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Haematological profile of *Heterobranchus bidorsalis* fingerlings fed processed *Delonix regia* seeds at different inclusion levels of diets

Oyegbile B.[1]*, Abdullahi B. A.[2] and Yola I. A.[2]

[1]Nuhu Bamalli Polytechnic, Zaria, Kaduna State, Nigeria.
[2]Bayero University Kano, Kano State, Nigeria.

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This study aimed at investigating the haematological profile of *Heterobranchus bidorsalis* fingerlings fed processed *Delonix regia* seeds at different inclusion levels of diets. Ten isonitrogenous diets (40% crude protein) were formulated with processed *D. regia* seed at 0% (Control), 10, 20 and 30% inclusion, respectively. The parameters analysed were pack cell volume (PCV), red blood cell (RBC), white blood cell (WBC), hemoglobin (HB), mean corpuscular volume (MCV), mean corpuscular heamoglobin (MCH) and mean corpuscular heamoglobin concentration (MCHC). Different among the groups were tested using analysis of variance. Raw *Delonix regia* seed meal had significant effect (P<0.05) on RBC, HB, MCV, MCH and MCHC respectively across the dietary treatments. RBC, MCV, MCH and MCHC differs significantly (P<0.05) across the dietary treatments for fish fed fermented *D. regia* seeds. All the haematological parameters differ significantly (P<0.05) across the dietary treatments with the exception of PCV, MCV and MCHC respectively for fish fed cooked *D. regia* seeds. It is therefore concluded that significant variations exist among the processing methods on the health status of the fish. It is recommended that inclusion of *D. regia* up to 20% will have no deleterious effect on their health status.

Key words: Haematological profile, *Delonix regia*, processing methods, *Heterobranchus bidorsalis*, inclusion levels.

INTRODUCTION

Fish is an important source of high quality protein, providing approximately 16% of the animal protein consumed by the world's population (FAO, 1997). Fish evolved after several years of genetic improvement, and their relevance and success as a relatively cheaper and steady source of animal protein hinges on their higher carcass yield. Much progress in the productivity indices of fish are now achieved through improvement of several environmental factors regarding their growth, health and maintenance. Among the Clariidae family, *Heterobranchus bidorsalis* is the second most important aquaculture species in Nigeria (Vanden Bossche and Bernacsek, 1990). *H. bidorsalis* is endemic to Africa and recent interest in culturing its species has been rising. *H.*

*Corresponding author. E-mail: bigm4real@gmail.com. Tel: +2348023741725.

bidorsalis, an active swimmer and predator (Fagbenro, 1992) which like all chariid catfishes is capable of aerial respiration. This species is found in turbulent or fast running streams, it feeds on fish, molluscs and alarming number of people, mostly in developing crustaceans. It attains a considerable size of over 120 cm. Physiological, including hematological, behavioral and biochemical parameters are useful diagnostic tools in the practice of veterinary medicine (Lemma and Moges, 2009). Haematological parameters are good indicators of the physiological status of animals under different conditions (Ambore et al., 2009). Heamatological studies are important when evaluating fish health diagnostically just as it is important in human health. Sampath et al. (1993) observed that studies on fish blood could reveal conditions within the body of fish long before an outward manifestation of disease or stress condition. Many hematological parameters can be used to assist in providing evidence and possible identification of any abnormality or disease condition. Hematological parameters have been acknowledge as valuable tools for monitoring fish health, confirming maturation and monitoring any changes in the quality of feed, water and related soil (Kumar et al., 2011). Low heamatological indices are indications of anemic conditions (Haruna and Adikwu, 2001). The quest for more economically viable, palatable and environmentally friendly feed among the fish farmers is highly desirable. This has redirected research interests toward the use of unconventional protein sources especially from plant products like leaves, seeds and other agricultural by products (Ali et al., 2003; Bake et al., 2009). In Nigeria, the high cost of formulated commercial fish feed is a major constraint to the growth and expansion of the aquaculture sector and this has prompted a concerted effort to seek for alternative feed ingredients. Hence, the objective of this study was to evaluate the haematological profile of *H. bidorsalis* fingerlings fed processed *Delonix regia* seeds at different inclusion levels of diets.

