ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 2, Issue 3, pp: (114-126), Month: July 2014 - September 2014, Available at: www.researchpublish.com

Stocking Densities And Zero Culture-Water Exchange Can Modulate Growth And Hemato- Immunological Functions In Juvenile GIFT Strain Tilapia, Oreochromis Niloticus L.

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Abstract: **The synergy effect of stocking densities and long term zero culture water exchange on growth and Hemato- immunological parameters of genetically improved farm tilapia (GIFT) juvenile (initial size 18.02±0.02g) was assessed. Water quality parameters; pH, dissolved oxygen (DO), temperature (T ^{o}C) and ammonia were recorded weekly. Results showed that weight gain, feed conversion rate (FCR), survival rate, final weight and SGR were negatively modulated (P<0.05). Hematological parameters including white blood cell (Wbc), red blood cell (Rbc), haemoglobin (Hb) and haematocrit (Ht); and non specific immune parameters including Lysozyme activity (LYS), Immune globulin M (IgM) and Superoxide dismutase (SOD) were significantly affected by the varied densities and long term zero culture water exchange rate (P<0.05). Serum biochemical parameters including total protein; TP, glucose (GUL), cholesterol (CHO), triglyceride (TG), Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) were significantly affected by varied densities and long term zero culture water exchange rate (P<0.05). Meanwhile, while TP, GUL, CHO, and TG decreased significantly (P<0.05); AST and ALT increased significantly (P<0.05). High levels of TP, TG and CHO were observed in the lowest density group (TI). Ammonia level exceeded optimum range for tilapia weeks later. Practically, water in tanks culture must be refreshed after a week.**

Keywords: **GIFT tilapia; stocking densities; zero culture water exchange; growth; Hemato- immunological functions**

I. INTRODUCTION

Tilapia aquaculture is rapidly expanding and has been estimated to increase to 8.89 million metric tons by 2020 (Tacon and Metian 2008). Presently in China, the increased culture of GIFT (Genetically Improved Farmed Tilapia) strain Nile tilapia is mainly due to its many advantages such as rapid growth rate, high fillet yield, and good disease resistance capability. The healthy cultivation of tilapia depends on nutritional status and rearing environmental conditions (Qiang et al. 2013). Stocking density is a major factor that affects aquatic animals' growth under cultured conditions (Di Marco *et al*. 2008; de Oliveira 2012). High stocking density results in stress leading to high energy demand. Studies have demonstrated the effect of stocking density on cultured fish welfare (Correa and Cerqueira 2007; Di Marco *et al*. 2008;

Caruso et al. 2011). The relationship between welfare and stocking density can be influence by variables such as food availability and water quality (Ellis *et al*. 2002). Zhang *et al.* (2011) acclaimed ammonia as third important respiratory gas after oxygen and carbon dioxide in ammoniotelic teleosts. Culture water is prone to ammonia toxicity if build up from fish wastes and uneaten feed are not put into check Studies have shown that, chronic/long term ammonia exposure can hinder fish growth (El-Shafai *et al*. 2004; Hegazi and Hasanein 2010), cause gill hyperplasia (Benli *et al*. 2008), liver tissue deterioration (Lease *et al*. 2003) and fish mortality. Most bony fishes are very sensitive to ammonia toxicity; when subjected to chronic ammonia stress fish antioxidant defense system will be damaged (Caruso et al. 2011), thus reducing the body's ability to clear free radicals (Romano and Zeng 2007). Low levels of ammonia nitrogen have been reported to have negative impact on fish health and growth rate (Foss *et al*. 2003; Biswas *et al*. 2006a, b; Remen *et al*. 2008). Sun et al. (2012) divulged that ammonia significantly restrained the antioxidant system in bighead carp *Hypophthalmythys nobilis*, thus making it susceptible to more pathogens. Chen *et al*. (2011) also reported that, the immune response of tilapia (*O. niloticus)* was restrained with exposure to ammonia toxicity.

Recent study probed into the effect of acute ammonia toxicity on fish immune system (Chen *et al*. 2011); however, little is known about the long term exposure of ammonia on fish immune responses (Lemarié *et a*l. 2004). Earlier studies on fish physiological responses to acute and chronic stressors in relation to welfare have been reported (Demers and Bayne 1997; Caruso *et al*. 2005; Caruso *et al*. 2008; Maricchiolo *et al*. 2008; Conte, 2004). Whiles acute stress can have different effects in fish; severe stress can have lethal consequences (Maricchiolo *et al*. 2008). This study therefore aimed at evaluating the effect of stocking density and long term zero culture water exchange on the Growth performance, Hemato-immunological and Biochemical Parameters in GIFT strain tilapia juvenile. This study provides insight to the indicative parameters fish farmer can use to assess the welfare of fish in tank based culture system.

