

Original Research Article

Haematological and serum biochemical profiles of *Clarias gariepinus* (Burchell, 1822) fed commercial and farm-made feeds

ABSTRACT

The present study was carried out to determine the haematological and serum biochemical profiles of *Clarias gariepinus* juveniles fed commercial and farm-made feeds and reared in intermediate bulk containers (IBC) tanks. Nine hundred juveniles ($15.15 \pm 3.48\text{g}$; $128.37 \pm 9.67\text{mm}$) were stocked in 09 IBC tanks (1m^3) at a density of 100 fish/tank in triplicate and fed with imported extruded (Le), locally pelleted (Lpe) and locally extruded (Lex) feeds thrice a day to satiation for 16 weeks. At the end of the experiment, blood samples were collected from 10 fish/ dietary treatment to determine haematological and biochemical indices. Results revealed that Red Blood Cells Count, Packed Cell volume and Haemoglobin concentration were highest ($p < 0.05$) in fish fed with "Le" feed while Mean Cell Haemoglobin Concentration, Mean Cell Haemoglobin, Mean Cell Volume, White Blood Cells Count, Lymphocytes, Monocytes, Granulocytes and Platelets showed no significant difference ($p > 0.05$) among the dietary treatments. As for serum biochemistry, Total Cholesterol and Glucose were highest ($p < 0.05$) in fish fed with "Le" feeds while Total Protein, Alanine Transaminase, Aspartate Transaminase and Alkaline Phosphatase were significantly higher ($p < 0.05$) in fish fed "Lpe" and "Lex" feeds. Conclusively, variation of dietary treatments did affect the haematological and biochemical indices without any adverse effect on the health condition of the studied fish.

Keywords: Haemato-biochemistry, serum, fish feed, *Clarias gariepinus*, IBC tanks

Comment [A1]: fishes

1. INTRODUCTION

World population is growing geometrically expecting to reach 9 billion in 2050 [1]. Projections show that feeding this population would require an increase of the overall food production by 70% [2]. Therefore, there is the need for safe and nutritious production techniques to meet the increasing diet needs of the world's population [3]. Aquaculture is one of the fast growing food production sectors accounting for 16.6% of animal protein consumed worldwide. As such, it is the ideal candidate for meeting the growing food demand in the future [4, 5].

The African catfish (*Clarias gariepinus*, Burchell, 1822) is one of the most cultured freshwater fish species in the tropics [6]. This is mainly due to its positive attributes: resistance to diseases, high fecundity, thriving on cheap feed, hardiness etc. [7]. However, its culture is faced with many problems amongst which the provision of nutritionally balanced cost effective feeds so as to reduce the cost of production which accounts for as much as 40-70% of overall expenditure of its culture venture [5, 8].

Comment [A2]: Please provide complete information

In recent years, fish nutrition has improved with the development of cheaper and readily available balanced diets that promote optimal growth and health of fish [8]. The advent of these improved feeds coupled with revised management protocols has greatly limited fry mortality; improved fingerlings productivity and has thereby enhanced survival and sufficient supply of fingerlings. These practices have been disseminated to fish farmers and have led to tremendous improvement in fingerlings production and eventually aquaculture production especially in Cameroon [9]. To this effect, Cameroon's aquaculture production in metric tonnes (MT) which stood at 50MT in the year 2000 [10] has increased significantly, now reaching over 9 000 tons [9].

Comment [A3]: As a result, Cameroon's aquaculture production has substantially expanded, today totaling over 9000 tonnes [9], from 50MT in the year 2000 [10].

Intensive production of *C. gariepinus* has increased in the last few decades [11] in most African countries including Cameroon and this has led farmers to now raise fish in out of pond holding systems (concrete tanks, fast tanks, tarpaulins, plastics, Water recirculating aquaculture systems etc.). This is a clear departure from the traditional earthen pond culture system which made fish production seasonal and unreliable in comparison to a more advanced reliable, intensive, and result-oriented culture system [12, 13]. With this level of intensive stocking density, there is therefore the need to monitor the health status of cultured fish to prevent the outbreak of devastating diseases [12].

Haemato-biochemical analyses are one of such studies that reliably monitor the physiological condition and health status of cultured fish [14, 15] since morphological and biometric parameters alone do not always give a complete picture [16]. Haematological and biochemical parameters have proven to be valuable tools in determining the health status of fish in response to dietary manipulations [17, 18]. In addition, Svobodova *et al.* [19] stated that haematological investigation can be used to appraise the suitability of new and unconventional feeds, to examine the effect of stress situation etc. Studies on haematological and biochemical responses of *C. gariepinus* to feed have been centred on responses to different: feedstuffs [11], dietary inclusion and substitution levels [20-24] and culture systems including: concrete tanks [25], tarpaulin tanks [26], water recirculating aquaculture systems [12, 27], earthen ponds [11, 28] and reservoirs [29, 30]. Based on literature, there is paucity of information on haematological and biochemical responses of *C. gariepinus* reared in plastic tanks and fed with industrially manufactured and locally formulated feeds. The aim of this study was thus; to investigate the effects of imported feeds and locally formulated feeds on haematological and biochemical parameters of *C. gariepinus* blood reared in IBC (Intermediate bulk containers) holding tanks.

