

Comparative Histological Studies in Vencobb Broiler Birds Supplemented with Kitchen Herbs

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ABSTRACT

Recently, the research on the avian brain has emerged as the most exploring area due to its role in cognitive behavior of the birds. The avian brain serves as a model for the neuroanatomists as they explore the basic concepts of the nervous system with regard to behaviour, development and molecular biology. Studies on the brain mechanisms underpinning diverse cognitive abilities, like as learning, memory, attention, and awareness, heavily rely on avian models. The present gross and histoarchitectural study was conducted on the brain of 120 numbers of healthy Vencobb broiler birds divided into four groups, with each group containing 30 chicks over three replicates (10 chicks per replicate). The three treatment groups received a basal diet supplemented with kitchen herbs at concentrations of 0.5% (T2), 1% (T3) and 1.5% (T4) of the basal diet, respectively, while the control group (T1) was maintained without these herbs. Basing on the development of wulst, population of neuronal cell bodies and neuroglial cells, heterogeneity of neurons in the hippocampus and optic lobe, the visual cognitive ability of the Vencobb broiler birds of T3 was found to be best followed by the birds of T2, T4 and T1 groups.

Key words: Visual cognition, birds, gross morphology, histoarchitecture, brain

INTRODUCTION

Recently, the research on the avian brain has emerged as the most exploring area due to its role in cognitive behavior of the birds. The avian brain serves as a model for the neuroanatomists as they explore the basic concepts of the nervous system with regard to behaviour, development and molecular biology. Studies on the brain mechanisms underpinning diverse cognitive abilities, like as learning, memory, attention, and awareness, heavily rely on avian models. Numerous issues in development, ecology, and physiology can be addressed using brain morphometry (Srivastava *et al.*, 2007). Large brains are linked to stronger relationships and higher survival in unfamiliar contexts. The behavioural repertoire, cognitive ability, and evolutionary background of a species are reflected in the shape of the brain. For assessing the level of brain growth, cognitive behaviour, and intellectual capacity, brain volume and weight are significant

parameters. The avian hippocampus plays a well-known role in spatial learning, memory, cognition, and navigation (Srivastava *et al.*, 2007). The well developed optic lobes of the avian brain are suggestive of their highly developed sense of vision. Varied birds have quite different brain morphologies. Larger birds exhibit elongated, reptilian-like brains, but smaller birds often have circular, avian-style brains.

Researchers from several academic backgrounds are increasingly collaborating on the developing topic of avian cognition research as future breeding programmes and policies might employ cognition as a valuable factor to select birds in order to produce birds with greater developmental potential. Little research has been published till date on the gross morphology, and histoarchitecture of the chicken brain that could be correlated with their cognitive behaviour. So, the present comparative study was undertaken to establish a correlation between the visual cognition with the gross morphology and histoarchitecture of different parts of the brain in the Vencobb broiler birds supplemented with different concentrations of kitchen herbs.

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MATERIALS AND METHODS

120 numbers of day-old Vencobb broiler chicks were divided into four groups, with each group containing 30 chicks over three replicates (10 chicks per replicate). The three treatment groups received a basal diet supplemented with kitchen herbs comprising of dried Coriander seeds, Garlic cloves and Fenugreek seeds at concentrations of 0.5% (T2), 1% (T3) and 1.5% (T4) of the basal diet, respectively, while the control group (T1) was maintained without these herbs. The birds were housed in a deep litter rearing system, segregated by replication in distinct sheds and the experiment diet was administered from the 7th day to the 35th day. For histomorphological study, the tissue samples were collected from the hippocampus and optic lobe of the brain from Vencobb broiler chicken of the above groups on 36th day and were fixed in 10% Buffered Neutral Formalin for 72 hours. After fixation, the tissue samples were processed by routine Paraffin technique (Bancroft and Stevens, 1996) to obtain 6µm thick paraffin sections by digital rotatory microtome. The cut sections were put in the water bath maintained at 37°C and then taken on clean grease free glass slides. The slides with cut tissue sections on them were air dried and subjected to the routine Haematoxylin and Eosin staining technique (Bancroft and Stevens, 1996) for recording the histomorphological characteristics of the hippocampus and optic lobe in the Vencobb broiler chicken of all groups under study. The observations were subsequently photographed under Trinocular research microscope with the photographic unit attached with the above microscope at the CIF, OUAT, Bhubaneswar.

