Trends in

Applied Sciences Research

ISSN 1819-3579

Academic
Journals Inc.

www.academicjournals.com

OPEN ACCESS Trends in Applied Sciences Research

ISSN 1819-3579
DOI: 10.3923/tasr.2019.271.277

Research Article
Haematology of Hybrid Catfish (*Heteroclarias*): Effect of Stocking Densities and Feeding Levels

Ofonime Edet Afia and Gift Samuel David

Department of Fisheries and Aquatic Environmental Management, University of Uyo, P.M.B. 1017, Uyo, Nigeria

Abstract

Background and Objective: Fish haematology is gaining increasing importance in fish culture because of its importance in monitoring the health status of fish. The present study compared different stocking densities and its effect on the blood parameters of hybrid catfish (*Heteroclarias*) when they are fed at 1 and 2% body weight, respectively. **Materials and Methods:** The experiment lasted 12 months using 30 tarpaulin tanks of 1 m^3 volume. The study was designed to have five different stocking densities, T_1 = 100 fish/m^2, T_2 = 75 fish/m^2, T_3 = 38 fish/m^2, T_4 = 18 fish/m^2 and T_5 = 9 fish/m^2 and two feeding levels, 1 and 2% body weight as treatments. These were replicated 3 times. The fish was fed three times daily using commercial feed at 1.0 and 2.0% body weight, respectively. The feed was adjusted monthly with increase in body weight. Blood profile was determined using 5-part differential Haematology Auto-analyzer (Mindray BC 5300 model). **Results:** At 1.0%, white blood cells count, haemoglobin concentration, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration were insignificant ($p>0.05$) for T_1-T_5. Red blood cells count and packed cell volume however showed significant variation ($p<0.05$) across treatments. At 2.0%, there was significance ($p>0.05$) in only mean corpuscular haemoglobin, all other blood parameters showed no significance ($p>0.05$) across treatments. **Conclusion:** Heteroclarias easily adapts to high stocking densities without any serious effect on the health status if adequate measures are put in place during management.

Key words: Hybrid catfish, *Heteroclarias*, stocking density, feeding level, haematology, blood profile

Citation: Ofonime Edet Afia and Gift Samuel David, 2019. Haematology of hybrid catfish (*Heteroclarias*): Effect of stocking densities and feeding levels. Trends Applied Sci. Res., 14: 271-277.

Corresponding Author: G.S. David, Department of Fisheries and Aquatic Environmental Management, Faculty of Agriculture, University of Uyo, P.M.B. 1017, Uyo, Nigeria Tel: +2347037617930

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Fish is a vital source of animal protein for many households. World per capita fish consumption had risen from 9.9 kg in 1960s and 14.4 kg in 1990s to 20 kg of animal protein intake[1] in 2014. Aquaculture is the rearing of fish and other aquatic organisms in man-made ponds, reservoirs, cages or other enclosures in lakes and coastal waters[2]. Due to increasing protein demand in Nigeria, fish farming practices have elevated drastically over the last five decades. Aquaculture in Africa has come a long way since it was first introduced. However, in comparison to the rest of the world, aquaculture production in Africa is still insignificant at the global level, accounting for about 2.32% of total global aquaculture level in 2014 according to FAO[1] report. Nigeria accounted for 0.42% of global aquaculture production[1] in 2014.

Fish farmers have the desire to produce table-sized fish within the shortest possible time[3], thus, the choice of species to culture is critical in the realization of this goal. In Africa, especially Nigeria, the species most cultured are *Clarias gariepinus*, *Heterobranchus* spp. and their hybrids[4]. To aquaculturists targeting high yields, acquiring fast growing fish seed have been a main constraint in the sub-saharan nation. Hybrid catfish production has witnessed rapid growth in the last few years and market demand is still increasing obviously. Aquaculture with emphasis to *Heteroclarias* has become an important sector in terms of its potentials for contributing to food and family income. It is very profitable as a result of their high resistance against diseases and environmental stress[5]. The blending of high survival rate and fast growth rate in the hybrid catfish (*Heteroclarias*) offers higher production prospects. Heteroclarias is an inter-specific hybrid of *Clarias gariepinus* and *Heterobranchus bidorsalis* which transfer or combine desirable traits of the two species[6]. Hybridization is one of the genetic improvements in aquaculture industry which has been recognized as a tool for stock improvement and management purposes. Some studies have demonstrated that *Clarias gariepinus* × *Heterobranchus bidorsalis* hybrid exhibit superior growth, improved survival and general hardiness than pure breed of either *Clarias gariepinus* or *Heterobranchus bidorsalis*[7,8]. These hybrids have been reported to show heterosis which makes it a very good aquacultural candidate[9].

