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Full Length Research

Haematological and serum biochemical parameters as biomarkers of growth performance in artificially spawned H*eterobranchus longifilis*

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ABSTRACT: This research aimed at assessing variation in haematological and biochemical profiles of F1 generation of *Heterobranchus longifilis* by growth. The juveniles were classified based on the total length of the individual fish at 8th week post-hatch according to their growth rates into "shooters" (> 15.5cm), "averages" and "runs" (< 12.5cm). Haematological analysis was carried out using the automated Sysmex Haematology and Coagulation systems; serum biochemical assay was conducted via an automated Serological Roche Hitachi system. The haematological values of juvenile *H. longifilis* F1 ranged from 11.0 to 17.2 (g/dL), 32.9 to 51.5 (%) and 1.8 to 5.08 (k/µL) for haemoglobin, packed cell volume and white blood cells, respectively. Biochemical profile of juvenile *H. longifilis* F1 across three growth classes had mean values of 10.13 ± 3.23 (IU/L), 28.69 ± 14.12 (IU/L) and 3.77 ± 0.49 (mg/dL) for aspartate aminotransferase, alkaline phosphatase and total cholesterol respectively. As the growth quality increased corresponding increases in neutrophil, white blood cell, haematocrit, haemoglobin and alanine phosphatase were observed. Alanine aminotransferase was observed to decrease with increase in growth quality. The shooters' were the healthiest. Haematological and serum biochemical parameters are good biomarkers of growth performance in *H. longifilis.* Further studies should be carried out on the association between growth factors, metabolic enzymes and sex hormones.

Key words: Biochemical, growth, haematological, *Heterobranchus longifilis*, juveniles.

INTRODUCTION

The profitability of the aquaculture industry has become widely known (Huda et al., 2002). There is increased global attention on aquaculture because of the need to augment fish production from the wild. This is particularly noticeable in populous countries like Nigeria where there is high protein demand (Owodeinde et al., 2011). *Clarias gariepinus* and *Heterobranchus longifilis* are two commonly cultured clariid fishes that are very popular among fish farmers and consumers in Nigeria (Ojutiku, 2008). They are reared all over the country especially in the south and have very good commercial value in Nigerian markets (Adewolu and Adoti, 2010). Qualities that make them suitable for aquaculture include: fast growth rate, hardiness, high yield potential, high fecundity and palatability (Offem et al., 2008). Oteme et al. (1996) stated that the fast growth rate of *H. longifilis* makes it a fish of the future of aquaculture.

Historically, biochemical parameters have been studied and baseline values have been established for fish species used in research or as food sources in Europe and North America. Data on haematological, biochemical and biological parameters of fish species, which are admitted to aquaculture in the developed countries, are available especially in relation to fish vaccinology programmes (Gudding and Van Muiswinkel, 2013). Information on the interaction of blood biochemical parameters and fish health, may prove useful as part of an integrated management system for fishery, especially in predicting the onset of disease, thereby allowing for the employment of appropriate intervention strategies to mitigate fish loss. Adeyemo et al. (2007) reported that haematological

indices are important parameters for evaluating the physiological status of fish. Certain physiological dysfunctions in the body are reflected as alterations in blood constituents, which can be used as diagnostic indicators.

Reports on the normal haematological and serum biochemical parameters of many fish species farmed in northern Nigeria, such as *H. longifilis* with respect to differential growth and deformity are scarce to come by. This research aimed at assessing variation in haematological and biochemical profiles of F1 generation of *H. longifilis* by growth.

MATERIALS AND METHODS

Description of the Study Area

The culture exercise was carried out at Sama fish farm, Mando. Mando is situated in Igabi Local Government Area of Kaduna, Kaduna State, Nigeria, which falls between latitude 10º 49' 06" N and longitude 6º 42' 00" E. The annual rainfall in Kaduna varies from 0.0 – 825.0 mm per month reaching its peak in August. Temperature during peaks of breeding season (June to September) ranges from 22-29 ºC and as low as 17 ºC in the dry season (Google Imagery, 2013).

Source, Selection and Management of Broodfish

Twelve (seven females**,** five males), twenty-four months old brooders of *H. longifilis* weighing about 2,000 to 3,000g were purchased from Aquatect Fish Farms, Makurdi, Benue State**.** The mature brooders were selected on the basis of their morphological and egg characteristics from a greater population of spawners that were reared from egg to maturity at the Aquatect Fish Farms, Makurdi. During the acclimatization period of 2 weeks, a fixed feeding regime of 40% crude protein (Madu et al., 2003) and 12kcal of energy, 12.5% lipid and 100mg of vitamin C kg^{-1} diet (Ibiyo et al., 2006) at 5% body weight per day divided into two and given between the hours of 0800 to 0900 h and 1600 to 1700h was adopted.

