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Hypophyseal acetylcholinesterase, and growth hormones of broiler chicken fed *Ficus carica* and vitamin C supplemented diets

David Olawale Ayodele *, Clement Oluwafemi Osowe, Olufemi Adesanya Adu and Clifford Adinma Chineke

Department of Animal Production and Health, The Federal University of Technology, Akure, Nigeria.

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Abstract

The research was conducted to investigate hypophyseal acetylcholinesterase and growth hormones of broiler chickens fed *Ficus carica* and Vitamin C supplemented diets. 160-day-old chicks were randomly assigned to 4 treatments supplemented with *Ficus carica* and vitamin C in 4 replicates and 10 birds per replicate. At the end of the 8th week, the brains and hypophyses of 3 randomly selected birds per treatment were removed and tested for Acetylcholinesterase activities and total protein determination while blood samples were collected for hormonal assay. Results showed that total protein of the whole brain (0.50 mg/dl) and cerebellum (0.12 mg/dl) were significantly ($p<0.05$) lower in birds fed control diet while that of the olfactory lobe was significantly higher than that of the control (0.25 mg/dl). However, the acetylcholinesterase activities (µmol/g tissue) of the optic lobe (0.36), Cerebellum (0.22), and medulla (0.25) of birds fed the control diet were significantly ($p<0.05$) higher than those fed supplemented diets. Also, all hormonal assays tested were significantly influenced by the treatments with the exception of thyroxine. Birds fed diet 4 had the highest record for most growth hormonal assay. From the results obtained, Diet IV could be recommended in broiler chickens' diets for superior growth promotion.

Keywords: Supplementation; Hypophyseal Acetylcholinesterase; Hormones; Ficus

1. Introduction

The use of antibiotics has been restricted in many parts of the world. However, 80% of raised livestock were fed some kind of antibiotics or growth promoters [1]. The improvement of growth rate resulted in high incidence of muscle abnormalities. The most recent and emerging muscle abnormalities are white striping (WS) and wooden abnormalities (WA) [2]. Recently, herbs extract was fed as non-traditional feed additive to replace the growth promoters and antibiotics [3]. Herbal extracts were used in the extensive livestock system to improve animals' general performance [4]. The positive effects of herb extracts were due to the improvement of feed intake, digestibility, activation of the immune system and the anthelmintic actions [1.] Feeding poultry with phytochemicals (products derived from plants such as essential oil, dried plant materials, pure isolated compound) has been reported as a reliable means of combating the negative effect of oxidative stress in heat-stressed poultry [5]. The current trend in the use of phytochemicals in animal production is not limited to being used as placement for antibiotic growth promoters, but also for other anabolic compounds used to improve animal performance [6]. Because health-conscious consumers are increasingly rejecting the use of inorganic substances in food production, plant-derived compounds with performance-enhancing activity, also known as phytogenic compounds, are becoming prominent in the feed additive market [7].

Among such herbs, the Fig tree (*Ficus carica)*, locally known as "opoto" is of important significance [8]. The leaf of *Ficus carica* is said to contain anti-parasitic, anti-pyretic, anti-bacterial, anti-diarrhea, anti-inflammatory, free radical scavenging activities [9], which are beneficial to health because they can act as antioxidants in a variety of ways, such as reducing agents, hydrogen donors, free radical scavengers, and singlet oxygen quenchers [10]. The supplementation

* Corresponding author: David Olawale Ayodele

of animal feed with vitamin C has been described as a cost-effective strategy to mitigate oxidative stress and improve antioxidant status and immune performance [11]. Studies have shown that supplementation of vitamin C in diets will enhance the antioxidant status of an organism by up to 30% which helps the body's natural defenses to combat inflammation [12]. [8] reported on the nutritional and antioxidant status of *Ficus carica* to be suitable as a supplement for animal production. This study was therefore designed to investigate the hormones, acetylcholinesterase, total protein of the brain of Arbor acre strain of broiler chickens fed *Ficus carica* leaf powder and vitamin C supplemented diet.

2. Material and methods

2.1. Collection, processing, and analysis of phytogens after ethical approval

The Research and Ethics Committee of the Department of Animal Production and Health, The Federal University of Technology, Akure, Nigeria, accepted the experiment's requirements and criteria for animal and animal protocol. *Ficus carica* leaves were freshly plucked and washed in clear running water before being drained and spread lightly on polythene in the shade for two weeks. FCLP was made from dried *Ficus carica* leaves. [8] investigated the proximate, phytochemical, antioxidant and mineral components of FCLP.

