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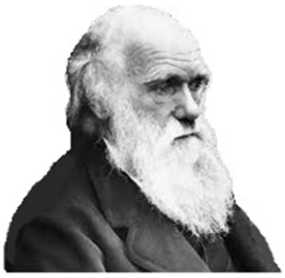

World News of Natural Sciences

An International Scientific Journal

WNOFNS 55 (2024) 50-59 EISSN 2543-5426

Haematological profile of *Clarias gariepinus* juveniles fed *Pueraria phaseoloides* leaf meal inclusion diets

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ABSTRACT

Haematology is used in assessing the health status of man and livestock. A 10-week feeding experiment was conducted to evaluate the haematological profile of *Clarias gariepinus* juveniles fed *Pueraria phaseoloides* leaf meal (PLM) inclusion diets. Five isonitrogenous diets (D1-D5) were formulated to contain 40% crude protein with inclusion of PLM at 0%, 5%, 10%, 15% and 20% levels, respectively. D1 (0% PLM) served as the control diet. One hundred and fifty *C. gariepinus* juveniles of initial mean weight of 79.87 ± 5.85 g were randomly allocated to five dietary groups (G1-G5) and three replicates each and fed with the five PLM formulated diets, respectively, at 5% biomass daily. Fifteen concrete tanks of $1m^3$ volume each were used for the experiment. Each tank was stocked with 10 juveniles. Results showed improved blood profile of fish in all the treatment groups as the inclusion levels of PLM in the formulated diets increased up to the 20% level. The present findings showed that PLM inclusion diets enhanced the blood profile of the experimental fish, hence can be used to ameliorate anemic conditions in African catfish.

***Keywords*:** Aquaculture, blood, fish, growth, nutrient, protein

(Received 08 May 2024; Accepted 03 June 2024; Date of Publication 05 June 2024)

1. INTRODUCTION

Fish is an excellent source of nutrients: providing essential amino acids, essential fatty acids, vitamins, and minerals in an easily accessible form. Global demand for food fish is rising due to population growth, urbanization, and increasing wealth (Godwin et al., 2021b). However, world capture fisheries continue to fall due to overfishing. Aquaculture seems to be a readily available alternative to the provision of food fish for the growing world population. A successful aquaculture business is dependent on the health of the fish and good water quality management. Fish haematology would be helpful in the evaluation of feed suitability, toxic effect of feedstuff, fish health conditions and disease diagnosis (Godwin, 2021). It is essential to develop reference intervals as a first step to determine the particular haematological changes that is linked to disease conditions (Hrubec and Smith, 2004; Akinrotimi et al., 2007). However, there is slow progress in the establishment of normal values of haematological parameters in fish and literature in fish haematology is often scanty.

Clarias gariepinus (African catfish) belongs to the family *Clariidae* and is one of the most popular fish for culture in Nigeria (Adeogun et al., 2007). The reason for preference of the *Clariids* in tropical aquaculture includes hardiness to adverse environmental conditions, the fast growth rate in captivity, easy procurement of seed, adaptation to artificial feed, and high consumer preference in the market (Akinrotimi, 2008; Godwin et al., 2021a).

Pueraria phaseoloides (Tropical kudzu) is a fast-growing plant species that belongs to the family *Fabaceae* and is used as a cover crop in the tropics. It is included in the Global Compendium of Weeds (Randall, 2012) and listed as one of the most aggressive weeds in the tropics (USDA-ARS, 2012). The growing season is all-year-round in the tropics and it does not have a dormant period. It is a twiner and climbs over other plants. It can become an invasive species (Soria *et al*., 2002) due to its fast growth, wide seed distribution, and ability to fully cover other plants. *P. phaseoloides* is ubiquitous and the leaves can easily be prepared as leaf meal (Godwin et al., 2021a).

The present study seeks to investigate the haematological profile of *Clarias gariepinus* juveniles fed diets formulated with varying inclusion levels of *Pueraria phaseoloides* leaf meal.

2. MATERIALS AND METHODS

Collection and preparation of *Pueraria phaseoloides* leaf meal

The *P. phaseoloides* leaf sample was sourced and collected from the premises of African Regional Aquaculture Centre (ARAC), Aluu, Rivers State, Nigeria. The sample was identified at the Department of Plant Science and Biotechnology, University of Port Harcourt with Herbarium Number UPH/V/1460. The leaves were shade-dried at room temperature for three weeks and ground into very fine particles. The ground sample was stored for later usage.

