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Full Length Research Paper

Effects of stocking density on haematological functions of juvenile African catfish (*Clarias gariepinus)* fed varying crude protein levels

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This study was carried out to evaluate the growth performance and some haematological functions of *Clarias gariepinus* fed different levels of crude protein under varying stocking densities. Fish of even sizes were randomly selected and stocked in plastic circular tanks (0.05 x 0.03 x 0.03 m) at three stocking densities of 10 fish/m^3 (control), 15 fish/m^3 and 20 fish/m^3. The fish of each stocking density were fed either a diet containing 40% Crude protein (CP) or 45% CP with a feeding rate of 3% body weight twice daily. The experiment was replicated thrice and it lasted for 12 weeks during which haematological parameters and plasma biochemistry were measured. From this study, haemoglobin (Hb) and Red Blood Cell (RBC) slightly increased in all treatments but the variation was not significant in relation to protein level in the diets. Plasma glucose increased significantly (P<0.05) in relation to the stocking densities. The plasma protein showed insignificant variation in relation to the stocking density but the variation was more pronounced (P<0.05) at the lowest stocking density as the protein level in the diets varied. It can be concluded that enhancing feed quality especially protein level in the fish diet may ensure faster growth, stress reduction and improve health status of the fish.

Key words: Crowding stress, haematological functions, *Clarias gariepinus*, diet quality.

INTRODUCTION

Environmental stress is an important factor responsible for limiting fish performance under aquaculture conditions (Ellis et al., 2002). When fish are subjected to adverse environmental conditions, some endocrine and physiological alterations occur, often resulting in change in ability of the fish to survive, grow and reproduce (Barton and Iwama, 1991). Overcrowding is a common chronic stressor in aquaculture that can induce a prolonged elevation of cortisol levels, which may cause damaging consequences, and suppressed growth (Rowland et al., 2006). This effect has been attributed to factors such as decreased food consumption. The high stocking density also imposes increased energy demands that require fish to cope with metabolic adjustments, such as changes of gluconeogenic and glucolytic activities. Under such conditions, food consumption is reduced; the extra expenditure energy has

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to be met by the reserve, resulting in reduced growth (Rowland et al., 2006).

Crowding is a common aquaculture practice used to manage water usage or increase fish stocking density (Baras and Lagardere, 1995). However, the use of high stocking density as a technique to maximize water usage and thus increase stock production has also been shown to have adverse effect on growth. In many cultured fish species, growth has indirect relationship to stocking density and this observation is mainly attributed to social interacttions (Holm et al., 1990; Haylor, 1991; Ma et al., 2006). Social interactions as a result of competition for food and/or space can negatively affect fish growth. On the other hand, the fish price is influenced by the market requirement such as size and production, which depends on their growth. In modern day commercial aquaculture the stocking density is an important indicator that determines the economic viability of the production system (Ellis et al., 2002).

Therefore by establishing the relationship between dietary protein level and crowding stress, monitoring of fish stocks and prediction on their haematological needs will be possible and enhanced. This study was carried out to assess the effects of crowding stress on the growth performance and some haematological functions of juvenile African catfish; *Clarias gariepinus* (a commercially important fish species in Africa) fed varying crude protein levels.

MATERIALS AND METHODS

Experimental procedure

This study was carried out in the Department of Wildlife and Fisheries Management laboratory, Faculty of Agriculture and Forestry, University of Ibadan. Four hundred *Clarias gariepinus* juveniles were purchased from a reputable farm - Rayak Fish farm Ibadan, Nigeria. The fish were transported in a well -oxygenated bag, which was half filled with fresh water to the Department of Wildlife and Fisheries Management laboratory. The average weight of the fish was 17.8 g±2.50 and their average length was 14.4 cm±1.20. The fish were kept to acclimatize for 2 weeks under laboratory condition in an indoor circular plastic tank. The fish were randomly distributed in 18 experimental tanks (each of 0.05x0.03x0.03 m) and the tank was filled with water at 60% capacity. The positioning of the tanks allowed a natural photoperiod of 12 ho sunlight and 12 hof darkness throughout the experiment. Water was changed daily to prevent fouling resulting from food residues. The source of water was the University of Ibadan Borehole Water Station. The fish were stocked into the tanks using three different stocking densities of 10 fish/m^3, 15 fish/m^3 and 20 fish/m^3 for treatments I and II respectively. The fish with lowest stocking density and fed with 40% CP diet served as the control based on recommendation of Akinwole (2007). The fish were fed with 40% crude protein and 45% crude protein diets for 12 weeks twice daily between the hours of 8.00 hand 1700 hat the rate of 3% body weight. Feeds were given in pellet form and the particle size increased periodically as fish grew. Fish were weighed initially using a weighing scale (g) before the commencement of the experiment and on a bi-weekly basis during the experiment. A calibrated measuring ruler (cm) was used to take the length of the fish before the experiment and bi-weekly during the experiment for 12 weeks.