Comment [A4]: Please provide complete information for this sentence

2. MATERIALS AND METHODS

2.1 Study area

This study was carried out at the grow-out production unit of the Fish Farming Demonstration Association (FIFADA) Yaoundé, Cameroon. The unit consisted of 09 IBC tanks for grow-out which were exposed to 12h of daylight and 12h of night.

2.2 Experimental fish

A total of nine hundred fingerlings of mean initial weight (15.15 ± 3.48 g) and length (128.37 ± 9.67 mm) were acclimated in 03 IBC (Intermediate Bulk Container) tanks ($1 \times 1 \times 1 \text{ m}^3$) containing 800L domestic water for two weeks before the commencement of the experiment using an imported extruded feed (Le Gouessant®).

2.3 Experimental diets

Locally available feed ingredients: Fishmeal, soybean cake, groundnut cake, wheat bran, cassava flour, palm kernel cake, premix, L-lysine, methionine and refined palm oil were purchased to formulate the locally pelleted feed (Lpe) by Pearson square method then enhanced with trial and error method for perfection. The ingredients were processed, mixed and pelleted with a flat die pelletizer (Capsfeed Ltd), sun-dried and stored in airtight containers at room temperature until further use. Locally extruded (Lex) and imported extruded (Le) feeds (Le Goussant®) were respectively purchased from a respectable feed producer (VicFeeds) and a retailer (AgroBio) in Yaoundé, Cameroon. The gross composition of ingredients of the experimental diets is presented on table 1. Proximate analysis carried out according to standard methods [31] revealed the following: imported feed (42.00% crude protein, 11.00% crude fats and 2.30% crude fibre), locally pelleted feed (32.55% crude protein, 5.15% crude fats and 6.21% crude fibre) and locally extruded (33.57% crude protein, 5.57% crude fats and 3.90% crude fibre).

Table 1: Gross ingredient composition of experimental diets

Ingredients	Le	Lpe	Lex
Fish meal	+	+	+
Soybean meal	+	+	+
Groundnut cake	-	+	+
Pig blood products	+	-	-
Rapeseed meal	+	-	-
Methionine	-	+	+
Lysine	-	+	+
Hydrolysed poultry feather meal	+	-	-
Maize	-	-	-
Corn gluten	+	-	-
Wheat	+	+	+
Wheat feed flour	+	-	-
Cassava flour	-	+	+
Fish oil	+	-	-
Rapeseed oil	+	-	-
Vegetable oil	-	+	+
Palm kernel cake	-	+	+
Other by-products of fermentation	+	-	-
Premix	-	+	+
Vitamins	+	+	+
Compounds of trace elements	+	-	-
Binders	+	-	-
Monocalcium phosphate	+	+	+
Oyster shell	-	+	+

Le: imported feed; **Lpe:** Locally pelleted feed; **Lex:** Locally extruded feed; **+**: Present; **-**: absent

2.4 Experimental design

The fish were randomly stocked in 09 IBC tanks at a density of 100 fingerlings per tank in triplicate and reared for 16 weeks. The juveniles were hand-fed experimental diets thrice daily (between 8:30-9:30am in the morning, between 14:30-15:30pm in the afternoon and between 20:30-21:30pm in the night) to satiation. Domestic water (tap) that was well de-chlorinated (left for 48h) and oxygenated was used. Water level in each tank was maintained at 800L throughout the study period. One third of water from each tank was replaced daily and completely changed every fortnightly throughout the experimental period to maintain a relative uniform water quality and prevent fouling from feed remnants and metabolic waste. Water parameters of importance to aquaculture were measured fortnightly (between 5am to 6am) according to APHA [32] and Boyd [33]. All the parameters were within standard limits for aquaculture as recommended by Boyd [33] except for dissolved oxygen which had low values (<3mg/L). This could be attributed to the time of sampling [34].

2.5 Haematological analysis

Ten live specimens of *C. gariepinus* were harvested from each dietary treatment and their weights and lengths measured to the nearest 1g and mm respectively. Mean weight and length obtained for fish fed with "Le" were 693.40±38.55g and 426.10±7.79mm respectively. Those fed with "Lpe" were respectively 261.54±22.08g and 302.50±11.28mm and those fed with "Lex" were respectively 297.27±25.27g and 322.30±6.42mm.

