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Biochemical and Haematological Response of *Clarias gariepinus* (Burchell, 1822) Juveniles Fed with Diet Containing *Mytilus edulis* Shell at Varying Level

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ABSTRACT

A twelve week feeding trial was carried out to assess the effect of *Mytilus edulis* shell on the biochemical and haematology parameters of *Clarias gariepinus* juveniles, in an attempt to substitute Dicalcium Phosphate (DCP) in the preparation of fish diets. Ten fish were randomly distributed in a plastic (High density polyethylene) tank in triplicates and fed 40% crude protein commercial feed. The test fish were fed with feed containing DCP replaced with *M. edulis* shell at different percentage 0, 25, 50, 75 and 100%. The fish fed with diet 5 which contain 100% *Mytilus edulis* shell had lower Feed Conversion Ratio (FCR) when compared to the fish fed with the control diet which indicate that *C. gariepinus* was able to effectively utilize the *M. edulis* shell in the production of fish flesh. Fish fed with feed containing 100% *M. edulis* showed a slight decrease in haematological values of Parked Cell Volume (PCV 0.19±0.11), Haemoglobin (Hb 83.36±0.47), White Blood Cell (WBC 8.64±0.55), Mean Corpuscular Volume (MCV 89.90±0.26), Mean Corpuscular Haemoglobin (MCH 39.23±1.96) and are slightly higher in the Red blood cell (RBC 2.13±0.11), Mean Corpuscular Haemoglobin Concentration (MCHC 0.44±0.23), compared to the values of fish fed the control diet, PCV(0.37±0.01), HB (85.76±5.0.12), WBC (9.3±0.02), MCV (128.3±1.7), MCH (52.96±3.5), RBC (1.4±0.10), MCHC (0.41±0.02). It is concluded that using *Mytilus edulis* shell as a premix in the diet of *C. gariepinus* showed a slight decrease in the haematological parameters but there was no significant changes ($p>0.05$) thereby having no negative impact on the health status of the specie. Therefore, direct use of *M. edulis* shell as a premix to fish feed should be encouraged.

Key words: Biochemical, haematology, *Clarias gariepinus, Mytilus edulis* shell

INTRODUCTION

The over-exploitation of fish resources and the ever increasing protein demand by the world population have posed problems to the fish supply from natural waters. The supply of proteinous food in Africa is very expensive, a problem that needs to be tackled very seriously, considering the limited available resources (Gabriel *et al*., 2007). Faced with a supply constraint, attention has now been drawn to aquaculture as a means to combat protein malnutrition in the developing countries. Fish production is relatively inexpensive when compared with other sources of animal protein such as cattle, pig and poultry their productions are very expensive due to low level of technology and poor pasture lands (Ayoola, 2010a).

One of the important problems in aquaculture is the development of practical diets for the rearing of larval and juvenile organisms in order to increase growth and survival rates. The development of better feeds and their more efficient use have resulted in the production of larger fish for which there is not only a higher demand but which also commands a premium price (FAO, 2010).

Tilapia species (*Oreochromis niloticus, Sarotherodon melanotheron*) and Clariidae catfishes (*Clarias* and *Heterobranchus* sp.), are the most widely cultured fish and are suited to low-technology farming systems in many other developing countries because of their fast growth rate, efficient use of natural aquatic foods propensity to consume a variety of supplementary feeds, omnivorous food habits, resistance to disease and handling, ease of reproduction in captivity and tolerance to wide ranges of environmental conditions. African catfish *Clarias gariepinus* is the most widely cultured fish in Nigeria (Omitoyin, 2004). Catfish grows fast, commands high market value, hardy and can survive where most other cultivable species cannot. Fish supplies over 50% of the total animal protein consumed in developing countries and less so in developed countries (Ayoola, 2010b).

However, Omitoyin *et al.* (2005) observed that some major problems militating against mass production of catfish in Nigeria are inadequate supply of fingerings, lack of quality brood stock and disease outbreak among others. With the present government initiative in aquaculture development in Nigeria, more intensive and semi-intensive commercial fish farms, hatcheries are fast developing. The development of small scale aquaculture depends on the use of availability of local ingredients which will reduce the feeding cost (Edwards and Allan, 2004). This study was conducted to investigate the effect on growth, haematological changes and plasma biochemical parameters of *Clarias gariepinus* fed with a diet containing various levels of *Mytilus edulis* shell as a substitute to Dicalcium Phosphate (DCP).

