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Growth, antioxidant activities and haemato-biochemical responses of Genetically Improved Farmed Tilapia (GIFT), *Oreochromis niloticus* fingerlings reared in Recirculating Aquaculture System (RAS) at different stocking densities fed on artificial feed

Anusha Patel[1], Sikendra Kumar[1]*, Tincy Varghese[1], Ashutosh D. Deo[1], Vijaykumar M.[1] and A.K. Verma[2]
[1]Fish Nutrition, Biochemistry and Physiology Division, ICAR-CIFE, Mumbai (Maharashtra), India.
[2]Division of Aquaculture, ICAR-CIFE, Mumbai (Maharashtra), India.

*(Corresponding author: Sikendra Kumar*sikendra@cife.edu.in, 8709802595)*
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ABSTRACT: A 6- weeks feeding trial was conducted by stocking Genetically Improved Farmed Tilapia (GIFT), *Oreochromis niloticus* fingerlings at different stocking densities in a recirculating aquaculture system (RAS) to estimate the growth, antioxidant activities and haemato-biochemical responses during the culture period. The water flow rate of 10litres per minute was maintained throughout the experiment. The GIFT tilapia fingerlings with an average weight of 15.34g ±0.59 were stocked at different stocking densities as T_1 (3833g/m^3, 250 fishes/m^3), T_2 (5500g/m^3, 366 fishes/m^3) and T_3 (7166g/m^3, 466 fishes/ m^3). Significantly ($P<0.05$) higher weight gain, weight gain %, specific growth rate and protein efficiency ratio were found in the T_1 group compared to T_2 and T_3. The T_3 manifested the lowest above growth parameters. The T_1 group reported a significantly ($P<0.05$) lower release of phosphorus 4.29mg/l compared to the T_2 and T_3 groups. The highest phosphorus level of 7.49 mg/l was reported in the T_3 stocked with 7166g/ m^3, which was comparable with the T_2. The release of total ammonia nitrogen did not vary ($P>0.05$) among the treatments. The T_1 group showed a lower dietary total ammonia nitrogen release than the T_2 and T_3. The T_3 group had significantly ($P<0.05$) elevated nitrite and nitrate levels. The serum total protein, albumin and globulin were significantly ($P<0.05$) higher in T_1, which was comparable to T_2. The superoxide dismutase and catalase activities were also analysed, and these parameters vary significantly ($P<0.05$) among the treatments. The T_1 group reported the highest survival percentage in comparison to other treatments. It can be concluded that in the present experiment at a stocking density of 3833g/m^3 (250 fishes/m^3) the optimal growth performance of GIFT tilapia was found in the RAS with the lower release of dietary phosphorus, total ammonia nitrogen and nitrite.

Keywords: Recirculating aquaculture system, GIFT tilapia, Growth, Stocking densities.

INTRODUCTION

Aquaculture is a growing sector which is reported to become more reliable in agriculture allied activities for providing quality food to the human. The intensive aquaculture technology of the recent recirculating aquaculture system (RAS) has evolved to upgrade the fish production from a unit area using less water. It is now possible to produce 60 MT fish from 1/6th ha from RAS, far better than the normal aquaculture of average production of 3-10 MT per ha (DAHDF, 2017). RAS allow the fish farmers to regulate all the water quality parameters completely. As RAS is a completely soil-independent system, there is a lack of a natural sink for dissolved water generated by the system. Thus, dissolved wastes such as ammonia and phosphorus need to be dealt with by the biological filters installed in the system. However, the waste produced should not exceed the handling capacity of the biological filter; hence, a proper stocking density is of paramount importance (Bhakta *et al.*, 2009). The recirculating systems maintain an energy-intensive flow of water to reuse it after filtration by passing through different kinds of filters. As the filtration and resultant water purification are continuous processes, RAS maintains high water quality; therefore, it can maintain a higher stocking density than traditional aquaculture. The optimisation of proper stocking density is very important in intensive aquaculture systems (Turnbull *et al.*, 2005) as it enables us to know the suitability of any species in a particular culture system. The stocking density of fish and the feed ratio has an important influence on fish growth and water quality like-total ammonia nitrogen, nitrite, nitrate and phosphorus, which affect the dissolved oxygen level of water. Higher stocking density also affects the feeding rate and biomass; therefore, higher net yields can be expected at higher densities when water quality in the RAS is appropriately managed (Al-Harbi and Siddiqui 2000).

