RESEARCH ARTICLE

Changes in serum lipid profiles of weaner rabbits fed diets fortified with processed *Garcinia kola* seed.

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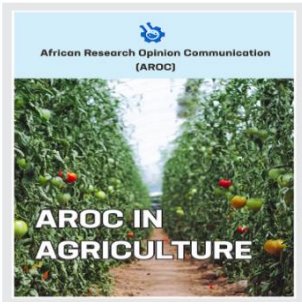

ABSTRACT

Background: A fifty-six (56) day experiment was conducted at the small animal experimental unit of Federal College of Wildlife Management, New-Bussa, Niger State to determine the effect of fortifying the feed of weaned rabbits with graded levels of Garcinia kola (G. kola). **Methods**: Forty unsexed New-Zealand white rabbits weighing between 0.90-0.92kg used for this experiment were randomly distributed into five dietary treatments tagged T1, T2, T3, T4 and T5 using a Completely Randomized Design (CRD). The experimental rabbits were fed with Iso-Caloric and Iso-Nitrogenous diets that are rich in lipids with a very high metabolizable energy (M/E). Rabbits in T1which served as the control were fed plain diets not fortified with G. kola. Rabbits in treatments 2 to 5 were fed diets fortified with graded level of processed G. kola seeds at 5g/100g, 10g/100g, 15g/100g and 20g/100g respectively and the animals were fed at 10% of their body biomass by monitoring the weight on weekly basis. **Results:** Data obtained from this study revealed that feeding of weaner rabbits with feed fortified with powdered G. kola seed produced a significant ($p<0.05$) dose-dependent serum-lipid lowering effects as compared to the control group, this may be mediated by some of the phytochemical and nutritional constituents of G. kola. The phytochemical analysis revealed that values of Tannin Flavonoids, Saponin and Alkaloids ranges from 5.94 ± 0.58%, 1.57 ± 0.37%, 3.46 ± 0.365% and 6.58 ± 0.38% respectively while the results of the Proximate analysis revealed that G. kola seed contained 8.51 ± 0.045% Moisture, 2.39 ± 0.420% Protein, 4.65 ± 0.365% Ash and 75.91 ± 2.265%NFE. **Conclusion**: This research work has revealed that G. kola seed has a hypolipidemic effect when consumed and that consumption of G. kola seed may help in the reduction of the incidence of cardiovascular.

Keywords: Serum-lipid; Phytochemical; *Garcinia kola*; Weaner Rabbits; Graded level.

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1.0 Introduction

The increase in price of conventional feed ingredients for animals has led researchers to work on alternative feed source which are non-conventional and less competed for between animals and humans (especially plants and animal by-products, kitchen wastes and left-over) to reduce the cost of production [1].

Feeding of livestock with these by-products comes with its limitations because some of them contains some nutrients which when consumed in excess can result into physiological problems which when treated with conventional commercial medications results to increase in cost of production [2].

Rabbit, as a micro-livestock, is an economic animal that could bridge the wide gap in dietary protein requirements in Nigeria because it is socially acceptable, requires less space for rearing and absence of religious taboo on its rearing and consumption as well as peculiar digestive physiology which permits the use of forages and agro-industrial by-products thus making it a less competitive species with man for cereal and legume grain [2]. In addition, rabbits are efficient converters of feed to meat and can utilize up to 30% fibre as against 10% fibre utilized by most poultry species [3].

Although rabbits can survive on all forage diets, but optimum performance can only be insured in a mixed feeding regime involving forage and formulated feed [4].

Survival of these animal species is highly dependent on their physiological status and diets which at times often leads to health issues like Atherosclerosis, (a major cardiovascular disease (CVD) in man and animal which can shorten the life span). Apart from mortality that may be recorded, the cost of medication will also lead to increase in the cost of production [5]. While the use of orthodox lipid lowering agents is expensive and associated with side effects like flatulence, constipation, and dyspepsia [4], the screening of herbs used in the traditional medicine in the management of CVD for lipid lowering effect has gained wide scientific interest in the last decades [5].

