

EFFECT OF GINGER (*Zingiver officinale*) and GARLIC (*Allium sativum*) SUPPLEMENTED DIET ON SOME HEAMATOLOGICAL INDICES OF *Clariasgariepinus* BURCHELL, 1822

Chiroma Yushau*

*Department of Fisheries and Aquaculture, Federal University Dutse, Jigawa State, Nigeria

Corresponding Author: Chiroma Yushau*, Department of Fisheries and Aquaculture, Federal University Dutse, Jigawa State, Nigeria

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Abstract

Nutrition is the main factor determining the potential of farmed fish to exhibit their growth capacity as a function of the protein content and certain additives contained in the diet. Thus, the present study was conducted to access dietary supplementation effect of Ginger and Garlic as feed additives of blood heamatological analysis for *Clarias gariepinus* fingerlings. In this experiment, following a week of acclimatization 180 fingerlings were randomly divided into triplicate in four treatment containing fifteen fingerlings each and fed for 56 days. In treatment 1(T1) fingerlings are fed with control basal diet, treatment 2(T2) are fed with basal diet containing 1% garlic and treatment 3(T3) are fed with basal diet containing 1% Ginger while treatment 4(T4) are fed with basal diet containing 1% mixture of Ginger and Garlic. The main water quality parameters were recorded on weekly basis. After the feeding trials the blood samples were analysed using NORTEK GENESIS MODEL HA6000 automatic haematology analyzer at laboratory department of federal medical center birnin kudu. Fish fed with basal diet containing 1% Ginger (T3) recorded the best heamatological result in terms of Red blood cells (RBC), white blood cells (WBC), Hemoglobin concentrations (HGB), monocytes, MVC, HCT, MVC and lymphocytes. In conclusion *Clarias gariepinus* fingerlings fed with 1% dietary inclusions level of Ginger has had a better heamatological analysis which has been attributed to its physiological, pharmacological properties and its nutritional effects.

Keywords: Ginger, Garlic, Supplementary Diet, Hematological Indices, *Clarias gariepinus*.

Introduction

Aquaculture has been introduced in sub-Saharan Africa, particularly in Nigeria since the 1960s, on the one hand to minimize the pressure exerted by fishing communities on wild fish stocks and on the other hand to ensure permanent availability of fish for a steadily increasing population. Despite this, aquaculture production remains very low to cope with increased market demand of fish, resulting in massive imports to meet domestic demand. Local importers must therefore invest about millions of naira. This represents huge financial losses and therefore an imbalance in the country's economy (Kouam et al., 2008). To solve this situation, local production of available fish species should be encouraged meeting the strong market demand for fish

and fish products. Among the endogenous species with high yield potential in Nigeria, there is *Clarias gariepinus*, which is a popular choice for local aquaculture; certainly because of its fast growth rate, omnivorous eating habits, the ability to feed on natural and complementary feeds, disease resistance, tolerance to low levels of

oxygen and to overpopulation as well as pH fluctuations (Beinga et al., 2016). However, one of the main constraints to the development of aquaculture production is lack of availability of fish seeds both quantitatively and qualitatively, as well as high quality, low-cost feeds (FAO, 2006). Seeds

production especially rearing of larvae and fry are the most sensitive steps in aquaculture production.

Aims and Objectives of the Study

The main aim of the research was to assess the effect of Ginger (*Zingiber officinale*) and Garlic (*Allium sativum*) supplemented diet on some Hematological parameters in juveniles *Clarias gariepinus* while the specific objectives were: Evaluate the effect of inclusion of Garlic and Ginger for Hematological parameters in juvenile *C. gariepinus* and to monitor some water quality parameters.

2. Methodology

The experiment was carried out indoors in plastics containers in hatchery units at Department of Fisheries Aquaculture, Faculty of Agriculture, Federal University Dutse, Jigawa State, Nigeria. Located on latitudes 11° 70' North and longitude 9° 33' East and altitude of 431m above sea level (Elevation-map, 2019).

