PHYTOGENIC EFFECTS OF NEEM LEAF ON BRAIN HEALTH: A STUDY ON PURKINJE CELLS IN BROILER CHICKENS

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ABSTRACT
Neem leaf (Azadirachta indica) was used as a phytogenic feed additive in a study to examine the effects on Purkinje cells in the cerebellum of broiler chickens. A total of 100 one-day-old Arbor Acres broiler chicks were assigned randomly to five treatments, T1 (0%), T2 (0.3%), T3 (0.4%), T4 (0.5%), and T5 (0.6%) inclusion of neem leaf respectively. Photomicrographs were taken using an Olympus Microscope at x400 magnification, examined for morphometrics using ImageJ software, and analyzed using Graph-Pad Prism 8.0.2. Completely Randomized Design (CRD) was the experimental design used in the study. GraphPad Prism version 8.0.2(263) was used to do a one-way analysis of variance (ANOVA) on data in order to ascertain the treatment effect. The same statistical package's Tukey test was used to analyze mean differences. The experimental design was completely randomized design (CRD), and differences in means were analyzed using Tukey test. Results showed there were significant difference (P<0.05) in Purkinje cells of chickens fed T2 and T5. Histological study revealed that chickens fed a control diet displayed a higher linear density of Purkinje cells compared to those fed Neem leaf supplementation. Notably, the highest inclusion levels T5 (0.6%) resulted in shrunken and irregularly shaped Purkinje cells, indicating possible neuro-degeneration. Behavioral observations in chickens fed T5 showed symptoms of lack of coordination and tremors, suggesting a connection between Neem supplementation and motor function impairment. Although brain weights of Neem-fed chickens were marginally greater than those in the control group, the density and shape of Purkinje cells prompted concerns about the potential detrimental consequences of excessive Neem leaf ingestion. These findings reinforce the need for careful assessment of Neem leaf supplementation in poultry diets, following the quest of sourcing alternatives to replace the use of synthetic substances in optimizing, growth, performance, and health.
Keywords: Broiler chickens, Cerebellum, Neem, Phytogenic, Purkinje cells

INTRODUCTION

The chicken brain, a vital part of the nervous system, plays a crucial role in movement, coordination, memory, cognition, and emotional processes. It comprises the cerebellum, cerebrum, and brainstem, protected by meninges. The cerebrum's smooth surface reflects evolutionary pressures. Chickens have a higher density of pyramidal neurons in their cerebrum, responsible for information processing, signaling, balance, temperature regulation, movement, and cognition compared to their wild ancestors (Kelsey *et al*., 2021). The cerebellum, a three-layered membrane structure, controls motor nerves, body balance, cognitive processes, and movement in chickens, aiding in learning and memory. It comprises of molecular layer, Purkinje cells, and granular layer, (Nangoy *et al.,* 2019) each with distinct cells for microscopy. The Purkinje cells layer, located in the middle layer of the cerebellum, is a large, flat, highly branched dendritic tree with a long axon that enters the cerebellar nuclei, inhibiting other neurons and regulating movement and balance.

The brain's biochemical and physiological functions, high energy and lipid demands, and continuous production of reactive oxygen species make it highly susceptible to free radical damage (Belles *et al*., 2017). According to Ferreira *et al.* (2014), oxidative stress is harmful to tissues or cells. The brain requires high oxygen levels for proper function, thus the production of free radicals to promote growth, development, memory, and cognitive activity. However, stressors, such as climate change, have caused livestock production to be greatly impacted by oxidative stress (Oke *et al*., 2022). Stressful conditions increase bird's vulnerability to the production of more free radicals than normal, whereas antioxidant enzymes operate as defensive mechanisms to scavenge free radicals (Miao *et al*., 2020). Inflammation and cellular damage are generated by the presence of reactive oxygen species (ROS) by altering biological molecules (Alzohairy, 2016; Kiranmai *et al*., 2011). To create a balance between the free radicals and antioxidant enzymes that will scavenge it, the use of synthetic substances has been in use for years. However, following the ban placed on the use of synthetic substances to improve growth, researchers have sourced natural alternatives that pose no threat of residual effect or resistance in the body. Herbs and spices, like garlic, ginger, and turmeric, can prevent infections and optimize poultry health and performance, despite being neglected due to focus on synthetic medications and antibiotics.

Neem leaf as a plant extract has been recognized through several studies to have shown immunomodulatory, anti-inflammatory, hyperglycemic, and antioxidant properties (Subapriya and Nagini, 2005; Paul *et al*., 2020). The use of phytogenic feed additives to improve performance, growth, welfare, and productivity in recent years has increased. This study was designed to investigate the impact of Neem leaf supplementation on the Purkinje cells in broiler chickens' cerebellar cortex, aiming to understand the physiology behind observable signs and symptoms, crucial for better production and management.

MATERIALS AND METHODS

Location of the study

The study was carried out at Poultry unit of the Teaching and Research farm (TRF) of the University of Ibadan, Ibadan located between the latitude 60^0 10 and 90^0 10 north of the equator, and longitude 30^0 and 60^0.

Experimental Animals and Design

One hundred un-sexed Arbor Acres broiler chicks were randomly assigned to five treatments, each replicated four times, under standard management practices under a deep litter system in a completely randomized design. Neem leaf was included into diet as follow; T1 (0%), T2 (0.3%), T3 (0.4%), T4 (0.5%), and T5 (0.6%).

