

HAEMATOLOGICAL AND BIOCHEMICAL STUDIES ON THE BLOOD OF *Clarias gariepinus* FINGERLINGS FED COOKED *Jatropha curcas* SEED MEALS

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Summary

The effects of replacing soybean meal with *Jatropha curcas* seed meal on haematology and biochemistry of the blood of African Catfish (*Clarias gariepinus*) were evaluated. Two hundred and twenty five (225) juveniles of *Clarias gariepinus* were acclimatized for a week, weighed and allotted into five dietary treatments; DT1, DT2, DT3, DT4 and DT5 containing 0, 25, 50, 75 and 100% *Jatropha curcas* replacement levels with soybean meal respectively. The diets were isonitrogenous and isolipidic. Each treatment was replicated three times with 15 fish per replicate. Fish were fed 5% body weight on two equal proportions per day. The results from the study indicated that the haematological and biochemical parameters of *Clarias gariepinus* exposed to different dietary treatments in this study was not significantly different between the control diet and test diet D2 (25% replacement level) except for WBC. Generally, there was a lack of trend in the haematological parameters in the blood of *Clarias gariepinus* exposed to different dietary treatments. However, the values of some haematological parameters recorded in this experiment were within the range of normal haematology of a healthy fish.

Keywords: *Jatropha curcas*, feed ingredients, *Clarias gariepinus*, blood biochemistry

Introduction

Haematological and serum biochemical parameters are important tools that can be used as indicators of monitoring physiological and pathological changes in fish (1). Blood analysis provides reliable information on metabolic disorders in farmed animals (2). Haematological indices have been used in assessment of suitability of feed and feed

mixtures as well as disease diagnosis (3; 4; 5). The nutritional state of fish could change the blood values (6). Haematological parameters have proven to be veritable tools in evaluating the health status of fish with respect to dietary manipulations (7). Investigating possible nutritional disease that may result from feeding lesser known feed ingredient to fish is a reasonable attempt to forestall factors that could negatively affect

aquaculture production and development (4). The seed of *Jatropha curcas* have been proven to have nutrient density similar to commonly used plant protein in aquafeed (8) but not much has been reported on the effect of feeding this seed meal on the blood parameters of *Clarias gariepinus*. This study attempts to investigate the possible impact of feeding *Jatropha curcas* on the health status of *Clarias gariepinus* using haematological and biochemical parameters as indices.

Materials and Methods

Sources and Processing of Ingredients

Table 1: Proximate composition (g/100g Dry Matter) of Feed Ingredients

Parameters	Fish meal	Soybean Meal	*JSM	Corn Meal
Moisture	9.75	10.70	3.90	10.48
Crude Protein	72.4	38.74	30.34	9.87
Crude Lipid	10.45	16.68	45.89	4.28
Crude Fibre	-	5.10	8.41	5.78
Ash	8.32	4.48	4.42	6.73
NFE	-	24.30	7.04	62.35

**Jatropha curcas* meal

Experimental Diets

The experimental diets were formulated (Table 2) using the nutrient composition of the protein feedstuffs (Table 1). The experimental diets contained full-fat soybean meal which was replaced by cooked *Jatropha curcas* seedmeal at the rate of 0, 25, 50, 75, and 100%. The diets were isolipidic and isonitrogenous containing 40% crude protein and 12% lipid with fish meal, full fat soybean

Sample of dried *Jatropha curcas* seeds (1kg) were obtained from Osogbo, Osun state. The *Jatropha curcas* seeds were de-kernelled, rinsed with water and boiled for 15 minutes after which they were sundried (29-31°C) for three days and then ground in a hammer mill to produce a meal. The meal was thereafter analyzed for their proximate composition (9). Fish meal, soybean meal and other feedstuffs obtained from commercial sources in Nigeria were separately milled and screened to fine particles size. Samples were taken in triplicates and analyzed for their proximate composition (9).

meal, fish oil, vitamin premix and starch serving as ingredients. Starch serves the purpose of binder and filler. The feedstuffs were ground and water was added to aid binding after which it was introduced into a pelleting and mixing machine to obtain a homogenous mass and then passed through a mincer to produce 2mm size pellet which was immediately sundried at 30 - 32°C. After drying for three days, the diet was kept in a cool place.

