**EFFECT OF PROCESSED SELECTED MEDICINAL PLANTSDIETS ON HAEMATOLOGICAL PARAMETERS OF *CLARIAS GARIEPINUS* (BURCHELL, 1822)**

**By**

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**ABSTRACT**

An experiment was conducted to test the effect of some processed medicinal plants on haematological parameters of *Clarias gariepinus.* The plants were processed with aqueous ethanol and hexane solvent. Thirteen diets with 3% and 5% inclusion level of the processed plants were formulated and diet with zero medicinal plant serving as a control. Water quality parameters measured were within the recommended standard range. The results of the tested diets were within the haematological recommended range. There were significant difference (P>0.05) among the tested diets. There were increase in RBC count 3% EAQ (4.00±0.20×106/l), (4.10±0.10×106/l) and 5% MHX (4.00±0.10×106/l) based diets. The RBC count of all the tested diets were observed to be significantly higher than control diet (2.40±0.20×106/l). There were varying increase in White Blood Cell (WBC) Count among the experimental based diets while 3% EET (10.30±0.30×109/l), 3% MAQ (10.60±0.20×109/l) and 5% EET (10.30±0.10×109/l) were least significant (P>0.05) among the tested processed Medicinal plants diets. There were significant difference (P>0.05) among haemoglobin (Hb), MCV and MCH while no significant difference were observed in PCV, MCHC counts. The tested shows haematological significant contribution to fish survival due to high immunity and resistance against anaemic condition. 3% and 5% inclusion levels of medicinal processed diet both have similar haematological positive impact on *Clarias gariepinus* but 3% is recommended for use due to the growth performance of the fish measured during the study.

**Keywords:** Haematology; Aqueous; Ethanol; Hexane; and *Clarias gariepinus*

**INTRODUCTION**

Aquaculture production in Africa has been on a steady increase, growing more rapidly in Sub-Saharan African countries than the rest of Africa countries on the continent (FAO, 2012). According to Halwart (2020), the contribution of Africa to the global production of aquaculture in 2018 was estimated at 21,296 metric tons representing an insignificant 2.67% and was mainly dominated by freshwater finfish production. Nigeria population as at July, 2021was estimated to be 211,400,708 and the highest demand for fish in Africa and has also been identified to have been the largest producer of African catfish (Adeleke *et al*., 2021).

However, during the past few years, aquaculture has encountered repeated problems of diseases emanated from feed due to poor availability of suitable constituent, lack of adequate knowledge on feed preparation, poor formulation and processing of ingredients and lack of knowledge and understanding of dietary requirement of targeted fish species (Fagbenro *et al*., 2013). The best way to curtail the challenges of fish losses in this regard is to improve their resistance to infections in addition to improving husbandry with good health management (Anjusha *et* *al*., 2019). Disease is a considerable constraint in aquaculture expansion, production and development. Recent research has been demonstrating the positive effect of medicinal plants incorporation with aqua-feed formulation in fish culture (Anjusha *et al*., 2019).

Haematology investigation serves mainly for diagnostic purpose. It can be used to appraise suitability of feeds and examine the stress situation of fish (Fagbenro *et al*., 2013). It is one of the diagnostic tools for identifying diseases. Normal variation from intrinsic or extrinsic factors or diseases affecting blood cells and counts may be evaluated by clinical haematology (Grant, 2015). Most of the fish have nucleated erythrocytes which play an important role in oxygen transport, which depends on the amount of haemoglobin concentration within the cell and the gas exchange mechanism (Fauci, 1993; Anjusha *et al*., 2019).