GIFT tilapia (*Oreochromis niloticus*) is one of the most widely used cultured finfish species, which accounts for approximately 80% of cultured tilapia worldwide (FAO, 2018), owing to its faster growth and better consumer acceptance. Growth performance of tilapia at different stocking densities in RAS is reported but large variations are found across the world. This variation

could be due to different types of microbes developed in the biological filter of the RAS system depending upon the environmental variables in different geographical locations. Wambua (2021) reported stocking density of tilapia in RAS is 5000g/m³. However, Menaga and Felix (2017) reported higher weight gain (%) and specific growth rate (SGR) of GIFT tilapia in the RAS at a stocking density of 9000g/m^3 fed with 39% crude protein and 8.0% lipid-based diet. Higher weight gain % and SGR of tilapia were reported at a stocking density of 480 g/m³ with 30% crude protein-containing feed (Bahnasawy, 2009). Large changes are reported in the stocking density of tilapia in RAS and its effects on growth performance.

Ramy *et al.* (2020) reported higher growth and serum protein of GIFT tilapia at a stocking density of 700g/m³ compared to a higher stocking density of 1400g/m³ in the RAS system.

Feed is the main source of phosphorus, total ammonia, nitrogen, and nitrite in the RAS system. The release of phosphorus, total ammonia nitrogen, nitrite and nitrate depends on the types of feed and stocking density in the culture system. The evaluation of the release of dissolved wastes in the RAS is necessary for monitoring the carrying capacity and fish production in the culture unit. Menaga and Felix (2017) reported phosphorus release of 4.01±0.34 mg/L in the RAS of GIFT tilapia fed with 39% crude protein and 8.0% lipid feed. Ekasari and Maryam (2012) reported the highest total ammonia nitrogen concentrations at a stocking density of 100 fish/m^3 (1.04 mg/L) in their experiment conducted on red tilapia culture in the RAS.

The proper stocking densities of GIFT tilapia will help to assess the production capacity and release of dissolve wastes- phosphorus, total ammonia, nitrite and nitrate in the RAS. Utilizing the above facts the present experiment was conducted to evaluate the growth and haemato-biochemical changes in GIFT tilapia fingerlings reared in the RAS at different stocking densities.

MATERIAL AND METHODS

A. Experimental Animal

GIFT-tilapia fingerlings were procured from the Hans Aquaculture farm in Raigad, Maharashtra. The fish were transported using a 100L capacity polythene bag provided with oxygen from the oxygen cylinder and transported to the experimental site. The animals were disinfected with potassium permanganate solution before placing them in the acclimatization tank. Then fishes were acclimatized for a period of 14 days in the freshwater. (After acclimatization, the fish attended an average weight of 15.34g ±0.59 g). During the acclimatization, fish were fed a 3% pellet diet (30.0% crude protein and 6% lipid) till satiation twice a day.

B. Experimental Setup

The recirculating aquaculture system was made with the help of a 0.5 HP motor fitted in individual plastic creates (75 L capacity) with continuous water recirculation. A total 9 experimental tanks were used in the experiment. (Before stocking, the tanks were dewatered and treated with bleaching powder for disinfection. Then the tanks were washed and sun-dried. Water was filled to 60 litres in all the tanks.

C. Experimental Design

A completely randomized design (CRD) was made for the experiments. Total three stocking densities as T_1 (3833g/m^3, 250 fishes/m^3), T_2 (5500g/m^3, 366 fishes/m^3) and T_3 (7166g/ m^3, 466 fishes/ m^3) in triplicates were made in the RAS. The design ensured that each fish was available to 3.5 to 4 L of water irrespective of their size. The fish were fed with floating pellet feed (30% Crude protein and 6.00 % Lipid) with 3% body weight twice a day throughout the experiment. Periodical growth and water quality parameters were evaluated.

Feeding. Fishes were fed with 3% body weight twice a day during the entire experimental duration. Feed was given in the morning and evening at 07.00 hrs and 17.00 hrs. The feed used in the experiment contained 30 % crude protein, 6.0% lipid, 6.25% crude fibre and 6.85% total ash. The proximate analysis of feed was performed according to the method of AOAC (1995). The unconsumed feed in every experiment tank was removed with the help of siphoning.

Chemicals, Glass Wares and feed. The glass wares, chemicals and kits used throughout the experiment were supplied by Genetix Biotech Asia Pvt. Ltd and Genex Life Sciences Pvt. Ltd. The feed used in the experiment was procured from Growel feed company.

