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Haematological and serum biochemical indices of male wistar albino rats fed raw and processed monkey cola (*Cola rostrata*) seed meal

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***Targeted audience:** Animal Nutritionists and Animal Biochemists and Farmers*

Abstract

This study was undertaken to evaluate the effect of feeding processed and raw Cola rostrata seed meal (CRSM) on the haematological and serum biochemical indices of some organs (liver, kidney, duodenum, jejunum and ileum) of male wistar albino rats. Cola rostrata seed was processed using different methods; boiling, boiling/fermenting, fermenting, and toasting to eliminate the anti-nutritional factors in it. Six (6) experimental diets were formulated with diet 1 (Control) containing 0% CRSM, while diets 2, 3, 4, 5 and 6 contained boiled, boiled/ fermented, fermented, toasted and raw CRSM respectively. Sixty (60) weanling male rats aged 6-7 weeks, were randomly divided into the six (6) dietary treatments groups, which were replicated twice with five (5) rats per replicate in a Completely Randomized Design (CRD). The experiment lasted for 21 days. The results indicated that there were significant differences ($P<0.05$) in packed cell volume (PCV) and Haemoglobin among the treatment groups. The raw seed (diet 6) resulted in the reduction of the serum total protein, serum albumin and serum globulin. It is concluded that Cola rostrata seed boiled for 30 minutes can be used as feed ingredient in replacing maize in animal diets without adverse effects on haematological and serum biochemical indices studied.

Keywords: *Cola rostrata* seed meal; haematology; serum biochemistry; and male wistar rats

Description of problem

Determining the safety of any new feed material to be used by humans and animals using the appropriate toxicity test approved by government regulatory agencies such as; National Agency for Food and Drug Administration Control (NAFDAC) and the United States Food and Drug Administration (USFDA) amongst others, cannot be over emphasized. The importance of evaluating the nutrient content of feed with animal assays as compared with alternative methods of analysis (i.e. chemical or microbiological) is well recognized. Researchers use animal feeding trials to test whether their products are safe for animal and human consumption. This necessitates the use of species such as albino rats that can be compared with human for factors such as metabolism, excretion, absorption and distribution of the test materials (1). Rats as one of the animal species, has proven to be extremely useful in toxicological/pharmacological research because of the many similarities they have with the human metabolic pathways. More so, many anatomical and physiological characteristics of rats and humans are similar and therefore allow for comparison in terms of absorption, excretion and distribution (2).

It was asserted (3) that the high cost of animal protein sources (meat and eggs) as a result of rising prices of animal feed stuff worldwide has engendered considerable efforts by researchers, to identify alternative low cost and readily available non-conventional livestock feedstuff that could relieve the conventional feedstuffs, especially maize (known to be in serious competition as food

for man and feed for animal). Monkey cola (*Cola rostrata*) seed meal can be said to be one of such non-conventional feedstuff that has a great potential for replacing maize in livestock diets. The mesocarps of monkey cola widely consumed in the southern parts of Nigeria as ordinary fruits, while the seeds are often thrown away (4 & 5). As reported by (6 & 7), the starch component of the seed is about 83.42% and therefore a potential energy source in animal diets. However, its use appears limited as a result of the anti-nutrients found in it (6 & 8).

Anti–nutritional contents of feedstuff have deleterious effect on human/animal nutrition mainly by aiding the risk of renal calcium absorption, causing corrosive gastro enteritis and renal damage and formation of kidney stones (9). Therefore, using appropriate methods of processing of the materials helps in reducing the anti-nutritional content of the feed material thus enhancing its usage in animal feed formulation. Works by several authors indicate that boiling, toasting and fermenting of seeds lower their anti-nutritional contents and increase their crude protein (CP), Nitrogen free extract (NFE) and crude fat (CF) contents (10,11 &12). Monkey cola *(Cola rostrata)* seed as a potential alternative and non-conventional animal feedstuff has received very little research attention and therefore, this study was carried out to determine the effect of feeding the raw and processed (boiled, boiled/fermented, fermented and toasted as a replacement for maize as energy source) seed meal on haematological and serum biochemical indices of male Wistar albino rats.