Stocking density affects growth and survival directly. Not withstanding the research on stocking density, it is still difficult to obtain information on better densities for each species, because the best densities are affected by different culture systems, fish species and fish age[10]. For aquaculture to succeed, there is need for proper species selection, adequate feeding, maintenance of water quality and general management[11]. Stocking density may affect fish growth performance, physiology and fish behaviour[12], influence feeding activities, metabolism distortion and digestive utility[13], feed utilization[14], hormonal alteration[15] and immunological activities[16]. Nevertheless, growth of Pisces is also determined by availability of space, enough food and other environmental factors. Farmed fish typically are fed 1-5% of their body weight per day, with highest performance observed[17] in fish fed 5%.

Haematological analysis have been routinely used in determining the physiological state of animals. Fish haematology is gaining increasing importance in fish culture because of its importance in monitoring the health status of fish[18,19] and the nutritional state of the fish is one of the most important variables altering the blood values[20]. Haematological characteristics of most fish have been studied with the aim of establishing normal value range and deviation from it may indicate a disturbance in the physiological process[21]. At times environmental and physiological factors are known to influence fish haematology, these include stress due to capturing, transportation, sampling, age and sex. However, in most cases, the knowledge of haematological characteristics of the fish is important in toxicological studies and its implication on final consumers which is man[22]. In culture fisheries these studies are usually associated with the feed input. Banerjee *et al.*[23] reported that blood composition is moderately constant under normal condition with little variation above limits. However, the composition of blood can be changed by dietary treatment, malnutrition and disease condition[24].

Studies on the effect of stocking densities on the growth response of cultivable species are inexhaustible. Reports on the effect of stocking densities on growth performance of *Heteroclarias* include[11,25,26]. Authors have evaluated the haematological response of catfish species to fish feeds[22,27-29], captured from the wild[30-32], different feeding levels[33], different ages[34], cultured with wild[35], different culture systems[36,37], different stocking densities and protein levels[38]. However, little is known on the impact of stocking densities on hematological indices of hybrid catfish (*Heteroclarias*) as such more research is needed since stocking density and dietary level can influence physiological and immunological responses of culturable fish species. Moreover, information from this study will enable aquaculturists predict haematological indices of the species.

Hence, the objective of the current study was to investigate the influence of different stocking densities on the blood profile of *Heteroclarias* when fed at 1 and 2% body weights.

MATERIALS AND METHODS

Study area: The research was conducted at the Fish Hatchery Complex of the Department of Fisheries and Aquatic Environmental Management, Faculty of Agriculture, University of Uyo (Annex Campus), Akwa Ibom state, Nigeria. This area lies between latitudes 4°52' S and 4°51' N and longitudes 7°54' W and 8°03' E. The study lasted 12 months (May, 2015 to June, 2016) using thirty tarpaulin tanks of 1 M^3 volume.

Experimental procedures and design: The fingerlings of *Clarias gariepinus*×*Heterobranchus longifilis* (hybrid catfish) used was obtained from a breeding exercise using two broodstock females (*C. gariepinus*) and two broodstock males (*H. longifilis*) according to method narrated by Ngugi *et al.*[39]. The selection of brood fish was based on external morphology and eggs characteristics. The initial weight of the fingerlings (2.06±0.48 g) was taken before stocking them in the various tanks which were randomly positioned. The study employed factorial experimental design. Thirty tarpaulin tanks measuring 1×1×1 m^3 were used. Each was designed with an outlet for easy drainage and was filled with 0.25 m^3 of water. The study was designed to have 5 different stocking densities (SD), T_1 = 100 fish/m^2, T_2 = 75 fish/m^2, T_3 = 38 fish/m^2, T_4 = 18 fish/m^2 and T_5 = 9 fish/m^2 and two Feeding Levels (FL), 1 and 2% body weight as treatments. These were replicated three times. The fish was fed three times daily using commercial feed at 1.0 and 2.0% body weight, respectively. The feed was adjusted monthly with increase in body weight.

Water quality monitoring: Water in each tank was refreshed at 100% daily by using a partial flow through mechanism. The various water quality parameters were monitored at monthly intervals. Temperature was determined using a mercury thermometer calibrated 0-50°C, pH using a pen type pH meter (pH-009 111) and dissolved oxygen using dissolved oxygen meter (HI 9461).

