

## Assessment of the nutritive value of toasted castor seed cake-based diets as a reflect on blood profile of weanling wistar albino rats



Agboola, A. F

Department of Animal Science, University of Ibadan, Ibadan, Nigeria

**Abstract** Corresponding author: adebisi.agboola@gmail.com, +234(8)022004830

Since the cost of feed is increasing due to the keen competition between humans and animals, there is a need to critically seek for a matching alternative. Castor seed cake, a residue after the extraction of oil from nutrients-rich castor seed constitutes a waste which can be converted into livestock feed. Thus, the study was carried out to investigate the effect of graded levels of castor seed cake diets on haematological and serum biochemical indices of weanling albino rats in an experiment that lasted 21 days. Thirty weanling Wistar albino rats were weighed individually and randomly assigned to 5 dietary treatments with 3 replicates of two rats in a completely randomized design. Diet 1 was a nitrogen-free diet (NFD), while diets 2, 3, 4, and 5 had 0% (without castor seed cake inclusion), 2, 4 and 6% castor seed cake replacing soyabean meal respectively. At day 21, blood samples were obtained from two rats per replicate for haematological and serum analysis. There were no significant differences ( $P > 0.05$ ) observed in all the parameters (packed cell volume, haemoglobin, red blood cell, white blood cell, lymphocyte, neutrophil, monocyte, eosinophil and platelet) measured. Similarly, diet had no effect on the serum biochemical indices (creatinine, urea, total protein and globulin) measured. Significant difference ( $P < 0.05$ ) was however observed in albumin (2.62 – 3.18g/dL) of rats on the different experimental diets. The albumin concentrations of weanling albino rats on dietary treatments were similar to those on the control diet but significantly ( $P < 0.05$ ) higher (3.09 – 3.18g/dL) than those on the nitrogen-free diet. It can be concluded that castor seed cake can replace soyabean meal up to 6% inclusion level without eliciting detrimental effect on blood profile of weanling wistar albino rats.

**Keywords:** Castor seed cake, Albino rat, Haematological parameters, Serum biochemical indices

### Introduction

Castor bean (*Ricinus communis L.*) is an oilseed shrub of significant economic importance because of its several potentials in industry and medicine. The plant, is a species of flowering plant in the spurge family, Euphorbiaceae and the sole species in the monotypic genus, *Ricinus*, and sub-tribe, Riciniinae. Castor is indigenous to the south eastern Mediterranean basin, Eastern African and India, but it is widespread throughout tropical regions (and widely grown elsewhere as an ornamental plant). It is abundant along water courses and flood plains, disturbed or waste land (Lima Da Silva *et al.*, 2006; Berman *et al.*, 2011;

Akande *et al.*, 2014). Castor seeds contain between 40% and 60% oil that is rich in triglycerides, mainly ricinolein, 38.58% crude protein (Akande *et al.*, 2012), is a good source of phosphorous, sodium, potassium, magnesium (Agbabiaka *et al.*, 2011) and calcium (Annongu and Joseph, 2008) necessary in both human and livestock diets but low in iron, copper and manganese. Threonine and methionine are the first limiting amino acids in castor seed meal (Puttaraj *et al.*, 1994) and other indispensable amino acids are isoleucine and lysine (Annongu and Joseph, 2008) compared to the standard plant protein source soyabean. However, utilization of castor bean cake in livestock feed is limited

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due to the presence of antinutritional factors such as: ricin, ricinine and thermostable allergen (Darby *et al.*, 2001; Olsnes, 2004) which are present in the seed and in lower concentrations throughout the plant. Various processing methods like genetic manipulation, chemical (lye, ammonia salt, blanching, sodium hydroxide treatment), physical (*germination, sprouting, fermentation*), heat (*boiling, steaming, autoclaving, microwave cooking*) and mechanical methods (*crushing, decortications, de-hulling*) (El-Adawy, 2002; Anandan *et al.*, 2005; Emiola *et al.*, 2007; Soetan, 2008; Ani and Okorie, 2009; Akande *et al.*, 2011; 2012; 2014) have been employed to detoxify the anti-nutritive factors in castor oil bean but with distinct inherent limitations. According to Akande *et al.* (2012), lye-treated castor bean cake had low calcium content. Germinated castor seed had low crude protein which was traced to nitrogen used up during germination (Akande *et al.*, 2014). Cooking treatments and/or germination caused decreased fat, total ash, carbohydrate fractions, minerals, B-vitamins and the concentrations of lysine, tryptophan, total aromatic and sulfur-containing amino acids (Soetan and Oyewole, 2009). However, castor bean has been suggested for use in livestock feed as an alternative protein source if properly detoxified.