Induced Breeding

Induced breeding activities were carried out in the rainy season in July, 2012. The brood stock used had their standard lengths (to the nearest 0.1cm) and weights (to the nearest 0.1g) with taken top loading balance (model: Gottinen V-240). Breeding involved triplicate crosses comprising two females and a male per cross. The synthetic hormone ovaprim Syndel® was administered intramuscularly as a single dose at the rate of 0.5 ml/kg in the flank just below the dorsal fin. Stripping and wet fertilization was carried out as described by Carballo et al. (2008). The pH, temperature and dissolved oxygen levels of incubation and up to the end of the experiment within the water recirculation system were determined and recorded weekly using a pH and temperature Hanna® combo meter and the Winkler's titrimetric method, respectively.

Rearing of the F1 Generation of *H. longifilis*

Three-day old larvae of *H. longifilis* from each mating combination in duplicate hatching troughs were fed live *Artemia* nauplii (shell free), predetermined as 54% crude protein, four times a day (0800-0900 h, 1200-1300 h, 1600-1700 h and 2000-2100 h) at an approximate rate of 50 live *Artemia* per fry per feeding time, as starter feed, for two weeks. After two weeks of feeding, the fry was transferred to nursery tanks and introduced gradually to an artificial dry diet of 45% crude protein from Durante Superior Fish Concentrate (0.5 mm pellet size) catfish feed. The pellet size was adjusted from 0.7, 1.0 to 1.8 mm, to suit the diameter of the fish's buccal cavity as the fingerlings grew into juveniles. At eight weeks, the juveniles were sorted, counted and measured.

Classification of juveniles by growth

The juveniles were classified according to their growth rates into "shooters", "averages" and "runs". The representatives of the growth classes were identified at the 8th week based on a 5% randomly selected sample via a stratified sampling technique, before sorting. Determination was based on the total length of the individual fish. A juvenile was considered to be a shooter if its total length was approximately equal to or greater than 15.5cm; and a run if its total length was less than12.5 cm at the end of the 8th week post-hatch (Ataguba et al., 2009). At 8th week post-hatch the fingerlings are referred to as juveniles and ready for stocking into grow-out ponds, at this stage, size variation which is a major causative factor of cannibalism is pronounced (Solomon and Udoji, 2011).

Determination of Haematologic Biochemical Analytes of F1 Generation of *H. longifilis*

Haematological analysis was conducted with the aid of a Sysmex Haematology and Coagulation Systems model KX-21N in the Haematology unit, Ahmadu Bello University Teaching Hospital, Zaria. Haematological analysis was conducted on the 'shooters', 'average-size' and 'runs' of 8-week old *H. longifilis*. The fish were bled in the Necropsy Laboratory, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. Blood samples were pooled from 7 juveniles per treatment in triplicates. The sampled fish were rinsed thoroughly in

Table 1. Haematological Values of Juvenile *Heterobranchus longifilis* F1

Parameter	Shooters	Averages	Runs
Haemoglobin (g/dL)	16.63 ±1.03a	12.33 ± 2.23b	11.27 ± 1.19c
Total Protein (g/dL)	6.80 ± 0.22a	6.53 ± 0.98a	6.73 ± 1.02a
Packed Cell Volume (%)	49.87 ± 3.11a	36.97 ± 2.43b	33.70 ± 2.26c
White Blood Cells (k/µL)	5.05 ± 0.23 a	4.33 ± 0.12b	1.87 ± 0.63c
Lymphocytes (IU/L)	97.83 ± 2.28a	97.17 ± 3.03b	98.33 ± 3.11a
Monocytes (k/µL)	0.1 ± 0.23a	0.2 ± 0.16a	0.1 ± 0.21a
Neutrophils (k/µL)	2.67 ± 0.82a	3.17 ± 0.76a	1.67 ± 0.27b

Mean of triplicate samples each pooled from 7 juveniles. Means with the same superscripts across a row are not significantly different ($P > 0.05$).

clean water and wiped dry with clean towels. Blood samples were collected from severed caudal peduncle in juveniles and from the intraperitoneal vein in adults, using 2 ml plastic syringes with 22-gauge needles treated with the anti-coagulant ethylene diamine tetra acetate acid (EDTA) and stored in labeled sample bottles.