Haematological analysis: After 52 weeks, haematological studies was carried out on the fishes. Samples of fish were taken out individually from each treatment tank using a plastic filter basket net and placed belly upward on a table. Blood samples of about 2.0 mL were collected from the ventral region near the anal opening using a 2.5 mL syringe and hypodermic needles. The blood samples were introduced into Heparinized Ethylene Diamine Tetraacetic Acid (EDTA) anti-coagulant tubes and capped sealed effectively to avoid escape for haematological analysis. The use of plastic syringe is a necessary precaution with fish blood because contact with glass will increase coagulation time and furthermore, the anti-coagulants decreases clotting time. The Packed Cell Volume (PCV), Haemoglobin Concentration (HGB), Red Blood Cell (RBC), White Blood Cell (WBC), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin concentration (MCHC) of each of the blood sample were determined in haematology laboratory of the University of Uyo Teaching Hospital (UUTH), Uyo, using 5-part differential Haematology Auto-analyzer (Mindray BC 5300 model).

Statistical analysis: Blood and water quality parameters were subjected to one-way analysis of variance (ANOVA) to evaluate mean differences at 0.05 significant levels. Results with $p \leq 0.05$ were considered significantly different[40]. Duncan multiple range test was used to compare significant difference among the treatments. The statistical analysis were done using IBM SPSS Inc. (Windows version 20).

RESULTS

Water quality parameters during the study: Table 1 shows water quality at different stocking densities fed at 1.0 and 2.0% feeding level. At feeding level 1.0%, temperature values were insignificant ($p>0.05$) dissolved oxygen showed significance ($p<0.05$) for T_1, while pH showed significance ($p<0.05$) for T_4 and T_5, respectively. At feeding level 2.0%, temperature, dissolved oxygen and pH showed no significance ($p>0.05$).

Haematological indices of studied heteroclarias: Table 2 shows the haematological profile of hybrid catfish during the study. At 1.0%, significance ($p<0.05$) was observed in RBC and PCV. Other indices were insignificant ($p>0.05$). At 2.0%, MCH showed significance ($p<0.05$). Other parameters were insignificant ($p>0.05$).

DISCUSSION

The study discovered hybrid catfish adapted to wide range of stocking densities and low feeding levels with no significant impact on the haematological parameters of the fish. Environmental parameters influence significantly the

Table 1: Water quality parameters for T_1-T_5 and at both feeding levels

	Mean±SE		
Stocking densities	Temperature (°C)	Dissolved oxygen (mg L^{-1})	pH
1% feeding level			
T_1	28.46 ± 0.19^{a}	5.42 ± 0.09^{a}	7.59 ± 0.16^{a}
T_2	28.26 ± 0.20^{a}	5.21 ± 0.09^{b}	7.62 ± 0.15^{a}
T_3	28.49 ± 0.15^{a}	5.20 ± 0.05^{b}	7.69 ± 0.15^{a}
T_4	28.74 ± 0.10^{a}	5.13 ± 0.06^{b}	7.48 ± 0.14^{ab}
T_5	28.42 ± 0.20^{a}	5.05 ± 0.05^{b}	7.08 ± 0.15^{b}
2% feeding level			
T_1	28.62 ± 0.07^{a}	5.03 ± 0.05^{a}	7.31 ± 0.15^{a}
T_2	28.70 ± 0.09^{a}	5.03 ± 0.06^{a}	7.63 ± 0.14^{a}
T_3	28.80 ± 0.11^{a}	4.97 ± 0.06^{a}	7.06 ± 0.15^{a}
T_4	28.68 ± 0.10^{a}	5.01 ± 0.05^{a}	7.28 ± 0.14^{a}
T_5	27.98 ± 0.75^{a}	5.00 ± 0.06^{a}	7.34 ± 0.15^{a}

Means within same column with same superscript are not significantly different (p>0.05, n = 3)