MATERIALS AND METHODS

The study was conducted at the aquaculture production technology unit of the skill acquisition and development centre, National Agricultural Extension and research Liason Services, Ahmadu Bello University, Zaria, located at latitude 11° 09 45.2 N and longitude 7° 38 17.9 E.

Matured and dry pods of *D. regia* containing the seeds were collected from the annex campus of Nuhu Bamalli Polytechnic Zaria. Seeds were collected by opening the pods manually.

Fermentation of *D. regia* seeds

The seeds were soaked in water for 12 h. The drained soaked seeds were allowed to ferment naturally by tying in polythene bag and kept in a dark cupboard for 72 h without the addition of yeast (Udensi et al., 2006). The fermented seeds were allowed to air dry for two days before grinding into homogenous powder using a hammer mill.

Cooking of *D. regia* seeds

The seeds were boiled at 100°C for 80 min and were allowed to cool by sun drying and later grounded to homogenous powder using a hammer mill (Bake et al., 2013).

Raw *D. regia* seeds

The raw seeds were sundried for two days and were milled into a homogenous powder using a hammer mill.

Determination of haematological parameters of experimental fish

At the end of 26 weeks of feeding trials, a total of ninety fishes were randomly selected from the ten treatments used in this study. Nine fish was selected per treatment. The blood was collected from live fish by putting it on a tray. It was handled carefully to minimize stress. A damp cloth was used to cover the head of the fish. Blood was collected in the morning hours to avoid diurnal variation. The blood was drawn from the caudal vein using syringe. The collected blood was transferred from the syringe into an anti- coagulant, ethylene diaminetetraacetic acid (EDTA) bottles for heamatological analysis.

Heamatological procedures

All the heamatological parameters were determined using standard techniques. The heamatological parameters determined include red blood cell count (RBC), white blood cell count (WBC), packed cell volume (PCV) and hemoglobin (HB).

Determination of red blood cell rbc and white blood cell (WBC) counts

Red blood cell counts (RBC) and white blood cell count (WBC) were determined by use of the Neubeur improved counting chamber (Kelly, 1979). The blood was diluted 1:200 with Dacies fluid (99 ml of 3% aqueous solution of sodium citrate and 1 ml of 40% formal dehydrate). This keeps and preserves the shape of red blood cell which was then estimated using the counting chamber for RBC. For the total white blood cell count the dilution was 1:20 using 2 to 3% aqueous solution of acetic acid to which a tinge of Gentian violet was added. The blood smear was stained using Wright-Giemsa stain, a total of 100 white blood cells were enumerated and differentiated (Schalm et al., 1975).

Determination of packed cell volume (PCV)

The packed cell volume was determined using a micro heamatocrit centrifuge. The blood was placed into capillary tubes and filled to ¾ of the tubes; one end was sealed with plasticine. They were centrifuged for 5 min at 12,000 rpm. The PCV was read by the use of heamatocrit reader.

Estimation of heamoglobin (HB)

The heamoglobin was estimated using the Cyanomethaemoglobin method as described by Schalm et al. (1975) and Kelly (1979). 0.02 ml of blood was added to 4 ml of modified Dabkin's solution (Potassium ferricyanide - 200 mg; potassium cyanide - 50 mg, potassium dihydrogen phosphate - 140 mg).

Table 1. Means ±standard error of heamatological profile in *Heterobranchus bidorsalis* fed raw *Delonix regia* seeds meal at different inclusion levels of diet.

Parameter	0% (Control)	R_{10}	R_{20}	R_{30}	LOS
PCV (%)	51.00±1.00^{a}	49.6±0.10^{a}	49.4±0.53^{a}	49.0±2.65^{a}	0.41^{ns}
RBC ($10^{6}mm^{-3}$)	4.20±0.20^{a}	3.13±0.15^{b}	3.00±1.00^{b}	2.60±0.20^{b}	0.03*
WBC (10^{9}/L)	6.80±0.60^{a}	6.00±1.00^{a}	6.23±0.25^{a}	6.87±0.35^{a}	0.31^{ns}
HB (g/dL)	16.00±1.00^{a}	14.80±0.20^{ab}	14.40±0.40^{b}	14.20±0.80^{b}	0.05*
MCV (fl)	121.43±0.77^{c}	160±10.00^{b}	164.67±6.42^{b}	188.46±0.04^{a}	<0.0001**
MCH (Pg)	38.10±0.10^{c}	47.74±0.02^{b}	48.00±2.00^{b}	54.62±0.02^{a}	<0.0001**
MCHC (g/dL)	31.37±0.03^{a}	29.84±0.03^{b}	29.15±0.03^{c}	28.98±0.03^{d}	<0.0001**

