



HAEMATOLOGICAL AND HISTO-BIOCHEMICAL INDICES OF AFRICAN CATFISH (*Heterobranchus bidorsalis*) FED *NIGELLA SATIVA* SEED EXTRACT

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ABSTRACT: The aim of this study was to determine the haematological and histo-biochemical indices of African catfish (*Heterobranchus bidorsalis*) juveniles fed *Nigella sativa* seed extract. The fishes were fed with a formulated diet supplemented with extract at 0.0g/kg, 1.0g/kg, 1.5g/kg, and 2.0g/kg inclusion level (representing treatment groups: T_C, T₁, T₂, and T₃, respectively) for 28 days under laboratory condition. Sampling was done on the 28th day; 15 fish (5 from each replicate) were harvested and anesthetized using clove oil; a blood sample was drawn from each group via caudal peduncle puncture using a disposable needle and syringe; later emptied into ethylene diamine tetraacetate (EDTA) bottles to prevent clotting and kept for haematological analysis. Haemoglobin (Hgb) was determined using the cyanomethemoglobin method. Red blood cells (RBC) and white blood cells (WBC) were counted by Neubauer's improved haemocytometer using Hyem's and Turk's solution as diluting fluids, respectively. The haematocrit method was used for the estimation of packed cell volume (PCV). The mean corpuscular haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), and mean cell volume (MCV) were calculated. Kidney and liver of the fish were excised, cleaned of extraneous tissues in phosphate buffer solution then immediately snap frozen in liquid nitrogen; then stored in an ultra-low freezer (-86°C) for biochemical analysis. Haemoglobin levels ranged from 11.97(g/l) to 13.12(g/l). Treatment groups T_C and T₁ showed no significant differences ($p > 0.05$) in the levels/concentrations of Hgb, RBC, WBC, and MCH; similarly, the treatment groups T₂ and T₃. Decreased histo-biochemical parameters (ALP, ALT, AST, creatinine, and urea nitrogen) levels in *H. bidorsalis* fed *Nigella sativa*-supplemented diets, when compared with those fed a basal diet, were recorded; this indicates that the treatment group (T₃-2 g/kg) was found to be most effective. This study, therefore, recommends the use of *Nigella sativa*-supplemented diets at an inclusion level of 2 g/kg in aquaculture practice.

Key words: Aquaculture, *Heterobranchus bidorsalis*, Biochemical Parameters, Haematological Indices, *Nigella sativa*.

INTRODUCTION

African catfish (*Heterobranchus bidorsalis*) is a very important aquaculture species and one of the most cultured aquaculture species in various regions in Nigeria and globally. It usually grows larger; may usually reach 64 to 150 cm (25 to 59 in); weighing up to 55 kg and taste sweeter than *Clarias gariepinus* being the largest completely freshwater fish in southern Africa (Ferraris, 2007; Froese and Pauly, 2011; Jabbi *et al.*, 2022; Enyidi *et al.*, 2024). The African catfish is highly appreciated as a good aquaculture species because of its relatively fast growth rate, resistance to disease, high fertility, the ability to tolerate a wide range of environmental parameters such as high temperature and low-level water, as well as dissolved oxygen, and can survive in a high stocking density (Jabbi *et*

al., 2022). These characteristics have determined its commercial use and made it an excellent fish species for aquaculture. Despite the wide acceptability of *H. bidorsalis*, its full potential as an aquaculture species has not yet been apprehended (Basira *et al.*, 2025). African catfish is cultured under semi-intensive polyculture system and is highly prestigious among other cultured fish species; consequently, it is highly demanded as a protein supplement in Nigeria and globally, hence, it is readily available in most local markets (Jabbi *et al.*, 2022). Plant extracts are potential alternatives to chemotherapeutic hormones and other synthetic drugs in aquaculture, as they offer useful active metabolites with innumerable benefits, such as appetite-stimulating effects, digestion enhancement, growth promotion, and immune system enhancement, among others, when properly administered (Zanuzzo *et al.*, 2015; Zahran *et al.*, 2022).