Fish Diet Formulation and Processing

Four (4) different diets was formulated using Pearson's square method of fish feed formulation to contain 40% crude protein. Ginger (*Z. officinale*) and garlic (*A. sativum*) in powder form which was added to the formulated fish diets at 0% (T0) as control diet, each treatment will contains 1% of either garlic, ginger or mixture of garlic and ginger. Feed ingredients used for the experimental diet include: Ginger, garlic, fish meal, soybean meal, maize meal, wheat bran, Methionine, Lysine, premix, bone meal and palm oil. The ingredients were grinded, measured and thoroughly mixed in bowl; palm oil was added and mixed manually to achieve a proper consistency. The resulting mixture was pelletized using pelletizer. The wet pellets were sun-dry, packaged into airtight containers and store at room temperature to be crumble before being used as a diet.

Experimental Design

One hundred and eighty fish (180) of African catfish fingerlings of average weight (weight: 5.32 ± 1.06 mg and length: 7.98 ± 0.9 cm) were purchase from Reputable Fish Farm, Ladane fish farm Kano and transported to the hatchery complex in 50L Jerry can. The fish was allowed to acclimatize for 7 days before the commencement of the experiment (Okoye and Sule, 2001).

The 12 units of plastics container were used for the study, which was randomly allocated to four different treatment diets (T0, T1, T2, T3) in duplicate of three and fish will be randomly distributed into the 12 plastics container at density

of fifteen (15) fingerlings per container and subjected to four feeding treatments. In Treatment 1 or control (T0), the fingerlings was fed with a normal control feed, while in Treatments 2 are fed with 1% Garlic inclusion, Treatment 3 with 1% ginger inclusion and Treatment 4 with 1% mixture of both Ginger and Garlic inclusion.

Water Quality Management

Physico-chemical parameters of the water in each container such as temperature (T°C) was measured using digital thermometer; transparency, using a Secchi disk, Dissolved Oxygen (D.O), using JBL Test Kits, pH. Water physico-chemical parameters will be recorded weekly before feeding.

Blood Collection

A scoop net, was used randomly to remove individual fish from the holding container and sedated using anesthetic solution (containing 2.5 ml of diluent solutions in 5 liter of water), for 4-6 min before the blood collection procedure. Complete sedation as confirm when a fish no longer responds to external stimuli, yet slight gill movements were still observed.

Blood collection was accomplished by removing completely sedated fish with the aid of a net from the anesthetic solution and place on a clean towel on its side. Using the towel, the fish was carefully lifted to a comfortable handling position with one hand. The insertion of the needle into the fish was at a perpendicular angle to the ventral surface (at approximately 90°), until some blood entered into the syringe or when the needle made contact with the vertebral column (hard impenetrable surface). Once the needle touches the vertebral column, it was withdrawn slightly, so that the blood vessel overlying the vertebral column could be sample easily and rapidly. This was done by gently pulling on the plunger by maintaining consistent pressure until the desired quantity of blood is drawn into the syringe. The collected blood sample was slowly but quickly transfers into collection tubes containing 0.2ml of anticoagulant. The sample fish was transfer to a recovery tank, containing clean, aerated water (Patty m. et al., 1993) Haematological Analysis

Fish sample was taken to the Department of Haematology, Federal Medical Center Birnin kudu, for haematological analysis. The samples were analyzed for haematological parameters. Haemoglobin (Hb), White blood cells (WBC), Red blood cells (RBC) and Packed cell volume (PCV) using the methods of Roberts, (1978), Mgbenka et al. (2003) and Shah and Altindag, (2004). Mean corpuscular volume

(MCV), Mean corpuscular haemoglobin concentration (MCHC) and Mean corpuscular haemoglobin (MCH) etc

Mean cell haemoglobin concentration (MCHC)

$$MCHC(\%) = Hb \times 100$$

Mean cell haemoglobin (MCH)

$$MCH(Pg) = Hb \times 10$$

Mean cell volume (MCV)

$$MCV(fl) = PCV \times 10$$

Data Analysis

Data on Hematological analysis were subjected to one way analysis of variance (ANOVA) to test for significant difference in the mean while means was separated using Duncan's multiple range test (Duncan's laws). Analysis was performed using the SPSS (statistical package for social science) version 23, significant level was $p < 0.05$, values were expressed as mean \pm SD.

