INTERNATIONAL JOURNAL OF INNOVATIVE RESEARCH & DEVELOPMENT

ISSN 2278 – 0211 (Online)

Effects of Boiled Gacinia Kola Seeds on Growth Performance, Haematology and Lipid Profile of Broiler Chickens

H. A. Ibekwe
Department of Animal Science, Cross River University of Technology, Obubra Campus, Nigeria
F. B. Agiop
Department of Animal Science, Cross River University of Technology, Obubra Campus, Nigeria

Abstract:
A study designed to investigate the effects of Garcinia kola seeds, on growth performance, haematology and lipid profile of broiler chicken was conducted in Cross River University of Technology (CRUTECH). Obubra Campus poultry farm. Thirty (30) broiler chickens of Anak 2000 breed were assigned into three treatments of ten birds each. Treatment one (control) received 0:00g of Garcinia kola seeds to basal feed. Treatments two and three were given 10:90g and 20:80g of the same feed respectively. The growth performance index of live body weight was taken weekly with coagulated and uncoagulated blood samples collected after a 24 hour overnight fast of the experimental birds. The coagulated blood sample was used for assay of lipid profile while the uncoagulated was used for haematology. The result obtained showed a significant (P<0.05) decrease in body weight of broilers treated with boiled Garcinia kola seeds whereas no significant(P>0.05) changes (increase or decrease) in haematology was observed at both 10:90 and 20"80g levels of inclusion of Garcinia kola seeds relative to control treatment and also between themselves. There were however, significant (P<0.05) reductions in serum levels of total cholesterol and low-density lipoprotein cholesterol in 10g inclusion of boiled G. kola seeds relative to control treatment. The 20g inclusion on the other hand produced a significant reduction in low density lipoprotein (LDL) cholesterol but not in serum level of total cholesterol. It was observed that both treatment (10g & 20g inclusions of boiled G. kola seeds) caused significant elevation in serum levels of high density lipoprotein cholesterol (HDL) but no significant difference between themselves. The serum triacylglycerol concentration was only significantly reduced at 20g inclusion of boiled G. kola seeds relative to control and 10g inclusions. The positive response to serum lipid can be utilized in lean broiler meat production whereas its non-detrimental effects to live body weight and haemotology of broilers lends credence to report in literatures that heat application can be utilized in deactivation of antinutritional factors present in raw G. Kola seeds.

Keywords: *boiled gercinia kola, growth performance, haematology, lipid profile, broilers*

1. Introduction

Garcinia kola (G. kola) popularly known as bitter cola belongs to plant family known as Guttiferaece (plowden, 1992). It is a medium size plant growing up to 12 meters tall and 1.5meters wide. It is usually found in the rainforest zone of Nigeria and it is widely distributed throughout West and Central Africa. Plant food for animal nutrition contains anti-nutritional factor which makes it difficult for the nutrient to be available to the animal. Plant protein such as soy beans contains trypsin inhibitor which limits the availability of plant protein contained in soy beans (Smith 1990). Cotton seed is a plant protein for poultry but contains gospel that is poisonous to birds if consumed raw in appreciable quantity (Smith, 1990). Species of the grass genus setaria contains high oxalate content (Williamson and Payne, 1978). Garcinia kola seed is reputable for its medicinal uses. The stem bark has been shown to contain a complex mixture of phenolic compounds such as biflavonoides, xanthones and benzophenones (Iwu and Igboko, 1982) reported its antimicrobial activity as Kolanone whereas Iwu (1993) made the same observation with Garcinia flavonone and kola flavanone. The seeds are used in trado-medicine for the treatment of cough, asthma, diarrhaea, intestinal colic and gastroenteritis (Braide, 1989). Further studies revealed antihepatotoxic effects against experimentally induced hepatotoxins (Iwu, 1985, Akintowa and Essien, 1990, Braide, 1991).

However, some deleterious reports have been made on this all-important seed. The presence of tannins and guttiterins in the seed of G. kola was reported by Etkin (1981). Ebana et al (1991) reported the presence of cardiac glycosides