^{abc}Means with different superscripts across the treatments differs significantly (P<0.05). ns, Not significant; PCV, packed cell volume; WBC, white blood cell; RBC, red blood cell; HB, haemoglobin; MCV, mean cell volume; MCH, mean cell haemoglobin; MCHC, mean corpuscular haemoglobin concentration.

The volume was made up to 1 L with distilled water. The mixture was allowed to stand for 3 min and the HB concentration was read photonutrically by comparing with a cyanomethaemoglobin standard with a yellow- green filter at 625 mm.

Erythrocytes indices which include mean corpuscular volume (MCV), mean corpuscular heamoglobin (MCH) and mean corpuscular heamoglobin concentration (MCHC) were calculated using the following formulae (Jain 1986; Adeyemo et al., 2007).

$$\text{MCV (fl)} = \frac{\text{PCV}}{\text{RBC}} \text{ X } 10$$

$$\text{MCH (pg)} = \frac{\text{Hb}}{\text{RBC}} \text{ X } 10$$

$$\text{MCHC (\%)} = \frac{\text{Hb (100mg blood)}}{\text{PCV}} \text{ X } 100$$

Determination of physicochemical parameters of experimental water

Water temperature was recorded daily in the morning using thermometer. Hydrogen ion concentration (pH) was taken with a pH meter model pH - 009(111). Dissolved oxygen (DO) was recorded using a dissolved oxygen model meter - DO 510. Turbidity of the water was determined by the method of AOAC (2003).

Statistical model

Model equation for analysis of variance used in this study includes:

$Y_{ijkl} = \mu + T_i + W_{ij}$

Where, μ is the effect population mean; T_i is the effect of treatments (Processing methods = cooking and fermentation), and W_{ij} is the random error associated with the record heamatology of the l^{th} fish.

Data analysis

Data obtained were subjected to one way analysis of variance (ANOVA) using general linear model (GLM of SAS 9.2, 2008). Duncan multiple range test (DMRT) was used to test difference between levels of means and mean separation was considered significant at P<0.05.

RESULTS

Table 1 shows the effect of raw *D. regia* at different inclusion levels of diets on the haematological parameters of *H. bidorsalis* fish. Raw *D. regia* seed meal had significant effect (P < 0.05) on RBC, HB, MCV, MCH and MCHC respectively across the dietary treatments. PCV and WBC were statistically similar (P > 0.05) across the dietary treatments. The fish fed control diet had higher numerical values (51.00 ± 1.00%) as compared to other dietary treatments for packed cell volume. The control group had significantly (P < 0.05) the highest volume of red blood cell as compared to R_{10}, R_{20} and R_{30} respectively. Fish fed raw *D. regia* seed meal at 30% inclusion level had highest concentration of WBC (6.87± 0.35) while the least concentration was recorded in R_{10} (6.00 ± 1.00 10^{9}/L).

Haemoglobin levels were higher in fish fed the control and R_{10} diets (16.00 ± 1.00 and 14.80 ± 0.20 g/dl) which were statiscally different (P < 0.05) from fish fed raw *D. regia* at 20 and 30% inclusion levels 14.40 ± 0.40 and 14.20 ± 0.80 g/dl). Fish fed raw *D. regia* at 30% inclusion level had the highest quantity of MCV (188.46±0.04 fl) while the control group recorded the least value (121.43 ± 0.77 fl). R_{30} had the highest concentration of MCH (54.62 ± 0.02) which was statistically significant (P < 0.05) from the control, R_{10} and R_{20} respectively. Fish fed the control diet (31.37 ± 0.03 g/dl) had the highest concentration of MCHC which was statistically different from control, R_{10}, R_{20} and R_{30}, respectively.

