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Influence of Aquazyme-Plus on Growth, Blood Profile and Nutrient Quality of *Clarias Gariepinus* Cultured in Net Hapas

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ABSTRACT: The study evaluated effects of an exogenous enzyme (AquaZyme Plus) on the growth performance, blood parameters, and flesh composition of *Clarias gariepinus* in a twelve-week feeding trial. The enzyme was incorporated in a common commercial catfish feed (Blue Crown) at the rate of 0.0g/kg (control), 0.5, 1.0, 1.5 and 2.0g/kg of the feed. These were designated as Diets 1 to 5 respectively. Fish were reared in a 1m x 1m x 1m floating net-hapas set on a concrete tank (10m x 8m x 1.8m). Triplicate groups of fish with mean weight of 4.30±0.3g were randomly stocked at 20fish per unit and fed the experimental diets at 5% of body weight. All analyses followed standard procedure. During the study period, there was no significant difference (p > 0.05) in water quality parameters among the treatments and were within the suitable range for catfish growth and development. Growth performances improved with increase in enzyme concentration in diets. Here, Diet 5 (2.0g/kg) yielded the best: mean weight gain (204.51g), specific growth rate (4.60%/day), average daily growth (2.44g/day), food conversion ratio (0.94) and protein efficiency ratio (2.33). A reverse trend was observed in carcass protein and lipid contents. However, moisture, ash, calcium, phosphorus and iron contents increased with enzyme concentration in diets. This study revealed that exogenous enzyme (AquaZyme Plus) inclusion in catfish feed led to increase growth and improved flesh quality.

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INTRODUCTION

Intensive aquaculture production requires effective and economical fish feed for growth and production of the aquatic species at all life stages. The preparation of fish feed requires that essential nutrients necessary for profitable growth, survival, and reproduction of the species be balanced in nutrient requirement of the individual species (Sampath *et al*., 2020). The formulation of fish feed is expected to make use of non-conventional ingredients of both plant and animal sources that are high in essential nutrients rather than through fish meal and fish oil (Effiong, 2015). However, some of these non-fish raw materials are composed of high molecular weight organic matter which may not be fully digested and metabolized by the animal which could result in partial or incomplete absorption of such nutrients by the animal, thereby leading to negative impact in the overall performance and growth of the species. Other studies by Ufodike *et al*. (2003); Ogunkoya *et al*. (2006); Kalhoro *et al*. (2018) Maas *et al.* (2019) also opined that fish may have digestibility problems with plant-based feed stuffs due to the presence of non-starch polysaccharides within plant cell walls. These had been shown to reduce digestive enzyme access to other nutrients thus, resulting in reduced feed efficiency and slow growth rate of fish (Francis *et al.*, 2001) . In order to address this digestibility problems, different exogenous enzymes such as glucose oxidase, phytase, lipase and lysozyme had been successfully used in aquafeed production (Okayi *et al*., 2010; Yigit and Olmez, 2011; Maas *et al*., 2020; Zheng *et al.*, 2020). The studies revealed that the addition of these enzymes in fish feed improved protein and carbohydrate digestibility resulting into low food conversion and fast growth responses. These enzymes are also known to increase the bioavailability of phosphorus and other minerals, and overall growth performance of cultured fish (Eyiwunmi *et al.*, 2017; Lemos and Tacon 2017). Furthermore, the addition of enzyme in fish feed has been reported to reduce aquaculture diseases and promote the health of farmed fish, improve intestinal health and inhibit harmful bacteria, which is of significance for the control and reduction of antibiotic use, improvement of the environment, and food safety in aquaculture (Yigit *et al*., 2011; Zheng *et al.*, 2020).