(bitter principles) in G. kola seed. Chronic ingestion of 10% G. kola seed powder induced histopathological changes in liver parenchyma cells, renal tubular epithelium and duodenal villi epithelium (Braide, 1990; Braide and Grill, 1990). Adedeji *et al* (2008) reported that bitter kola did not cause any deleterious effects to the birds even at 80g/kg of diet. Adedeji *et al*(2006) obtained highest feed efficiency from broiler chicks fed 25g/kg diet of feed. Osifo *et al* (2011) observed significant ($P<0.05$) reduction in body weight of rabbit administered with 1500mg/kg and 1800mg/kg suspension of dried bitter kola relative to control. He however did not observe any significant change in body weight of rabbit administered 1200mg diet suspend and below relative to control experiment. Aliyu and Mundi (2013) observed no significant change in final body weight of broilers exposer to 10g/kg weight of diet or basal feed. The cardiovascular health of many animals is always carried out by examining the lipid profile of the animal. Ibekwe *et al*(2008) reported a significant ($P<0.05$) reduction in total cholesterol and low-density lipoprotein of albino wistart rats treated with crude flavonoid extract of Garcinia kola. But no significant decrease in triacylglycerol relative to control experiment. Haematological indices are examined to ascertain the general state of health of the experimental animal or draw conclusion with respect to the treatment administered to the experimental animal. Ibekwe *et al.*(2009) observed a non-significant ($P>0.05$) changes in haematological indices of broiler chicks exposed to different doses of aqueous extract of Garcinia kola. The literature so far reviewed concentrated more on seed extract, dried seed powered or fresh seed with little or death of information on boiled seed on body weight of animal and other parameters. This research is therefore; set to investigate the effects of boiled Garcinia kola seeds on growth performance, haematology and lipid profile of broiler chickens.

2. Material and Methods

Preparation of Garcinia kola boiled diet. Peeled Garcinia kola seeds weighing 1000g were subjected to heat treatment by pouring them into boiling water at 100^{o}Cfor10 minutes. The seeds were removed from the boiling water and sliced with sharp kitchen knife to increase the surface area of the seeds exposed to drying. The drying was carried out in an electric oven (Gallen Kamp grade U.S.A). The dried seeds were ground to powder by a motor powdered milling machine. The Garcinia kola seed diet was prepared by mixing 10grams of boiled Garcinia kola seed powder with 90grams of basal feed (Boiler starter and Broiler finisher) phase of broiler production and used as treatment two. Treatment three was prepared by mixing 20grams of boiled dried Garcinia kola seeds with 80grams of basal feed (vital broiler starter and finisher) for starter and finisher phases of broiler production. These proportions were maintained even as the birds were growing to maturity.

2.1. Animal and Animal Treatment

A total of 30 broiler chicks of Anak, 2000 strain were assigned into three treatments of 10 birds each. The pens were properly demarcated to prevent birds mixing themselves together. The actual treatment commenced 3 weeks after brooding. Treatment group one received 0.0 gram of Garcinia kola seed powder and 100.0 grams of vital starter and finisher mash. Treatment group two received 10.0 grams of boiled dried Garcinia kola seed powder and 90.0 grams of vital starter and finisher mash. Treatment three receives 20.0 grams of boiled dried Garcinia kola seed powder and 80.0 grams of vital starter and finisher mash. Weekly live body weights of birds were taken for the 4 weeks the treatment lasted. The treatment was terminated with a 24hour overnight fast prior to collection of blood sample.

Collection of blood uncoagulated sample tubes containing ethylene diamine tetra active acid (EDTA) were prepared for blood sample collection for haematological indices examination. Another 30 sample tubes without anticoagulants were prepared for blood sample collection for lipid analysis. A 5.0ml syringe and needles was used to collect the blood through a venipuncture at the external jugular vein of the neck region. A 2½ml into the tubes without anticoagulant.

2.2. Determination of red Blood Cell Count

This was carried out using autohaematology analyzer (BC-22600) of Shenzhen mind ray Biomedical Electronic company China. Pre-diluted sample were used for the determination of red cell count. A 20µliter of capillary blood sample was diluted by 1.6ml of diluent. This was presented to the sample probe by pressing the aspirator key which aspirated 0.7ml of the sample into the analyzer. The counting proper was monitored by an inbuilt device in the autohaematology unit consist of metering tube with two optical sensors mounted on it. This tube ensures that a precise amount of diluted sample is measured during each count cycle. The exact amount is determined by the distance between the two optimal sensors. The count cycle starts when the meniscus reaches the upper sensor and stopped when the meniscus reaches the lower sensor. The number counted were expressed in 10^{12}/L.

2.3. Determination of Haemoglobin Concentration

A 1.6ml of diluent thoroughly mixed and aspirated was bubbled mixed with certain amount of lyse which converts haemoglobin to haemoglobin complex that is measurable at 525nm. The haemoglobin concentration was expressed in g/dl using the colorimetric method.

$$\text{Haemoglobin (HGB) g/dl} - \text{constant} \times \text{Log}_{10}\frac{\text{(Blank Photocurrent)}}{\text{Sample Photocurrent}}$$

2.4. Determination of Parked Cell Volume (PCV).

This was measured with the QBC II centrifugal haematology system of Becton (Dicinson C. U.S.A.)

