

ALTERATIONS IN THE BLOOD PARAMETERS OF *Clarias gariepinus* (Clariidae) FEED VARYING INCLUSION OF LEVELS OF RUBBER SEED MEAL (*Havea brasiliensis*)



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ABSTRACT

Haematological changes in *Clarias gariepinus* juveniles fed diets containing Rubber (*Havea brasiliensis*) Seed Meal (RSM) at varying inclusion levels (0%, 5%, 10%, 15%, 20%) was evaluated in a ninety-day feeding trials. Four iso nitrogenous and iso caloric diets containing the fish feed diet showed significant ($P < 0.05$) difference over fish feed control diet with respect to WBC, Hb, MCH mean corpuscular haemoglobin concentration (MCHC). Fish feed control diet had the lowest WBC, Hb, MCH, and MCHC. There was no significant difference ($P > 0.05$) in the fish fed control diet and test diets with respect to RBC. Hence it can be concluded that the haematological parameters of *Clarias gariepinus* is not significantly ($P > 0.05$) changed with increase dietary inclusion of rubber seed meal.

KEYWORDS: Haematological profile, *Clarias gariepinus*, Rubber Seed Meal

INTRODUCTION

Aquaculture possesses a strong potential for both human and economic growth. Fish among other products, is a product of aquaculture. It contains some bioactive compound with therapeutic properties that are beneficial to human health (Nnaji et al., 2010; Lordan et al., 2011). Aquaculture provides employment, alleviates poverty, etc (William et al., 2007). *Clarias gariepinus* is the most cultured fish in Nigeria and indeed Africa (Kara, 2012). It is hardy, acceptable and highly fecund, in addition to other characteristics which makes it to be highly favoured in the industry. The success of commercial aquaculture depends mainly on the availability of suitable diets which provide required nutrients for optimal growth. This has made the use of supplementary feed inevitable. In fact, significant effort has been directed towards evaluating the nutritive value of different non-conventional feed resources. These resources, such as aquatic and terrestrial macrophytes including rubber (*Havea brasiliensis*) seed are used to formulate nutritionally balanced and cost-effective diet for fish and other livestock (Licari et al., 2019; Roy and Pal, 2015). Previous researchers have attempted to utilize non-conventional plant and animal materials to partially or wholly replace conventional ingredients which appear to be very costly. The high cost of these commodities has necessitated the need to search for an alternative source of ingredients which are widely available, less competitive and affordable. Rubber (*Havea brasiliensis*) seed fits into this consideration because it has been proven to enhance growth in animals, it is widespread and more often than not considered as a waste product (Makkar et al., 2014; Mmereole, 2008; Sharma et al., 2014).

Ichthyohaematological investigations were adopted mainly for diagnostic purpose and is also used to appraise the suitability of feeds and feed mixture pellets to examine the effect of stress situation (Fagbenro et al., 2003). Studies have shown that blood analysis is a valuable means of evaluating the physiological conditions of cultured fish in order to

determine the effect of diets and other stress factors on its health. Variations in blood parameters of fish in response to stressing factor are indicators of stress condition in fish (Bello-Olusoji et al., 2006; George et al., 2007). The present study was conducted to investigate the status of haematological characteristics of *Clarias gariepinus* fed with rubber seed meal-based diets.

MATERIALS AND METHODS

Sources and Processing of Ingredients:

A ninety-day feeding trials was conducted on *Clarias gariepinus* using dehulled rubber (*Havea brasiliensis*) seeds which were obtained from the Rubber Research Institute of Nigeria (RRIN) Iyanono, Benin, Nigeria. They were dried in an oven at 105°C to make them suitable for grinding. They were later ground in a mechanical grinder to powder form, sieved and stored in an air tight container for further use. The other ingredients such as fish meal, soybeans, corn, flour, etc were procured from local market. Five iso nitrogen and iso caloric diets were prepared (Table 1) with the inclusion of rubber seed meal (RSM) at level 0% (for control diet), 5%, 10%, 15% and 20% and used as experimental diets. The proximate composition of the experimental diets (Table 2) was determined using standard methods (AOAC, 1985).

