



HAEMATOLOGICAL RESPONSE OF *Clarias gariepinus* (BURCHELL, 1822) FINGERLINGS TO VARYING LEVELS OF BETAINE/ β – GLUCAN FEED ADDITIVE

*^{1,2}Ali, Mark Eka, ²Sogbesan, Olukayode Amos, ³Abubakar, Kotos Abdulrahman and ¹Salisu, Sylvia Ali

¹National Biotechnology Research and Development Agency, Billiri, Gombe State, Nigeria.

²Department of Fisheries, Modibbo Adama University, Yola, Adamawa State, Nigeria.

³Department of Zoology, Modibbo Adama University, Yola, Adamawa State, Nigeria.

*Corresponding authors' email: mealikumbo@gmail.com Phone: +2348034935792

ABSTRACT

This study was designed to evaluate the haematological response of *Clarias gariepinus* fingerlings to varying levels of betaine/ β – glucan feed additive. Combination of betaine/ β – glucan as feed additive was included into formulated feed with 40% crude protein at; 0.0g/100g (BBG0 i.e. control), 0.325g/100g (BBG1), 0.75g/100g (BBG2), 1.125g/100g (BBG3) and 1.50g/100g (BBG4) in triplicates. Feed was fed to *C. gariepinus* fingerlings (n = 300, 10.0 \pm 0.00g) in fifteen (15) plastic tanks (n = 20) at a fixed feeding rate of 3% body weight twice daily between the hours of 8:00 – 9:00am and 4:00 – 5:00pm at regular interval and adjusted after every two (2) weeks of sampling for a period of twelve (12) weeks. Blood samples were collected from three (3) fish at the commencement and also from three (3) fish from each treatment and the control at the termination of the feeding trial for hematological analysis. Data collected was statistically analysed using one - way analysis of variance (ANOVA) at P = 0.05. PCV values (41.3 \pm 3.3 – 42.7 \pm 1.9 %), RBC (2.56 \pm 0.6 \times 10⁶ – 2.82 \pm 0.1 \times 10⁶ dL⁻¹), WB (249.8 \pm 14 \times 10³ – 266.5 \pm 16 \times 10³ dL⁻¹), Hb (12.1 \pm 2.2 – 13.5 \pm 2.4 g dL⁻¹), platelet (27.75 \pm 1.5 \times 10⁴ – 29.20 \pm 1.4 \times 10⁴ dL⁻¹), MCV (136.7 \pm 7.9 – 155.1 \pm 11.0 μ m⁻³), MCH (40.7 \pm 2.2 – 50.1 \pm 2.5 μ g dL⁻¹), MCHC (31.2 \pm 2.1 – 33.1 \pm 2.6 g dL⁻¹), neutrophil (32.10 \pm 3.2 – 33.70 \pm 2.0 %), lymphocyte (95.7 \pm 6.4 – 97.6 \pm 8.3 %) and monocytes (2.85 \pm 0.2 – 3.70 \pm 0.3 %) respectively were recorded. Combination of betaine/ β – glucan feed additive at varying levels used in this study did not result in statistically significant changes (p>0.05) in haematological responses of *C. gariepinus*.

Keywords: Haemoglobin, Lymphocytes, Monocytes, Packed Cell Volume, Platelet

INTRODUCTION

Haematology is the science of studying the anatomical, physiological and pathological aspects of blood (Elezu, 2016). Haematological profile has for long been used in clinical and pathological diagnosis in human and livestock (Ayoola, 2011). Ichthyo - haematology would be useful in the appraisal of suitability of feeds, fish condition, toxic effect of substances and diagnosis of diseases, haematological responses are also important in analyzing the health status of animals exposed to toxicants (Elezu, 2016). Osuigwe *et al.* (2005) reported that the application of haematological indices has become a valuable tool for fishery biologist in assessing the health of fish and monitoring stress response. Blood parameters such as packed cell volume (PCV), red blood cell (RBC), white blood cell (WBC), haemoglobin concentration (Hb) and the serum biochemical constituents are used to assess the health status of fish. Decrease in the PCV, RBC and Hb values are indicators of reduced fish activity and is unhealthy for the fish (Adeyemo, 2005; Osuigwe *et al.*, 2005). The reduction in PCV and Hb values are possible reason for anaemia. The same author stated that the most common haematological variables measured during stress are; the RBC, WBC, Hb and haematocrit which is also known as the PCV values. Fish haematological changes are often determined as indices of their health status (Oshode *et al.*, 2008).