3. Results And Discussion

4.1 Result of Hematological Analysis

Table 1 shows the hematological indices of the fish fed with supplemented Ginger and Garlic based diet. The LYM values are significantly different across all the treatments with T4 recording the highest value (94.4 ± 0.10) and T1 recording the lowest value (84.90 ± 0.10). MON is not significantly different across the treatments except T4 with different values. T2 recorded the highest value (1.10 ± 0.00) and T4 records the lowest values (0.30 ± 0.10). NEU T2 and T1 are significantly different while T3, T4 are not. T2 recorded the highest value (7.70 ± 0.10) and T1 recording the lowest value ($1.70 \pm 0.00a$). EOS all treatments are not

significantly different with T1 recording the highest value (12.40 ± 0.00) and T2 with lowest value (0.30 ± 0.10). BAS T2 is significantly different while T1, T2 and T3 are not. T2 recorded the highest value (0.73 ± 0.06) and T1 having the lowest value (0.30 ± 0.00). ALY is significantly different across treatments with T1 recording the highest value (0.73 ± 0.06) and T3 recording the lowest value (0.30 ± 0.00). IMM T1 and T3 are not significantly different, T2 and T4 are significantly different with T2 recording the highest value (0.80 ± 0.10) and T2, T1 recording the lowest value. RBC T4 and T1 are not significantly different T3 and T1 are

was determined using the method describe by Blaxhall and Daisely (1973).

significantly different. T3 recorded the highest value (1.92 ± 0.00) and T4 records the lowest value (0.68 ± 0.01). HGB values are significantly different across all treatments with T3 recording the highest value (7.70 ± 0.10) and T4 recording the lowest value. HCT value are significantly different across all treatments T3 recording the highest value (25.00 ± 0.10) and T4 recording the lowest value (7.40 ± 0.10). MCV values are not significantly different across the treatments with T3 recording the highest value (130.30 ± 0.00) and T4 recording the lowest value (109.10 ± 0.00).

MCH-T2&T3 are not significantly different but significantly different with T1 and T4. T1 recorded the higher value (50.00 ± 0.00) and T3 recorded the lowest value (40.10 ± 0.00). MCHC are significantly different across the treatments with T4 recording the highest value (41.37 ± 0.46) and T3 recording the lowest value (30.90 ± 0.10). RDW -CV value are significantly different with T2 recording the highest value (18.70 ± 0.00) and T3 recording the lowest value (13.10 ± 0.00). RDW-SD is not significantly different across the treatments with

T2 recording the highest value (105.00 ± 0.00) and (64.60 ± 0.00).

Table 1: Result of Hematological Analysis. T3 recording the lowest value

T2 recording the highest value T3 th lowest
(105.00 ± 0.00) and recording value
(64.60 ± 0.00). g

Table 1: Result of Hematological Analysis.

| Parameter | T1 | T2 | T3 | T4 |
|-------------------------|-------------------|-------------------|-------------------|-------------------|
| LYM($\times 10^3/uL$) | 84.90 \pm 0.10d | 89.00 \pm 0.10c | 90.70 \pm 0.10b | 94.40 \pm 0.10a |
| MON($\times 10^3/uL$) | 0.60 \pm 0.10c | 0.50 \pm 0.10b | 1.10 \pm 0.00a | 0.30 \pm 0.10d |
| NEU($\times 10^3/uL$) | 1.70 \pm 0.00d | 7.70 \pm 0.10a | 2.20 \pm 0.00c | 2.30 \pm 0.10b |
| EOS($\times 10^3/uL$) | 12.40 \pm 0.00a | 2.1 \pm 0.00d | 5.50 \pm 0.00b | 2.50 \pm 0.00c |
| BAS($\times 10^3/uL$) | 0.40 \pm 0.00c | 0.50 \pm 0.00d | 0.50 \pm 0.00b | 0.50 \pm 0.00b |