**MATERIALS AND METHODS**

Feeding trial was conducted at the indoor hatchery of the farm complex of the Department of Fisheries and Aquaculture, Bayero University Kano, Nigeria. Latitude 11.978422°N and longitude 8.424395°E. *Eucalyptus globulus* and *Moringa oleifera* leaf meals were subjected to Aqueous, Ethanol and Hexane processing methods. Each was measured 250g into 1000 ml labelled bottle using Ohaus sensitive weighing balance (PA313). The meals were soaked with 500 ml of the aforementioned solvents (Suleiman *et* *al*., 2018) in a tightly covered bottles s and agitated at interval of 8 hours for a period of 72 hours and filtered using muslin (Ezearigo *et* *al*., 2014). The filtrate was evaporated to dryness under pressure at 45°C using rotary evaporator (RE300). The extracts were labelled as Eucalyptus Aqueous (EAQ), Eucalyptus Ethanol (EET), Eucalyptus Hexane (EHX), Moringa Aqueous (MAQ), Moringa Ethanol (MET) and Moringa Hexane (MHX) respectively. The extracts were preserved at -4°C until further usage (Chakraborty *et al*., 2018).

**Diet Formulation**

Thirteen diets (40% Crude Protein) were formulated for the experiment. It was done based on the determination of proximate compositions of the ingredients using AOAC (2010) with incorporation of 3% and 5% inclusion levels of processed plants (Table 1a and 1b). The extract were dissolved in 350 ml of warm water which was used to form a uniform dough-like paste. The feeds were pelleted to 2 mm size using improvised manual pelletiser and dried at 26°C for 72 hours (Ochang *et al*., 2015; Jiomh *et al*., 2022).

**Table 1a: Feed Formulation Showing Inclusion Levels of Processed Medicinal Plants (Eucalyptus and Moringa) Diets**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Ingredients** | **D1****ZMD** | **D2****(3%EAQ)** | **D3****(3%EET)** | **D4****(3%EHX)** | **`D5****(3%MAQ)** | **D6****(3%MET)** | **D7****(3%MHX)** |
| **FM (61.45%)** | 25.71 | 24.90 | 24.90 | 24.90 | 24.90 | 24.90 | 24.90 |
| **SBM (40.05%)** | 51.42 | 49.80 | 49.80 | 49.80 | 49.80 | 49.80 | 49.80 |
| **MM (9%)** | 17.87 | 17.30 | 17.30 | 17.30 | 17.30 | 17.30 | 17.30 |
| **Moringa** | 0.00 | 0.00 | 0.00 | 0.00 | 3.00 | 3.00 | 3.00 |
| **Eucalyptus** | 0.00 | 3.00 | 3.00 | 3.00 | 0.00 | 0.00 | 0.00 |
| **Oil** | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 |
| **Lysine** | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| **Methionine** | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| **Bone Meal** | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| **VMP** | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| **Total** | **100** | **100.00** | **100.00** | **100.00** | **100.00** | **100.00** | **100.00** |
| **Proximate Compositions (%) of the Experimental Diets formulated at 3% and 5% Inclusion Levels of Medicinal Plants** |
| **Ash (%)** | 12.63 | 12.66 | 12.52 | 12.38 | 12.65 | 9.45 | 11.92 |
| **Crude Fibre (%)** | 4.07 | 4.17 | 4.4.00 | 4.63 | 2.28 | 5.83 | 6.05 |
| **Fat (%)** | 16.52 | 11.76 | 11.97 | 12.18 | 8.95 | 10.84 | 10.54 |
| **Crude Protein (%)** | 43.22 | 38.05 | 40.01 | 39.72 | 42.34 | 42.00 | 41.50 |
| **Moisture (%)** | 7.05 | 5.45 | 5.31 | 5.63 | 5.93 | 5.03 | 6.10 |
| **Carbohydrate (%)** | 16.51 | 27.91 | 25.79 | 25.46 | 24.85 | 26.85 | 23.89 |
| **Total** | **100.00** | **100.00** | **100.00** | **100.00** | **100.00** | **100.00** | **100.00** |