Table 1: Percentage Composition of Experimental Feed stuff

Ingredients	Percentage Inclusion of Rubber Seed Meal				
	0%	5%	10%	15%	20%
Rubber Seed Meal (RSM)	0.00	5.00	10.00	15.00	20.00
Fish Meal (FM)	28.00	28.00	28.00	28.00	28.00
Soy Bean Meal (SBM)	44.80	42.93	41.04	39.17	37.27
Corn Flour	19.20	16.09	12.96	9.83	6.73
Starch	1.00	1.00	1.00	1.00	1.00
Fish Oil	5.00	5.00	5.00	5.00	5.00
Salt	1.00	1.00	1.00	1.00	1.00
Vitamin C	0.25	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25
Total	100	100	100	100	100

Table 2: Proximate Composition (%) of Experimental Diet

Experimental Diet	% Moisture	% Crude Protein	% Crude Lipid	% Ash	% Crude Fibre
0%	8.16±0.06	32.15±0.09	5.08±0.18	5.36±0.93	3.19±0.33
5%	8.12±0.05	32.12±0.25	5.15±0.25	5.33±0.13	3.26±0.10
10%	8.28±0.09	32.37±0.14	5.43±0.06	5.40±0.04	3.25±0.33
15%	8.12±0.06	32.86±0.59	5.21±0.28	5.39±0.04	3.08±0.35
20%	8.19±0.07	32.01±0.39	5.62±0.16	5.21±0.06	3.16±0.84

Experimental fish

The African Catfish *Clarias gariepinus* species has been one of the most cultured species of fish in Nigeria (Alarape et al., 2015). It was selected for the present study because it has excellent growth rate, easily available and very acceptable and of high commercial important, etc. 300 Juveniles of *Clarias gariepinus* were collected from Almond fish farm, a reputable farm in Uyo, Akwa Ibom State, Nigeria. They were kept in a large tarpaulin pond and climatised for 15 days on a control feed in order to habitated them from artificial feeding. Groups of 20 juvenile catfish (10.00±0.11 g) were stocked into 15 Hapa (1m x 1m x 1.5m). Each diet was fed to the catfish in triplicate Hapa twice daily (09.00 and 16.00h) at 5% body weight of fish for 90 days. Fish were removed from each tank every 14 days and batch-weighted and the amount of feed was adjusted accordingly, sub samples of catfish per treatment were sacrificed at the beginning and end of the feeding trials respectively.

Haematological Studies

The blood analyses were determined using the methods described by Svobodova et al.. (1991). The following parameters were determined. Blood analysis; 5 – 10 ml. blood samples were collected from cardiac puncture using 2ml disposable heparinised syringe treated with EDTA as anti-coagulant. Blood Cell Count: - Haemocytometer was used in blood cell count. The blood diluting fluid was prepared as described by Svobodova et al. (1991). The blood cells were counted on the counting chamber of Haemocytometer with the aid of a compound microscope.

Haemoglobin Estimation: Haemocytometer was used for haemoglobin estimations based on acid haematin method (SAHLI).

$$\text{Haemoglobin} = \frac{\text{Value Obtained} \times 17.2\text{mg}/100\text{ml}}{100}$$

Packed Cell Volume: The packed cell volume was measured after placing sealed microhaematocrit tube in a centrifuge at 10,500rpm using microhaematocrit reader and expressed as percentage.

Mean Corpuscular Haemoglobin (MCH): This was expressed in pictograms (pg).

$$\text{MCH} = \frac{H \times \Phi}{E}$$

Mean Corpuscula Haemoglobin Concentration (MCHC): This was obtained using the formula

$$\text{MCHC} = \frac{H \times \Phi}{\text{PCV}}$$

Statistical Analysis:

Data obtained from the analyses were expressed in mean ± SD and it was subjected to one-way analysis of variance (ANOVA) using SPSS 16.0 where the ANOVA reveals significant difference (P,0.05) and Duncan multiple range test was used to compare difference among individual treatment means.