The African catfish, *Clarias gariepinus* is the most preferred cultured freshwater species in Nigeria and has been described as a high-protein, low-fat fish (Effiong, 2015). The ability to accelerate the production and development of the species at commercial level requires pre-knowledge of essential nutrients to meet up protein demand for the ever- increasing human population. The successful culture of this species relies solely on the use of nutritionally balanced and economically viable non-conventional feed ingredients. Increasing trend in catfish farming in Nigeria has expanded significantly in recent times involving several feed producing industries where ingredients from non-conventional sources are used to reduce feed cost and still maintain feed quality (Ufodike *et al.*, 2012; Effiong and Ufodike, 2015; Eyidi and Nwosu, 2023). Plants-based ingredients had also been successfully used to replace fish meal in fish feed production after employing various processing techniques to remove anti-nutritional factors in them (Ufodike *et al*., 2011; Olaniyi *et al*., 2013; Effiong *et al*., 2017; Enyidi and Oyazi, 2021). This fish has gain popularity among indigenous fish farmers due to its large size, fast growth, hardy nature, high resistance to infection and the ability to thrive well on artificial feed (Effiong *et al*., 2014). Its high food value also commands high consumer preference and market demand of this fish. However, many catfish farmers in Nigeria lack the knowledge of incorporating exogenous enzymes in fish feed to improve feed digestibility. Therefore, the present study was conducted to evaluate effects of an exogenous enzyme (AquaZyme) mixed with a commercial catfish feed (Blue Crown) on the growth, blood parameters and flesh composition of the African catfish, *Clarias gariepinus*.

MATERIALS AND METHODS

Experimental Site and Design

The research was conducted at the United Nations Development Programme (UNDP) pilot fish farm located at No.6A Phenson Street, Federal Housing Estate, Uyo, Akwa Ibom State, Nigeria. Experimental fishes were obtained from the hatchery complex of the same farm. The experimental design was made up of a module consisting of 8.5m x 6.5m bamboo raft with twelve 1.5m x 1.5m apartments. These were fitted with 1m x 1m x 1m net-hapas placed on a 10.0m x 8.0m concrete tank. Each hapa was rigged and suspended to maintain a depth of 0.75m in water and a free board of 0.25m was provided. The float lines were tied to the four corners of each compartment using kuralon rope (No15) as described by Effiong (2015).

Experimental Diets Preparation

The commercial catfish feed (Blue Crown) and the enzyme (AquaZyme Plus) were procured from a reputable Agrovet shop within Uyo Capital City, Akwa Ibom State. The enzyme was incorporated at the rate of 0.0 (control), 0.5, 1.0, 1.5 and 2.0 g of enzyme/kg of feed. These were designated as Diets 1 to 5 respectively. The enzyme was accurately weighed out and mixed manually with the feed. Dried pellets were labeled according to diet, stored in airtight plastic bags and placed in deep freezer at - 4°C for feeding trials.

Experimental Fish Culture

The fish (*C. gariepinus*) with initial average weight 4.30±0.3g were acclimated for 14 days before commencement of feeding trials. Twenty (20) fish were randomly sampled and stocked in each hapa and raised for 12 weeks. The stocked fish were fed at 5% biomass which was divided into two portions and fed at 8.00hr and 18hr daily. Each treatment feed was replicated three times. Fish were counted and weighed fortnightly and readings obtained were used to compute growth parameters. New fish weights were used to adjust the amount of feed supplied. Leftover feed was collected by siphoning, dried and then weighed. The values were then subtracted from amount supplied to estimate feed consumption.

Determination of Feed Utilization Parameters

Growth and nutrient utilization parameters were evaluated using standard formulae (Effiong, 2015).

*Percentage weight gain (PWG) (%) =Mean final weight–Mean initial weight/ Mean initial weight × 100

*Daily weight gain (DWG) (g/day) = Mean final weight–Mean initial weight/ Culture period (day)

*Survival rate (%) = Number of fish survived/ Number of fish stocked × 100

*Protein efficiency ratio (PER) = Mean final weight – Mean initial weight/ Crude Protein fed

*Apparent net protein retention (ANPR) = Final carcass protein- Initial carcass protein/ Crude protein fed x 100

*Specific growth rate (%/day) = Loge W2–LogeW1/ T2 – T1 × 100

Where: W2 = Weight gain at time T2; W1 = Weight gain at time T1

*Feed conversion ratio (FCR) = Mean final weight–Mean initial weight/ Total feed consumed

*Condition factor (CF) of *C. gariepinus* was determined using the following formula:

$CF = 100W/L^3$ (Gupta and Gupta, 2010)

Where W = Weight (g) and L = Length (cm).