RESULTS

The neuroglial cells such as astrocytes, oligodendrocytes and microglia are well identified by routine Haematoxylin and Eosin stains. The nucleus of astrocytes was large, rounded or oval and vesicular in nature with finely dispersed chromatin (Fix *et al.*, 1996). The nucleus of oligodendrocyte was perfectly round and intensely dark stained due to densely packed chromatin (Fix *et al.*, 1996). Similarly, the nucleus of microglia

was small, elongated and intensely dark stained due to densely packed chromatin (Fix *et al.*, 1996).

T1 GROUP

The majority of the neuronal cell bodies were pyramidal like and pyramidal shaped in both the hippocampus (Fig.1) and optic lobe. The population of pyramidal neurons per unit field of microscope was comparatively high as compared to T4, but less than that of T2 and T3 in both the hippocampus and optic lobe. Very few multipolar neurons were noted in both the hippocampus and optic lobe. The astrocytes were very fair in number in both the hippocampus and optic lobe. The population of both oligodendrocytes and microglial cells per unit field of microscope was certainly higher as compared to that of T4, but less than that of T2 and T3 in both the hippocampus (Fig.2) and optic lobe (Fig.3).

T2 GROUP

The large sized pyramidal neurons were predominant in different regions of the hippocampus and optic lobe. The population of pyramidal like neurons was also more as compared to that of T1 and T4, but less than that of T3 in both the hippocampus and optic lobe. Large sized multipolar neurons were also frequently noted in the different regions of hippocampus (Fig.4) and optic lobe (Fig.5). The astrocytes were abundant in number in both the hippocampus and optic lobe. The population of both oligodendrocytes and microglial cells per unit field of microscope were certainly higher as compared to that of T1 and T4, but less than that of T3 in both the hippocampus (Fig.6) and optic lobe (Fig.7).

T3 GROUP

The large sized pyramidal neurons were predominant in both the grey matter and white matter of the hippocampus and optic lobe. The population of pyramidal and pyramidal like neurons per unit field of microscope in both the hippocampus (Fig.7) and optic lobe was also more as compared to rest of the groups under study. Large sized multipolar neurons were also frequently noted in the different regions of hippocampus and optic lobe (Fig.8). The astrocytes were abundant in

number in both the hippocampus and optic lobe. The population of both oligodendrocytes and microglial cells per unit field of microscope were certainly higher as compared to rest of the groups under study in both the hippocampus (Fig.9) and optic lobe (Fig.10).

T4 GROUP

The majority of the neuronal cell bodies were granular in both the hippocampus (Fig.11) and optic lobe. The population of pyramidal like shaped and pyramidal neurons per unit field of microscope in both the hippocampus and optic lobe was found to be least in this group. The multipolar neurons were not observed in both the hippocampus and optic lobe. The majority of the neuroglial cells were oligodendrocytes in both the hippocampus and optic lobe. The amount of astrocytes and microglial cells were very few in numbers in both the hippocampus and optic lobe (Fig.12).

DISCUSSION

The population of neuroglial cells as oligodendrocytes, astrocytes and microglia per unit field of microscope was found to be more in both the hippocampus and optic lobes of the brain in the birds in T3 group followed by T2, T1 and T4. The astrocytes not only play very important role in regulation of blood flow to the brain, but also help in transferring mitochondria to neurons and supplying the building blocks of neurotransmitters, which fuel the neuronal metabolism. It also has antioxidant property, which is mediated through reactive gliosis. All this activities help in the proper development of the brain mass and neurons, which could be directly correlated with the cognitive ability of the birds. Similarly, the microglial cells regulate the brain development, maintain the neuronal networks and play a vital role in injury repair as they act like stem cell. So, the increase in microglial cell population is also desirable, which is positively related with the cognition.

The glial cells are involved in both short term and long term synaptic plasticity (Citri and Malenka, 2008). There is growing realization that glia may be involved in some forms of short-term plasticity (Haydon, 2001). With their intimate

association with synapses, astrocytes and perisynaptic Schwann cells are well positioned to regulate synapses. They have an established role in clearance of neurotransmitter and may participate in synaptic plasticity by controlling the speed and extent of such clearance (Danbolt, 2001). This can in turn impact on the degree of postsynaptic receptor activation and desensitization. Another way that glia may be involved in synaptic plasticity is by sensing extracellular messengers and then releasing substances that in turn can directly affect synaptic efficacy (Haydon, 2001). For example, glia express many different neurotransmitter receptors (eg, glutamate receptors), which when activated result in the release of substances (eg, ATP) that can then act on presynaptic terminals to regulate neurotransmitter release. The glial cells also produce a protein, i.e. tumor necrosis factor α (TNF α), enhances synaptic efficacy by increasing surface expression of AMPA receptors. Thus, the continual presence of TNF α is required for preservation of synaptic strength at excitatory synapses. Through its effects on AMPA receptor trafficking, TNF α may play roles in synaptic plasticity and modulating responses to neural injury (Beattie *et al.*, 2002).