The blood contains myriad of metabolites and other constituents, which provide a valuable medium for clinical investigation and nutritional status of animals. According to Church (1991), dietary components have measurable effects on blood constituents such that significant changes in their values could be used to draw inference on the nutritive value of feeds offered to the animals. It was

therefore the aim of this study to determine the effect of castor seed cake diets on the haematological and serum biochemical indices of weanling wistar albino rats.

### **Materials and methods**

#### ***Experiment site***

The experiment was carried out at the rats house (rat metabolic cages), Department of Animal Science, University of Ibadan, Ibadan, Nigeria.

#### ***Preparation of test ingredient, experimental diets and management of animals***

The castor oil bean used was harvested along watercourses and floodplains, disturbed on waste land in Ibadan Metropolis, Akinyele Local Government Area, Oyo state. The castor seeds were toasted, by spreading out the seeds in an even layer, heated over medium heat and shaken often until the seeds were golden brown and removed from pan to cool for about 20 minutes. The oil was then extracted with the use of mechanical screw press. The resultant cake from oil extracted processed castor seeds was autoclaved at 120°C for 15min.

Thirty weanling wistar albino rats were purchased from a reputable rat farm in Ibadan, Nigeria. The metabolic rats' house was cleaned and disinfected prior to arrival day. The animals were randomly allotted to five treatments comprising three replicates of paired rats per replicate. Each group received one of the experimental diets (Table 1) and was fed for 21 days. Diet 1 was a nitrogen-free diet, diet 2 was the basal diet without castor seed cake. Diets 3 was the basal diet with 2% castor seed cake inclusion while diets 4 and 5 contained the basal diet with inclusion of 4 and 6% castor seed cake respectively in a completely randomized design. The rats were fed *ad libitum* and had access to clean water.

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### **Blood collection**

Blood samples were obtained through ocular puncture from two rats per replicate at day 21 of the experiment into a bottle containing EDTA (ethylene diamine tetra acetic acid) for haematological studies while another set of bottles were without EDTA for subsequent serum separation.

### **Haematological parameters of albino rats fed toasted castor seed cake diets**

The packed cell volume (PCV) and haemoglobin (Hb) were determined using

the micro haematocrit method and cyanmethaemoglobin method respectively as described by Mitruka and Rawnsley (1981). Erythrocyte count (RBC) and Leukocyte count (WBC) were determined using the improved Neubauer haemocytometer after the appropriate dilution (Schalm *et al.*, 1975). Differential leukocyte counts were determined by scanning Giemsa's stained slides in the classic manner (Schalm *et al.*, 1975).

**Table 1: Gross composition (g/100g DM) of experimental diets fed to rats**

Ingredients	Treatments				
	NFD	0% CSC	2% CSC	4% CSC	6% CSC
Corn	0.00	40.00	38.00	36.00	34.00
Soyabean meal	0.00	16.50	16.50	16.50	16.50
Castor seed cake	0.00	0.00	2.00	4.00	6.00
Corn starch	77.00	20.00	20.00	20.00	20.00
Dextrose	10.00	10.00	10.00	10.00	10.00
Soya oil	5.00	5.00	5.00	5.00	5.00
Cellulose	5.00	5.00	5.00	5.00	5.00
*Premix	0.25	0.25	0.25	0.25	0.25
Lysine	0.00	0.25	0.25	0.25	0.25
Methionine	0.00	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25
DCP	1.50	1.50	1.50	1.50	1.50
Limestone	1.00	1.00	1.00	1.00	1.00
Total	100	100	100	100	100
<b>Calculated nutrients</b>					
Crude protein (%)	0.00	10.93	11.33	11.73	12.13
Metabolizable energy(Kcal/kg)	3424.6	3280.8	2552.65	2561.44	2569.99
Crude fibre	4.50	6.78	6.79	6.78	6.78