Materials and Method

Experimental sites

The study was carried out in the Post Graduate Laboratory of the Department of Animal Science in Akwa Ibom State University, Obio-Akpa Campus, Oruk Anam Local Government Area, Akwa Ibom State, Nigeria. Obio Akpa which is located within the hot humid tropics in the southern zone of Nigeria at a Latitude of 4^{O}50N of the Equator and Longitude of 7^{O}45E is and 7^{O} 55E of the Greenwich Meridian has a climate characterized by two seasons (rainy and dry seasons). The rainy season spans between April and October while the dry season spans between November and March. Temperatures are uniformly high throughout the year ranging between 26^{O}C and 28^{O}C. Solar radiation ranges from 4.11 to 4.95mm, partly because of the high values of insulation and temperature **(**13**).**

The proximate composition of Monkey c*ola* seed meal samples and the experimental diets were determined by the method described by (14). From the proximate analysis, the Gross Energy (GE) of the feed was calculated according to (16) by applying the equation;

G.E= 5.72 (%CP) + 4.79 (%CF) + 9.50 (%EE) + 4.03 (%NFE)…….....(1)

The Metabolizable Energy (ME) was calculated according to **(**15**)**, by applying the equation M.E= 37(%CP) + 81.8 (%EE) + 35.5 %NFE)…….....…(2)

Sample Preparation

Mature monkey cola (*Cola rostrata*) seeds (yellow variety) which were obtained from Obio Akpa community and used in this experiment were washed in clean tap water to remove dirt and thereafter chopped into chips for easy drying and spread under the sun. These sundried chips were then divided into five batches. The first batch of the chips was the raw *Cola rostrata* seed, while the remaining four (4) batches were processed using locally adaptable methods (boiling, toasting and fermenting) to engender the elimination or reduction of anti-nutritional factors (oxalate, phytate, tannin, caffeine,

hydrocyanide) in the raw seed as was reported by (6&8). From these four batches, one of them was processed, by boiling for 30 minutes in a metal pot with water that was pre-heated at a temperature of 100^0C. The next batch was toasted in a metal (with sand that was pre-heated to 100^0C) pot for 30 minutes. Another batch of the chips was boiled in a metal pot for 30 minutes at 100^0C and then soaked in a plastic container with tap water for 3 days at room temperature to ferment. The last batch was also only soaked for 3 days in plastic containers with tap water at room temperature for fermentation to occur.

Table 1: Proximate composition of experimental diets

	Processing Method/Treatment					
Ingredients	Control	Boiled	Boiled/fermented	Fermented	Toasted	Raw
Maize	91.49					
Monkey Cola (Cola rostrata)	0	88.26	87.90	88.04	87.90	87.79
Soybean	1.41	3.99	4.28	4.17	4.28	4.37
Fish meal	0.35	1.0	1.07	1.04	1.07	1.09
Palm oil	3.0	3.0	3.0	3.0	3.0	3.0
Salt	0.25	0.25	0.25	0.25	0.25	0.25
Oyster shell	1.0	1.0	1.0	1.0	1.0	1.0
Bone meal	2.0	2.0	2.0	2.0	2.0	2.0
Vitamin premix*	0.5	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100	100
Calculated Nutrients						
Crude Protein (%)	10	10	10	9.99	10	10
Metabolizable Energy (ME)(Kcal/g)	3239.90	3256.52	3177.48	3177.14	3194.50	3175.04
Analysed nutrients (%)						
Crude Protein (CP)	10.09	10.02	9.95	9.97	10.08	9.91
Ether Extract (EE)	2.18	3.11	2.98	2.90	3.02	2.83
Crude fibre (CF)	1.36	1.08	1.27	1.16	1.33	1.87
Calcium (Ca)	0.97	1.07	0.99	0.95	0.86	0.94
Phosphorus (P)	2.04	2.57	2.31	1.93	2.84	2.15
Nitrogen Free Extract (NFE)	77.19	78.93	76.18	76.04	75.86	75.73
Metabolizable Energy (ME)(Kcal/g)	3078.90	3427.16	3316.30	3305.53	3313.03	3286.58

