Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.5, No.3, 2015

Haematological and Serum Biochemical Characteristics of Rabbit Bucks Fed Diets Containing Garcinia Kola Seed Meal

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Abstract
The effect of diets containing *Garcinia kola* seed meal on blood characteristics of 36 growing rabbit bucks of about 3 months old were investigated in an experiment that lasted for 3 months. The animals were randomly assigned to 3 treatments of 3 replicates each. Three experimental diets, T_1, (control; containing 0 % *G. kola* seed meal), T_2 (2.5 % *G. kola* seed meal) and T_3 (5 % *G. kola* seed meal) were administered *ad libitum* to the animals. The haematological parameters evaluated were; packed cell volume (PCV), haemoglobin (Hb), white blood cell (WBC) and differentials. There were significant ($P<0.05$) proliferation of total WBC and lymphocyte counts in T_2 than in T_1 and T_3 which were similar ($P>0.05$). Serum biochemical analysis of total protein, albumin, globulin, aspartate amino transaminase (AST), alanine amino transaminase (ALT) and alanine phosphate (ALP), recorded significantly ($P<0.05$) higher aspartate amino transaminase (AST) in T_2 (81.67$\pm$ 4.41 IU/L) than in T_1 (61.0$\pm$2.52 IU/L) and T_3 (68, 67$\pm$ 3.48 IU/L) which were similar ($P>0.05$). Alanine amino transaminase (ALT) were similar ($P>0.05$) in T_2 (59.0$\pm$2.65 IU/L) and T_3 (54.0$\pm$1.0 IU/L) but significantly ($P<0.05$) higher than T_1 (42.33$\pm$4.63 IU/L). The results of this study indicate that *G. kola* seed meal increases lymphocyte count in rabbit bucks which also gives rise to a corresponding total white blood cell count. Serum biochemical characteristics showed a possible mild organ degenerations as evident in the significant ($P<0.05$) increase in aspartate amino transaminase (AST) and alanine amino transaminase (ALT) of animals consuming diets containing *Garcinia kola* seed meal.
Keywords: *Garcinia kola*, haematological, biochemical, rabbits.

Introduction

Garcinia kola (bitter kola) belongs to the family of plants called *Guttiferae*, the genus is known as *Garcinia* (Iwu, 1993). It is a perennial crop growing in the forest, distributed throughout West and Central Africa (Iwu, 1993). *G. kola* is also found distributed in the forest zone of Sierra Leone, Ghana, Cameroon and other West African countries; particularly in Nigeria it is common in the South Western States and Edo State of Nigeria (Eka, 1971). It is mainly grown on homesteads in Southern Nigeria (Uko *et al*., 2001); a detailed description and distribution of the plant has been documented (Iwu, 1993).

It has been found that *Garcinia kola* contains a lot of valuable constituents useful to humans and animals (Adedeji *et al*., 2008a). An important constituent of *G. kola* seed is biflavonoid (kolaviron) having anti-inflammatory properties (Braide, 1993) and a natural antioxidant (Olatunde *et al*., 2002; Terashima *et al*., 2002). Other constituents of *G. kola* seed include 1-3, 8-11 benzophenones, *Garcinia* biflavonones (GB-1, GB-2) and kolaflavonone (Cotterih *et al*., 1978). Apigenin based flavonoids represent 60% of the total flavonoids present in the diethyl ether fraction of *G. kola* seeds (Iwu and Igboko, 1982). Phenols, alkaloids, tannins and saponins are other phytochemical constituents of *G. kola* seeds, and they exert various beneficial effects in humans and animals (Okwu, 2005).

The biological activities of flavonoids include action against allergies, inflammation, free radicals, hepatoxins (Terashima *et al*., 2002). However, excessive ingestion of *G. kola* nuts can result in some adverse effects. Histological alterations in the liver, kidney and duodenum of rats fed diets containing 10 % *G. kola* nut have been reported (Braide and Grill, 1990). Similarly, Oluwole and Obatomi (1992) observed an increase in both basal and histamine-mediated gastric acid secretion of rats fed *G. kola*.

The inclusion of antibiotics in livestock ration has been discouraged. This is because of the residual effect in livestock products and development of resistant strains of micro-organisms to drug therapy (Oyekunle and Owonikoko, 2002). *G. kola* can serve as alternative substance to antibiotics in livestock feeds and be used as a growth promoter (Adedeji *et al*., 2008b). It can also be employed in livestock industry to effect some changes in egg quality characteristics of laying hens (Adedeji *et al*., 2008a), and as a contraceptive and fertility control agent in female Sprague-Dawley rats (Akpantah *et al*., 2005).

G. kola can also serve as raw material for pharmaceutical industries (Iwu, 1989) and also not elucidating its use in herbal medicine (Hertog *et al*., 1993; Manimi *et al*., 1994; Chairungsrilerd *et al*., 1996).