Garcinia kola which belongs to the family of *Guttiferae* also known as''Bitter kola'' in English are known to have hypolipidemic effects on serum lipids, thus it can serve the lipid the lipid lowering purpose in the serum. The seed is commonly found in the West African countries and its natural habitat is subtropical or tropical moist low land forests [1]. The growing tree and roots of *Garcinia kola* provide chew-sticks and the seeds are eaten raw [6]. The extract of the seed has been proven scientifically to have several pharmacological activities which include anti-inflammatory, analgesic, molluscidal, anti-atherogenic, antioxidant and hepato-protective activities [6,7,8,9]. The present work was aimed at evaluating the effect of fortifying the feed of rabbits with graded level of processed *G. kola* seed on the serum lipid profile of weaner rabbits fed lipid rich diets.

2.0 Materials and Methods

2.1 Study Location

The study was conducted at the small animal experimental Unit of the Department of Animal Health and Production Technology, Federal College of Wildlife Management, New-Bussa, Niger State, Nigeria between the months of February and May 2022.New- Bussa lies on latitude 9^0 52' 59.99'' N and Longitude 4^0 30' 59.99'' E.

2.2 Plant Material

The freshly harvested fruits of *G. kola* (Bitter kola) were bought from Wawa market, Borgu Local Government Area of Niger State, Nigeria in the month of January 2022 and were authenticated in the herbarium of Nigeria Institute of Science Laboratory Technology (NISLT), Samonda, University of Ibadan Road, Ibadan, Oyo State, Nigeria with Laboratory number 20121164 – 20121165 and Code number460/2021.

2.3 Sample Preparation

The outer testa of each *G. kola* seed was removed washed and air dried for about twenty-four (24) hours. Each seed was cut into smaller pieces and the resulting pellets were dried in hot air oven (Gallenkamp) for about twelve (12) hours at 40ºC as described by [5] and the dry seed pellets were ground to fine powder. The resulting powder was stored in an airtight container throughout the experimental period.

2.4 Experimental Animals

Forty (40) unsexed New Zealand white breed of Weaner rabbits weighing 0.90 ± 0.02Kg used for this experiment were sourced from the small animal unit of the department of Animal Health and Production Technology, Federal College of Wildlife Management, Forestry Research Institute, New-Bussa in Niger State. They were fed with the compounded and pelleted (to prevent aspiration pneumonia) experimental diet *ad-libitum* in well-ventilated cages as shown in table 1. They were supplied with clean drinking water throughout the experimental period. Ethical conditions governing the conduct of experiments with live animals were strictly observed as recommended by [10].

2.5 Experimental Design

The experimental animals were randomly divided into five treatments containing eight rabbits per treatment and each treatment replicated four times with two rabbits per replicate in a Completely Randomized Design (CRD) and were labeled T_1, T_2, T_3, T_4 and T_5 respectively. All the rabbits were fed on the same diet which is lipid rich with a very high metabolizable energy (M/E). T_1 served as the control and received plain feed without seed sample. Treatments 2 to5 were fed feeds fortified with graded level of the processed *G. kola* seeds at 5g/100g, 10g/100g, 15g/100g and 20g/100grespectively. The animals were fed at 10% of their body biomass for fifty-six (56) days.

2.6 Proximate Analysis of the Experimental Diets

Standard methods of the Association of Official Analytical Chemists [3] were used to determine the moisture, crude protein (CP), ether extract (EE), ash, nitrogen free extract (NFE) and crude fiber (CF) contents of the experimental feed samples as described by [5]. Each analysis was carried out in quadruplets and the result is as shown on table 1.

Table 1.0: Percentage and proximate Composition of the experimental diets

Ingredients (kg)	T_1	T_2	T_3	T_4	T_5
Maize	40.00	40.00	40.00	40.00	40.00
Maize offal	30.00	30.00	30.00	30.00	30.00
Full fat soya	25.00	25.00	25.00	25.00	25.00
Blood meal	1.50	1.50	1.50	1.50	1.50
Oyster shell	1.00	1.00	1.00	1.00	1.00
Bone meal	2.00	2.00	2.00	2.00	2.00
Salt	0.25	0.25	0.25	0.25	0.25
Vitamin premix *	0.25	0.25	0.25	0.25	0.25
Garcinia kola seed	-------	5.00	10.00	15.00	20.00
Total	100.00	105.00	110.00	115.00	120.00
Analyzed content (%)					
Dry matter (DM)	90.45	92.13	94.27	96.55	97.89
Crude protein (CP)	15.13	15.69	15.93	16.11	16.24
Crude fibre (CF)	9.63	9.88	9.97	10.01	10.13
Ether extract (EE)	4.10	3.86	3.49	3.18	3.03
NFE	54.51	55.64	55.85	56.94	57.74
Ash	6.49	6.58	6.79	6.93	7.42
M.E (MJ/Kcal/DM)	2,800	2,835	2,898	2,965	3,002

