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EFFECT OF GRADED DOSES OF AQUEOUS EXTRACTION OF GARCINA KOLA SEEDS ON GROWTH PERFORMANCE AND HAEMATOLOGICAL RESPONSE OF BROILER CHICKS

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ABSTRACT
This study on the effects of graded doses of aqueous extract of *Garcina kola* seeds on growth performance and haematological response of broiler chicks was carried out to investigate the effects of different dosage regimen of the extract on growth performance and haematological indices of body homeostasis. A total of 30 broilers Chicks of anak 2000 strain were assigned into 3 treatment groups of ten birds each. Group A (control) received 0.0ml of aqueous extract of *Garcina kola* seeds / kg body mass of bird. Group B and C received1.0 and 2.0 ml extract of *Garcina kola* seeds per kilogram body mass of birds. The growth performance indices of feed consumption, live body mass were taken on weekly basis while dressed body mass and selected organs mass were taken at the end of experiment. Uncoagulated blood was collected 12 h post treatment. The result obtained showed a significant ($p<0.05$) dose dependent reduction in growth performance whereas there were no significant ($p<0.05$) changes in haematological indices of body homeostasis except for percentage parked cell volume, haemoglobin concentration, eosinophils, lymphocyte and monocyte (agranulocyte) counts which revealed a significant ($p<0.05$) increase at both doses. The above findings imply that aqueous extract of *Garcinia kola* seeds should not be used above 0.5ml (100mg/kg) body mass even for medicinal purposes in broilers.

KEY WORDS: graded doses, aqueous extract, *Garcinia kola* seeds, growth performance and haematological response.

INTRODUCTION

The poor economic status and relatively low cost of medicinal plant product have enhanced their patronage by traditional African society. *Garcina kola* seeds and their extract have enjoyed high reputation among trado-medical practitioners and listed medicinal plants in Africa. The detailed description and distribution in Africa have been documented (Iwu, 1993). *Garcina kola* stem bark has been shown to contain a complex mixture of phenolic compounds such as biflavonoides, xanthones and benzophenones (Iwu and Igboko, 1982). Husain *et al* (1982) reported its antimicrobial activity as kolanone whereas (Iwu, 1993) made the same observation with *Garciniaflvanone* and *kolaflavanone*. The seeds are used in trado-medicine for the treatment of cough, asthma, diarrhoea, intestinal colic and gastro-enteritis (Dalziel, 1956; Braide, 1989).

Further studies revealed antihepatotoxic effects against experimentally induced hepatotoxins (Iwu, 1985; Akintowa and Essien, 1990; Braide, 1991). Similarly a significant increase in both basal and histamine-mediated gastric acid secretion of albino rats were reported (Oluwole and Obatomi, 1992). The 16th International Botanical Congress in St. Louis USA (1999) revealed that *G. kola* compound is capable of halting the multiplication of deadly Ebola virus(Iwu,1999)..

However, some deleterious reports were made pertaining 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 principle) in *G. kola* seeds. Chronic ingestion of 10% of *G. kola* seeds powder induced histopathological changes inn live parenchyma cells, renal tubular epithelium and duodenal villi epithelium (Braide, 1990; Braide and Grill, 1990). Udoh (1998) reported adverse effects of *G. kola* seed on testicular tissue of which testicular atrophy, degeneration of spermatozog, reduced testicular weight and spematogenesis arrest were the most pronounced. Available reports (Uko *et al.* 2001;

Adediji *et al*, 2006) maintain no-significant changes in haematological parameters assayed except for lymphocyte and monocyte which showed a significant increase in number.

The report of Adedeji *et al* (2006) that pullets can tolerate (2-5)% inclusion levels of *Garcinia kola* seed in the feed without compromising the body mass calls for more clarification since birds are food (meat/egg) producing animal. The conflicting reports of this seed on body mass of animal when different concentration and dosages were used make this study imperative. Broiler enterprise is solely embarked upon for meat production purposes. Therefore any material intended for feed replacement or theraputic uses which compromise body mass and homeostasis must be handled with caution by determining the optimum dose range of administration.