Blood samples were collected via caudal puncture with a hypodermic needle inserted to a plastic syringe and 5ml of blood was extracted. 2.5ml was decanted into plastic tubes (Eppendorf) containing Ethylene Diamine Tetra-Acetic acid (EDTA) as the anticoagulant and the remaining 2.5ml stored in dry tubes for biochemical analysis. The site chosen for puncture was wiped dry with tissue paper to avoid contamination with mucus. The blood samples were rocked gently in the tubes to allow thorough mixing of its contents with the anticoagulant. Thereafter, the samples were taken to the Haematology Laboratory of the "Chantal Biya" International Reference Centre for haematological analysis. Haematological parameters of interest to this study were: Red blood cells (RBCs) count, Packed cell volume (PCV), haemoglobin (Hb) concentration, Mean cell haemoglobin concentration (MCHC), Mean corpuscular

haemoglobin (MCH), Mean cell volume (MCV), White blood cells (WBCs) count, lymphocyte (LYM), monocytes (MON), granulocytes (GRA) and platelet (PLT).

2.6 Biochemical analysis

The second portion of the collected blood was dispensed into dry tubes. This was then centrifuged for five minutes to obtain serum for colorimetric measurement of Total Cholesterol, Total protein, Aspartate aminotransferase, Alanine aminotransferase and Glucose with LABKIT kits (CHEMELEX, S.A.) as well as of alkaline phosphatase with BIOLABO kits following the manufacturers' instructions.

Comment [A5]: Total cholesterol, total protein, aspartate aminotransferase, alanine aminotransferase and glucose

2.7 Data analysis

Results were analysed using the statistical software GraphPad Prism® (version 8.1.0) and a one-way analysis of variance (ANOVA). Tukey HSD test was used to assess significance of differences at 0.05.

Comment [A6]: Tukey's test (Honestly Significant Difference, HSD)

3. RESULTS

3.1 Haematological profile

Results of the mean haematological parameters of *Clarias gariepinus* fish fed with the three experimental diets are presented in table 2. Most of the blood parameters of the fish did not differ significantly ($p>0.05$) between different treatments except for red blood cells count (RBC), packed cell volume (PCV), haemoglobin concentration (Hb) and mean corpuscular volume (MCV). Imported and locally pelleted feeds showed significant difference ($p<0.05$) in terms of RBC and Hb. Fish fed with imported and locally extruded feeds showed significant difference ($p<0.05$) in terms of RBC, PCV, Hb and MCV. No significant difference ($p>0.05$) in all the studied blood parameters was observed between fish fed with locally pelleted and locally extruded feeds except for MCV.

Comment [A7]: Table 2

Comment [A8]: haemoglobin (Hb) concentration

Table 2: Haematological parameters of *C. gariepinus* fed with different dietary treatments

Parameters	Le	Lpe	Lex
RBCs ($\times 10^6/\text{mm}^3$)	5.67 $\pm$ 0.47 ^a	3.57 $\pm$ 0.31 ^b	3.36 $\pm$ 0.37 ^b
PCV (%)	43.22 $\pm$ 1.46 ^a	37.49 $\pm$ 1.50 ^{ab}	35.34 $\pm$ 1.64 ^b
Hb (g/dl)	14.80 $\pm$ 0.74 ^a	11.29 $\pm$ 0.78 ^b	10.76 $\pm$ 0.67 ^b
MCHC (g/dl)	32.70 $\pm$ 1.14 ^a	30.56 $\pm$ 0.83 ^a	29.91 $\pm$ 1.00 ^a
MCH (pg)	31.77 $\pm$ 0.99 ^a	29.26 $\pm$ 1.01 ^a	29.02 $\pm$ 1.51 ^a

MCV (fl)	88.33±1.80 ^a	82.40±2.16 ^a	77.20±2.30 ^b
WBCs (x10 ³ /mm ³)	7.71±0.20 ^a	8.05±0.26 ^a	7.95±0.26 ^a
LYM (%)	27.47±2.28 ^a	38.81±5.6 ^{89 a}	32.96±4.53 ^a
MON (%)	6.04±0.77 ^a	7.99±1.43 ^a	7.51±0.99 ^a
GRA (%)	53.91±6.06 ^a	41.89±5.71 ^a	55.13±6.86 ^a
PLA (x10 ³ /mm ³)	435.00±11.05 ^a	445.70±8.10 ^a	449.10±9.02 ^a

Values are expressed as mean ± SEM. Means in each row with different superscript are significantly different at p<0.05.

3.2 Biochemical profile

Results of the biochemical analysis of fish serum per studied diets are presented in table 3. All studied biochemical parameters differed significantly (p<0.05) between fish fed with imported and locally pelleted feeds. Only total cholesterol (TC), Aspartate amino transferase (ASAT) and Alkaline Phosphatase (ALP) differed significantly (p<0.05) between fish fed with imported and locally extruded feeds. Of the studied parameters, only total cholesterol (TC) and total protein (TP) showed no significant difference (p>0.05) between locally pelleted and locally extruded fed fish.