Vitamin premix is to provide the following per kg diet: Vit, A, 1500IU; Vit E, 11.0mg; Riboflavin, 9.0mg; Biotin, 0.25; Pantothenic acid, 11.0mg; Vit k , 3.0mg; B_1 , 2.5mg; B_2, 0.3mg; B_6 , 8.0mg; Nicotinic acid, 8.0 mg; Fe, 5.0mg; Mn, 10.0mg; Zn, 4.5mg; Co,0.2mg; Se,0.01mg.NFE: Nitrogen free extract**ME: Metabolizable Energy.*

2.7 Sample Collection

Twenty-four (24) hours after the last feed administration, blood sample for sera preparation were collected by direct cardiac puncture into sterile plain sample bottles. The serum samples were separated from the clot by centrifugation at 3000rpm for 5min using bench top centrifuge (MSE minor, England®). Serum samples were harvested into sterile plain sample bottles and stored in refrigerator for serum lipid profile analysis.

2.8 Biochemical Analysis

The serum lipid profiles of the rabbits were evaluated using a commercially available assay kit (Randox, UK) as described by [5]. The serum level of total cholesterol (TC) was measured by enzymatic hydrolysis and oxidation method as described by [11]. The serum triglyceride (ST) level was determined after enzymatic hydrolysis of the sample with lipases as described by [12]. The serum level of High Density Lipoprotein Cholesterol (HDL-C) was measured by the method of [13]. The serum Very Low-Density Lipoprotein Cholesterol (VLDLC)was calculated as 1/5 of the ST [14], while the serum Low Density Lipoprotein Cholesterol (LDLC) was calculated using the formula described by [15].

2.9 Photochemical Analysis of *G. kola*

Tannins, Saponins, Flavonoids and Total alkaloids of the seed samples were determined using the method described by [16]and [17] and the result is as shown on table 2.

2.9.1 Determination of Alkaloids

Five (5) grams of the plant sample was placed in a 250ml beaker and 200ml of 10% ethanoic acid in ethanol was added. The mixture was covered and allowed to stand for 4 hours. It was then filtered and the filtrate was concentrated on a water bath until it reaches a quarter of its original volume. Concentrated ammonium hydroxide was added until precipitation was complete. The mixture was allowed to settle and the precipitate collected on a weighed filter paper and washed with dilute ammonium hydroxide. The precipitate, alkaloid, was dried and weighed. The percentage alkaloid was calculated by difference [5].

2.9.2 Determination of Flavonoids

Ten (10) grams of plant sample was repeatedly extracted with 100ml of 80% aqueous methanol at room temperature. The mixture was then filtered through a filter paper into a pre-weighed 250ml beaker. The filtrate was transferred into a water bath and allowed to evaporate to dryness and weighed. The percentage flavonoid was calculated by difference [5].

2.9.3 Determination of Saponins

Twenty (20) grams of seed sample was weighed into a 250ml conical flask. One hundred milliliters of 20% ethanol was added. The mixture was heated over a hot water bath for 4 hours with continuous stirring at about 55ºC. It was then filtered with a Whatman No.1 paper. The residue was re-extracted with another 200ml of 20% ethanol. The combined extract was reduced to 40ml over a water bath at

about 90ºC. The concentrated extract was then transferred into a 250ml separator funnel and 20ml of diethyl ether was added to the extract and shaken vigorously. The aqueous layer was recovered while the diethyl ether layer was discarded. This purification process was repeated. Sixty (60) ml of n-butanol was added and the combined n-butanol extract was washed twice with 10ml of 5% sodium chloride. The remaining solution was then heated on a water-bath in a pre-weighed 250ml beaker. After evaporation the residue was dried in a Gallenkamp moisture extraction oven (Size 1) to a constant weight. The % Saponin was calculated by difference [5].

