Haematological Characteristics of *Heterobranchus Bidorsalis* Geoffroy St. Hilaire (1809) Fed Varying Inclusion Levels of Aqueous and Ethanolic *Moringa Oleifera* Leave Extracts

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ABSTRACT

The application of haematological techniques is a valuable tool in fisheries health management and is equally essential procedure for diagnosing and monitoring of fish physiological, pathological and nutritional status. The use of phytogenic feed additives (PFAs) is a modern innovation in achieving sustainable agquaculture, However, there is limited report on the effects of PFAs such as Moringa oleifera leave extract on the haematological profile of *H. bidorsalis* broodstock Hence, this study on hematological characteristics provide useful information in this regard. The solvent and method of extraction of feed additives, the age as well as the sex influence the blood differentials of fish. In this study the haematological analyses of the male and female broodstock of *H.* bidorsalis were investigated following dietary administration of diets containing graded inclusion levels of aqueous *Moringa oleifera* Leave Extract (AMOLE) and Ethanolic *Moringa oleifera* Leave Extract (EMOLE). The formulated test diets contained 0.00/100g (control), 1.0g/100g, 2.0g/100g and 3.0g/100g inclusion levels of the extracts while *H. bidorsalis* samples (n=216; 800.00±150.00g; 37.50±1.5cm) were randomly distributed in triplicate into 24 concrete tanks of size 6m x 4m x1.3m in a completely randomized 2x4 factorial design and fed at 5% body weight twice daily for 16 weeks. The haematological profile of the male and female broodstock samples were evaluated after the nutritional experimental feed trial following standard procedures. Analysis of variance (one-way ANOVA with post hoc (LSD) comparison) was used to determine and compare haematological data among all reared fish. All Treatment means were separated by Least Significant Differences (LSD) at $P < 0.05$ using SPSS 20 software. Data was considered significant when $\alpha < 0.05$. Blood indices values observed revealed that the varying additive levels of *M. oleifera* leave extract (1.0g/100g – 3.0g/100g) used had no negative physiological stress on the health status of the fish studied and haematological values observed were seen to be best in the group fed 3.0/100g Aqueous and Ethanolic *Moringa oleifera* leave extracts. Thus, the use of M. *oleifera leaves* extracts as phytogenic feed additives and nutrients booster should be encouraged and sustained towards sustainable fish health management.

Key Words: Phytogenic Feed Additives, *Moringa Oleifera* Leave, *Heterobranchus bidorsalis*, Haematology

INTRODUCTION

Blood is an active transport medium in higher animals, especially in the vertebrates and it is explained to be a medium that constantly bathes all the organs and tissues of the body, enabling exchange of materials between the internal and external environment of the organs and tissues (Ramalingam, 2011). Any change in the constituents of a blood sample when compared to the normal values could be used to interpret the metabolic state of the animal and the influence of the treatment given to the animal (Babatunde *et al.*, 1992). Haematological characteristics are widely used in clinical diagnosis and pathological examinations in human beings, domestic animals and livestock and they indicate responses to stress (Osuigwe *et al.*, 2005). Sotolu (2008) reported that haemotology has been developed and well utilized in assessing the health conditions of man and livestock. Like the study of blood and its components, haemotology deals with the nature, functions and diseases of blood. Olukunle (1996) reported that application of haematological techniques is a valuable tool in fish biology in the assessment of fish health and response to stress and is equally an essential procedure for the diagnosis of infections in animals. Hence, its parameters are routinely used for the evaluation of physiology

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environment and husbandry stressors in fishes (Rainza-Palva *et al*., 2000). Haematological component of blood is also valuable in monitoring feed toxicity especially with feed constituents that affect the formation of blood in culture fisheries (Oyawoye and Ogunkunle, 2004). Many studies concerning the hematology of different fish species (Adeyemo, 2007; Adedeji & Adegbile 2011; Suleiman & Abdullahi, 2016) had been carried out and they had been used among the few tools available to monitor health status in different species of fish to detect acute and chronic patho-physiologic changes attributable to nutrition, water quality, toxicants, and disease. Also, Svobodova *et al.*, (1991) recommended that ichtyohaematology (fish haematology) would be useful in the assessment of suitability of feeds and feed mixture evaluation of fish conditions, determination of toxic effects of substances and diagnosis of disease. This agrees with the recommendations of Ogunji *et al.*, (2005) on the use of feed ingredients containing one or more anti-nutritional substance in livestock feed preparation. In addition, nutrition, age, sex, disease, genetics (bread and cross-breading), reproduction, housing, starvation, climate, environmental factors, stress and transportation are known to affect haematological parameters in animals (Coles, 1986). These factors are also believed to play major roles in the differences in haematological parameters observed between tropical and temperate animals (Ogunrinade *et al.*, 1981; Kaggwa *et al*., 1984).