Table 2: Gross composition of experimental diets (g/100g) containing *Jatropha curcas* Seedmeal fed to *Clarias gariepinus*

Feed Ingredients	D1 (0%)	D2(25%)	D3(50%)	D4(75%)	D5(100%)
Fishmeal	33.33	33.33	33.33	33.33	33.33
Yellow maize	10.00	10.00	10.00	10.00	10.00
Full fat JSM	-	12.36	24.72	37.08	49.44
Full fat SBM	50.00	37.50	25.00	12.50	-
Fish premix	5.00	5.00	5.00	5.00	5.00
Starch	1.67	1.81	1.95	2.09	2.23
Total	100	100	100	100	100

i. JSM- *Jatropha curcas* Seedmeal ii. SBM- Soybean meal
Each 1Kg Premix Contains:: Vitamin A ;4000000 IU, Vitamin D3;800000 IU, Vitamin E; 40000 IU, Vitamin K3; 1600mg, Vitamin B1; 4000mg, Vitamin B2; 3000mg, Vitamin B6; 3800mg, Vitamin B12; 3mcg, Nicotonic acid;18000mg, Pantothenic acid; 8000mg, Folic acid; 800mg, Biotin; 100mcg,Cholin chloride; 120000mg, Iron; 8000mg, Copper; 800mg, Manganese; 6000mg, Zinc; 8000mg, Iodine; 400mg, Selenium; 400mcg, Vitamin C(coated); 40mg, Inositol; 60000mg, Cobalt; 10000mg, Lysine; 150mg, Methionine; 10000mg, Anti-oxidant; 25000mg.

Experimental Fish and System

The experiment was conducted at the nutrition unit of the Department of Fisheries

Technology, Federal College of Animal Health and Production Technology, Moor Plantation Ibadan. Two hundred and twenty five (225) *Clarias gariepinus* fingerlings (2.61±0.02g)

were obtained from a reputable fish farm in Ibadan, Oyo state and transported live to the project site in an aerated bag. The *Clarias gariepinus* fingerlings were acclimated for 7 days prior to the feeding trial while being fed on a commercial pelleted diet. Fifteen (15) fingerlings were allotted into each of the fifteen 25 litres rectangular tanks containing 20 litres of water. Experimental diets were assigned randomly to the tanks with three replicates per treatment. Fish in each tank were fed 5% body weight per day in two equal proportions between 9.00 –10.00am and 5.00 – 6.00 pm for 56 days. The experiment was carried out at fish nutrition wet laboratory of the Fisheries Technology Department, Federal College of Animal Health and Production Technology, Ibadan.

Haematological Examination

One test organism was removed from each tank for blood analysis. Five to ten millilitre (5-10ml) blood per fish was collected from cardiac puncture in juvenile using 2ml disposable heparinised syringe treated with ethylene diamine tetra acetic acid (EDTA) as anti-coagulant. The blood was stored at -4°C in refrigerator prior to analysis. The blood analysis follows the method described by (10).

Packed Cell Volume (PCV)

Non-clotted blood was drawn by capillary action into micro haematocrit tubes; one end of the tube was sealed with synthetic sealant. The sealed tube was centrifuged in a micro haematocrit centrifuge. Centrifugation lasted for 5 minutes at 10500 rpm. The packed cell volume was measured using micro haematocrit reader and expressed as percentage.

Mean Corpuscular Haemoglobin Concentration (MCHC)

This refers to the percentage of haemoglobin in 100ml of red blood cell. This was calculated by dividing the haemoglobin content (in g/100ml) by the PCV/100ml of red blood cell according to the formulae:-

$$\text{MCHC} = \frac{\text{Hb}}{\text{PCV}} \times 100 \text{ (gm/dl)}$$

Mean Corpuscular Volume (MCV)

The value of the mean corpuscular volume was calculated from the haematocrit value (PCV) (%) and the Red Blood Count (RBC) ($10^6/\text{mm}^2$), according to the following formulae:

$$\text{MCV} = \frac{\text{PCV}}{\text{RBC}} \times 100 \text{ (fl)}$$

Mean Corpuscular Haemoglobin (MCH)

Mean corpuscular haemoglobin concentration, expressed the concentration of haemoglobin in unit volume of erythrocyte. It was calculated from the haemoglobin value (Hb) and from the red blood cells according to the following formulae:-

$$\text{MCH} = \frac{\text{Hb}}{\text{RBC}} \quad (\text{Picogramme}) \text{ (pg)}$$

Blood Cell Count (Red Blood Cell (RBC) and White Blood Cell (WBC))

Haemocytometer was used in blood cell counts. The apparatus consists of a counting chamber; a cover slit white and red blood and a plastic mouth piece for drawing the fluid into pipette. The blood diluting fluid was prepared as described by (10). The blood cells were counted on the counting chamber of haemocytometer with the aid of compound microscope.

$$\text{RBC} = \text{Number of cells counted} \times 3 \times 10 \times 200 \text{ (} 10^6 \text{ mm}^3 \text{)}$$

$$\text{WBC} = \text{number of cells counted} \times 0.25 \times 10 \times 20 \times 20 \text{ (} 10^3 \text{ mm}^3 \text{)}$$

Biochemical Tests

The serum total protein was determined by the Biuret method (11) using a commercial kit (Randox Laboratories Ltd, U.K), while albumin value was obtained by bromocresol green method (12). The globulin and albumin-globulin ratio were determined according to

the method of (13). Blood glucose was determined according to the method of (14).