Formulation and preparation of experimental diets

The feed ingredients used in this study were sourced locally from Modern Agro Enterprises, Rumuokoro, Port Harcourt, Rivers State, Nigeria. These include: wheat bran, soya bean meal, fish meal, bone meal, commercial fish premix, lysine, methionine, and vitamin C. Iodized salt, palm oil, and garri (binder) were purchased from Rumuokoro market. All the feed ingredients measured out summed up to 100% as shown in Table 1.

Table 1. Percentage composition of experimental diets

Ingredients	Diet 1 (0% PLM)	Diet 2 (5% PLM)	Diet 3 (10% PLM)	Diet 4 (15% PLM)	Diet 5 (20% PLM)
PLM	0.00	5.00	10.00	15.00	20.00
Wheat Bran	19.76	15.02	10.27	5.54	0.79
Soybean Meal	33.80	33.67	33.54	33.41	33.28
Fish Meal	33.80	33.67	33.54	33.41	33.28
Palm Oil	5.00	5.00	5.00	5.00	5.00
Garri (Binder)	5.00	5.00	5.00	5.00	5.00
Bone Meal	1.50	1.50	1.50	1.50	1.50
Salt	0.50	0.50	0.50	0.50	0.50
Premix	0.25	0.25	0.25	0.25	0.25
Lysine	0.15	0.15	0.15	0.15	0.15
Methionine	0.15	0.15	0.15	0.15	0.15
Vitamin C	0.10	0.10	0.10	0.10	0.10
Total (%)	100	100	100	100	100

PLM = *Pueraria phaseoloides* leaf meal

Five isonitrogenous diets of 40% crude protein were formulated with varying inclusion levels of PLM. The control diet (D1) contained 0% PLM, D2 contained 5% PLM, D3 contained 10% PLM, D4 contained 15% PLM, and D5 contained 20% PLM. The feeds were formulated using Pearson Square method. The dough of each experimental diet was pelletized separately using ARAC pelletizer through a 4mm die to produce pellets and sun-dried separately for 72 hours and more. The dry pellets were stored in air-tight plastic buckets and labeled accordingly.

Project location

The feeding trial was carried out at the African Regional Aquaculture Centre (ARAC), a Department of the Nigerian Institute for Oceanography and Marine Research (NIOMR), Aluu, Rivers State, Nigeria.

Proximate analyses

Proximate analyses of the dry leaf sample and the formulated diets were carried out at the Plant Anatomy and Physiology Research Laboratory, University of Port Harcourt, Rivers State,

Nigeria. Moisture content, ash content, crude protein, crude fat, crude fibre, and total carbohydrates were determined. All analyses followed the Association of Official Analytical Chemists (AOAC) method (2006).

Water quality analyses

Water in all the experimental tanks was sourced from a borehole at ARAC Family Testing Unit. The water quality parameters determined include pH, temperature, dissolved oxygen, ammonia, nitrite, and total hardness. The temperature of the water was taken with a mercury-in-glass thermometer, while pH, dissolved oxygen, ammonia, nitrite, and total hardness were determined using LaMotte Fresh Water Aquaculture Test Kit (Code 3633-05, USA).

Source of experimental fish/acclimatization

One hundred and fifty (150) juveniles of *C. gariepinus* of mean weight 79.87 ± 5.85 g were obtained from the ARAC Catfish Hatchery. The fish were acclimatized for two weeks and fed twice daily with ARAC catfish feed at 5% biomass.

Experimental design, rearing units, and stocking of fish

The design of the experiment was a Completely Randomized Design with five treatment levels and three replicates each. A total of 15 concrete tanks of $1m^3$ volume each at the ARAC Family Testing Unit were used for the experiment. Each tank was stocked with 10 juveniles. A total of 150 juveniles were stocked.

Feeding of experimental fish

The juveniles were handfed twice daily at 09:00 hours and 16:00 hours. The daily ration of 5% biomass was divided into two; half fed to fish each time. The weight of feed fed was adjusted every two weeks to accommodate weight gain by fish. The fish were cultured for 10 weeks.

Collection of blood samples

Blood samples for haematological analyses were collected and preserved in sterile ethylenediaminetetraacetic acid (EDTA) bottles, 15 in number, and labeled based on each experimental treatment for easy identification. EDTA salt was used as the anticoagulant because it produces excellent results with preserved blood (Ariweriokuma et al., 2016). The blood sample was drawn from the caudal vein situated near the vertebrae column in the caudal part of the fish (Godwin, 2021). A hand net was used to capture individual fish from the experimental tanks and blood samples were collected using 5ml disposable syringes and 21-G hypodermic needles.