However, Babale. (2016) reported that the history of applying haematological methods as diagnostic aids in episodes of diseases in confined and free - living populations of fish is quite inadequate. The major reason for the insufficiency, as compared with mammalian medicine is the variability of data. Fish are subject to many environmental

influences, which alter the healthy haemogram, that is, the baseline data for cellular and plasma components (Elezu, 2016). Terry *et al.* (2000) reported that haematology could serve as aids in fish disease diagnosis but stated that caution should be applied in doing so.

Terry *et al.* (2000) also stated that there are no “normal” values because of the responsiveness of blood vascular system to external stimuli. The same researchers indicate that the most appropriate approach is to establish the baseline data for the fish in a specific situation and monitor the population for changes in the haematological profile. They concluded that if good techniques are applied and interpretations are guarded, haematological methods have value in fish health management. Therefore, this study was designed to evaluate the haematological response of *Clarias gariepinus* (Burchell, 1822) fingerlings to varying levels of betaine/ β – glucan feed additive.

MATERIALS AND METHODS

Study Area

The study was carried out at Lay – Joy Fish Farm, Gombe – Yola road, Billiri local government area (LGA), Gombe State Nigeria. Billiri LGA lies within Lat. 9°50'N; 11°09'E and Long. 9.833°N 11.150°E. It covers an area of 737km² (285 sq. m) and is 50 km away from Gombe the State capital.

Experimental Fish

Three hundred (300) *C. gariepinus* fingerlings with mean initial weight (10.0 \pm 0.00g) were stocked at twenty (20) fingerlings per tank in triplicates per treatment after one (1) week of acclimatization, the study lasted for a duration of twelve (12) weeks.

Experimental Feed

The formulated feed contained; fish meal (FM), soybean meal (SBM), yellow maize meal (YMM), groundnut cake meal (GNCM) and combination of betaine/ β – glucan. All ingredients were grounded into a fine powder using a hammer mill and sieved by a 0.25 mm sieve. Fish meal, soybean meal, groundnut cake meal and yellow maize meal were obtained from commercial suppliers in Gombe, the vitamin/mineral premix, fish oil and chromic oxide (Cr_2O_3) were purchased from TTS Integrated Farms Lagos, while the betaine powder

naturally derived from sugar beets (*Beta vulgaris*) and the β – glucan (β – 1,3/1,6 – D – glucan) powder naturally derived from baker's yeast (*Saccharomyces cerevisiae*) were obtained from Bon – Amour. Pharmacy Limited, Lagos. Experimental diet was prepared by incorporating the combination of betaine/ β – glucan into formulated feed with 40% crude protein as recommended by Ali et al. (2024) at 0.0g/100g (BBG0 i.e. control), 0.325g/100g (BBG1), 0.75g/100g (BBG2), 1.125g/100g (BBG3) and 1.50g/100g (BBG4) feed as shown in Table 1.

Table 1: Ingredient % (g/100g) of Formulated Feed with Combination of Betaine/ β – Glucan

Ingredients (%)	BBG0	BBG1	BBG2	BBG3	BBG4
Fish meal	20.00	20.00	20.00	20.00	20.00
Soybean Meal	21.50	21.50	21.00	21.00	21.00
GNC meal	23.00	22.625	22.75	22.375	22.00
Yellow maize	30.00	30.00	30.00	29.75	30.00
Betaine/ β – glucan	0.00	0.373	0.75	1.125	1.50
Fish oil	1.00	1.00	1.00	1.00	1.00
Vegetable oil	1.00	1.00	1.00	1.00	1.00
Starch	1.00	1.00	1.00	1.00	1.00
Lysine	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25
*Vitamin/premix	1.00	1.00	1.00	1.00	1.00
Salt	0.50	0.50	0.50	0.50	0.50
Cr_2O_3	0.50	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00	100.00

Keys: BBG0 – Betaine/ β – glucan (0.0g/100g), BBG1 – Betaine/ β – glucan (0.375g/100g), BBG2 – Betaine/ β – glucan (0.75g/100g), BBG3 – Betaine/ β – glucan (1.125g/100g), BBG4 – Betaine/ β – glucan (1.5g/100g).