Comment [A9]: alkaline phosphatase (ALP)

Table 3: Serum biochemical parameters of *C. gariepinus* fed with different dietary treatments

Parameter	Le	Lpe	Lex
TC (mmol/L)	2.44±0.10 ^a	2.00±0.11 ^b	2.03±0.10 ^b
TP (mg/dL)	342.41±8.44 ^a	403.64±14.38 ^b	391.47±18.94 ^{ab}
ALAT (IU/L)	11.49±0.59 ^a	14.02±0.60 ^b	11.45±0.67 ^a
ASAT (IU/L)	152.98±8.85 ^a	198.48±9.53 ^b	241.82±9.48 ^c
ALP (IU/L)	136.67±8.29 ^a	261.29±14.60 ^b	202.76±11.92 ^c
Glu (mg/dL)	110.71±4.45 ^a	86.76±2.54 ^b	107.99±5.26 ^a

Values are expressed as mean ± SEM. Means in each row with different superscript are significantly different at p<0.05.

4. DISCUSSION

4.1 Haematological profile

Red blood cells (RBCs) also called erythrocytes are the most abundant blood cells (98–99%) in fish [35]. The highest RBCs count observed in fish fed with imported feed could be attributed to its highest crude

protein content. In fact, Ahmed and Ahmed [8] observed that high dietary protein levels resulted in high RBCs counts indicating a well oxygen transport in the body thereby improving health status and increasing growth performance. Erythrocyte counts observed in this study were higher than those obtained previously for *Clarias gariepinus* cultured in out of pond productions systems (concrete, tarpaulin and plastic tanks) and fed different commercial (floating) and farm made (sinking) feeds [26, 36]. A similar trend was observed in previous studies with *C. gariepinus* cultured in freshwater earthen ponds [11]. Witeska *et al.* [37] observed that erythrocytes depend greatly on environmental and biological factors such as water quality and type, fish activity, age, sex, nutritional status, reproductive status and can differ among various populations of the same species, corroborating the differences observed between this study and previous ones. However, erythrocyte values obtained from the studied diets were within the normal range for a healthy fish [38, 39]. In addition, Rastogi [39] stated that erythrocyte counts greater than $1.00 \times 10^6/\text{mm}^3$ was considered high and indicative of high oxygen carrying capacity of the blood, which is characteristic of fishes capable of aerial respiration and high activity.

Comment [A10]: were

Comment [A11]: of the high

Packed cell volume (PCV) also known as haematocrit is the simplest measure of erythrocyte content in blood as a percentage of erythrocytes in blood volume [37]. It is a useful tool in fisheries and aquaculture management for checking anaemic condition in aquatic species [26] and effect of stressors on the health of fish [40]. The low PVC of fish fed with locally formulated diets could be attributed to anti-nutritional factors in the feed ingredients during processing since the reduction in the concentration of haematocrit in the blood usually suggests the presence of toxic compounds (e.g. haemagglutinin) which generally affects blood formation and growth. Nonetheless, these values were higher than those observed by Bake *et al.* [26] and Mogaji *et al.* [36] working on the same fish species in similar conditions and also those of Abdel-Hay *et al.* [11] and Fagbenro *et al.* [23], working in different conditions. Though Alwan *et al.* [41] stated that low PCV in fish could be attributed to a reduction in RBC volume caused by osmotic changes, the values obtained were within normal range (20- 38%) for catfishes [28].

Haemoglobin concentration is a good indicator of anaemic conditions in fish according to Blaxhall and Daisley [42] as it reflects organisms' oxygen supply which should be maintained stable as much as possible [43]. The lower haemoglobin levels in fish fed with both locally pelleted and locally extruded feed

could be explained by the fact that the feeds' ability to provide sufficient oxygen to the tissues was restricted considerably [44]. However, these values were higher than those obtained by Afia and Gift [26] and Mogaji *et al.* [36] working on the same fish species in similar conditions and also those of other authors [11, 23, 27, 43] working in different conditions.

Haematological indices: Mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV), have a particular importance in the diagnosis of anaemia in most animals [45]. MCHC is a good indicator of red blood cell swelling [46]. That MCHC values did not vary significantly between the studied diets suggest that MCHC was not affected by dietary treatments. That MCH values did not also vary significantly between dietary treatments also suggest that MCH was not affected by dietary treatments. However, only imported feed showed values within reference interval (30.21 - 46.74pg) for catfishes [12]. Diyaware *et al.* [47] suggested that higher MCH indicates a good volume of haemoglobin which indicates effective oxygen transportation in the bloodstream required for optimal wellbeing of the fish. That MCV values were significantly lower in locally extruded feed could be due to lower iron content of the feed which could result in anaemic conditions. In fact, Adesina [20] observed that MCV indicates the status or size of RBCs and reflects normal or abnormal cell division during RBC production. Thus reduced MCV values could be linked to shrinkage of RBCs produced in low iron environment or by destruction of RBCs leading to anaemia as reported by Afia and Gift [26] and Alwan *et al.* [41].

The leukogram (White blood cells count, lymphocytes, monocytes and granulocytes) is an indicator of health in fish [48] and is used to get a picture of the status of an animal's immune system [49]. The insignificant changes in the leukogram of fish fed with the studied diets suggest that any possible stress-related factors (handling during sampling, anti-nutrients etc.) were too low to induce pathological changes in the fish immune status [23].