Glia can affect both the encoding and consolidation of memory (Fields *et al.* 2014). Activation of intracellular Ca²⁺ signaling in astrocytes can augment or suppress both excitatory and inhibitory synaptic transmission and influence state dependent changes in cortical activity during, for example, sleep (Halassa *et al.*, 2009) and working memory. The astrocytic signaling participates in some forms of synaptic plasticity (i.e., cholinergic-induced long-term potentiation (LTP) in cortex and hippocampus (Navarrete *et al.*, 2012); spike-timing dependent cortical plasticity, hippocampal long-term depression (LTD) and working memory (Han *et al.*, 2012)). Transient disruption of the flow of energy substrates from astrocytes to neurons has little effect on learning but severely impairs the later formation of long term memory (Suzuki *et al.*, 2011). Astrocytes associated with blood vessels shuttle ions and energy substrates between neurons and the blood stream and regulate local blood flow, which in turn

modulates neuronal function (Moore and Cao, 2008). Moreover, microglia, oligodendrocytes, and astroglia are primary sources for extracellular matrix components now thought to play critical roles in late stages of LTP and memory consolidation (Babayán *et al.*, 2012). Recently, humanized chimeric mice, in which a large proportion of mouse astrocytes were replaced with human astrocytes, were found to exhibit increased synaptic plasticity and faster learning. The oligodendrocytes are also involved in the formation of myelin sheath around the axons of nerves in the central nervous system forming insulation around the nerve fibers helping the faster conduction of nerve impulse thorough the axon. The high population of astrocytes and oligodendrocytes in both the hippocampus and optic lobes of the birds in T3 group might be attributable to the cognitive ability of the birds as compared to the other groups under study.

The weight of the brain was more in the Vencobb broiler birds of T3 followed by T2, T1 and T4. The number of neurons and neuroglial cells per unit field of microscope in both the hippocampus and optic lobes were quite more in the Vencobb broiler birds of T3 followed by T2, T1 and T4. The high neuronal and neuroglial cell population along with high glial cell-neuronal ratio (GNR) in both the hippocampus and optic lobes of the Vencobb broiler birds in T3 led to better cognitive ability of the birds as compared to the other groups under study, which was similar to the findings of Bartheld *et al.* (2016) in human being.

The neuronal density was found to be more in the hippocampus and optic lobes of Vencobb broiler birds in T3 followed by T2, T1 and T4. The presence of high neuronal density might have formed more computational units to process and store information resulting in better cognitive ability of the birds T3 group as compared to the other groups under study (Galakhova *et al.*, 2022).

The neurons were comparatively bigger in size in the hippocampus and optic lobes of Vencobb broiler birds in T3 followed by T2, T1 and T4. The population and size of pyramidal, pyramidal like and multipolar neurons per unit field of microscope in both the hippocampus and optic lobes of Vencobb broiler birds was more in T3 group

followed by T2, T1 and T4 suggesting of highest neuronal heterogeneity in both the hippocampus and optic lobe of the birds in T3. The large sized pyramidal, pyramidal like and multipolar neurons comprised of longer and larger branches and complexed dendrites forming numerous long-range connections or complexed synaptic circuits leading to increased ability of the neuron to receive more inputs from surrounding cells. Further, the larger spines were more stable and long-lasting (Kasai, 2010). This had resulted in better cognitive ability of the birds in T3 as compared to the other groups under study (Galakhova *et al.*, 2022).