The anticoagulated blood sample was mixed using the automatic mixer; the well mixed blood was then aspirated into the machine by ensuring that the probe was dipped inside the blood sample. The green background (start switch) was pressed; a clicking sound indicated that it had sucked the appropriate volume of blood required for the analysis. The tube was then removed from the probe, recapped and kept in the test tube rack. Results for white blood corpuscles, haemoglobin, haematocrit, total protein counts; number and percentage lymphocytes, monocytes, neutrophils were printed automatically.

Determination of Prognostic and Diagnostic Values of Serological Markers of the F1 Generation of *H. longifilis*

Serum biochemical assay was conducted via an automated Serological Roche Hitachi model 902 machine in the Chemical Pathology Laboratory, Ahmadu Bello University Teaching Hospital, Zaria. Pooled blood samples were put in labeled sample bottles and centrifuged (Triac Clinical Centrifuge Saitexiangyi, model TG12MX) at 1,400 rpm. The sera obtained were fed into the automated Serological machine to obtain values for aspartate aminotransferase, alanine aminotransferase, bilirubin, alkaline phosphatase and total cholesterol, following the automated methods recommended by the International Federation of Clinical Chemistry (Baisk and Panteghini, 2006). The results obtained were printed automatically.

Statistical Analyses

Statistically significant differences was determined by setting the aggregate type I error at 5% ($P \leq 0.5$) for each comparison. The one-way ANOVA was used to test for any significant differences between treatments (the various growth classes) in terms of their haematological and biochemical values. Differences between the means, where present, were ranked using the Duncan's Multiple Range Test. Correlation-based principal components analysis was used to establish the relationship between growth rate and the haematologic and biochemical analytes.

RESULTS

Haematological Values of Juvenile *H. longifilis* F1

The haematological values of juvenile *H. longifilis* F1 ranged from 11.0 to 17.2 (g/dL), 6.2 to 7.2 (g/dL), 32.9 to 51.5 (%) and 1.8 to 5.08 × 10^3/mm^3 for haemoglobin, total protein, packed cell volume and white blood cells, respectively. Significant differences ($P \leq 0.05$) were observed amongst the differential growth classes with respect to haemoglobin, packed cell volume and white blood cells, which decreased significantly from the "shooters", to the "averages" and to the "runs" (Table 1). An analysis of variance revealed that the differences observed amongst the differential growth classes were not significant ($P > 0.05$) for total protein. Differential on the corpuscular components revealed variation in abundance of lymphocytes, monocytes, and neutrophils.

Biochemical Profile of Juvenile *Heterobranchus longifilis* F1

Biochemical profile of juvenile *H. longifilis* F1 across three growth classes had mean values of 20.81 ± 2.37 (IU/L), 10.13 ± 3.23 (IU/L), 28.69 ± 14.12 (IU/L) and 3.77 ± 0.49 (mg/dL) for alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and total cholesterol respectively. The values ranged from 17 to 24.2 (IU/L), 7.3 to 15 (IU/L), 11 to 47 (IU/L), 2.8 to 4.3 (mg/dL) for ALT, AST,

Table 2. Serological Values of Juvenile *Heterobranchus longifilis* F1

Parameter	Shooters	Averages	Runs
Alanine aminotransferase (IU/L)	18.33 ± 3.23[c]	20.60 ± 2.62[b]	23.50 ± 2.24[a]
Aspartate aminotransferase (IU/L)	8.53 ± 3.12[b]	7.53 ± 2.83[b]	14.33 ± 3.41[a]
Alkaline Phosphatase (IU/L)	44.33 ± 11.02[a]	29.73 ± 15.42[b]	12.00 ± 14.32[c]
Total Cholesterol (mg/dL)	4.23 ± 0.63[a]	3.63 ± 0.38[a]	3.43 0.49[a]

Mean of triplicate samples each pooled from 7 juveniles. Means with the same superscripts across a row are not significantly different ($P > 0.05$)