FM = Fishmeal, SBM = Soybean Meal, MM = Maize Meal, VMP = Vitamin and Mineral Premix, ZMD = Zero Medicinal Diet, EAQ = Eucalyptus Aqueous, EET = Eucalyptus Ethanol, EHX = Eucalyptus Hexane, MAQ = Moringa Aqueous, MET =Moringa Ethanol and MHX = Moringa Hexane

**Table 1b: Feed Formulation Showing Inclusion Levels of Processed Medicinal Plants (Eucalyptus and Moringa) Diets**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Ingredients** | **D8****(5%EAQ)** | **D9****(EET5%)** | **D10****(5%EHX)** | **D11****(5%MAQ)** | **D12****(5%MET)** | **D13****(5%MHX)** |
| **FM** | 24.36 | 24.36 | 24.36 | 24.36 | 24.36 | 24.36 |
| **SBM**  | 48.71 | 48.71 | 48.71 | 48.71 | 48.71 | 48.71 |
| **MM** | 16.93 | 16.93 | 16.93 | 16.93 | 16.93 | 16.93 |
| **Moringa**  | 0.00 | 0.00 | 0.00 | 5.00 | 5.00 | 5.00 |
| **Eucalyptus** | 5.00 | 5.00 | 5.00 | 0.00 | 0.00 | 0.00 |
| **Oil**  | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 |
| **Lysine** | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| **Methionine** | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| **Bone Meal** | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| **VMP** | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| **Total** | **100** | **100** | **100** | **100** | **100** | **100** |
| **Proximate Compositions (%) of the Experimental Diets formulated at 3% and 5% Inclusion Levels of Medicinal Plants** |
| Ash (%) | 13.38 | 13.88 | 12.54 | 12.75 | 10.31 | 13.92 |
| Crude Fibre (%) | 6.09 | 6.09 | 4.64 | 5.35 | 4.88 | 4.45 |
| Fat (%) | 12.22 | 12.22 | 12.91 | 14.24 | 12.65 | 13.09 |
| Crude Protein (%) | 41.60 | 41.80 | 38.36 | 39.2 | 38.82 | 40.36 |
| Moisture (%) | 4.76 | 4.79 | 5.93 | 5.40 | 5.48 | 6.09 |
| Carbohydrate (%) | 21.95 | 21.22 | 25.62 | 23.06 | 27.86 | 22.09 |
| **Total** | **100** | **100** | **100** | **100** | **100** | **100** |

**Experimental Design**

*Clarias gariepinus* fingerlings (9.66 ± 0.15 g) were acclimatize to experimental condition for two weeks before being distributed to various experimental units. Fish were starved 24 hours prior to the commencement of feeding trials in order to increase their appetite and eliminate variation in weight due to residual feed content that may be left in their gut (Ochang *et al*., 2015). Fish are weighed equally and distributed into various experimental units using an electronic digital sensitive weighing balance (MP300) at a stocking density of 20 fish per experimental unit. Water quality parameters were monitored weekly, they were fed 5% of their body weight daily with equal meals being fed between 10 am and 16 pm.

**Blood Sample Collection and Haematological Determination**

Haematological parameters was conducted before and after the experiment suing (Kelly, 1979) procedures. Fish were carefully caught randomly, one at a time with least disturbance. Each fish was held at the head region, carefully lifted with one hand to a comfortable handling position while the other hand was used to hold 2 ml syringe and needle which was inserted into the fish at a perpendicular angle of 45° to the anal fin to the vertebral column and desirable blood sample was collected and transferred into anticoagulant Ethylene Di Amine Tetra Acetic Acid (EDTA) labelled tubes (Argungu *et al*., 2017). The tubes containing the samples were gently mixed with the anticoagulant after covering them and kept in a sample box containing ice prior to haematology analysis.

**Statistical Analysis**

The experimental data were subjected one way Analysis of Variance (ANOVA) using Statistical Package for Social Scientists (SPSS) version 16.0 to test the effect among the treatment means at 0.05 significant difference. Multiple comparisons among the treatments were achieved using “R” statistical packages.

