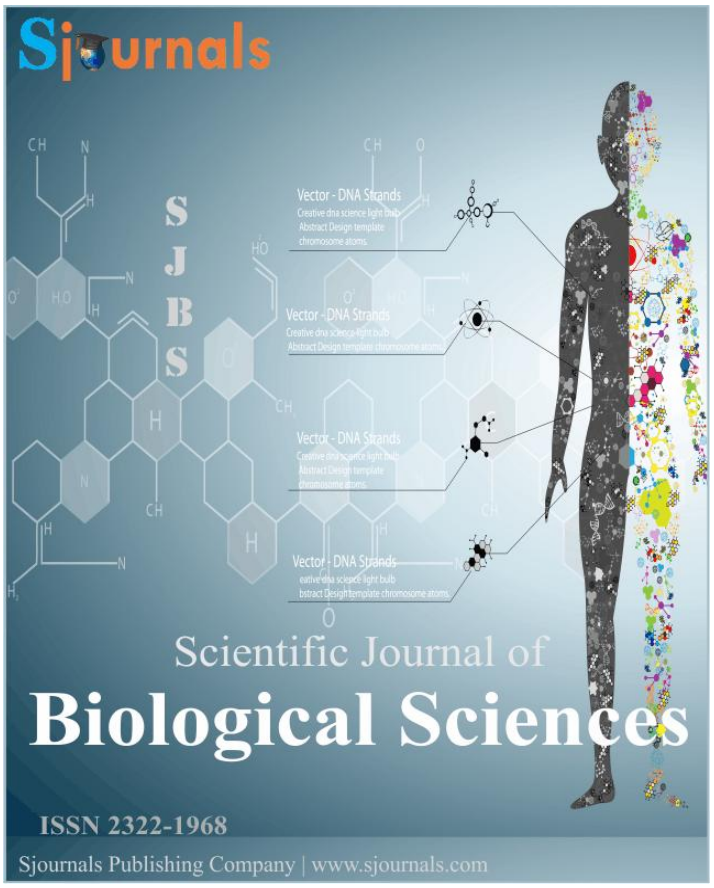

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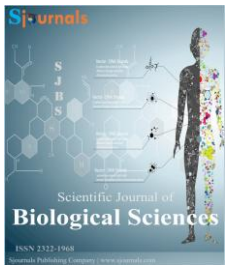

Original article

Morphogenesis of the cerebrum of grey breasted helmeted guinea fowl (*Numida meleagris galeata*) at incubation period

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ABSTRACT

This study was carried out to investigate the development of the brain in the grey breasted helmeted guinea fowl in Nigeria. Domestication of this species of bird is in an increase, but scanty documentation on the development of cerebrum and how it relates to the neurobiology of this bird is lacking. A total of seventy four (74) fertilized grey breasted helmeted guinea fowl eggs purchased from National Veterinary Research Institute (NVRI) Vom, Jos, Plateau State, Nigeria and other local breeders within Jos and its environs were used for this study. Grossly, the cerebrum of the helmeted guinea fowl appeared first on day 4 of incubation and was lissencephalic throughout the incubation period. The mean weight of the cerebrum was established to be 0.010 ± 0.003 g on day 5 pre-hatch. The weights progresses steadily but weighed less than 3.0 g on day 28 prior to hatching. At day 5 of incubation, there were numerous neuroblast migrating from the neuroepithelium of the lateral ventricles. Blood vessel was the first organ to be formed. The cerebrum was first observed to appear on day 4 of incubation. The cerebrum was fully formed void of olfactory lobe at pre-hatch period.

1. Introduction

The grey breasted helmeted guinea fowl (*Numida meleagris galeata*) is a native to Africa and belongs to the Phylum, *Chordata*; Subphylum, Vertebra; Class, Aves; Order, Galliformes; and Family, Numidae. It is widely distributed in the Guinea Savannah vegetation zone of Nigeria (Ayeni, 1983) and estimated at 44 million in captivity (Ayeni, 1980). 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 is spreading to other small-holder farming areas (Ayorinde, 1987). 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). The brain being part of the nervous system is made up of several parts (cerebrum, diencephalon, mesencephalon, pons, cerebellum and medulla oblongata) which all obtain information about the internal and external environment, analyze and respond to information, store information and coordinate outgoing motor impulses to the viscera and skeletal muscles (Northcutt, 2001). Developmentally, in avian, the central nervous system develops from the neural plate, an epithelial sheet that arises from the dorsal ectoderm of the developing embryo (Hallonet et al., 1990). After neural tube closure, series of vesicles can be clearly distinguished morphologically at the anterior end indicate an anterior-posterior axis development (Joyner, 2002). The most anterior end of the neural tube gives rise to the procencephalon forebrain consisting of the telencephalon and diencephalon.