Table 2: Haematological parameters of hybrid catfish to different SD and FL

	Mean±SE				
Blood parameters	T_1	T_2	T_3	T_4	T_5
1% feeding level					
WBC (x10^9 L^{-1})	5.49 ± 1.33^{a}	7.81 ± 0.71^{a}	3.81 ± 0.58^{a}	3.65 ± 0.62^{a}	5.71 ± 2.29^{a}
RBC(x10^{12} L^{-1})	2.43 ± 0.04^{b}	2.94 ± 0.02^{a}	2.71 ± 0.17^{ab}	2.57 ± 0.10^{ab}	2.59 ± 0.15^{ab}
HGB (g dL^{-1})	11.33 ± 0.33^{a}	13.73 ± 0.18^{a}	12.47 ± 0.79^{a}	15.13 ± 3.19^{a}	11.80 ± 0.62^{a}
PCV (%)	37.83 ± 3.10^{ab}	45.80 ± 1.76^{a}	40.23 ± 2.04^{ab}	37.47 ± 3.43^{ab}	35.50 ± 2.35^{b}
MCV (fl)	155.17 ± 10.58^{a}	155.23 ± 6.75^{a}	150.47 ± 9.92^{a}	148.23 ± 7.72^{a}	137.07 ± 1.44^{a}
MCH (pg)	45.90 ± 1.69^{a}	46.03 ± 0.61^{a}	46.20 ± 1.44^{a}	46.00 ± 0.45^{a}	45.63 ± 0.32^{a}
MCHC (g dL^{-1})	29.83 ± 2.06^{a}	29.43 ± 0.88^{a}	30.83 ± 1.04^{a}	31.50 ± 1.37^{a}	33.33 ± 0.54^{a}
2% feeding level					
WBC (x10^9 L^{-1})	6.38 ± 1.64^{a}	3.84 ± 0.41^{a}	4.19 ± 0.73^{a}	6.10 ± 1.74^{a}	4.42 ± 0.33^{a}
RBC(x10^{12} L^{-1})	2.78 ± 0.08^{a}	2.54 ± 0.03^{a}	2.79 ± 0.37^{a}	2.90 ± 0.17^{a}	2.75 ± 0.10^{a}
HGB (g dL^{-1})	12.33 ± 0.59^{a}	11.63 ± 0.38^{a}	11.83 ± 1.81^{a}	13.30 ± 0.70^{a}	12.60 ± 0.72^{a}
PCV (%)	41.13 ± 3.70^{a}	40.10 ± 1.48^{a}	39.60 ± 3.52^{a}	43.50 ± 1.27^{a}	39.83 ± 1.49^{a}
MCV (fl)	147.47 ± 9.61^{a}	153.17 ± 0.29^{a}	143.40 ± 5.66^{a}	150.83 ± 6.55^{a}	144.77 ± 2.47^{a}
MCH (pg)	44.30 ± 0.96^{ab}	44.93 ± 1.44^{ab}	42.20 ± 1.25^{b}	45.97 ± 0.50^{a}	45.70 ± 0.95^{a}
MCHC (g dL^{-1})	30.23 ± 1.41^{a}	29.37 ± 0.92^{a}	29.57 ± 1.82^{a}	30.60 ± 1.36^{a}	31.57 ± 0.81^{a}

Means within same row with same superscript are not significantly different (p>0.05, n = 3), WBC: White blood cell, RBC: Red blood cell, HGB: Haemoglobin concentration, PCV: Packed cell volume, MCV: Mean corpuscular volume, MCH: Mean corpuscular haemoglobin, MCHC: Mean corpuscular haemoglobin concentration

maintenance of a healthy aquatic environment and production of natural food organisms[41]. Temperature was within acceptable range for aquaculture[42] <40°C. Results from this study agrees with reports of Dienye and Olumuji[22], Afia and David[28], Akinrotimi *et al.*[36] and Afia *et al.*[43]. Ekubo and Abowei[44] stated that pond fish would die if exposed for long period to less than 0.3 mg/L DO. The range of DO was within acceptable range for the survival of heteroclarias. The DO results was similar with findings of Akinrotimi *et al.*[36] and Afia *et al.*[43] but disagreed with reports of Dienye and Olumuji[22] and Afia and David[28]. Differences could be attributed to the daily water exchange in treatment tanks for the current study. Ekubo and Abowei[44] stated that pH of 7.0-8.5 is ideal for biological productivity as fish become stressed in water with pH ranging from 4.0-6.5 and 9.0-11.0. The pH values were within tolerable range. The pH result is in consonance with reports of Afia and David[28], Akinrotimi *et al.*[36] and Afia *et al.*[43]. Result was slightly higher than report of Dienye and Olumuji[22].