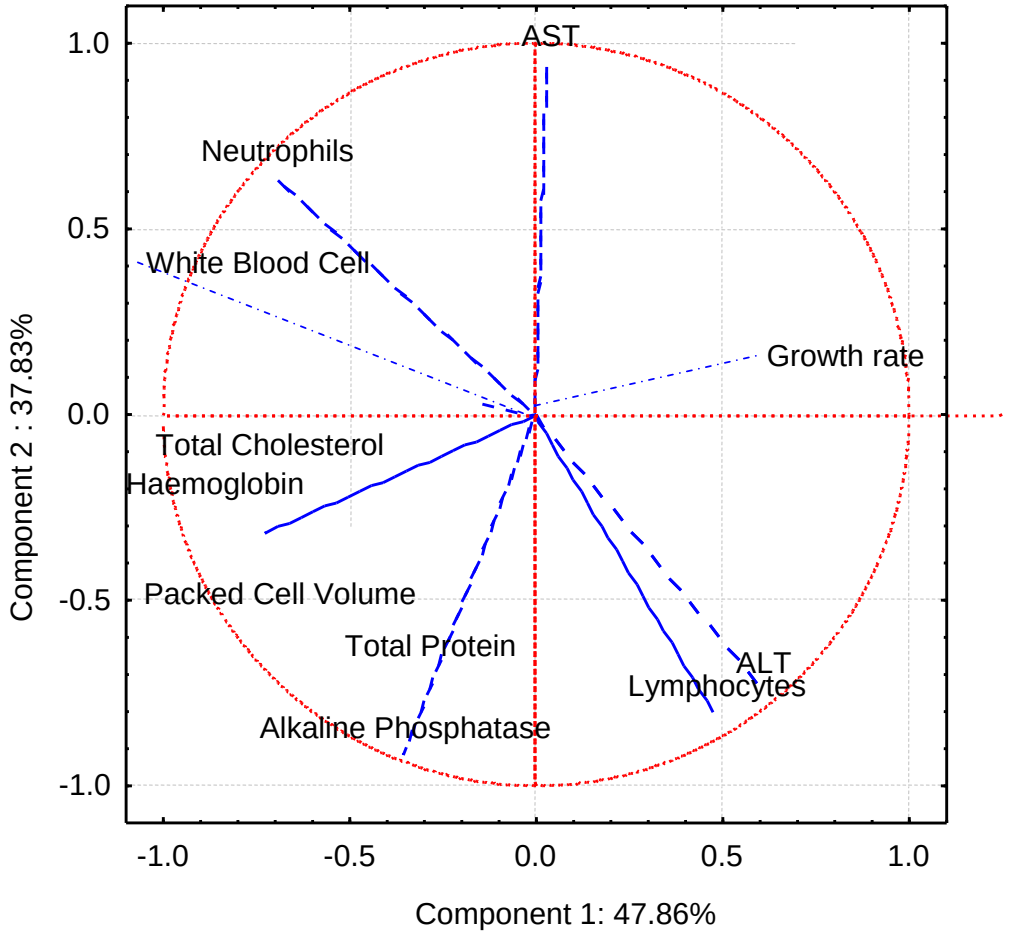

Figure 1. Principal components analysis (PCA) biplot showing the relationship of growth rate with haematological and serological analytes

ALP and total cholesterol, respectively. Statistical analysis revealed no significant difference with respect to total cholesterol. Other parameters showed differences across the growth classes (Table 2).

Relationship between Growth Class and Haematologic and Biochemical Analytes

A correlation based principal component analysis is shown in Figure 1. Growth class showed significant correlation along the x-axis. As the growth quality increased corresponding increases in neutrophil, white blood cell, haematocrit, haemoglobin and alanine phosphatase were observed. Alanine aminotransferase was observed to decrease with increase in growth quality. The growth quality showed no significant relationship with aspartate aminotransferase.

Physico-chemical parameters were within the recommended ranges for the culture of *Heterobranchus longifilis* in the tropics. Mean values for pH, temperature and dissolved oxygen were 6.45, 27.25°C and 5.25mg/l, respectively.

DISCUSSION

The significant decrease ($P \leq 0.05$) observed in the 'shooters', 'averages' to 'runs' in juvenile *H. longifilis* with respect to haemoglobin and packed cell volume suggests that the 'shooters' were the least stressed and most healthy (Gabriel et al., 2004). The relatively lowest haemoglobin and hematocrit values in the 'runs' indicates a predisposition to anaemia, while the relatively lowest white blood cell count in the 'runs' is a pointer to a compromise in the immune system making them less able to fight infections and other disease conditions, invariably resulting in slower growth. A significant decrease in haemoglobin with fungi infestation was reported by Chauhan et al. (2014) for *Channa marulius*. Low leucocyte count has been associated with the presence of a disease process.