Nigella sativa, popularly known as black seed, is a medicinal plant belonging to the family Ranunculaceae. It has been used traditionally for over 2000 years in the treatment of certain diseases (Ziaee *et al.*, 2012). *N. sativa* seeds extract has been used to treat a variety of disorders affecting the cardiovascular system, digestive tract, immune system support, kidney, liver functions, respiratory system, and overall well-being (Ahmad *et al.*, 2013). *N. sativa* seeds extract has been used in aquaculture as a feed supplement since it possesses a rich bioactive profile comprising of antifungal, antimicrobial, antioxidant, hepato-protective, immune-modulatory, and nephron protective properties, which enhance production and health in fish (Abdel-Tawwab and Ahmad, 2020). Research on using *N. sativa* seed extract as a supplement in aquaculture diets has yielded promising outcomes in terms of fish body composition, feed utilisation, growth, and survival. The pharmacological properties of the *N. sativa* plant are mainly attributed to its seeds, which have several bioactive constituents such as dithymoquinone, nigellidine, thymol, and thymoquinone (Ahmad *et al.*, 2013). Thymoquinone is an active biomolecule in *N. sativa* that has been demonstrated to improve stress resilience, reduce fat deposition, and increase protein metabolism (Reverter *et al.*, 2021). Moreover, *N. sativa* was found to enhance physico-chemical properties of water significantly; an important aspect in aquaculture through the maintenance of pH, temperature, and dissolved oxygen within operational ranges, hence creating an optimal environment for culture (Rahman *et al.*, 2020). In relation to such benefits, the incorporation of *N. sativa* extract into aqua-feeds could be one of the tactical options for augmenting the production and welfare of reared fish species like *Anabas testudineus*, *Clarias gariepinus*, *Heterobranchus bidorsalis*, *Oreochromis niloticus*, *Oncorhynchus mykiss*, *Sparus aurata*, and *Cyprinus carpio* by improving their growth performance, biochemical, and immuno-haematological parameters (Altunoglu *et al.*, 2017; Kotb *et al.*, 2018).

Haematological analyses are commonly used to assess the influence of environmental factors as well as fish health under aquaculture conditions (Fazio, 2019; Witeska *et al.*, 2022). Blood indices are sensitive and fast-reacting biomarkers of various environmental impacts, including water pollution by toxic agents such as detergents and herbicides (Jabbi *et al.*, 2022). Blood parameters reflect a wide range of physiological conditions (adaptive and disruptive), and provide far-reaching information on various physiological functions as reliable biomarkers of an organism's performance. The basic haematological parameters include packed cell volume (PCV), haemoglobin concentration (Hgb), haematocrit (Ht), red blood cell (RBC) or erythrocyte count, and white blood cell (WBC) or leucocyte count. Some derived blood parameters, such as the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) can also be determined using PCV, Hgb, Ht, and RBC values with appropriate formulae. Stained blood smears can be used for quantitative evaluation of the erythrocyte and leucocyte populations to calculate the percentage of immature erythrocytes (erythroblasts), erythrocyte cellular and nuclear anomalies, differential leucocyte count (DLC), which provides the proportions of various types of leukocytes, and thrombocyte count (TC). These parameters are useful for evaluating cytotoxic and genotoxic effects, erythropoietic activity, and the status of the immune system (Witeska *et al.*, 2022).

This study was conducted to determine the effect of *N. sativa* seed extract supplementation on the haematological and histo-biochemical parameters of *H. bidorsalis* juveniles. Results obtained from this study may contribute to the expansion of eco-friendly feeding strategies to ensure the long-term development of the aquaculture industry.

MATERIALS AND METHODS

Collection and Identification of Experimental Fish

One hundred and eighty healthy *H. bidorsalis* fingerlings weighing 3.72 ± 0.05 g were obtained from the Millennium Development Goal farm at Damba-Gusau, Zamfara State, Nigeria. They were transported to the laboratory in an

open plastic container with aerated water. Identification was done using morphological characteristics of the experimental fish (ISSG, 2014).