The experimental diets were analyzed for proximate composition by using the methods prescribed by Association of Official Analytical Chemists (AOAC, 1990).

Haematological and biochemical analyses

This was carried out at the haematological laboratory of Veterinary Medicine Department University of Ibadan. Fish were starved for 24 h prior to sampling. Fish were anaesthetized with buffered MS222 (50 mg/l) and blood was collected with a hypodermic syringe from the caudal vein. Blood collection lasted less than 3 min in order to avoid stress-induced situation during sampling. The extracted blood was divided in two sets of eppendorf tubes. One set contained heparin, used as anticoagulant, for haematology (haemoglobin, hematocrit and red blood cell counting). The second set, without anticoagulant, was left to clot at 4°C and subsequently centrifuged at 5000 rpm for 10 min at room temperature. The collected serum was stored at –20°C for further assays (Stoskopf, 1993).

Physiological measurements

Red blood cells (RBCs, cells/μl) were counted under the light microscope using a Neubauer hemocytometer after blood dilution with phosphate-buffered saline solution. White blood cells were counted by using diluting fluids (3% aqueous solution of acetic acid plus 1% aqueous solution of gentian violet up to 1%). A 1:20 dilution was made and charged into Neubauer hemocytometer chamber for counting. Glucose was determined colorimetrically using glucose kits according to Trinder (1969). Total protein content in blood plasma was determined colorimetrically according to Henry (1964).

Statistical analysis

The data obtained were subjected to two-way ANOVA test and the differences between means were at the 5% probability level using Duncan's new multiple range test (DMRT). The software SPSS, version 10 (SPSS, Richmond USA) was used as described by Dytham (1999).

RESULTS

Gross composition of experimental diets at 40% and 45% CP is presented in Table 1 while Table 2 shows the haematological parameters of the test fish at various stocking density fed varying crude protein diets. Result from this study showed that there is no variation in the RBCs level with increasing stocking density; the RBCs level however increased significantly ($P< 0.05$) with increase in diet protein level. Also the Hb and Hcrt followed the same pattern as observed for the RBC in relation to the stocking density but however did not show any appreciable reduction with increase in diet protein level.

The plasma protein was significantly influenced by the crude protein elevation in the diets while high stocking density gave no significant variation in the plasma protein level. Generally, the plasma glucose level ranged from 90.00 to 126.28 mg/dl with lowest value recorded at lowest stocking density in the fish fed 40% crude protein diet. The plasma glucose level significantly increased

Table 1. Gross composition and chemical analysis of experimental diets using 40% and 45% crude protein.

Ingredient	(40% CP)	(45% CP)
Fish Meal	8.38	9.95
Groundnut Cake	25.14	29.85
Soybean Meal	33.52	39.81
Wheal offal	10.49	6.30
Yellow Maize	20.97	12.59
Salt	0.5	0.5
Vitamin Premix	0.25	0.25
Vegetable Oil	0.5	0.5
Mineral Premix	0.25	0.25
Total (kilogram) 100	100	
Chemical analysis (%)		
Moisture	8.61±0.02	8.52±0.03
Crude Protein	41.28±1.01	45.43±0.57
Crude Fat	4.79±0.002	5.86±0.02
Crude Fiber	2.97±0.02	2.84±0.02
Ash	14.88±0.001	15.34±0.01
Dry Matter	91.36±0.02	91.46±0.01
Nitrogen Free Extract	27.44±0.02	27.98±0.01

Vitamin premix contains; Vit. A, 20000000 I.U; D_3, 2000000 I.U; E, 200000 mg; K_3, 8000 mg;B_2, 30000 mg; B_1, 30000; B_6, 12000mg; B_{12}, 50 mg; C, 500000 mg; Niacin, 150000; pantothenic acid, 50000 mg; folic acid, 4000 mg; Biotin H12, 800 mg; choline chloride, 600000 mg; cobalt, 2000mg; copper, 4000 mg; iodine, 5000 mg; inositol, 200000 mg; iron, 40000 mg; manganese, 30000 mg; selenium (organic), 200 mg; zinc, 40000 mg; lysine, 100000 mg; methionine, 100000 mg and Antioxidant, 100000 mg.