II. MATERIAL AND METHODS

Fish and acclimatization process

Sixteenth generation GIFT tilapia juveniles, bred by Freshwater Fisheries Research Center of the Chinese Academy of Fishery Sciences in China, were used as test object in this trial. Prior to the official trial, fish were acclimatized for 7days in 12 m^2 concrete tank with water depth 0.8m. During the acclimatization process, water temperature was 26 ±0.3 °C as continuous aeration ensured right through. Fish were fed floating commercial feed (35% crude protein) to near satiation, twice daily. The Fish subsisted under 12hr: 12hr (light: dark), water pH; 8.0 ±0.2, and ammonia level 0.01mg/l; under regulated water inflow and out flow system.

Experimental design and animal rearing conditions

Twelve tanks (1.5m^3 each) were impounded with water up to 1m level and stocked with the acclimatized GIFT tilapia juvenile; initial weight 18.02±0.02g, in triplicate densities of 8fish/m^3, 15fish/m^3, 20fish/m^3 and 25fish/m^3 corresponding to TI, TII, TIII and TIV respectively. Fish were fed (3% total biomass twice daily; 08:00 and 16:00) commercial diet (Crude protein 35%; Fiber 8.0%; Ash 18%; Moisture 12%; Calcium 1.0%; total phosphorus 0.5%; NaCl 3.0%; Lysine 1.7% per kg feed) from Tian Bang Freshwater fish feed industry, Ningbo, China. Fish were grouped weighed on a weekly basis for readjustment of feeding amount. The total amount of feed consumed by each group was subsequently calculated as summation of given feed during the course of the trial. Solid wastes and uneaten floated feed 15min after fish have been served were removed from the respective tanks using a finely meshed scoop net. The experiment was carried out in a hatchery building under natural photoperiod; 12hr: 12hr (light: dark). Cultured waters in all the tanks were not refreshed. To minimize loss from evaporation as well as maintain equal volume of water in the respective tanks every week; equal volume of water (1L each) from the respective treatment was obtained for water quality analysis but replaced with tap water ((pH; 8.0 ±0.2, ammonia level; 0.01mg/l). Cultured water temperature in all the treatment tanks was subjected to changes in ambient temperature. Water in the culture tanks was continuously aerated as fish were cultured under the above conditions for 30days.

Sample collection and analytical methods

At the end of the 4-week feeding trial, fish in each tank were individually weighed and sampled for tissue analysis 24 h after the last feeding.

Blood analysis

Blood samples were drawn from the caudal vein of five fish from each tank with heparinized needles and centrifuged at 4°C, 3000 *g*, for 15 min to obtain the serum. Whole blood sample was used for white blood cell count, red blood cell count, hematocrit and hemoglobin content measurement. White blood cell count, haematocrit, and red blood cell count were determined using an automatic blood analyser (Hitachi 7170A). The Hemoglobin content (Hb) level was measured using the Diagnostic Kit (Sekisui medical, Tokyo,Japan), with complete conversion to cyan-methemoglobin and read at 540 nanometers (nm).

Blood serum was used for lysozyme activity, superoxide dismutase activity, immune globulin M (IgM) concentration, Serum glucose (GUL), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) activities, Cholesterol (CHO), Triglyceride (TG) and Total protein (TP) level measurement.The blood serum samples were quickly frozen and kept at -80°C until analysis.

Serum lysozyme activity was determined based on lysis of the lysozyme-sensitive Gram-positive bacterium, *Micrococcus lysodeikticus* (Sigma). The dilutions of hen egg white lysozyme (Sigma) ranging from 0 - 20 µg/ml (in 0.1 M phosphate citrate buffer, pH 5.8) were taken as standard and evaluated against the test serum (25µl) in 96-wells of flat-bottomed microtitre plates with 175 µl of *M. lysodeikticus*. After rapid mixing and change in turbidity, it was measured after every 30 s for 5 min at an approximate temperature of 20 °C using a microplate reader at 450 nm.

Superoxide dismutase (SOD) activity was measured based on its ability to inhibit superoxide anion generated by xanthine and xanthine oxidase reaction system according to Wang and Chen (2005); using SOD detection kit (Nanjing Jiancheng Bioengineering Institute, China).

Serum immune globulin (IgM) level was measured by an ELISA assay using a commercial kit (Cusabio, Wuhan, Hubei, China), as described by Sun et al. (2010); flat-bottomed 96-well plates were coated with serum samples for 2 h at 37 °C and the liquid were removed. The samples were blocked with 100ml of biotin-antibody for an hour at 37 °C. Each well was aspirated and washed three times using wash buffer (350ml). Samples were incubated with 100ml of horseradish peroxidaseeavidin (HRPeavidin) working solution for an hour at 37 °C and developed with TMB for 30 min at 37 °C. Each well was aspirated and washed three times with wash buffer (350ml). The reaction was stopped by adding 50ml of stop solution per well. The plates were read at 450 nm in a plate reader. Negative controls included samples without biotin-antibody. The mean absorbance of the negative controls for each plate was then subtracted from the optical density at 450 nm. All assay kits are specially designed for fish detection.