**Results**

Table 2a and 2b shows the haematological parameters of the *Clarias gariepinus* fingerlings that were fed 3% and 5% inclusion levels of processed medicinal plants diets. Definite variations were dictated among the various treatments. High levels of Red Blood Cell (RBC) count were observed in 3% EAQ (4.00±0.20×106/l), 3% MAQ (4.10±0.10×106/l) and 5% MHX (4.00±0.10×106/l) as they were not significantly different (P>0.05) from each other. The RBC count of all the tested diets were observed to be significantly higher than the control diet ZMD (2.40±0.20×106/l). There were varying increase in White Blood Cell (WBC) Count among the experimental based diets while 3% EET (10.30±0.30×109/l), 3% MAQ (10.60±0.20×109/l) and 5% EET (10.30±0.10×109/l) were least significant (P>0.05) among the tested processed Medicinal plants diets. Haemoglobin (Hb) in all the tested diets were observed to be significantly (P>0.05) higher than the control diet (6.60±0.20×10g/dl). The Mean Cell Volume (MCV) and Mean Cell Haemoglobin (MCH) counts in this study appeared to be significantly (P>0.05) different among all the tested diets. However, the Mean Cell Haemoglobin Concentration (MCHC) count for all the experimental diets were not significantly (P>0.05) different from each other. The water quality parameters measured during the experiments are Temperature (27-30°C), pH (6.6 – 8.4) and Dissolved Oxygen (3-5 mg/l).

**Table 2a: Haematological Parameters of *Clarias gariepinus* fed Processed Medicinal Plants Diets**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Parameters** | **D1****ZMD** | **D2****(3% EAQ)** | **D3****(3%EET)** | **D4****(3%HEX)** | **D5****(3%MAQ)** | **D6****(3%MET)** | **D7****(3%MHX)** |
| **RBC×106/l**  | 2.40±0.20ef | 4.00±0.20ab | 3.27±0.15cd | 2.10±0.10f | 4.10±0.10a | 3.60±0.20abc | 2.80±0.00de |
| **WBC×109/l** | 14.60±0.10de | 16.80±0.20cd | 10.30±0.30f | 18.07±0.15bc | 10.60±0.20f | 13.73±0.31e | 15.93±0.15cde |
| **PCV (%)** | 20.00±1.00j | 34.00±0.00a | 29.00±1.00bcd | 22.00±1.00**ij** | 30.00±1.00bc | 33.00±1.00ab | 25.00±1.00gh |
| **Hb (g/dl)** | 6.60±0.20h | 11.30±0.10ab | 9.60±0.20def | 7.30±0.20h | 10.00±0.10de | 11.00±0.10bc | 8.30±0.20g |
| **MCV(fl)** | 83.97±11.21bc | 85.17±4.25bc | 89.03±7.27b | 104.77±0.25a | 73.23±4.25c | 91.83±4.57b | 89.30±3.60b |
| **MCH (pg)** | 27.67±3.15bcd | 28.30±1.20bcd | 29.43±2.02bc | 34.8667±2.60a | 24.43±0.85d | 30.63±1.63ab | 29.63±0.75bc |
| **MCHC (%)** | 33.03±0.65a | 33.20±0.30a | 33.13±0.45a | 33.27±2.40a | 33.33±0.75a | 33.33±0.75a | 33.23±0.55a |

Average values on the same row with similar superscripts are not significantly different (P>0.05) from each other. RBC = Red Blood Cell, WBC = White Blood Cell, PCV = Pack Cell Volume, Hb = Haemoglobin,