This study is therefore set up to ascertain the effects of graded doses of aqueous extract of *Garcinia kola* seed on growth performance and haematological response of broiler chicks with the aim of determining the optimum dose range.

MATERILS AND METHODS:

i. Preparation of Garcinia kola seed powder and extract:

Garcinia kola seed weighing 1000grams were purchase from Marian market, Calaba in June 2004.. The seed were sun dried for 24 h to ease the removal of the testa. The seeds were sliced with sharp kitchen knife to increase the surface area of the seeds exposed to drying. The drying was consummated in an electric oven (Gallenkamp grade USA). The dried seeds were ground to powder by a motor powered milling machine.

Fifty grams of the powder was soaked in 250ml of clean water and left to stand for 48 h but was 250ml subjected to vigorous steering every 12 h interval. The fifty grams of *Garcinia kola* seed powder to250ml is equivalent to 1 gram in 5ml or 1000mg in 5ml of water. The mixture was filtered using a musclin cloth and the volume of filtrate and mass of residue recovered. The filtrate was evaporated to dryness weighed, and redissolve in 50ml of water ready for administration to animals.

ii. Animal and animal treatments:

A total of 30 broiler chicks anak 2000 strain were assigned into 3 treatment groups of 10 broilers each. Group A received 0.0ml aqueous extract of *Garcinia kola*. Group B and C received 1.0ml or 200mg/ml and 2.0ml or 400mg/ml of aqueous extract of *Garcinia kola* respectively. The extract was administered orally to each bird once a day using 5ml syringe without needle. The administration lasted for 4 weeks and live mass and feed consumption were recorded while the dressed mass and selected organ's mass were taken after the birds were slaughtered and dressed.

iii. Collection of blood Sample:

Blood was drawn from each broiler chick 12 hours post administration of extract by decapitation and carefully transferred into bijou sample bottles containing ethylenediamine tetra acetic acid (EDTA) anticoagulant. Haemoglobin concentration (Hbmg/ml) was determined by the cyanomethaemoglobin method using a Beckman model spectrophotometer. Erythrocytes (RBC) were counted with an improved Neuberger's haemcytometer. Total leucocyte (WBC) count and parked cell volume (PCV) were measured with the QBCII centrifugal haematology system (Becton Dickinson Co. USA). The differential leucocyte count was carried out by staining the blood smear with Leishman's stain and the number of each cell counted. The erythrocyte indices of mean corpuscular volume (MCV), mean corpuscular haemglobin (MCH)and mean corspucular haemoglobin concentration (MCHC) were calculated according to (Jain, 1986).

STATISTICAL ANALYSIS

The Student's T-test was used to compare the significance of differences between control and treated groups while analysis of variance was used to compare significance of difference among and within groups of treatments (Nwabuoike, 1986).

RESULTS

Table 1: Proximate composition of basal feed.

Proximate fraction	Percentage composition
Moisture content	10.50
Dry matter	89.59
Crude protein	10.00
Crude fibre	7.20
Ether extract	7.00
Crude Ash	1.20
Nitrogen free Extract	64.10
Metabolizable Energy	13.15KJ/g

Table 2: Proximate composition of *Garcinia kola* seed

Proximate Fraction	Percentage Composition
Moisture content	14.60
Dry matter	85.40
Crude protein	0.58
Crude fibre	0.10
Ether extract	3.00
Crude Ash	5.00
Nitrogen free Extract	76.72

Table 3: Mass and Yield of *Garcinia kola* seed.

Powder	50g
Residue	38g
Filtrate	12g
Extract	10g
Reconstitution	10g in 50ml of water
Concentration	200mg/ml

Table 4: Weekly Average feed intake (kg)

Treatment	A	B	C
Age (weeks)			
4	0.61	0.60	0.61
5	0.95	0.95	0.96
6	1.14	1.11	1.04
7	1.26	1.28	1.26
Mean	$0.99^{a}\pm0.28$	$0.99^{a}\pm0.29$	$0.97^{a}\pm0.27$

Treatments (A: 0.00ml; B: 1.00ml; C: 2.00ml)

The mean in the same row with different superscript differ significantly ($p<0.05$).

Table 5: Average final live Mass of birds.