Knowledge of the haematological characteristics can be used as an effective and sensitive index to monitor physiological and pathological changes in fishes[45]. Blood analysis is a useful means of evaluating the physiological condition of cultured fish with respect to determining the effect of diets and other stressed factors on fish health[19,33]. Low haematological indices are indicators of anaemic conditions[46], as this is used by fish scientists' in determination of fish health and responses to stress. When fish is stressed, HGB, PCV and RBC reduces while WBC tends to increase. White blood cells are the defense cells of the fish body. The white blood cells showed no variation in the mean values (p>0.05). The increased levels of WBC observed at increasing level of stocking density might be attributed to the increase in leucocytes synthesis as a defense mechanism against the destruction of erythrocytes; a protective response to stress as well as consequence of tissue damages[47]. Similar findings were reported by Afia and David[28], Oyegbile *et al.*[29] and Onyia *et al.*[31]. Reports by Dienye and Olumuji[22], Onyia *et al.*[32], Okorie-Kanu and Unakalamba[34] and Akinrotimi *et al.*[36] were dissimilar. Erythrocyte count greater than 1.00×10^6 mm^{-3} is considered high and indicative of high oxygen carrying capacity of the blood, which is characteristic of fishes capable of aerial respiration and with high activity[48]. The result of RBC counts for both feeding levels were slightly higher than that and were within the range of normal haematology of a healthy fish[49]. The RBC count compares well with reports of Dienye and Olumuji[22], Onyia *et al.*[32] and Ajani *et al.*[38]. However, they fell below results obtained from Onyia *et al.*[31], Okorie-Kanu and Unakalamba[34] and Akinrotimi *et al.*[36]. Haemoglobin is linked directly to oxygen carrying ability and this is essential for fish survival. However, Teixeria *et al.*[50] cited by Afia and Ofor[33] stated that referenced values determined for haematological tests should be carefully interpreted once there is a wide range of physiological variations since these variations might be influenced by environmental conditions, sex, age, feeding system and feeding. Low haemoglobin level might decrease the ability of fish to enhance its activity in order to meet occasional demands[51]. The range of HGB were higher than reports of Dienye and Olumuji[22], Afia and David[28] and Ajani *et al.*[38]. Nevertheless, HGB compared less favourably with reports of Oyegbile *et al.*[29], Onyia *et al.*[31,32] and Akinrotimi *et al.*[36]. Packed Cell Volume (PCV) otherwise called haematocrit is a useful tool in fisheries and aquaculture management for checking anaemic condition in aquatic species. Abnormally low PCV (i.e., a decrease in total amount of RBC) may suggest anaemia and the presence of toxic factors which affects blood formation and growth generally. The PCV was slightly lower than normal range for human (men = $47\pm5\%$ and women = $42\pm5\%$)[52]. Result from the current study was better when compared with reports of Dienye and Olumuji[22], Afia and David[28], Okorie-Kanu and Unakalamba[34] and Akinrotimi *et al.*[36]. However, they compared less to results of Oyegbile *et al.*[29] and Onyia *et al.*[31,32]. The MCV from the study compared favourably with results of Dienye and Olumuji[22], Afia and David[28], Onyia *et al.*[31], Okorie-Kanu and Unakalamba[34] and Akinrotimi *et al.*[36]. The present result was different from the reports of Oyegbile *et al.*[29] and Onyia *et al.*[32]. The MCH compared favourably with results of Dienye and Olumuji[22], Afia and David[28] and Oyegbile *et al.*[29], was higher than results of Onyia *et al.*[31], Okorie-Kanu and Unakalamba[34] and Akinrotimi *et al.*[36]. The MCHC disagreed with reports of Afia and David[28] and Akinrotimi *et al.*[36]. This might be as a result of the different feed form, culture environment, species, age and size of the fishes which consequently influenced blood parameter values. However, MCHC value was similar to reports of Dienye and Olumuji[22], Oyegbile *et al.*[29], Onyia *et al.*[31,32] and Okorie-Kanu and Unakalamba[34]. Variations observed in some of the blood parameters when compared with other haematological studies imply that age, size, species, feed type, feed forms, culture period and different management systems affect haematological values of fishes. The authors recommend further research in evaluating the haematology of this species in water recirculatory aquaculture system and biofloc technology, as these systems permits even greater stocking densities. Hence, raising hybrid catfish is encouraged.

CONCLUSION

Hybrid catfish could be raised in high stocking densities at 1 and 2% feeding levels as reported in this study since haematological parameters were not off charts of previous studies. The different treatments did not impose much variation among haematological indices. However, for best result, stocking densities T_3-T_5 are recommended. It is concluded that hybrid catfish can easily adapt to high stocking densities without any serious effect on the health status if adequate management measures are put in place during the culture period.