Proximate Analysis of Fish Carcass

All proximate analyses of the fish samples were carried out in triplicate and reported in percent (Effiong *et al.*, 2019). The analysis of ash, crude protein (N × 6.25), moisture, crude fat crude fibre and carbohydrate (by difference) were determined in accordance with AOAC (2004). Caloric value was estimated based on physiological fuel values (0.2364 KJ/g for protein; 0.3954 KJ/g for lipid and 0.1715 KJ/g for carbohydrate) as described by (Effiong and Yaro, 2020).

Estimation of Minerals in Fish Carcass

The trace elements (Calcium, Phosphorus and Iron) in catfish fillets were estimated using flame atomic absorption spectrophotometer as described by (Effiong and Akpan, 2015).

Haematological Analysis of Fish

A total of five fish samples were randomly collected from each hapa for blood profile analysis. 10ml of blood was collected from vertebral blood vessel using 2ml EDTA treated disposable syringe and needle. The haematological analysis was conducted based on the methods described by (Effiong *et* al., 2019). All haematological parameters were estimated at Haematological Unit of the University of Uyo Teaching Hospital, using automated haematology analyzer (SYSMEX, model: KX–21N, USA, 2012) based on the reference method described in International Federation of Clinical Chemists (Schwartz *et al.*, 1985).

Statistical Analysis

Data were computed using Microsoft Excel version 2013. Analysis of variance (ANOVA) was used to determine variation among different treatment diets while Duncan Multiple Range Test was used to separate the means. All analyses were performed at 95% confidence interval. Statistical Analysis were done using Statistical Package for Social Sciences (SPSS) software (Version 22.0 for Windows; SPSS Inc., Chicago, IL, USA).

RESULTS

Growth Performance and Feed Utilization

The results of growth performances and feed utilization of *C. gariepinus* fed AquaZyme Plus fortified diets are presented in Table 1. Results revealed that the highest growth: final fish weight (208.81±6.3g), mean weight gain (204.51±4.3g), specific growth rate (4.60±0.61%/day), food conversion ratio (0.94±0.23), protein efficiency ratio (2.33±0.45) and average daily growth (2.44±0.21g) were recorded in fish that received diet fortified with the highest concentration of exogenous enzyme. The condition of fish was not significantly different ($p > 0.05$) among tested diets. Survival rates of fish fed the different diets were statistically similar ($p > 0.05$).

Table 1: Growth performance and feed utilization of *C gariepinus* fed AquaZyme Plus fortified diets

Variables	Diet 1 (control)	Diet 2 (0.5g/kg feed)	Diet 3 (1.0g/kg feed)	Diet 4 (1.5g/kg feed)	Diet 5 (2.00 g/kg feed)
IFW (g)	4.30±0.3	4.30±0.3	4.30±0.3	4.30±0.3	4.30±0.3
FFW (g)	87.79±1.3	124.68±3.1	158.11±2.3	187.72±3.4	208.81±6.3
MWG (g)	83.49±4.3	120.38±4.4	153.81±6.3	183.42±6.3	204.51±4.3
SGR (%/day)	3.59±0.08	4.01±0.05	4.29±0.13	4.50±0.07	4.60±0.61
FCR	1.11±0.01	1.5±0.05	1.31±0.15	0.97±0.21	0.94±0.23
PER	0.91±0.01	1.38±0.09	1.72±0.04	1.99±0.06	2.33±0.45
ADG (g/day)	1.00±0.03	1.43±0.33	1.83±0.007	2.23±0.48	2.44±0.21
SR (%)	95.00	95.00	90.00	95.00	95.00
Condition factor	1.32±0.01	0.83±0.02	0.78±0.01	0.93±0.01	0.89±0.01

Data are mean ± standard error: means with different superscript within a row are significantly different ($p < 0.05$).Where: IFW=initial fish weight; FFW=final fish weight; MWG=mean weight gain; SGR=specific growth rate; FCR=food conversion ration; PER=protein efficiency ratio; ADG=average daily growth; SR=survival rates.