Serum glucose (GUL), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) activities, Cholesterol (CHO), Triglyceride (TG) and Total protein levels (TP), were measured by colorimetric method, using Mindray Auto Bio-chemical Analyzer (BS-400, Mindray, P.R. China) and test kit from Mindray Bio Medical Co., Ltd.

Water sampling and analytical methods

Prior to stocking the fish, triplicate water samples from all the tanks were collected for initial analysis of ammonia concentration. Initial and all water samples were collected and Analyzed following (SEPA, 2002). After initial sample collection, further sampling was on weekly basis. Water samples from the respective treatments were normally sampled in the morning at 06: 00h and immediately stored in a refrigerator at -18^{o}C pending analysis. All water samples collected were analyzed within three days. The pH of culture water was measured using pH meter (pHS-3TC, Shanghai reaches instrument Co., Ltd, Shanghai, China), whiles water temperature and dissolved oxygen were measures by a dual probe, SG6-FK10CN; Mettler Tory instruments Co., Ltd, Shanghai China.

Calibration of standard curve

Standard or calibration curve was made using standard solution and reagents. Volumes; 0.0ml, 0.5ml, 1.0ml, 3.0ml ,5.0 ml,7.0 ml and 10.0ml of ammonium standard liquid were respectively transferred into six different 50ml colorimetric tubes, followed by addition of different volumes of distilled water reaching the 50ml mark on colorimetric tubes. Thereafter, 1.0ml of potassium tartrate solution of sodium was added followed by the addition of 1.5ml of sodium Grignard reagent. The mixture was then gently mixed and absorbance of the respective solution in the six different tubes measured after 10min, via optical path using 10mm cuvette inserted in the UV-1200 spectrophotometer at a wavelength of

420 nanometers (nm), using distilled water as blank reference. Corrected absorbance was obtained by subtracting the zero-density (blank absorbance) from the measured absorbance of the respective solutions and final value obtained for calibration curve plot.

Analysis of Ammonia level in water samples

Each replicate sample of all treatment groups was first filtered using a suction vacuum pump with Buchner filter membrane. The filtered water collected was then analyzed (using Nessler reagent, GB7479-87) and Spectrophotometer, according to (SEPA, 2002). Equal volume (25ml) of filtered water sample from the respective treatment group, were transferred into 50ml colorimetric tubes followed by addition of 25ml of distilled water to make a total of 50ml followed by the addition of 1.0ml of potassium tartrate solution of sodium, mixed, followed by an addition of 1.5ml Sodium Grignard reagent. After gently mixing them together absorbance of the liquid mixture was then measured after 10 min via 10mm cuvette in a spectrophotometer at a wavelength of 420nm, using distilled water as blank reference. The sample actual absorbance was computed by subtracting the blank absorbance from the respective absorbance of the treatment groups. Absorbance was then inputted into the regression equation of the standard curve to obtain the mass/content (mg).

The calorimetric formula: NH_3-N (mg/l) = m / V × 1000; was use to calculate the concentration (mg/l); where: m, by calibration check was ammonia nitrogen content (mg) in the water sample; V= volume (ml) of water sample used.

Calculations and statistical analysis

The parameters for growth performance assessment were calculated as follows:

Feed conversion ratio (FCR) = dry feed fed (g)/ Wet weight gain (g)

Specific growth rate; SGR (%/d) = $[(\ln W_2 - \ln W_1) / (t_2 - t_1)]\times 100$

Weight gain (WG; %) $=100\times (W_2 - W_1) / W_1$

Survival rate; SR (%) = N_2/N_1 x 100

Where, W_1, W_2 and N_2, N_1 were body weights (g) and total number of fish at starting (t_1) and ending time (t_2) respectively.

Results were expressed as mean±SD. Furthermore, data were subjected to one-way analysis of variance. When significant differences occurred, the group means were further compared with Duncan's multiple-range tests. All statistical analyses were performed using the SPSS 19 (SPSS, IL, USA).

III. RESULTS

The results of growth performance GIFT tilapia juvenile are presented in Table 1. The results showed that weight gain decrease with increase in stocking density ($P<0.05$), on the contrary, feed conversion rate (FCR) increase with increased stocking density ($P<0.05$). Specific growth rate (SGR) and survival rate dwindled with increased stocking density ($P<0.05$). There was a significant difference in weight gain, FCR, survival rate, final weight and SGR among the treatment groups under the trial conditions ($P<0.05$). The highest survival rate was recorded in TI followed by TII and TIII respectively; TIV had the least survival rate.