**Table 2b: Haematological Parameters of *Clarias gariepinus* fed Processed Medicinal Plants Diets**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Parameters** | **D1****ZMD** | **D8****(5%EAQ)** | **D9****(5%EET)** | **D10****(5%EHX)** | **D11****(5%MAQ)** | **D12****(5%MET)** | **D13****(5%MHX)** |
| **RBC×106/l**  | 2.40±0.20ef | 3.33±0.49cd | 2.80±0.10**de** | 3.10±0.10**cd** | 3.10±0.10**cd** | 3.47±1.10**bc** | 4.00±0.10**ab** |
| **WBC×109/l** | 14.60±0.10de | 10.30±0.10**f** | 20.10±0.10**b** | 24.20±0.20**a** | 24.20±0.20**a** | 15.57±4.99**de** | 16.30±0.30**cd** |
| **PCV (%)** | 20.00±1.00j | 28.00±1.00**ef** | 23.00±1.00**hi** | 27.00±1.00**fg** | 27.00±1.00**fg** | 31.00±5.20**bc** | 33.00±1.00ab |
| **Hb (g/dl)** | 6.60±0.20h | 9.30±0.10**ef** | 7.30±0.10**h** | 9.00±0.00**fg** | 9.00±0.00**fg** | 10.30±1.73**cd** | 11.00±0.10**bc** |
| **MCV(fl)** | 83.97±11.21bc | 84.93±9.63**bc** | 82.13±0.65**bc** | 87.10±0.40**b** | 87.10±0.40**b** | 93.13±17.72**ab** | 82.50±0.40**bc** |
| **MCH (pg)** | 27.67±3.15bcd | 28.30±4.09bcd | 26.10±1.30cd | 29.03±0.95bc | 29.03±0.95bc | 30.97±5.83**ab** | 27.53±0.95bcd |
| **MCHC (%)** | 33.03±0.65a | 33.23±1.55a | 31.77±1.80a | 33.33±1.25a | 33.33±1.25a | 33.20±0.00a | 33.37±1.30**a** |

**Discussion**

The water quality parameters measured in this study were within the optimal recommended range as observed by Afia and Ofor (2016) the temperature of fish changes according to its environment which affect metabolism, dissolved oxygen is needed to aid aerobic generation of energy for body maintenance and physiological functions of aquatic organisms. According to Fagbenro *et al* (2013) haematological Parameters on catfish species are to determine their health status with well establish ranges. The RBC count were in disagreement with Eyiwumi *et al* (2018). The latter reported that decrease in low RBC were due to decrease in inclusion levels of Moringa Leaf Meal (MLM) while in this research the lower RBC were within the medicinal diets processed with hexane solvents for 3% and 5% inclusion level (Table 2) and may be as a result of non-polar nature of the hexane solvent.

The Increased values recorded in WBC count contributed to the better survival rate of the experimental fish in this study and were in agreement with Eyiwumi *et al* (2018) who discovered that 15% MLM diet recorded high increase in WBC and Lymphocyte count increases fish survival rate which also increases the iron content of the diet fed as major sources of Haemoglobin in the fish diets. WBC are the defence cells of the fish body. Therefore, the increase recorded attributed to increase in leucocytes synthesis as defence mechanism against the destruction of erythrocytes (Oniya *et al*., 2013; Afia and Ofor, 2016; Suleiman *et al*., 2018). All the PCV counts recorded in this study may likely be as a result of sign of healthier fish with high immunity which is an indication that the increase in PCV of fish under study had high immunity or resistance anaemic condition. The values recorded for the experimental based diets were higher than control-based diets and were in opposition to Eyiwumi *et al* (2018) who reported low PCV counts on fish fed experimental diets of 0.5% and 1% MLM inclusion levels. The highest MCV (104.77±0.25 fl) was recorded in 3% Eucalyptus Hexane (EHX) based diet which was also an indication that the fish under study had high immunity or resistance to disease (Fagbenro *et al*., 2013). It however concluded that 3% and 5% inclusion levels of medicinal processed diet both have similar haematological positive impact on *Clarias gariepinus* but 3% is recommended for use due to the growth performance of the fish measured during the study.

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