Experimental Design

The *C. gariepinus* fingerlings were cultured in fifteen (15) rectangular white plastic tanks (flow - through system) with a water holding capacity of one thousand litres (1,000L) each in a complete randomized design (CRD). Each tank was washed thoroughly with salt, filled to just a little over 1/3 (350 litre) capacity and stocked with twenty (20) fingerlings of *C. gariepinus* with mean initial weight ($10.0 \pm 0.00\text{g}$). The *C. gariepinus* fingerlings were fed the experimental diet at 3% body weight two (2) times daily between the hours of 8:00 – 9:00am and 4:00 – 5:00pm for a period of twelve (12) weeks. The quantity of feed was adjusted accordingly after every two (2) weeks of sampling for growth performance and survival rate (mean body weight and mortality). Water temperature, pH, dissolved oxygen, and ammonia were measured at the beginning of the experiment after which they were measured weekly throughout the period of the experiment. Water temperature, dissolved oxygen and pH were measured using Horiba U-22 XD multi - parameter water quality checker while ammonia was measured using freshwater aquaculture test kit (Model AQ-2, Code 3633-03, Lamotte U. S. A.

Determination of Haematological Parameters

Blood samples were collected from three (3) fish at the commencement and also from three (3) fish from each treatment and the control at the termination of the feeding trial according to the method as described by Elezuo (2016) for haematological analysis. The fish blood samples were analysed at the haematology laboratory of the Specialist Hospital Gombe, Gombe State for haematological parameters such as packed cell volume (PCV), red blood cell (RBC) count, white blood cell (WBC) count, haemoglobin concentration (Hb), platelet count, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), neutrophil,

lymphocytes, monocytes, eosinophil and basophils using standard haematological procedures,

Blood Collection and Preservation

A 2ml plastic syringe needle was inserted at the ventral midline just posterior to the anal fin of the fish at angle 450 until it penetrated the caudal vessel lying between adjacent haemal arches. This was accomplished by inserting the needle until it stopped at the backbone. Blood was then drawn slowly into the syringe and preserved in a labelled Ethylene diminitetra acetate (EDTA) bottles.

Haematological Parameters

Packed cell volume (PCV)

The heparinized capillary tubes were $\frac{3}{4}$ filled with whole blood and one end sealed with plasticine. The tubes were centrifuged for 5min in a micro haematocrit centrifuge at 12,000 rpm. The PCV was read by the use of haematocrit reader (Terry et al., 2000).

Red blood cell (RBC) and white blood cell (WBC) counts

The RBC and total WBC count was carried out by the use of the Neubauer improved counting chamber as described by Teixeira (2000).

Red blood cell (RBC) count

For red blood cell count, blood was diluted 1:200 with Dacies fluid (99 ml of 3 % aqueous solution of sodium citric; and 1ml of 40 % formaldehyde) which was kept and preserved the shape of the red blood cell for estimation in the counting chamber (Shah and Altindag, 2004).

White blood cell (WBC) count

For white blood cell count, the dilution was 1:20 using 2 - 3% aqueous solution of acetic acid of which tinge of Gentian violet was added. Thin blood smears were stained with Wright

- Giemsa stain (Robinson *et al.*, 2001). A total of 100 white blood cells were enumerated and differentiated.

Haemoglobin concentration (Hb)

The cyanomet haemoglobin method as described by Osuigwe (2005) was used in the determination of haemoglobin concentration (Hb). Well - mixed blood of 0.02mL was added to 4mL of modified Dabkin's solution (potassium ferricyanide, 200mg; potassium cyanide, 50mg; potassium dihydrogen phosphate (140mg). The volume was made up of 1L with distilled water at pH of 7.0. The mixture was allowed to stand for 3min and the Hb concentration was taken photometrically by comparing with a cyanomet haemoglobin standard with a yellow - green filter at 625nm.

Platelet count

Platelet count determination was carried out by putting diluting fluid into the bulb of an erythrocyte diluting pipette, and excess fluid was expelled. Blood was drawn from EDTA embedded vial to the 0.5 marks, followed by diluting fluid to the 101 marks. Pipette content was mixed for some minutes and several drops were discarded to fill both sides of the counting chamber. Content was allowed to stand for 10 minutes for erythrocytes to settle. The light partially reduced; platelets were counted in the entire central ruled of each side of the counting chamber (Akinrotimi *et al.*, 2016).