SIGNIFICANCE STATEMENT

Previous studies reported no significant effect on haematological indices when fish are reared in low stocking densities and fed higher feeding levels. High stocking densities and low feeding levels employed in this study did not also have significant impact on haematology of hybrid catfish. This study revealed that hybrid catfish (*Heteroclarias*) can be cultured at high stocking densities and fed at low feeding rate without compromising the health status of the fish. The study may be helpful as a tool to monitor the health status of *Heteroclarias* and other related fish species. Moreover, evaluating these haematological indices provides useful data for future research as it can serve as a biomarker in relation to stress factors. It is critical to fish farmers to understand the relationship between stocking density and the health of this fish. This has been a problem for aquaculturists as low stocking densities usually lead to low profit maximization. This study is beneficial for fish farmers as they can now rear *Heteroclarias* at high stocking densities to maximize profit without fear of endangering the fish health.

REFERENCES

1. FAO., 2016. The State of World Fisheries and Aquaculture 2016: Contributing to Food Security and Nutrition for All. Food and Agriculture Organization, Rome, Italy, ISBN: 9789251091852, Pages: 200.
2. Suleiman, M.A. and R.J. Solomon, 2017. Effect of stocking on the growth and survival of *Clarias gariepinus* grown in plastic tanks. Direct Res. J. Vet. Med. Anim. Sci., 2: 82-92.
3. Ekelemu, J.K. and P.A. Ekokotu, 1999. Principle and Practice of Fish Seed Production. In: Issues in Animal Science, Omeje, S.I. (Ed.). Reykenedy Scientific Publishers, Enugu, pp: 182-196.
4. Adewolu, M.A., A.O. Ogunsanmi and A. Yunusa, 2008. Studies on growth performance and feed utilization of two Clariid catfish and their hybrid reared under different culture systems. Eur. J. Sci. Res., 23: 252-260.
5. Khaleg, M.A., 2000. Fisheries resources of Rajshahi division. Department of Fisheries, University of Rajshahi, Rajshahi, Bangladesh.
6. Bartley, D.M., K. Rena and A.J. Immink, 2000. The use of inter-specific hybrids in aquaculture and fisheries. Rev. Fish Biol. Fish., 10: 325-337.
7. Nwadukwe, F.O., 1995. Hatchery propagation of five hybrid groups by artificial hybridization of *Clarias gariepinus* (B) and *Heterobranchus longifilis* (Val.) (Clariidae) using dry, powdered carp pituitary hormone. J. Aquacult. Trop., 10: 1-11.
8. Madu, C.T. and P.O. Aluko, 1999. Hybrid mudfish production comparative growth and survival of hybrids and putative parents. Proceeding of the 12th Annual Conference of the Biotechnology Society of Nigeria, April 13-17, 1999, New Bussa, Nigeria, pp: 89-94.
9. Anibeze, C.I.P. and A. Eze, 2000. Growth rates of two African catfishes osteichthys: (Clariidae) in homestead concrete ponds. J. Aquat. Sci., 15: 55-58.
10. Ellis, T., B. North, A.P. Scott, N.R. Bromage, M. Porter and D. Gadd, 2002. The relationships between stocking density and welfare in farmed rainbow trout. J. Fish Biol., 61: 493-531.
11. Ofor, C.O. and O.E. Afia, 2015. Effect of stocking densities on growth and feed utilization of hybrid catfish (*Clarias gariepinus* x *Heterobranchus longifilis*) fed at 1% body weight. Am. J. Biol. Life Sci., 3: 211-217.