Biweekly Increment in Fish Size

Biweekly increment in size of catfish fed diets fortified with exogenous enzyme for 12 weeks are shown in Figure 1. Results revealed that growth increased linearly with enzyme concentration in diets. At Week 2, significant increase in growth rates were recorded with the least increment (6.78±0.21g) recorded in the control group while the highest growth (11.14±0.61g) was obtained in Diet 5. Similar patterns of growth were observed throughout the feeding period. When mean weight gain of fish was plotted against specific times, at which the weights were measured, the graph appeared as sigmoid curves, illustrating the change in the weight of the body through time (i.e. rate of growth). The growth curves rise up slowly with an increasing slope, representing an increasing rate of growth up to the end of the feeding trial. The growth curve revealed that in the first two weeks of the experiment, growth rate was low. This may probably mean that fish were acclimating to the formulated diets. Thereafter, all the fish picked up utilizing the diets better and this was reflected in higher growth rate observed in subsequent weeks.

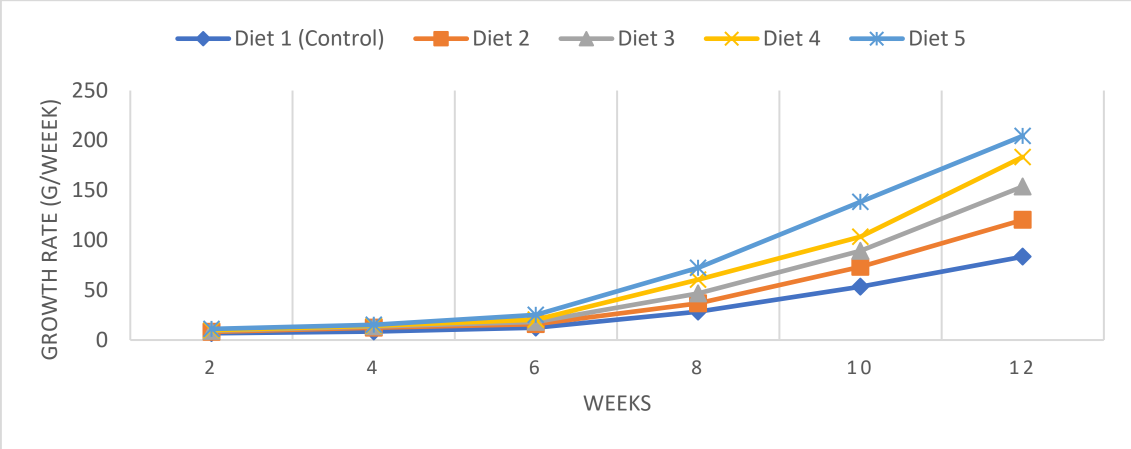

Figure 1: Biweekly growth curve of *Clarias gariepinus* fed AquaZyme Plus fortified diets for 12-weeks

The results of nutrient analysis of catfish fillet following treatment with exogenous enzyme are presented in Table 2. The highest percentage dry matter contents of fish were 50.87% for protein (Diet 5) and 9.02% for lipid (control diet). Mean carbohydrate ranged from 20.25% in Diet 5 to 23.29% in Diet 1. This may mean that the fish belongs to a high-protein-low-lipid fish category. The data further revealed that the levels of calcium, phosphorus and iron increased with increasing concentration of enzyme in diets.

Table 2: Carcass nutrient composition of Catfish fed diets fortified with AquaZyme Plus