2.5. Assy of Serum Lipid Profile

- Estimation of total cholesterol
  Total cholesterol in serum was estimated by Chop-PAP method of Richmond 1973. This method was based on the understanding that cholesterol esterase catalysis the hydrolysis of cholesterol esters into free cholesterol and fatty acids
- Estimation of triacylglycerol
  This was carried out by GPO-PAP method of Trinder (1969). Triacylglycerols were determined after enzymatic hydrolysis with lipase. The indicator is quininemine formed from hydrogen peroxide, 4 - aminophenazone and 4 – chlorophenol under catalytic influence of peroxidases
- Determination of high density lipoprotein cholesterol. This was done according CHOP-PAP method of Richmond (1973). The same procedure as that of total cholesterol estimation was employed.
- Estimation of low density lipoprotein cholesterol LDL. By the Friedwalds relationship (Friedwald *etal.*1972) low density lipoprotein cholesterol is derived from the difference between the total serum cholesterol and the sum of high density lipoprotein cholesterol with very low-density lipoprotein cholesterol
- Estimation of verylow-density lipoprotein cholesterol.
  The verylow-density lipoprotein cholesterol was obtained by dividing the serum triacylglycerol value by 5. This relationship is based on the fact that the ratio of triacylglycerol and very low-density lipoprotein in human serum is fixed relatively at 1:5 in fasting subject with triacylglycerols concentration not exceeding 400mg/dL.
  Data collection and statistical analyses.This was done using one-way analysis of variance (ANOVA).

3. Results and Discussion

The result of this study as presented in table 1 showed non-significant($p>0.05$) difference in live weight of broiler chicks fed with 10g of boiled Garcinia kola seed powder/100g of basal feed and 20g of boiled Garcinia kola seed powder/100g of basal feed relative to control. This observation agrees with the report of Ibekwe *et al*(2010) on evaluation of growth performance and haematological response of broiler chicks to raw and boiled Garcinia kola seed diet where he reported a non-significant difference in live weight of broiler chicks fed with 10% boiled Garcinia kola seed diet relative to control. This is also in consonance with the report of Esiegwu and Undedibie (2009) who reported a non-significant difference in feed intake of broiler chicks that received 0,2.5, 5.0 and 7.5% Garcinia kola seed meal inclusions. They however reported a heavier liver organ weight and superior feed conversion ratio at 2.5% inclusion level but in raw Garcinia kola seed meal. Esiegwu *et al*(2012) reported a no treatment effect on body weight, feed intake, feed conversion ratio, weight gain, and egg quality indices of laying hen fed graded levels of Garcinia kola. This observation was however at variance with report of Adedeji *et al*(2008) who reported significant ($P<0.05$) increase in hen-day production and albumen weight in laying hens fed 10g of G. kola/trial that include 20g/kg diet, 40g/kg diet and 80g/kg weight of feed in addition to a control diet without bitter kola inclusion. The same author, Adedeji *et al*(2006[b])reported once growth performance in growth of rats fed different doses of bitter kola. Dada and Ikuerowo (2009) reported that fish fed 1g/kg diet of Garcinia kola ethanolic extract had best weight gain than those fed on control diet. All the above reports are at variance with the observation in the current study seeing that the study concentrated on boiled Garcinia kola seed. The two doses of G. kola in this study showed that the live weight of birds on 20g of boiled G. kola/100g of basal diet reduced the body weight more than 10g of boiled Garcinia kola diet. The decrease in live weight of birds may possible be explained from angle of substrate concentration on activities of enzymes. Where the substrate concentration continues to increase, utilization of active centres on the enzyme becomes maximal and no further increase in reaction rate (Verma 2006). This may lead to constant weight or decreased weight in body weight of experimental birds.

Final live weight (kg/bird)			
Birds Treated	**Control Txt 1** 0:100g inclusion level	**Txt 2** 10:90g inclusion level	**Txt 3** 20:80g Inclusion level
1	1.80	2.00	1.60
2.	2.00	2.50	2.60
3	1.60	1.90	1.90
4	2.40	1.80	1.50
5	2.20	2.00	2.00
6	2.00	2.50	1.90
7	1.80	1.90	2.00
8	2.20	2.00	1.50
9	2.40	1.80	2.60
10	1.60	2.00	1.60
Total	**2.00 ± 0.32^{a}**	**2.04 ± 0.27^{a}**	**1.92 ± 0.43^{a}**

Table 1: Effects Of Boiled Garcinia Kola Seeds On Live Weight Of Broiler Chicks
Mean is ± SD. Mean values on the same row with different superscripts are significantly (P<0.05) different

The haematological indices determined revealed non-significant (increase or decrease) changes for 10g boiled Garcinia kola seed/100g of basal feed and 20g of boiled Garcinia kola seed/100g of basal feed. This observation is in agreement with the report of Uko *et al*(2001) on no significant difference between blood samples from the control and experimental rats for haemoglobin, parked cell vol. (PCV), and red blood cell count (RBC). This was further supported by Adedeji *et al* (2005) reported no-significant difference in haematologial incises of broiler chicks placed on 0% (w/w), 5%, 10% and 20% basis of Garcinia kola seeds. This is also in consonance with the report of Ibekwe *et al*(2010) who reported no-significant difference in haematology of broiler chicks exposed to raw and boiled Garcinia kola seeds at 10% inclusion levels. Haematology indices assess general state of health of experimental birds or assess effect of treatment on the experimental animal. This is presented in table 2.