The results of haematological and non specific immune parameters of the GIFT tilapia juvenile exposed to varied stocking densities and long term zero culture water exchange rate are presented in Table 2. Hematological parameters including Wbc, Rbc, Hb and Ht as well as the non specific immune parameters including LYS, IgM and SOD of GIFT strain tilapia juvenile were significantly affected by the varied densities and long term zero culture water exchange rate ($P<0.05$). Among the haematological parameters, only Wbc was increasing with increasing stocking density ($P<0.05$); the rest including Rbc, Hb and Ht decreased with increased stocking density ($P<0.05$). Meanwhile, non specific immune parameters including LYS, IgM and SOD in this study were decreasing significantly ($P<0.05$) as stocking density increased under the zero culture water exchange rate system of the trial.

Serum biochemical parameters including total protein; TP, glucose; GUL, cholesterol CHO, triglyceride TG, AST and ALT were significantly affected by the varied densities and the long term zero culture water exchange rate ($P<0.05$). Meanwhile, while TP, GUL, CHO, and TG decreased significantly ($P<0.05$); AST and ALT increased significantly

($P<0.05$) under the trial conditions. High levels of TP, TG and CHO were observed in the lowest density group (TI), as opposed to AST and ALT.

Weekly change in pH, dissolved oxygen (DO), temperature and ammonia are presented in Figs 1, 2, 3 and 4 respectively. pH (Fig. 1) and temperature (Fig. 2) values tended to increase with culture duration; on the contrary, DO levels decrease with stocking density and long term zero culture water exchange. The high pH values were recorded in the higher density groups. During the first two weeks, DO levels were the same; but marked differences were observed on the third and fourth week respectively (Fig 1). There was a gradual increase in temperature during the first two weeks, followed by an abrupt drop (wk. 3). The culture water temperature (Fig. 3) was influenced by daily ambient temperature. Ammonia levels in the first week were somewhat the same. After the first week (wk 1) a differences in trends were observed ($P<0.05$) among the treatment groups (Fig. 4).

IV. DISCUSSION

In the present study, weight gain and specific growth rate significantly decreased with increased stocking density and long term zero culture water exchange; higher stocking density caused higher accumulation of ammonia. Mean specific growth rates is one way of quantifying the effect of stocking density on growth (Correa and Cerqueira, 2007; d'Orbcastel *et al*. 2010; Ellis *et al*. 2002). Higher stocking densities have been reported to aggravate stress leading to reduced feeding efficiency and specific growth rate (Montero *et al*. 1999; Yousif, 2002). Our results are in agreement with the finding in spotted wolfish Anarhichas minor (Foss et al. 2003), turbot *S. maximus* (Person-Le Ruyet 2003; Foss et al. 2009) and Atlantic halibut *Hippoglossus hippoglossus* (Paust et al. 2011), and was mainly due to water quality related stress initiated by higher stocking density per meter cube of culture water environment. Previous studies have shown that fish exposed to stressors of this nature tend to show high glucose mobilization in the glycolysis pathways, and causing lipids and proteins to be utilized as an energy source (Mommsen et al. 1999), leading to little or no energy for growth purposes. Feeding appetite of fish in the higher density groups significantly reduced under the trial conditions which resulted to higher FCR values (Table 1) in TII, TIII, and TIV respectively.

Fish hematological parameters are important tool for monitoring fish health status (Hrubec *et al*. 2001; Qiang et al. 2013). In this study, haematological parameters including Wbc, Rbc, Hb and Ht as well as the non specific immune parameters including LYS, IgM and SOD of GIFT strain tilapia juvenile were significantly affected by the varied densities and long term zero culture water exchange rate. Rbc, Hb and, Ht decreased as stocking density was increasing (Table 2), and could have been caused by the reduced feed intake suffered by the fish which gives credence to the explanations of d`Orbcastel *et al*. (2010), Paspatis *et al*. (2003), Lambert and Dutil (2001); that long term/chronic stress from high stocking density can affect fish feeding behavior. On the other hand, Wbc was increasing with increased stocking density under long term zero culture water exchange. The increased Wbc may have been provoked by the innate immune system of the fish under varied stocking density and the long term zero culture water exchange conditions. Moreover, physiological responses to chronic/long term stress are mediated by stress hormones that may have caused activation of metabolic pathways that may have led to the modulation of hematological parameters (Bernier 2006). Higher density groups of fish may have suffered from weakened immunity that resulted to their death (Mehrim 2009; Paspatis *et al*. 2003); and informed the low values of survival rates in (TIII and TIV); Table 1. Mehrim (2009) did similar study correlating stocking density and dietary probiotic, and observed that, at optimal density, the probiotic improved fish immunity, but when stocking density went beyond optimal, positive effect of probiotic was suppressed which led to reduced levels of hematological parameters and survival rate.

