# CHANGES IN HAEMATOLOGICAL PARAMETERS OF RABBIT BUCKS FED COTTONSEED CAKE BASED-DIETS SUPPLEMENTED WITH OR WITHOUT *KIGELIA AFRICANA* FRUITS.

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**ABSTRACT**
*In this experimental study, 60 rabbit bucks were randomly assigned to five dietary treatments in a completely randomized design. The treatments included a control group without cottonseed cake (CSC) (T1), CSC-based diets without supplementation (T2), powder of Kigelia africana fruits supplementation (T3), extract of Kigelia africana fruits supplementation (T4), and a combination of powder and extract supplementation (T5). After a 14-week feeding period, blood samples were collected for hematological parameter analysis. Results showed significant decreases (P<0.05) in packed cell volume (PCV), red blood cell (RBC) count, hemoglobin concentration (Hb), and white blood cell (WBC) count in bucks fed CSC diets without Kigelia africana fruits supplementation. However, supplementation with Kigelia africana fruits extract and powder-extract combination significantly increased (P<0.05) these parameters. The study concludes that CSC negatively impacted certain hematological parameters in rabbit bucks, but supplementation with Kigelia africana fruits showed potential corrective effects*.

**Keywords: Rabbit bucks, Cottonseed cake, Haematology, *Kigelia africana* fruits, supplementation.**

## INTRODUCTION

The global challenges in animal husbandry, marked by feed resources and escalating prices have compelled animal nutritionists to explore cost effective alternatives. Cottonseed cake stands out as a promising substitute for soybean meal, renowned for its protein and fibre content. Despite is potential, CSC has been underutilized in non-ruminant diets due to the presence of gossypol, a polyphenolic compound with physiological implications (Amao et al 2014: Amao and Akanbi 2017). Gossypol originating from cotton plants, readily binds to various substances including minerals and amino acids. Meanwhile *Kigelia africana* recognise for its application in traditional medicine, offers diverse benefits such as anticancer, antiulcer, antioxidant properties and more. The study aimed to examine haematological components as vital indicators of an organism's physiological, nutritional and health status.

## MATERIALS AND METHODS

*Experimental site*

The experiment was conducted at the Rabbit Research and Production Unit of the Teaching and Research farm, Osun State University, College of Agricultural Sciences and Management Ejigbo, Campus, Ejigbo.

***Cottonseed cake and Kigelia africana fruits preparation***

Cottonseed cake (CSC) was obtained from a cotton milling industry in Kano, Nigeria. The material was sundried, milled and the initial moisture content was determined by drying until constant weight at $110^0$C in hot-air oven. The matured ripe fruits of *Kigelia africana* were collected based on ethno-pharmacological information from the forest in Oko-Ara town area, Osun State. The botanical identification of the plant and fruits was done at the Forestry Research Institute of Nigeria, Ibadan where a voucher specimen number- FHI/08257 was recorded for ease of identification.

***Powder preparation***

The matured *Kigelia africana* fruits were harvested, washed, peeled cut into small pieces; sun-dried into a constant weight then ground into fine powder particles by using a grinding machine. The

powder of *Kigelia africana* fruits was weighed and kept for further phytochemical analysis. The powdered fruit were incorporated into four experimental diets.

***Extract preparation.***

Aqueous extraction was performed according to the method of Gwatido *et al.* (2018) with minor modification. A mass of 10g of powdered *Kigelia africana* fruits were mixed with 50mL of water, analytical grade in a 250mL of volumetric flask. The sample was shaken for 30 minutes on a horizontal shaker and then filter using Whatman's number one filter paper. The filtrate was collected for further analysis. The maceration process was repeated twice more with 30mL and 20mL with water. The collected filtrate for the whole process was mixed to obtain the aqueous extract.

***Animals and management***

Obtained from a reputable breeding farm, sixty weaned rabbit bucks, aged 6-8 weeks and averaging 868.52g, were acquired for the study. Randomly assigned to four dietary treatments, each group comprised twelve bucks serving as individual replicates. Housed in wooden metabolic cages with quarter-sized rods and wire mesh floors, the rabbits underwent a three-week acclimatization period receiving a soybean meal-based diet, water-based anti-stress, and antibiotics. Subsequently, they were fed treatment diets containing *Kigelia africana* fruit powder (100g/100kg of feed) and K. *africana* fruit extract (100mg/kg body weight) for 14 weeks. Feed and clean water were provided ad-libitum, with feeding twice daily at 8:00 am and 4:00 pm, allowing 120g/animal per day.

*Experimental design*

This experiment was laid out in Completely Randomized Design (CRD).