MATERIALS AND METHODS

Proximate Analysis of *Moringa Oleifera* leaf Extract samples: *Moringa oliefera* leaf extract and other samples from fish carcass and eggs were analyzed according to the method of AOAC (2012). The following nutrients were determined: Moisture, Ash, Fat, Fibre, Protein and Carbohydrate (Nitrogen Free Extract):

$$\text{Moisture \%} = \frac{\text{Wet weight – dry Weight}}{\text{Wet weight of sample}} 100$$

$$\text{\% Ash} = \frac{\text{Weight of sample remaining}}{\text{Weight of original Sample}} 100$$

$$\text{Fat \%} = \frac{(W2 - W1)}{(VA \times SW)} Vc\ 100$$

Where: W2 is the weight of glass tube and dried extract (g), W1 is the weight of empty dried glass tube (g), Vc is the total volume of chloroform (ml), VA is the volume of extract dried (ml), and SW is the weight of the sample in grammes.

$$\text{\% Fibre} = \frac{W1 - W2}{\text{Weight of the Sample}} 100$$

Where: W1= Weight of initial dried sample, W2= Weight of final dried sample

Crude Protein (%): The percentage of protein content was calculated according to the AOAC, 2000.

Carbohydrate (%): Carbohydrate content was calculated as a difference from the amount of other proximate parameters measured (AOAC, 2000). Carbohydrate = 100% - (% Moisture + % Ash + % Protein + % Fat)

Haematological analysis

The fish samples were taken out individually using a small hand net and were placed belly upward on a table. Following Soetan *et. al*. (2013) method, blood samples of about 2ml were collected from the actual caudal peduncle (a quick, easy, non-invasive and widely used method of obtaining a blood sample in fish biology) with the aid of a 2cm3 plastic syringe and the blood was dispensed into ethylene diamine tetra-acetic acid (EDTA) anticoagulant bottle (to prevent blood clotting) for haematological studies. The haematological indices of the Mean Cell Haemoglobin Concentration (MCHC), Mean Cell Haemoglobin (MCH) and Mean Cell Volume

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(MCV) were determined using the total Red Blood Cell count (RBC), Haemoglobin Concentration (Hb), and Hematocrit (Hct) values. The cyano-haemoglobin method was used to determine haemoglobin (Hb) using diagnostic kits from Sigma diagnostics USA, and packed cell volume (PCV) was determined by the microhaematocrit method. Red blood cell (RBC) count was determined with the improved Neubauer haemocytometer according to Dacie and Lewis (1991). White blood cells (WBC) was determined with the improved Neubauer counter, while differential counts such as neutrophils, lymphocytes and monocytes were determined on blood film stained with Giemsa stain. Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were derived from the RBC, PCV and Hb using standard formulae (Svobodova *et al*., (1991). MCV was calculated in femtoliters = PCV/RBC x 10, MCH was calculated in picograms = Hb/RBC x10 and MCHC = (Hb in 100mg blood / Hct) x 100.

RESULTS

Proximate: The extraction methods had significant influence on the proximate composition of the MOL. There was no significant difference ($p > 0.05$) in the ether extract of MOL between the aqueous and ethanol extraction methods. The ash and crude protein contents in ethanol extraction methods were significantly lower ($p < 0.05$) than the aqueous extraction method. However, the lowest significant ($p < 0.05$) crude fiber content in MOL were recorded in aqueous extraction method compared to the ethanol extraction method. Moreover, the crude protein and crude ether values of the MOLE diets in this study which ranges from 35.73 - 38.83 and 6.77 - 7.91 respectively are similar but lower to the values of the findings of Tijani (2017) where the crude protein ranges 39.93 - 42.01 and lipid content of the diets ranged from 11.45 to 13.02 % which could be as a result of the replacement of the fish oil with vegetable oil in the compounded diet. These are comparable to and within the value range of 38.28 - 42.23, 11.43 - 13.65 reported for crude protein and crude lipid respectively by Ochang (2007) for African Catfish (C. *gariepinus*) broodstock.

Table 1: Proximate composition of experimental diet containing graded levels of aqueous extract of *Moringa oleifera* Leaves

Proximate composition	0g/100g	1g/100g	2g/100g	3g/100g
Crude protein	37.24±0.50	37.81±0.79	38.84±0.17	38.83±0.35
Ash	$16.00±0.15^{c}$	$17.34±0.14^{b}$	$20.01±0.27^{a}$	$17.27±0.09^{b}$
Ether extract	$6.77±0.09^{bc}$	$7.08±0.09^{ab}$	$7.21±0.06^{a}$	$6.72±0.06^{c}$
Crude fiber	$3.16±0.07^{c}$	$3.45±0.04^{ab}$	$3.19±0.05^{bc}$	$3.59±0.08^{a}$
Nitrogen Free Extract	$36.84±0.66^{a}$	$34.31±0.99^{ab}$	$30.75±0.31^{c}$	$33.60±0.41^{bc}$
Moisture	$92.43±0.05^{b}$	$93.22±0.08^{a}$	$90.28±0.25^{c}$	$90.00±0.06^{c}$

