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EFFECT OF GRADED DIETARY LEVELS OF *GARCINIA KOLA* SEED MEAL ON PERFORMANCE, INTESTINAL MICROBIAL LOAD, HAEMATOLOGICAL AND SERUM BIOCHEMICAL PROFILE OF RABBITS.

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ABSTRACT
Effect of dietary Garcinia kola seed meal (GKSM) (bitter kola) on the performance, microbial activities and blood constituents of rabbits were investigated. Four rabbit grower mash were prepared such that diet T_0 (control) contained no Garcinia kola seed meal while diets T_1, T_2 and T_3 contained 2.5%, 5.0% and 7.5% Garcinia kola seed meal, respectively, partly replacing maize. Each diet was fed to a group of 9 grower rabbits for 56 days, in completely randomized design (CRD) experiment. Each group was further subdivided into three replicates of 3 rabbits each. Faecal samples were collected from four rabbits per treatment at the first and last weeks of the experiment and used to determine intestinal microbial load of the rabbits. At the end of the feeding trial, blood samples were randomly collected from four rabbits per treatment and used to determine heamatological and serum biochemical profile of the rabbits. Body weight gain, daily body weight gain and feed conversion ratio (g feed/g gain) of the groups on GKSM diets decreased significantly ($P < 0.05$) while the feed intake increased ($P < 0.05$). GKSM had no significant effects ($P<0.05$) on the parasite, cysts of Isospora belli, but significantly eradicated Salmonella and Streptococcus species. Haematological indices (RBC, HB, PCV, MCV, MCHC, MCH, WBC and platelets) were not affected by the treatments ($P > 0.05$). There were no traces of eosinophils, basophils and monocytes. Biochemical indices (serum cholesterol, serum protein, serum albumin, serum globulin) were also not significantly affected ($P > 0.05$) by the treatments. The glucose levels of GKSM fed groups were significantly ($P < 0.05$) reduced. The electrolytes (potassium, sodium and chloride) as well as calcium and bicarbonate levels were not affected by the treatments ($P > 0.05$). Cost of production (₦/Kg gain) was highly increased with increase in dietary GKSM.

Key words: Diets, *Garcinia kola*, grower rabbits, performance.

INTRODUCTION
There is no doubt that the costs of providing feed and drugs in livestock and poultry industry in Nigeria are steadily increasing. These costs affect the final consumers of the products. South-eastern agro-ecological zone of Nigeria is highly endowed with plants possessing nutritional and medicinal properties. It has therefore become necessary to investigate such plants for possibilities of incorporating their leaves or seeds in livestock and poultry feeds to serve as feed ingredients and/or prophylactic agents. Earlier studies at our station have shown that leaves of *Alchornia cordifolio* (Udedibie and Opara, 1998) and *Azadirchta indica* (Esonu *et al.*, 2005; Obikaonu, 2009) could be of value in poultry diets.

Garcinia kola, Heckel, commonly called bitter kola is an indigenous medicinal tree belonging to the family, *guttiferae*. It is highly valued because of its medicinal properties (Hertog *et al.*, 1993). The seeds are chewed as an aphrodisiac or used to cure cough, dysentery or chest cold in herbal medicine (Irvine, 1961). Phytochemical studies have shown that the seed constituents include biflavoids, xanthones and benzophenones and which have anti-microbial, anti-inflammatory, anti-bacterial and anti-viral properties (Akoachere *et al.*, 2002). Esiegwu and Udedibie (2009) reported that broilers placed on diets containing *Garcinia kola* seed meal produced heavier body weight and superior feed conversion ratio at 2.5% dietary level. Adedeji *et*

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al. (2008) also reported that it improved egg production performance of laying hens. However, no information is available on its nutritional value for rabbits although Uko *et al.* (2001) reported that its water extract had depressive effect on feed intake of rats.

The study herein reported was therefore designed to determine the effects of graded levels of *Garcinia kola* seed meal on the performance, intestinal microbial load and blood constituents of grower rabbits.