4.2 Biochemical profile

Analyses of serum biochemical constituents give useful information for detecting and diagnosing metabolic disturbances and diseases in fishes [50].

That total cholesterol was higher in fish fed with imported feed could be due to the higher proportion of fat in the chemical composition of the test diet [51]. The low total cholesterol observed in local feeds could be due to its high plant composition in comparison with imported feed. In fact, plant ingredients have been reported to possess phytosterols which compete with cholesterol for adsorption in the digestive system resulting in reduced levels of blood cholesterol [52, 53].

Serum total protein concentration provides critical information on the functional status of various organs and/or systems [54]. Proteins maintain colloid osmotic pressure and serve as catalyst in biochemical processes [55]. Increased concentrations of total protein observed in fish fed with locally pelleted and extruded feeds could be explained by structural liver alterations with reduced amino transferase activity, and concurrent reduced de-amination capacity [56]. Nonetheless, these concentrations were by far lower than those obtained by Adesina [20] and Bello *et al* [22] working on the same species.

Hepatic enzymes (transaminases and alkaline phosphatase) are important enzymes for monitoring the health status of fish [57]. These enzymes are synthesized in the liver and in hepatocellular or cholestatic liver injuries; they are liberated into the serum [55]. Increased levels of transaminases in the blood serum of fish are usually associated with dying or damaged liver cells while a decrease could suggest leakage of enzymes into the serum [58, 59]. The values of Alanine amino transferase (ALAT) in this study were lower than the reference range (38.19-39.40 UI/L) reported for *Heterobranchus longifilis* by Okorie-Kanu and Unakalamba [60] but were within the range (3.50-50.20 UI/L) for *Synodontis membranacea* as reported by Owolabi [61]. Values of Aspartate amino transferase (ASAT) and Alkaline Phosphatase (ALP) were higher than values obtained by Okorie-Kanu and Unakalamba [60] and Owolabi [61]. This difference observed could be attributed to difference in the fish species, culture media and feeding.

Serum glucose is regarded as an exceptional indicator of the fish health due to its rapid response to the various kinds of stressors [62]. Artacho *et al*. [63] stated that glucose in serum is a major metabolite of carbohydrate metabolism, thus the higher glucose concentration observed in fish fed with imported and locally extruded feeds could be attributed to their higher glycogen reserves.

5. CONCLUSION

The study revealed that with regards to haematological parameters, red blood cells count, packed cell volume and haemoglobin level differed among the test diets whereas parameters of erythrocytes indices (mean corpuscular haemoglobin concentration, mean corpuscular haemoglobin and mean corpuscular volume) and leucogram (white blood cells, lymphocytes, monocytes and granulocytes) did not differ significantly among dietary treatments. With regards to serum biochemistry, total cholesterol and glucose were higher in fish fed with imported feed while total protein, alanine amino transferase, aspartate amino transferase and alkaline phosphatase were higher in fish fed with locally pelleted and locally extruded feeds. Thus, variation in dietary treatments was not detrimental to the health status of *C. gariepinus* reared in plastic IBC tanks.

CONSENT

It was not applicable

ETHICAL APPROVAL

It was not applicable

REFERENCE

1. Béné C, Barange M, Subasinghe R, Pinstrup-Andersen P, Merino G *et al.* Feeding 9 billion by 2050 – Putting fish back on the menu. *Food Security* 2015; 7(2): 261-274. Doi: 10.1007/s12571-015-0427-z.
2. FAO. Global agriculture towards 2050. High level expert forum, 13- October 2009, Rome. 2009; 04p. Accessed on January 26 2022.
3. Mylona K, Maragkoudakis P, Miko L, Bock AK, Wollgast J, Caldeira S *et al.* Future of food safety and nutrition-seeking win-wins, coping with trade-offs. *Food Policy*. 2018; 74: 143-146. doi: 10.1016/j.foodpol.2017.12.002.

