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Effects of Omega-3 Fatty Acids in Fish Feeds on Haematological Profile of *Heterobranchus bidorsalis* (Geoffrey St. Hillaire, 1809)

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ABSTRACT: The effect of different levels of inclusion of omega-3 fatty acid in fish feeds on haematological profile of *Heterobranchus bidorsalis* fish was investigated. One hundred and forty juveniles of *H. bidorsalis* with mean weight 32.02±10g and total length 15.2±3.5cm purchased from a commercial fish farm were used for the study. After acclimation, ten fish each were separated into four tanks labelled A_1, A_2 & A_3 (control), B_1, B_2 & B_3, C_1, C_2 & C_3 and D_1, D_2 & D_3 representing two replicates respectively. *H. bidorsalis* fed different levels (1000mg, 2000mg and 3000mg) of omega-3 fatty acid in fish feeds showed a gradual reduction in values of haematological parameters with increasing levels of omega-3 in fish feeds. Fish fed on feeds containing 3000mg omega-3 fatty acid had significantly ($P<0.05$) lower PCV, Hb level, RBC and WBC counts in comparison with other levels of treatment and control. Fish fed on feeds containing 2000mg and 1000mg omega-3 had significantly ($P<0.05$) lower RBC and WBC counts as compared with control. However, a slight but not significant ($P>0.05$) increase in PCV was observed in fish fed 1000mg omega-3 when compared with controls. Fish in control tank had higher values of haematological parameters than fish fed on feed containing omega-3. Mean corpuscular volume (MCV) was lower and mean corpuscular haemoglobin (MCH) was significantly ($P<0.05$) higher in fish fed on feeds containing omega-3 than in control fish. The mean corpuscular haemoglobin concentration (MCHC) of *H. bidorsalis* was similar in the control and treatment fish. The inclusion of omega-3 in fish feeds led to anaemic conditions which may result in reduced immunity in fish.

Key words: haematological profile, fish, omega-3 fatty acid.

INTRODUCTION

The paucity of reliable reference on the normal condition of fish has been one of the difficulties in assessing the state of health of natural fish populations. The physiology of haematological parameters has been considered as an index to the general health status in a number of fish species [1]. Changes in haematological variables have continued to be valuable tool in the clinical diagnosis of fish to determine the effect of external stressors. The determination of haematological values of fish is carried out for a variety of purposes especially to establish a normal range of blood parameters and to investigate any alterations in blood values [2]. It has been reported that haematological data could vary with strain, temperature, season of the year, diet and nutritional status of fish [3]. Fish diet rich in essential nutrients such as fatty acids ensure proper growth of fish [4].

Cat fish has been noted to have the ability to synthesize most of their fatty acid requirements except for the essential fatty acids which they require in small amounts [5]. Fish has been observed to display reduced growth, survival and poor food conversion efficiency

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when fed experimental diets deficient in essential fatty acids [6]. However, the incorporation of fish oil, rich in omega-3 fatty acid, into fish feeds has helped to promote fish growth and provide energy [4, 7]. Fatty acids can be utilized to substitute protein in aquaculture feeds serving as transporters for fat soluble vitamins [8]. Addition of omega-3 fatty acid in fish feeds enhances the immunity of fish. Omega-3 fatty acids have been observed to stimulate blood circulation, increase the breakdown of fibrin to improve clotting formation and reduce mortality rate [9]. Regular monitoring of haematological parameters of farmed fish can therefore be used to enhance fish production. Though the essential fatty acid requirement of cat fish and most warm water fish has not been precisely defined, this study investigates the effect of different levels of omega-3 fatty acid in fish feeds on haematological parameters of fish.

MATERIALS AND METHODS

One hundred and forty juveniles of *Heterobranchus bidorsalis* fish with mean weight 32.02±10g and having total length 15.2±3.5cm purchased from Aviara Fish Farm in Isoko South, Nigeria was used for the study which lasted six weeks. Fish samples were acclimated for seven (7) days in stock tank of 6ft x 4ft x 4ft dimension containing 120litres of borehole water.

Table 1: Composition of fish feeds for feeding juveniles of *H. bidorsalis* in different tanks.