\*Premix contains Vit. A: 12,500,000.00 (I.U), Vit. D<sub>3</sub>: 2,500,000.00 (I.U), Vit. E: 40,000.00 (mg), Vit. K<sub>3</sub>: 2,000.00 (mg), Vit. B<sub>1</sub>: 3,000.00 (mg), Vit. B<sub>2</sub>: 5,500.00 (mg), Niacin: 55,000.00 (mg), Calcium Pantothenate: 11,500.00 (mg), Vit. B<sub>12</sub>: 25.00 (mg), Choline Chloride: 500,000.00 (mg), Folic Acid: 1,000.00 (mg), Biotin: 80.00 (mg), Maganese: 120,000.00 (mg), Iron: 100,000.00 (mg), Zinc: 80,000.00 (mg), Copper: 8,500.00 (mg), Iodine: 1,500.00 (mg), Cobalt: 300.00 (mg), Selenium: 120.00 (mg), Anti-oxidant: 120,000.00 (mg).  
NFD – Nitrogen-free diet, CSC – Castor seed cake, DCP – Dicalcium phosphate

### **Serum biochemical indices of albino rats fed toasted castor seed cake diets**

Glucose, cholesterol and triglyceride were assayed by the methods of Roschlan *et al.* (1974). Serum urea was determined by the kit (Quinica Clinica Spam), a uricase method as described by Wootton (1964). The biuret method was utilized in the determination of the total protein fraction

while the serum was subjected to the direct colorimetric method for albumin with bromocresol green (BCG) as the dye as described by Peters *et al.* (1982).

### **Statistical analysis**

Data were subjected to a one-way analysis of variance using SAS (2008) software and the mean separated by Tukey's HSD option of the same software package.

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### Results

#### *Haematological indices of albino rats fed toasted castor seed cake diets*

Results of the haematological indices of albino rats fed toasted castor seed diets are shown in Table 2. There were no significant

differences ( $P>0.05$ ) observed in all the parameters (packed cell volume, haemoglobin, red blood cell, white blood cell, lymphocyte, neutrophil, monocyte, eosinophil and platelet) measured.

**Table 2: Haematological parameters of albino rat fed with different levels of toasted castor seed cake diets**

Parameters	Treatments					SEM	P value
	NFD	Control	2% CSC	4% CSC	6% CSC		
PCV (%)	43.00	39.67	42.00	39.33	40.33	1.21	0.2283
Hb (g/dL)	14.37	13.50	13.90	13.07	13.47	0.38	0.2355
RBC ( $\times 10^{12}/L$ )	7.34	6.70	7.08	6.61	6.65	0.23	0.1678
WBC ( $\times 10^9/L$ )	6.03	7.52	10.07	5.25	7.82	1.04	0.0617
Lymphocyte ( $\times 10^3/mm^3$ )	66.00	68.33	56.00	66.33	66.67	3.58	0.1876
Neutrophil ( $\times 10^3/mm^3$ )	31.00	27.00	38.67	29.67	29.00	3.32	0.2011
Monocyte ( $\times 10^3/mm^3$ )	1.67	2.00	2.33	2.33	1.67	0.54	0.8149
Eosinophil ( $\times 10^3/mm^3$ )	1.33	2.67	3.00	1.67	2.67	0.65	0.3558
Platelet ( $\times 10^3/mm^3$ )	134.67	154.00	180.67	157.67	158.00	19.45	0.6056

Mean along the same row with the same superscripts are not significantly ( $P>0.05$ ) different

NFD – Nitrogen-free diet; Hb - Haemoglobin, RBC - Red blood cell; WBC - White blood cell, PCV - Packed cell volume, CSC – Castor seed cake, SEM- Standard error mean

#### *Serum biochemical indices of albino rats fed toasted castor seed cake diets*

The serum biochemical indices of rats fed different levels of toasted castor seed cake are shown in Table 3. Diet had no effects on serum biochemical indices (creatinine, urea, total protein and globulin) measured.

However, there was significant difference ( $P<0.05$ ) observed in serum albumin concentration of rats on experimental diets. The albumin of weanling albino rats on dietary treatments were similar to those fed the control diet but significantly ( $P<0.05$ ) higher than those fed nitrogen-free diet.

**Table 3: Serum biochemical indices of albino rats fed on different levels of toasted castor seed cake diets**

Parameters	Treatments					SEM	P value
	NFD	Control	2% CSC	4% CSC	6% CSC		
Creatinine(mg/dL)	1.02	1.07	0.92	0.97	1.01	0.08	0.6949
Urea (mg/dL)	13.75	23.09	20.44	20.69	18.75	3.26	0.3903
Total Protein (g/dL)	5.46	6.12	5.42	5.74	5.36	0.37	0.6051
Albumin (g/dL)	2.62 <sup>b</sup>	3.11 <sup>a</sup>	3.14 <sup>a</sup>	3.09 <sup>a</sup>	3.18 <sup>a</sup>	0.09	0.0139
Globulin (g/dL)	2.84	3.01	2.28	2.65	2.18	0.42	0.6005