Mean corpuscular volume (MCV)

This represents the mean volume of a single red cell in a blood sample and is determined according to Akinrotimi *et al.* (2016) by:

$$MCV(fl) = \frac{\text{Haematocrit}(\text{Packed Cell Volume}) \times 100}{\text{RBC}(\text{Red Blood Cell})}$$

Mean corpuscular haemoglobin (MCH)

MCH was derived from the relationship between the haematocrit concentration (PCV) and the erythrocyte count (RBC) and is determined according to Akinrotimi *et al.* (2016) by:

$$MCH(pg) = \frac{\text{Haematocrit Conc.}}{\text{Erythrocyte count (RBC)}}$$

Mean corpuscular haemoglobin concentration (MCHC)

MCHC was derived from the relationship between the haemoglobin concentration and the haematocrit expressed in pictograms (10^{-12}) and is determined according to Akinrotimi *et al.* (2016) by:

$$MCHC(g/d) = \frac{\text{Haemoglobin content} \times 100}{\text{Haematocrit (PVC)}}$$

Leucocyte differential count

Two drops of blood were placed on a slide, it was left to dry and was made into a thin smear with another slide. The smear was fixed with an absolute methanol, then stained with Giemsa stain and 170ml buffered distilled water. It was allowed to stand for about 20 – 30 minutes after which the slide was rinsed with buffered distilled water and allowed to dry for reading the differential count of neutrophils, lymphocytes, monocytes, eosinophils and basophils (Robinson *et al.*, 2001).

Statistical Analysis

Data obtained from the study was statistically analysed using one – way analysis of variance (ANOVA) at $P = 0.05$, where significant differences were detected, mean values were separated using least significant difference (LSD).

RESULTS AND DISCUSSION

The results of haematological response of *C. gariepinus* fingerlings to varying levels of betaine/ β – glucan feed additive is presented in Table 2. PCV value of the experimental fish was 25.2 ± 2.3 %. This increased in all the fish fed diet with combination of betaine/ β – glucan and the control diet (BBG0) final values. The PCV values ranged from 41.3 ± 3.3 – 42.7 ± 1.9 %. Initial RBC count value was $2.1 \pm 0.7 \times 10^6 \text{ dL}^{-1}$. This increased in all the fish fed diet with combination of betaine/ β – glucan and the control diet (BBG0) final values. The RBC count values ranged from $2.56 \pm 0.6 \times 10^6$ – $2.82 \pm 0.1 \times 10^6 \text{ dL}^{-1}$. Initial WBC count value was $191.2 \pm 10 \times 10^3 \text{ dL}^{-1}$. This increased in all the fish fed diet with combination of betaine/ β – glucan and the control diet (BBG0) final values. The WBC count values ranged from $249.8 \pm 14 \times 10^3$ – $266.5 \pm 16 \times 10^3 \text{ dL}^{-1}$. Initial Hb value was $10.0 \pm 2.0 \text{ g/dL}^{-1}$. This increased in all the fish fed diet with combination of betaine/ β – glucan and the control diet (BBG0) final values. The Hb values ranged from 12.1 ± 2.2 – $13.5 \pm 2.4 \text{ g/dL}^{-1}$. Initial platelet count value was $20.90 \pm 2.1 \times 10^4 \text{ dL}^{-1}$. This increased in all the fish fed diet with combination of betaine/ β – glucan and the control diet (BBG0) final values. The platelet count values ranged from $27.75 \pm 1.5 \times 10^4$ – $29.20 \pm 1.4 \times 10^4 \text{ dL}^{-1}$. Initial MCV value was $106.9 \pm 7.3 \mu\text{m}^{-3}$. This increased in all the fish fed diet with combination of betaine/ β – glucan and the control diet (BBG0) final values. The MCV values ranged from 136.7 ± 7.9 – $155.1 \pm 11.0 \mu\text{m}^{-3}$. Initial MCH value was $38.0 \pm 2.0 \mu\text{g/L}^{-1}$. This increased in all the fish fed diet with the combination of betaine/ β – glucan and the control diet (BBG0) final values. The MCH values ranged from 40.7 ± 2.2 – $50.1 \pm 2.5 \mu\text{g/L}^{-1}$. Initial MCHC value was $26.2 \pm 2.8 \text{ g/dL}^{-1}$. This increased in all the fish fed diet with combination of betaine/ β – glucan and the control diet (BBG0) final values. The MCHC values ranged from 31.2 ± 2.1 – $33.1 \pm 2.6 \text{ g/dL}^{-1}$. Initial neutrophil value was 21.10 ± 1.0 %. This increased in all the fish fed diet with combination of betaine/ β – glucan and the control diet (BBG0) final values. The neutrophil values ranged from 32.10 ± 3.2 – 33.70 ± 2.0 %. Initial lymphocytes value was 84.0 ± 12.0 %. This increased in all the fish fed diet with combination of betaine/ β – glucan and the control diet (BBG0) final values. The lymphocytes values ranged from 95.7 ± 6.4 – 97.6 ± 8.3 %. Initial monocytes value was 2.70 ± 0.2 %. This increased in all the fish fed diet with combination of betaine/ β – glucan additive and the control diet (BBG0) final values. The monocytes values ranged from 2.85 ± 0.2 – 3.70 ± 0.3 %. Eosinophils and basophils were not recorded from the initial and final values of all the *C. gariepinus* fed diet with combination of betaine/ β – glucan additive and the control diet (BBG0) used in this study. However, there was no significant difference ($p > 0.05$) between the PCV, RBC count, WBC count, Hb, platelet count, MCV, MCH, MCHC, neutrophil, lymphocytes, monocytes, values recorded from the fish fed diet with combination of betaine/ β – glucan additive at different levels compared with that of the fish fed control diet (BBG0).