MATERIALS AND METHODS

Source and processing of *Garcinia kola seeds*

The *Garcinia kola* seeds used in the study were bought from Omuma market in Oru East Local Government Area of Imo State, Nigeria. The seeds were cut into small pieces and sun-dried until they became crispy. They were then milled in a hammer mill with 2mm sieve to produce *Garcinia kola* seed meal (GKSM). Samples of the meal were subjected to proximate and mineral analysis according to AOAC (1990).

Experimental diets

Four grower rabbit diets were compounded, incorporating *Garcinia kola* seed meal at 0%, 2.5%, 5.0% and 7.5% inclusion levels, partly replacing maize. The diets were designated T_0, T_1, T_2 and T_3, respectively, with T_o serving as the control. The ingredient and nutrient compositions of the diets are shown in Table 1.

Experimental rabbits and design

Thirty-six eight weeks old grower rabbits of mixed breeds were randomly divided into four groups of nine rabbits each which were randomly assigned to one of the four experimental diets, using completely randomized design (CRD). Each group was further sub-divided into three replicates of three (3) rabbits each. The rabbits were individually housed in 0.7m x 1.0m hutches. Feed and water were supplied *ad libitum* in separate earthen troughs. The trial lasted for 8 weeks (56 days).

Data collection and analysis

The rabbits were weighed at the beginning of the experiment to obtain their initial body weights and then weekly thereafter. Daily feed intake was determined by subtracting the weight of left-over feed from the weight of the feed fed the previous day. Feed conversion ratio was determined by dividing average daily feed intake by average daily body weight gain. Cost of production was determined by multiplying cost of feed (₦/kg) by feed conversion ratio (kg feed/kg gain).

Microbial studies

Faecal samples were collected from 4 rabbits per treatment at the first and last weeks of the experiment and analysed for presence of parasites. The faecal samples were cultured according to the method of Monica (2000) for presence of *Salmonella specie, Escherichia coli, Streptococcus specie and Staphylococcus aureus*.

Haematological and Serum Biochemical Analysis

Blood samples were collected at the end of the 56-day feeding trial from 4 rabbits per treatment through neck slitting and used for determination of haematological/serum biochemical indices. Blood was collected from each rabbit and about 2 ml of each sample was put into well labeled sterilized bijon bottles containing EDTA as anti-coagulant, while another 6 ml was put in bottles without EDTA to produce serum for determination of serum biochemical indices. Blood samples were analysed within three hours of collection for haemoglobin (Hb) level, white blood cell (WBC), red blood cell (RBC), packed cell volume (PCV), platelets, and differential white cell counts (percentage neutrophils, basophils, eosinophils, lymphocytes and monocytes) using standard methods (Monica, 1984). Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated. Serum biochemical indices analysed included blood glucose, serum total protein, globulin, albumin, serum cholesterol, sodium, potassium, calcium, chloride and bicarbonate.

RESULTS AND DISCUSSION

Proximate and mineral composition.

The proximate and mineral compositions of *Garcinia kola* seeds used for the experiment are shown in Table 2.

With the exception of crude fiber, which was 20.51%, other values (crude protein, ether extract and ash) were in agreement with the values earlier reported by Ibekwe (2007). The values for Mn, Mg, Ca and K were close to the values of 2.01 mg/100g for Mn, 0.42 mg/100g for Mg, 0.80 mg/100g for Ca and 2.50 mg/100g for K respectively, reported by Okwu (2005). The value for Zn (0.03mg/g) tended to agree with the values of 2.30 mg/100g and 2.30 mg/kg reported by Okwu (2005) and Odebunmi *et al.* (2009), respectively. It is therefore more of a source of carbohydrates

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Table 1: Ingredient composition of the experimental diets.