<sup>ab</sup> Mean along the same row with different superscripts are significantly ( $P<0.05$ ) different

NFD - Nitrogen free diet, SEM- Standard error mean, CSC – Castor seed cake

### Discussion

#### *Haematological indices of albino rats fed toasted castor seed cake diets*

The act of interpreting blood profile in normal and diseased condition of animal is part of the primary objectives of haematologic studies (Khan and Zafar,

2005). The results on haematological parameters of albino rats indicated that dietary inclusion had no significant effect on the blood parameters measured. This is in agreement with the study carried out by Oso *et al.* (2004) who assessed effect of feeding graded levels (50, 100 and 150 gkg<sup>-1</sup>) of

fermented castor oil seed meal to Nera strain chicks. The authors reported no significant effect on packed cell volume (PCV), haemoglobin (Hb), red blood cell (RBC), neutrophil, and eosinophil. Adedeji *et al.* (2006) reported similar findings in rabbits fed 0, 10, 15, 20 and 25% inclusion levels of boiled castor seed cake. The authors asserted that diets had no effect on Hb, PCV, RBC, white blood cell (WBC), neutrophils, eosinophils and lymphocytes of the rabbits. Also, Colette *et al.* (2013) observed that inclusion of dried rumen content and castor oil seed cake to diets of broilers had no significant effect on RBC and WBC, although PCV was affected. In a study conducted by Apata *et al.* (1999), 50 and 100g/kg detoxified castor seed cake in weaner pigs' diets had no effect on haematological parameters and serum biochemical indices measured. The authors averred that the similarity in the various blood constituents in pigs on treatments further substantiated the nutritional adequacy and safety of detoxified CSC diets up to inclusion level of 100g/kg.

However, a contrary opinion was asserted in the findings by Akande and Odunsi (2012). The authors observed that PCV, Hb and WBC values were influenced by moist heated and fermented or lye treated diets fed to 150 day-old Anak broiler chicks. Momoh *et al.* (2012) recorded reduced PCV and increased WBC values in albino rats fed castor seed oil extract. Shindang *et al.* (2012) also reported a decrease in PCV and increase in WBC when dried ground *Ricinus communis* var minor seeds were fed to female albino rabbits.

#### ***Serum biochemical indices of albino rat fed with toasted castor seed cake diets***

Blood parameters could be used to indicate the physiologic, pathologic and nutrition status of an animals (Colette *et al.*, 2013). The detection of organ disease in animal

and availability of protein in the diets are revealed in the serum (Iyayi and Tewe, 1998). Adeyemi *et al.* (2000) reported that the quality of diet is positively correlated to serum biochemistry. Serum biochemical indices measured were remarkably similar except albumin. Deldar (1994) reported that albumin is the most common protein in blood plasma. Albumin of rats on nitrogen-free diet was significantly lower than those on other diets. This is probably an indication of the absence of protein in their diet. Serum creatinine levels indicate the extent of muscle degradation in animals. When the values are high, it shows a high degradation of muscle phospho-creatinine to form creatinine (MacWilliams *et al.*, 1982). Creatinine values recorded in this study had no difference and were higher than the normal reference range while the results of blood urea nitrogen observed were within the normal range according to Mitruka and Rawnsley (1981). The higher level of creatinine in this study is an indication of muscle wastage in the albino rats due to the treatment imposed. The serum urea test measures the amount of nitrogen in the serum. High serum urea level is probably an indication of kidney dysfunction, because protein intake and kidney functioning are affected by quantity of blood urea nitrogen. Diet had no influence on the blood urea of the experimental rats. The values obtained were within the normal reference range of albino rat (Mitruka and Rawnsley, 1981) implying that the kidney was unaffected and there was a better utilization of protein since blood urea can be attributed to protein breakdown. A contrary view was observed in the findings of Akande *et al.* (2013) who reported influence of treated castor bean cake on urea and creatinine levels in Anak broiler chicks. However, Oso *et al.* (2004) findings agreed with the result of the present study except for serum albumin which was

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not affected fermented castor oil seed meal up to 150g/Kg in diets of Nera broiler chicks while Adedeji *et al.* (2006) had similar findings on serum biochemical indices when weaner rabbits were fed graded levels (0, 10, 15, 20 and 25%) of boiled castor seed cake.

#### **Conclusion**

Results from this study showed that castor seed cake can replace soyabean meal up to 6% inclusion level without eliciting any deleterious effect on blood profile of weanling wistar albino rats.

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