Ingredients	Tanks $A_1A_2A_3$ (control)	Tanks $B_1B_2B_3$	Tanks $C_1C_2C_3$	Tanks $D_1D_2D_3$
Maize (g)	4.60	4.60	4.60	**4.60**
GNC (g)	24.00	24.00	24.00	**24.00**
Soya bean cake (g)	22.00	22.00	22.00	**22.00**
Palm kernel cake (g)	13.00	13.00	13.00	**13.00**
Wheat offal (g)	9.80	9.80	9.80	**9.80**
Fish meal (g)	24.10	24.10	24.10	**24.10**
Bone meal (g)	1.00	1.00	1.00	**1.00**
Starch (g)	1.00	1.00	1.00	**1.00**
Lysine (g)	0.20	0.20	0.20	**0.20**
Methionine(g)	0.20	0.20	0.20	**0.20**
Vitamin premix (g)	0.10	0.10	0.10	**0.10**
Omega-3 Fatty acid (mg)	**-**	**1000**	**2000**	**3000**

Ten fish each were separated into smaller tanks labelled Tanks A_1, A_2 & A_3 (control), Tanks B_1, B_2 & B_3, Tanks C_1, C_2 & C_3 and Tanks D_1, D_2 & D_3 representing two replicates respectively. Two millilitres of blood was drawn from the posterior caudal vein of fish and dispensed into tubes containing lithium heparin anti-coagulant. Haematological parameters of sample fish in all experimental tanks were determined. Blood parameters investigated were packed cell volume (PCV), red blood cell (RBC), white blood cell (WBC) total, haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) [10, 11]. Fish feeds containing different concentrations of omega-3 fatty acid were compounded as in table 1. Data obtained were analysed using analysis of variance at P=0.05. Significant means were separated with Duncan Multiple Range Test (DMRT) using SAS [12].

RESULTS

Heterobranchus bidorsalis-fed different levels (1000mg, 2000mg and 3000mg) of omega-3 fatty acid in fish feeds show variations in values of haematological parameters as compared with values in fish-fed feeds without omega-3 (tables 2 and 3). A gradual reduction in values of haematological parameters was observed with increasing levels of omega-3 fatty acid in fish feeds. Fish fed on 3000mg omega-3 in fish feeds had significantly ($P<0.05$) lower PCV, Hb level, RBC and WBC counts in comparison with other levels of treatment and control (table 4). Fish fed on fish feeds containing 2000mg and 1000mg omega-3 had significantly ($P<0.05$) lower RBC and WBC counts as compared with control. However, a slight but not significant ($P>0.05$) increase in PCV was observed in fish fed on fish feeds containing 1000mg omega-3 when compared with controls. Fish in control tank had higher values of haematological parameters than fish fed feeds containing omega-3. The haematological indices of *H. bidorsalis* juveniles before and four weeks after feeding with fish feeds containing omega-3 are presented in tables 5 and 6. The haematological indices of *H. bidorsalis* juveniles show that mean corpuscular volume (MCV) was lower in fish fed feeds with different levels of omega-3 and mean corpuscular haemoglobin (MCH) was significantly ($P<0.05$) higher fish fed feeds with different levels of omega-3 than in the control fish (table 7). The mean corpuscular haemoglobin

concentration (MCHC) of *H. bidorsalis* juveniles was similar in the control and treatment fish.
H. bidorsalis-fed feeds without omega-3 in its feed had slightly increased value of erythrocytic parameters while *H. bidorsalis*-fed fish feeds with omega-3 had reduced values of PCV, Hb, RBC and WBC counts resulting in anaemic condition. Values of haematological parameters reduced with increasing levels of omega-3 in fish feeds.

Table 2: Mean haematological parameters of *H. bidorsalis*-fed feeds without omega-3 fatty acid and control.

Haematological Parameters	Tank A Control fish	Tank B	Tank C	Tank D
PCV (%)	31.00	35.50	32.50	**29.00**
RBC (μ/mm^2)	2.29	2.45	2.45	**2.46**
WBC (μ/mm^2)	1.21	1.49	1.47	**1.30**
Hb (g/dl)	**10.33**	**11.83**	**10.83**	**9.67**

Table 3: Mean haematological profile of *H. bidorsalis* 4 weeks after feeding with different levels of omega-3 fatty acid in fish feeds and control.

Haematological Parameters	Control		Treatments	
	Tank A (0mg)	Tank B (1000mg)	Tank C (2000mg)	**Tank D (3000mg)**
PCV %	32.20	33.50	30.50	**27.50**
RBC (μ/mm^2)	2.43	1.66	1.64	**1.60**
WBC (μ/mm^2)	1.24	1.39	1.37	**1.10**
Haemoglobin (Hb) g/dl	**10.73**	**11.16**	**10.16**	**9.17**

Table 4: Mean difference in haematological profile of *H. bidorsalis* 4 weeks after feeding on different levels of omega-3 fatty acid in fish feeds and control.