2.2. Experimental Site

The research work was carried out at the Poultry Unit of the Teaching and Research Farm of the Federal University of Technology, Akure, Nigeria. The geographical coordinates of the location are between 7° 17' North and 5° 9' East. The farm is located in the rainforest vegetation belt of Nigeria which is characterized by two rainfall peaks and high humidity during the raining season [13].

2.3. Experimental Diets

Basal diets were formulated for the starter and finisher (Table1) phases to meet the NRC [14] requirement. Thereafter, basal diets were thoroughly mixed and divided into four (4) parts. The four parts were designated as: Diet 1 (Control/basal diet), Diet 2 was supplemented with 1g *Ficus carica*/kg of feed, Diet 3 was supplemented with 200mg vitamin C/kg of feed and Diet 4 was fortified with 200mg of Vitamin C + 1g *Ficus carica* /kg of feed.

2.4. Experimental Birds and Management

A total of 160-day-old broiler chicks were randomly assigned to the four dietary treatments. Each treatment was replicated 4 times with 10 birds per replicate in a completely randomized design. The experimental diets were fed to the birds from the Starter Phase to the Finisher phase. The feed and water were supplied *ad libitum* throughout the experimental period.

2.5. Data Collection

2.5.1. Hormonal Assay

At the end of the 8th week of the experiment, three birds per replicate were randomly selected, tagged, weighed, stunned and slaughtered by cutting the jugular veins in their neck region with sharp, clean and dry stainless knife. Blood was allowed to flow into plain bottle and EDTA bottle for analysis from the clotted blood inside the tubes, serum was separated following centrifugation for about 10 minutes at 3000 revolution per minute (rpm) and was stored at -10 to -40°C. The serum samples were analyzed to determine the levels of the plasma corticosterone, insulin, insulin – like growth factor, growth hormone, plasma thyroid hormone levels, triiodothyronine (T3) and thyroxine (T4) using radioimmunoassay to monitor the levels of these hormones as indicated on the manufacturer's kit.

2.5.2. Brain and Hypophyseal AChE Activities and Total Protein Determinations

At the end of the 8th week, the brains and hypophyses of the slaughtered birds were removed immediately, freed of all adhering meninges and blood vessels. The brains and the hypophyses obtained were dissected on ice-cold porcelain tile into the pons, cerebellum, amygdala, hippocampus, hypothalamus, cerebral cortex, mid brain, medulla oblongata, adenohypophysis and neurohypophysis as described by [15]. The brain and hypophyseal acetylcholinesterase (AChE) activities and total protein concentrations were determined by colorimetric method according to [16] using a Randox commercial kit. Thereafter, the brain and hypophyseal samples from each animal were homogenized (1%, weight per volume (w/v)) with a Potter-Elvehjem homogenizer in 0.1mole (ice-cold phosphate buffer containing 0.1% Triton X-

100 (Sigma)). The AChE activity of each sample was divided by its total protein concentration to give the specific acetylcholinesterase (SAChE) activity in µmole/g protein /min.

2.5.3. Statistical Analysis

All data collected were subjected to Completely Randomized Design procedures of SAS version 9.2 (17). The analysis of variance indicated significant treatment effect and the means were compared using Duncan Multiple Range Test of the same software.

Table 1 Composition of the experimental diets

Ingredients (g/kg)	Starter feed (%)	Finisher diet (%)
Maize	51.00	50.00
Soyabean meal	15.00	13.00
Groundnut cake	15.00	13.00
Fish meal (72%)	5.00	0.00
Corn bran Rice bran	10.60 0.00	6.00 13.30
Limestone	1.00	1.00
Bone meal	1.50	2.50
Salt	0.35	0.35
Broiler Premix	0.25	0.25
Methionine	0.2	0.30
Lysine	0.10	0.30
Total	100	100