4. FAO. The State of World Fisheries and Aquaculture 2018- Meeting the sustainable development goals. Food and Agriculture Organization of the United Nations, Rome; 2018.
5. World Bank. Fish to 2030: Prospects for Fisheries and Aquaculture. Washington, DC: World Bank; 2013.
6. Chepkirui-Boit VCC, Ngugi J, Bowman E, Oyoo-Okoth J, Rasowo J, Mugo-Bund J *et al.* Growth performance, survival, feed utilization and nutrient utilization of African catfish (*Clarias gariepinus*) larvae co-fed Artemia and a micro-diet containing freshwater atyid shrimp (*Caridina nilotica*) during weaning. *Aquaculture Nutrition*. 2011; 17(2): e82–e89. Doi.10.1111/j.1365-2065.2009.00737.x.
7. Hogendoorn H. "Controlled propagation of the African catfish, *Clarias lazera* (C. & V.)—III. Feeding and growth of fry (Artemia)," *Aquaculture*. 1980; 21(3): 233–241.
8. Ahmed I, Ahmad I. Effect of dietary protein levels on growth performance, hematological profile and biochemical composition of fingerlings rainbow trout, *Oncorhynchus mykiss* reared in Indian Himalayan region. *Aquaculture Reports*. 2020; 16: 1-10. <https://doi.org/10.1016/j.aqrep.2019.100268>.
9. Ministry of Livestock, Fisheries and Animal Industries (MINEPIA). Situation of productions and imports in livestock, fisheries and animal industries subsectors in 2019. Publication from Studies, Planning, Cooperation and Statistics Division. 2020. 25p.
10. WorldBank. Aquaculture production (metric tons)-Cameroon. Accessed on January 26th 2022. Available: <https://tradingeconomics.com/cameroon/aquaculture-production-metric-tons-wb-data.html>.
11. Abdel-Hay AHM, Elsayy MY, Emam W, Eltras W, Mohamed RA. Haematological and biochemical blood Profile of African catfish (*Clarias gariepinus*) cultured in ponds of different Water depth and fed sinking versus Floating diet. *Biotechnology In Animal Husbandry*. 2021; 37(2):117-126. <https://doi.org/10.2298/BAH2102117A>
12. Akinrotimi OA, Bekibele DO, Orokotan OO. Select Hematological Values of the African Catfish (*Clarias gariepinus*) Raised in a Water Recirculating Aquaculture System. *International Journal of Recirculating Aquaculture*. 2011; 12: 1-12.

13. Gabriel UU, Akinrotimi OA, Orokotan OO. Water recirculatory system. A revolutionary tool for sustainable aquaculture development in Nigeria. *International Journal of Agriculture and Rural Development*. 2009; 12:121-133.
14. Hrubec TC, Cardinale JL, Smith SA. Haematology and plasma chemistry reference intervals for cultured tilapia (*Oreochromis* hybrid). *Veterinary and Clinical Pathology*. 2000; 29: 7-12.
<http://www.ncbi.nlm.nih.gov/pubmed/12070816>.
15. Fazio F. Fish hematology analysis as an important tool of aquaculture: a review. *Aquaculture*. 2019; 500:237–242. <https://doi.org/10.1016/j.aquaculture.2018.10.030>.
16. Tavares-Dias M, Moraes FR. Haematological and biochemical reference intervals for farmed channel catfish. *Journal of Fish Biology*. 2007; 71(2): 383-388.
17. Ferreira J, Hawkins A, Bricker S. Management of productivity, environmental effects and profitability of shellfish aquaculture-the Farm Aquaculture Resource Management (FARM) model. *Aquaculture*. 2007; 264: 60-174. doi:<https://doi.org/10.1016/j.aquaculture.2006.12.017>.
18. Mohammed AK, Sambo AB. Haematological assessment of the Nile tilapia *Oreochromis niloticus* exposed to sublethal concentrations of Portland cement powder in solution. *International Journal of Zoological Research*. 2007; 4(1): 48–52.
<http://docsdrive.com/pdfs/academicjournals/ijzr/2008/48-52.pdf>.
19. Svobodova Z, Pravda D, Palackova J. Unified methods of haematological examination of fish. Czechoslovakia: Research Institute of Fish culture and Hydrobiology; 1991.
20. Adesina SA. Haematological and serum biochemical profiles of *Clarias gariepinus* juveniles fed diets containing different inclusion levels of mechanically extracted sunflower (*Helianthus annuus*) seed meal. *Applied Tropical Agriculture*. 2017; 22(2): 24-35.
21. Agbabiaka LA, Madubuike FN, Ekenyem BU. Haematology and serum characteristics of African catfish (*Clarias gariepinus*, Burchell, 1822) fed graded levels of Tiger nut based diet. *American Journal of Experimental Agriculture*. 2013; 3(4): 988-995.
22. Bello OS, Olaifa FE, Emikpe BO. Haematological and blood biochemical changes in African catfish, *Clarias gariepinus* fed Walnut (*Tetracarpidium conophorum* Mull Arg) leaf and Onion