| | | | |
|----------------------------------|---------------------|----------------|-----------------|
| ALY($\times 10^3/\mu\text{L}$) | 0.73 \pm 0.06a | 0.30 \pm 0.0 | 0.50 \pm 0.00 |
| | 0.40 \pm 0.10c | 0d | b |
| IMM($\times 10^3/\mu\text{L}$) | 0.10 \pm 0.00c | 0.10 \pm 0.0 | 0.50 \pm 0.00 |
| | 0.80 \pm 0.10a | 0c | b |
| RBC($\times 10^6/\mu\text{L}$) | 0.92 \pm 0.01c | 1.92 \pm 0.0 | 0.68 \pm 0.01 |
| | 1.31 \pm 0.00b | HGB(g/dL)0a | d |
| | 4.60 \pm 0.10c | | |
| | 5.30 \pm 0.10b | HCT(%) | 7.70 \pm 0.1 |
| | 11.60 \pm 0.00c | 0a | 3.10 \pm 0.00 |
| | 14.60 \pm 0.00b | 25.00 \pm 0. | 7.40 \pm 0.10 |
| MCV(fL) | 126.40 \pm 0.00 | b | d |
| | 111.30 \pm 0.00 c | 130.30 \pm | 109.10 \pm 0. |
| | | 0.00 a | 00 d |

Note: Average values on the same row carrying similar superscripts are not significantly different ($p>0.05$).

Keys:

LYM=Lymphocytes,
 MON=Monocytes, NEU=Neutrophils, EOS=Eosinophils, B
 AS=Basophils, ALY=Alymphoplasia,
 IMM=Immunoglobulins, PCV=Packed cell volume;
 WBC=White blood cell, RBC=Red blood cell;
 HGB=Haemoglobin, HCT=Heamatocrite, MCHC=Mean
 corpuscular haemoglobin concentration, MCH=Mean
 corpuscular haemoglobin, MCV=Mean corpuscular
 volume, RDW-SD=Red cells distribution width(co-efficient
 of variation), RDW-SD=Red cell distribution
 width(Standerd deviation).

4.3 Water Quality Parameters

The result of water quality parameters examined are within the recommended range for fish wellbeing, and thus the water is in good condition as shown in table 2 below

Table 2: Water quality parameters examined

| | T1 | T2 | T3 | T4 |
|------------------------|------------------|------------------|------------------|------------------|
| Temperatu re(oc) | 31.71 \pm 1.03 | 28.18 \pm 0.91 | 30.88 \pm 1.25 | 30.38 \pm 0.17 |
| Dissolve oxygen(m g/l) | 6.28 \pm 0.95 | 5.25 \pm 0.96 | 5.24 \pm 0.54 | 5.48 \pm 0.42 |
| pH | 7.76 \pm 0.23 | 6.42 \pm 0.36 | 6.86 \pm 0.25 | 6.26 \pm 0.82 |

4.2 Discussion

Heamatological analysis of peripheral blood is an inexpensive and very useful tool for evaluating fish physiological status and health Blaxhall (1972). Some of the Heamatological parameters are very sensitive to the changes of environmental factors and provide information about physiological disturbances before the development of their external symptoms. All preanalytic and analytic factors may affect the results, therefore experience and care is necessary to obtain reliable Heamatological data.

Heamatological parameters are routinely used for the evaluation of physiological environment and husbandry stressors in fishes (Rainza-Paiva et al., 2000). In recent years, good management practices have been advocated as effective ways of reducing stress in fish culture (Gabriel et al., 2007). The change in the blood characteristics of *C. gariepinus* caused by stress due to exposure to environmental pollutants, diseases or by pathogens have been studied by a number of workers especially in capture fisheries (Onusiriku and Ufodike, 2000; Ezeri, 2001; Gabriel et al., 2001).

Heamatological components of blood are also valuable in monitoring feed toxicity especially with feed constituents that affect the formation of blood in culture fisheries (Oyawoye and Ogunkunle, 1998).