3. Results

3.1. Brain total protein of broiler chicken fed *Ficus carica* and vitamin C supplementations

Table 2 present the brain total protein concentration of broiler chicken fed *Ficus carica* and vitamin C supplemented diets. The result revealed that treatment effect was significant (P<0.05) for total protein concentration of the brain sections measured in this study with the exception of pineal body, optic lobe and medulla. Birds fed *F. carica* + vitamin C had the highest value for whole brain (0.64g/dL) and medulla (0.18g/dL), while birds fed the control diet and vitamin. C supplemented diet had the lowest value for the whole brain (0.50g/dL) and medulla (0.12g/dL) respectively. Also, the highest total protein concentration for the olfactory lobe (0.25g/dL) was observed in birds fed the control diet while birds fed vitamin C and *F. carica* + vitamin C diets recorded the lowest value for the olfactory lobe total protein concentration (0.17g/dL). In addition, birds fed *F. carica* recorded the highest value for the pineal body total protein concentration (0.26g/dL) while birds fed vitamin C had the lowest pineal body total protein concentration (0.21g/dL). Furthermore, the highest value for the optic lobe total protein concentration (0.25g/dL) was recorded in birds fed vitamin C while the lowest value (0.18g/dL) was observed in birds fed *F. carica* supplemented feed. The value for the cerebellum total protein concentration was highest (0.17g/dL) in birds fed *F. carica* + vitamin C while the lowest value (0.11g/dL) was recorded in birds fed vitamin C.

3.2. Hypophyseal Acetylcholinesterase (AChE) of broiler chicken fed *Ficus carica* and vitamin C supplementations

Table 3 presents the hypophyseal acetylcholinesterase activities of broiler chicken fed *Ficus carica* and vitamin C supplemented diets. The result revealed that there was significant (P<0.05) effect on all the parameters measured in response to the supplemented diets except for olfactory lobe and pineal body. Acetylcholinesterase activity was highest in the olfactory lobe (0.32µmol/g), optic lobe (0.36 µmol/g) and cerebellum (0.22µmol/g) of birds fed the control diet, while birds fed vitamin C supplemented diet recorded the lowest AChE activity in the olfactory lobe (0.15 µmol/g) and optic lobe (0.16µmol/g). Furthermore, birds fed vitamin C, *F. carica* and *F. carica*+ vitamin C supplemented diets had the same value for cerebellum AChE (0.19µmol/g). Also, highest medulla AChE (0.27µmol/g) was recorded in birds fed *F. carica* while the lowest (0.20µmol/g) was recorded in birds fed *F. carica* + vitamin C supplemented diets. The acetylcholinesterase activity of the pineal body was highest (0.21 µmol/g) in birds fed *F. carica*+ vitamin C supplemented diet while the lowest (0.17µmol/g) was recorded in birds fed vitamin C supplemented diet.

3.3. Growth Hormone of broiler chicken fed *Ficus carica* and vitamin C supplemented diets

Growth hormone concentration of broiler chicken fed *Ficus carica* and vitamin C supplemented diets are as presented in Table 4. The result revealed that treatment effect was significant (P<0.05) for all parameters measured in response to the supplemented diets. Birds fed *F. carica* + vitamin C supplemented diet recorded the highest insulin (4.29µIU/mL), growth hormone (130.00ng/mL), insulin-like growth factor (30.24ng/mL),T3 (1.72ng/mL) and T4 (16.71ng/mL) concentration. The highest cortices (590.83nmol/L) was recorded in birds fed *F. carica* supplemented diet while birds fed *F. carica* + vitamin C supplemented diets recorded the lowest value for cortices (528.67nmol/L). e ratio of T3 and T4 was the same in birds fed vitamin C, *F. carica* + vitamin C supplemented diet and the control diet (0.10) while the lowest ratio (0.09) was recorded in birds fed *F. carica* supplemented diet.

Table 2 Brain Total Protein (g/dL) of broiler chicken fed *Ficus carica* and vitamin C supplemented diets

Parameters	Control	Vitamins C	*F. carica*	*F. carica* + Vit C	p-value
Whole Brain	0.50 ± 0.05^{b}	0.64 ± 0.02^{a}	0.59 ± 0.03^{ab}	0.64 ± 0.01^{a}	0.02*
Olfactory lobe	0.25 ± 0.02^{a}	0.17 ± 0.02^{b}	0.19 ± 0.01^{ab}	0.17 ± 0.03^{b}	0.04*
Pineal body	0.23 ± 0.02	0.21 ± 0.04	0.26 ± 0.02	0.25 ± 0.01	0.64
Optic lobe	0.23 ± 0.05	0.25 ± 0.05	0.18 ± 0.05	0.19 ± 0.03	0.66
Cerebellum	0.12 ± 0.00^{b}	0.11 ± 0.01^{b}	0.15 ± 0.01^{a}	0.17 ± 0.00^{a}	0.01*
Medulla	0.15 ± 0.01^{ab}	0.12 ± 0.02^{b}	0.16 ± 0.02^{ab}	0.18 ± 0.02^{a}	0.12

Table 3 Hypophyseal Acetylcholinesterase (AChE) (µmol/g tissue) of broiler chicken fed *Ficus carica* and vitamin c supplemented diets