The effect of fermented *D. regia* seed meal at different inclusion levels of diet on the haematological parameters of *H. bidorsalis* are shown in Table 2. RBC, MCV, MCH and MCHC differs significantly (P < 0.05) across the dietary treatments. PCV, WBC and HB were statistically similar across the dietary treatments.

Fish fed fermented *D. regia* seed meal at 10% inclusion level had higher numerical concentration of PCV (51.50 ± 0.20%), WBC (7.00 ± 1.00 10^{9}/L) and HB (17.27 ± 0.35 g/dL) across the dietary treatments, though they were similar (P > 0.05) across the treatments.

Table 2. Means ±standard error of heamatological profile in *H. bidorsalis* fed fermented *D. regia* seeds meal at different inclusion levels of diet.

Parameter	0% (Control)	F_{10}	F_{20}	F_{30}	LOS
PCV (%)	51.00±1.00^{a}	51.50±0.20^{a}	51.10±0.10^{a}	50.00±5.00^{a}	0.90^{ns}
RBC (10^6mm^{-3})	4.20±0.20^{a}	4.27±1.15^{a}	4.20±0.10^{a}	3.60±0.05^{b}	<0.0001**
WBC (10^9/L)	6.80±0.60^{a}	7.00±1.00^{a}	6.67±0.35^{a}	6.00±0.50^{a}	0.34^{ns}
HB (g/dL)	16.00±1.00^{a}	17.27±0.35^{a}	16.60±0.40^{a}	16.33±0.65^{a}	0.19^{ns}
MCV (fl)	121.43±0.77^{b}	119.77±0.03^{c}	121.67±0.03^{b}	138.89±0.04^{a}	<0.0001**
MCH (Pg)	38.10±0.10^{d}	40.23±0.07^{b}	39.52±0.08^{c}	45.28±0.04^{a}	<0.0001**
MCHC (g/dL)	31.37±0.03^{c}	33.59±0.50^{a}	32.29±0.03^{b}	32.63±0.06^{b}	<0.0001**

^{abc}Means with different superscripts across the treatments differs significantly (P<0.05). ns, Not significant; PCV, packed cell volume; WBC, white blood cell; RBC, red blood cell; HB, haemoglobin; MCV, mean cell volume; MCH, mean cell haemoglobin; MCHC, mean corpuscular haemoglobin concentration.

Table 3. Means ±standard error of heamatological profile in *H. bidorsalis* fed cooked *D. regia* seeds meal at different inclusion levels of diet.

Parameter	0% (Control)	C_{10}	C_{20}	C_{30}	LOS
PCV (%)	51.00±1.00^{b}	52.50±0.25^{a}	52.37±1.00^{a}	52.00±1.00^{a}	0.01**
RBC (10^6mm^{-3})	4.20±0.20^{a}	4.40±0.30^{a}	4.30±0.20^{a}	4.30±0.20^{a}	0.82^{ns}
WBC (10^9/L)	6.80±0.60^{a}	6.40±0.20^{a}	6.77±0.25^{a}	6.90±0.20^{a}	0.39^{ns}
HB (g/dL)	16.00±1.00^{b}	17.6±0.40^{a}	17.6±1.00^{a}	17.20±0.30^{ab}	0.09^{ns}
MCV (fl)	121.43±0.77^{ab}	119.32±0.03^{c}	121.86±0.04^{a}	120.93±0.03^{b}	0.0002**
MCH (Pg)	38.10±0.10^{a}	40.0±5.00^{a}	40.93±0.04^{a}	40.0±1.00^{a}	0.60^{ns}
MCHC (g/dL)	31.37±0.03^{d}	33.52±0.03^{b}	33.59±0.02^{a}	33.08±0.02^{c}	<0.0001**

^{abc}Means with different superscripts across the treatments differs significantly (P<0.05). ns, Not significant; PCV, packed cell volume; WBC, white blood cell; RBC, red blood cell; HB, haemoglobin; MCV, mean cell volume; MCH, mean cell haemoglobin; MCHC, mean corpuscular haemoglobin concentration.