During blood collection, the head of each fish was covered with a piece of moist cloth for physical restriction with minimal stress (Nwadukwe and Ayinla, 2004; Godwin, 2021). The needle was inserted perpendicularly on the surface of the fish at a point slightly above the openings of the genital papilla. As the needle pierced the fish vein, blood flowed easily into the syringe and 1.5ml of blood was collected and transferred into EDTA bottles. The blood samples were analyzed for haematological parameters at the Lively Stones Medical Laboratory, Choba, Port Harcourt, Rivers State, Nigeria.

3. DATA ANALYSIS

Data obtained from the study were subjected to Analysis of Variance (ANOVA) using SPSS (version 21) and comparisons were done at 0.05 significance level. Values were expressed as mean ± standard deviation (mean ± S.D) of multiple determinations.

4. RESULTS

Proximate composition of the leaf sample

The results of the proximate composition of dry *P. phaseoloides* leaf sample are shown in Table 2. Moisture content was 5.23 ± 0.23 %; ash was 5.35 ± 0.75 %; crude protein was 18.31 ± 2.62 %; crude fat was 7.00 ± 0.00 %; crude fibre was 43.96 ± 4.09 %; and total carbohydrate was 20.15 ± 0.57 %.

Table 2. Proximate composition of *P. phaseoloides* leaf sample

Nutrients	% Composition
Moisture	5.23±0.23
Ash	5.35±0.75
Crude Protein	18.31±2.62
Crude Fat	7.00±0.00
Crude Fibre	20.15±0.57
Carbohydrate	43.96±4.09

Values are mean ± standard deviation of triplicate determinations.

Proximate composition of the formulated diets

Table 3 shows the results of proximate composition of the five formulated diets. Moisture content ranged from 7.75 ± 1.61 % to 10.34 ± 1.55 %; ash ranged from 9.31 ± 0.36 % to 11.35 ± 0.05 %; crude protein ranged from 39.21 ± 0.29 % to 41.44 ± 1.21 %; crude fat ranged from 7.96 ± 0.65 % to 9.45 ± 0.35 %; crude fibre ranged from 5.12 ± 0.65 % to 11.03 ± 1.19 %; and total carbohydrate ranged from 19.45 ± 2.19 % to 26.96 ± 0.90 %.

Physico-chemical analyses of water in the experimental tanks

The water quality parameters in the experimental tanks showed that pH values ranged from 6.61±0.08 to 6.68±0.02. Temperature ranged from 27.27±0.01 ºC to 27.29±0.01 ºC. Dissolved oxygen ranged from 6.59±0.02 mg/l to 6.62±0.01 mg/l. Ammonia ranged from 0.10±0.01 mg/l to 0.12±0.02 mg/l. Nitrite ranged from 0.29±0.01 mg/l to 0.30±0.02 mg/l. Total hardness ranged from 43.45±0.01 mg/l to 43.48± 0.02 mg/l (Table 4).

Table 3. Proximate composition of the formulated diets

Sample Identity	% Moisture	% Ash	% Crude Protein	% Crude Fat	% Crude Fibre	% Carbohydrate
D1 (control)	7.75±1.61[a]	11.35±0.05[a]	39.37±0.87[a]	9.45±0.35[a]	5.12±0.65[a]	26.96±0.90[a]
D2	10.34±1.55[a]	10.74±0.06[a]	39.21±0.29[a]	7.97±0.15[a]	6.48±3.02[a]	25.26 ±1.54[a]
D3	9.37±2.72[a]	9.91±0.24[a]	41.07±1.61[a]	7.96±0.65[a]	7.79±1.54[a]	23.90 ±1.15[b]
D4	9.68±0.00[a]	9.31±0.36[b]	39.69±1.31[a]	8.80±0.70[a]	9.72±0.98[b]	22.80±1.13[b]
D5	9.04±0.55[a]	10.76±1.80[a]	41.44±1.21[a]	8.28±1.68[a]	11.03±1.19[b]	19.45±2.19[b]

Values are mean ± S.D. of triplicate determinations. Values with similar superscript letters along the same column are not significantly different ($p>0.05$) compared to the control (D1).