Note: Each 2.5kg of grower finisher vitamins and mineral premix contains 800,000 iu of Vit. A, 1600,000 iu of Vit. D3; 5,000 iu of Vit. E; 2000 mgr of Vit. K; 1500 mgr of B1; 4000 mgr of B2; 80 gr of manganese, 50 gr of Zinc; 20gr of Iron; 5 gr of Copper, 15000 mg of Niacin; 10mg of B12; 5000mg of Panthothenic acid, 5000mgr of Folic acid, 20 mgr of Biotin, 125 mgr of Antioxidant; 200gr of Selenium; 200mgr of Cobalt and 200 mgr of Choline chloride

The thermally processed seeds (boiled and toasted) were spread on a clean corrugated iron roofing sheet to cool. Thereafter, the raw and all the processed portions were sun dried, milled using a 0.05 mm laboratory hammer mill and thereafter stored in a well labelled container for feed formulation.

Feed formulation

A total of six experimental diets were formulated as shown in Table1. Diet 1 which served as control was prepared with maize as the major source of energy with no *Cola rostrata* seed meals. Diet 2 was prepared with the boiled seed meal while Diet 3 was

formulated with boiled then fermented seeds and Diet 4 was prepared with only fermented seeds. Diets 5 and 6 were prepared with toasted and raw seeds respectively. The differently processed *Cola rostrata* seed meals completely replaced maize as energy source in those diets. The protein sources used in the formulation were Soybean and fish meal. All the diets were formulated to furnish the required 10% crude protein required by rats for optimum growth.

Management of experimental animal and design

Sixty weanling male albino rats age between 6 and 7 weeks were purchased from the Animal house of the Department of Biochemistry, University of Calabar and used in the 21 days feeding trial. The rats were allotted separately to each experimental diet in a completely randomized design (CRD) and each treatment had two replicates with five rats each. The rats were housed in metabolic cages with access to clean water and treatment diets *ad-libitum* under standard condition of 27^0C ambient temperature and 45% relative humidity daily with a 12hr light day cycle.

Haematology and Serum chemistry evaluations

At the completion of the feeding period, all the rats were sedated by chloroform asphyxiation and bled. Five millilitres (5mls) of blood samples were collected into an Ethylene diamine tetra-acetic acid salt (EDTA) labelled bottles for the haematological parameters; Packed Cell Volume (PCV), Haemoglobin (Hb), Red Blood Cell (RBC) and White Blood Cell (WBC) counts, Neutrophils, Lymphocytes, Monocytes, Eosinophils, Basophils, mean Corpuscular volume (MCV) and mean Corpuscular Haemoglobin (MCH). The second set of blood samples were collected in heparin tubes for serum chemistry evaluation which included total protein, albumin, globulin, thiocinide, urea, creatinine, Aspartate amino-transferase (AST), Alanine amino-transferase (ALT), Alkaline phosphatase (ALP), glucose and cholesterol. The cell counts were carried out by the use of haemocytometer; Hb and PCV were determined using standard methods of (17) while the serum chemistry indices were determined as described by (18).

Statistical analysis

All the data generated from the haematological and serum biochemical studies were subjected to a One-way Analysis of Variance (ANOVA) and the Duncan's Multiple Range Test (DMRT) was used for mean separation where differences occurred (19).