Table 1: Composition of Experimental Diets at Starter and Finisher Phases

Ingredients	Starter Phase (0-28 days)	Finisher Phase (29-42 days)
Maize	42.00	50.00
Soya bean Meal	39.60	30.10
Wheat	14.50	14.25
Fish meal	2.00	2.00
Palm oil	0.50	0.50
Bone Meal	0.50	2.00
Premix	0.25	0.25
Limestone	0.25	0.50
Methionine	0.10	0.10
Lysine	0.10	0.10
Salt	0.20	0.20
Total	**100.00**	**100.00**
Calculated Analysis		
Metabolizable Energy(Kcal/kg)	3191.20	3143.20
Crude Protein (%)	23.11	19.47
Crude Fibre (%)	4.23	3.75
Dry Matter (%)	87.43	87.48
Ether Extract (%)	3.46	3.59

Histological processing

Twenty birds, (four birds per treatments) were sacrificed and brains were cut open and preserved for analysis. The brains were split into halves, dehydrated, and sectioned using a microscope. Morphology and distribution were revealed using Nissl stain. The tissue was differentiated and examined under a light microscope to identify changes in morphology, damage, or degeneration. The study aimed to understand the brain's structure and function.

Data collection

Photomicrographs were taken using an Olympus Microscope at x400 magnification, and morphometric examination was performed using ImageJ software and Motic plus on processed tissues.

Statistical analysis

Completely Randomized Design (CRD) was the experimental design used in the study. GraphPad Prism version 8.0.2 (263) was used to do a one-way analysis of variance (ANOVA) on data in order to ascertain the treatment effect. The same statistical package's Tukey test was used to analyze mean differences.

RESULTS AND DISCUSSION

Results shown in Table 2 revealed that, the brain weights of chickens administered neem leaf at 0.3%, 0.4%, and 0.6% neem leaf T2, T3, and T5 respectively were higher than those fed 0% (T1-control) and 0.4% (T3). However, there was no statistically significant difference between the treatment groups and the control group, despite T2, T4, and T5 exhibiting higher brain weight than T1 (control) and T3. The Purkinje cell density in the cerebellum of broiler chickens administered Neem leaf as a phytogenic feed addition was evaluated; observations indicated that T1 (control) had the highest density of Purkinje cells compared to the other groups. T5, which exhibited the highest degree of Neem leaf inclusion, demonstrated the lowest number of Purkinje cells. A statistical difference was observed between T1 and T2 ($P<0.05$), as well as between T1 and T5, in Purkinje cell density.

Table 2: Effect of Neem leaf supplemented as phytogenic feed additives on brain weight, body weight, and Purkinje cell linear density in broiler chickens

Variables	T1	T2	T3	T4	T5	SEM	P-value
Brain Wt.(g)	2.57	2.64	2.57	2.64	2.69	0.03	0.516
Body Wt.(kg)	1.39	1.59	1.51	1.46	1.60	0.03	0.172
Purkinje length	164.38	201.13	230.68	233.35	235.70	11.19	0.197
Purkinje cell count	6.50	5.00	7.00	6.00	4.50	0.39	0.242
Purkinje linear density	0.04^{a}	0.03^{a}	0.03^{ab}	0.03^{ab}	0.02^{b}	0.00	0.008

DISCUSSION

At sacrifice, the body weight of broiler chickens fed Neem leaf as phytogenic feed supplement was higher than that of control. This is comparable to previous study done by Ansari *et al.,* (2012) stating that, broiler hens fed supplemental neem leaf meal had greater body weight, higher feed efficiency, and dressing percentage. It was also noted that, across treatment groups, there were similarities in brain weight after the sacrifice of broiler chicks. Brain weight of birds fed Neem leaf as phytogenic feed additive were higher than those fed control diet. Roth and Dicke (2005) asserted that animals exhibiting a greater brain-to-body size ratio are frequently correlated with enhanced Intelligence Quotient, cognition, and favorable social behavior. The Purkinje cells are responsible for motor learning and cognition processes. The Purkinje cell linear density in broiler chickens fed a control diet is higher than those fed Neem leaf-supplemented diets. Observation showed that Purkinje cells appeared slightly darker and more condensed, suggesting stress or degeneration in birds fed T3, although the cerebellum appeared well-maintained. However, in T4 (0.5%), Purkinje cells appeared smaller, with more highly stained cells, indicating pyknotic or degraded cells. This is comparable to the findings of Fikra *et al*., (2022) although the study was not on broiler chicks and Neem, but on curcumin in albino rats. Treatment 5 (T5-0.6%) revealed undersized, darkly pigmented, and irregularly shaped Purkinje cells. This study claims that birds administered T5 displayed evident evidence of lack of coordination, tremor, and paralysis; these signs could be as a result of some of the Purkinje cell death or degeneration identified in the Purkinje cell layer of the cerebellum in birds fed this medication

CONCLUSION

The study shows that neem leaf, as a phytogenic feed additive, significantly affects the morphology and density of Purkinje cells in broiler chickens' cerebellum. Control diets showed higher density of Purkinje cells, suggesting potential neuro-protective effects. However, Neem-supplemented chickens showed degeneration, with shrunken, darkly pigmented, and irregularly shaped cells. This suggests that excessive Neem leaf intake may negatively impact motor function. The study recommends further research on dosage, long-term effects, mechanics, comparative studies, and behavioral assessments to better understand the effects of Neem leaf supplementation on broiler chickens.

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