Table 4. Physico-chemical analysis of water in the experimental tanks

Parameters	Experimental Tanks				
	Tank 1 (control)	**Tank 2**	**Tank 3**	**Tank 4**	**Tank 5**
pH	6.68±0.02[a]	6.66±0.01[a]	6.61±0.08[a]	6.62±0.03[a]	6.62±0.02[a]
Temperature (ºC)	27.27±0.01[b]	27.28±0.01[b]	27.28±0.02[b]	27.29±0.01[b]	27.29±0.01[b]
Dissolved Oxygen (mg/l)	6.62±0.01[c]	6.59±0.02[c]	6.60±0.02[c]	6.59±0.02[c]	6.60±0.02[c]
Ammonia (mg/l)	0.10±0.01[d]	0.11±0.02[d]	0.12±0.02[d]	0.12±0.02[d]	0.12±0.02[d]
Nitrite (mg/l)	0.29±0.01[e]	0.29±0.01[e]	0.29±0.01[e]	0.30±0.02[e]	0.29±0.02[e]
Total Hardness (mg/l)	43.46±0.02[f]	43.48±0.02[f]	43.47±0.02[f]	43.47±0.03[f]	43.45±0.01[f]

Values are mean ± S.D. of triplicate determinations. Values with similar superscript letters along the same row are not significantly different ($p>0.05$) compared to the control (Tank 1).

Haematological parameters of *C. gariepinus* juveniles fed the formulated diets

Table 5 shows the haematological parameters of the experimental fish fed the formulated diets for 10 weeks. Packed cell volume (PCV) ranged from 36.00 ± 3.61 % to 52.00 ± 2.00 %; haemoglobin (Hb) ranged from 12.00 ± 1.18 g/dl to 17.33 ± 0.65 g/dl; red blood cells (RBC) counts ranged from $5.27 \pm 0.68 \times 10^{12}$ to $6.27 \pm 0.40 \times 10^{12}$; white blood cell (WBC) counts ranged from $9.73 \pm 2.05 \times 10^{9}$ to $11.47 \pm 1.86 \times 10^{9}$; platelets (PLA) counts ranged from $210.00 \pm 7.00 \times 10^{9}$ to $266.33 \pm 10.23 \times 10^{9}$; neutrophils (NEU) ranged from 24.33 ± 6.03 % to 49.33

± 3.21 %; lymphocytes (LYM) ranged from 46.67 ± 4.16 % to 64.00 ± 5.29 %; eosinophils (EOS) ranged from 2.67 ± 0.58 % to 4.00 ± 1.73 %; monocytes (MON) ranged from 6.00 ± 1.73 % to 8.33 ± 1.53 %; whereas basophils (BAS) were absent in the blood of the fish in all the dietary groups.

Table 5. Haematological parameters of *C. gariepinus* juveniles fed the formulated diets for 10 weeks

Parameters	Group 1 (control)	Group 2	Group 3	Group 4	Group 5
PCV (%)	36.00±3.61^{a}	42.00±2.65^{b}	45.00±2.00^{b}	48.00±2.00^{b}	52.00±2.00^{b}
Hb (g/dl)	12.00±1.18^{a}	14.00±0.89^{b}	15.00±0.70^{b}	16.00±0.70^{b}	17.33±0.65^{b}
RBC (x10^{12})	5.27±0.68^{a}	5.93±0.40^{a}	6.10±0.36^{b}	6.13±0.15^{b}	6.27±0.40^{b}
WBC (x10^{9})	9.73±2.05^{a}	9.87±0.76^{a}	10.13±1.10^{a}	11.27±1.97^{a}	11.47±1.86^{a}
PLA (x10^{9})	210.00±7.00^{a}	228.33±16.20^{a}	233.33±11.72^{a}	245.33±22.19^{b}	266.33±10.23^{b}
NEU (%)	24.33±6.03^{a}	35.67±4.04^{a}	38.00±6.56^{a}	44.00±3.61^{a}	49.33±3.21^{a}
LYM (%)	46.67±4.16^{a}	50.00±2.00^{a}	53.00±6.56^{a}	56.00±5.29^{b}	64.00±5.29^{b}
EOS (%)	2.67±0.58^{a}	3.00±0.00^{a}	3.00±1.00^{a}	4.00±1.00^{a}	4.00±1.73^{a}
MON (%)	6.00±1.73^{a}	6.67±1.53^{a}	7.67±0.58^{a}	7.67±0.58^{a}	8.33±1.53^{a}
BAS (%)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Values are mean ± standard deviation (n=10). Values with similar superscript letters along the same row are not significantly different (p>0.05) compared to the control (Group 1).