CONCLUSION

Avian species have developed highly sensitive visual systems adapted to their living environment after more than a hundred million years of evolution. Compared with mammals, they are better at tracking small objects moving at high speed, and at identifying targets under low contrast and luminance conditions. The remarkable visual cognitive abilities have attracted an increasing number of researchers devoted to revealing the underlying neural mechanisms. Study on avian brain will usher a path to the neuro-anatomists to investigate general principles of the nervous system in respect to development behavior, physiology, anatomy and molecular biology. The avian models can be used to decipher many unknown facts about neuronal mechanism underlying various cognitive functions such as memory, learning, consciousness and attention. Brain morphology reflects the cognitive capacity, behavioral repertoire and evolutionary process of a species. The present study provided a brief overview on the visual cognitive ability of the birds with reference to the histoarchitecture different parts of the brain. The above correlation could be validated by doing on field trial by providing training to the birds for a specific period of time. Further, the Duncan's encephalization quotient and Cuvier's encephalization quotient can be calculated for determination of the intelligence and cognitive levels of the birds. Basing on the population of neuronal cell bodies and neuroglial cells, heterogeneity of neurons in the hippocampus and optic lobe, the visual cognitive ability of the Vencobb broiler birds of T3 was found to be best followed by the birds of T2, T4 and T1 groups.

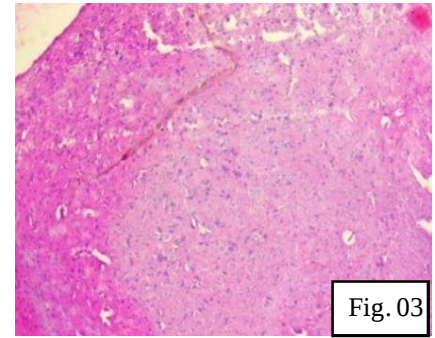
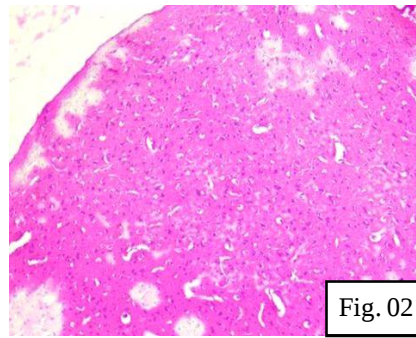
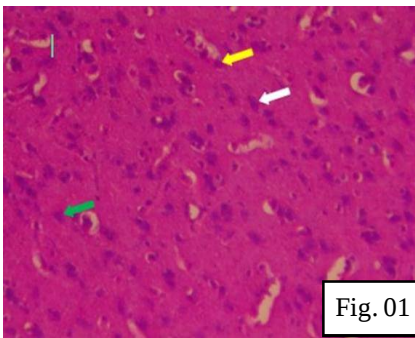


Fig.1: Photomicrograph showing few granular neurons (Yellow arrow) and numerous pyramidal like (White arrow) and pyramidal (Green arrows) neurons in the grey matter of hippocampus in Vencobb broiler chicken of T1; H & E, x400, **Fig.2:** Photomicrograph showing less neuronal density and less population of neuroglial cells in the grey matter of hippocampus in Vencobb broiler chicken of T1; H & E, x100, **Fig.3:** Photomicrograph showing less neuronal density and less population of neuroglial cells in the grey matter of optic lobe in Vencobb broiler chicken of T1, H & E, x100.

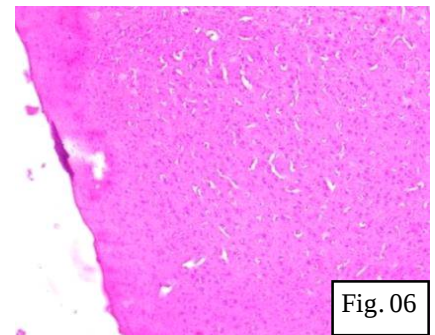
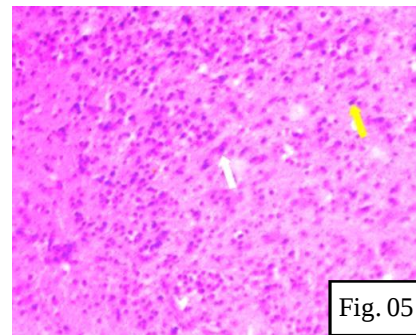
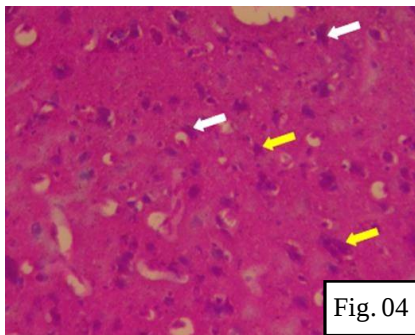


