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The Effect of Garlic (Allium sativum) on Growth and Haematological Parameters of *Clarias gariepinus* (Burchell, 1822)

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Abstract

The effect of different concentrations of garlic (*Allium sativum*) supplement in fish diet on growth and haematological parameters of *Clarias gariepinus* fingerlings was investigated. Fish were separated into four experimental groups of 0% (controls), 0.5%, 1.0% and 3% concentrations of garlic in diet and fed at 3% body weight per day in a renewal static bioassay system. Garlic supplemented diet did not have any significant effect on weight gain of *C. gariepinus* when compared to fish in the control diet. However, final fish weight was significantly ($P<0.05$) higher than the initial fish weight in all concentrations of garlic in fish diet and in the controls. No significant ($P>0.05$) difference in total length was observed in fish fed different concentrations of garlic at the end of 12 weeks. An increase in total length was observed in fish with garlic supplemented diet over fish in the control diet. Fish fed different concentrations of garlic in diet showed slight differences in total length but did not significantly ($P>0.05$) differ from each other in total length. Fish in 0.5% garlic diet had higher weight gain and total length than fish in the rest of the treatment groups and the controls. Final white blood cell (WBC), red blood cell (RBC), packed cell volume (PCV), haemoglobin (Hb) were observed to be significantly ($P<0.05\%$) higher than the initial counts in the treatment groups as compared to the controls. RBC, PCV and Hb were however, significantly higher in fish fed 0.5% garlic supplemented diets than other concentrations. Plasma protein was observed to increase significantly ($P<0.05$) only in fish fed 0.5% and 1.0% garlic in diet. This study has shown that 0.5% (0.5g/kg) garlic supplement in fish feeds elicited more increase in fish total length and volume of haematological parameters of *C. gariepinus.* Garlic inclusion in fish diet at 0.5% (0.5g/kg) concentration is therefore beneficial for use in aquaculture to enhance the disease resistant status of *C. gariepinus*.

Keywords: *Clarias gariepinus,* garlic, fish growth, haematological parameters

1. Introduction

Disease outbreaks have been a major problem in aquaculture (Yunxia et al., 2001). Disinfectants and antimicrobials have been used in preventing or curing aquatic diseases but with limited success (Subasinghe, 1997). The use and abuse of disinfectants and antimicrobials have led to the emergence of resistance bacteria and drug residues in treated fish (FAO/WHO/OIE, 2006). Administration of vaccines has also been used as a measure for combating aquatic diseases. However, vaccines for emerging fish diseases have been found to be rather too expensive for fish producers and also of limited availability (Raa et al., 1992).

Immuno-stimulants are now used to enhance the activities of non-specific defence mechanisms and increasing disease resistance in aquaculture (Dalmo & Seljelid, 1995; Raa, 1996). Garlic (*Allium sativum*) is one of the known medicinal plants used as an immuno-stimulant (Lewis & Elvin-Lewis, 2003). Garlic has been used to control pathogenic bacterial and fungi in animals including fish (Adetumbi et al., 1986; Rees et al., 1993; Corzo-Martnez et al., 2007). Harris et al. (2001) reported that garlic has antibacterial, antiviral, antifungal and antiprotozoal and also has beneficial effects on the cardiovascular and immune systems.

Garlic inclusion in fish feeds has also been reported to increase growth performance in fish (Metwally, 2009). According to Sheela & Augusti (1992) and Dias et al. (2002) garlic has the ability of enhancing catalase activity in serum and lowering the levels of plasma glucose in fish. The non-specific defence system of *Oreochromis niloticus* has been improved by the inclusion of garlic in fish feed (Dias et al., 2002). Garlic as a natural product and an immuno-stimulant may be useful in combating diseases in *Clarias gariepinus* which is one of the

commonly farmed catfishes in Nigeria due to its ability to tolerate adverse environmental conditions (Holden & Reeds, 1972).

This study investigated the effect of garlic on growth and haematological parameters of *C. gariepinus* fingerlings with the aim of ascertaining the importance and level of garlic supplementation in fish feeds especially in *C. gariepinus*.