Mean±SD in the same row with different superscripts are significantly different at $p < 0.05$

Table 2: Proximate composition of experimental diet containing graded levels of ethanolic extract of *Moringa oleifera* Leaves

Proximate Composition (%)	0g/100g	1g/100g	2g/100g	3g/100g
Crude protein	35.73±0.17	37.04±0.09	38.44±0.07	38.64±0.27
Ash	$16.71±0.18^{b}$	$17.08±0.13^{b}$	$18.33±0.04^{a}$	$17.20±0.17^{b}$
Ether extract	$7.94±0.05^{a}$	$7.66±0.02^{b}$	$7.48±0.04^{c}$	$7.91±0.01^{a}$
Crude fiber	$3.73±0.01^{c}$	$4.02±0.05^{b}$	$4.35±0.09^{a}$	$4.00±0.06^{b}$
Nitrogen Free Extract	$35.89±0.14^{a}$	$34.21±0.24^{b}$	$31.41±0.25^{c}$	$32.25±0.18^{c}$
Moisture	$93.22±0.08^{a}$	$90.28±0.25^{c}$	$90.00±0.06^{c}$	$92.43±0.05^{b}$

Mean±SD in the same row with different superscripts are significantly different at $p < 0.05$. The CP was not significantly different

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HAEMATOLOGY

For the aqueous extracted *Moringa oleifera* leave based diet (AMOLE) raised Male fish, the following ranges were obtained in the parameters: the PCV or HCT (29.67±3.76 - 36.00±3.51), HB (8.90±1.25 - 12.23±1.33), RBC (2.40±0.29 - 3.03±0.15), WBC (1.38±0.89 - 2.85±0.35), PLT (1.36±0.04 - 1.74±0.03), MCH (36.90±1.50 - 47.90±6.35), MCHC (32.87±0.32 - 35.07±0.04), MCV (105.27±4.42 - 145.40±17.90), Neutrophil (51.00±4.36 - 65.00±1.73), Eosinophil (1.00±0.58 - 2.33±0.33), Basophil (2.00±0.58 - 3.00±0.58), Monocyte (4.67±0.33 - 6.33±0.67), Lymphocyte (23.00±0.58 - 41.00±4.62). While the range values for the female fish fed with aqueous extracted *Moringa oleifera* leave based diet (AMOLE) is thus: PCV (29.00±1.73 - 43.67±0.88), HB (8.97±0.49 - 14.33±0.64), RBC (2.50±0.23 - 3.70±0.06), WBC (1.38±0.89 - 2.70±0.23), PLT (1.29±0.10 - 1.62±0.12), MCH (39.15±1.18 - 46.80±6.81), MCHC (32.70±0.69 - 34.2±0.12), MCV (89.47±8.63 - 142.37±21.88), Neutrophil (54.00±3.21 - 59.67±1.45), Eosinophil (1.00±0.58 - 1.67±0.1.20), Basophil (2.00±0.58 - 3.00±0.58), Monocyte (6.00±0.58 - 7.00±0.58), Lymphocyte (27.33±2.40 - 30.33±1.45).

Whereas, for the ethanolic extracted *Moringa oleifera* leave based diet (EMOLE) raised Male fish, the parameter values ranged as follows: the PCV or HCT (38.33±3.76 - 42.67±0.88), HB (13.00±1.21 - 14.30±0.29), RBC (2.92±0.06 - 4.81±0.63), WBC (1.57±0.02 - 2.95±0.78), PLT (1.59±0.09 - 1.82±0.04), MCH (33.40±4.70 - 45.92±4.58), MCHC (33.26±0.21 - 33.82±0.02), and MCV (99.21±14.08 - 137.83±12.88), Neutrophil (56.00±2.89 - 70.00±0.58), Eosinophil (0.00±0.00 - 0.67±0.33), Basophil (2.00±0.00 - 4.00±0.58), Monocyte (7.67±0.88 - 8.33±0.88), and Lymphocyte (18.33±1.45 - 30.00±0.58) While the range values for the female fish fed ethanolic extracted *Moringa oleifera* leave based diet (EMOLE) is thus: PCV or HCT (34.67±2.60 - 39.33±2.03), HB (11.63±0.90 - 13.40±0.69), RBC (3.06±0.34 - 4.13±0.28), WBC (1.68±0.01 - 1.98.00±0.13), PLT (1.55±0.06 - 1.74±0.02), MCH (32.54±0.57 - 38.57±1.44), MCHC (33.72±0.03 - 34.73±0.11), MCV (95.99±1.68 - 108.18±1.16), Neutrophil (47.00±0.58 - 67.00±0.58), Eosinophil (1.67±0.33 - 2.67±0.88), Basophil (3.67±0.33 - 6.67±0.33), Monocyte (8.33±0.33 -10.00±0.58), Lymphocyte (9.67±4.33 - 35.67±1.76).