Haematological Parameters	Control		Treatments	
	Tank A (0mg)	Tank B (1000mg)	Tank C (2000mg)	**Tank D (3000mg)**
PCV %	2.2850^a	2.4450^b	2.4450^b	**2.4550^c**
RBC (μ/mm^2)	2.4250^a	1.6550^b	1.6550^b	**1.6500^b**
WBC (μ/mm^2)	1.2650^a	1.3900^b	1.3900^b	**1.1500^c**
Haemoglobin (Hb) g/dl	**9.8000^a**	**10.1500^b**	**10.2000^b**	**8.3500^c**

Means on same row with different superscripts are significantly different (P<0.05).

Table 5: Mean difference in haematological indices of *H. bidorsalis*-fed feeds without omega-3 fatty acid and control.

Haematological Indices	Tank A Control fish	Tank B	Tank C	Tank D
MCV (μ/m^3)	135.37	144.89	132.65	**117.89**
MCH (μ/mg)	45.11	48.29	44.25	**39.31**
MCHC (%)	33.33	33.33	33.32	**32.67**

Table 6: Mean haematological indices of *H. bidorsalis* 4 weeks after feeding on different levels of omega-3 fatty acid and control.

Haematological Indices	Tank A Control fish	Tank B (1000mg)	Tank C (2000mg)	Tank D (3000mg)
MCV (μ/m^3)	14.00	21.75	21.50	**18.50**
MCH (μ/mg)	33.00	65.60	65.50	**55.60**
MCHC (%)	0.35	0.30	0.30	**0.30**

Table 7: Mean difference in haematological indices of *H. bidorsalis* 4 weeks after feeding on different levels of omega-3 fatty acid in fish diet and control.

Haematological Indices	Tank A Control fish	Tank B (1000mg)	Tank C (2000mg)	Tank D (3000mg)
MCV (μ/m^3)	14.0000^a	21.7500^b	21.5000^b	**18.5000^c**
MCH (μ/mg)	0.3500^a	0.2500^a	0.2500^a	**0.3000^a**
MCHC (%)	0.3500^a	0.3000^a	0.3000^a	**0.3000^a**

Means on same row with different superscripts are significantly different (P<0.05).

DISCUSSION

This study has shown that the inclusion of omega-3 fatty acid in fish feeds resulted in anaemic conditions in fish by reducing PCV, Hb levels and RBC counts. This finding is similar to an earlier study which reported that omega-3 decreased levels of erythrocytic parameters [13]. It has been reported that erythrocyte counts together with PCV and haemoglobin estimation are a

highly valuable blood parameters that could be used as a tool in aquaculture and fishery management for checking anaemic conditions in fish [14]. It was observed that male albino rats fed different concentrations of omega-3 inclusion in feed had decreased PCV, RBC and WBC counts [15]. According to a report [11], blood is a good indicator for determining the health of an organism. It also provides a pathological reflection of the whole body, hence its usage in diagnosing the functional status of animals exposed to toxicants. Seasonal variations, nutrition, size, genetic properties, population density, lack of food supply and environmental stress have been reported to affect the haematological data of fish [3]. This fact is however of less importance as the use of haematology as a diagnostic technique is based on the premise that the parameters measured are not substantially influenced by the sample procedures [3]. The normal levels of haematological variables are important in the utilization of haematological parameter for detecting any disturbance introduced into the fish environment. Haematological parameters are important in detecting the status of immunity of animals. A reduction in levels of haematological parameters depicts a reduction in immunity. As a result the immunity of the experimental fish is affected negatively.

Haematological indices have also been reported to be useful diagnostic tool for determining anaemia [16]. It was been reported the use of mean corpuscular haemoglobin concentration and other haematological indices of fish as important in determining the status of health of fish [2]. MCV reflects the size of red blood cells while MCH and MCHC are a reflection of the haemoglobin content of red blood cells which are used to diagnose anaemic conditions [17]. Fish oil has been used as a component in aquaculture feed. More than 50% of the world's fish oil used in aquaculture feed is fed to farmed salmon [18]. Catfish has been found to require small amount of omega-3 fatty acid feeds for normal growth [5]. Omega-3 fatty acid has also been reported to enhance growth performance in *H.bidorsalis* fish [7]. Low dose fish oil extract administration has been found to improve the function of platelets and red blood cells and may help prevent cerebrovascular diseases in elderly humans without any side effects [19].

The inclusion of omega-3 in fish feeds may lead to anaemic conditions which may lead to reduced immunity in fish. There is a need for further investigation to set acceptable levels of omega-3 inclusion in fish feeds to promote growth and at the same time prevent anaemic conditions in fish.

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