MATERIALS AND METHODS

This study was carried out in the Department of Marine Sciences, University of Lagos, Nigeria. The juveniles were bought from a commercial fish farm (Duton fish farm) at Bariga, Lagos.

Mytilus edulis shell was collected from the Lagos Lagoon from the shoreline. The shell was washed properly to remove any toxic and sand found on the shells and after which, was dried for 3 days to remove the moisture content. After being dried, the shell was milled to powdery form.

The experimental fishes were first acclimatized to laboratory conditions for two weeks (14 days) at a temperature of 28.60±0.51°C and a 12L/12D photoperiodic regime. They were fed with a commercial fish feed (40% crude protein) twice daily 7 am and 5 pm on 5% of the total biomass.

During the next 12 weeks the fish were fed with feed containing crushed *Mytilus edulis* shell collected from the Lagos Lagoon and the control fish with diet containing Dicalcium Phosphate (DCP) (Table 2). The experimental fish were fed with *Mytilus edulis* at a rate of 0, 25, 50, 75 and 100%. One hundred and eighty healthy farmed *Clarias gariepinus* juveniles (both male and female) with a mean weight of 126.66±11.54 g and mean length of 12.60±0.31 cm were randomly selected for this study. The juveniles' stages were used because they are more sensitive to toxicity tests than adults. Water was continuously supplied from a borehole at the back of the laboratory. Water level in the tanks was maintained at a level of 15 cm throughout the experimental period. Water quality of the rearing environment such as temperature, Dissolved Oxygen (DO), pH, of the

experimental setup was measured. While the proximate composition of *Mytilus edulis* shell and control diets were determined using the AOAC (1990) methods. After which, the blood samples were collected and taken for analysis.

Experimental set up: Fifteen plastic tanks each with 34 cm height, 33 cm width and 50 cm length were used for the experiment. Each of the tanks filled with water were stocked with 10 juveniles. Water was filled to 2/3 of the volume of each tank. The mean weight gain and length of the specimen in each of the experiment tanks were obtained at the end of every week. The tanks were labeled, $T0_a$, $T0_b$, $T0_c$, $T1_a$, $T1_b$, $T1_c$, $T2_a$ $T2_b$ $T2_c$, $T3_a$, $T3_b$, $T3_c$, $T4_a$, $T4_b$, $T4_c$.

Diet preparation and feeding regime: The dicalcium phosphate content of the control diet was replaced with *Mytilus edulis* shell meal in the test diet. All feed ingredients (maize, indomine, wheat offal, soya bean meal, palm kernel cake, fishmeal, vitamin C and fish premix), were milled to pass through a 2 mm diameter sieve, mixed together in a mixer and pelleted. The juveniles were fed at least two times daily. The tanks labeled $T0_a$, $T0_b$ and $T0_c$ feed were not treated with the *Mytilus edulis* shell, the other tanks , $T1_a$, $T1_b$, $T1_c$, $T2_a$ $T2_b$ $T2_c$, $T3_a$, $T3_b$, $T3_c$, $T4_a$, $T4_b$, $T4_c$ feed were treated with *Mytilus edulis* at different percentage. The percentage substitutions in formulating feeds for diet 1-5 are presented in Table 1 and also composition of the formulated diet are represented in Table 2.

Determination of physico-chemical parameter: Temperature measurement was determined in situ by means of simple mercury in glass thermometer, calibrated in degrees Celsius (°C). A highly sensitive pH meter (Jenway model 3050, serial No. 42010) was used to determine the pH by dipping the probe into the water sample and reading off the values from the meter.