Variables	Diet 1 (control)	Diet 2 (0.5g/kg feed)	Diet 3 (1.0g/kg feed)	Diet 4 (1.5g/kg feed)	Diet 5 (2.00 g/kg feed)
Crude protein	53.21±0.9	52.18±1.2[c]	51.19±1.03[d]	51.03±1.03[e]	50.87±.1.71[f]
Crude lipid	9.02±0.18[b]	8.31±0.32[d]	7.28±0.41[a]	6.43±.34[bc]	6.01±0.21[bc]
Moisture	7.31±0.13[b]	7.81±0.12[b]	8.03±0.03[b]	8.42±0.34[b]	8.61±.0.08[b]
Ash	5.14±0.21[b]	7.06±0.18[b]	9.83±0.14[b]	10.21±0.31[b]	12.18±0.12[b]
Crude fibre	2.03±0.02[b]	2.13±0.03[b]	2.04±0.03[b]	2.31±0.14[b]	2.08±0.01[b]
Carbohydrate	23.29±0.4[b]	22.51±0.3[b]	21.63±0.28[b]	21.60±0.31[b]	20.25±0.23[b]
Calcium (mg/kg	192.4±.5[b]	201.5±1[b]	197.6±.2[b]	208.6±4[b]	198.8±.1[b]
Phosphorus (mg/kg)	68.0±3[b]	69.7±8[b]	70.4±.6[b]	69.2±4[b]	70.6±.3[b]
Iron (mg/kg)	6.5±0.5	6.53±0.8	7.03±0.7	7.12±0.31	6.92±0.56

The results in Table 3 showed haematological profile of catfish after dietary treatment with AquaZyme Plus fortified diets for 12 weeks. The counts of WBC, RBC, HB, HCT, MCV, MCHC and PLT all increased with increase in enzyme concentrations in diets. At the end of the feeding trial, the values of all the haematological parameters measured were statistically higher ($p < 0.05$) than the initial levels. The group treated with 2.0g/kg enzyme in diet had the highest levels of all the aforementioned haematological indices. The values recorded for total protein did not follow any particular trend.

Table 3: Haemato-biochemical profile of *Clarias gariepinus* fed AquaZyme Plus fortified diets for 12-weeks

Variables	Initial	Diet 1 (control)	Diet 2 (0.5g/kg feed)	Diet 3 (1.0g/kg feed)	Diet 4 (1.5g/kg feed)	Diet 5 (2.00 g/kg feed)
WBC (x10^3/µl)	102.5±.3[a]	215.32±8[b]	231.4±.2[c]	248.7±.6[d]	269.3±8[e]	283.5±.6[f]
RBC (x10^6/µl)	1.38±.2[a]	2.37±.4[b]	2.68±.1[d]	2.73±.1[a]	2.91±.4[bc]	2.74±.1[bc]
HGB (g/dl)	6.27±.1[a]	11.86±.5[b]	12.05±.2[b]	11.28±.1[b]	10.98±.9[b]	11.85±.1[b]
HCT (%)	18.75±.5[a]	35.34±.3[b]	36.09±1[b]	35.61±.5[b]	34.0±0.9[b]	34.75±.1[b]
MCV (fl)	109.3±.8[a]	123.71±.4[b]	126.85±4[b]	121.0±.3[b]	124.01±5[b]	122.17±.1[b]
MCH (pg)	29.90±.6[a]	38.4±.3[b]	35.9±1[b]	36.2±.1[b]	36.8±4[b]	37.1±.1[b]
MCHC (g/dl)	30.82±.4[a]	40.20±.1[b]	39.15±1[b]	38.6±.2[b]	37.72±4[b]	36.8±.1[b]

PLT($x10^3/\mu l$)	93.1±.2[a]	134.0±3[b]	138.2±8[b]	141.6±.5[b]	138.2±5[b]	133.6±.3[b]
Total protein	7.01±0.2	8.2±0.3	7.3±0.4	8.02±0.5	8.07±0.2	7.18±0.6

Data are mean ± standard error: means with different superscript within a row are significantly different ($p < 0.05$).Where: WBC=white blood cell, RBC=red blood cell, HGB=haemoglobin, HCT=haematocrit, MCV=mean corpuscular volume, MCH=mean corpuscular haemoglobin, MCHC=mean corpuscular haemoglobin concentration, PLT=platelet