Collection, Identification, and Preparation of *Nigella sativa* Seeds

Nigella sativa dried seeds were obtained from traditional herbalist in the central market Gusau, Zamfara State - Nigeria. These were later identified by a Taxonomist from the Herbarium, Department of Biological Sciences, Federal University, Gusau with voucher number FUGUS-BIO-HER-097, later the seeds were washed, shade-dried for 48 hours, and ground into powder with an electric blender. One hundred grams of this powder was soaked in 500 ml of aqueous solvent for 72 hours, with constant shaking (AOAC, 2019). The solution was filtered, and the filtrate was concentrated by drying at 40°C for 8 hours (Sani *et al.*, 2025). The extracted sample was stored in a refrigerator before analysis.

Experimental Diet Preparation and Analysis

Ingredients used for the formulation of the diet included: soya beans, maize, palm oil and *Nigella sativa*. Other ingredients include vitamin premix, lysine, methionine, salt and binder (Table 1). Then all the ingredients were mixed and pelleted into 2 and 4 mm diameter pellets, which were used for the first six weeks and last six weeks, respectively. The feeds were shade-dried for one week. A basal diet containing 35% crude protein was formulated and added with the crude extracts of *Nigella sativa* seeds at inclusion levels of 0.0g/kg, 1g/kg, 1.5g/kg, and 2g/kg making four experimental diets (T_C, T₁, T₂ and T₃) and kept until the feeding trial (Sani *et al.*, 2025).

Table 1: Formulation of Experimental Diets

Ingredient	Composition (%)
Soya bean meal	49.44
Fish meal	24.72
Maize	15.84
Palm oil	3.5
Lysine	2
Methionine	2
Binder	1
Salt	0.8
Vitamin premix	0.7
Total	100

Fish Culture

The experiment was conducted at the Zoology Laboratory, Department of Biological Sciences, Federal University Gusau, Nigeria from September 2024 to February, 2025. *Heterobranchius bidorsalis* fingerlings were acclimatized under laboratory conditions for two weeks in plastic holding tanks (Jabbi *et al.*, 2022). They were fed twice daily at 5% of body weight with commercial feed containing 40% crude protein during acclimatization and later were fasted for 16hr to enable gut evacuation of the feed (Basira *et al.*, 2025). The fishes were randomly distributed into four groups (15 fingerlings/group) in 12 well-aerated plastic aquaria (36 x 25 x 25 cm) in triplicates. They were fed twice daily at 5% of body weight with formulated feed containing 35% crude protein, and ratio size was adjusted on weekly basis after each sampling. The water levels and quality were maintained appropriately (Sani *et al.*, 2025). The experiment was a completely randomized design (CRD) consisting of four treatments (0.0g/kg, 1g/kg, 1.5g/kg, and 2g/kg) designated as T_C, T₁, T₂, and T₃ respectively with T_C serving as control. The trial lasted for 28 days, as described by Latif *et al.* (2021). Sampling were done weekly and aquaria water was replaced every day with well-aerated ones while used water was diluted and drained into a septic tank for proper disposal as effluent and uneaten feeds were daily siphoned before changing water (Jabbi *et al.*, 2022).

Water Quality Parameters

Some water physico-chemical parameters viz; temperature, dissolved oxygen (DO), pH, electrical conductivity (EC), and total dissolved solids (TDS), were monitored on a daily basis to ensure optimal conditions for fish culture

and to assess the effect of *N. sativa* extract on these parameters. DO was measured with an oxygen meter (Hanna model H1-9142) while, temperature, pH, EC, and TDS were measured using a digital water quality meter (model EZ-9910). (Jabbi *et al.*, 2022).