2. Materials and Methods

2.1 Study Station

The study which lasted for a period of three months from April to June in 2011, was conducted in the Research farm of the Department of Fisheries of Delta State University, Asaba Campus, Asaba, Nigeria.

2.2 Collection of Samples

2.2.1 Fish Samples

Sixty *Clarias gariepinus* fingerlings aged four weeks old ranging in size from 18.1 cm to 20.1 cm in total length and 51.0 g to 52.5 g in weight were purchased from a local fish farm in Asaba, Nigeria.

2.2.2 Garlic Samples

Fresh garlic bulbs were purchased from a local market in Asaba, Nigeria. One gram garlic was sun-dried and ground to powder and incorporated into fish diet at 0%, 0.5%, 1.0% and 3.0% concentrations.

2.3 Experimental Design

Sixty fingerlings of *C. gariepinus* were held in stock tank of glass aquarium (90cm x 45cm x 45cm) containing 50 litres of bolehole water for 14 days and fed *ad libitum* with commercially available pelleted feeds. Stock tank was well aerated and held at 25^0C and half of the water was changed on alternate days. Fifteen fish were then separated into each of the four experimental groups of 0% (controls), 0.5%, 1.0% and 3% concentrations of garlic in diet and fed at 3% body weight per day in a 50% renewal static bioassay system. Fish were fed equal portions twice daily (10.00 and 18.00 hrs). Fish feeding was increased with increase in body weight. The experiment was carried out in three replicates.

2.4 Experimental Diet

Fish feed was formulated according to Ndong and Fall (2011) with garlic in diet (Table 1). The diets were pelleted and oven-dried for 8 hours at 35^0C. The experimental diet was analyzed for proximate composition based on AOAC (1984) standard methods. Crude protein was determined using Kjeldahl method, crude lipid by Soxhlet apparatus [chloroform - methanol (2:1, v/v) extraction method (Folch et al. 1957)], crude fibre by fibretec system M1020 hot extractor (Foss Tecator). Ash and moisture were determined by conventional methods using muffle furnace at 505^0C and oven at 105^0C.

Table 1. Composition of experimental diet

Ingredients	Concentration of garlic in diet			
	0%	0.5%	1.0%	3.0%
Fish meal (g)	25.55	25.55	25.55	25.55
Groundnut cake (g)	14.43	14.43	14.43	14.43
Soya bean meal (g)	17.03	17.03	17.03	17.03
Wheat bran (g)	32.99	32.99	32.99	32.99
Garlic (g)	0.00	0.50	1.00	3.00
Oil (ml)	2.25	2.25	2.25	2.25
Salt (g)	1.00	1.00	1.00	1.00
Methionine (g)	0.50	0.50	0.50	0.50
Lysine (g)	0.50	0.50	0.50	0.50
Vitamin C (g)	0.50	0.50	0.50	0.50
Bone meal (g)	2.75	2.75	2.75	2.75
Starch (g)	2.50	2.50	2.50	2.50

2.5 Fish Growth

Weekly fish growth was measured in total length and weight. Percentage weight gain was expressed as: Weight gain (%) = 100 (Final Body Weight - Initial Body Weight).

2.6 Collection of Blood

Haematological parameters examined include white blood cell (total WBC), red blood cell (RBC) counts, pack cell volume (PVC), haemoglobin (Hb) and plasma protein. Blood samples were collected from three fish in each of the four experimental groups before and at weekly intervals of the study using a 2ml syringe and immediately transferred to an EDTA bottle to prevent coagulation. Total WBC and RBC counts were determined by using the Neubaeur haemocytometer. PCV was analyzed using a micro haematocrit centrifuge, Hb was determined according to Masser and Jensen (1991) and plasma protein by placing a drop of centrifuged blood on a refractometer and value read off.

2.7 Analysis of Data

Data collected were analyzed using analysis of variance at P=0.05 and means were separated by Duncan Multiple Range Test.

3. Results

3.1 Effect of garlic on the Growth of C. gariepinus

Garlic supplemented diet did not have any significant effect on weight gain of *C. gariepinus* when compared to fish in the control diet. However, final fish weight was significantly (P<0.05) higher than the initial fish weight in all concentrations of garlic in fish diet and in the controls (Figure 1). Also, no significant (P>0.05) difference in total length was observed in fish fed different concentrations of garlic at the end of 12 weeks. An increase in total length was observed in fish with garlic supplemented diet over fish in the control diet. Fish fed different concentrations of garlic in diet showed slight differences in total length but did not significantly (P>0.05) differ from each other in total length (Figure 2).