	Dietary levels of GKSM			
Ingredients (%)	T_0 (0.00%)	T_1(2.5%)	T_2 (5.0%)	T_3 (7.5%)
Maize	47.00	44.50	42.00	39.50
GKSM	0.00	2.50	5.00	7.50
Soya bean meal	9.00	9.00	9.00	9.00
Fishmeal	2.00	2.00	2.00	2.00
Blood meal	1.00	1.00	1.50	1.50
Wheat offal	26.00	26.00	25.50	25.50
Palm kernel cake	11.00	11.00	11.00	11.00
Bone meal	3.00	3.00	3.00	3.00
Common salt	0.25	0.25	0.25	0.25
Vit./Tm premix*	0.25	0.25	0.25	0.25
L-lysine	0.25	0.25	0.25	0.25
L-methionine	0.25	0.25	0.25	0.25
Calculated Chemical Composition (% of DM)				
Cp	16.31	16.15	15.99	15.84
CF	5.71	6.15	6.60	7.05
EE	4.47	4.61	4.74	4.88
Ash	3.72	3.72	3.71	3.70
NFE	69.79	69.37	68.96	68.53
Ca	1.24	1.24	1.24	1.24
P	1.12	1.11	1.10	1.09
Lysine	1.03	1.03	1.02	1.01
Methionine,	0.55	0.54	0.54	0.53
ME (mcal/kg)	2.54	2.53	2.51	2.50

*Provided the following per kilogram of diet: Vit. A, 10,000 iu; Vit. D_3, 2000 iu; Vit. E, 5 iu; Vit. K, 2 mg; riboflavin, 4.20 mg; Vit. B_{12}, 0.01 mg; panthotenic acid, 5 mg; nicotinic acid, 20 mg; folic acid, 0.5 mg; chloride, 3 mg; Mg, 56 mg; Fe, 20 mg; Cu, 10 mg; Zn, 50 mg; Co, 125 mg.

Table 2: Proximate and mineral composition of *Garcinia kola* seeds (DM basis)

Proximate composition (% of DM)	
Dry matter	92.70
Crude protein	2.64
Crude fibre	20.51
Ether extract	9.47
Ash	1.07
Nitrogen free extract	57.54
Macro elements (mg/g)	
Magnesium	0.28
Calcium	0.25
Potassium	1.61
Phosphorus	0.057
Sodium	0.282
Chlorine	0.400
Sulphur	0.073
Micro elements (mg/g)	
Fe	0.21
Zinc	0.03
Cupper	0.52
Manganese	0.12

Performance of the Experimental Rabbits

Data on the performance of the experimental rabbits are presented in Table 3.

Body weight gain, daily body weight gain and feed conversion ratio of the groups on GKSM diets were significantly ($P < 0.05$) reduced. These findings corroborate the report of Uko *et al.* (2001) that water extract from *Garcinia kola* caused poor feed utilization efficiency and weight gain of rats. The poor performance of the rabbits on GKSM diets could be attributed to the presence of anti-nutritional factors in *Garcinia kola*, especially tannin (Esiegwu and Udedibie, 2009). Condensed tannin from the browse plant *Robin's Pseudoacacia* has been reported to cause reduced growth and coprophagy in rabbits (Raharjo *et al.*, 1990) and reduced protein digestibility in rats (Horigone *et al*., 1988). Lebas *et al*. (1997) reported that through coprophagy, rabbits obtain a significant amount of water soluble vitamins, up to 20% of its crude protein (CP) requirement, 30% of its energy requirements as volatile fatty acids (VFA) and 18% of its dry matter intake. Feed intake of the rabbits, however, was significantly ($P < 0.05$) increased by *Garcinia kola* seed meal. This finding is in agreement with the report of Esiegwu and Udedibie (2009) on broilers fed graded levels of *Garcinia kola* seed meal but contradicts that of Uko *et al.* (2001) that rats fed water extract from *Garcinia kola* hard depressed appetite and water intake. Feed costs increased as dietary levels of *Garcinia kola seed* meal increased due to the high cost of the test material. This invariably resulted in very high cost of production of the rabbits as the dietary levels of the meal increased.