Table 1: The percentage substitutions in formulated feed

Diet	Percentage substitutions
1	100% DCP (Control feed)
2	75% DCP and 25% *Mytilus edulis* shell
3	50% DCP and 50% *Mytilus edulis* shell
4	25% DCP and 75% *Mytilus edulis* shell
5	100 *Mytilus edulis* shell

Table 2: Composition of the formulated diet

Composition	0%	25%	50%	75%	100%
Maize	15.0	15.0	15.0	15.0	15.0
Wheat offal	14.0	14.0	14.0	14.0	14.0
Groundnut cake	20.0	20.0	20.0	20.0	20.0
Soya bean cake	13.0	13.0	13.0	13.0	13.0
Palm kernel cake	10.0	10.0	10.0	10.0	10.0
Fish meal	15.0	15.0	15.0	15.0	15.0
Dicalcium phosphate	2.0	1.5	1.0	0.5	_
Mytilus edulis shell	_	0.5	1.0	1.5	2.0
Fish premix	0.75	0.75	0.75	0.75	0.75
Vitamin C	0.25	0.25	0.25	0.25	0.25
Indomine	10.0	10.0	10.0	10.0	10.0

The amount of dissolved oxygen was determined using a sensitive DO meter. The probe was dipped into the water and the sample reading was recorded.

Blood collection: The fishes were taken from the different tanks. The fishes were labeled T_0, T_1, T_2, T_3, T_4. Each fish was held firmly then bled from the ventral region near the anal opening and also through the gills using sterile syringe, 2 mL of blood was collected and immediately transferred into ethylenediaminetetracetic acid bottle to prevent clotting which have been labeled according. The blood samples were taken immediately to the Nigeria Institute for Medical Research laboratory (NIMR), Yaba, Lagos, for analysis of Packed Cell Volume (PCV), erythrocyte count, Urea, Creatinine, Total protein, Albumin, Globulin, Triglyceride and Alkaline phosphatase.

Blood analysis: The blood samples were dispensed into tubes containing Lithium heparin anticoagulant to obtain plasma for biochemical analysis. Haematological values were measured following standard methods (Joshi *et al*., 2002).

Statistical analysis: The data obtained were statistically evaluated using student test. Data obtained were subjected to one way analysis of variance. Data includes mean, standard deviation. While the means was compared for significant differences using Duncan's multiple range tests using Statistical Package for the Social Sciences (SPSS) 10 packet programs.

RESULTS

Proximate analysis of *Mytilus edulis* shell: The proximate compositions of the *Mytilus edulis* shell are presented in Table 3. The values showed that the crude protein content is 6.75 and having 33.39 of calcium.

Water quality parameters: The mean water quality parameters of the cultured environment of the tested *Clarias gariepinus* are represented in the Table 4. The mean temperature values ranges from 28.20±0.46 to 26.66±0.2°C while the pH values ranges from 6.99±0.21 to 7.42±0.25. The values range from 7.45±0.57 to 7.93±0.59.

Growth response, feed and protein utilization by *Clarias gariepinus* are shown in Table 5. The best overall weight gain was found in the fish fed with diet 1 and the least weight gain in the tested

Table 3: Proximate analysis of *Mytilus edulis* shell

Composition	Values
Crude protein (%)	6.75
Fat (%)	0.50
Fiber (%)	15.69
Ash (%)	61.58
Moisture (%)	29.05
Energy ($kcal^{-1}$)	291.00
Calcium (%)	33.39

Table 4: Water quality indices of the cultured environment

	Parameters		
Diet	Temperature (°C)	pH	DO (mg L^{-1})
1	28.60±0.51[a]	7.42±0.25[a]	7.61±0.62[a]
2	28.41±0.39[a]	7.09±0.23[a]	7.8±0.58[a]
3	28.20±0.46[a]	6.99±0.21[a]	7.45±0.57[a]
4	28.33±0.40[a]	7.15±0.15[a]	7.93±0.59[a]
5	26.66±0.2[a]	7.06±0.25[a]	7.82±0.66[a]

Means with the same superscripts along the vertical roll are not significantly different (p>0.05)

Table 5: Growth response, feed and protein utilization by *Clarias gariepinus* with the various diets