Water Quality Monitoring

Water quality was monitored every week throughout the feeding trials. Water temperature (°C) and pH were measured daily with a combined digital pen meter, while dissolved oxygen (mg/l) was measured using Dissolved Oxygen (DO) meter.

Statistics

Data obtained from the experiment were expressed in mean $\pm$ SD and were subjected to

one way Analysis of Variance (ANOVA) using SPSS 16.0 version. Where the ANOVA reveals significant difference ($P < 0.05$). Duncan multiple range test was used to compare differences among individual treatment means.

Results

The results of proximate composition as presented in table 3 reveal the diet to be isonitrogenous and isolipidic as there was no significant variation in the proximate composition parameters among the different dietary treatments.

Table 3: Proximate composition (g/100g dry matter) of the experimental diets containing boiled *Jatropha curcas* seedmeal fed to *Clarias gariepinus*

	D1 (0%)	D2 (25%)	D3 (50%)	D4 (75%)	D5 (100%)
Moisture	9.76 $\pm$ 0.26	9.88 $\pm$ 0.62	9.70 $\pm$ 0.47	9.99 $\pm$ 0.72	9.61 $\pm$ 1.39
Crude protein	40.20 $\pm$ 0.20	40.18 $\pm$ 0.19	40.23 $\pm$ 0.34	40.13 $\pm$ 0.19	40.10 $\pm$ 0.13
Crude lipid	12.03 $\pm$ 0.05	12.04 $\pm$ 0.07	12.02 $\pm$ 0.09	12.12 $\pm$ 0.18	12.19 $\pm$ 0.13
Crude fibre	5.06 $\pm$ 0.08 ^c	5.10 $\pm$ 0.09 ^{bc}	5.43 $\pm$ 0.29 ^b	5.83 $\pm$ 0.11 ^a	5.99 $\pm$ 0.24 ^a
Ash	4.24 $\pm$ 0.25	4.19 $\pm$ 0.57	4.21 $\pm$ 0.21	4.12 $\pm$ 0.07	4.22 $\pm$ 0.19
NFE	28.72 $\pm$ 0.26	26 $\pm$ 0.77	26.41 $\pm$ 0.74	27.80 $\pm$ 0.82	27.89 $\pm$ 1.14

Mean on the same row without superscript are not significantly different ($p > 0.05$) from each other.

Table 4 shows the hematological changes in the blood of *Clarias gariepinus* fed diets containing *Jatropha curcas* seedmeal. The PCV of the blood of fish fed diet D5 was the highest which was significantly different ($p < 0.05$) from the PCV of the blood of fish exposed to other dietary treatments. There was reduction trend in the PCV of fish as dietary inclusions of *J. curcas* increased up to 75% replacement level with soybean. There was no significant difference ($p > 0.05$) in the PCV of the blood of fish fed D1 (control) and fish fed D2. Same trends of results as obtained for PCV were recorded for Hb. The RBC of fish fed D5 was the highest which was not significantly different ($p > 0.05$) from the RBC of fish fed D1 and D2. There was no significant difference ($p > 0.05$) in the WBC of

the blood of fish exposed to different dietary treatments.



Table 4: Heamatological changes in the blood of *Clarias gariepinus* fed diets containing *Jatropha curcas* seedmeal

	D1 (0%)	D2 (25%)	D3 (50%)	D4 (75%)	D5 (100%)
PCV	12.66±0.50 ^b	12.00±0.00 ^{bc}	11.00±0.00 ^c	11.67±0.58 ^{cd}	14.67±0.50 ^a
Hb	4.13±0.11 ^b	4.03±0.56 ^b	3.83±0.58 ^c	4.00±0.10 ^{cd}	5.00±0.00 ^a
RBC	1.24±0.02 ^a	1.15±0.05 ^{ab}	1.05±0.05 ^{bc}	1.14±0.12 ^c	1.25±0.04 ^a
WBC	1.78±0.02 ^a	1.17±0.03 ^c	0.81±0.03 ^d	0.71±0.06 ^e	1.34±0.03 ^b
MCV	99.9±4.13 ^b	103±5.19 ^{ab}	105±5.00 ^{ab}	103.33±1.47 ^{ab}	117.67±1.15 ^a
MCH	33.47±0.51	35.13±1.13	36.56±2.27	35.43 ±4.82	40.17±1.36
MCHC	32.63±0.58 ^b	33.60±0.52 ^{ab}	34.83±0.58 ^{ab}	34.33 ±1.11 ^{ab}	34.17±1.33 ^a

Row means with different superscript are significantly different ($p < 0.05$) from each other.