Table 3: Performance of grower rabbits fed graded levels of *Garcinia kola* seed meal

Parameters	Dietary levels of *GKSM* (%) T_0 (0.0%)	T_1(2.5%)	T_2 (5.0%)	T_3 (7.5%)	SEM
Initial body wt. (g)	566.57	577.78	555.56	533.33	14.72
Av. final body wt. (g)	1864.47^{a}	1609.18^{b}	1710.26^{ab}	1664.7	36.56
Av. body wt gain (g)	1297.80^{a}	1031.40^{c}	1154.70^{b}	1131.30^{bc}	32.14
Av. daily body wt. gain (g)	14.42^{a}	11.46^{c}	12.83^{b}	12.57^{bc}	0.36
Av. daily feed intake (g)	64.99^{b}	77.99^{a}	85.59^{a}	84.55^{a}	3.19
Feed conversion ration (g feed/g gain)	4.78^{b}	6.77^{a}	6.69^{a}	6.74^{a}	0.32
Feed cost (₦/kg)	68.92	95.57	124.04	150.69	
Cost of production (₦/kg gain)	329.44	647.01	829.83	1012.64	-
Mortality (number)	0.00	0.00	0.00	0.00	-

^{abc}Means within the same row with different superscripts are significantly different ($P<0.05$).

Table 4: Parasitological observations on the rabbits fed *Garcinia kola* seed meal

Parasites	Dietary levels of *GKSM* T_0(0.0%)	T_1(2.5%)	T_2(5.0%)	T_3(7.0%)
First week of experiment				
Cysts of isospora	+	++	+	+
Ascaris lumbricoides	-	-	-	-
Last week of experiment				
Cyst of isospora belli	+	+	+	+
Ascaris lumbricoides	-	-	-	-

(+): indicates presence and levels of presence
(-): indicates absence

Table 5: Bacterial observations on the rabbits fed graded levels of *Garcinia kola* seed meal

Bacteria	Dietary levels of *GKSM* T_0(0.0%)	T_1(2.5%)	T_2(5.0%)	T_3(7.5%)
First week of experiment				
Salmonella spp	++	++	+	-
Staphylococcus aureus	+	-	++	+
Escherichia coli	+	++	++	+
Streptococcus spp	-	-	+	-
Last week of experiment				
Salmonella spp	++	-	-	-
Staphylococcus aureus	+	+	-	++
Escherichia coli	+	+	++	+
Streptococcus spp	-	-	-	-

(+): indicates presence and levels of presence
(-): indicates absence

Table 6: Haematological and Serum biochemical indices of grower rabbits fed graded levels of *Garcinia kola* seed meal

Parameters	Dietary levels of *GKSM* (%) T_0(0.0%)	T_1(2.5%)	T_2(5.0%)	T_3(7.5%)	SEM
Haematological indices					
RBC ($x10^6$/ul)	6.45	6.10	6.20	6.97	0.21
Hb (g/dl)	11.95	11.50	11.70	12.00	0.26
PCV (%)	33.85	35.60	35.85	36.00	0.64
WBC (MM^3)	10350	11950	12000	12133	911.04
Platelets (MM^3)	292000	269000	330000	387667	34897.38
Lymphocytes (%)	71.50^a	54.50^b	52.00^b	45.33^b	4.08
Eosinophils (%)	28.00^b	45.50^a	48.00^a	54.67^a	4.15
Neutrophil	0.00	0.00	0.00	0.00	0.00
Basophils	0.00	0.00	0.00	0.00	0.00
Monocytes	0.00	0.00	0.00	0.00	0.00
MCV (fl)	55.50	58.40	54.70	51.80	1.27
MCHC (g/dl)	33.30	32.20	32.75	33.37	0.54
MCH (pq)	18.45	18.80	17.85	17.30	0.45
Serum Biochemical indices					
Glucose	150.67^a	57.00^b	76.00^b	65.00^b	15.57
Cholesterol	96.67	86.67	96.67	110.00	6.64
Proteins	5.23	6.53	5.47	6.00	0.21
Albumin	4.40	4.20	4.63	4.97	0.21
Globulin	2.13	1.03	0.83	1.03	0.26
Potassium	6.18	5.27	6.75	6.41	0.27
Sodium	138.73	135.33	138.30	134.80	0.88
Chloride	109.00^{ab}	104.03^a	114.97^a	105.77^b	1.59
Bicarbonate	16.67	17.33	18.00	17.33	0.41
Calcium	11.67	11.47	13.80	11.47	0.63

^{ab}Means within a row with different superscripts are significantly ($P < 0.05$) different.