2.9.4 Tannin determination

The seed sample, 500mg, was weighed into a 50ml plastic bottle. 50ml of distilled water was added and shaken for 1h in a mechanical shaker. This was filtered into a 50ml volumetric flask and made up to the mark. Then 5ml of the filterate was pipetted out into a test tube and mixed with 2ml of 0.1M ferric chloride in 0.IN hydrochloric acid and 0.008M potassium ferrocyanide. The absorbance was measured at 700nanometers within 10minutes [5].

2.9.5 Proximate Analysis of Garcinia kola

The values of moisture content, crude protein (CP), Ether extracts (EE), Nitrogen Free Extracts (NFE) and Crude fibre of the seed samples were determined by using the Standard methods described by the Association of Official Analytical Chemists [18].

2.9.6 Determination of the Moisture content

Moisture content was determined by heating 2.0g of each fresh sample to a constant weight in a crucible placed in an oven maintained at 105ºC. The dry matter was used in the determination of the other parameters.

2.9.7 Determination of the Crude Protein (CP)

Crude protein (% total nitrogen X 6.25) was determined by the Kjeldahl method, using 2.0g samples. Ether extract was obtained by exhaustively extracting 5.0g of each sample in a Soxhlet apparatus using petroleum ether (boiling point range 40º-60ºC) as the extractant.

2.9.8 Ash content determination

Ash was determined by the incineration of 2.0g sample placed in a muffle furnace maintained at 550ºC for 5h. Crude fiber was obtained by digesting 2.0g of sample with 1.25% w/v Sulphuric acid and 1.25% w/v sodium hydroxide, the fiber was filtered, dried at 120ºC, weighed and incinerating the residue in a muffle furnace maintained at 550ºC for five hours (5hrs).

2.9.9 Determination of Nitrogen Free Extracts (NFE)

Nitrogen free extracts (NFE) which represents soluble carbohydrates and other digestible and easily utilizable non-nitrogenous substances in feed were determined by mathematical calculation. It was obtained by subtracting the sum of the percentages of all the nutrients already determined from 100 by using the formula below;

%NFE=100-(%moisture+%CF+%CP+%EE+%Ash)

2.9.10 Statistical Analysis

Data obtained were analyzed using one-way analysis of variance (ANOVA) and the variant means were separated by least significant difference (LSD) of the different groups. Significance ($p<0.05$) was accepted at 5% level.

3.0 Results and Discussion

3.1 Phytochemical Composition

The result of the phytochemical analysis obtained for the seed sample of *G. kola* in this research work is not in agreement with the reports of [19] who reported the values of 0.342% for Tannins, 2.471% for Saponin, 0.645% for Alkaloids and 2.041% for flavonoids, but the results are in agreement with the works of [5], that reported the values of 5.08 ± 0.2% for Tannin, 0.93± 0.03% for Flavonoids, 5.13 ± 0.67% for Alkaloids and 2.54±0.01% for saponin. The difference in the values might be due to the fact that the seeds were sourced from different locations and they grew in different environment.

Table 2: Phytochemical Composition of G. kola seeds.

Phytochemical	Mean values ± SEM (%)
Tannin	5.94 ±0.58
Flavonoids	1.57 ±0.37
Saponins	3.465 ± 0.365
Alkaloids	6.58 ± 0.38

3.2 Proximate Composition

Results of proximate nutrient composition of *G. kola* on table 3shows that the values of the moisture content, Crude protein, Ash content and NFE varies between 8.51 ± 0.045, 2.39 ± 0.420, 4.65 ± 0.365 and 75.91 ± 2.26 respectively. This result is not in agreement with the works of [9] who reported that *G. kola* seed contained 97.31, 39.52, 43.25, 11.40,

114.02 and 694.48 g/kg dry weight of moisture, CP, EE, ash, CF and NFE respectively. Also, [5] reported that G. kola seed contained 60.48, 2.48, 4.51, 0.79, 5.23 and 35.64% of moisture, CP, EE, Ash, CF and NFE respectively. These differences might not be unconnected with the source of the materials used [5].

Table 3: Proximate compositions of G*. kola* seed*.*

Proximate	Mean values ± SEM (%)
Moisture	8.51 ± 0.045
Protein	2.39 ± 0.420
Ash	4.65 ± 0.365
NFE	75.91 ± 2.265

3.3 Serum lipid profile

The results of this study shown on table 5 revealed that feeding of the New-Zealand breed of weaner rabbits with feed fortified with powdered *G. kola* seed produced a significant ($p<0.05$) dose-dependent serum-lipid lowering effects which may be mediated by some of its phytochemical and nutritional constituents, this in agreement with the works of [20] who fortified the feed of Albino Winstar Rat with *G. kola* and obtained similar results. Though the mechanism of lipid lowering effect is not known, it could be either through reduction in absorption of cholesterol from the gut or by reduction in the biosynthesis of cholesterol [5].