*Proximate analysis*

Samples of experimental diets, CSC and phytoconstituents of *Kigelia africana* were analyzed for proximate composition according to AOAC, (1990) Free gossypol was determined by high performance liquid chromatography (HPLC).

**Table 1: Gross composition of experimental diets and calculated nutrients**

| Ingredients (%) | TR1 | TR2 | TR3 | TR4 | TR5 |
|---|---|---|---|---|---|
| Maize | 43.57 | 43.24 | 43.23 | 43.24 | 43.24 |
| Soybean meal | 20. 43 | - | - | - | - |
| Cottonseed cake | - | 20.76 | 20.76 | 20.76 | 20.76 |
| Rice husk | 21.00 | 21.00 | 21.00 | 21.00 | 21.00 |
| Brewers dried grain | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 |
| Fish meal 72%) | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| Oyster shell | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| Bone meal | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Premix | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Salt | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 |
| Lysine | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| Methionine | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 |
| Total | 100 | 100 | 100 | 100 | 100 |
| **Calculated Nutrient** | | | | | |
| Crude protein (%) | 16.34 | 15.72 | 15.72 | 15.72 | 15.72 |
| Crude Fibre (%) | 10.22 | 11.57 | 11.57 | 11.57 | 11.57 |
| ME (Kcal/kg) | 2582 | 2436 | 2436 | 2436 | 2436 |
| Lysine | 0.86 | 0.64 | 0.64 | 0.64 | 0.64 |
| Methionine | 0.27 | 0.25 | 0.25 | 0.25 | 0.25 |

*Premix composition (per kg of diet): vitamin A, 12,500 IU; vitamin D3,2500 IU; vitamin E, 50.00k3, 2.50mg; vitamin B1, 3.00mg; vitamin B2, 6.00mg; vitamin B6. 6.00mg; niacin, 40mg; calcium pantothenate, 10mg; biotion, 0.08mg; vitamin B12,0.26mg; folic acid, 1.00mg; chlorine chloride, 300mg; manganese, 100mg; iron, 50mg; zinc, 45mg; copper, 2.00mg; meal. BDG- Brewer's dried grain, ME-Metabolizable Energy, T1- Control (SBM-based diet), T2- Cotton seed cake without *K. africana*, T3- Cotton seed cake with *K. africana* powder, T4- Cotton seed cake with *K. africana* extract, T5- CSC with K. *africana* fruits powder and extract.

***Collection of blood samples***

At the 14th week of the experiment, three rabbits per replicate were randomly selected from each treatment for blood collection. Blood samples were obtained from the marginal ear vein of the bucks

using sterilized needle and syringe. Two (2) mL of blood was collected into each plastic bottle containing anticoagulant, ethylene Diamine Tetra-acetic Acid (EDTA) for haematological examination.

***Heamatological parameters and determination***

The following haematological parameters were analyzed: Packed cell volume (PCV (%)). Hemoglobin concentration (Hb)-(g/dL), Red blood cell count (RBC ($x10^9/mm^3$), white blood cells count (WBC) ($x10^6/mm^3$), Mean corpuscular hemoglobin (MCH), Mean Corpuscular Volume (MCV), Mean corpuscular hemoglobin concentration (MCHC), White blood cell differential count (%) namely monocytes, lymphocytes, neutrophils, eosonophils and basophils. Packed Cell Volume (PCV) was determined by micro-haematocrit method (Dacie and Lewis, 1991). Heamoglobin concentration was determined by the calometry-cyanmethaemoglobin method. Red Blood Cell (RBC) counts was determined by improved Neubauer haemocytometer method (Igene and Iboh, 2004). While mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentrations (MCHC) were computed according to the method of Jain (1986). The differential white blood cell (WBC) counts will be obtained by making a differential smear stained with Wright's stain and the percentage counts taken for segmented neutrophils and lymphocytes (Dacie and Lewis, 1991).

*Statistical analysis*

Data obtained were subjected to one way analysis of variance (ANOVA) using SAS, (2000). Means were separated by Duncan's multiple range option of the same statistical package.