Garcinia kola possesses anti-bacterial (Madubunyi, 1995; Adefule-Ositelu *et al*., 2004), anti-hepatoxic (Akintowa and Essien, 1990; Braide,1990), antioxidant (Olatunde *et al*.,2004), hypoglycemic (Iwu *et al*.,1990a; Odeigha *et al*.,1999) and aphrodisiac properties (Ajibola and Satake, 1992) which makes it highly valued in traditional African medicine for the treatment of various ailments and diseases. The seeds are chewed as an aphrodisiac and also used to cure cough, dysentery, head or chest cold in herbal medicine (Irvine, 1961). Among the people of Eastern Nigeria, the raw stem bark of *G. kola* is used as a purgative, and powdered bark is applied to malignant tumours (Iwu, 1989). The sap is used to cure parasitic skin diseases and dermatological disorders associated with melanin pigmentation (Okunji *et al*., 2007), the latex or gum is used internally against gonorrhea and applied externally on fresh wounds (Iwu, 1989). *Garcinia* seed is also used in the treatment of cirrhosis and hepatitis (Iwu, 1986; Ogu and Agu, 1995). Other known medicinal uses include guinea worm remedy (Lewis, 1977), anti-atherogenic effects (Adaramoye *et al*., 2005), and antilipoperoxidative effects (Emerole *et al*., 2005). The plant has been shown to posses even antiviral activity as it halts the replication of the deadly Ebola virus in its tract in laboratory tests and it has been suggested that if the anti-Ebola compound proves successful in animal clinical trials, it will be the first medicine to successfully treat the virus that causes Ebola hemorrhagic fever; an often fatal condition (Tebekeme and Ibiba, 2008).

This study will be investigating the effects of *G. kola* seedl on haematological characteristics and serum biochemical characteristics of rabbit bucks fed diets containing *Garcinia kola* seed meal.

Materials and methods

Location of study

This study was carried out at the Michael Okpara University of Agriculture, Umudike Teaching and Research Farm (Rabbitry Unit). The University and the farm is located on an elevation of about 120m above sea level at latitude 5°21' North and Longitude 7°29' East. Umudike falls within the rainforest zone of Nigeria which is characterized by hot and humid climate. The mean annual rainfall is about 2177mm, mean annual relative humidity is about 90 % and that of temperature is 22 °C to 36 °C depending on the season.

Management of animals

A total of 36 growing rabbit bucks of about 3 months old were used for this study. The hutches for the animals were thoroughly cleaned and disinfected. On arrival, the animals were given Piper dewormer and allowed one week to acclimatize to the environment before administering the experimental treatments, prior to commencement of treatment. The animals were randomly assigned to 3 experimental diets containing 0 %, 2.5 % and 5 % *Garcinia kola* seed meal, respectively. Each treatment had 12 rabbits (3 replicates of 4 rabbits each) with feed and water given *ad libitum*.

Plant materials

Nuts of *Garcinia kola* were purchased from 'Afo Enyiogugu' market in Aboh Mbaise LGA, Imo State, Nigeria; and processed by removing the thin layer covering, chopped into pieces, air-dried and ground as described by Uko *et al* (2001).

Experimental diets

The diets were formulated using the feed materials in Table 1. *Garcinia* seed meal were included at three different levels in the diets, T_1 is the control diet and contained 0 % level of *Garcinia kola* seed meal, while T_2 and T_3 contained 2.5 % and 5 % *Garcinia kola* seed meal, respectively.

Table 1: Nutrient composition of treatment diets.

Component	**T_1**	**T_2**	**T_3**
Ingredients (%)			
Maize	54.90	51.50	47.96
Brewers dried gram	36.60	37.50	38.54
Groundnut cake	1.75	1.75	1.75
Fish Meal	3.00	3.00	3.00
Oyster Shell	2.00	2.00	2.00
Bone Meal	1.00	1.00	1.00
Vitamin/Mineral premix	0.25	0.25	0.25
Salt	0.50	0.50	0.50
Garcinia kola	0.00	2.50	5.00
Total	**100.00**	**100.00**	**100.00**
Crude protein content (%)$^+$	17.07	16.77	16.79
Metabolisable energy (Kcal/kg)$^+$	2741.95	2707.16	2706.35

+ Calculated.

Haematological parameters

Blood samples were taken fortnightly from the animals. The haematological parameters were determined as follows: The total white blood cell (WBC) counts were determined by the haemocytometer method while the differential count smears were prepared and stained by the Leishman Technique and enumerated by the longitudinal counting method (Schalm *et al*., 1975). The packed cell volume (PCV) was determined by the Microhaematocrit method and the haemoglobin (Hb) was determined by the Cyanomethaemoglobin method (Schalm *et al.*, 1975; Thrall and Weiser, 2002).