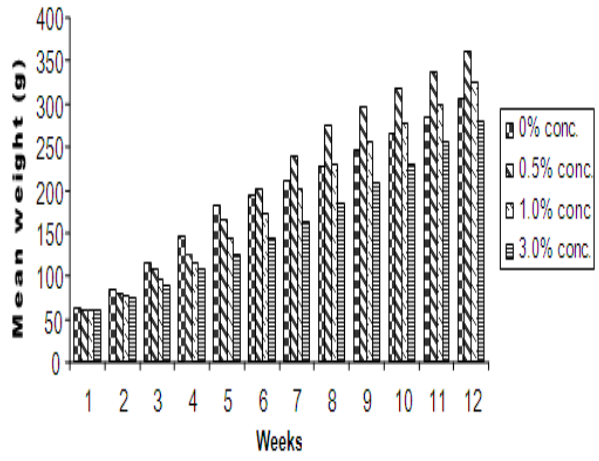

Figure 1.Weekly mean weights of *Clarias gariepinus* fed different concentrations of garlic in diet

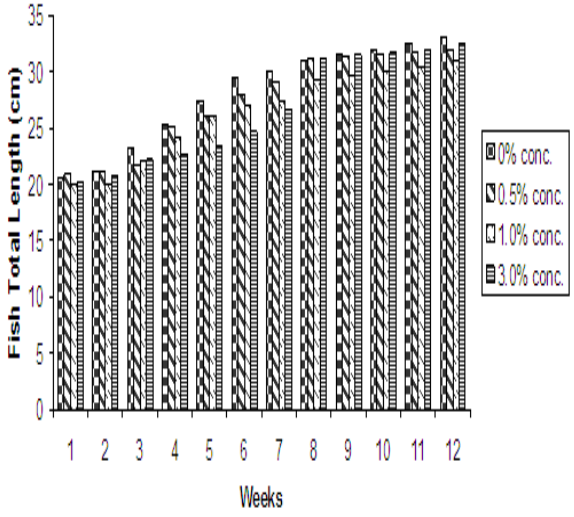

Figure 2. Weekly mean Total length of *Clarias gariepinus* fed different concentrations of garlic in diet

3.2 Effect of Garlic on Haematological Parameters

There was a progressive weekly increase in the WBC counts in all garlic supplemented diets except fish in the controls (0%) which had little or no change in WBC counts throughout the study. WBC counts of fish fed 0.5% garlic supplemented diet were higher than WBC counts of fish fed 1.0% and 3.0% (Figure 3). Other haematological parameters were differently affected by the inclusion of garlic in fish diet. Final RBC counts were observed to be significantly (P<0.05%) higher than initial counts in treatments as compared to the controls. RBC counts were however, higher in fish fed 0.5% garlic than the other concentrations (Figure 4). PCV was

significantly (P<0.05%) higher in the treatment groups than in the controls (Figure 5). PCV of fish fed with 1.0% and 3.0% garlic supplemented diet were not significantly (P<0.05) different, while fish fed with 0.5% garlic had a significantly (P<0.05) higher PCV. Final Hb value was significantly (P<0.05%) different from the initial Hb. Final Hb value was higher in fish fed 0.5% garlic supplement in diet than in the other concentrations (Figure 6). Plasma protein was observed to increase significantly (P<0.05) in fish fed with 0.5% and 1.0% garlic in diet but it was reduced in fish fed with 3.0% garlic (Figure 7).