5. DISCUSSION

Physico-chemical analysis of water in the experimental tanks

Fishes completely depend on water for feeding, respiration, excretion, growth, reproduction, etc. Thus, a successful aquaculture business is dependent on good water quality management plus fish health (Godwin, 2021). In the present study, the water quality parameters showed no significant differences (p>0.05) in all the experimental tanks. Therefore, whatever differences observed in the performance of the experimental fish was not a result of the culture water.

Proximate composition of the formulated diets

The moisture content, crude protein, and crude fat showed no significant differences (p>0.05) in all the experimental diets. Hence, the formulated diets were isonitrogenous and contained the same amount of crude protein, which is the most important nutrient in fish feed

as it helps the fish to grow to its full potentials. However, ash content, crude fibre, and total carbohydrate varied significantly ($p<0.05$) across the dietary groups. In the present study, all the formulated diets were accepted by *C. gariepinus* juveniles, which indicated that the levels of inclusion of *P. phaseoloides* leaf meal did not affect the palatability of the diets.

Haematological parameters of *C. gariepinus* juveniles fed the formulated diets

Packed cell volume (PCV), haemoglobin (Hb), red blood cells (RBC) counts, white blood cells (WBC) counts, platelets (PLA) counts, neutrophils (NEU), lymphocytes (LYM), eosinophils (EOS), and monocytes (MON) values increased as the inclusion levels of PLM in all the treatment groups increased; whereas basophils (BAS) were absent in the blood of the fishes in all the dietary groups. PCV and Hb values in groups 2, 3, 4, and 5 were significantly different ($p<0.05$) compared to the control values. RBC values in groups 3, 4, and 5 varied significantly ($p<0.05$) to the control value. PLA and LYM values in groups 4 and 5 were significantly different ($p<0.05$) compared to the control values. However, there were no significant differences ($p>0.05$) in the WBC, NEU, EOS, and MON values compared to the control values.

Certain variables (age, sex, diet, stress, environment, etc.) can alter blood parameters in fish and further confound the haematological data (Ariweriokuma et al., 2016; Godwin, 2021). In the present study, as the inclusion levels of PLM in the formulated diets varied, the blood parameters of the experimental fish were altered. Hence, the present study was in line with the findings of Ariweriokuma et al. (2016).

Packed cell volume (PCV) is the level of erythrocytes in the whole blood, whereas haemoglobin (Hb) is the oxygen-carrying pigment of the red blood cells (RBCs). PCV and Hb are useful in anaemia diagnosis (Brown, 2002). Increased PCV, Hb and RBCs values in the blood of fish fed the PLM inclusion diets indicated increased oxygen circulation in the tissues of the fish for aerobic respiration and oxidative phosphorylation. Also, it is known that erythrocytes are dependable and most important stress indicators in fish; based on earlier haematological studies of dietary effects (Osuigwe et al., 2005) and pollutants (Gabriel et al., 2007c), stress is known to decrease the erythrocytes content of fish blood. Hence, increased erythrocytes content as observed in the present study showed that PLM functions as anti-stress indicator in the experimental diets.

According to Ezeri et al. (2004), PCV appeared to be a sensitive indicator of vitamins status more than growth or survival rates; it is also an easy way to measure the present vitamin status of fish that may be used to predict future growth performance. Deficiencies in essential fatty acids, vitamins and minerals are known to decrease erythrocytes count, packed cell volume and haemoglobin. Hence, increased RBCs, PCV and Hb as observed in the present study showed that the formulated diets were rich in essential fatty acids, vitamins and minerals.

6. CONCLUSION

In the present study, all the PLM inclusion diets were accepted by *C. gariepinus* juveniles, which showed that the levels of inclusion of the leaf meal did not affect the palatability of the diets. Also, all the experimental fish fed PLM inclusion diets showed improved blood profile compared to those fed the control diet. Considering the all-year-round availability of *P. phaseoloides* leaf, its cheapness, high protein content, and the easy method of its preparation,

the need to include PLM in the diets of *C. gariepinus* is highly imperative. Since the haematological profile of fish fed D5 (containing 20% PLM) were better than those fed the control diet (D1, containing 0% PLM), the results showed that PLM can be added as a protein ingredient in *C. gariepinus* feed up to 20% level without compromising feed quality and acceptability, and can be used to ameliorate anaemic conditions in African catfish.

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