Serum biochemical parameters

Serum for the biochemical parameters were obtained from blood samples collected by the Orbital Technique (Stone, 1954). Determinations of serum activity of alanine amino transaminase (ALT), aspartate amino transaminage (AST), alkaline phosphate (ALP), total protein (TP), albumin (Alb) and globulin (Glb) were carried out (Coles, 1986; Meyer *et al.,* 1992; Evans, 1996), using Quimica Clinica Aplicada (QCA) Test Kits (Quimica Clinica Aplicada, Spain) and a Spectrophotometer (Spectrum lab, England).

Experimental design and statistical analysis

The experiment was carried out in a completely randomized design (CRD). The statistical model for this experiment was:

$$Yij = \mu + Ti + e_{ij}$$

Where:

Yij = Individual observation

μ = Overall mean

T_i = Effect of ith treatment

e_{ij} = Error term

The data collected were subjected to analysis of variance (ANOVA) to determine significant differences among treatment means according to Steel and Torrie (1980). Where there were significant differences between means, the means were separated using the Duncan's Multiple Range Test.

Results

Haematological Characteristics:

The haematological parameters of the experimental animals are presented in Table 2. No significant difference ($P>0.05$) was recorded in packed cell volume (T_1 = 28.80±0.79 %, T_2=29.10±308 %; T_3 = 28.40±1.64 %), haemoglobin concentration (T_1 = 9.60 ±0.26 g/100ml; T_2 = 9.70±1.03 g/100ml; T_3 = 3.74±0.40 g/100ml), monocytes (T_1 = 0.07±0.05 x 10^3/mm^3 ; T_2 = 0.15±0.04 x 10^3/mm^3; T_3 = 0.05±0.03 x 10^3/mm^3) and eosinophil counts (T_1 = 0.01±0.01 x 10^3/mm^3; T_2 = 0.08±0.04 x 10^3/mm^3; T_3 = 0.02±0.02 x 10^3/mm^3). However, animals on T_2 recorded significantly ($P<0.05$) higher total white blood cell (WBC) and lymphocyte counts of 7.67±0.23 x 10^3/mm^3 and 3.35±0.04 x 10^3/mm^3, respectively, than animals on T_1 (6.43±0.19 x 10^3/mm^3 and 2.34±0.24 x 10^3/mm^3) and T_3 (5.70±0.32 x 10^3/mm^3 and 1.89±0.30 x 10^3/mm^3), which were similar ($P>0.05$). All the mean values obtained for the haematological parameters of the experimental animals were in the order of $T_2 > T_1 > T_3 >$, except for eosinophils which were in the order of $T_2 > T_3 > T_1$.

Table 2: Haematological parameters of rabbit bucks fed diets containing *Garcinia kola* seed meal.

Parameters	T_1	T_2	T_3
PCV (%)	28.80±0.79	29.10±3.08	28.40±1.64
Hb (g/100ml)	9.60±0.26	9.70±1.03	9.47±0.55
Total WBC (x 10^3/mm^3)	6.43±0.19^b	7.67±0.23^a	5.70±0.32^b
Lymphocytes (x 10^3/ mm^3)	2.34±0.24^b	3.35±0.04^a	1.89±0.30^b
Neutrophils (x 10^3/ mm^3)	4.01±0.17	4.09±0.24	3.74±0.40
Monocytes (x 10^3/ mm^3)	0.07±0.05	0.15±0.04	0.05±0.03
Eosinophils (x 10^3/ mm^3)	0.01±0.01	0.08±0.04	0.02±0.02

a,b: Means on the same row bearing different superscripts are significantly different ($P<0.05$).

Biochemical Characteristics:

The total protein (T_2 = 6.33±0.41 mg/100ml; T_3 = 5.97± 0.32 mg/100ml), albumin (T_2 4.10 ±0.10 mg/100ml; T_3 = 3.87±0.19 mg/100ml), globulin (T_2 = 2.23±0.49 mg/100ml; T_3 = 2.10 ±0.49 mg/100ml) and alkaline phosphate (T_2 =2.23±0.49 IU/L; T_3 = 72.0±3.06 IU/L) of T_2 and T_3 were not significantly ($P>0.05$) higher than 5.50±0.26 mg/100ml, 3.73±0.27 mg/100ml, 1.77±0.13 mg/100ml and 66.67±2.85 IU/L recorded in T_1 for total protein, albumin, globulin and alkaline phosphate, respectively (Table 3). On the other hand, the aspartate amino

transaminase (AST) of T_2 (81.67±4.41 IU/L) was significantly (P<0.05) higher than that of T_1 (61.0±2.52 IU/L) and T_3 (68.67±3.48 IU/L) which were similar (P>0.05). Furthermore, alanine amino transaminase (ALT) of T_2 (59.0±2.65 IU/L) and T_3 (54.0±1.00 IU/L) were similar (P>0.05) but they were significantly (P<0.05) higher than that of T_1 (42.33±4.63 IU/L). It is observed that all the biochemical parameters except alanine phosphate (ALP) followed the order of T_2> T_3> T_1.