The cerebrum in birds plays a role in processing visual information, auditory processing, emotion, learning and voluntary movement of body parts (Ulinski, 1990). The avian forebrain displays no lamination that corresponds to the mammalian neocortex, hence lamination does not seem to be a requirement for higher cognitive functions (Metzger, 1996). Several research has been done to study the development of the avian brain, which includes; structural organization of grey breasted helmeted guinea fowl a pre-hatch study (Wanmi et al., 2016), the immunoreactivity of glial cells (Maximina et al., 1998), glial cells in the CNS of healthy Passeriformes birds (Peer, 2012) and the development of chicken cerebellar cortex (Serdar and Emrah, 2010). Despite these studies, there exist dearth of information on the histomorphogenesis of the grey breasted helmeted guinea fowl in Nigeria. In this investigation an attempt will be made to find out the variations in the developmental anatomy of the cerebrum in the grey breasted helmeted guinea fowl with regard to their developmental gross structure and histogenesis, which may be helpful in the understanding of the neurobiology of this species of bird despite an increase awareness on its domestication.

2. Materials and methods

2.1. Experimental design

A total of seventy four (74) fertilized grey breasted helmeted guinea fowl eggs purchased from National Veterinary Research Institute (NVRI) Vom, Jos, Plateau State, Nigeria and other local breeders within Jos and its environs were used for this study. The eggs were transported to a hatchery, still 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 according to method modified by Moreki et al. (2012).

2.2. Extraction of embryo

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 made on the egg until a crack was formed. Then, the crack was gently widened manually and the embryo collected in a transparent dish, the procedure as modified by Salami (2009).

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/body weight.

Thereafter, decapitation was made and the heads fixed in 10 % neutral buffered formalin for 3-5 days. After 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 is 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. Some of the extracted brains were fixed in Bouin's solution for routine staining.

2.4. Separation of the cerebrum

The cerebellum is located on the dorsal portion of the brain stem with three peduncles: the restiform body connected to the medulla, the brachium pontis that connects cerebellum to the Pons and the brachium conjunctivum that connects cerebellum to the midbrain. These peduncles were severed using a scalpel blade to expose the entire brainstem. After the midbrain removal, the anterior portions left are the diencephalon and the cerebrum. At the boundary between the diencephalon and cerebrum, an incision was made revealing the cerebral hemispheres held together by the corpus callosum. Each hemisphere was freed by a transverse incision into the longitudinal fissure using scalpel and blade (Baumel et al., 1993).

2.5. Gross anatomy and morphometry

Seven brain samples were used for gross study at pre-hatch period and 68 cerebrum were used for morphometric study at pre-hatch period were collections were done from days 5, 8, 1, 14, 17, 20, 23, 26 and 28. A small opening was made on the large air space area and the entire egg dropped into a labeled container of 10 percent buffered formalin for proper fixing (Gossomji, 2014). The weight of the cerebrum was taken using digital electronic balance; (Model JJ1000, Max. 1000g, d=0.01g, e=10d, No. 211011011098, Made in China and Analytical Weighing balance, Adventure QHAUS Corporation, Item No. AR3130, Max. Capacity=310g Readability=0.001g, Made in China). 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). Weight was recorded in grams (g).

2.6. Data analysis

Morphometric data on the whole body and brain were analyzed using Statistical Package for Social Science (SPSS) version 17.0. In the analysis, the descriptive statistics was expressed as Mean ± Standard Error of the Mean.