Table 3: Interaction Effect of Sex and Inclusion Levels on the Haematological Parameters of *H. bidorsalis* Fed Aqueous Extracted MOL Based-diet

	0g/100gM	1g/100gM	2g/100gM	3g/100gM	0g/100gF	1g/100gF	2g/100gF	3g/100gF
PCV (%)	35.33 ± 3.71^{ab}	36.00 ± 3.51^{ab}	29.67 ± 3.76^{b}	31.33 ± 3.33^{ab}	31.67 ± 0.33^{ab}	29.00 ± 1.73^{b}	34.00 ± 2.31^{ab}	43.67 ± 0.88^{a}
Hb (gdl^{-1})	12.23 ± 1.33^{ab}	11.40 ± 0.26^{ab}	9.80 ± 1.25^{b}	11.23 ± 0.50^{ab}	10.70 ± 0.23^{ab}	8.97 ± 0.49^{b}	11.30 ± 0.64^{ab}	14.33 ± 0.64^{a}
RBC ($x10^{6}/\mu l$)	2.40±0.29	2.83±0.15	2.70±0.46	3.03±0.15	2.50±0.23	3.20±0.40	2.50±0.23	3.70±0.06
WBC ($x10^{3}/\mu l$)	1.95 ± 0.03^{abc}	2.85 ± 0.35^{a}	1.93 ± 0.31^{abc}	1.80 ± 0.12^{bc}	2.70 ± 0.23^{ab}	1.77 ± 0.44^{bc}	1.38 ± 0.89^{c}	1.42 ± 0.88^{c}
PLT ($x10^{3}/\mu l$)	1.36 ± 0.04^{ab}	1.74 ± 0.03^{a}	1.42 ± 0.08^{ab}	1.63 ± 0.06^{ab}	1.46 ± 0.12^{ab}	1.36 ± 0.00^{ab}	1.29 ± 0.10^{b}	1.62 ± 0.12^{ab}
MCH (pg)	36.90±1.50	40.27±2.68	37.93±1.67	47.90±6.35	44.87±4.01	41.93±9.61	46.80±6.81	39.15±1.18
MCHC (gdl^{-1})	35.07 ± 0.04^{a}	33.50 ± 0.58^{ab}	34.00 ± 0.12^{ab}	32.87 ± 0.32^{b}	34.2 ± 0.12^{ab}	32.70 ± 0.69^{b}	33.10 ± 0.29^{ab}	32.80 ± 0.64^{b}
MCV (fl)	105.27±4.42	122.13±10.25	111.37±4.53	145.40±17.90	131.00±12.18	89.47±8.63	142.37±21.88	119.37±1.24
Neutrophil ($x10^{6}/\mu l$)	65.00 ± 1.73^{a}	63.67 ± 2.33^{ab}	61.00 ± 1.53^{ab}	51.00 ± 4.36^{b}	59.00 ± 4.51^{ab}	54.00 ± 3.21^{ab}	58.00 ± 0.58^{ab}	59.67 ± 1.45^{ab}
Eosinophil ($x10^{2}/\mu l$)	2.33±0.33	1.67±0.33	1.33±0.33	1.00±0.58	1.33±0.67	1.00±0.58	1.67±0.1.20	1.33±0.33
Basophil ($x10-^{2}/\mu l$)	2.00±0.58	2.00±0.58	3.00±0.58	2.67±0.33	3.00±1.00	2.00±0.58	3.00±0.58	3.00±0.58
Monocyte ($x10-^{2}/\mu l$)	6.33±0.67	5.67±0.33	4.67±0.33	5.33±0.33	6.00±0.58	6.33±0.0.67	7.00±0.58	6.00±0.58
Lymphocyte ($x10^{3}/\mu l$)e	23.67 ± 1.20^{b}	25.00 ± 2.31^{b}	23.00 ± 0.58^{b}	41.00 ± 4.62^{a}	29.00 ± 1.73^{ab}	31.67 ± 4.81^{ab}	27.33 ± 2.40^{ab}	30.33 ± 1.45^{ab}

Mean ± SE in the same row with different superscripts are significantly different at $p < 0.05$

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KEY: F= Female, M= male. Parameters without superscripts shows no significant difference at all levels (RBC, MCH, Eosinophil, Basophil and Monocyte)

Table 4: Interaction Effect of Sex and Inclusion Levels on the Haematological Parameters of *H. bidorsalis* fed Ethanol Extracted MOL Based-diet