Table 2. Haematological parameters of African catfish (*C. gariepinus*) fed different protein levels at two stocking densities.

Parameter	Treatment					
	40%CP			45% CP		
	SD1 (10 fish/tank)	SD2 (15 fish/tank)	SD3 (20 fish/tank)	SD1 (10 fish/tank)	SD2 (15 fish/tank)	SD3 (20 fish/tank)
RBCs (10^6/mm^3)	1.68^a± 1.05	1.82^a± 0.04	1.94^a± 0.04	1.74^a± 0.13	1.88^a± 0.14	2.05^a± 0.05
Hb (g/dl)	7.02^a± 0.82	7.50^a± 0.54	7.62^a± 0.54	7.90^a± 0.42	8.22^a± 0.20	8.90^a± 0.45
Glucose (mg/dl)	90.00^c± 8.25	102.24^b± 6.25	112^b± 3.42	122.40^a± 10.00	125.20^a± 8.22	126.28^a± 10.25
Protein (g/dl)	3.84^d± 0.20	4.10^c± 0.05	4.25^c± 0.45	4.90^c± 0.32	5.10^b± 0.58	6.05^a± 0.35

The same letter in the same row is not significantly different at P<0.05. Means with the same superscript along the same row are not significantly different (P>0.05).

(P<0.05) in relation to stocking densities at 40% CP. A significant increase (P< 0.05) was however observed in the plasma glucose level between the two protein diets with higher values recorded in the fish fed diet containing 45% CP. There was significant elevation (P< 0.05) in the plasma glucose level in relation to stocking density as it increases with the increasing stocking density.

DISCUSSION

The use of heamatological parameters in assessing the stress level of teolost fish has been widely adopted in recent times (Barton and Iwama, 1991; Ajani, 2008). The use of these immune system parameters in the determination of alterations in fishes as a result of stress relating issues and the renew interest in understanding the fish body defense mechanism comes from the need to develop a good healthy management tools toward the sustenance of the rapidly growing aquaculture industry (Mehdi et al., 2010). The RBCs, Hb, and Hcrt levels in the *C. gariepinus* increased as the stocking density increased. This may be linked to the issue of length of

study, space and water volume used in this study, which resulted in low level of fish activity during the experiment. This observation agreed with the report of McFarlane et al. (2004) and Akungur et al. (2007) who confirmed that any chronic stress induced by high fish density may be limited, but if the fish density increased for a long time or the fish growth was faster, the stress effect may be higher. It was observed that under longer stress time, growth reduction would be recorded especially at higher fish density, as fish will spend more energy on swimming activity at the expense of growth. Mehdi et al. (2010) reported that length of time may be a factor affecting the physiological functions as a result of crowding-related social stress due to increase in biomass with time.

Cortisol and glucose are two of the most common stress indicators but due to their high variability, they must be complemented with other stress indicators in order to have a more complete profile about the stress status of any fish (Martinez-Porchas et al., 2009) and its levels are enhanced under adverse situations (Grutter and Pankhurst, 2000). The plasma glucose of the fish increases with increase in stocking density. This increase may be attributed to the mobilization of glucose by the fish in response to crowding stress; this is generally used as a means of providing extra energy resources so at to enable the fish to overcome the disturbance (Arends et al., 1999; Carragher and Rees, 1994). Alterations in glucose metabolism are a common response to stress (Barton and Iwama, 1991; Braley and Andersson, 1992). On the other hand, the direct effect of the higher metabolic energy demands during stress is driven by an increase in oxygen transport efficiency by elevation of the haematocrit and red blood cell numbers (Acerete et al., 2004). Farghaly et al. (1973) and Wedemeyer and Yasutake (1977) observed that plasma proteins contribute significantly to the maintenance of the blood volume and the water content of the fluid in tissue. The colloidal plasma protein cannot diffuse through capillary membranes to the surrounding relatively protein-free tissues. In this way, they exert an osmotic pressure thus allowing a minimum liquid volume to be maintained in the capillary blood vessels (Verdegem et al., 1997). Plasma protein in this study reflected the protein feeding levels. Schippers et al. (1994) in their works with freshwater-reared rainbow trout did not find significant differences in total plasma protein levels in fish fed different protein levels.

Therefore from the results of this study, it can be concluded that African catfish can easily adapt to high stocking density without any serious effect on the health status or big response of the fish if the correct management procedures are adopted.

Conflict of interests

The authors did not declare any conflict of interest.

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