Water quality parameters. Water quality parameters such as dissolved oxygen, pH, phosphorus, total ammonia, nitrate and nitrite were evaluated at the end of the experimental period as per the standard procedures (APHA, 2005).

The temperature was measured using a digital thermometer (Thermoscientific, USA). Dissolved oxygen was measured using Winkler's method.

Total ammonia. Total ammonia concentration before exchange of water was estimated spectrophotometrically at 635 nm wavelength by phenate method (APHA, 2005) and the concentration was estimated from the standard graph and expressed as mg/L.

Nitrite-NO_2-N. Nitrite-N concentration was estimated spectrophotometrically at 543 nm wavelength (APHA, 2005). The concentration was estimated from the standard graph and expressed as mg/L.

Nitrate-NO_3-N. Nitrate-N concentration was estimated spectrophotometrically at 543 nm wavelength (APHA, 2005). The concentration was estimated from the standard graph and expressed as mg/L.

Growth Analysis. The growth of fish was estimated at the beginning and the end of the experiment by collecting the fish using hand net. During sampling, 25% of the stock was collected from each tank to record the weight of the individual fishes.

Percentage weight gain. Percentage weight gain was calculated using the following formula:

$$\text{Percentage weight gain} = \frac{\text{Final weight (g)} - \text{Initial weight (g)}}{\text{Initial weight (g)} \times 100}$$

Specific growth rate (SGR). The specific growth rate was calculated by the following formula:

$$SGR = \frac{\ln \text{Final weight} - \ln \text{Initial weight}}{\text{Number of days} \times 100}$$

Feed conversion ratio (FCR). The feed conversion ratio was calculated by the following formula

$$\text{Feed Conversion Ratio} = \frac{\text{The feed given (dry weight)}}{\text{Body weight gain (wet weight)}}$$

Feed efficiency ratio (FER). The feed efficiency ratio was calculated by the following formula

$$\text{Feed Efficiency Ratio} = \frac{\text{Body weight gain (wet weight)}}{\text{The feed given (dry weight)}}$$

8.5 Protein efficiency ratio (PER)

The protein efficiency ratio was calculated by the following formula

$$\text{Protein Efficiency Ratio} = \frac{\text{Body weight gain (wet weight)}}{\text{Crude Protein fed in g}}$$

Survival rate. At the end of the experiment, all the experimental tanks were dewatered and the number of experimental animals in each tank was counted and the survival rate (%) was calculated by the following formula:

Total number of animals harvested

$$\text{Survival (\%)} = \frac{\text{Total number of animals harvested}}{\text{Total number stocked}} \times 100$$

Enzyme assays

Sample preparation. A total of five fish were collected from each experimental tank and anaesthetized with clove oil (50 µl/L) for growth study, blood collection, serum preparation and tissue collection. After dissection of fishes, tissues (liver& gill) were removed carefully and weighed. A 5% tissue homogenate was prepared in a chilled sucrose solution (0.25 M) in a plastic 15 ml centrifuge tube using a Teflon-coated high-speed homogenizer (Miccra-D, Germany). The tube was kept in ice during homogenisation to avoid heating. Centrifugation of the homogenized samples was done in a cooling centrifuge machine at 5000 rpm for 10 min at 4°C. The supernatant was collected in a plastic vial and stored at -20°C until use.

Tissue protein estimation: Quantification of protein in the different tissue homogenates was carried out by Lowry's method (Lowry *et al.*, 1951).

Activities of protein metabolic enzymes

Aspartate aminotransferase (AST). AST activity in different tissues of GIFT was analysed by the procedure, as described by Wooton (1964) and the activity was expressed as nanomoles oxaloacetate formed/mg protein/min at 37°C.

Alanine aminotransferase (ALT). The process was the same as that of AST activity except that the substrate comprised 0.2 M D, L-alanine instead of aspartic acid. The ALT activity was expressed as nanomoles of pyruvate formed/mg protein/min at 37°C**.**

Activities of antioxidant enzymes

Superoxide dismutase (SOD). Superoxide dismutase activity in different tissues of GIFT was analysed by the procedure described by Misra and Fridovich (1972) and the activity was expressed as the mg of protein required for 50% inhibition of epinephrine auto-oxidation/minute.

Catalase (CAT). Catalase activity in GIFT fingerlings was analysed by the procedure described by Takahara *et al.* (1960) and expressed as nano-moles H_2O_2 decomposed/min/mg protein.