- (*Allium cepa* Linn.) bulb supplemented diets. American Journal of Experimental Agriculture. 2014; 4(12): 1593-1603.
23. Fagbenro OA, Adeparusi EO, Jimoh WA. Haematological profile of blood of African catfish (*Clarias gariepinus*, Burchell, 1822) fed sunflower and sesame meal based diets. Journal of Fisheries and Aquatic Sciences. 2013; 8(1): 80-86.
 24. Lawal MO, Aderolu AZ, Adewumi GA, Mudiaga A. Growth, nutrient utilization, haematology and biochemical parameters of african catfish (*Clarias gariepinus*, Burchell, 1822) fed with varying levels of *Bacillus subtilis*. Agrosearch. 2019; 19(1): 13-27.
<https://dx.doi.org/10.4314/agrosh.v19i1.2>.
 25. Bake GG, Theophilus R, Abdullahi A, Nwangwu DC, Aliyu MH. Growth Response and Haematological Indices of Hybrid Catfish Fingerlings Fed Varying Inclusion Levels of Fermented Sword Bean (*Canavalia Gladiata*) Seed Meal in a Concrete Tank. International Journal of Innovative Studies in Aquatic Biology and Fisheries. 2019; 5(2): 21-30. DOI: <http://dx.doi.org/10.20431/2454-7670.0502004>
 26. Afia OE, Gift SD. 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. Appl. Sci. 2017; 4(6): 213-222.
 27. Genç E, Genç MA, Kaya A, Seçer FS, Qaranjiki A, Güroy D. Effect of prebiotics on the growth performance, haematological, biochemical, and histological parameters of African catfish (*Clarias gariepinus*) in recirculating aquaculture system. Turk. J. Vet. Anim. Sci. 2020; 44: 1222-1231. Doi:10.3906/vet-2005-106.
 28. Erundu ES, Nnubia C, Nwaduikwe FO. Hematological studies in four catfish species raised in fresh water ponds in Nigeria. Journal of Applied Ichthyology. 1993; 9:250-256.
 29. Ezeri GNO, Gabriel UU, Opabunmi, OO. Hematological response of cultured and wild *Clarias gariepinus* to acclimation. Environment and Ecology. 2004; 3:628-632.
 30. Sowummi AA. Hematology of the African catfish (*Clarias gariepinus*) from Eleyele Reservoir, Ibadan South-West Nigeria. The Zoologist. 2003; 2:85-91.

31. Association of Official Analytical Chemists (AOAC). International official methods of analysis. 18th ed. AOAC International, Gaithersburg, MD; 2005. Available on: <http://academicjournals.org/journal/JPHE/article-xml/FF7324C50865>.
32. American Public Health Association (APHA). Standard Methods for Examination of Water and Wastewater. 21th edn., Washington, DC, USA; 2000.
33. Boyd CE. Water quality in warmwater fish ponds. Alabama: Craft Master Printers Inc.; 1979.
34. Godoy AG, Rovigatti –Chiavelli LU, Oxford JH, Romulo BR, Ferreira IO, Marcondes AS *et al*. Evaluation of limnological dynamics in Nile Tilapia farming tank. *Aquaculture and Fisheries*. 2021; 6(5): 485-494.
35. Fange R. Blood cells, haemopoiesis and lymphomyeloid tissues in fish. *Fish Shellfish Immunol*. 1994; 4(6): 405–411. <https://doi.org/10.1006/fsim.1994.1036>.
36. Mogaji OY, Robert EA, Mogaji NO. Haematological and latrical response of *Clarias gariepinus* (Burchell, 1822) fed different commercial feed and farm-made feed. *World J. Agri.& Soil Sci*. 2021; 7(2): 1-4. Doi:10.33552/WJASS.2021.07.000660.
37. Witeska M , Kondera E , Ługowska K, Bojarski B. Hematological methods in fish-Not only for beginners. *Aquaculture*. 2021; 547:737498. <https://doi.org/10.1016/j.aquaculture.2021.737498>.
38. Fagbenro OA, Adedire CO, Owoseeni EA, Ayotunde EO. Studies on the biology and aquaculture of feral catfish *Heterobranchus bidorsalis* (Geoffroy St. Hillarie 1809) (Clariidae). *Tropical Zoology*. 1993; 6:67-79. <http://doi/abs/10.1080/03946975.1993.10539209>.
39. Rastogi SC. Essentials of animal physiology. New Delhi: New Age International; 2007.
40. Munkittrick KR, Leatherland JF. Haematocrit values in feral goldfish, *Carasius auratus* L., as indicators of the health of the population. *J. Fish Biol*. 1983; 23: 153 - 161.
41. Alwan SF, Hadi AA, Shokr AE. Alterations in haematological parameters of freshwater fish, *Tilapia zillii*, exposed to Aluminium. *Journal of Science and Its Applications*. 2009; 3(1): 12-19.
42. Blaxhall PC, Daisley KW. Routine haematological methods for use with fish blood. *J. Fish Biol*. 1973; 5 (6): 771–781. <https://doi.org/10.1111/j.1095-8649.1973.tb04510.x>.
43. Olanike KA. Haematological Profile of *Clarias gariepinus* (Burchell, 1822) Exposed to Lead. *Turkish Journal of Fisheries and Aquatic Sciences*. 2007; 7: 163-169.