Table 2: Haematological Response of *C. gariepinus* Fingerlings to Varying Levels of Betaine/β – Glucan Feed Additive

Parameters	Initial	BBG0	BBG1	BBG2	BBG3	BBG4
PCV (%)	25.2±2.3 ^b	42.1±3.7 ^c	41.7±2.8 ^c	42.7±1.9 ^c	41.3±3.3 ^c	41.5±2.6 ^c
RBC (x10 ⁶ dL ⁻¹)	2.1±0.7 ^a	2.80±0.3 ^b	2.59±0.4 ^b	2.82±0.1 ^b	2.56±0.6 ^b	2.57±0.3 ^b
WBC (x10 ³ dL ⁻¹)	191.2±10 ^b	265.7±12 ^c	257.3±10 ^c	266.5±16 ^c	249.8±14 ^c	252.6±12 ^c
Hb (gdL ⁻¹)	10.0±2.0 ^c	13.1±2.1 ^d	12.7±2.2 ^d	13.5±2.4 ^d	12.1±2.2 ^d	12.3±2.1 ^d
Platelet (x10 ⁴ dL ⁻¹)	20.90±2.1 ^a	28.10±1.9 ^b	28.00±1.7 ^b	29.20±1.4 ^b	27.75±1.5 ^b	27.90±1.7 ^b
MCV (µm ⁻³)	106.9±7.3 ^c	149.7±5.8 ^a	140.3±3.6 ^{ab}	155.1±11.0 ^a	136.7±7.9 ^b	138.2±8.6 ^b
MCH (µg ⁻¹)	38.0±2.0 ^b	48.0±2.3 ^d	47.6±1.8 ^d	50.1±2.5 ^d	40.7±2.2 ^d	44.2±2.7 ^d
MCHC (gdL ⁻¹)	26.2±2.8 ^a	32.1±1.7 ^b	31.8±2.1 ^b	33.1±2.6 ^b	31.2±2.1 ^b	31.5±3.2 ^b
Neutrophil (%)	21.10±1.0 ^b	33.00±2.1 ^c	32.70±2.5 ^c	33.70±2.0 ^c	32.10±3.2 ^c	32.40±2.3 ^c
Lymphocytes (%)	84.0±12.0 ^a	97.1±9.0 ^b	96.2±6.7 ^b	97.6±8.3 ^b	95.7±6.4 ^b	96.0±4.6 ^b
Monocytes (%)	2.70±0.2 ^a	3.20±0.7 ^a	3.10±0.4 ^a	3.70±0.3 ^a	2.85±0.2 ^a	3.00±0.1 ^a
Eosinophils (%)	0.00	0.00	0.00	0.00	0.00	0.00
Basophils (%)	0.00	0.00	0.00	0.00	0.00	0.00

Mean values in each row with similar superscripts are not significantly different (p>0.05).

Keys: BBG0 – Betaine/β – glucan (0.0g/100g), BBG1 – Betaine/β – glucan (0.375g/100g), BBG2 – Betaine/β – glucan (0.75g/100g), BBG3 – Betaine/β – glucan (1.125g/100g), BBG4 – Betaine/β – glucan (1.5g/100g).