Haematological Analysis

Blood sample being a good bio-indicator to determine the health of an organism was drawn from each treatment group via caudal peduncle puncture using a disposable needle and syringe as described by Alarape *et al.* (2024) and later emptied into ethylene diamine tetraacetate (EDTA) bottles to prevent clotting and kept for haematological analysis. Haemoglobin (Hgb) was estimated using cyanmethemoglobin method. Red blood cells (RBC) and white blood cells (WBC) were counted by Naubauer's improved haemocytometer using Hyem's and Turk's solution as diluting fluids, respectively. The haematocrit method as described by Joshi *et al.* (2002) was used to estimate packed cell volume (PCV). A standard formula described by Soyinka and Bofo (2015) and Alarape *et al.* (2024) in equations (i) to (iii) was used to determine the mean corpuscular haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), and mean cell volume (MCV).

$$\text{MCH (pg)} = \frac{\text{Hb (g/dl)} \times 10}{\text{RBC}(10^6/\mu\text{l})} \dots \dots \dots (i)$$

$$\text{MCHC (\%)} = \frac{\text{Hb (g/dl)} \times 100}{\text{PCV}} \dots \dots \dots (ii)$$

$$\text{MCV (fl)} = \frac{\text{PCV} \times 10}{\text{RBC} (10^6/\mu\text{l})} \dots \dots \dots (iii)$$

Determination of Kidney and Liver Histo-Biochemical Parameters

Sampling was done in the fourth week (i.e., on the 28th day). During the sampling, 15 fish (5 from each replicate) were harvested using a hand net. The fish were anesthetized using clove oil, and their length-weight was recorded before their dissection. They were sacrificed, and later, the kidneys and livers of the fish were excised, cleaned of extraneous tissues in phosphate buffer solution, then immediately snap frozen in liquid nitrogen, and then stored in an ultra-low freezer (-86°C) until further analysis.

Tissues homogenates were prepared in 0.1M sodium phosphate buffer (pH 7.4) using microbiome tissue homogenizer (Omni TH - SKU:TH115). A portion of tissues homogenate was stored at -20°C , which was used for assessing lipid peroxidation and remaining portion of homogenates were centrifuged at 4°C / 13,500rpm for 30 min using Fisher Scientific centrifuge (Marathon 3000R Refrigerated) to obtain post mitochondrial supernatant (PMS). The PMS was stored at -20°C in clean labelled eppendorf until further biochemical assays.

Liver and kidney tissues homogenates were used for Biochemical estimates of the hepatic-nephric enzymes levels viz; alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate aminotransferase (AST), creatinine, and urea nitrogen were determined using standard methods (Fawcette and Scott, 1960; Folin and Wu, 1920). The Randox colorimetric kits (UK) were used for performing all these biochemical analysis and optical density was noted using UV-visible spectrophotometer (Shimadzu UV-1700) following manufacturer instructions.

Statistical Analysis

Growth performance, feed utilization and water quality parameters were subjected to one-way analysis of variance (ANOVA) using the Statistical Analysis System (University Edition). Means were separated using the New Duncan's Multiple Range Test at 5% level of probability.

RESULTS AND DISCUSSION

Water Quality Parameters

The results of this study showed that the mean temperature of water is within the range of 26.15°C and 22.42°C, which showed no significant difference ($p>0.05$) between diet groups. Electrical Conductivity ranged from 30.92 μ S/cm to 33.21 μ S/cm. Mean dissolved oxygen varied from 3.49mg/l to 3.81mg/l, EC and DO values reflect that there is no significant difference ($p>0.05$) between diet groups T_C and T_3 while these diet groups showed significant difference ($p>0.05$). Total Dissolved Solids ranged between 22.65mg/l and 25.12mg/l: diet group T_C showed significant difference ($p>0.05$) with other groups. pH values ranged from 6.85 to 7.00, this revealed no significant difference diet groups (Table 1). All the water quality parameters measured in this study were within the permissible limits recommended for aquaculture practices including pisciculture as reported by Boyd (1983) and Viveen *et al.* (1985).