IgM plays an important role in defending the host from infectious diseases (Li *et al*. 2007). In this trial, IgM levels decrease as stocking density increased under the trial conditions. Immune defense mechanisms in fish is of vital importance since a direct relationship between the functioning of the immune system and the ability to counteract disease outbreaks has been established (Blaxhall 1972), which informs therefore that immunodepression effect of stress conditions could increase susceptibility of the animals to pathologies (Pickering *et al*. 1987; Maule *et al*. 1989; Ellis 1999). The lower survival rates in the higher density groups (TIII and TIV) can be explained based on the poor fish resistance after long term exposure to the trial conditions. Lysozyme also has been documented as important defense element which causes lysis of bacteria (Jollès and Jollès 1984); its act as a vital bio-defense effectors of innate immunity (Simser *et al*. 2004). In this study, incessant aeration was ensured right through but it did not improve immunity of the

fish; accumulated level of ammonia could have affected oxygen intake of the fish, which led to the reduced haemoglobin count (Singh *et al*. 2013; Tilak *et al*. 2005). Meanwhile, the oxygen intake of fish may have been affected by elevated nitrite and ammonia levels in the culture water (Datta *et al*. 2005; Remen *et al*. 2008; Singh *et al.* 2013; Tilak *et al*. 2005) in culture system which may have caused the physiological imbalances in the fish (Iwama *et al*. 2000, Randall and Tsui 2002); especially in high density treatments. The antioxidant enzyme, Superoxide dismutase (SOD) is vital in reducing oxidative stress (Lushckak 2011), it plays a crucial role in responding against oxygen radicals and converts superoxide to H_2O_2 (winston and Giulio 1991). SOD in this trial decrease with increased stocking density under zero culture water exchange system. Wdziaczek et al. (1982), studied SOD activity in erythrocytes and liver of different fish species; their results showed that younger fish have higher antioxidant activity than older fish; in this trial, juvenile fish were but SOD modulation was caused by stocking density and water quality related stresses.

Biochemical parameters including total protein; TP, glucose; GUL, cholesterol CHO, triglyceride TG, AST and ALT were significantly modulated in this trial. Stress in fish has been correlated with changes in biochemical parameters (Casillas *et al.* 1983; Svobodova *et al*. 2006). In this trial, GUL, TG, CHO and TP levels decrease with increasing stocking density (Table 3). Triglyceride and cholesterol are energy based substances basically derived from lipid absorption in the intestines and liver fatty acid metabolism (Di Marco *et al.*, 2008); their levels in blood serum have been associated with stress management (Lupatsch *et al*. 2010; Pérez-Casanova et al. 2008). Vijayan *et al.* (1990) reported a reduction in triglyceride level when brook charr (*Salvelinus fontinalis*) was exposed to a stressful situation that triggered higher energy demand; and was further supported by Da Rocha *et al*. (2004), who also reported a change in the above parameter in matrinxã *(Brycon cephalus)* after handling and acute crowding stress. In the present trial, the change in GUL, TP, TG and CHO levels could be attributed to the long term exposure to varied densities and zero culture water exchange exposure; the animals could have utilized substantial amount of metabolizable energy responding to the stressful condition. Our result is similar to what was observed in Senegalese sole; *Solea senegalensis* (Costas *et al*. 2011). ALT is an important liver enzyme closely related to metabolism of protein, fats and carbohydrate; this enzyme will be released in blood when the liver is damaged (Zhang *et al*. 2007). Under normal condition AST can be found in soluble cytosol of liver cells, with relatively low activity, and may increase in blood serum when cells are damaged (Hu *et al*., 2012; Ming *et al*., 2012). According to Casillas *et al.* (1983), serum total protein, AST and ALT activities will inform liver damage in fish. In this trial, increased AST and ALT activities could have resulted from the long stay in chronic ammonia (Mona and Hegazi 2011) set-in by both fish wastes and uneaten feed. Ruane et al. (2002) reported marked decrease in plasma total protein in common carp (*Cyprinus carpio*) when held at high density; moreover, Biswas et al. (2006a) further reported decrease plasma total protein in red sea bream after short term handling stress. Stress has a wide range of negative impacts on fish growth and wellbeing (Øverli 2005; Trenzado 2003). In this present study, the decrease in total protein (TP) may have been caused by cumulative stresses from stocking density and accumulated ammonia, under the zero culture water exchange condition. On the contrary, Caipang *et al*. (2009) did not observe such change after exposing Atlantic cod (*Gadus morhua*) to short-term high stocking density stress. This discrepancy can be attributed to the differences in experimental design, stress duration and the test objects. In our trial, GIFT strain tilapia was used as test object and was held under the trial conditions for thirty days.