3. Results and discussion

3.1. Pre-hatch features

In the grey breasted helmeted guinea fowl, two types of yolk were observed and these were the milk and yellow coloured yolks (Plate I). At day 2 of incubation, area of encircled blood clot appeared on the side of the yolk indicating the position of the future embryo (Plate II). At day 4 of incubation, the embryo of the helmeted guinea fowl appeared as J-shaped figure or inverted "9" on the surface of the yolk with visible blood vessels. When exteriorized, the embryo was observed to have an oval rostral projection as the brain; a black spot as the lens placode (head region) and a caudal tail (Plate III). At day 5, blood vessels were elaborate and the developing embryo at this point was seen as a round object, still on the surface of the yolk. It was observed that the cerebrum including the longitudinal fissure, components of brain stem and spinal cord has been formed (Plate IV).

At day 8 of incubation, the optic lobes were first seen to appear caudal to the cerebral hemispheres as two opposing crescents that had dorsal and lateral convex surfaces. The optic lobes were separated from each by a longitudinal fissure and from the cerebrum by a transverse fissure. Between the cerebral hemispheres and the optic lobes, there exists a clear space in the form of diamond shape. The cerebral hemispheres and the optic lobes were flattened dorsally (Plate V). Dorsally, the cerebrum was observed to be pushed rostrally thereby reducing its size and altering its shape, while the optic lobes bulged out to increase its size. This bulging narrowed the large diamond-shaped space that initially existed between it and the cerebrum. At day 11 of incubation, diamond-shaped clear space reappeared between the cerebrum and the optic lobes (Plate VI). At day 21 of incubation, those major features of the cerebrum have been developed except the olfactory lobes, which was found to be grossly evidence after incubation period (Plate VII).

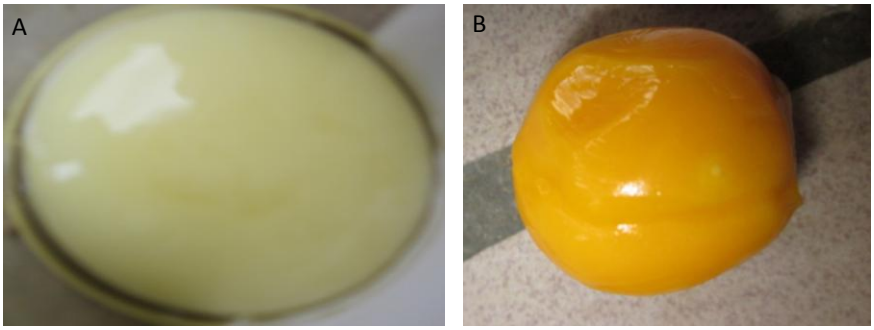

Plate I. Two forms of egg yolk, Milk-coloured egg yolk, A; Yellow-coloured egg yolk, B; (Day 1 of incubation) X12.1

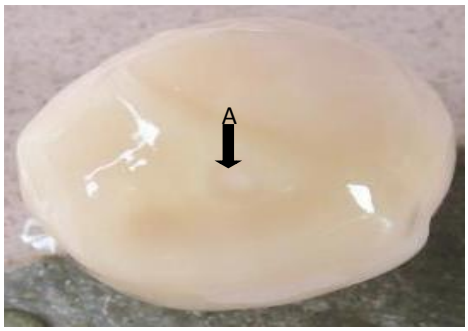

Plate II. Yolk of helmeted guinea fowl indicating: An encircled point of future embryo development, A; (Day 2 of incubation) X12.1

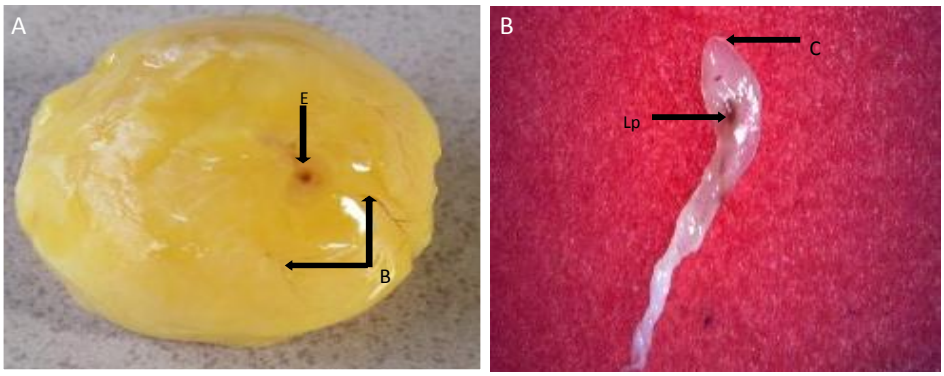

Plate III. Embryo of the helmeted guinea fowl showing: J-shaped embryo, E; Blood vessels, B; The brain, C; Lens placode, LP; (Day 4 of incubation) X12