Table 1: Water Quality Parameters under Different *Nigella sativa* Treatment Levels

Parameter	Treatment Group (Dietary <i>Nigella sativa</i> Inclusion Levels)			
	T_C (0g/kg)	T_1 (1g/kg)	T_2 (1.5g/kg)	T_3 (2g/kg)
Temperature (°C)	26.15 $\pm$ 0.11 ^a	26.42 $\pm$ 0.31 ^a	26.39 $\pm$ 0.43 ^a	26.32 $\pm$ 0.05 ^a
Electrical Conductivity (μ S/cm)	33.21 $\pm$ 0.25 ^a	31.19 $\pm$ 0.76 ^{ab}	32.87 $\pm$ 0.29 ^a	30.92 $\pm$ 0.96 ^{ab}
Dissolved Oxygen (mg/l)	3.81 $\pm$ 0.23 ^a	3.53 $\pm$ 0.53 ^{ab}	3.75 $\pm$ 0.12 ^a	3.49 $\pm$ 0.22 ^{ab}
Total Dissolved Solids (mg/l)	25.12 $\pm$ 1.52 ^a	22.85 $\pm$ 0.10 ^{ab}	22.65 $\pm$ 0.11 ^{ab}	23.03 $\pm$ 0.02 ^{ab}
pH	7.00 $\pm$ 0.10 ^a	6.98 $\pm$ 0.33 ^a	6.85 $\pm$ 1.07 ^a	6.96 $\pm$ 0.21 ^a

Note: ^{abc}Means $\pm$ S.E. with the same superscript across the same row are not significantly different ($p>0.05$)

Haematological Parameters of *Heterobranchus bidorsalis* Juveniles

Haematological parameters estimated are presented in Table 2. Haemoglobin levels ranged from 11.97(g/l) to 13.12(g/l). Treatment groups T_C and T_1 showed no significant differences ($p>0.05$) in the levels/concentrations of Hgb, RBC, WBC, and MCH; similar to the treatment groups T_2 and T_3 . Treatment groups T_C and T_1 differed significantly from treatment groups T_2 and T_3 in terms of concentrations of Hgb, RBC, WBC, and MCH. RBC concentrations range from 18.55(10^{12} /l) to 24.18(10^{12} /l), that of WBC range from 3.95(10^9 /l) to 5.11(10^9 /l). PCV concentrations range from 32.97% to 39.27%. MCH range from 50.21(10^{-12} g/cell) to 82.30(10^{-12} g/cell), while MCHC range from 29.89(g/l) to 39.00(g/l) (Table 2). MCV values shows significant differences ($p>0.05$) between all the treatment groups; with highest values (209.41(10^{-15} l/cell)) recorded in treatment group T_3 and lowest (158.45(10^{-15} l/cell)) recorded in treatment group T_1 . Haematological parameters are sensitive and reliable indicators of environmental impacts on fish and other aquatic animals, including those from toxic agents. They may show either the negative effects of toxicity, such as anaemia and immunosuppression, or compensatory effects, such as an increase in blood oxygen transport capacity or inflammation. (Witeska *et al.*, 2022).

Table 2: Haematological Indices of the *Heterobranchus bidorsalis* Juveniles under Different *Nigella sativa* Treatment Levels