Discussion

PCV value also known as haematocrit indicates the oxygen carrying capacity of blood and the degree of stress on animal health (Bichi et al., 2021). The PCV values, 41.30 – 42.70 % recorded from this study were comparable with the values, 34.23 – 44.01 % reported by Babale. (2016) for *C. gariepinus* juveniles fed various inclusion levels of processed water melon (*Citrullus lanatus*) seed cake diets and the values, 28.82 – 40.35 % reported by Inya et al. (2020) for *C. gariepinus* juveniles fed beniseed, (*Sesame indicum*) seed meal as replacement for soybean. The PCV values recorded from this study were within the recommended ranged of 20 – 50 % reported for *C. gariepinus* (Tiamiyu et al., 2019). The RBC value of a fish indicates the dissolved oxygen carrying capacity of the blood (Melefa and Okoloye, 2023). The RBC count values, $2.56 \times 10^6 - 2.82 \times 10^6$ dL⁻¹ were comparable with the values, $2.62 \times 10^6 - 3.01 \times 10^6$ dL⁻¹ reported by Anyanwu et al. (2017) for *C. gariepinus* fed *Enterococcus faecium* (prebiotics) additive diets and the values, $1.96 \times 10^6 - 2.74 \times 10^6$ dL⁻¹ reported by Lawal et al. (2016) for *C. gariepinus* fed *Spondias mombin* and *Morinda lucida* leaves extracts additive diets. WBC are the defence cells of the fish body (Ofonime and David, 2017). The WBC count values, $249.8 \times 10^3 - 266.5 \times 10^3$ dL⁻¹ were higher than the values, $164.1 \times 10^3 - 243.7 \times 10^3$ dL⁻¹ reported by Nya (2009) for rainbow trout (*Oncorhynchus mykiss*) fed ginger additive diet. The higher final WBC count values (within the normal ranged) recorded from the *C. gariepinus* used from this study indicated that the fish were able to utilize the combination of betaine/β – glucan additive diets and the control diet (BBG0) in producing more WBC to boost its immune system. This was in agreement with the findings of Adesina (2017) who reported higher final WBC count values for *C. gariepinus* fed diets containing different inclusion levels of mechanically - extracted sunflower seed meal than the initial value. The Hb values, 12.1 – 13.5 gdL⁻¹ were comparable with the values, 8.66 – 13.33 gdL⁻¹ reported by Akinde et al. (2020) for *C. gariepinus* fingerlings fed turmeric (*Curcuma longa*) additive diet. The high ranged of Hb values recorded from this study could be attributed to large anaerobic metabolism capacity of *C. gariepinus*. The platelet count values, $27.75 \times 10^4 - 29.20 \times 10^4$ dL⁻¹ were comparable with the values, $11.00 \times 10^4 - 25.50 \times 10^4$ dL⁻¹ reported by Lawal et al. (2016) for *C. gariepinus* fed varying inclusion of *Spondias mombin* and *Morinda lucida* leaves extracts additive diets and the values, $20.40 \times 10^4 - 31.80 \times 10^4$ dL⁻¹ reported by Elezuo (2016) for

C. gariepinus Clarias juveniles fed processed almond (*Terminalia catappa*) kernel meal. The MCV values, 136.7 – 151.1 µm⁻³ were comparable with the values, 143.90 – 152.00 µm⁻³ reported by Soyinka and Bofo (2015) for *C. gariepinus*. The MCH values, 40.7 – 50.1 µg⁻¹ were higher than the values, 23.00 – 36.86.0 µg⁻¹ reported by Olasunkanmi (2011) for *C. gariepinus*. The MCHC values, 31.2 – 33.1 gdL⁻¹ were comparable to the values, 32.04 – 33.58 gdL⁻¹ reported by Elezuo (2016) for *C. gariepinus*. Normal ranged of MCHC values are 32.00 – 36.00 gdL⁻¹. High MCHC values are always associated with low blood and liver abnormalities while low levels might be as a result of little iron in the body or gastrointestinal tract tumors. The neutrophil values, 32.10 – 33.70 % were comparable with the values, 32.50 – 37.10 % reported by Nya (2009) for rainbow trout (*Oncorhynchus mykiss*) fed allicin additive diet. The lymphocytes values, 95.7 – 97.6 % were comparable with the values, 93.00 – 95.00 % reported by Agbabiaka et al. (2016) for *C. gariepinus* fed African star apple kernel diets. The monocytes values, 2.85 – 3.70 % were comparable with the values, 2.17 – 3.25 % reported by Udoh et al. (2017) for male *C. gariepinus* broodstock fed diet enriched with bitter leaf additive. Eosinophils and basophils were not recorded from the initial and final values of all the *C. gariepinus* fed diet with combination of betaine/β – glucan additive and the control diet (BBG0) used in this study. Under the conditions employed in this study, no basophils or eosinophils, nor their precursors, could be found in blood smears of the *C. gariepinus*. Basophils and eosinophils are generally lacking in fish blood (Bittencourt et al., 2003).

