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Growth, Haematology and Immunology of Heteroclarias Juveniles Fed Varying Dietary Inclusion Levels of Vitamin E

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Abstract

Effect of vitamin E inclusion in diets of hybrid catfish (Heteroclarias) juveniles on its growth, feed Utilization, hematological responses and immunological response was examined. Three levels of vitamin C supplemented diets with 60 (T1), 120 (T2), and 180 (T3) mg.kg^{-1} vitamin E equivalent and a control basal diet without vitamin C were fed to Heteroclarias juveniles (triplicate group) twice daily for 12 weeks. Result showed that growth parameters, feed utilization efficiency values, haematological and immunological parameters of the treatments groups when compared to control (vitamin E depleted diet), increased significantly ($p<0.05$). Furthermore, growth, heamatological and immunological parameters increases as inclusion level of dietary vitamin E increases. Therefore, inclusion of up to 150 mg.kg^{-1} vitamin E in diets may be beneficial for the optimal growth and survival of hybrid catfish.

Keywords: Vitamins E, growth, feed Utilization, haematological parameters, immunological parameters.

Introduction

In comparison to the amount supplied, global demand for fish and fish products is extremely high. Increased aquaculture operations have resulted from a decrease in supplies from ocean fisheries as a result of overexploitation of fish stock, habitat loss, and pollution[1]. Fish contributes significantly to the supply of protein, vitamins and minerals in the world, particularly in developing countries[2]. Catfish, tilapia and carp are among the most common species cultivated in Nigeria. Catfish, are however, the most resistant and widely accepted fish in Nigerian aquaculture[3].

Catfish occupies a dinstinct and important position in the aquaculture industry as it is valued for its taste, hardiness and tolerance to poor water quality conditions[4]. Catfish can easily be produced into large size[4], has an efficient feed conversion rate, and attracts high market price[5]. Heteroclarias is obtained from inter-generic hybridization of two Clariid catfishes; male Heterobranchus species and female Clarias species, and increasing attention has is being paid to the hybrids of the cross[6,7].

However, there are constraints on the culture and growth of this fish species. The most prominent of these constraints is the formulating diets of acceptable quality as regards to the required vitamin composition which is important for fish growth. Vitamins are important essential nutrients which are important for the wellbeing, development and management of all fauna including fish[8]. Vitamin deficiency is known to induce biochemical dysfunction in cultured fish, which can manifest clinically at the tissue and cellular scale[9].

α-tocopherol (vitamin E) is an essential antioxidant for majority of cultures fish species and have been acknowledged as a vital dietary supplement that has effects on fish immunity, development and survival[8,10-12]. The antioxidantory effect of vitamin E have been well documented[13]. It protects phagocyte membranes against peroxidative damage, thereby making it an essential nutrient in the fish immunity[14].

In many fish species, vitamin E deficiency have been reported suppress immune responses[15-19]. Supplementing diets of fish with vitamin E has been reported to improve the immunity and cytotoxic production of leucocytes in some fish species[20,21]. However, there is little knowledge about vitamin E requirement of hybrid catfish, Heteroclarias.

As a result, the aim of this study is to assess the growth rate, feed utilization, haematology, and immunology of hybrid catfish fed varying levels of vitamin E.

Material and methods

Experimental diets: Table-1 shows the basal diet formula used in this study. The control diet (D_1) had no vitamin inclusion, while D_2, D_3 and D_4 contained 50, 100 and 150mg/kg of vitamin E respectively. Dry ingredients were reduced into particle in a miling machone before being added together as diet component. Following that, using a Hobart pelleting system (model A200), the diets were cold pelleted into 2mm diameter pellets.

The pellets were sundried for two days and stored separately in an airtight container prior to use and 5g each of the experimental diets was taken to the laboratory for analysis of its proximate composition.

Table-1: The experimental diet's composition (dry weight).