Parameters	Control	Vitamins C	Carica	Carica + Vit C	p-value
Olfactory lobe (µmol/g tissue)	0.32 ± 0.06^{a}	0.15 ± 0.02^{b}	0.21 ± 0.04^{ab}	0.21 ± 0.03^{ab}	0.06
Pineal body (µmol/g tissue)	0.18 ± 0.01	0.17 ± 0.01	0.19 ± 0.01	0.21 ± 0.03	0.31
Optic lobe (µmol/g tissue)	0.36 ± 0.08^{a}	0.16 ± 0.02^{b}	0.17 ± 0.02^{b}	0.21 ± 0.04^{b}	0.02*
Cerebellum (µmol/g tissue)	0.22 ± 0.00^{a}	0.19 ± 0.01^{b}	0.19 ± 0.01^{b}	0.19 ± 0.01^{b}	0.04*
Medulla (µmol/g tissue)	0.25 ± 0.02^{ab}	0.20 ± 0.02^{b}	0.27 ± 0.03^{a}	0.20 ± 0.01^{b}	0.05*

Table 4 Growth Hormone of broiler chicken fed *Ficus carica* and vitamin C supplemented diets

Parameters	**Control**	**Vitamins C**	**Carica**	**Carica + Vit C**	**p-value**
Cortices (nmol/L)	529.17 ± 4.72^{b}	567.83 ± 11.67^{ab}	590.83 ± 28.45^{a}	528.67 ± 3.41^{b}	0.02*
Insulin (μIU/mL)	4.10 ± 0.15^{ab}	3.88 ± 0.07^{b}	3.75 ± 0.09^{b}	4.29 ± 0.18^{a}	0.04*
Growth hormone (ng/mL)	125.33 ± 3.07^{ab}	117.33 ± 4.45^{bc}	114.83 ± 3.36^{c}	130.00 ± 1.91^{a}	0.01*
IGF(ng/mL)	29.78 ± 0.49^{a}	27.20 ± 0.76^{b}	26.10 ± 1.34^{b}	30.24 ± 0.17^{a}	0.01*
T3(ng/mL)	1.70 ± 0.03^{ab}	1.60 ± 0.04^{bc}	1.53 ± 0.04^{c}	1.72 ± 0.03^{a}	0.01*
T4(ng/mL)	16.70 ± 0.02^{a}	16.56 ± 0.11^{ab}	16.33 ± 0.19^{b}	16.71 ± 0.03^{a}	0.07
T3:T4	0.10 ± 0.00^{a}	0.10 ± 0.00^{b}	0.09 ± 0.00^{b}	0.10 ± 0.00^{a}	0.01*

4. Discussion

4.1. Hypophyseal Acetylcholinesterase of the Broiler Chicken Fed *Ficus carica* and Vitamin C Supplementations

The primary enzyme responsible for the hydrolytic metabolism of the neurotransmitter acetylcholine (AChE) into choline and acetate is acetylcholinesterase (AChE). It plays a vital function in the central nervous system (CNS) and also implicated in behavioural, learning and memory as well as neurodegenerative diseases. Both abnormal increase and decrease in the AchE concentrations have negative impact on the brain. Excessive accumulation of acetylcholinesterase at the neuromuscular junctions and synapses was explained to cause symptoms of both muscarinic and nicotinic toxicity [18]. These include cramps, increased salivation, lacrimation, muscular weakness, paralysis, muscular fasciculation, diarrhea, and blurry vision. Conversely, a low level of acetylcholinesterase has been associated with memory impairment and brain disorders in humans but contributes to behavioral and learning deficits in animals.

The significant increase observed in the acetylcholinesterase activities of the same regions of the brain among the broiler fed diets containing *Ficus carica* without vitamin C in the present study could be attributed to the fact that *Ficus carica* could cause an overstimulation of the AChE, and thereby leading to high concentration of AChE in the brains of the broiler which may eventually cause brain disorders. The data obtained from the broilers fed diets with vitamin C in this study showed that the acetylcholinesterase activity was reduced when compared with broilers fed diet without vitamin C. It can, therefore, be concluded that vitamin C inhibited the action of the elevated acetylcholinesterase. This action could promote cholinergic neurotransmission and thus availability of acetylcholine at the synapse [19]. The insignificant effect in the AChE activities of the pineal body of the broiler chickens showed that dietary *Ficus carica* and vitamin C at the supplemental levels as well as the interaction of the two treatments and levels did not interfere with melatonin production thereby did not affect negatively the modulation of sleeping pattern and behavior of the birds [20]. Moreso, the significant effect of the activities of the AChE of the olfactory lobes of the broilers indicated that their sense of smell was not also negatively affected by the interactions which are attributed to the fact that birds have the least sense of smell and do not use smell extensively to make most of their decisions [21].