**Table 2. Haematological parameters of rabbit bucks fed of cottonseed cake-based diet supplemented with or without *K. africana* fruits.**

| Parameters | TR1 | TR2 | TR3 | TR4 | TR5 | SEM |
|---|---|---|---|---|---|---|
| PCV (%) | $44.00^{a}$ | $32.00^{c}$ | $39.00^{b}$ | $43.50^{a}$ | $45.08^{a}$ | 1.28 |
| RBC ($x10^{12}$/L) | $4.75^{a}$ | $3.50^{c}$ | $4.30^{b}$ | $4.60^{ab}$ | $4.78^{a}$ | 0.13 |
| HB (g/dL) | $14.50^{a}$ | $10.70^{c}$ | $12.85^{b}$ | $14.45^{a}$ | $14.52^{a}$ | 0.39 |
| MCH (pg) | $30.54^{b}$ | $30.55^{b}$ | $31.10^{ab}$ | $31.44^{a}$ | $31.45^{a}$ | 0.14 |
| MCV (fl) | $92.59^{b}$ | $91.37^{bc}$ | $94.25^{a}$ | $94.63^{a}$ | $94.69^{a}$ | 0.47 |
| MCHC (%) | $32.99^{b}$ | $33.44^{a}$ | $33.80^{a}$ | $33.22^{b}$ | $33.54^{a}$ | 0.18 |
| WBC ($x10^3$/µl) | $4.05^{c}$ | $2.55^{d}$ | $4.98^{b}$ | $6.05^{a}$ | $6.09^{a}$ | 0.27 |
| Neutrophil (%) | $53.00^{c}$ | $46.50^{d}$ | $64.50^{a}$ | $60.50^{b}$ | $63.05^{a}$ | 1.49 |
| Lymphocytes (%) | $41.00^{b}$ | $49.50^{a}$ | $33.00^{d}$ | $38.00^{c}$ | $35.00^{bc}$ | 1.38 |
| Basophils (%) | $2.00^{a}$ | $1.00^{b}$ | $1.00^{b}$ | $0.50^{b}$ | $0.7^{ab}$ | 0.17 |
| Monocytes (%) | $3.00^{a}$ | $2.00^{b}$ | $1.00^{cd}$ | $0.50^{d}$ | $0 .50^{d}$ | 0.19 |
| Eosiniphils (%) | $1.50^{a}$ | $1.00^{b}$ | $0.00^{c}$ | $0.00^{c}$ | $0.00^{c}$ | 0.12 |
| Platelets($x10^3$/µl) | $197.00^{c}$ | $209.50^{b}$ | $234.50^{a}$ | $237.00^{a}$ | $238.00^{a}$ | 4.59 |

$^{abcd}$ Means with different superscript along the same row are significantly different ($P<0.05$). SEM-Standard Error of Mean, TR1- Control (SBM-based diet), TR2- Cotton seed cake without *K. africana*, TR3- Cotton seed cake with *K. africana* powder, TR4- Cotton seed cake with *K. africana* extract, TR5- CSC with *K. africana* powder and *K. africana* extract, PCV- Packed Cell Volume, RBC- Red Blood Cell, HB- Haemoglobin,MCH- Mean Corpuscular Haemoglobin, MCV-Mean Corpuscular Volume, MCHC-Mean Corpuscular Haemoglobin Concentration, WBC- White Blood Cells

**DISCUSSION**

The decline in packed cell volume (PCV) and hemoglobin (Hb) levels in male rabbits fed cottonseed cake (CSC) diets without supplementation indicates a harmful impact of CSC-derived gossypol, potentially failing to meet the animals' nutritional needs and compromising their health. Despite this, PCV and Hb levels remained within normal ranges for male rabbits. Reduced red blood cell (RBC) count in bucks on CSC-based diets without supplementation suggests mild anemia due to insufficient protein intake, although hemopoiesis wasn't hindered. Similar findings were reported with vitamin E supplementation, linking RBC reduction to residual toxic effects of CSC-gossypol (Amao et al., 2012). Rabbits given CSC diets with *Kigelia africana* fruit extract exhibited increased RBC values, suggesting adequate vitamin and mineral intake for hemoglobin synthesis. Lower leukocyte counts in CSC-based diets indicate potential immune compromise without reaching dangerous levels, while higher

leukocyte values in the *Kigelia africana* fruit extract treatment imply improved phagocytic properties against gossypol toxicity, aligning with previous findings in broilers (Adeyemo and Longe, 2007). Mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) values were higher than normal for young adult buck rabbits, while mean corpuscular hemoglobin concentration (MCHC) fell within the normal range, consistent with earlier studies in rabbit bucks fed CSC (Amao et al., 2012). Elevated monocyte count in CSC diets without *Kigelia africana* fruit supplementation suggests gossypol's proliferative impact, whereas lower monocyte values in supplemented groups hint at the fruit's potential to inhibit gossypol's adverse effects. The observed antimicrobial actions of macrophages, mediated by dietary antioxidants, suggest that bucks on CSC diets with *Kigelia africana* fruits are more likely to effectively combat infections based on monocyte count. Flavonoids' free radical scavenging properties may play a protective role.

## CONCLUSION.

The findings also underscore the health risks associated with feeding CSC diets without K. *africana* fruit supplementation.

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