Microbial Observations

Data from bacteriological and parasitological studies are presented in Tables 4 and 5. Clinical evaluation of the faecal samples revealed the presence of *Isospora belli* at the beginning and end of the experiment (Table 4), indicating that *Garcinia* kola has no effect on *Isospora belli* in rabbits. Bacteriological analysis of the faecal samples at the beginning and end of the experiment (Table 5) revealed that *Garcinia kola* may be bactericidal to *Salmonella* and *Streptococcus species*, supporting the report of Ebana *et al*. (1991) and Akoachere *et al*. (2002) that the seeds of *Garcinia kola* to some extent have anti-bacterial and anti-microbial properties. *Garcinia kola* had no significant anti-bacterial effect ($P > 0.05$) on *Staphylococcus aureus* and *Escherichia coli.*

Haematological and Serum Biochemical Indices

Data on haematological and serum biochemical indices of the rabbits are presented in Table 6. There were no significant differences ($P > 0.05$) in most of the indices evaluated (RBC, Hb, PCV, WBC, MCV, MCHC, MCH, platelets as well as the differential white cell counts, neutrophils, basophils and monocytes). However, GKSM significantly ($P < 0.05$) reduced lymphocytes and enhanced eosinophils

of the rabbits. The values of the indices were, however, within the ranges reported as normal for rabbits (MacNamee and Sheeley, 1952; Mitruka and Rawnsley, 1977; Kathy, 2003; Ahamefule *et al*., 2006). The findings are in agreement with the earlier reports of Uko *et al.* (2001) and Adedeji *et al.* (2005) on the effects of *Garcinia kola* seeds on blood profile of rats. With exception of glucose which was significantly ($P < 0.05$) reduced by dietary *Garcinia kola* seed meal, serum biochemical indices were not affected by the treatments ($P > 0.05$). The values obtained were, however within the ranges reported as normal by CCAC (1980) for rabbits, an indication that *Garcinia kola* seed meal has no negative or deleterious effects on blood profile of rabbits

CONCLUSION AND RECOMMENDATION

The results of the study have shown that *Garcinia kola* seed meal has negative effect on growth performance of rabbits. Even though it can eliminate *Salmonella species* and has no deleterious effects on blood profile of rabbits, it cannot be recommended as feed ingredient or additive in view of the poor growth performance of the rabbits and high cost of production.

REFERENCES

Adedeji, O.S., Farinu, G.O., Ameen, S.A. and Oyewope, A.O. (2005). The effects of different dietary inclusion levels of bitter kola (*Garcinia kola*) on blood profiles of laboratory rats. Tropical Veterinarian, 23 (2): 56 – 60.

Ahamefule, H., Eduok, G. O., Usman, A., Ahamefule, K. U., Obua, B. E. and Oguike, S. A. (2006). Blood biochemistry and haematology of rabbits fed sun-dried ensiled and fermented cassava peel-based diets. Journal of Nutrition, 5(3): 248 - 253.

Akoachere, J.F., Ndip, R.N., Chenwi, E.B., Ndip, L.M., Njock, T.E. and Anong, D.N. (2002). Antibacterial effect of *Zingiber officinale* and *Garcinia kola* on respiratory tract pathogens. East Afr. Med. J., 79 (11): 588 - 592.

AOAC. (1990). Association of Official Analytical Chemists, Official Methods of Analysis, 13th Edition, Washington D.C., USA.

Canadian Council on Animal Care, CCAC (1980). *Guide to the care and use of experimental animals*. CCAC, 1: 82 - 90.

Ebana, R.U., Madunagu, B.E., Ekpe, E.D. and Otung, I.N. (1991). Microbiological exploitation of cardiac glycosides and alkaloids from *Garcinia kola*, *Borreria ocymoides, Kola nitida and Citrus aurantifolia.* J. Appli. Bacteriol., 71(5): 398 – 401.