Results and Discussion

Haematology

Although the result in Table 2 shows significant differences in most of the haematological indices of the rats, some of the treatments groups recorded values that were within the normal range reported by (20). Packed cell volume (PCV) which measures the percentage volume of blood was significantly lower ($p<0.05$) in the raw (37.67%) than the control and boiled groups (43.33% and 44.33%) respectively. The low PCV in the raw seed group might be indicating the poor protein supply of the seed. According to (6) cola seed contain phytate (an anti-nutrient) that binds protein and makes it unavailable to the animal.

Table 2: Haematological indices of rats fed raw and differently processed Monkey cola (*Cola rostrata)* seed meal (mg/100g)

	PROCESSING METHODS						
PARAMETERS	**Control**	**Boiled**	**Boiled/ Fermented**	**Fermented**	**Toasted**	**Raw**	**SEM**
Packed Cell Volume (PCV) %	43.33^{a}	44.33^{a}	41.00^{b}	37.67^{b}	37.67^{b}	37.67^{b}	0.46
Haemoglobin (Hb) mg/dl	8.23^{b}	10.06^{a}	9.57^{a}	8.61^{b}	8.37^{b}	9.37^{b}	0.30
Red blood Cell (RBC) x10^{3}mm	9.97^{a}	10.90^{a}	10.23^{a}	10.17^{a}	9.37^{b}	8.50^{b}	0.22
White Blood Cell (WBC) x10^{3}mm	3.00^{b}	3.17^{b}	3.60^{b}	3.76^{b}	4.50^{a}	4.90^{a}	0.20
Neutrophils (x10^{3}µl)	9.00^{b}	11.00^{b}	11.33^{b}	11.67^{b}	16.33^{a}	20.00^{a}	0.73
Lymphocytes (x10^{3}µl)	78.69^{b}	80.23^{b}	86.67^{a}	86.67^{a}	87.00^{a}	89.00^{a}	0.88
Monocytes (x10^{3}µl)	0.00	1.67^{b}	1.67^{b}	1.67^{b}	2.00^{b}	3.33^{a}	0.36
Eosinophils (x10^{3}µl)	0.00	0.00	0.00	0.00	0.67^{a}	1.33^{a}	0.14
Neutrophils (x10^{3}µl)	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean Corpuscular Vol. (MCV) f/l	44.27^{a}	45.00^{a}	44.63^{b}	41.58^{b}	41.58^{b}	41.58^{b}	1.29
Mean Corpuscular Haemoglobin (MCH) mg/dl	19.23^{b}	25.07^{a}	23.31^{a}	20.47^{a}	19.90^{b}	21.60^{a}	0.81

Note: ^{abc} Means along the rows with different superscripts are significantly different (P<0.05)

Apart from the rats on the toasted seed treatment, the Hb of the other treatment groups were significantly (p<0.05) higher than those on the control diet. The results also, showed a similar pattern with respect to MCV and MCH. The cola seed have pro-vitamin A, which probably can be responsible for the blood pigmentation (6).

. The WBC in the raw and toasted seed groups were significantly (p<0.05) higher than the control and boiled groups. The neutrophils, lymphocytes, eosinophils monocytes and basophils (these are types of white blood cells) also followed the same pattern as the WBC. Since the WBC helps to fight infection, the elevated levels therefore raised a red flag of the probability of the animals being infected.

The control and boiled seed treatment groups in this study recorded values of parameters that were all within the range reported by (20).

Serum biochemistry

The result of serum biochemistry of the experimental rats is presented in Table 3. Total protein was significantly (p<0.05) lower in the raw group than the control and boiled groups respectively. This is an indication of the poor quality of the protein it supplies. (21) stated that protein reserve in an animal gives an indication of the total protein value in the diet.