Hematological Parameters	Treatment Schedule		
	Control $T_1$0 :100g inclusion level	$T_2$10 :90g inclusion level	$T_3$20 :80g Inclusion level
PCV (%)	37.67±1.53[a]	36.67±1.15[a]	36.67±2.08[a]
Haemoglobin (g/dl)	12.67±37.042[a]	12.03±0.32[a]	12.13±0.58[a]
RBC (10^{12}/L)	4.10±0.20[a]	3.97±0.12[a]	3.97±0.29[a]

Table 2: Effect Of Boiled Garcinia Kola Seeds On Haematology Of Broiler Chicks
Mean is ± SD. Mean values on the same row with different superscripts are significantly (P<0.05) different

The lipid profile of broiler chicks fed with 10g of boiled G. kola seed/100g of basal diet and 20g of boiled G.kola seed/100g of basal feed is presented in table 3. The result showed a significant reduction in total cholesterol of birds fed 10g of boiled Garcinia kola seed/100g of basal seed relative to control but not with 20g of boiled Garcinia kola seed100g of basal feed. The antioxidant property of Garcinia kola seed as reported by Iranloye *et al* (2006) may be part of plausible explanation for the reduced serum total cholesterol produced by boiled Garcinia kola seeds. The presence of sterol in most plants including Garcinia kola probably may be part of explanation to the reduced total cholesterol observed in this study since sterols inhibit cholesterol and bile acid absorption in animals. They can have appreciable effects on low density lipoprotein (LDL) cholesterol levels even at relatively low intake (Heinemann *et al*., 1986). This report gives credence to the significant reduction in low density lipoprotein cholesterol observed in this study at both 10g of boiled Garcinia kola seed/100g of basal feed and 20g/100g of basal feed. There is however no significant difference in serum LDL between the two treatments. This was also supported by the report of Iranloye *et al* (2006) who reported that kola-viron, a biflavoniod of G. kola possesses antioxidant property. The serum high density lipoprotein (HDL) cholesterol is however significantly elevated in this study by both treatments without any significant difference between them. These treatments maintain the normal inverse lipoprotein relationship which exist between LDL and HDL. A good lipidemic agent is the one that lowers serum LDL while increasing or elevating the serum HDL FAO/WHO (1993) which was manifested by boiled Garcinia kolaseed at both levels of feeding. Seeding that most of the observation made in this study was either supported or disagreed by previous researchers who solely worked on raw G. kola seeds, one then asks what the essence of boiling the seed was. Heat treatment renders anti-nutritional factor or agents ineffective and makes bound protein available for the experimental birds (Smith, 1990). The absence of tannin in boiled G. kola seed was reported by Ibekwe *et al* (2007). Tannins and glycosides when hydrolyzed binds to almost any available protein making this nitrogenous source indigestible or unpalatable to birds maintained on such feed ingredients (Peter and Richard, 1999). Therefore, heat treatment was only to deactivate the anti-nutritional agents that may be present in the unconventional feed source.

Lipid Indices (MMOL/L)	Treatments		
	Control $T_1$0 :100g inclusion level	$T_2$10 :90g inclusion level	$T_3$20 :80g Inclusion level
Total Cholesterol	3.54±0.02[a]	2.59±0.04[b]	2.81±0.06[ab]
Triacylglycerol	0.70±37.042[a]	0.74±0.04[a]	0.53±0.02[b]
Low Density Lipoprotein (LDL)	1.61±0.04[a]	1.23±0.03[b]	1.11±0.11[b]
High Density Lipoprotein (HDL)	1.29±0.11[a]	1.45±0.09[b]	1.45±0.09[b]

Table 3: Effect Of Boiled Garcinia Kola Seeds On Lipid Profile Of Broiler Chicks
Mean is ± SD. Mean values on the same row with different superscripts are significantly (P<0.05) different

4. Conclusion

The positive response of boiled Garcinia kola seeds to lipid profile of broiler chicks in this study is manifest. The haematology and live weight of birds fed at the two levels of G. kola seed inclusions neither showed positive nor negative response due to non-significant changes observed. For maximum butritional benefits therefore, heat treatment (boiling) will not be out of place since heat application deactivates, anti-nutritional agents (tannins) commonly found in feed sources.

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