12. Holm, C.J., T. Refstie and S. Bo, 1990. The effect of fish density and feeding regimes on individual growth rate and mortality in rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 89: 225-232.
13. Vijayan, M.M., J.S. Ballantyne and J.F. Leatherland, 1990. High stocking density alters the energy metabolism of brook charr, *Salvelinus fontinalis*. Aquaculture, 88: 371-381.
14. Alanara, A. and E. Brannas, 1996. Dominance in demand-feeding behaviour in Arctic charr and rainbow trout: The effect of stocking density. J. Fish Biol., 48: 242-254.
15. Kebus, M.J., M.T. Collins, M.S. Brownfield, C.H. Amundson, T.B. Kayes and J.A. Malison, 1992. Effects of rearing density on the stress response and growth of rainbow trout. J. Aquat. Anim. Health, 4: 1-6.
16. Tort, L., J. Sunyer, E. Gomez and A. Molinero, 1996. Crowding stress induces changes in serum haemolytic and agglutinating activity in the gilthead sea bream *Sparus aurata*. Vet. Immunol. Immunopathol., 51: 179-188.
17. Ashley-Dejo, S.S., O.J. O laoye, O.A. Adelaja and I. Abdulraheem, 2014. Effects of feeding levels on growth performance, feed utilization and body composition of African catfish (*Clarias gariepinus* Burchell 1822). Int. J. Biol. Biol. Sci. Acad. Res. J., 3: 12-16.
18. Hrubec, T.C., J.L. Cardinale and S.A. Smith, 2000. Hematology and plasma chemistry reference intervals for cultured tilapia (*Oreochromis* Hybrid). Vet. Clin. Pathol., 29: 7-12.
19. Jimoh, W.A., F.O. Ajasin, A.A. Ayeloja, A.O. Rifhat and M.O. Shodamola, 2013. Haematological changes in catfish (*Clarias gariepinus*) fed diets containing water melon seedmeal (*Citrullus lanatus*). Proceedings of the 47th Annual Conference of the Agricultural Society of Nigeria, November 4-8, 2013, Ibadan, Nigeria, pp: 1234-1241.
20. Congleton, J.L. and T. Wagner, 2006. Blood-chemistry indicators of nutritional status in juvenile salmonids. J. Fish Biol., 69: 473-490.
21. Rainza-Paiva, M.J.T., C.M. Ishikawa, A.A. das Eiras and N.N. Felizardo, 2000. Haematological analysis of chara *Pseudoplatystoma fasciatum* in captivity. Euro. Aquacult. Soc. Special Pub., 28: 590-592.
22. Dienye, H.E. and O.K. Olumuji, 2014. Growth performance and haematological responses of African mud catfish *Clarias gariepinus* fed dietary levels of *Moringa oleifera* leaf meal. Net J. Agric. Sci., 2: 79-88.
23. Banerjee, S.K., B.C. Patra, P. Bandyopadhyay and A. Tewary, 2002. Changes of the blood parameters in an Indian major carps, *Catla catla* Ham. due to Myxozoan parasites infection. Indian J. Aquat. Biol., 17: 79-84.
24. Feist, S.W. and M. Longshaw, 2000. Myxosporidiosis of fish and the bryozoan link with proliferative kidney disease (PKD) of salmonids. Fish. Vet. J., 5: 37-46.
25. Oguguah, N.M., F. Nwadukwe, C.I. Atama, J.I. Chidobem and J.E. Eyo, 2011. Growth performance of hybrid catfish (*Heterobranchus bidorsalis* (♀) × *Clarias gariepinus* (♂)) at various stocking densities in varied culture tanks. Anim. Res. Int., 8: 1419-1430.
26. Okeke, P.A., 2014. Studies on the effects of stocking densities on growth performance and survival level of *Heteroclarias* (Hybrid). Int. J. Agric. Biosci., 3: 136-140.
27. Agbabiaka, L.A., F.N. Madubuike and B.U. Ekenyem, 2013. Haematology and serum characteristics of African catfish (*Clarias gariepinus* Burchell) fed graded levels of Tigernut based diet. Am. J. Exp. Agric., 3: 988-995.