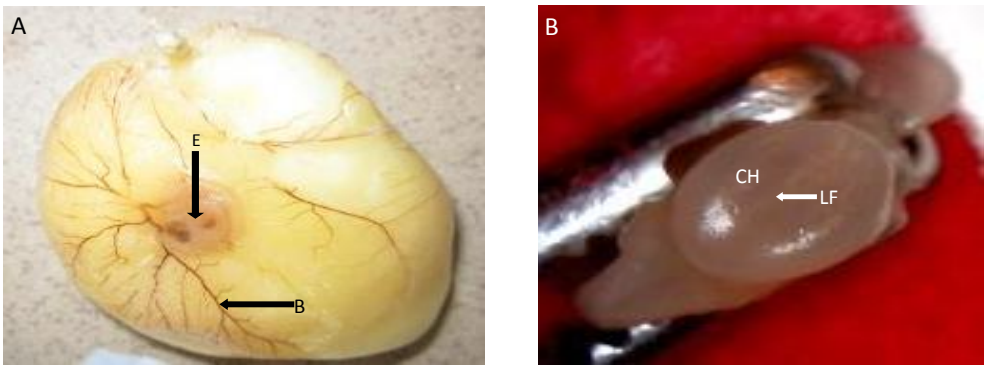

Plate IV. Developing embryo of the helmeted guinea fowl showing, An oval shape embryo, E; Blood vessels, B; Cerebral hemisphere, CH; Longitudinal fissure, LF; (Day 5 of incubation) X12.1

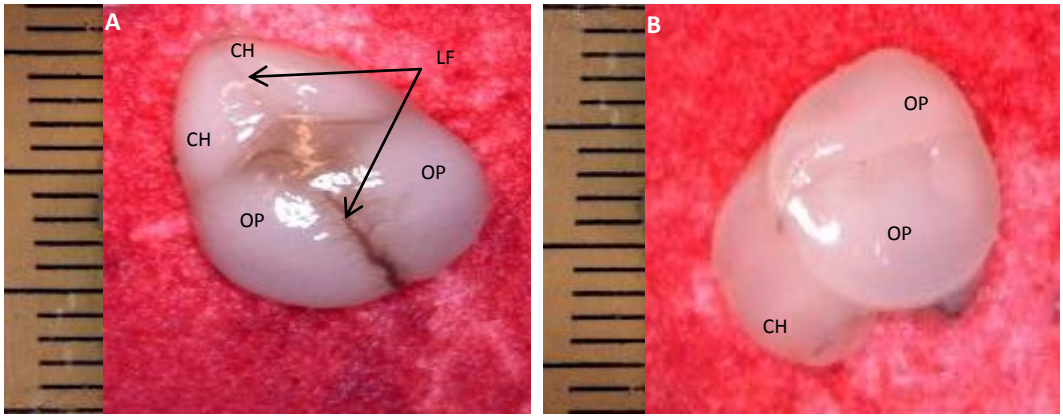

Plate V. Dorsal and lateral views of the brain of the embryo, Cerebral hemisphere, CH; Optic lobe, OP; Longitudinal fissure, LF; (Day 8 of incubation) X500