Fish fed the control diet was significantly ($P < 0.05$) higher (4.20 ± 0.20 10^6 mm^{-3}) than fish fed fermented *D. regia* seed meal at 10, 20 and 30% inclusion levels respectively. Fish fed 30% inclusion level of fermented *D. regia* seed meal had the highest concentration of MCV (138.39) and MCH (45.28 ± 0.04 Pg) which differs significantly from other dietary treatments. Fish fed 10% inclusion level of fermented *D. regia* seed meal had significantly ($P < 0.05$) higher concentration (33.59 ± 0.50 g/dL) of MCHC from fish fed control diet, F_{20} and F_{30} respectively.

The effect of cooked *D. regia* seed meal at different inclusion levels of diets on the haematological parameters of *H. bidorsalis* are presented in Table 3. All the haematological parameters differs significantly ($P < 0.05$) across the dietary treatments with the exception of PCV, MCV and MCHC respectively. The highest concentration of PCV was recorded in the fish fed cooked *D. regia* seed meal at 10, 20 and 30% inclusion levels respectively which were statistically different ($P < 0.05$) from the control (51.0± 1.00%). C_{20} and the control group had the highest levels of MCV (121.86 ± 0.04 and 121.43 ± 0.77 fl) while C_{10} recorded the least concentration (119.32± 0.03 fL). Fish fed cooked *D. regia* seed meal at 20% inclusion levels had the highest quantity (33.59 ± 0.02 g/dL) of MCHC which was statistically different ($P < 0.05$) from fish fed diets containing cooked *D. regia* seed meal at 0, 10 and 30% inclusion levels.

DISCUSSION

The use of heamatological parameters for on the spot assessment of health status of few tropical African catfish species are well documented (Fagbenro et al., 2013; Etim et al., 1999). The result of PCV (49.0 to 52.50%) obtained in this study were higher than the 28.3 to 29.5% reported by Gayatri and Prafulla (2012) for *Claris batrachus.* The difference could be due to differences in species and *H. bidorsalis* seems to have more blood volume than the other species. The result of the RBC count of this work which ranged between 2.60 × 10^6/mm^3 and 4.40 × 10^6 mm^3 was within the range of 2.41 to 2.89 × 10^6 mm^3 reported by Gayatri and Prafulla (2012) but lower than the range of 5.05 ± 0.17 × 10^6 to 5.2 ± 0.26 × 10^6 mm^3 as reported by Onyia et al. (2013).

The higher values of RBC count in the fish fed cooked and fermented *D. regia* seeds could be linked to the

higher activity of the seeds during processing which degraded the antinutritional factors in the seed. The white blood cell count in this study (6.00 - 7.00 × 10^3/mm of blood) was lower than the range of 8.59 ± 0.27 × 10^3 and 9.71 ± 0.43 × 10^3/mm of blood reported by Gayatri and Prafulla (2012) in *Clarias betrachus* (Linnaeus 1758).

The result of Hb count (14.20 - 17.60 × 10^3/mm of blood was higher than the 8.70 g/100 ml for *Clarias gariepinus* (Sowunmi, 2003; Gayatri and Prafulla, 2012). The MCV value reflects the size of red blood cells by expressing the volume occupied by a single red blood cell. The higher MCV and MCH values in fish fed raw *D. regia* seed meal as compared to fish fed cooked and fermented *D. regia* seed meal indicates higher likelihood of occurrence of macrocytic anaemia in fish fed raw *D. regia* seed. The range of 28.98 to 33.59 g/dl in HB was similar to the range of 32.41 ± 0.40 to 32.79 ± 0.59) in male and female *C. batrachus* as reported by Gayatri and Prafulla (2012). The higher concentration of MCHC in the fish fed cooked and fermented *D. regia* seeds implies more HB in a unit of RBCs (Robbins, 1974) as compared to fish fed raw *D. regia* seed meals.

Conclusion

It is therefore concluded that significant variations existed among the processing methods on the health status of the fish, though values were within the normal range reported for healthy fish. It is recommended that inclusion of *D. regia* up to 20% will have no deleterious effect on their health status.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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