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Average initial weight	126.67±11.5[a]	126.67±11.5[a]	126.67±20.8[a]	126.67±10.4[a]	126.67±16.0[a]
Average final weight	358.33±2.8[a]	341.67±10.4[a]	351.67±7.63[a]	346.67±11.5[a]	353.33±7.63[a]
Mean weight gain	231.67±5.77[a]	215±13.22[a]	225±21.79[a]	220±13.22[a]	226.67±23.6[a]
Specific growth rate	1.2±0.03[a]	1.15±0.09[a]	1.19±0.18[a]	1.17±0.09[a]	1.19±0.16[a]
Feed conversion ratio	2.21±.02[a]	2.07±0.15[a]	2.16±0.2[a]	2.17±.15[a]	2.18± 1.6[a]
Average daily growth	2.69±0.06[a]	2.5±15[a]	2.61±.25[a]	2.55±0.15[a]	2.63±0.27[a]
Voluntary feed intake	0.25±.01[a]	0.25±0.01[a]	0.25±.01[a]	0.24±0.01[a]	0.24±0.01[a]
Relative growth rate	183.02±8.61[a]	171.03±22.8[a]	182.32±42.9[a]	174.80±21.9[a]	182.26±39.3[a]
Feed intake	104.73±1.43[a]	103.5±3.77[a]	104.10±2.98[a]	101.30±1.69[a]	102.2±0.8[a]
Protein intake	41.89±0.57[a]	41.4±1.50[a]	41.64±1.19[a]	40.52±0.67[a]	40.88±0.32[a]
Protein efficiency ratio	5.52±0.06[a]	5.19±0.38[a]	5.41±0.63[a]	5.43 ±0.39[a]	5.54±0.59[a]

Means with the same superscripts along the vertical roll are not significantly different (p>0.05)

fish fed with diet 2. The least PER was observed in the fish fed with diet 2 and best PER in the fish fed with diet 5.

The mean values of the biochemical parameters of the tested fish with the various diets: The biochemical parameters are represented in table 6. The mean urea ranges from 1.53±0.76 to 9.96±3.34 mg dL^{-1} while mean creatinine values has 0.39±0.14 mg dL^{-1} has its lowest value and 0.64±0.43 mg dL^{-1} has it highest value. Mean total protein has its values ranging from 32.5±18.4 to 38.1±18.49 g dL^{-1} also, Albumin has its lowest value as 3.86±0.3 and 4.06±0.15 (g L^{-1}) as it highest value. Triglyceride values range from 3.86±0.3 to 4.11±0.23 mg dL^{-1}, alkaline phosphatase has its value ranging from 5.52±2.76 to 12.88±13.04 (IU L^{-1}).

The mean values of the haematological parameters of the test fish with the various diets: Table 7 shows the haematological changes in test fish and the control fish. The mean Haemoglobin (Hb) ranges from 58.33±1.15 to 85.76±0.12 g L^{-1} while Packed Cell Volume (PCV) mean ranges from 0.14±0.10 to 0.36±0.01 L L^{-1}. The values of the mean Red blood cell, White Blood Cell (WBC) value ranges from 8.64±0.55 (G L^{-1}) to 10.89±0.30 (G L^{-1}). Mean Corpuscular Haemoglobin (MCH) has its mean values ranging from 37.76±3.90 to 52.96±3.50 (pg). Mean Corpuscular Haemoglobin Concentration (MCHC) mean values ranges from 0.41±0.02 to 0.44±0.23 l L^{-1}. Mean Corpuscular Volume (MCV) mean values ranges from 88.08±10.21 to 128.3±1 L L^{-1}.

Table 6: Plasma biochemical parameters of the tested *Clarias gariepinus* with various diets

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Urea (mg dL^{-1})	1.53±0.77^{a}	3.83±5.31^{a}	5.11±3.62^{a}	9.96±3.34^{a}	1.53±0.7^{a}
Creatinine (mg dL^{-1})	0.45±0.23^{a}	0.39±0.14^{a}	0.42±0.17^{a}	0.64±0.43^{a}	0.59±0.45^{a}
Total protein (g dL^{-1})	34.06±0.74^{a}	33.17±3.1^{a}	38.1±7.63^{a}	37.21±10.6^{a}	32.70±1.06^{a}
Albumin (g dL^{-1})	4.01±0.28^{a}	4.06±0.15^{a}	3.86±0.3^{a}	4.11±0.23^{a}	4.01±0.02^{a}
Triglyceride (mg dL^{-1})	82.79±3.5^{a}	114±17.13^{a}	97.23±12^{a}	100.12±37^{a}	95.4±3.27^{a}
Alkaline Phosphatase (IU L^{-1})	8.40±2.94^{a}	7.36±4.21^{a}	9.2±4.21^{a}	4.6±3.18^{a}	5.50±2.76^{a}
Globulin (g L^{-1})	30.04±0.79^{a}	29.11±3.1^{a}	34.22±7.6^{a}	30.72±7.60^{a}	28.62±0.8^{a}