The physiological state resulting from inclusion of *Zingiber officinale* and *Allium sativum* in the diets was clearly reflected by significant differences ($p>0.05$) in the red blood cell count (RBC) and packed cell volume (PCV) observed in this study. This is in line with the observation of Obasa et al. (2013), feeding the African catfish with fermented African breadfruit seed meal based diets. RBC range ($1.82 \times 10^{12} - 1.98 \times 10^{12}$) observed in this study is fairly comparable with ($1.70 \times 10^{12} - 4.00 \times 10^{12}$) Bhasker and Rao (1990) and (2.24×10^{12}

- 2.49×10^{12}) Sotolu and Fatureti (2009). However, the increase in RBC may be ascribed to the higher concentration of anti metabolite in the diets containing more of *z. Offinale* than *A.sativum*.

Haemoglobin (HGB) range (3.8- 7.7g/100ml) compared well with (8.70g/100ml) for *Clarias gariepinus* (Sowunmi,

2003). These values were also within the range (4.46g/100ml) reported for *Heterotis niloticus* (Fagbenro et al., 2000). The optimum concentration in the HB concentration could imply that diets having higher substitutions contained high quality protein therefore resulting to transportation of oxygen from the respiratory organs to the peripheral tissue (Robert et al., 2000).

The significant difference in the WBC values among the treatments probably signified that inclusion of *A. sativum* and *Z. officinale* even at high level of inclusion in diet was not toxic to *Clarias gariepinus*, and did not have any influence on its immune status. Allen (1994) observed increased WBC (leucocytes) counts in *Oreochromis aureus*. Maheswaran et al. (2008) also observed increase in WBC when *Clarias batrachus* were exposed to mercuric chloride.

Also, Bhatt and Farswan (1992) also observed that Red blood Cell, Total White Blood Cell, Haemoglobin (Hb), packed cell volume (PCV) decreases with exposure of *Barilius bendalensis* (Ham) to plant toxicants.

The mean corpuscular volume (MCV) range (109.05 to 126.00 fl) recorded in this experiment was higher than 79.20 to 105.32 fl reported for *Heteroclaris* (Anyanwu et al., 2011), meanwhile the mean corpuscular haemoglobin concentration (MCHC) range (30.60 to 41.10%) recorded in this study for fish fed *A. sativum* and *Z. officinale* based diets is better compared with (30.70%) reported for *C. gariepinus* from Asejire dam (Adedeji and Adegbile 2011).

The mean corpuscular haemoglobin (MCH) results showed that the fish fed control diet recorded the highest values for both stages of the experiment. The MCH range (40.80 - 50.05 pg) obtained in this study was high than the range (20.82 to 26.60 pg) reported for *Heteroclaris* fed *Carica papaya* leaf meal incorporated feed (Anyanwu et al., 2011). RDW- CV (13.0-18.70) and RDW-SD (64.0-104.40) are all high compared to 55 reported for *Heteroclaris* fed with *Carica papaya* leaf meal (Anyanwu et al., 2011).

4. Conclusion And Recommendations

Conclusion

The assessment of the blood parameter of the experimental catfish used in this study was carried out using NORTEK GENESIS MODEL HA6000 automatic haematology analyzer. With regards to the automated blood cell count result obtained, catfish treatment T2 presented the highest result.

From the four treatment applied both *Allium sativum* and *Zingiber officinale* has no negative health effect on the

haematological parameter of the experimental fish fed the experimental diet, in terms of the count and concentration of Red blood cell (RBC), White Blood Cell (WBC), Packed cell volume (PCV) and Haemoglobin (HGB). Finally in conclusion *Clarias gariepinus* juveniles fed with 1% inclusion level of Ginger has had a better haematological analysis which is attributed to anti-oxidant, anti-inflammation and attractants properties.

Recommendation

From the findings of this study the following recommendations were made:

1. The Ginger and garlic should be included in the diet of *Clarias gariepinus* at the range of 1-3% to increase disease fighting mechanisms of the fish.
2. Both Ginger and garlic are recommended to serve as attractant, anti-oxidant and anti-inflammatory to the fish diet.

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