Row means without superscript are not significantly different ($p > 0.05$) from each other.

The MCV of the blood of fish fed D5 was the highest which was not significantly different ($p > 0.05$) from the MCV of the blood of fish exposed to other dietary treatments except control. MCHC follow the same trends of results as recorded for MCV. There was no significant difference ($p > 0.05$) in the MCV of the blood of fish exposed to different dietary treatments.

The biochemical changes in the blood of *Clarias gariepinus* fed diets containing *J. curcas* seedmeal is presented in Table 5. The total protein of fish exposed to D1 was the highest which reduced as the level of *J. curcas* increased up to 50% replacement level. There was no significant difference ($p > 0.05$) between the total protein of blood of fish fed D1 and D5.

Table 5: Biochemical changes in the blood of *Clarias gariepinus* fed diets containing *Jatropha curcas* seedmeal

	D1 (0%)	D2 (25%)	D3 (50%)	D4 (75%)	D5 (100%)
Total protein	5.37±0.15 ^{ab}	5.20±0.15 ^b	3.63±0.15 ^c	4.93±0.11 ^b	5.00±0.20 ^a
Albumin	3.77±0.15 ^{ab}	3.63±0.15 ^b	2.57±0.12 ^c	3.57±0.06 ^b	3.90±0.01 ^a
Glucose	89.67±18.50 ^a	68.33±7.63 ^{bc}	62.33±2.51 ^c	83.90±0.10 ^{ab}	92.67±6.46 ^a

Row means with different superscript are significantly different ($p < 0.05$) from each other.

Similarly no significant variation existed between the total protein of the blood of fish fed D1 and D2. However, significant variation existed between the total protein of the blood of fish fed D5 and D2. Same trends of results as recorded above were noted for Albumin. Blood glucose level of fish fed D5 was the highest which was not significantly different ($P > 0.05$) from the blood glucose of fish fed D1

(control). Blood glucose level reduced as the *J. curcas* increased up to 50% replacement level.

Discussion

The haematological and biochemical parameters of *Clarias gariepinus* exposed to different dietary treatments in this study was not significantly different ($p > 0.05$) between the control diet and test diet D2 (25%

replacement level) except for WBC. There was a lack of trend in the haematological parameters in the blood of *Clarias gariepinus* exposed to different dietary treatments. However, the values of some haematological parameters recorded in this experiment were within the range of normal haematology of a healthy fish (15). RBC greater than $1 \times 10^6/\text{mm}^3$ was reported as a normal feature of fishes capable of aerial respiration and with high activity (16). Although the same trends of result for rainbow trout (*Onchorhynchus mykiss*) fed the same seedmeal (8), the value of PCV recorded in this study differ from the values recorded for *Clarias gariepinus* by (17), for Asian catfish, *heteropneustesfossilis* by (18) and (19). The PCV values recorded in this study was below the normal range of 20-38% reported by (20). The decrease in haematological parameters with increasing level of incorporation of JSM agreed with the observation in previous studies (21, 22, 23 and 24). The concentration of blood plasma protein is a good pointer to assessment of general health condition of fish (15). There was significant reduction of plasma protein of the blood of fish exposed to different dietary treatments in this study with increasing replacement level of JSM except for fish fed diet D5. A reduction in plasma protein is an indicator of the effect of toxins in the kidney, spleen and liver (26). Albumin and globulin plays a significant role in the immune response of fish (8). Significant reduction of albumin of the blood of fish exposed to different dietary treatments in this study was also observed with increasing replacement level of JSM. Similarly there was decrease in the blood glucose of the fish under study with increasing replacement levels of JSM. Same observation was made by (8). Reduction in blood glucose level with increasing replacement levels of JSM might be as a result of hypoxic condition induced by feeding diets containing higher concentration of anti-nutrients (27). It is evident from the foregoing that feeding diets containing *Jatropha curcas* seedmeal did not impact much negative effect on haematology and blood chemistry of *Clarias gariepinus* when compared to control; soybean based diet.

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