Fig.4: Photomicrograph showing fair population of pyramidal (Yellow arrows) and frequent occurrence of multipolar neurons (White arrows) in the grey matter of hippocampus in Vencobb broiler chicken of T2, H & E, x400, **Fig.5:** Photomicrograph showing fair population of pyramidal neurons (Yellow arrow) and frequent occurrence of multipolar neurons (White arrow) even in the granular region of grey matter of optic lobe in Vencobb broiler chicken of T2 .H & E, x400; **Fig.6:** Photomicrograph showing comparatively high neuronal density and high population of neuroglial cells in the grey matter of hippocampus in Vencobb broiler chicken of T2; H & E, x100

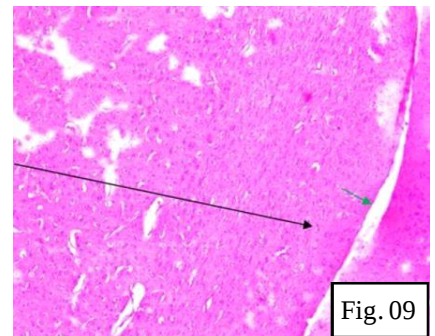
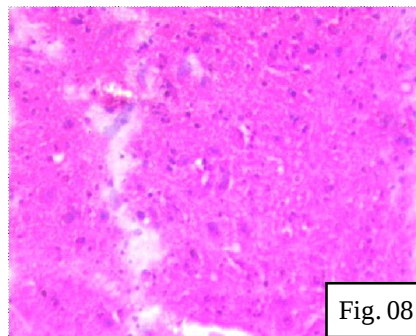
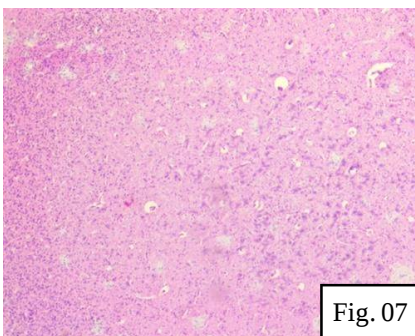


Fig.7: Photomicrograph showing comparatively high neuronal density and high population of neuroglial cells in the grey matter of optic lobe in Vencobb broiler chicken of T2 ,H & E, x100; **Fig.8:** Photomicrograph showing abundance of multipolar neurons (Yellow arrows) in the grey matter of optic lobe in Vencobb broiler chicken of T3, H & E, x400; **Fig.9:** Photomicrograph showing high neuronal density and high population of neuroglial cells in the grey matter region (Black arrow) and white matter region (Green arrow) of hippocampus in Vencobb broiler chicken of T3, H & E, x100.

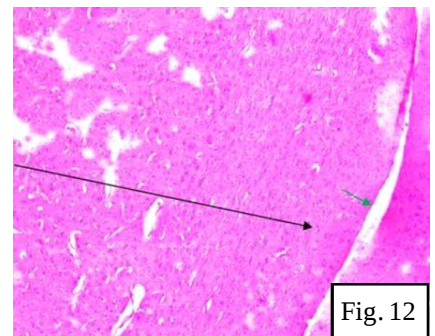
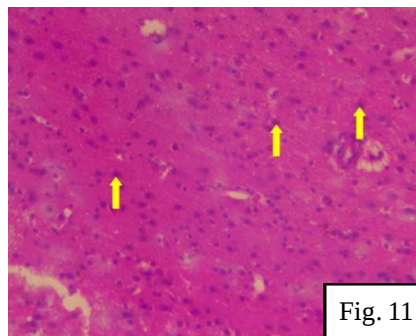
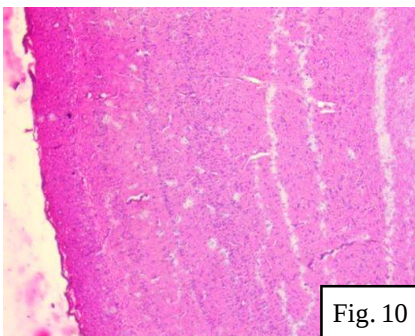


Fig.10: Photomicrograph showing high neuronal density and high population of neuroglial cells in the grey matter of optic lobe in Vencobb broiler chicken of T3, H & E X 100; **Fig.11:** Photomicrograph showing abundance of granular neurons (Yellow arrows) in the grey matter of hippocampus in Vencobb broiler chicken of T4, H & E, x400; **Fig.12:** Photomicrograph showing ventricle (Red arrow), less neuronal density and less population of neuroglial cells in the grey matter region (Black arrow) and white matter region (Green arrow) of optic lobe in Vencobb broiler chicken of T4 ,H & E, x100.

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