CONCLUSION

Findings from this study shows that inclusion of the combination of betaine/β – glucan feed additive into *C. gariepinus* fingerlings feed at varying levels applied in this study did not result in statistically significant changes (p>0.05) in the haematological responses of *C. gariepinus*. Further study may be required to assess the suitability of the combination of betaine/β – glucan and other feed additives, its correlation with the various biological impacts, and the possible implication for human consumers.

REFERENCES

Adesina, S. A. (2017). Haematological and serum biochemical profiles of *Clarias gariepinus* juveniles fed diets containing different inclusion levels of mechanically

- extracted sunflower (*Helianthus annuus*) seed meal. *Journal of Applied Tropical Agriculture*, 22 (2): 24 – 35.
- Agbabiaka, L. A., Atanda, A. O. and Nwankpa, R. (2016). Growth and haematological characteristics of African catfish (*Clarias gariepinus*, Burchell, 1822) fed African star apple kernel diets. *International Journal of Zoology Studies*, 1(3): 25 – 29.
- Akinde, A.O., Joda, O. A. and Ibidapo - Obe, E. O. (2020). Growth performance, haematology and intestinal microflora of *Clarias gariepinus* (Burchell, 1822) fingerlings fed turmeric (*Curcuma longa*) supplemented diets. *Proceedings of the 35th Annual Conference of Fisheries Society of Nigeria (FISON)*. Pp. 97 - 102.
- Akinrotimi, O. A., George, A. and Nwokoma, U. K. (2017). Haematological changes in African catfish (*Clarias gariepinus*) exposed to mixture of atrazine and metolachlor in the laboratory. *Journal of Fisheries Sciences.com*, 11(3): 48 – 54.
- Ali, M. E., Danba, E. P., Sani, T., and Danzaria, A. (2024). Evaluation of the carcass proximate composition of *Clarias gariepinus* (Burchell, 1822) fingerlings fed different levels of maca (*Lepidium meyenii*, Walp.) root powder as phyto – additive. *African Journal of Agricultural Science and Food Research (AJASFR)*, 15(1): 107 – 117.
- Anyanwu, C. N., Osuigwe, D. I., Nwaka, D. E., Anyanwu, D. C. and Chukwuotito, P. C. (2017). Evaluation of growth and body composition of *Clarias gariepinus* fingerlings fed graded levels of *Enterococcus faecium*. *Proceedings of the 32nd Annual Conference of Fisheries Society of Nigeria (FISON)*. Pp. 16 – 19.
- Ayoola, S. (2011). Haematological characteristics of *Clarias gariepinus* (Buchell, 1822) juveniles fed with poultry hatchery waste. *Iranica Journal of Energy and Environment*, 2(1): 18 – 23.
- Babale, M. Y. (2016). Growth performance at various growth stages of *Clarias gariepinus* (Burchell, 1822) fed various inclusion levels of processed water melon (*Citrullus lanatus*, mansf.) seed cake diets. Published Ph.D. Thesis, Department of Biological Sciences, Ahmadu Bello University Zaria, Zaria, Nigeria. Pp. 164.
- Bichi, K. A., Yusif, B. B., Mustapha, S. B., Jibrin, Y. U. and Thomas, P. S. (2021). Evaluation of hematological parameters in albino rats exposed dermally to tannery effluent from Challawa industrial estate Kano, Nigeria. *FUDMA Journal of Sciences (FJS)*, 5(1): 372 – 376.
- Bittencourt, N. L. R., Molinari, L. M., Scoaris, D. O., Pedroso, R. B., Nakamura, C. V., Ueda - Nakamura, T., Filho, B. A. A. and Filho, B. P. D. (2003). Haematological and biochemical values for Nile tilapia *Oreochromis niloticus* cultured in semi - intensive system. *Acta Scientiarum Biological Sciences*, 25 (2): 385 – 389.
- Elezuo, K. O. (2016). Growth performance OF *Clarias gariepinus* juveniles fed processed almond (*Terminalia catappa* L.) kernel meal. Ph.D. Thesis, Department of Aquaculture and Fisheries Management, University of Ibadan, Ibadan, Nigeria. Pp. 197.
- Inya, F. U., Okey, I. B., Ochang, S. N. and Ekpenyong, J. J. (2020). Effects of replacing soybean with beniseed, (*Sesame indicum*) seed meal on some haematological parameters of *Clarias gariepinus* juveniles. *Proceedings of the 35th Annual Conference of Fisheries Society of Nigeria (FISON)*. Pp. 75 – 80.
- Lawal, M. O., Aderolu, A. Z. and Nwobodo, T. U. (2016). Effects of stoolwood (*Alstonia boonei*), Hogplum (*Spondias mombin*) and brimstone (*Morinda lucida*) leaves extracts on growth and haematology of catfish, *Clarias gariepinus* juveniles. *Proceedings of the 31st Annual Conference of Fisheries Society of Nigeria (FISON)*. Pp. 156 – 159.
- Melefa, T. D. and Okoloye, U. (2023). Haematological and biochemical profile of *Clarias gariepinus* from Opi lake in Nsukka local government area, Enugu state. *FUDMA Journal of Sciences (FJS)*, 7(6): 391 – 395.
- Nya, E. J. (2009). Studies on dietary supplements for the control of *Aeromonas hydrophila* infection in rainbow trout (*Oncorhynchus mykiss*, Walbaum). Published Ph.D. Thesis, Centre for Marine Biodiversity and Biotechnology, Heriot Watt University, Edinburgh, UK. Pp. 179.
- Ofonime, E. A. and David, G. S. (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. *American Journal of Innovative Research and Applied Sciences*, 4(6): 213 – 222.
- Olasunkanmi, J. B. (2011). Nutrient utilization and growth performance of *Clarias gariepinus* fed differently processed *Mucuna utilis* meals as a replacement for soya bean - based diet. Published Ph.D. Thesis, Department of Wildlife and Fisheries Management University of Ibadan, Ibadan, Nigeria. Pp. 195.
- Oshode, O. A. I., Bakare, A. A., Adeogun, A. O., Efuntoye, M. O. and Sowunmi, A. A. (2008). Ecotoxicological assessment using *Clarias gariepinus* and microbial characterization of leachate from municipal solid waste landfill. *International Journal of Environmental Research*, 2(4): 391 – 400.
- Osuigwe, D. I., Obiekezie, A. I. and Onuoha, G. C. (2005). Some haematological changes in hybrid catfish (*Heterobranchus longifilis* x *Clarias gariepinus*) fed different dietary levels of raw and boiled jackbean (*Canavalia ensiformis*) seed meal. *African Journal of Biotechnology*. 4 (9): 1017 – 1021.
- Robinson, E. H., Li, M. H. and Manning, B. B. (2001). *A practical guide to nutrition, feeds and feeding of catfish*. Bulletin 1113. Mississippi Agriculture and Forestry Experiment Station, Mississippi State University, Starkville, Mississippi, U.S.A.
- Shah, S. L. and Altindag, A. (2004). Haematological parameter of tench (*Tinca tinca*) after acute and chronic exposure to lethal and sub - lethal mercury treatments. *Bulletin of Environmental Contamination Toxicology*, 13(73): 911 – 918.
- Soyinka, O. O. and Boafo, F. O. (2015). Growth performance, haematology and biochemical characteristics of *Clarias*