Ingredients	Control (D_1)	D_2	D_3	D_4
Fishmeal	15.2	15.2	15.2	15.2
Soybeanmeal	43.0	43.0	43.0	43.0
Groundnut cake	19.0	19.0	19.0	19.0
Maize	16.3	16.3	16.3	16.3
Cod liver oil	5.0	5.0	5.0	5.0
Premix (Vitamin E free)	0.5	0.5	0.5	0.5
Starch	1.0	1.0	1.0	1.0
Vitamin E (mg kg^{-1})	0.00	50.00	100.00	150.00

Experimental feeding trial: The feeding trial was for a period of 12 weeks, divided into nine experimental treatments in triplicates. Three hundred (300) juveniles of hybrid catfish (Heteroclarias) with average initial weight of 36+0.01g were stocked into thirty (30) glass aquaria tanks (70×45×30cm) at 10 fish per tank in three replicates. Fish were fed two times in a day (0800 and 1600) with feed of 3 percent of their average body weight, with weekly adjustment to the amount of feed given based on body weight. Every two weeks, the average weight of the fish per experimental tank was determined so as to adjust the feed quantity. All through the experiment, hydrobiological parameters of the water were monitored.

Assessment of Growth Parameters: Measurement of fish growth indices was conducted biweekly to monitor growth parameters. Prior to measurement, fish were anesthetized in tricaine methane sulphonate (100ppm of MS 222). After the experiment, the following growth parameters of fish were measured: specific growth rate (SGR), weight percent gain (WPG), net protein utilization (NPU), protein efficiency ratio (PER), feed conversion ratio (FCR), and survival rate (SR).

SGR = ((Log Wf - Log Wi) x 100)/ Time (days)
WG= ((Wf- Wi) / Wi) × 100
NPU % = (Protein gain/ Protein consumed) × 100%
PER = Weight gain /Amount of Protein consumed (g).
FCR= F/(Wt-Wi)
SR (%) = (Final fish count / Initial fish count) x 100%
Wt is final weight, Wi is the initial weight, TL is total length, and F is feed consumed.

Assessment of Hematological Parameters: At the conclusion of the 12-week trial, samples of blood were obtained from the caudal peduncle with a heparinized syringe lazed with anticoagulant potassium salt of ethylene diamine tetra-acetic acid (EDTA) to examine the haematological parameters. Collected blood samples were quickly delivered to the laboratory for RBCs, WBCs, Hct, Hb, IgM, TIg, and Lysozyme analysis.

Microhaematocrit reader was used for measurement of PCV values and was expressed in percentage. Using a haemoglobinometer, Hb values were measured based on the acid haematin procedure. Heamoglobin value was obtained using the relationship Haemoglobin = (obtained value/100) × 17.2 (mg/100ml). With the aid of a Neubauers haemocytometer, chamber method was used to determine the values of RBC and WBC[22,23]. The MCHC and MCH values were calculated as follows:

MCHC (g.dl-1) = (haemoglobin concentration) / (haematocrit value) × 100
MCH (pg) = Hb/RBC × 10 (μ^3)

Immunological Analysis: Immunological Response analysed include immunoglobulin, IgM, Total Protein, Globulin and Albumin. Serum concentrations of IgM were measured with C-ELISA kit. To measure the immunoglobulin and protein determination, blood serum was collected from the caudal aorta, into a non-herparinised bottle and was allowed to clot overnight at room temperature before taken to the laboratory for analysis[24]. The total protein in serum was calculated using Biuret's procedure and with the aid of Total protein kit. (BIOLABO.FR). Albumin kit was used to estimate albumin using the bromocresol green binding process. Albumin values were subtracted from overall plasma protein to measure globulin. By finding the ratio of albumin values to globulin values, the albumin/globulin ratio was determined.

Statistical Analysis: Data obtained was analyzed using SPSS software (version 21). Data were subjected to analyses of variance (ANOVA) at ($P \leq 0.05$). When ANOVA revealed significant F-ratios, follow up test (Turkey) was applied to discern the significant differences at 0.05.

Results and discussion

Results in Table-2 presents the growth and feed utilization parameters of juvenile Heteroclarias fed differing amounts of vitamin E supplemented diets. Fish that were fed on diets with vitamin E supplements showed significantly higher WG, FCR, SGR, NPU and PER. specific growth rate ($p<0.05$) compared with those fed the control diet. Significantly ($p<0.05$) higher values of growth and feed utilization indices was observed with increasing (50mg kg^{-1}, 100mg kg^{-1}, 150mg kg^{-1}) dietary inclusion of vitamin E in this study. The smallest growth and performance indices was observed for fish that were fed on diets without vitamin E supplementation, followed by fish fed diets with lower inclusion of vitamin E. Fish that were fed on diets with the highest inclusion of vitamin E had the highest growth and performance (1.5 percent).

Table-2: Growth and Feed Utilization Parameters of Juvenile Heteroclarias fed diets containing varying levels of Vitamin E.