Esiegwu, A.C. and Udedibie, A.B.I. (2009). Growth performance of and anti-microbial activities in broilers fed supplementary bitter kola (*Garcinia kola*). Animal Production Research Advances, 5(1): 20 - 24.

Esonu, B. O., Emenalom, O.O., Udedibie, A.B.I., Anyanwu, G.A., Madu, U. and Inyang, A.O. (2005). Evaluation of neem (*Azadirchta indica*) leaf meal on performance, carcass characteristics and egg quality of laying hens. Intl. J. Agric. Rural Devt., (6): 208 – 212.

Hertog. M.G.I., Fejkeen, E.J.M., Hokman, C.H. and Katan, A. (1993). Dietary antioxidant flavonoids and risk of coronary heart disease, de zutphen elderly study. Lancet, 342: 2007 – 1011.

Horigone, T., Kumar, R. and Okamoto, k. (1988). Effects of condensed tannins prepared from the leaves of fodder plants on digestive enzymes in vitro and in the intestine of rats. British Journal of Nutrition, 60: 275 – 285.

Ibekwe, H.A., Eteng, M.U. and Antigha, E. (2007). Proximate and phytochemical composition of *Garcinia kola* and *Vernonia amygdalina.* Proceedings of the 32nd Annual Conf. of the Nig. Soc. for Anim. Prod., Calabar, March 18 – 21, 2007, pp. 255 – 258.

Irvine, F. R. (1961). *Woody Plants of Ghana with Special Reference to Their Uses*. Oxford University press, London.

Kathy, S. (2003). *Rabbit Health in the 21st Century. A Guide to Bunny Parents.* 2nd edition. Pp. 131 - 140.

Lebas, F., Corring, T. and Courtot, D. (1997). Equipment enzymatique du pancrease exocrine chez le lapin, mise en place et evolution de la naissance au servage. Relation avec la composition du regime alimentaire. Animal Biochemistry and Biophysics, 11: 399 - 413.

MacNamee, J. K. and Sheely, R. W. (1952). The use of the small laboratory animal for repeated clinical pathological studies. Inc. Book, Anim. Vet. Med. Assoc. 89th Inc. Session, 138.

Mitruka, B. M. and Rawnsley H. M. (1977). *Clinical, Biochemical and Haematological Reference Values in Normal Experimental Animals*. Masson Publishing USA Inc. NY, Pp. 21 - 84, 278, 106 - 112.

Monica, C. (1984), *Medical Laboratory Manual for Trop. Countries*. Vol. 11, Microbiology ELBS ed.

Monica, C. (2000). *District Laboratory Practice in Tropical Countries* (part 2). Cambridge University Press.

Obikaonu, H.O. (2009). Production Performance of and Anticoccidial Effects in deep litter managed chickens fed neem leaf meal. Ph.D Thesis, Fed University of Technology, Owerri – Nigeria.

Odebunmi, E.O., Oluwaniyi, O.O., Awolola, G.V. and Adediji, O.D. (2009). Proximate and nutritional composition of kola nut *(Cola nitida)*, bitter cola (*Garcinia kola*) and alligator pepper *(Afromomum melegueta)*. African Journal of Biotechnology, 8(2): 308 - 310.

Okwu, D.E. (2005). Phytochemical, vitamin and mineral contents of two Nigerian medicinal plants. International Journal of Molecular Medicine and Advance Sciences, 1(4): 375 – 381.

Raharjo, Y.C, Cheeke, P.R. and Patton, N.M. (1990). Effect of cecotrophy on nutrient digestibility of alfalfa and blank locust leaves. Journal of Applied Rabbit Research, 13: 56 – 61.

Udedibie, A.B.I. and Opara, C.C. (1998). Response of growing broilers and laying hens to dietary inclusion of leaf meal from *Alchornia cordifolia*. Anim. Fd. Sci. Tech., 71: 157 – 164.

Uko, O.J., Usman, A. and Ataja, A. M. (2001). Some biological activities of *Garcinia kola* in growing rats. Vet. Arhiv., 71: 287 – 297.