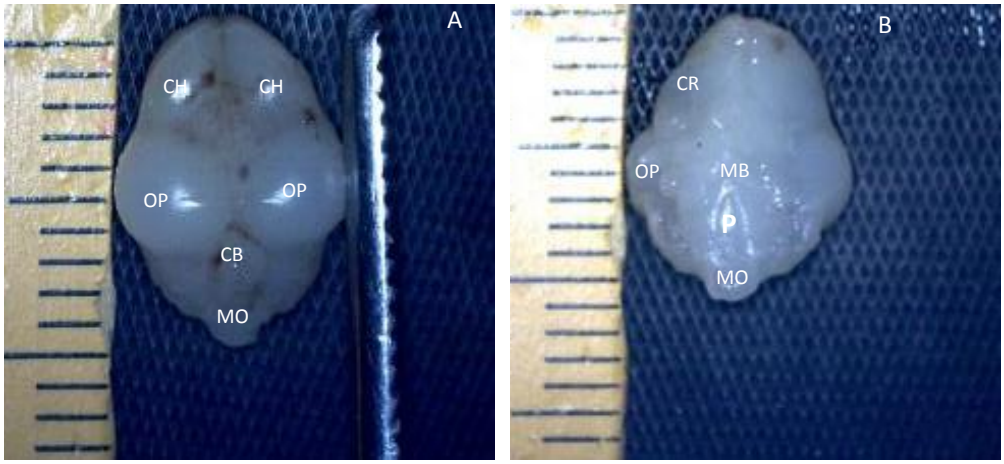

Plate VI. Developing brain of the helmeted guinea fowl, dorsal and ventral aspect, Cerebral hemisphere, CH; Optic lobe, OP; Cerebellum, CB; Medulla oblongata, MO; Cerebrum, CR; Midbrain, MB; Pons, P; (Day 11 of incubation) X500

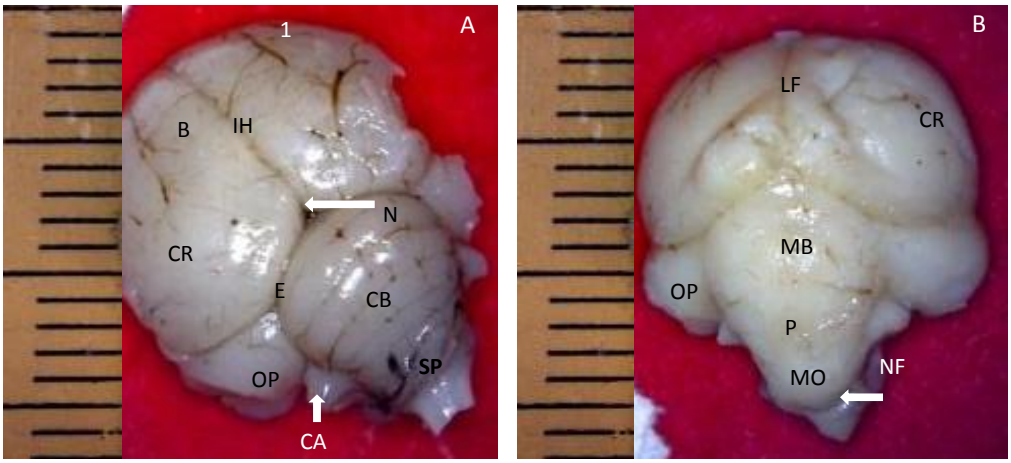

Plate VII. Whole brain of the guinea fowl showing, Dorsal and ventral aspects, Wulst, B; Cerebrum, CR; Interhemispheric fissure, IH; Notch, N; Transverse fissure, E; Spinal cord, SP; Optic lobe, OP; Cerebellum, CB; Cerebellar auricle, CA; Vallecula, 1; Medulla Oblongata, MO; Midbrain, MB; Pons, P; Nuchal flexure, NF; (Day 21 of incubation) X500

3.2. Morphometric studies

3.2.1. Pre-hatch morphometry

The mean weight of the cerebrum on day 5 of incubation was 0.010 ± 0.003 g. The mean weights on day 14 and 20 were 0.031± 0.022 g and 0.085 ± 0.074 g respectively. On days 23 and 28, there mean weights were 0.102 ± 0.074 g and 0.214 ± 0.121 g, respectively (Table 1). Their mean weights increase with number of days. From the chart, from days 5 to 17, the weight of the cerebrum was less than 1 gram. At days 25 and 28, the weights of the cerebrum were above 1 g and 2 g respectively (Fig. 1).

Table 1
Mean weights of the helmeted guinea fowl cerebrum at days 5-28 of incubation (in gram), (n=7).

Days	Minimum	Maximum	Mean ± SEM
5	0.0056	0.01470.010 ± 0.003	
8	0.0061	0.0284	0.013 ± 0.007
11	0.0115	0.0501	0.026 ± 0.015
14	0.0065	0.0758	0.031 ± 0.022
17	0.0262	0.0848	0.050 ± 0.019
20	0.0101	0.2236	0.085 ± 0.074
23	0.0203	0.3202	0.102 ± 0.074
26	0.0720	0.3684	0.189 ± 0.097
28	0.0623	0.4083	0.214 ± 0.121

n; number of birds used per day, SEM; Standard Error of Mean.