	Inclusion							
	0g100gM	1g100gM	2g100gM	3g100gM	0g100gF	1g100gF	2g100Gf	3g100gF
PCV (%)	40.00±2.89	40.67±1.45	38.33±3.76	42.67±0.88	39.33±2.03	36.67±0.33	34.67±2.60	35.67±2.60
Hb (gdl^{-1})	13.30±1.04	13.73±0.46	13.00±1.21	14.30±0.29	13.40±0.69	12.63±0.20	11.63±0.90	12.37±0.95
RBC ($x10^6/\mu l$)	$2.92±0.06^{b}$	$3.65±0.28^{ab}$	$3.82±0.23^{ab}$	$4.81±0.63^{a}$	$4.13±0.28^{ab}$	$3.40±0.02^{ab}$	$3.06±0.34^{b}$	$3.28±0.20^{b}$
WBC $x10^3/\mu l$	2.95±0.78	1.60±0.06	1.65±0.06	1.57±0.02	1.78±0.01	1.88±0.13	1.98.00±0.13	1.68±0.01
PLT ($x10^3/\mu l$)	1.82±0.04	1.59±0.09	1.61±0.04	1.67±0.04	1.56±0.03	1.55±0.06	1.74±0.02	1.72±0.09
MCH (pg)	$45.92±4.58^{a}$	$37.90±1.62^{ab}$	$33.80±1.17^{ab}$	$33.40±4.70^{b}$	$32.54±0.57^{b}$	$36.64±0.03^{ab}$	$38.57±1.44^{ab}$	$37.57±0.52^{ab}$
MCHC (gdl^{-1})	$33.26±0.21^{c}$	$33.82±0.02^{b}$	$33.77±0.10^{b}$	$33.68±0.05^{bc}$	$33.90±0.01^{b}$	$34.13±0.06^{b}$	$33.72±0.03^{bc}$	$34.73±0.11^{a}$
MCV (fl)	$137.83±12.88^{a}$	$112.06±4.73^{ab}$	$100.11±3.77^{b}$	$99.21±14.08^{b}$	$95.99±1.68^{b}$	$107.35±0.30a^{b}$	$114.4±4.36^{ab}$	$108.18±1.16^{ab}$
Neutrophil ($x10^6/\mu l$)	$70.00±0.58^{a}$	$57.00±0.58^{b}$	$65.00±1.15^{a}$	$56.00±2.89^{b}$	$67.00±0.58^{a}$	$58.33±1.45^{b}$	$47.00±0.58^{c}$	$56.67±0.88^{b}$
Eosinophil ($x10^2/\mu l$)	$0.00±0.00^{b}$	$0.67±0.33^{ab}$	$0.67±0.33^{ab}$	$0.67±0.33^{ab}$	$1.67±0.33^{ab}$	$1.67±0.33^{ab}$	$2.67±0.88^{a}$	$1.67±0.33^{ab}$
Basophil (x10-2/µl)	$3.33±0.33^{b}$	$4.00±0.58^{ab}$	$2.67±0.33^{b}$	$2.00±0.00^{b}$	$4.67±1.45^{ab}$	$4.33±0.33^{ab}$	$6.67±0.33^{a}$	$3.67±0.33^{b}$
Monocyte ($x10^{-2}/\mu l$)	8.00±0.58	8.33±0.88	8.00±0.00	7.67±0.88	7.67±0.33	9.00±0.58	10.00±0.58	8.33±0.33
Lymphocyt (x103/µl)e	$18.33±1.45^{ab}$	$30.00±0.58^{ab}$	$25.67±1.76^{ab}$	$23.67±10.59^{ab}$	$9.67±4.33^{b}$	$25.67±1.45^{ab}$	$35.67±1.76^{a}$	$30.00±1.15^{ab}$

Mean ± SE in the same row with different superscripts are significantly different at $p < 0.05$.

KEY: F= Female, M= male. Parameters without superscripts show no significant difference at all levels (PCV, Hb, WBC, PLT and Monocyte)

Table 5: Main Effect of Extraction, Sex and Inclusion levels of Dietary MOL on the Heamatology and RBC Morphology of *H. bidorsalis*