Parameters	Control	T1	T2	T3
Initial Mean Weight (g)	36.60±0.00	36.57±0.05	36.57±0.05	36.57 ±0.05
Final Mean Weight (g)	79.73±0.25^{d}	89.20±0.49^{c}	98.17±0.6^{b}	100.93±0.31^{a}
Mean Weight Gain (g)	43.33±0.25^{d}	52.60±0.43^{c}	61.60±0.65^{b}	64.37±0.35^{a}
Specific Growth Rate	0.92±0.00^{d}	1.06±0.00^{c}	1.17±0.01^{b}	1.20±0.00^{a}
Feed Conversion Ratio	1.28±0.00^{d}	1.57±0.01^{b}	1.34±0.01^{c}	1.65±0.01^{a}
Net Protein Utilization (%)	54.20±0.14^{c}	58.97±0.20^{b}	62.75±0.28^{a}	63.77±0.15^{a}
Protein Efficiency Ratio	1.08±0.01^{d}	1.51±0.01^{b}	1.29±0.01^{c}	1.58±0.01^{a}
% survival Rate	90.02±0.01	90.33±0.01	90.29±0.05	90.12±0.01

Means±SD on same row with different superscript are significantly different (P>0.05).

Table-3: Haemotological parameters of Heteroclarias Juveniles fed on varying levels of vitamin E.

Parameters	Control	T1	T2	T3
RBC (%)	1.81±0.03^{d}	2.82±0.03^{c}	3.04±0.04^{a}	2.95±0.04^{b}
WBC (%)	24.52±0.02^{d}	7.81±0.03^{c}	8.44±0.05^{a}	7.92±0.03^{b}
Hb (g dL^{-1})	6.9±0.02^{d}	31.14±0.03^{c}	31.57±0.01^{b}	32.17±0.03^{a}
PCV	32.23±0.05^{a}	26.22±0.02^{b}	25.82±0.02^{c}	26.02±0.02^{d}
Lymphocyte	52.34±0.03^{d}	63.86±0.02^{b}	66.18±0.01^{a}	62.08±0.02^{c}
MCH	37.31±0.02^{a}	27.20±0.02^{d}	27.44±0.02^{c}	28.22±0.02^{b}
MCHC	21.39±0.01^{d}	24.72±0.02^{c}	25.09±0.02^{b}	26.86±0.02^{a}

Means±SD on same row with different superscript are significantly different (P>0.05).

Results obtained from heamatological analysis of Heteroclarias juvenile fed on diets containing varying levels of vitamin E are presented in Table-3. Significant differences (P>0.05) was observed in the RBC, HB, WBC, Lymphocyte, MCH and MCHC values among the treatments. Higher values (P>0.05) of analyzed parameters except PCV was detected in fish that were fed on diets with vitamin E inclusion when compared with the control. Values for RBC, WBC and Lymphocyte recorded in T2 (100mg kg^{-1} inclusion of vitamin E) was observed to be the highest (Table-3). The greatest HB and MCHC values were observed for fish fed T3 which contain 100 mg $kg^{-1}$of vitamin E, while the highest value for PCV was recorded for the control treatment (Table-3).

Results of analyzed immunological parameters in Figure-1 shows that IgM, total protein, albumin, globulin were higher in fish that were fed vitamin supplemented diets compared to those fed control (P<0.05), However, there is significant difference (P<0.05) in A/G ratio of fish fed on control compared to those fed with vitamin supplemented feeds. Fish that were fed on T3 containing 100 mg $kg^{-1}$of vitamin E had the highest IgM, total protein, albumin, and globulin. (Figure-1), while those fed on control have the lowest values of these parameters (P<0.05).

