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Growth performance, hematology and serum biochemical indices of growing rabbits fed diets containing graded levels of *Cola rostrata* (monkey cola) seed meal

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Abstract

The study investigated the growth performance, hematology and serum biochemical indices of growing rabbits fed diets containing graded levels of Cola rostrata (monkey cola) seed meal (CRSM). Sixty (60) six to seven weeks old cross breed (Chinchilla and New Zealand white) growing rabbits were grouped into six treatments, and each treatment had two replicates containing five 5 rabbits per replicate in a Completely Randomized Design (CRD). Diets containing 0, 20, 40, 60, 80 and 100 percent of CRSM were formulated and denoted as T_1 T_2, T_3, T_4, T_5, and T_6 respectively. T_1 and T_2 had significantly ($p<0.05$) higher feed intake than other treatment groups. The final body weight, total weight gain and feed conversion ratio of the animals on T_3 (40% inclusion level of Cola rostrata) were significantly higher than the control and other treatment groups. T_3 and T_4 had significantly ($p<0.05$) higher values for PCV, Hemoglobin, red blood cells, total protein, albumin and globulin. T_3 also had significantly ($p<0.05$) lower urea and creatinine levels than other treatments. The ALT, AST and Cholesterol values obtained in this study were statistically ($p<0.05$) higher for T_1 than the groups with CRSM, while serum glucose was statistically ($p<0.05$) higher for T_3 than other treatment groups. In conclusion, for improved feed conversion ratio, enhanced serum protein bio-availability that would culminate in better final body weight and body weight of growing rabbits, diet that contains 40% Cola rostrata a replacement for maize, is recommended.

Key words: *Growth Performance, Hematology, Serum Biochemical Indices Rabbits and Cola rostrata*

Description of Problem

The Nigerian animal industry is hindered by the limited supply and high cost of conventional feed resource and therefore, there is urgent need to search for and exploit other alternative feed resources with appreciable amount of nutrients (energy and protein) which are cheap and available and can replace the costly feed ingredients especially maize. Studies by (1), (2), (3) and (4) had earlier reported the potential and utilization of some non-conventional feed resources in the feed formulation for animals. However, *Cola rostrata* seed meal which is one of such ideal non-conventional feedstuff has not been well utilized as feed ingredient for animal production especially rabbit in Nigeria.

Cola rostrata (Monkey cola) has been utilized as food by humans in a number of West African countries where it has been found. According to (5) the plant *Cola rostrata* belongs to the family *sterculiaceace*, genus *Cola* and species; *rostrata.* Fruits obtained from *Cola rostrata* tree are important supplement to the diet of humans especially children (6).

The seed of C*ola rostrata* is produced by an evergreen perennial tree called *Cola rostrata* plant (7). There are about 50 known

species of *Cola* plants in West Africa and over 600 species have been described in Africa (5). *Cola rostrata* is one of the popular species found in the lowland rain forest and coastal areas in Nigeria while others include; *Cola nitida,* and *Cola accuminata* (8). The mesocarps of *Cola rostrata* are widely consumed in the southern parts of Nigeria as ordinary fruits however; the seed left after the removal of the juicy pulp is of no importance to the consumers and therefore discarded as waste (6). The starch component of the seed is about 83.42% and gives an indication that the seed has a nutritional potential as an energy source (6). However, its use is limited due to the anti-nutritional components present in the seed.

As it is common with most non-conventional feedstuffs, a relatively large number of anti-nutritional substances have been reported in *Cola rostrata* seed. Various anti-nutritional constituents associated with *Cola rostrata* seed include; oxalic acid, phytic acid, hydrocyanic acid, tannin and caffeine. (9, 8 & 10) but (1) and (11) reported that boiling the seeds for 30 minutes will eliminate the anti-nutrients and enhance its inclusion in animal diets as replacement for maize. The seed meal has been used as replacement for maize in the diets of quails and snails by (1) and (11) with impressive results, however, its use for rabbits in general and growing rabbits specifically have not been reported. This study was therefore carried out to assess growth performance, hematology and serum biochemical indices of growing rabbits fed diets containing graded levels of *cola rostrata* (monkey cola) seed meal.