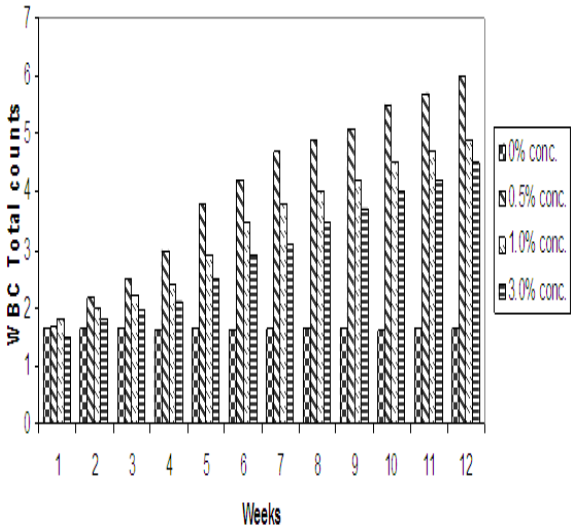

Figure 3. Weekly Total WBC Counts x10^{12} (u/mm^2) of *Clarias gariepinus* fed different concentrations of garlic in diet

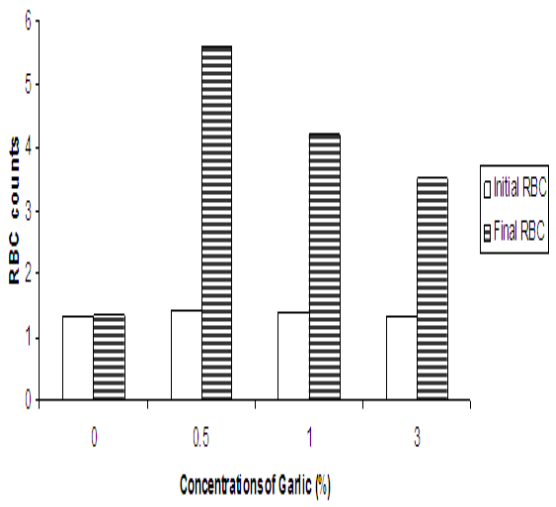

Figure 4. Initial and Final RBC Counts x10^{12} (u/mm^2) of *Clarias gariepinus* fed different concentrations of garlic in diet

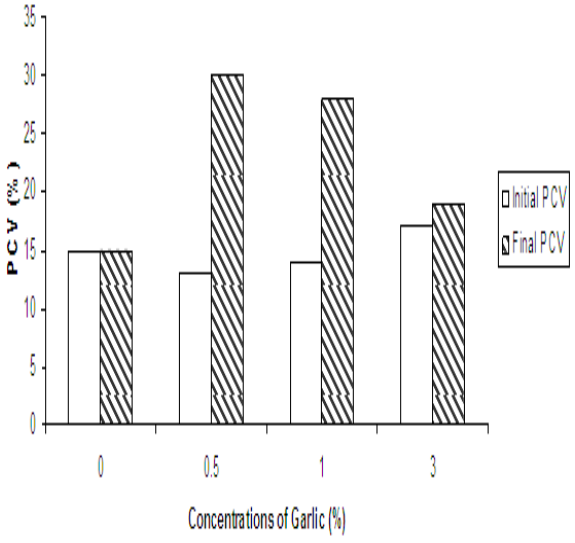

Figure 5. Initial and Final PCVof *Clarias gariepinus* fed different concentrations of garlic in diet

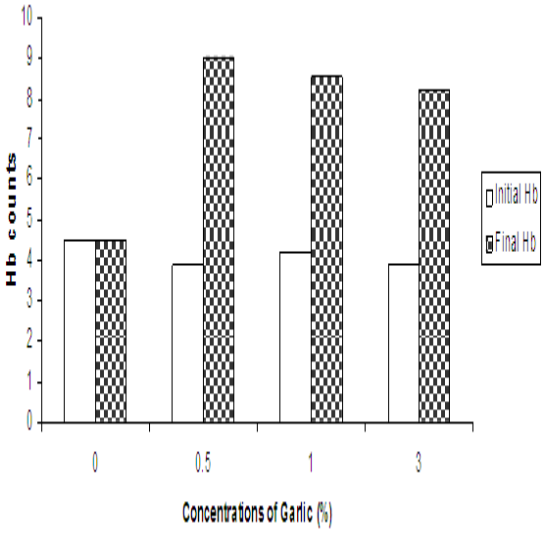

Figure 6. Initial and final Hb (g/dl) of *Clarias gariepinus* fed different concentrations of garlic in diet