Mean with the same superscripts along the vertical roll are not significantly different ($p<0.05$)

Table 7: Haematological parameters of the tested *Clarias gariepinus*

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Hb (g L^{-1})	85.76±0.12^{b}	83.66±0.72^{a}	85.10±4.61^{a}	58.33±1.15^{c}	83.36±0.47
PCV (G L^{-1})	0.36±0.01^{a}	0.19±0.01^{a}	0.20±0.15^{b}	0.14±0.10^{a}	0.19±0.11^{a}
RBC (T L^{-1})	1.40±0.10^{a}	2.23±0.25^{c}	1.73±0.15^{b}	1.30±0.10^{c}	2.13±0.11^{a}
WBC (G L^{-1})	9.30±0.02^{a}	10.89±0.30^{e}	10.34±0.17^{d}	3.41±0.01^{a}	8.64±0.55^{c}
MCV (fl)	128.30±1.7^{a}	88.08±10.21^{a}	120.03±15.8^{bc}	107.66±0.6^{b}	89.90±0.26^{c}
MCH (pg)	52.96±3.5	37.76±3.90^{a}	49.43±6.80^{a}	44.96±2.71^{a}	39.23±1.96^{a}
MCHC (L L^{-1})	0.41±0.02^{a}	0.44±0.20^{a}	0.40±0.25^{a}	0.41±0.20^{a}	0.44±0.23^{a}

Means with different superscripts in column are significantly different ($p<0.05$)

DISCUSSION

The feeding trials revealed that *Clarias gariepinus* responded to all the diets, irrespective of their composition. The fish fed with diet 5 which contain 100% *Mytilus edulis* shell had lower Feed Conversion Ratio (FCR) when compared to the fish fed with the control diet which indicate that *C. gariepinus* was able to effectively utilize the *M. edulis* shell in the production of fish flesh. The water quality parameters of both experimental set up for both treatment were similar and within the optimum range recommended for culture of *C. gariepinus* by Omitoyin *et al.* (2006) when he worked on haematological changes in the blood of *C. gariepinus* (Burchell, 1822) juveniles fed poultry litter. The values of the plasma excretory products are higher than that of (Omitoyin *et al.*, 2006). The increase in total protein and globulin levels in the fish fed with diet 2 may depend on dehydration due to diminished feed and water intake which is similar to the work of Uyanik *et al.* (2001) on changes in some biochemical parameters and organs of broilers exposed to cadmium and effect of zinc and cadmium induced alterations. Total protein values were slightly lower than those reported by Ayoola (2011) and the values were slightly lower than those reported by Omitoyin *et al.* (2005), this may be due to environmental condition of rearing facilities and handling.

Gabriel *et al.* (2004) further reported no significant differences in haematological values for apparently healthy *C. gariepinus* before and after acclimation which was similar to the observation in this study. The decrease in the White Blood Cell (WBC) in the fish fed *Mytilus edulis* shell showed that the fish was in a good state of health, this is in agreement with the work done by Al-Dohail *et al.* (2011) on Evaluating the use of *Lactobacillus acidophilus* as a bio-control agent against common pathogenic bacteria and the effects on the haematology parameters and histopathology in African catfish *C. gariepinus* juveniles. The increase in the total White Blood Cell (WBC) as observed in the fish fed with diet 2 is attributed to increased production of leucocytes in the hematopoietic tissue of the kidney perhaps the spleen, this agreed with work of Omitoyin (2006). The results of this present study provides baseline values for some haematological

and biochemical parameters for *C. gariepinus* which according to Omitoyin (2006), can be used to assess fish health.

In conclusion, the present study revealed that using *Mytilus edulis* shell as a premix in the diet of *C. gariepinus* feed showed a slight decrease in the haematological and biochemical parameters but there was no significant changes ($p>0.05$) thereby having no negative impact on the health status of the specie. Therefore, direct use of *M. edulis* shell as a premix to fish feed should be encouraged.

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