The mean haemoglobin value of 13.41 $\pm$ 2.50 g/dL obtained in this study is comparable to the 7.53 $\pm$ 0.92g/dL recorded for juvenile *C. gariepinus* of mean weight 24.04g by Ochang et al. (2007) and 10.62g/100mL reported in the juveniles of the hybrid (*H. longifilis* × *C.gariepinus*) by Osuigwe et al. (2005), but relatively higher than the 82.13 $\pm$ 0.68 g/L in *O. niloticus* of mean weight 24.3 $\pm$ 2.85g by Younis et al. (2012). There are no standard haematological values for tropical fish species but higher haemoglobin values indicate higher rate of transportation of oxygen to and removal of carbon (iv) oxide from the body tissues. This results in higher metabolism and growth. Low haemoglobin suggests a predisposition to anaemia. Giardina et al. (2004) reported that fishes are group of animals with the highest number of multiple haemoglobins. Haematological values give a better understanding of the physiological and pathological state of fish (Satheeshkumar et al., 2011). Protein examination is indicated in perturbations involving the liver immunosuppressions, parasitic disease and or starvation. The total protein concentrations are especially valuable in determining a fish's state of hydration.

The mean percentage PCV of 40.18 $\pm$ 7.53 recorded in this study is relatively higher than the 22.00% and 35.50% for juvenile *C. gariepinus* (Ochang et al., 2007) and juvenile hybrid (*H. longifilis* × *C.gariepinus*) (Osuigwe et al., 2005). The mean white blood cell count of 3.75 $\pm$ 1.45 k/µL in this study compares favourably with the 4.40 $\pm$ 0.41 × 10^3/µL recorded by Ochang et al. (2007) in juvenile *C.gariepinus* but is dissimilar to the 20.42 × 10^3/mm^3 reported by Osuigwe et al. (2005) for juvenile hybrid (*H. longifilis* × *C.gariepinus*). The lower mean white blood cell value in this study could be as a result of the low values for the "runs" (1.85 k/µL) compared to that of the "shooters" 5.05 k/µL. Low white blood cells count indicates a compromise to the immune system due to infection or a disease process.

The mean value for alanine aminotransferase (ALT) (20.81 $\pm$ 2.37) for juvenile *H. longifilis* in this study is relatively higher than 18.62 $\pm$ 0.87 (IU/L) obtained in *O. niloticus* fingerlings with mean weight of 24.3 $\pm$ 2.85g (Younis et al., 2012). The mean value of ALT in this study is also relatively higher than 11.30 $\pm$ 0.20 (IU/L) in *C. gariepinus* juvenile reported by Ozovehe (2013). ALT is primarily liver-specific therefore an elevation in blood content suggests liver disease, the higher ALT values obtained in this study for the "runs", does not necessarily indicate a disease condition, rather it probably implies that the "runs" were relatively more physiologically stressed compared to other growth classes. This is in tandem with the higher value of 26.81 $\pm$ 0.54 (IU/L) obtained for *O. niloticus* fingerlings following short term exposure to zinc by Younis et al. (2012).

Growth quality had a positive import on the values of neutrophil, white blood cell, haematocrit, haemoglobin and alkaline phosphatase (ALP). This indicates that better growth is associated with better health condition and immunodefense system. This suggests that increase in alanine phosphatase and decrease in alanine aminotransferase over time within a culture system indicates rapid growth. Alkaline phosphatase levels was found to decline markedly during starvation due to some factors like a fall in the rate of synthesis caused by lowered metabolic demands and to electrolyte imbalance caused by tissue over hydration. The difference in ALP between the shooters and other growth classes observed in this study is related to the trend that growing animals tend to have higher ALP values due to development of bony tissue. In addition, differential food intake may be responsible. Serological values may differ in the same application depending on the diet, season and presence of environmental stressors. Variations in the activities of these enzymes in fish species are due amongst other factors to diet (Sakamoto et al., 2001). Though the values of cholesterol in this study (3.95 to 4.7 mg/dL) is far lower than the 291.69 $\pm$ 10.2, 208.04 $\pm$ 2.01 and 253.16 $\pm$ 8.25 for 235 to 410 grams apparently healthy *L. calcarifer*, *M. cephalus* and *C. chanos* respectively from Vellar estuary, India (Satheeshkumar et al., 2011).

Conclusion

Haematological and serum biochemical parameters are good biomarkers of growth performance in *H. longifilis.* The decrease observed from the 'shooters', 'averages' to 'runs' with respect to haemoglobin, packed cell volume and white blood cells indicate that the 'shooters' were the healthiest. Higher ALP values in the 'shooters' is associated with greater development of bony tissues in this growth class. High values for ALT and bilirubin in fishes with stunted growth indicate hepatic conditions, infections or inflammation that involved hemorrhaging, atrophy or lysis of cells.

Recommendation

Further studies should be carried out in relation to the

association between growth factors, metabolic enzymes and sex hormones.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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