Materials and Methods

Experimental site

The experiment was conducted at the Rabbitary unit of the Teaching and Research Farm of the Akwa Ibom State University Obio Akpa Campus, Oruk Anam Local Government Area of Akwa Ibom State, Nigeria. Obio Akpa is located within the southern zone of Nigeria at Latitude of $4^0$50'N of Equator and Longitude of $7^0$45'E and $7^0$55'E. The area is in the hot humid tropics with a climate that is characterized by two seasons (Rainy and Dry seasons) (12).

Preparation of experimental feed ingredients.

Cola rostrata seed were collected from Obio Akpa Village and were washed in cleaned tap water to remove dirt. The seeds were boiled for 30 minutes in a pot of water that had been pre-heated to boiling point. The boiled seeds were thereafter spread on corrugated roofing sheets to cool-off before cutting them into sizeable chips for easy drying in the sun. The dried seeds were then milled using Hammer mill (with 0.5 mm screen) and stored for diet formulation.

Forages and their preparation

The forages used for feeding the rabbits were *Tridax procumbens* (Tridax), *Calopogonium mucunoides* (Calopo leaves) and *Centrosema pubescens* (Centrosema leaves). The forages were harvested within the vicinity of the University, washed to remove dirt, drained and weighed with a Salter electronic scale before being fed to the animals.

Formulation of diet and treatment groups

Six (6) experimental diets were formulated for growing rabbits. The control diet (T_1)contained maize as the main energy source and zero (0) percent *Cola rostrata* seed meal. The other five test diets (T_2. T_3, T_4, T_5, and T_6) were formulated to contain 20, 40, 60, 80 and 100 percent C*ola rostrata* seed meal, respectively in replacement for maize.

Samples of the six diets used in the feeding trials experiment were subjected to proximate analysis using the methods of (13). Samples were analyzed in triplicates.

Table 1: Composition of concentrate diets fed to weaner rabbits

Feed ingredient (%)	T_1 (0%)	T_2 (20%)	T_3 (40%)	T_4 (60%)	T_5 (80%)	T_6 (100%)
Soyabean meal	26.64	26.64	26.64	26.64	26.64	26.64
Fish meal	6.66	6.66	6.66	6.66	6.66	6.66
Cola rostrata seed meal	0.00	8.47	16.94	25.41	33.88	42.35
Maize	42.35	33.88	25.41	16.94	8.47	0.00
Wheat offal	15.00	15.00	15.00	15.00	15.00	15.00
Bone meal	2.50	2.50	2.50	2.50	2.50	2.50
Oyster shell	3.00	3.00	3.00	3.00	3.00	3.00
Salt	0.10	0.10	0.10	0.10	0.10	0.10
Palm oil	3.00	3.00	3.00	3.00	3.00	3.00
Methionine	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin premix	0.25	0.25	0.25	0.25	0.25	0.25
Total	100	100	100	100	100	100

Note: Each 2.5kg of grower finisher vitamins and mineral premix contains 10,000,000 iu of Vit A. 2,000,000 iu of Vit D3; 10,000 iu of Vit. E. 2,200 mg of Vit. K; 100 g of Vit. C; 1500 mg of B1; 80 g of manganese, 50 g of Zinc; ; 5 g of Copper, 15500 mg of Niacin; 10mg of B12; 5000mg of Panthothenic acid, 500mg of Folic acid, 20 mg of Biotin, 125 mg of Antioxidant; 200g of Selenium; 1.5mg of Iodine 200mg of Cobalt and 200 mg of Choline chloride

Experimental animals, management and design

Sixty (60) six to seven weeks old cross breed (Chinchilla and New Zealand white), growing rabbits (12 males and 48 females) supplied by a farm in Uyo, Akwa Ibom State, Nigeria were randomly assigned to the six dietary treatments comprising ten (10) growing rabbits per treatment replicated twice (5*2 * 6) over a period of 13 weeks. The animals were managed intensively in a metal three tiers cage of 6 cells per tier with dimensions of each cell being 1.5m X 0.6 m X 0.6m and were equipped with proper and adequate drinking and feeding troughs. Feeding of the rabbits was done twice daily (morning and evening). The concentrates were moistened to reduce dustiness and weighed with Slater Electronic scale before being fed to the animals. The forages were also fed to the rabbits. A weighed portion of the forages and concentrates fed to the animals were kept in an empty space in the hutch and were re-weighed in the morning to obtain the moisture loss. This was deducted from the left over feed to account for the weight loss due to moisture loss and also to ascertain the actual amount of feed consumed by the rabbits.