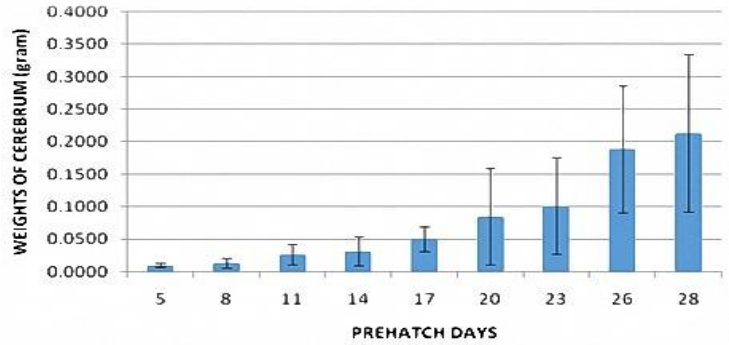

Fig. 1. Mean weight of the cerebrum helmeted guinea fowl during days 5-28 pre-hatch period in gram.

The grey breasted helmeted guinea fowl had two types of egg yolks; the yellow coloured and milk coloured types. The egg yolk played a significant role during pre-hatch development of the embryo by primarily providing it with the necessary nutrients required for the development of the embryo (Naber and Squires, 1993). At day 2 of incubation, blood clot was first noticed, and by the fifth day, blood vessels were prominent. In the grey breasted helmeted guinea fowl, one of the first organ systems to develop is the vascular system with blood vessels being visible on day 4 of incubation. Blood islands were seen soon after day 1 of incubation and the circulation was established on day 2 of incubation in the chicken (Bellairs and Osmond, 2005). This supports the fact that vascular system is the first to be formed during embryogenesis of the galliformes. The embryo of the helmeted guinea fowl appeared first on the fourth day of incubation with a rostral projection of the brain and an eye spot referred to as lens placode, and a caudal tail with a stripped middle part as the body. This developmental order was reported in the chicken by Eyal-Giladi and Kochav (1975). Gosomji (2014) reported that the gastrointestinal tract (GIT) first developed on the 8th day of incubation and an osteogenic development in grey breasted guinea fowl on day 10 of incubation (Salami, 2009). This indicated that during avian embryogenesis, the circulatory system is the first organ to appear and subsequently, the nervous system developed alongside with it in the Galliformes.

In all the fissures of the brain, the interhemispheric fissure was the first to be formed during the formation of the cerebrum. Throughout the developmental phases of the cerebrum, its surfaces were smooth, void of gyration.

This is in agreement with Pearson (1972) whose findings showed that most brain of birds are lissencephalic and not gyricephalic.

From this present study, the mean weights of the cerebrum and optic lobe on the first day, were found to be high while the midbrain was the least. This is in agreement with the findings of Wanmi (2012) in the domestic pigeon where he recorded a forebrain weight that was consistently higher in the female and male domestic pigeons (*Columbia livia domestica*), making up 67.13 % of the total brain weight. This increase in the weight of the forebrain was attributed to the lengthening nature of the cerebral hemisphere, which covered almost three quarters of the entire length of the brain. The cerebrum of the grey breasted helmeted guinea fowl on the fifth day of incubation was made up of neuroepithelium with neuroglia cells making up the major cells. Thereafter, the lateral ventricle was observed to divide the Wulst into hyperpallium dorsally and the mesopallium ventrally containing similar neuronal cells at pre-hatch. The neuronal cells formed correspond to those of the mammals (Karten and Hodes, 1967). The dorsal ventricular ridge as concluded by Husband and Shimizu (1999) was said to be unique to birds and serves as the centre of learning and intelligence and were best developed in Canaries, Passeriformes but less developed in the pigeon, dove and fowl. Based on this fact, it can be said that the helmeted guinea fowl possess some degree of learning and intelligence. Apparently mammals have developed a particular form of cerebral hemispheres, relying heavily on the spreading out of neurones in the cortical sheet over the surface, while in the birds functionally similar neurones were compressed into a more restricted region in the hyperstriatum (Wulst).

4. Conclusion

Grossly, the cerebrum of the grey breasted helmeted guinea fowl appeared first on day 4 of incubation and was lissencephalic throughout the incubation period. As a major finding, the olfactory bulb is one of the parts that was not formed during the incubation period.

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