44. Wepener W. the effects of heavy metals at different pH on the blood physiology and metabolic enzymes in *Tilapia sparmanii* (Cichlidae). M.Sc. Thesis, Rand Afrikaans University, Johannesburg, South Africa; 1990.
45. Coles EH. Veterinary Clinical Pathology (4th Ed); W. B. Saunders Co. Philadelphia; 1986.
46. Wepener W, Wan Vuren JHJ, Du Preez HH. Effects of manganese and iron at neutral pH values on the haematology of the banded tilapia (*Tilapia sparmanii*). Bull. Environ. Contam. Toxicol. 1992; 49: 613-619.
47. Diyaware MY, Haruna AB, Abubakar KA. Some haematological parameters of intergeneric hybrid of African catfish (*Clarias anguillaris* x *Heterobranchus bidorsalis*) juveniles and their pure lines in North-Eastern Nigeria. Journal of Fisheries and Aquatic Science. 2012; 8(1): 33-42.
48. Fox HE, White SA, Koa MF, Fernald RD. Stress and dominance in a social fish. The Journal of Neuroscience. 1997; 16(17): 6463-6469.
49. Fagbenro OA, Adedire CO, Ayotunde EO, Faminu EO. Haematological profile, food consumption and enzyme assay in the gut of the African bony-tongue fish *Heterotis (Clupisudis) niloticus* (Cuvier 1829) (Osteoglossidae). Tropical Zoology. 2000; 13: 1-9.
<http://www.tandfonline.com/doi/pdf/10.1080/03946975.2000.10531125>.
50. Ferrari A, Venturino A, Pechén AM, D'Angelo. Effects of carbaryl and azinphos methyl on juvenile rainbow trout (*Oncorhynchus mykiss*) detoxifying enzymes. Pesticide Biochemistry and Physiology. 2007; 88(2):134-142.
51. Bolade A, Ndidi E. Haematological and serum biochemical reference intervals of juvenile African bonytongue fish (*Heterotis niloticus*. Cuvier, 1829) sampled from the river Benue, Nigeria. Mansoura Veterinary Medical Journal. 2021; 22(2): 82-90. Doi: 10.35943/mvmj.2021.56537.1024.
52. Anonymous: Cleveland Clinic. Boost your cholesterol lowering potential with phytosterols. 2022. Accessed on February 16 2022. <https://my.clevelandclinic.org/health/articles/17368-phytosterols-sterols--stanols>.
53. Kortner T, Björkhem I, Krasnov A, Timmerhaus G, Krogdahl, Å. Dietary cholesterol supplementation to a plant-based diet suppresses the complete pathway of cholesterol synthesis

- and induces bile acid production in Atlantic salmon (*Salmo salar* L.). British Journal of Nutrition. 2014; 111(12): 2089-2103. Doi: 10.1017/S0007114514000373.
54. Knowles S, Hrubec TC, Smith SA, Bakal RS. Haematology and plasma chemistry reference intervals for cultured short Nose Sturgeon (*Acipenser brevirostrum*). Vet. Clin. Pathol. 2008; 35: 434- 440. <https://doi.org/10.1111/j.1939-165X.2006.tb00160.x>.
 55. Wagner T, Congleton JL. Blood chemistry correlates of nutritional condition, tissue damage, and stress in migrating juvenile chinook salmon (*Oncorhynchus tshawytscha*). Can. J. Fish Aquat. Sci. 2004; 61: 1066–1074. <https://doi.org/10.1139/f04-050>.
 56. Burtis CA, Ashwood ER. Tietz fundamentals of clinical chemistry. WB Saunders, Philadelphia; 1996.
 57. Racicot JG, Gaudet M, Leray C. Blood and liver enzymes in rainbow trout (*Salmo gairdneri* Rich.) with emphasis on their diagnostic use: Study of CC14 toxicity and a case of *Aeromonas* infection. Journal of Fish Biology. 1975; 7: 825-835.
 58. Ozovehe BM. Growth performance, haematological indices and some biological enzymes of juvenile *Clarias gariepinus* (Burchell, 1822) fed varying levels of *Moringa oleifera* meal diet. Aquaculture Research and Development. 2013; 4: 1-6.
 59. Yilmaz E, Akyurt I, Mutlu E. Effects of energetic diets on growth, blood chemistry and liver pathology of African catfish *Clarias gariepinus* (Burchell, 1822). Israeli Journal of Aquaculture, Bamidgeh. 2006; 58: 191-197.
 60. Okorie-Kanu, CO, Unakalamba NJ. Haematological and blood biochemistry values of cultured *Heterobranchus longifilis* in Umudike, Abia state, Nigeria. Animal Research International. 2014; 11(2): 1987 – 1993.
 61. Owolabi OD. Haematological and serum biochemical profile of the upside down catfish, *Synodontis membranacea* Geoffrey Saint Hilaire from Jeba Lake, Nigeria. Comparative Clinical Pathology. 2011; 20: 163 – 172.
 62. Polakof S, Mommsen TP, Soengas JL. Glucosensing and glucose homeostasis: From fish to mammals. Comp. Biochem. Physiol. - B Biochem. Mol. Biol. 2011; 160: 123–149. <https://doi.org/10.1016/j.cbpb.2011.07.006>.

63. Artacho P, Soto-Gamboa M, Verdugo C, Nespolo RF. Blood biochemistry reveals malnutrition in black-necked swans (*Cygnus melanocoryphus*) living in a conservation priority area. *Comp. Biochem. Physiol.* 2007; 146:283-290.

UNDER PEER REVIEW