Continuous aeration of cultured water in all treatments group was ensured, but (DO) gradual decrease with stocking density and culture duration. This decrease in DO may have occurred through respiration by fish and through decomposition of organic substances by micro organisms. Despite this gradual decrease however, the least DO value in this study was around 5mg/l, the minimum requirement for most fish. Ammonia is the main nitrogenous waste material in teleosts, and is generated from protein catabolism. Under intensive culture conditions, if water flow is restricted or inadequate, uneaten feed and fish waste can lead to elevated ammonia level in the water.

Ammonia occurs in water in two forms; un-ionized (NH3) and ionized (NH4+), and the relative proportion of each form is dependent on pH, temperature and salinity. Unionized ammonia in the range of 0.02-0.2mg/l is toxic to fish because excessive ammonia in water tends to block O_2 transfer from gills to the blood (Smart 1978). Toxic NH_3 level in culture water is a function of water temperature and pH. Under zero water exchange system, pHs of the culture water tended to increase given that culture water temperature was modulated by ambient temperature. Ammonia levels in high density groups (TIII and TIV) were markedly elevated compared to the other treatment groups. A sub-optimal level of unionized ammonia in the range of 0.1-0.42 mg/l, can cause considerable variation in growth performance (Datta et al. 2005). Meanwhile optimal ammonia level for tilapia was estimated to be below 0.05 mg/l (El-Sherif and EL-Feky 2008). In this

study, after two weeks of commencement, ammonia levels in the high density groups (TIII and TIV) exceeded that value and could have caused the imbalances in response parameters. Elevated ammonia is a limiting factor in fish farming (Randall and Tsui 2002). Irrespective of the hardy nature of GIFT strain tilapia, it is suggested that; in practice, culture water must be refreshed at most after a week, so as to buffer the effects of water quality problems in tank culture system.

V. CONCLUSION

In conclusion, stocking densities and long term zero culture water exchange system in the trial caused chronic stress conditions that modulated the growth performance and the haemato- immunological functions in GIFT tilapia juvenile. Growth parameters (weight gain, final weight and SGR), haematological parameters (Rbc, Hb, and Ht), non specific immune parameters (IgM, LYS and SOD), biochemical parameters (TP, CHO, GUL and TG); all decreased with increased stocking density under the zero culture water exchange system. Meanwhile, FCR, AST and ALT in the trial increased with stocking density and long term zero water exchange system. Water quality deteriorated because of the elevated ammonia initiated by the zero water exchange system. In general, all the parameters assayed would be very useful for assessing growth and state of wellbeing of tilapia under tank culture system. Culture water must be refreshed at most after a week, so as to buffer the effects of water quality problems in tank culture system.

ACKNOWLEDGEMENTS

The study was supported by Special Fund for Agro-scientific Research in the Public Interest (200903046-02) and Postgraduate Scientific Research Innovation Program of Jiangsu Ordinary Higher Colleges and Universities (CXLX11-0708). The authors are grateful to Mr. Zhu Zhixiang of Yixing Experimental Base of Chinese Academy of Fishery Sciences, for providing biological material, site and other technical assistance. Lastly, anonymous reviewers are greatly appreciated for their brilliant suggestions.

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Appendix – A

Tables And Figures:

Table 1: Growth response of GIFT tilapia juvenile exposed to varied stocking densities and long term zero water exchange.

Item	Treatment			
	TI	TII	TIII	TIV
Initial weight (g)	18.10±0.03	18.02±0.02	18.06±0.01	18.12±0.06
Final weight(g)	53.3 ± 1.97^{d}	46.5 ± 1.90^{c}	29.3 ± 1.82^{b}	23.1 ± 0.77^{a}
FCR	1.42 ± 0.21^{a}	1.77 ± 0.15^{a}	3.15 ± 0.57^{b}	3.21 ± 0.20^{b}
Weight gain	194.6 ± 10.9^{d}	158.1 ± 10.4^{c}	62.4 ± 10.2^{b}	27.3 ± 3.81^{a}
Survival rate	98.7 ± 2.31^{d}	$90.7\pm2.31d^{c}$	72.0 ± 4.00^{b}	54.7 ± 8.33^{a}
SGR (% /day)	1.54 ± 0.05^{d}	1.35 ± 0.06^{c}	0.69 ± 0.09^{b}	0.34 ± 0.04^{a}

Data are represented as mean ±SD, n=3. Means with the same letter in the same column for each parameter are not significantly different. Significant difference ($P<0.05$). SGR= Specific growth rate, FCR= Feed conversion rate.

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 2, Issue 3, pp: (114-126), Month: July 2014 - September 2014, Available at: www.researchpublish.com

Table 2: Responses of haematological and non specific immune parameters of GIFT tilapia juvenile exposed to varied stocking densities and long term zero culture water exchange.