Treatments		Body Mass
A	:	2.40±0.65[a]
B	:	2.10±0.55[b]
C	:	2.00±0.53[b]

Mean with the same row with different superscript differ significantly ($p<0.05$).

Table 6: Dressed and selected organ Mass (kg)

	Treatments		
Parameters	A	B	C
Dressed bird:	1.75±o.0.05[a]	1.36±0.01[b]	1.40±0.05[b]
Gizzard:	0.065±0.01[a]	0.065±5.0[a]	0.050±0.01[a]
Heart:	0.065±5.0[a]	0.050±0.01[a]	0.050±0.01[a]

Mean on the same row with different superscripts are significantly ($p<0.05$) different.

Table 7: Haematology

	Treatments		
parameters.	A	B	C
Hb conc. (g/100ml)	5.33±0.29[a]	5.40±0.31[a]	5.73±0.22[b]
PCV (%)	16.07±1.09	17.00±1.40	17.33±0.43
WBC ($\times 10^3/mm^3$)	6.09±0.09	6.091±0.09	7.09±0.09
ESR (mm/hr)	16.67±3.76[a]	10.55±1.70[b]	10.00±3.70[b]
RBC ($\times 10^6/mm^3$)	6.09±3.09	6.09±3.09	4.09±1.09
Neutrophils (%)	55.67±6.98[a]	53.67±3.67	39.67±1.67[b]
Lymphocytes (%)	43.67±7.20[a]	41.33±5.70[a]	52.67±3.71[b]
Eosinophils (%)	0.00±0.00[a]	2.33±0.88[b]	2.00±1.15[b]
Monocytes (%)	0.67±0.07[a]	2.67±1.20[b]	5.67±3.18[b]

Tables 1 and 2 showed the chemical composition of basal feed as well as *Garcinia kola* seeds. The *Garcinia kola* seed has very poor nutrient composition as can be seen in its crude protein, crude fibre and ether extract contents. This is an indication that this important medicinal plant may not be good in feed replacement trials.

Presented in Table 3 is the yield of *Garcinia kola* seed powder after soaking in water for 48hours. Fifty gram of powder from this seed was soaked in 250ml of water and filtered after 48hours harvesting 12 gram of filtrate and 38 grams of residue. The filtrate was subjected to evaporation in an oven which finally yielded 10 grams of aqueous extract. The extract was grounded to powder and reconstituted in 50ml of 10,000mg/50ml of water or 200mg/ml of water.

This concentration was used in this study in graded doses of 0.0ml for control (A), 1.0ml and 2.0ml for treatment B and C respectively.

Table 4 showed weekly feed intake of broiler chicks orally dosed with graded levels of aqueous extract of *Garcinia kola* seed. There was no significant ($p>0.05$) change in feed consumption of treated birds (0.99±0.29; 0.97±0.13)kg relative to control (0.99±0.28kg) birds. This method of administration circumvents the bitter taste and sticky nature of fresh seed of *Garcinia kola* which results to refusal and utilization of feed by birds.

Table 5 showed the final live mass in kg of broiler chicks orally fed with graded dose of aqueous extract of *Garcinia kola* seeds. There was a significant ($p<0.05$) reduction in live mass of treated (2.10±0.55; 2.00±.0.63) kg /birds when compared with the control (2.40±0.65kg).

Table 6 equally showed a significant ($p<0.05$) reduction in dressed body mass of birds treated with aqueous extract of *Garcinia kola* seed in relation to control treatment. There was however no-significant ($p>0.05$) changes in the mass of selected organs of these birds when compared with the control. This indicates that the action of *Garcinia kola* seed extract is more on the skeletal muscles than other soft tissues.

Presented in Table 7 are the haematological indices of homeostasis and their response to the graded doses of aqueous extract of *Garcinia kola* seeds. There were generally no-significant ($p>0.05$) changes in most of the haematological parameters assayed except for haemoglobin concentration (g/100ml) which increase significantly ($p<0.05$) at both doses and decrease in ESR, and Neutrophil. The agranulocytes increased significantly whereas there was no-significant ($p>0.05$) changes in total RBC and WBC counts relative to control birds.