	Extraction		Sex		Inclusion			
	Aqueous	Ethanol	Male	Female	0g/100g	1g/100g	2g/100g	3g/100g
PCV (%)	$33.83±0.90^{b}$	$38.5±0.90^{a}$	36.75±0.90	35.58±0.90	36.58±1.27	35.58±1.27	34.12±1.27	38.33±1.27
Hb (gdl^{-1})	$11.25±0.27^{b}$	$13.05±0.27^{a}$	12.38±0.27	11.92±0.27	$12.41±0.39^{xy}$	$11.68±0.39^{xy}$	$11.43±0.39^{y}$	$13.06±0.39^{x}$
Rbc ($x10^6/\mu l$)	$2.86±0.08^{b}$	$3.63±0.08^{a}$	1.90±0.08	1.73±0.08	1.76±0.11	1.76±0.11	1.72±0.11	2.02±0.11
WBC ($x10^3/\mu l$)	1.98.00±0.86	1.88.33±0.86	2.04.50±0.86	1.820±0.86	$2.34±0.12^{x}$	$2.02±0.12^{xy}$	$1.74±0.12^{y}$	$1.61±0.12^{y}$
MCH (pg)	$41.97±1.42^{a}$	$37.04±1.42^{b}$	39.25±1.42	39.76±1.42	40.06±2.01	39.19±2.01	39.28±2.01	39.50±2.01
MCHC (gdl^{-1})	$33.53±0.11^{b}$	$33.88±0.11^{a}$	33.75±0.11	33.66±0.11	$34.11±0.15^{x}$	$33.54±0.15^{xy}$	$33.65±0.15^{xy}$	$33.52±0.15^{y}$
MCV (fl)	$120.80±3.53^{a}$	$109.39±3.53^{b}$	$116.67±3.53^{m}$	$113.51±3.53^{n}$	117.52±4.99	107.75±4.99	117.06±4.99	118.04±4.99
PLT ($x10^3/\mu l$)	$1.49±0.03^{b}$	$1.66±0.03^{a}$	1.61±0.03	1.54±0.03	$1.55±0.37^{xy}$	$1.56±0.37^{xy}$	$1.52±0.37^{y}$	$1.66±0.37^{x}$

Mean±SE in the same row within the same category with different superscript are significantly different at $p<0.05$

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DISCUSSION

All the haematological parameters measured in this study were within the recommended physiological ranges reported for Catfish according to the previous studies carried out by some researchers (Erondu *et al.,* 1993; Adeyemo *et al.,* 2014, Okorie- Kanu *et al.,* 2014). Aletor and Egberongbe (1998) reported that red blood cell counts and packed cell volume (PCV) are mostly affected by dietary treatment. PCV ranging from 23.67 ± 0.33% to 34.67 ± 0.67% observed in this study were within the range of 20 to 50% reported for African Catfish. The value of 7.67 ± 0.18 to 12.03 ± 0.26 g/dl recorded for Hb concentrations were within the normal range reported by some researchers (Omitoyin, 2006; Osuigwe *et al.,* 2007) for African Catfish. PCV and Hb are major and reliable indicators of various sources of stress (Rainza-Paiva *et al,* 2000) and these parameters decrease in the presence of anti-nutritional factors (Osuigwe *et al.,* 2007). Reduction in the concentration of the PCV in the blood usually suggests the presence of toxic factor example of which is haemagglutin which has adverse effect on blood formation (Oyawoye and Ogunkunle, 1998). The increase in WBC may be due to increase in leucopolesis as a means of combating stressor in the body system of the fish, similar findings were recorded by Gabriel *et al.* (2004) in Clarias gariepinus acclimatized for 7 days.

The main effect of Sex and Inclusion Levels of Aqueous Extracted MOL Based-diet revealed that there was no significant difference in haematological parameters between the male and the female fed aqueous extracted MOL based-diet except in WBC and MCHC where the values of these parameters in male were significantly higher than female. The Hb, RBC, PLT and Lymphocyte were highest when aqueous extracted MOL was included at 3g/100g of feed. Conversely, the highest significant WBC and MCHC were in the control group (0g/100g) while the lowest recorded was in 3g/100g aqueous extracted MOL based-diet. However, the values recorded in PCV, MCV and MCH of the fish fed 3g/100g aqueous did not differ significantly from the fish fed 2g/100g, 1g/100g and 0g/100g respectively. While there was no significant difference ($p>0.05$) in the RBC, the PCV and Hb of the female fish fed 3g/100g, the aqueous extracted MOL were highest significantly. The PCV and Hb were lowest significantly in female fish fed 1g/100g aqueous extracted MOL which was not significantly different from male fish fed 2g/100g aqueous extracted MOL based-diet. The WBC and PLT were highest significantly in male fish fed 1g/100g aqueous extracted MOL based-diet and were lowest significantly in female fish fed 2g/100g aqueous extracted MOL based-diet. However, the WBC in female fish fed 2g/100g aqueous extracted MOL based-diet did not differ significantly from female fish fed 3g/100g aqueous extracted MOL based-diet.

The MCH and MCV did not differ significantly among the treatment groups. There was no significant difference in the MCHC recorded among the male fish fed 3g/100g, female fish fed 1g/100g and female fish fed 3g/100g aqueous extracted MOL based-diet. The values of the MCHC in these groups were lowest significantly while the highest significant MCHC was recorded in male fish fed 0g/100g aqueous extracted MOL based-diet.