Treatments	T_C	T_1	T_2	T_3
Hgb (g/l)	13.12 $\pm$ 0.18 ^a	13.56 $\pm$ 0.01 ^a	12.25 $\pm$ 0.21 ^b	11.97 $\pm$ 0.85 ^b
RBC (10^{12} /l)	24.18 $\pm$ 1.21 ^a	23.41 $\pm$ 0.42 ^a	19.62 $\pm$ 3.01 ^b	18.55 $\pm$ 2.67 ^b
WBC (10^9 /l)	3.95 $\pm$ 0.15 ^a	4.08 $\pm$ 0.91 ^a	4.87 $\pm$ 0.36 ^b	5.11 $\pm$ 0.04 ^b
PCV (%)	39.27 $\pm$ 0.32 ^c	33.67 $\pm$ 0.91 ^b	30.53 $\pm$ 0.83 ^b	32.97 $\pm$ 0.16 ^a
MCH (10^{-12} g/cell)	52.03 $\pm$ 0.12 ^b	50.21 $\pm$ 1.22 ^b	78.93 $\pm$ 0.92 ^a	82.30 $\pm$ 1.07 ^a
MCHC (g/l)	30.90 $\pm$ 0.11 ^c	29.89 $\pm$ 0.94 ^c	34.69 $\pm$ 0.21 ^b	39.00 $\pm$ 0.28 ^a
MCV (10^{-15} l/cell)	167.35 $\pm$ 1.07 ^a	158.45 $\pm$ 1.04 ^a	189.44 $\pm$ 1.26 ^b	209.41 $\pm$ 1.51 ^c

Note: ^{abc}Means $\pm$ S.E. values with the same superscript across the same row were not significantly different ($p>0.05$)

It is crucial to note that haematological parameters are indispensable tools that are used as indicators for monitoring physiological status and changes in aquaculture (Ogueji *et al.*, 2017). These parameters provide reliable information on metabolic disorders, stress and health status before and after clinical examination of specimens (Bahmani *et al.*, 2001). Generally, red cell indices are central for the diagnosis of anaemia in most animals, including fish (Cole, 1986). Alterations in the red blood cell indices (MCV, MCH, MHCH) may indicate macrocytic anaemia (Dacie and Lewis, 2011; Iheanacho *et al.* 2017).

Kidney and Liver Histo-Biochemical Parameters

The results obtained showed decreased histo-biochemical parameters (ALP, ALT, AST, creatinine, and urea nitrogen) levels in *H. bidorsalis* fed *Nigella sativa*-supplemented diets when compared with those fed a basal diet (Table 3). Furthermore, the treatment group- T₃ (2g/kg) recorded the highest decrease in the histo-biochemical parameters, while treatment group- T₁ (1g/kg) recorded the least; this indicates that treatment group- T₃ (2g/kg) was found to be the most effective in boosting the pisciculture productivity. The decreased trend in all the biochemical parameters across the treatments in comparison with control is in line with the findings of Latif *et al.* (2021).

Table 3: Effects of *Nigella sativa* Seeds Extract on the Liver and Kidney Histo-Biochemical Parameters in *Heterobranchus bidorsalis* Juveniles

Treatment Group	Key-Functioning Enzymes				
	ALP (U/l)	ALT (U/l)	AST (U/l)	Creatinine (mg/dl)	Urea (mg/dl)
T _C (0g/kg)	531.2±0.53 ^a	27.25±0.13 ^a	29.13±0.23 ^a	0.154±0.81 ^a	3.151±1.54 ^a
T ₁ (1g/kg)	495.3±0.25 ^a	26.51±0.34 ^a	28.11±0.79 ^a	0.149±0.28 ^a	3.010±0.28 ^b
T ₂ (1.5g/kg)	432.1±0.36 ^b	26.13±0.29 ^a	23.27±0.83 ^b	0.109±0.74 ^a	2.983±0.84 ^{bc}
T ₃ (2g/kg)	409.5±0.23 ^b	25.64±0.78 ^a	22.43±0.96 ^b	0.093±0.91 ^a	2.541±0.31 ^c

Note: ^{abc} Means ± S.E. values with the same superscript across the same column were not significantly different (p>0.05)

CONCLUSION

This study indicated that the use of *Nigella sativa*-supplemented diets in aquaculture to boost productivity is encouraging. Thus, the diets of *Heterobranchus bidorsalis* Juveniles can be supplemented with up to 2 g/kg of *Nigella sativa* without adverse effect on their health and wellbeing as indicated by the haematology and Liver and Kidney Histo-Biochemical Parameters.

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