Item	Treatment			
	TI	TII	TIII	TIV
Wbc10^{-9}/L	280.3±9.95^{a}	305.1±13.8^{b}	350.7±13.9^{cd}	364.6±13.7^{d}
Rbc10^{-12}/L	2.87±0.11^{b}	2.62±0.12^{b}	1.99±0.19^{a}	1.71±0.23^{a}
Hb g/L	93.9±6.56^{b}	82.5±8.50^{b}	67.9±5.25^{a}	62.3±10.19^{a}
Ht%	35.3±4.53^{c}	27.0±2.00^{b}	21.3±1.53^{a}	17.3±1.53^{a}
LYS (U/ml)	13.93±1.64^{b}	12.25±1.64^{ab}	11.36±1.56^{ab}	11.36±1.56^{ab}
IgM(mg/l)	29.47±1.45^{c}	27.13±0.60^{b}	24.10±1.35^{a}	23.53±1.17^{a}
SOD	123.25±8.26^{c}	112.12±8.33^{b}	77.91±6.61^{a}	71.54±6.09^{a}

Values are presented as mean±SD (n=3); different letter superscripts mean significant difference (P<0.05). Wbc= White blood cell; Rbc= Red blood cell; Hb=haemoglobin; Ht=hamatocrit; IgM =Immune globulin M; LYS= Lysozyme activity; SOD= Superoxide dismutase.

Table 3: Responses of some biological parameters in GIFT tilapia juvenile exposed to varied stocking densities and long term zero culture water exchange.

Item	Treatment			
	TI	TII	TIII	TIV
TP (gL^{-1})	33.27±4.50^{c}	25.56±3.95^{b}	18.68±1.49^{a}	17.31±2.41^{a}
GUL ($mmol^{L-1}$)	2.52±0.43^{b}	2.27±0.46^{b}	1.38±0.43^{a}	1.16±0.43^{a}
CHO ($mmolL^{-1}$)	3.13±0.11^{c}	2.63±0.19^{b}	2.44±0.11^{a}	2.37±0.02^{a}
TG ($mmolL^{-1}$)	1.29±0.12^{d}	0.83±0.02^{c}	0.63±0.03^{b}	0.52±0.01^{a}
AST(UL^{-1})	153.86±16.25^{a}	166.82±19.58^{a}	222.99±17.22^{b}	224.47±17.17^{b}
ALT(UL^{-1})	74.38±7.39^{a}	80.31±6.44^{a}	111.03±7.51^{b}	115.88±6.67^{b}

Values are presented as mean±SD (n=3); different small letter superscripts mean significant difference (*P*<0.05).GUL= Glucose; CHO= Cholesterol; TG= Triglyceride; TP= Total protein; ALT= Alanine aminotransferase; AST= Aspartate aminotransferase.

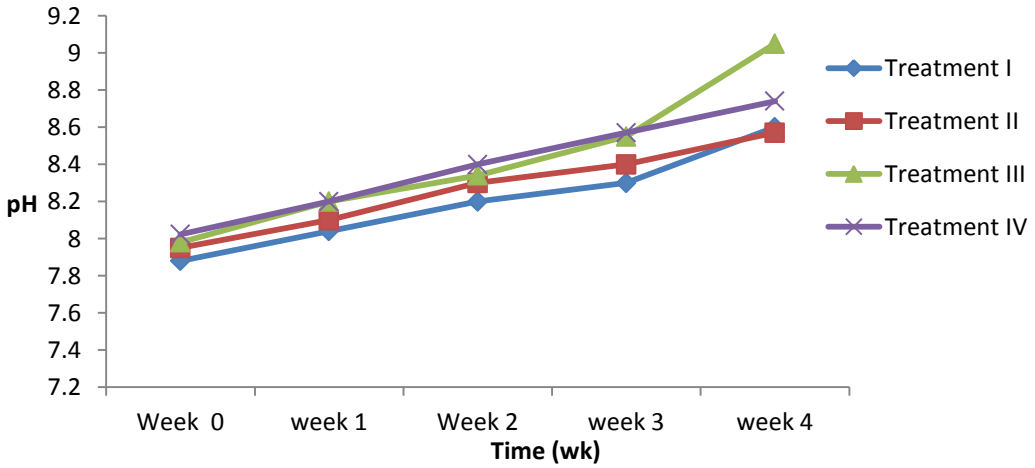

Fig.1 pH dynamics in tank culture water stocked with GIFT strain tilapia under zero water exchange.