The neutrophil in the male fish fed 0g/100g aqueous extracted MOL based-diet was highest significantly while the lowest was recorded in male fish fed 3g/100g aqueous extracted MOL based-diet. Conversely, the lymphocyte in the male fish fed 3g/100g aqueous extracted MOL based-diet was highest significantly and was lowest in the male fish fed 2g/100g aqueous extracted MOL based-diet which did not differ significantly from the male fish fed 0g/100g aqueous extracted MOL based-diet. There was no significant variation in the eosinophil, basophil and monocyte among the treatment groups. Whereas for the EMOLE diet, The Hb, RBC, WBC and PLT did not significantly differ between the male and the female fish fed ethanol extracted MOL based-diet. However, the PCV was significantly higher in male than female fish fed ethanol extracted MOL based-diet. The inclusion levels of ethanol extracted MOL based-diet did not significantly alter PCV, Hb, RBC, WBC and PLT across treatment groups. While there was no significant difference in the MCH and MCV between the male and female fish, the MCHC was higher significantly ($p < 0.05$) in female fish than the male fish. The inclusion level of ethanol extracted MOL based-diet that resulted in the highest significant MCHC in the fish was recorded in 3g/100g while the lowest was recorded in 0g/100g ethanol extracted MOL based-diet.

Moreover, the method used in the extraction of the bioactive compound in MOL, the sex of the fish and the inclusion level of MOL in the diet of the fish did significantly interact ($p <0.05$) to alter the hematological indices of the fish fed MOLE based diet except MCHC. While extraction method did not significantly influence ($p>0.05$) the WBC of the fish fed MOL based-diet, the fish fed ethanol extracted MOL based diet had a significant higher

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($p<0.0$) PCV, Hb, RBC, MCV and PLT when compared with fish fed aqueous extracted MOL based-diet. However, the values of these indices differ significantly between the male and the female. The MCV was significantly higher in male fish than female while also significantly higher ($p<0.05$) in fish fed aqueous extracted MOL based-diet. Whereas the highest significant PCV and PLT was recorded in fish fed MOL diet included at 3g/100g, there was no significant variability ($p > 0.05$) in the PCV, RBC, MCH and MCV among the treatment groups.

For the male fish samples investigated, the PCV was highest (36.00±3.51%) in the fish fed 1g/100g AMOLE diet and lowest (29.67±3.76%) in the fish fed 2.0/100g AMOLE diet while the female fish recorded higher values (43.67±0.88%) PCV in the 3.0g/100g AMOLE dietary treatment and lower (29.00±1.73%) value of PCV in 1.0/100g inclusion level of AMOLE. Whereas for the Ethanolic *Moring oleifera* leave extracted diet, the male fish recorded higher PCV (42.67±0.88%) at 3.0/100g inclusion level while the least PCV (38.33±3.76%) values was noticed at the 2.0/100g inclusion level of EMOLE diet. On the other hand, the female fish fed without EMOLE diet had the highest PCV value (39.33±2.03%) when compared with least PCV value (34.67±2.60) noticed at the fish fed 2.0/100g of EMOLE diet. The Hb (14.30±0.29 gdl^{-1}) and RBC (4.81±0.68 $x10^6$/µl) Values were higher in male fish fed 3.0/100g and lower (11.63±0.90 gdl^{-1}, 3.06±0.34 $x10^6$/µl) respectively in the fish fed the control (0.0/100g) diet. This was unlike the female fish which had higher Hb (13.40±0.69 gdl^{-1}) and RBC (4.13±0.2$x10^6$/µl) values in the control (0.0/100g) than the female fish fed 2g/100g (11.63±0.90 gdl^{-1}) and (3.06±0.34 $x10^6$/µl) EMOLE diets respectively.

A significant increase in the values of PCV and Hb recorded in the group of male and female fish fed with 1g/100g and 3.0g/100g AMOLE diet indicated that these additives added at that inclusion levels to the feed improved the blood level of the experimental fish. Blaxhall and Daisley (1973) reported the essence of using haematocrit to detect anaemic condition in fishes. The experimental fish were not anaemic as suggested by the improved values of PCVs recorded. The higher Haemoglobin values reported for the male fish fed 3.0/100g of Aqueous Moringa oleifera leave extracted diet indicate higher rate of transportation of oxygen to and removal of carbon (iv) oxide from the body tissues resulting in higher metabolism and growth. This is in agreement with the higher haemoglobin, haematocrit and RBC values haemoglobin: 11.27g/mm^3; haematocrit: 34.675%; erythrocyte count: 1.65$x10^6$/mm^3 of *Heterobranchus longifilis* reported by Erondu *et al*., (1993).According to Atamanalp and Yanık (2002) low Hb levels may impair oxygen supply to the various tissues and result in slow metabolic rate and low energy production. Satheeshkumer, *et al*, (2011) also reported low activity associated with low Hb value which suggests a predisposition to anaemia. The RBC values from both extracted dieatary treatments administered at 1.0g/100g -2.0g/100g were within the of 1.5 x 106 µl and 2.3 - 2.9 x 106 µl described for catfish by Adeyemo *et al.*, 2003 and Gabriel *et al.*, 2004 respectively. Higher RBC (4.81±0.68 $x10^6$/µl) Value found in male fish fed 3.0/100g was higher to the finding of Dada and Ikuerowo (2009) who recorded the value of 3.50 ± 0.35 x 106 µl when ethanoic extracts of Garcinia kola seeds were fed to Clarias gariepinus brood stock.