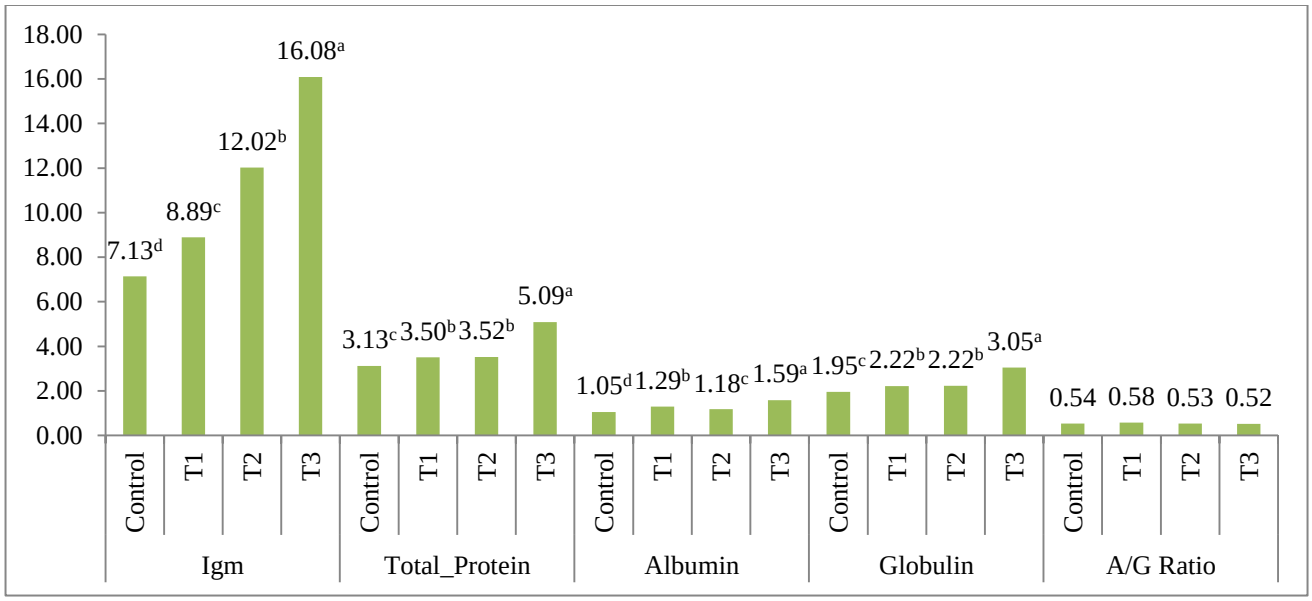

Figure-1: Immunological parameters of fingerlings of Heteroclarias fed on various levels of vitamin E.

Values on same row with the same superscript are not significantly different (P>0.05)

Discussion: There is paucity of information on vitamin E requirements of heteroclarias. In this study, fish that were fed on vitamin supplemented diets showed significantly higher growth parameters than those fed on the control diet. In this respect, fish fed 150mg.kg^{-1} Vitamin E had the highest WG, SGR, FCR, NPU, and PER. According to previous studies, vitamin E requirements of fish are species-specific[25] and is also influenced by other factors including temperature, stocking density and some other conditions[26]. The result of this study agrees with previous studies that reported a redundant growth due to the absence or reduced vitamin E inclusion in diets of various fish species[27-29]. Vitamin E plays a significant role in reduction of metabolic cost, prevention of muscle atrophy and protection of collagen tissues from damages thereby promoting growth[30-32].

In fish, haematological parameters are often used as indicators of health status[33]. WBCs, RBCs, and Hb levels were higher in diets supplemented with vitamin E as compared to the control sample. This agrees with the findings of Miar *et al.*[25]; and Esmaeli and Khara[34] who reported significantly higher heamatological parameters in trout fed vitamin E supplemented diet than trout fed with vitamins deficient diet. Similar finding was reported by Bai and Lee[35] with Korean rockfish, *Sebastes schlegeli*, and Taveekijakam *et al.*[36] with Amago salmon, *Oncorhynchus masou*. Vitamin E being an antioxidant prevents oxidative stress to cell membranes, like red blood cells[37].

Findings from this study indicates that vitamin E stimulates the immunity of Heteroclarias juvenile with observed elevated levels of WBCs, IgM, total protein, albumin and globulin in treatment groups that were fed on vitamin E supplemented diets. This immune stimulatory influence of vitamin E were reported in many fish species such as rainbow trout[38] and gilthead seabream[20,21]. Vitamin is believed to play several roles in fish immunity by enhancing the inherent immune responses and cytotoxic activity of leucocytes[20,21]. Due to the higher values (p>0.05) of IgM, total protein, albumin, and globulin relative to the control, the innate immune response of Heteroclarias juvenile is believed to be enhanced by dietary inclusion of vitamin E. Improvements in serum total protein, including albumin and globulin, are considered to be linked to a greater immune response in fish[39].

Conclusion

Dietary Vitamin E improves the growth and immunity of Heteroclarias juveniles, as shown by this study. In this respect, providing Heteroclarias juveniles with a dietary dose of up to 150mg.kg^{-1} vitamin E may be a good choice for ensuring proper growth and survival.

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