RESULTS AND DISCUSSION

The summary of haematological parameters of *Clarias gariepinus* fed rubber seed meal-based diets at varying inclusion levels is presented in Table 3.

Table 3: Haematological profile blood of *Clarias gariepinus* fed Rubber Seed Meal (RSM) diets.

Parameters	Percentage Inclusion in Rubber Seed Meal (RSM)				
	0%	5%	10%	15%	20%
White blood cell count (WBC X 10 ⁴ mm ³)	a 119.45±30.28	ab 1241.30±0.00	ab 1233.60±9.24	ab 1236.06±11.83	b 261.16±5.33
Red blood cell count (RBC X 10 ⁶ mm ³)	1.89±0.48	1.30±0.58	2.30±0.52	2.28±0.41	2.06±1.04
Haemoglobin (Hb g/dl ⁻¹)	c 8.43±1.56	a 9.05±1.12	ab 12.45±1.10	bc 10.63±0.00	Abc 14.10±0.69
Haematocrit (Ht %) (PVC)	26.93±7.83	17.30±7.36	28.66±6.02	27.93±4.69	36.70±1.21
(Hb/Ht)	3.04±0.45	2.69±0.47	2.28±0.42	2.71±0.65	2.60±0.88
MCH (Pgc ⁻¹)	46.87±4.77	56.81±8.13	49.52±4.64	48.79±7.11	52.99±7.44
MCHC	34.42±5.49	39.65±7.42	46.85±8.21	40.73±8.28	38.50±1.27

N/B. Means with the same superscript in the same row are not significant difference (P≥0.05).

The leucocytes count (WBC) ranged between $1261.16 \times 10^4 \text{ mm}^3$ in fish fed 20% Rubber Seed Meal (RSM) to $1194.45 \times 10^4 \text{ mm}^3$ in fish fed control diet (CTR). WBC increased with increment in levels of rubber seed meal. A remarkable increase in the haematological parameter was observed by Barros et al.; (2002) who fed channel catfish with cotton seed and Jimoh et al. (2013) that fed *Clarias gariepinus* with *Chrysophyllum albidum* seed meal to replace maize. Increase in WBC count as observed in this study could be attributed to increase in production of leucocytes in the haematopoietic tissue of the kidney and perhaps the spleen. Therefore, it can be deduced that limited stress was placed on the health of *Clarias gariepinus* fed RSM at the given inclusion levels. The Red Blood Cells (RBC) counts also showed a similar trend as observed in WBC, although there was no significant difference ($P > 0.05$) in fish fed control diets and fish fed test diets. The observed increase in Hb, Ht, and MCH in *Clarias gariepinus* = fed RSM in this study corroborates with the results given by Jimoh et al. (2012) and Tacon (1992) that nutritionally rich diets can cause increase in haemoglobin content, increased CV and increase RBC count.

Based on the results above, it is undoubtable that not much stress is placed on the health of *Clarias gariepinus* fed rubber seed meal. It therefore, shows that the fish under study had high immunity or resistance to disease. The high RBC count recorded in the study indicates high oxygen absorption carrying capacity of the blood which is characteristic of fishes capable of aerial respiration with high capacity. The erythrocyte counts greater than $1 \times 10^6 \text{ mm}^3$ is considered high and while the PCV values with the 20-30% for fish said to be within normal range. The results of this study therefore fall within the normal range and agrees with reports by Clarke et al. (1979) and Erondy et al. (1993).

CONCLUSION

This study reveals that the haematological parameters of *Clarias gariepinus* does not impact serious stress on the health of the fish even at 20% inclusion level of Rubber Seed Meal (RSM) in its diet. Although the optimal inclusion level for good growth has been found to be 5%, the results, also showed that the present level of anti-nutrients or in the RSM could not induce any pathological change in the fish.

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