Serum albumin and globulin which are also types of protein followed the same pattern with total protein. They were significantly (p<0.05) lower in the raw seed group than the control and boiled groups. The lower values of the albumin and globulin of rats in the raw seed diet is a reflection of the inadequacy of the dietary protein. This reduction can be attributed to the presence of an anti-nutrient (phytate) which binds protein and makes it unavailable to the animals. Thiocinide was significantly (p<0.05) higher in the raw (21.62mg/dl) and other groups than the control and boiled groups (18.16 and 18.84 mg/dl) respectively.

Table 3: Serum biochemistry of experimental rats fed raw and variously processed Monkey cola *(Cola rostrata)* seed meal (MCSM)

	PROCESSING METHODS						
PARAMETERS	Control	Boiled	Boiled/ Fermented	Fermented	Toasted	Raw	SEM
Total Serum (mg/di)	4.90[a]	4.83[a]	4.83[b]	4.47[b]	4.43[b]	3.43[b]	0.20
Serum Albumin (mg/dl)	2.43[a]	2.40[a]	2.17[a]	1.87[a]	1.70[a]	1.17[b]	0.14
Serum globulins (mg/dl)	6.67[a]	2.97[b]	2.77[b]	2.47[b]	2.27[b]	2.03[b]	0.20
Serum thiocinide (mg/dl)	18.10[b]	18.84[b]	19.65[a]	20.40[a]	20.83[a]	21.61[a]	3.01
Creatinine (mg/dl)	0.90[b]	0.93[b]	1.03[b]	1.07[b]	1.07[a]	1.73[a]	0.28
Urea (mg/dl)	6.42[b]	6.60[b]	7.84[a]	8.33[a]	8.91[a]	9.48[a]	1.27
AST (iu/L)	132.00[b]	135.33[b]	163.00[b]	212.00[a]	215.67[a]	217.33[a]	12.66
ALT (iu/L)	10.00[b]	17.33[b]	42.00[a]	45.67[a]	46.74[a]	65.00[a]	9.59
ALP (iu/L)	95.67[b]	101.00[b]	112.00[b]	120.00[a]	123.33[a]	130.67[a]	7.35

Note: [abc] Means along the rows with different superscripts are significantly different (P<0.05)

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The presence of cyanide in the seed according to (6) might be responsible for the formation of thiocinide which binds cyanide and thiosulphate. Thiocinide decreases the transport of iodine, hence the amount of thyroxine produced. The reduction of this parameter in the boiled group is an indication that the boiling processing method was effective in reducing this anti-nutrient

The result which also shows a reduction in serum urea value recorded by rats in the control and boiled seed treatments respectively compared with the raw seed treatment (9.48g/dl) indicate no observable muscular wastage as a result of inadequate protein. Creatinine was significantly higher in the raw (1.73mg/dl) than the control and boiled groups (0.90 and 0.93) mg/dl respectively. High creatinine is a pointer to impaired kidney function that is; malfunction or failure of the kidney. Preliminary work by (6) showed that the raw cola seed contain high amount of oxalate which could probably be the cause of high creatinine in the group of rat receiving this treatment diet.

Alanine Aminotransferase (ALT), Alanine Phosphotase (ALP) and Aspartate Aminotransferase (AST) which is also known as Serum Glutamic oxaloacetic Transferase (SGOT) are pointers of the condition of the liver. They are low in the blood when the liver is normal and high when the liver is damaged. Rats in the raw seed treatment had significantly ($p<0.05$) higher values of ALT, ALP, and AST compared with the control and boiled seed group. This indicated that the livers of the animals eating the raw seed meal were threatened or damaged (22). The elevated liver damage pointers may also be attributed to the presence of oxalate in the raw seed.

Conclusion and Application

1. The presence of anti-nutritional factors in raw *Cola rostrata* (Monkey cola) seeds give an indication that its use in the diets of rats would negatively affect their organs considering the values of haematological and serum biochemical indices obtained in this study.
2. However, processing the seed by boiling for at least 30 minutes engenders the reduction of these anti-nutrients and therefore allows for its use as replacement for maize in animal diets without

deleterious effect on the animal's organs and tissues.

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