4.2. Growth Hormones of the Broiler Chicken Fed *Ficus carica* and Vitamin C Supplementations

Hormones secreted by the bird's thyroid gland, like mammals is effective to control metabolism as well as growth of body tissues [22]. Previous studies indicated that normal level of thyroid hormones indirectly affected bird's growth, due to the stimulatory effect of growth factors such as insulin like growth factor [23]. Based on the results of different studies, usage of *Ficus carica* and vitamin C in broiler's diet improved feed conversion and increased weight of broilers [24]. The results of this study showed that the amount of *Ficus carica* used in this experiment has effect on thyroid hormones concentrations. Cold weather is considered as the most important factor in stimulating the production of thyroid hormones as documented by [25]. More than 90% of thyroid hormones produced by the thyroid gland of chickens are T4 and T3. The amount of *Ficus carica* and vitamin C used in this study had significant effect on blood T3. Previously researchers reported that T3 hormone is less influenced by appetite stimulant and growth [23]. However, usage of vitamin C significantly increased blood T4 compared to the *Ficus carica* group. It seemed that increased effect of vitamin C used in this study on production and secretion of T4 was due to the positive impact on increased digestibility and nutrient absorption in the digestive tract and increase of nitrogen retention materials [26]. Previous investigations indicated that under optimum condition of the digestion and absorption of nutrients for synthesis of thyroid hormones in poultry diet, thyroid gland is more successful in T4 than T3 production.

Level of growth hormone directly led to the growth of bones, muscles and other body tissues and indirectly improved bird's growth by stimulating the production and secretion of somatomed such as Insulin-Like growth factor 1 (IGF-1) especially from the liver tissue [27]. Biological traits such as growth hormone secretion are influenced by environmental factors. Since growth hormone gene expression is the first stage in the process of protein synthesis, therefore, molecular studies in this stage of protein synthesis could provide useful information about the effect of different factors on growth hormone synthesis.

The result showed that the *Ficus carica* and vitamin C supplementation used in this study has effect on growth hormone. According to the studies carried out by other researchers, *Ficus carica* and vitamin C usage, which improved digestion and absorption of nutrients in birds, could play an important role on the biological factors associated with growth [28]. T4 is considered as an effective factor on bird's growth [29]. It seems that in this study significant increase in T4 concentration relate to growth hormone.

4.3. Brain Total Protein of the Broiler Chicken Fed *Ficus carica* and vitamin C Supplemented diets

The significantly lower concentration of total protein in the total brain, olfactory lobe and cerebellum of birds fed diets containing *Ficus carica* and vitamin C is indicative of the interference of dietary *Ficus carica* and vitamin C with neural mechanisms involved with protein synthesis. The insignificant decrease in the total protein concentration in the medulla of the birds confirms the possibility of the interference of *Ficus carica* and vitamin C with protein synthesis in some brain sections. The significantly lower concentrations of total protein (TP) in the various brain parts of the broiler chicken are indicative that genetic makeup could play a main role in the neural mechanisms involved with protein synthesis. This means the genetic makeup of the broiler chicken could possibly favour and enhances the neural mechanisms in protein synthesis. The significant increase in the TP observed in the supplementation with vitamin C was indicative of the antioxidant role of vitamin C against possible oxidative stress which might have been induced in the various brain regions of the broilers fed *Ficus carica* diets without vitamin C inclusion. The significant differences observed in the combination of the two treatments (*Ficus carica* and vitamin C), clearly indicated that their interactions also affected protein synthesis in each of the brain parts of the broiler chickens fed the treatment diets.

5. Conclusion

Based on the findings of this research, it was concluded that the supplementation of diet with *F. carica* and Vitamin C improved hypophyseal acetylcholinesterase activities and growth hormones concentration of broiler chickens while vitamin C supplemented diet improved the total protein concentration of the various parts of the brain examined. Thus, *F. carica* and vitamin C could be used as herbal supplements for broiler chicken.

Compliance with ethical standards

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Disclosure of conflict of interest

There was no conflict of interest in the course of carrying out this study

Statement of ethical approval

The Research and Ethics Committee of the Department of Animal Production and Health, The Federal University of Technology, Akure, Nigeria, accepted the experiment's requirements and criteria for animal and animal protocol.

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