Data collection

Data were collected on initial body weight and weekly body weight, while weekly feed intake per rabbit in the treatments were computed from the daily feed intake. The weekly feed intake and weight gain were used to determine the Feed Conversion Ratio (FCR) for each treatment group.

Blood collection:

At the end of the experimental period, four rabbits were randomly selected from each treatment groups and 5mls of blood samples were collected from lateral Saphenous vein located in the lower rear hind leg as recommended by (14). Prior to the blood collection the rabbits were restrained and the hair from the lateral Saphenous vein was shaved making Saphenous vein visible. It was cleaned and dried with alcohol and sterile gauze. A sterilized disposable 20 gauge needle on a 5mls syringe was inserted into the occluded vessel and blood aspirated into the syringe pre-filled with air according to the procedure described by (15). Three (3mls) of samples were transferred to labeled sample collection tubes containing ethylene diamine-tetra–acetic acid (EDTA) as anticoagulants for hematological analysis. Blood samples were also collected in plain bottles without anticoagulants for serum biochemical analysis.

Proximate and Statistical Analysis

The six diets formulated and the mixtures of forages were subjected to proximate analysis using the method of (13). All the data collected were subjected to one-way analyses of variance (ANOVA). . Duncan's New Multiple Range test (16) was carried out to separate the means of the parameters measured. The statistical analysis was done using statistical software of (17).

Results and Discussion

Proximate composition of the experimental diets from (Table2) indicated a decrease in crude protein levels and increase in crude fiber, ash and nitrogen free extract as the level of inclusion of *Cola rostrata* seed meal in the diet increases. The result agrees with the report of (6) that *Cola rostrata* is a poor protein source and a higher fiber, mineral and energy source than maize.

Table 2: Proximatea Analysis of diets

S/N	Crude Protein (CP %)	Ether Extract (EE %)	Crude Fibre (CF %)	Ash (%)	Nitrogen Free Extract (NFE %)	Residual Moisture	SEM
T_1	19.76^{a}	6.34^{a}	5.25^{d}	7.00^{c}	45.50^{d}	11.2	1.06
T_2	19.68^{a}	6.30^{a}	9.0^{cd}	7.20^{c}	47.25^{d}	11.4	2.11
T_3	19.62^{a}	6.30^{a}	10.75^{c}	7.24^{c}	50.32^{c}	0.88	
T_4	19.25^{b}	6.30^{a}	12.50^{b}	7.50^{b}	51.43^{c}	11.4	1.73
T_5	19.25^{b}	6.30^{a}	13.25^{b}	7.69^{b}	58.00^{b}	11.0	1.26
T_6	19.05^{b}	6.30^{a}	14.75^{a}	9.04^{a}	61.00^{a}	11.4	2.10

Table 3: Performance of growing rabbits fed grade levels *Cola rostrata* seed meal (CRSM)

	T_1	T_2	T_3	T_4	T_5	T_6	SEM
Initial Body Weight (IBW) (g)	720.80^{a}	720.20^{a}	720.80^{a}	720.80^{a}	720.80^{a}	720.40^{a}	54.81
Mean Feed Intake (MFI) (g)	575.58^{a}	543.44^{a}	428.34^{c}	532.41^{a}	487.92^{b}	470.34^{b}	10.30
Final Body Weight (FBW) (g)	1244.60^{b}	1324.40^{b}	1539.60^{a}	1482.40^{b}	1246.00^{b}	1274.60^{b}	3.30
Body Weight gain (BWG) (g)	523.80^{b}	604.20^{b}	818.80^{a}	761.60^{a}	525.20^{b}	554.20^{b}	9.35
Feed Conversion Ratio (FCR) (g/day/animal)	1.10^{a}	0.78^{a}	0.53^{b}	0.70^{a}	1.01^{a}	0.85^{a}	2.50

^{abc} Means on the same row with different superscript are significantly different

Although the initial body weight of the animals were the same at the commencement of the experiment, the final body weight and the total weight gain of the animals on T_3 (which had 40% inclusion level of CRSM) was significantly ($p<0.05$) higher than the control (T_1) and other diets (T_2,T_4,T_5 &T_6) . Feed intake reduced with increased level of inclusion of *Cola rostrata* which probably could be attributed to increase in the levels of energy (NFE value from Table 2; 45,50, 47.25, 50.32 51.43, 58.00 and 60,00 for T_1 T_2, T_3, T_4,T_5 and T_6 respectively) contributed by CRSM in the diet. In addition, the Feed Conversion ratio (FCR) of T_3 was significantly ($p<0.05$) different among the treatment groups. The results from this study agrees with the findings of (18), who reported that dietary energy level is a major factor influencing feed intake and feed conversion ratio . They added that, animals will consume more of low energy feed at a constant growth rate while they consume less of higher energy feed to obtain it nutrient requirements thereby improving feed conversion ratio.