The possible mechanism of reducing dietary cholesterol absorption from the gut is by binding of the phytochemical constituent such as Phytosterols to the cholesterol receptor site in the gut mucosa [21].

Table: 4 Serum Lipid Profile (Mean ± SEM) of Rabbits Fed Graded level of *G. kola*.

Treatment group	**T_1 (0% *G. kola*)**	**T_2 (5% *G. kola*)**	**T_3 (10% *G. kola*)**	**T_4 (15% *G. kola*)**	**T_5 (20% *G. kola*)**
Cholesterol (mg/dl)	94.74±1.92	40.00	72.81±0.63*	67.14±0.70*	65.66±1.49*
HDL-C (mg/dl)	38.64±0.27	83.26±1.83*	36.35±0.29*	44.97±0.42*	50.34±0.27*
Triglyceride (mg/dl)	119.23±4.43	50.38±2.45*	45.11±2.43*	41.70±3.63*	24.81±2.33*
VLDL-C (mg/dl)	27.28±0.83	13.49±0.61*	12.57±0.35*	11.88±0.53*	8.33±0.49*
LDL-C (mg/dl)	40.96±2.23	32.41±1.63*	28.64±0.42*	20.39±0.72*	17.21±1.41*

**Significant difference (P<0.05) exists in all the values of measured parameters across the treatments) when compared to the values obtained from the negative control…HDL-C= High Density Lipoprotein Cholesterol, VLDL-C= Very Low-Density Lipoprotein Cholesterol, LDL-C= Low Density Lipoprotein Cholesterol.*

Garcinia kola has been shown to possess several pharmacological activities such as; anti-inflammatory, analgesic, molluscidal, anti-atherogenic, antioxidant and hepato-protective activities, which have been attributed to some of its phytochemical compositions [22,23].

Another possible mechanism through which the powdered seed sample of G. kola may have caused the lipid lowering effect may be by binding to bile acid in intestine, which will impede their reabsorption. This will subsequently deplete the bile acid pool leading to up regulation of cholesterol 7-α-hydroxylase and increased conversion of cholesterol to bile acids. This causes an increased demand for cholesterol in the liver cells, resulting in the dual effect of increasing transcription and activity of the cholesterol biosynthetic enzyme, HMG-CoA reductase and increase in the number of hepatic LDL receptors. These compensatory effects result in increased clearance of LDL-C from the blood, resulting in decreased serum LDL-C levels. Serum TG levels may increase or remain unchanged [24]. According to Evans and Trease [24], plants may grow well in different situations but fails to produce the same constituents. Plant growth and development, and often the nature and quantity of secondary metabolites are affected by temperature, rainfall, length of day (quantity of light) and altitude. Light determines the amount of glycosides or alkaloids present in a plant. Also, continuous rain can lead to a loss of water-soluble substances from leaves and roots by leaching; this is known to apply to some plants producing alkaloids, glycosides and even volatile oils [24].

4.0 Conclusion

In conclusion, this research work has revealed that G. kola seed has a hypolipidemic effect when consumed and that consumption of *G. kola* seed may help in the reduction of the incidence of cardiovascular diseases in man and animals and further research should be carried out to determine the tolerable level of *G. kola* or a dose that is not lethal and mechanism behind its hypolipidemic effect.

Author's Contributions

Author FBO is a Veterinary Doctor and a Principal researcher with Forestry Research Institute of Nigeria, he organized the research work with the co-authors and jointly carried out the research work. **Author BKO** renowned industrial Chemist and also

a researcher with the Forestry Research Institute of Nigeria designed and performed the proximate analysis and chemical and biochemical analysis of the experimental diets and samples, while **author BOO** renowned statistician and also a researcher with the Forestry Research Institute of Nigeria, designed and work on the statistical Analysis of the data obtained and also contributed to the results and in the discussion of the results.

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Conflict of Interest: The author declared that no conflict of interest exists

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