4. Discussion

There was no significant weight gain in *Clarias gariepinus* fed with different concentrations of garlic in the diet compared to the controls. However, final fish weight was higher than the initial weight. The similar body weight gain among treatment groups fed garlic supplemented diet implies that garlic had no effect on growth

performance in terms of increased fish weight. Qureshi et al. (1983) reported no differences in final body weights from initial body weights of pullets fed diets with various garlic products at levels equal to about 50 kg/l of added garlic bulb. Ndong and Fall (2011) reported that garlic supplemented diet resulted in decreased body weight gain in juvenile hybrid tilapia (*Oreochromis niloticus* x *Oreochromis aureus*) fed diets supplemented with 0.5g/kg garlic over 4 weeks. Weekly increment in fish total length was observed in treatments and control fish. Fish in the treatment groups had slightly higher growth in total length than in the control. Higher growth performance has been recorded for fish fed garlic supplemented diets (Dias, 2002; Metwally, 2009).

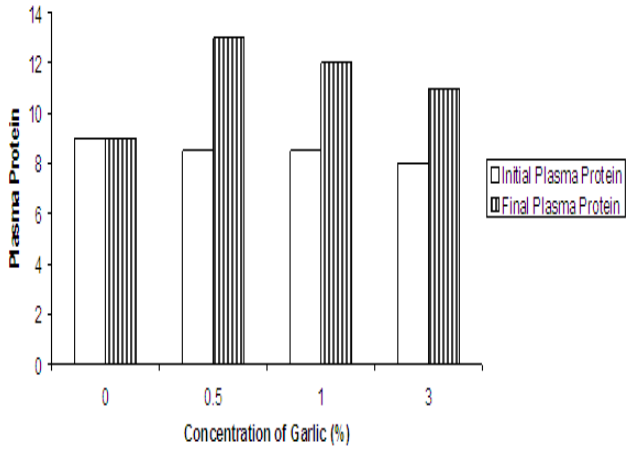

Figure 7. Initial and Final Plasma Protein (g/dl) of *Clarias gariepinus* fed different concentrations of garlic in diet

WBC counts increased significantly by weeks in fish in all treatment groups except in the control which had the same WBC counts. This result agrees with the findings of Ndong and Fall (2011) who reported that WBC counts increased significantly in juvenile hybrid tilapia fish fed 1% and 0.5% garlic supplemented diet. Also, the astronomic increase of total WBC counts in the 0.5% garlic in diet than in the other treatments suggests that 0.5g/kg is the concentration that stimulates and improves the immunologic functions of the blood. This concentration is appropriate for the overall increase in resistance of fish to environmental stressors. RBC counts in fish increased significantly in the treatment groups compared with controls. This finding agrees with the work of Lee et al. (1999) who reported that garlic increased glutathione levels in red blood cells. An increase in RBC counts indicated increase in haemoglobin levels. Haemoglobin levels increased significantly in the treatment groups, while Hb levels remained almost the same in the controls. Seeley et al. (1992) reported that the primary function of RBC in fish is to transport oxygen from the gills to the various tissues of the body, an action carried out by the haemoglobin found in RBC. According to Dias (2002) garlic increases super oxide dismutase (SOD) activity in blood serum and that this phenomenon has been used in fish farming to enhance the activity of non-specific defense system in *Oreochromis niloticus*.

Plasma protein was observed to increase significantly in fish fed 0.5% and 1.0% and reduced slightly but not significant in fish fed with 3.0% garlic supplemented diets. Level of plasma protein in the control fish was least. This indicates a drop in the level of plasma protein with increase in the concentration of garlic in fish diet. This finding is similar to earlier reports that total plasma protein in fish could vary from 2-8gd^{-1} (Ravi & Jithender, 2005). Shalaby et al. (2006) also reported increased levels of plasma protein in *Oreochromis niloticus* fed with different concentrations of garlic in diet.

This study has shown that 0.5% (0.5g/kg) garlic supplement in fish feeds elicited more increase in fish total length and volume of haematological parameters of *C. gariepinus*. Garlic inclusion in fish diet at 0.5% (0.5g/kg) concentration is therefore beneficial for use in aquaculture to enhance the disease resistant status of *C. gariepinus*.

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