Hematology of growing rabbits fed experimental diets

The hematological results of the rabbits fed graded levels of CRSM are presented on Table 4. The values obtained from this study are good indicators of the physiological status of animals in relation to the treatment given to them and the values were within the normal range for rabbit as reported by (19) and (20).

Table 4: Haematological profile of growing rabbits fed graded levels of *Cola rostrata* seed meal (CRSM).

Parameter	T_1 (0%)	T_2 (20%)	T_3 (40%)	T_4 (60%)	T_5 (80%)	T_6 (100%)	SEM
PCV (%)	22.50^{c}	20.00^{cd}	25.50^{a}	27.00^{a}	23.00^{b}	22.00^{c}	1.44
Haemoglobin (g/dl)	7.25^{b}	7.40^{b}	8.70^{a}	7.05^{b}	7.25^{b}	6.65^{c}	1.97
RBC (x106/µL)	7.65^{a}	8.00^{a}	8.90^{a}	8.50^{a}	8.25^{a}	8.20^{a}	1.45
WBC(x103/µL)	8.20^{b}	8.60^{b}	8.85^{b}	10.10^{a}	8.50^{b}	8.75^{b}	0.65
Neutrophils (%)	60.00^{a}	54.50^{b}	56.50^{b}	52.00^{b}	55.50^{b}	61.00^{a}	29.58
Lymphocytes (%)	38.50^{b}	40.00^{b}	40.50^{b}	45.00^{a}	37.50^{b}	34.00^{b}	6.57
Monocytes (%)	1.50^{d}	5.00^{a}	3.00^{c}	2.00^{cd}	2.00^{cd}	4.00^{b}	1.49
Eosinophils (%)	0.00^{c}	0.50^{b}	0.00^{c}	1.00^{a}	0.00^{c}	1.00^{a}	0.46
Basophils (%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00
MCV (fl)	32.25^{b}	36.05^{a}	28.80^{c}	24.25^{d}	27.05^{c}	32.50^{b}	5.49
MCH (pg)	104.00^{b}	129.45^{a}	99.15^{b}	82.95^{c}	88.75^{c}	81.70^{c}	16.87
MCHC (g/dl)	322.20^{b}	368.20^{a}	340.30^{b}	288.25^{cd}	318.50^{c}	301.25^{c}	24.37

abcd Means different superscript on the same row are significantly difference ($P>0.025$)

SEM- standard error of mean, PCV= Packed cell volume, RBC= Red blood cell, WBC= White blood cell, MCV= Mean corpuscular volume, MCH= Mean corpuscular haemoglobin, MCHC= Mean corpuscular haemoglobin concentration.

The PCV values recorded for the various treatment groups is viewed as coefficient of efficiency of various graded levels of CRSM in enhancing the animals' performance. The PCV was significant ($p<0.05$) and higher for T_3 and T_4 when compared with other treatments. (21) opined that animals with good blood concentration (PCV) are likely to show good performance.

Hemoglobin, the red blood cells and

their mean concentration were also significantly ($p<0.05$) higher in the T_3 and T_4 treatments among the treatment groups. The result would imply that these treatments were efficient in the synthesis of the iron-containing protein in the blood of the experimental rabbits. The white blood cells and their differentials (neutrophils, eosinophils, basophils, lymphocytes, monocytes) major functions are to fight infections, defend the body by phagocytocis against invasion by foreign organisms and to produce or at least transport and distribute antibodies in immune response. Although, there were significant differences in these parameters, their values were within the normal ranges for rabbits. This shows that the treatments were not deleterious to the experimental animals.