garipepinus (Burchell, 1822) juveniles fed quail eggshells as replacement for dicalcium phosphate. *Nigerian Journal of Fisheries and Aquaculture*, 3(1&2): 49 – 54.

Teixeira, M. A. (2000). Haematological and bio - chemical profiles of rats (*Rattus norvegicus*) kept under micro - environmental ventilation system. *Brazilian Journal of Veterinary Resources and Animal Science*, 37:50 – 55.

Terry, C., Hrubec, T. C. and Stephen, A. S. (2000). Haematology of fish. *Journal of Veterinary Clinical Pathology*, 23(174): 1120 – 1125.

Tiamiyu, A. M., Olatoye, I. O. and Adedeji, O. B. (2019). Blood indices of African catfish (*Clarias garipepinus*) following dietary administration of *Talinum triangulare*. *International Journal of Research - Granthaalayah*, 7:4: 185 – 198. ISSN - 2350-0530 (O), ISSN-2394-3629(P) DOI: <https://doi.org/10.29121/granthaalayah.v7.i4.2019.888>

Udoh, J. P., Emah, A. U., George, I. E. and Philip, A. E. (2017). Growth performance and haematological response of *Clarias garipepinus* broodstock fed diets enriched with bitter leaf meal. *AAFL Bioflux*, 10(5): 1281 – 1296.



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