28. Afia, O.E. and G.S. David, 2017. Haematological profile and growth response of African sharptooth catfish (*Clarias gariepinus*, Burchell 1822) fingerlings to locally formulated and commercial pelleted diets in tarpaulin tanks. Am. J. Innov. Res. Applied Sci., 5: 213-222.
29. Oyegbile, B., B.A. Abdullahi and I.A. Yola, 2017. Haematological profile of *Heterobranchus bidorsalis* fingerlings fed processed *Delonix regia* seeds at different inclusion levels of diets. Int. J. Fisher. Aquacult., 9: 81-85.
30. Adam, H.M. and H. Agab, 2008. Haematological and biochemical indices of *Clarias garipeinus* collected from River Nile, Sudan. Sudan Acad. Sci. J., 2: 91-97.
31. Onyia, L.U., K.G. Michael and B. Ekoto, 2013. Haematological profile, blood group and genotype of *Heterobranchus bidorsalis*. Net. J. Agric. Sci., 1: 69-72.
32. Onyia, L.U., M.Y. Diyaware, K.G. Michael, M. Musa and I.J. Ochokwu, 2015. Comparison of haematological indices, blood group and genotype of *Clarias gariepinus* (Burchell, 1822) and *Clarias anguillaris* (Linneaus, 1758). J. Fish. Aquat. Sci., 10: 392-399.
33. Afia, O.E. and C.O. Ofor, 2016. Haematological indices of the *Clarias gariepinus* × *Heterobranchus longifilis* (hybrid catfish-*Heteroclarias*) reared at different feeding levels. Niger. J. Agric. Food Environ., 12: 6-11.
34. Okorie-Kanu, C.O. and N.J. Unakalamba, 2015. Normal haematological and blood biochemistry values of cultured *Heteroclarias* hybrid in South East Nigeria. Comp. Clin. Pathol., 24: 1015-1020.
35. William, A., M. Zakariah, V.A. Miana, M.M. Chana, I. Wiam and P.A. Onyeyili, 2015. Studies on the hematological differences of pond and river African catfish (*Clarias gariepinus*) in Maiduguri, Borno State, Nigeria. Int. J. Fish. Aquat. Stud., 4: 351-354.
36. Akinrotimi, O.A., D.O. Bekibele and O.O. Orokotan, 2011. Select hematological values of the African catfish (*Clarias gariepinus*) raised in a water recirculating aquaculture system. Int. J. Recirculat. Aquacult., 12: 1-12.
37. Hastuti, S. and S. Subandiyono, 2018. Haematological parameters of the North African catfish *Clarias gariepinus* farmed using biofloc technology. AACL Bioflux, 11: 1415-1424.
38. Ajani, E.K., S.B. Setufe and O.O. Oyebola, 2015. Effects of stocking density on haematological functions of juvenile African catfish (*Clarias gariepinus*) fed varying crude protein levels. Afr. J. Food Sci., 9: 65-69.
39. Ngugi, C.C., J. Bowman and B.A. Omolo, 2007. A New Guide to Fish Farming in Kenya. Oregon State University, Oregon, USA., ISBN: 978-0-9798658-0-0, Pages: 95.
40. Zar, J.H., 2010. Biostatistical Analysis. 5th Edn., Prentice-Hall, Upper Saddle River, New Jersey, Englewood, USA., ISBN-13: 9780131008465, Pages: 960.
41. Haque, M.M., N.T. Narejo, M.A. Salam, S.M. Rahmatullah and M.A. Islam, 2003. Determination of optimum stocking density of *Macrobrachium rosenbergii* in carp polyculture in earthen pond. Pak. J. Biol. Sci., 6: 898-901.
42. APHA., 1998. Standard Methods for the Examination of Water and Wastewater. 20th Edn., American Public Health Association, Washington, DC., USA., ISBN-13: 9780875532356, Pages: 1220.
43. Afia, O.E., G.S. David and I.B. Effiong, 2018. Comparative effect of different stocking densities of heteroclarias on plankton abundance in tarpaulin tanks. J. Aquat. Sci. Mar. Biol., 1: 5-12.
44. Ekubo, A.A. and J.F.N. Abowei, 2011. Review of some water quality management principles in culture fisheries. Res. J. Applied Sci. Eng. Technol., 3: 1342-1357.
45. Bahmani, M., R. Kazemi and P. Donskaya, 2001. A comparative study of some hematological features in young reared sturgeons (*Acipenser persicus* and *Huso huso*). Fish Physiol. Biochem., 24: 135-140.
46. Haruna, A.B. and I.A. Adikwu, 2001. Haematological response to non-familiar diets: A study of the African mud catfish *Clarias gariepinus*. J. Arid Zone Fish., 1: 12-22.
47. Adeyemo, O.K., 2007. Haematological profile of *Clarias gariepinus* (Burchell, 1822) exposed to lead. Turk. J. Fish. Aquat. Sci., 7: 163-169.
48. Jimoh, W.A., F.O. Ajasin, M.D. Adebayo, O.T. Banjo, A.O. Rifhat and K.D. Olawepo, 2014. Haematological changes in the blood of *Clarias gariepinus* fed *Chrysophyllum albidum* seedmeal replacing maize. J. Fish. Aquat. Sci., 9: 407-412.
49. Fagbenro, O.A., C.O. Adedire, E.A. Owoseeni and E.O. Ayotunde, 1993. Studies on the biology and aquaculture potential of feral catfish *Heterobranchus bidorsalis* (Geoffroy St. Hilaire 1809) (Clariidae). Trop. Zool., 6: 67-79.
50. Teixeira, M.A., L.D.C.A.G. Chaguri, A.S. Carissimi, N.L. de Souza and C.M.C. Mori *et al*., 2000. Hematological and biochemical profiles of rats (*Rattus norvegicus*) kept under microenvironmental ventilation system. Braz. J. Vet. Res. Anim. Sci., 37: 341-347.
51. Joshi, P.K., D. Harish and M. Bose, 2002. Effect of lindane and malathion exposure to certain blood parameters in a fresh water teleost fish *Clarias batrachus*. Pollut. Resour., 21: 55-57.
52. Purves, W.K., D. Sadava, G.H. Orians and H.C. Heller, 2004. Life: The Science of Biology. 7th Edn., Sinauer Associates, Sunderland, Mass, ISBN-13: 9780716798569, Pages: 1121.