DISCUSSION

Aqueous extract of *Garcinia kola* seed caused a significant ($p<0.05$) dose dependent reduction in body mass of birds. There was equally a significant ($p<0.05$) reduction in dressed body mass of birds though not dose dependent.
A plausible explanation to this finding may be due to presence of tannin an antinutrtional factor (Etkin 1981). Tannins when hydrolysed bind to almost any available protein making this nitrogenous source indigestible or unpalatable to birds feeding on such feed ingredient (Peter and Richard, 1999).

This observation agreed with the report of Uko *et al*. (2001) on depressive effect of *G. kola* seeds on appetite and water intake of wister rats administratered with 220mg/ml of *G. kola* seed extract. The findings in this study with respect to the body mass are in consonance with the report of Dairo and Ogunmodede (2000) on decreased live body mass, dressed body mass, thigh, drum stick and total protein of broiler chick fed with graded levels of fermented Copra meal. There was however no significant ($p>0.05$) changes in body mass with the birds which received the graded dose of aqueous extract of *Garcinia kola* seed.

The formation of metallic- flavonoid complex with iron by *Garcinia kola* seed (Siegenberg, 1991) slows the biosynthesis of haemoglobin and hence tissue oxidation is impaired for lack of oxygen. The poor proximate composition of G. kola seed (Ibekwe *et al*, 2007) revealed that the seed contains 0.58% crude protein, 0.10% crude fibre and 3.0% ether extract and may not likely improve the body mass of birds expose to them. The body mass findings of this study is however at variance with the findings of Adedeji *et al* (2006) who reported an improved body mass of pullet fed with (2-5)% graded doses of *G. kola* seeds. The (2-5)% used by Adedeji and co is however below 10% or (100mg/ml) inclusion levels recommended by (Braide and Grill, 1990) above which there may be decrease in body mass of animal receiving the seed. The dosage used in this study is far more than 10% and hence justifying the reduction in body masses of birds administered with 200mg/ml and 400mg/ml of the extract of *Garcinia kola* seeds. The no – significant changes in most of the parameters monitored within treated groups in this study lend credence to non-toxic property of *Garcinia kola* seeds. This implies that any dose above the optimum causes a decrease in parameters with little or no toxic effect on doubling such doses. The no- significant changes in hematological indices of homeostasis as observed in this study could not be attributed soley to treatment

effect since even repeated evaluation of these parameters in the same treatment will show such variation. These differences are normal with such parameters (Conley, 1974). The findings of this study are at variance with the direct variation existing among total erythrocyte count, haemoglobin concentration and parked cell volume (Schalm *et al*, 1975) as there was no – significant changes in erythrocyte count. This may be attributed to the complexing power of flavonoid with metal like iron, zinc and Copper thereby making them unavailable (Siegenberg, 1991). Therefore since iron is needed for synthesis of erythrocyte it becomes difficult to have increased number of erythrocyte where there is deficiency of iron.

The observed significant ($p<0.05$) increase in agranulocyte (lymphocyte and Monocyte) count concurred with the findings of Uko *et al*, (2001) who reported a significant proliferation of total leucocyte counts in blood plasma of experimental rats treated with 220mg/ml of *G. kola* seed extract. This also lends credence to anti-inflammatory and immune booster properties of flavonoid extract of *G. kola* seed previously reported by (Mascolo *et al*, 1987).

Having observed no – significant changes in most parameters within the birds treated with graded dose of *G. kola* seeds one may therefore be correct to say that graded dosage is very necessary where optimum dosage (100mg/ml) is not exceeded in order to achieve optimum result.

CONCLUSION

Higher doses of *G. kola* seed extract always cause significant ($p<0.05$) body mass decrease irrespective of therapeutic benefits. Broiler enterprise is geared toward meat production and so graded doses may be employed to arrive at the optimum dose range with maximum therapeutic benefits. Doses higher than the optimum will always result in diminished alteration in haematological indices of growth performance and body haemeostasis and hence should be avoided in broiler production even when used for medicinal purposes. Dosage regimen not exceeding 10% level of powder in the feed or 100mg/ml of extract per kilogram body mass of birds is recommended for medicinal purposes in broiler production.

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