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 2, Issue 3, pp: (114-126), Month: July 2014 - September 2014, Available at: www.researchpublish.com

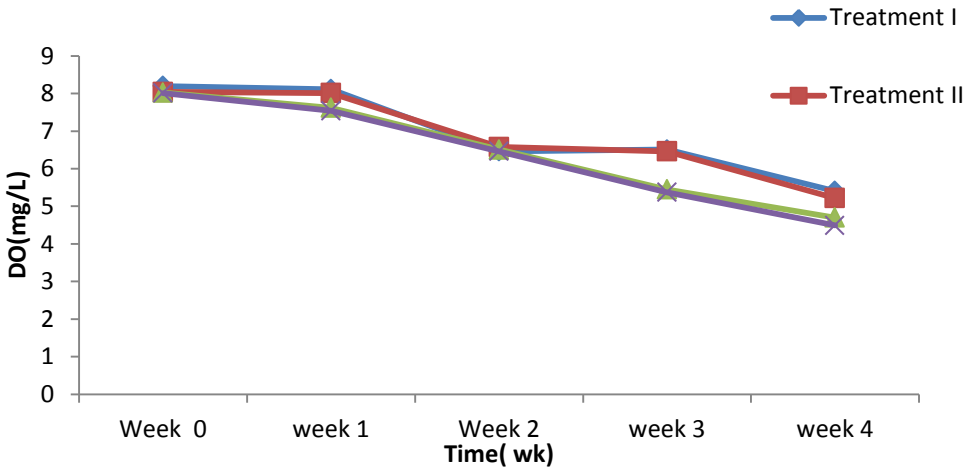

Fig.2. Dissolved oxygen (DO) dynamics in tank culture water stocked with GIFT strain tilapia under zero water exchange.

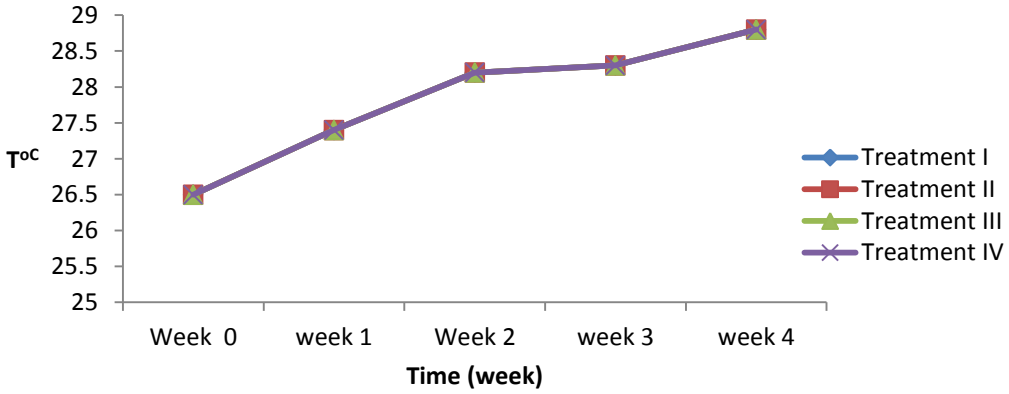

Fig. 3 Ambient Temperature dynamics in tank culture water stocked with GIFT strain tilapia under zero water exchange.

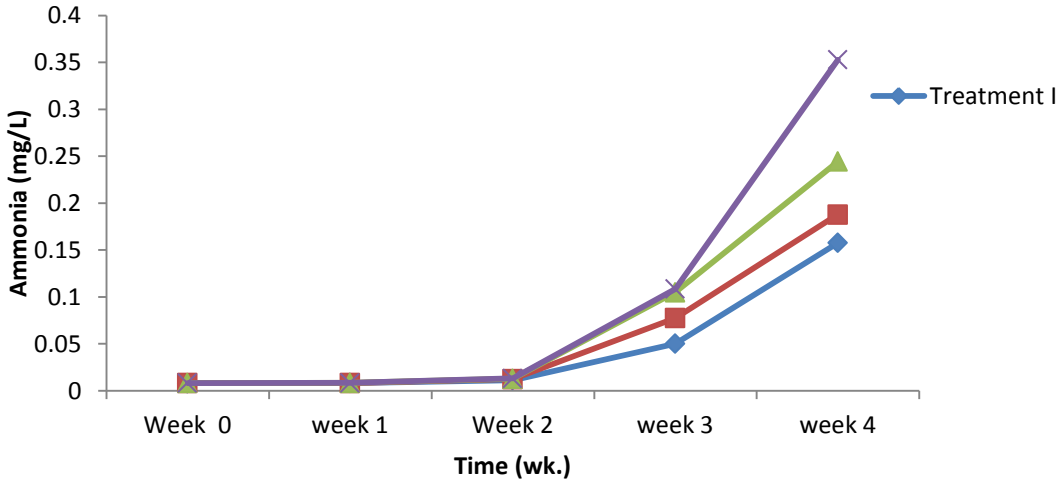

Fig.4. Ammonia dynamics in tank culture water stocked with GIFT strain tilapia under zero water exchange.