DISCUSION

The results of the present study revealed the effectiveness in incorporating enzyme in aqua feed production and the nutritional value present. The fish fed with the highest enzyme concentration showed a gradual increase in weight having the highest specific growth and the best food conversion ratio. Generally, growth responses were significantly better in all the fish group that received enzyme addition in feed when compared to the control group with no enzyme incorporation. Similar reports were given by Adeoye *et al*. (2016) and Kemigabo *et al*. (2018) when exogenous phytase was incorporated in diets of Nile tilapia, *Oreochromis niloticus* and African catfish, *Clarias gariepinus* respectively. These group of fish also recorded the best fillet protein and other essential nutrients such as calcium, phosphorus and iron. Proteins are essential to all life forms. In animals, like the African catfish, they help to form supporting and protective structures such as cartilage, skin and muscles. They are major constituents of enzymes, antibodies, hormones, and some important body fluids such as blood (Effiong *et al*., 2019). Calcium is the most abundant mineral in the human body and it is essential for growth, bone formation, blood coagulation, milk formation and vitamin D absorption. The deficiency of calcium leads to rickets, osteomalacia and osteoporosis (Islam *et al.,* 2013). Phosphorous is a major constituent of all animal cells. Though primary dietary deficiency of phosphorous may not occur in man, insufficient amount in food may lead to secondary phosphate depletion which may result into muscle weakness and other bone related illnesses (Eyiwunmi *et al.,* 2017)**.** In this study, catfish treated with the enzyme contained adequate phosphorus level similar to that reported in *Clarias batrachus* by (Islam *et al*., 2013). Iron is an essential life supporting macronutrient in animals and human. Iron plays an important role in cellular metabolism as an active component of various enzymes, especially those associated with the respiration chain of mitochondria. Iron deficiency – anemia is widely prevalent especially in children (Gehring *et al.,* 2011).

The haematological indices examined (white blood cell, red blood cell, hematocrit, etc) in catfish fingerling were significantly improved with the effect of dietary AquaZyme Plus in fish diets. The counts of these blood variables obtained in fish fed enzyme diets were significantly higher than those fish fed diets without enzyme incorporation. The red blood cell is a function of oxygen absorption and transportation within a living cell, and depletion in the count may weaken and lead to death in fish. Catfish fingerlings fed diets with high enzyme in diets had correspondingly high red blood cell counts. Haemoglobin concentration reflects the oxygen supply in the blood and a decrease level had been associated to developing anaemia. Hence, haemoglobin concentration in the blood is a rapid method of detecting disease conditions in fish. The results of the present study showed that mean haemoglobin concentrations were high. Haematological indices (MCV, MCH and MCHC) have been reported to indicate secondary responses of an organism to irritants (O'Neal and Weirich, 2001). The MCV is useful in the estimation of the size of the red blood cell while MCH are used to estimate the concentration of haemoglobin in fish blood and MCHC, a good indicator of red blood cell swelling (Wepener *et al.,* 1992). A low level of MCV, MCH and MCHC signifies normal condition of the blood of the fish. In this study, the values of MCV, MCH and MCHC decreased slightly with increasing enzyme levels. This indicated that high enzyme levels favoured these indices. The erythrocyte count is a routine parameter in haematology and increases or decreases can provide valuable hints for diagnosis. In combination with the haematocrit, haemoglobin concentration and the erythrocyte indices, it can provide information about the type of disease present in an organism. The levels of these variables obtained in this study have not been associated with any detrimental health effect in catfish. This explained the efficacy of AquaZyme Plus diets in maintaining good and healthy condition in catfish. The results of the present study revealed that supplementation of exogenous AquaZyme Plus (a non-starch polysaccharides degrading enzyme) in Blue Crown (a commonly used catfish commercial feed in Nigeria) improved digestion of the feed by the test fish, thereby improving feed efficiency and growth performances. Thus, the addition of AquaZyme Plus may be a smart solution for improving the profitability of aquaculture venture for catfish farmers in Nigeria.

CONCLUSION

The results revealed that the addition of exogenous AquaZyme Plus to commercial catfish feed was effective in releasing most of the bound nutrients for optimum utilization. This resulted into improve growth performance and normal blood condition in fish. The results showed that increase in enzyme level in diet resulted in increase in fish growth, nutritional status and health condition of catfish. Thus, the addition of AquaZyme Plus may be a smart solution for improving the profitability of aquaculture venture for catfish farmers in Nigeria.

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