Table 3: Biochemical parameters of rabbit bucks fed diets containing *Garcinia kola* seed meal.

Parameters	T_1	T_2	T_3
Total protein (mg/100ml)	5.50±0.26	6.33±0.41	5.97±0.32
Albumin (mg/100ml)	3.73±0.27	4.10±0.10	3.87±0.19
Globulin (mg/100ml)	1.77±0.13	2.23±0.49	2.10±0.49
AST (IU/L)	$61.0±2.52^b$	$81.67±4.41^a$	$68.67±3.48^b$
ALT (IU/L)	$42.33±4.63^b$	$59.0±2.65^a$	$54.0±1.00^a$
ALP (IU/L)	66.67±2.85	70.67±3.18	72.0±3.06

(AST= Aspartate amino transaminase, ALT= Alanine amino transaminase, ALP= Alkaline phosphate).
a,b:Means on the same row bearing different superscripts are significantly different (P<0.05).

Discussion

Haematological Characteristics:

Table 2 shows the average treatment means for the haematological parameters. All parameters measured showed no significant difference (P>0.05), except for the total white blood cells and lymphocyte counts that showed significant (P<0.05) proliferation in the animals that were receiving 2.5 % *Garcinia kola* seed meal (T_2). Since other differentials (Neutrophils, Monocytes and Eosinophils) were not significantly (P>0.05) different among the treatments, it becomes obvious that the significant (P<0.05) proliferation in total white blood cell count were as a result of the significant (P<0.05) proliferation in lymphocyte counts. Adedeji *et al*. (2008b) also recorded similar proliferation with broiler chicks, which they attributed to the white blood cells and its differentials identifying the active ingredient in the *G.kola* as foreign substance. This could also be probably responsible for the antimicrobial (antiviral, antibacterial and antiprotozoan) activity of *G.kola* (Iwu, 1993) as the lymphocytes were scientifically known to play key role in the immune defense system of the body both in man and domestic animals. One of the major functions of lymphocytes is their response to antigens (foreign substances) by forming antibodies that circulate in the blood or in the development of cellular immunity (Frandson, 1981). *Garcinia kola* has also been reported to possess the ability to enhance some elements of the immune system (Tebekeme and Ibiba, 2008).

Increasing the dietary level of *G.kola* seed meal to 5 % in the diet of the experimental rabbits significantly (P<0.05) reduce the means of the white blood cell and lymphocyte counts of the animals below that obtained from animals on 2.5 % level of inclusion, but not significantly (P>0.05) lower than those on control diet. This could be attributed to a corresponding increase in the level of certain compounds that leads to inferior haematological parameters present in *G. kola* (Eleyinmi *et al.,* 2006).

Biochemical Characteristics

The significant (P<0.05) elevation in aspartate amino transaminase (AST) and alanine amino transaminase (ALT) in rabbits fed 2.5 % dietary level of *Garcinia* seed meal may reflect liver and heart toxicity, which may be mild but sufficient to permit leakage of the cellular enzymes through the cell membranes with no appreciable effect on cellular functions. Whereas ALT concentrations are higher in liver of rabbits, AST occurs in a wide variety of tissues, but with high concentrations in muscular tissues and in liver (Kaneko, 1980; Bush, 1991; Dial, 1995). The increase in AST and ALT activity could therefore be ascribed to both myocardial and liver degeneration in the experimental animals because of a consistent decrease in relative weight of their hearts and livers. Surprisingly, at 5 % dietary inclusion of *Garcinia kola* seed meal, the mean values of AST and ALT fell below those obtained from 2.5 % dietary inclusion. Though the reduction were significant (P<0.05) in AST but not in ALT, the whole biochemical parameters (expect alkaline phosphate) were in the order of T_2 > T_3 >T_1. It can be suspected that this could be partially due to the active ingredients in *Garcinia kola* being functionally relative to each other in respect of quantities available (Noboru, 2001) and partially due to homeostatic mechanisms of the animals.

Conclusion

This study has demonstrated that *Garcinia kola* seed meal increases the white blood cell count of rabbit bucks; especially the lymphocytes, thereby increasing their immunity. However, dietary inclusion should be limited to

2.5 %, and chronic ingestion avoided in young rabbits to avoid organ degenerations, especially in the liver and kidney of the animals.

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