White blood cells (WBC) and lymphocytes are reported to be the defense cells of the body and Douglas and Jane (2010) demonstrated that the amount present in the body of the animal has implication in immune responses and the ability of the animal to fight infection. High WBC count is usually associated with microbial infection or the presence of foreign body or antigen in the circulating system (Oyawoye & Ogunkunle, 1998). The WBC recorded in the male fish fed Amole diets ranged (1.38±0.89 - 2.85±0.35 $x10^3$/µl) while the female fish range values was (1.38±0.89 - 2.70±0.23 $x10^3$/µl), Whereas the male fish fed the EMOLE diet had WBC range values: 1.57±0.02 - 2.95±0.78 $x10^3$/µl and female fish, 1.68±0.01 - 1.98.00±0.13 $x10^3$/µl. This implies that the additive inclusion levels of the aqueous and ethanolic Moring oleifera leave extracts did not affect the fish nutrition or methabolism. The decreased levels of WBC signifies that there was negligible external/internal stressors and or infection capable of affecting the health of the fish. Comparatively, Tiamiyu *et al.,* 2019 reported that results obtained for WBC which ranged from 11.55 ± 0.29 to 12.93 ± 0.58 x 103 µl in treated groups, show no indication of stimulation of the immune system in response to toxicity of feed additive, or otherwise could be explained that T. triangulare powder added to the feed was not toxic. Often the increase in WBC may be due to recruitment of more cells to combat the stressor (Ajani et al., 2007).

In the present study, the eosinophil and the basophil were significantly higher in female fish than male fish. Conversely, the neutrophil was higher significantly in male fish than female fish fed ethanol extracted MOL

based-diet. The monocyte and lymphocyte did not differ significantly between the male and female fish fed ethanol extracted MOL based-diet. There was no significant difference in the neutrophil among the treatment groups when ethanol extracted MOL was included at 1g/100g, 2g/100g and 3g/100g. The neutrophil in these groups were however significantly lower than those fed 0g/100g ethanol extracted MOL based-diet. Conversely, the lymphocyte was lowest significantly at 0g/100g from the lymphocyte of fish fed 1g/100g, 2g/100g and g/100g ethanol extracted MOL based-diet which were not significantly different from each other. The highest significant basophil was recorded in 2g/100g ethanol extracted MOL based-diet while the lowest recorded in 3g/100g.

Moreover, the Neutrophil mean vales ((54.00±3.21 - 59.67±1.45)) obtained for fish raised with AMOLE diets and the Neutrophil range values (47.00±0.58 - 67.00±0.58) for the EMOLE dietary treated fish in corroboration with (Spielman, 2000) which stated that Neutrophils are the most numerous of all white cells differentials and they make up 65% of the total white blood cell count in the blood of a healthy animal. They provide the host's first line of defense against invading factors especially bacteria and also have the ability to destroy or inactivate fungi, algae, viruses and other parasites as determined by in *vitro* testing. This was closer to the findings of Tiamiyu *et al.*, 2029 which discovered lymphocytes mean value of 49.33 ±5.24% to 70.33±5.24%, adjudged to be numerous than any other differential cells but typical of most fishes according to Owolabi, (2011). The abundance of neutrophils recorded in this fish species compared with the value of monocyte is also typical of most fishes (Owolabi, 2011). The Monocytes values (4.67±0.33- 10.00±0.58 x10-^{2}/µl) obtained in this study were higher than the Monocytes range of Tiamiyu *et al.*, 2019 (2.33± 0.67 to 3.67± 0.88%). This is also typical of most fish species.

CONCLUSION

The results of this research provides the knowledge of the characteristics of haematological indices of African Clariid Catfish, *Heterobranchus bidorsalis* fed with Aqueous and Ethanolic *Moringa oleifera* Leave Extracts as feed additive. Moreover, from our findings it was noted that incorporation of MOLE feed additive did not affect the palatability of the diets as all the experimental diets were accepted by *H. bidorsalis* broodstock and blood of the experimental fish improved. The Red blood cell counts (RBCs), white blood cell counts (WBCs), lymphocytes, MCV, MCH and MCHC recorded among the groups, were within standard for *Heterobranchus bidorsalis*, therefore they were adjudged not to indicate a negative physiological effect on the experimental fish. Blood indices values observed revealed that the varying additive levels of *M. oleifera* leave extracts used had no negative physiological stress on the health status of the fish studied and haematological values observed were seen to be best in the group fed 3.0/100g Aqueous and Ethanolic *Moringa oleifera* leave extract. It could be recommended that 3.0g/100g of MOLE be included in the diet of *Heterobranchus bidorsalis* for boosting of the animal blood and treatment of disease conditions such as anaemia in the fish.

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