Serum biochemistry of the growing rabbits fed graded levels of experimental diets

The results of serum biochemistry of the rabbits fed graded levels of CRSM are presented on Table 5. All the serum biochemical parameters measured were in normal ranges for rabbits as stated by (22). The normal range for these serum biochemical indices is an indication that the treatments were not deleterious to the experimental animals. T_3 and T_4 had significantly ($p<0.05$) higher total protein than other treatments. This probably shows that this group received optimal and maximal level of protein which culminated in tissues synthesis and increase in the body weight of the animals.

Table 5: Serum biochemical indices of growing rabbits fed graded levels of *cola rostrata* seed meal

Parameter	T_1(0%)	T_2(20%)	T_3(40%)	T_4(60%)	T_5(80%)	T_6(100%)	SEM
Total protein (g/dl)	5.95^{c}	7.30^{b}	8.35^{a}	8.75^{a}	7.25^{b}	6.45^{c}	0.56
Albumin (g/dl)	4.10^{c}	5.00^{b}	5.45^{a}	6.75^{a}	4.90^{b}	4.50^{b}	0.81
Globulin (g/dl)	1.85^{b}	2.30^{b}	2.90^{a}	2.00^{b}	2.35^{b}	1.95^{b}	0.43
Urea (mg/dl)	9.80^{a}	9.05^{a}	7.85^{b}	7.70^{b}	9.70^{a}	9.45^{a}	0.92
Creatinine (mg/dl)	1.15^{a}	1.10^{a}	0.50^{bc}	1.00^{a}	1.05^{a}	1.05^{a}	0.17
AST (U/L)	52.00^{a}	45.00^{b}	46.50^{b}	45.50^{b}	50.50^{a}	44.00^{b}	5.38
ALT (U/L)	27.00^{a}	23.50^{b}	20.00^{c}	24.50^{b}	17.00^{d}	21.00^{c}	3.73
Cholesterol (mg/dl)	95.50^{a}	91.50^{b}	89.00^{bc}	80.50^{d}	92.00^{b}	88.00^{c}	4.12
Glucose (mg/dl)	8.00^{b}	8.55^{b}	11.25^{a}	10.50^{a}	10.75^{a}	7.50^{c}	0.93

$^{a, b, c, d:}$means different superscript on the me row are significantly difference (P>0.025)

SEM- standard error of mean, AST= Aspartate aminotransferase, ALT= Alanine aminotransferase.

Total protein measures the combined amount of two types of proteins, albumin and globulin. The control (T_1) had significantly ($p<0.05$) lower albumin and globulin values than other treatment groups and this is a pointer to why the group had the least final body weight. Albumin helps in tissue synthesis and healing (22). T_3 had significantly ($p<0.05$) lower urea and creatinine level than other treatments. A normal hydrated animal will have low values of urea and creatinine (22).

The author further added that very high values of urea and creatinine is a pointer to over hydrated (too much of water) or a challenge of the kidney. The Alanine-amino tranferase (ALT) and Aspartate aminotransferase (AST) also known as serum glutamic oxaloacetate (GOT) and glutamic pyruvic transaminase (GPT) respectively are normally supposed to be low (23) and (22).

Treatment three ($T_{3)}$ had significantly ($p<0.05$) low values for these parameters

than other treatment groups. This is an indication that rabbits on this treatment were healthier than other groups it is the reason this group had the best performance in terms of final body weight and body weight gain.

The blood cholesterol of the T_1 group was significantly ($p<0.05$) higher than the treatments with CRSM although not threatening to animals' organs because it was within the normal range for rabbits. Cholesterol is biosynthesized by all animal cells and is an essential structural component of animal cell membranes. More so, cholesterol also serves as a precursor for the biosynthesis of steroid hormones (24). The membrane remains stable and durable allowing for normal rigidity, growth of the experimental animal. Glucose was also statistically ($p<0.05$) higher for T_3 and other treatments with CRSM than the control. Glucose is a source of energy in cell for proper functioning, and the regulation of metabolism (24). Adequate glucose in T_3 probably supported the high body weight of rabbits in this treatment group.

Conclusion and Application

1. CRSM inclusion in diets of rabbits at 20% to 100% can enhance their growth performance.
2. However, growth performance, hematology and serum biochemical indices were better for rabbits fed 40% CRSM in replacement for maize in the diet of growing rabbits.
3. It is concluded that up to 40% CRSM can be included in the diets of growing rabbits for optimum performance of growing rabbits.

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