Serum protein profile

Collection of serum. Each fish was anaesthetized with clove oil (50 µL of clove oil per litre of water) before taking blood from the fish. The caudal vein of the fish was ruptured to collect the blood using a medical syringe. The blood was immediately transferred to a dried eppendorftube. The tubes were allowed to stand in a tilted position at room temperature for an hour, which allowed the blood to clot. After clotting the blood, the yellow straw colour serum was carefully collected and transferred to another eppendorftube and stored at -20°C until analysis**.**

Serum total protein. Serum total protein estimation was done by the biuret method (Reinhold, 1953) using a commercially available kit (ERBA, Manheim, Germany). The absorbance of standard (S) and test (T) was measured against the blank (B) in a spectrophotometer at 546 nm. The calculation was done as follows:

$$\text{Serum protein(g/dl)} = \frac{\text{Concentration of standard} \times \text{absorbance of the test (T)}}{\text{The absorbance of the standard} \quad \text{Concentration of standard} = 6\ \text{g/dl}}$$

Serum albumin. Serum albumin estimation was done by the bromocresol green binding method (Doumas *et al.*, 1971) by using the ERBA KIT. The absorbance of standard (S) and test (T) was measured immediately against blank (B) in a spectrophotometer at 630 nm. The calculation was done as follows:

$$\text{Serum albumin(g/dl)} = \frac{\text{The absorbance of test} \times \text{Con. of standard}}{\text{Concentration of standard} = 3.6\ \text{g/dl absorbance of standard}}$$

Serum globulin. Serum globulin was calculated by subtracting albumin values from total protein as follows:

Globulin (g/dl) = Total protein (g/dl) – Albumin (g/dl)

Serum albumin to globulin ratio (A: G. Serum albumin value was divided by globulin value albumin to globulin ratio (A: G) as follows:

$$A : G = \frac{\text{Albumin (g/dl)}}{\text{Globulin (g/dl)}}$$

Statistical analysis. The data were subjected to one-way analysis of variance (ANOVA) using IBM SPSS statistics (version 22) for Windows to calculate means and standard error values. Duncan's multiple range test (DMRT) under post hoc was used for observing significant differences among the mean values at a 5% probability level (p<0.05). The analysed data were expressed as means ± standard error (SE).

RESULTS AND DISCUSSION

Physio-chemical parameters of water. The total ammonia nitrogen content of water in all the experimental tanks was in the range of 0.06 to 0.070

mg/L during the experimental period (Table 1). The nitrite nitrogen content in the water of all treatment tanks ranged between 0.006 to 0.012 mg/L. The nitrate-nitrogen values in the water of all experimental tanks were recorded within the range of 1.612 to 1.618 mg/L throughout the experimental period. Phosphorus values in the water of all experimental tanks were recorded within the range of 4.29 to 7.49 mg/L throughout the experimental period.

***Weight gain (%)*:** The body weight of the experimental groups was recorded at the end of the experiment and shown in Fig. 1. There was a significant difference ($P<0.05$) in the body weight gain % among different treatment groups at the end of the experimental period. Significantly ($P<0.05$) higher body weight gain per cent was observed in the T_1 group. However, there was no significant difference in the body weight gain %between the T_2 and T_3 groups.

Specific growth rate (SGR.). SGR of the experimental groups was recorded at the end of the experiment and shown in Fig. 2. There was a significant difference ($P<0.05$) in the SGR value among different treatment groups at the end of the experimental period. Significantly ($P<0.05$) higher SGR was observed in the T_1 group. However, there was no significant difference in the SGR between the T_2 and T_3 groups.

Feed conversion ratio (FCR). FCR of the experimental groups was recorded at the end of the experiment and shown in Fig. 3. At the end of the experimental period, there was a significant difference ($P<0.05$) in the FCR among different treatment groups. Significantly ($P<0.05$), the lowest FCR was observed in the group T_1. However, there was no significant difference in the FCR among the T_2 and T_3 groups.

Protein efficiency ratio (PER)

The PER of the experimental groups was recorded at the end of the experiment and shown in Fig. 4. There was a significant difference ($P<0.05$) in the PER found among different treatment groups at the end of the experimental period. Significantly ($P<0.05$) higher PER was observed in the group T1. However, there was no significant difference in the PER between the T_2 and T_3 groups.

Survival percentage. No significant variation in the survival percentage of the GIFT tilapia fingerlings was found at the end of the experiment (Fig. 5).

Aspartate transaminase. Liver aspartate aminotransferase (AST) activity varied non-significantly ($P>0.05$) among the treatments. T_1 reported the highest AST activity followed by T_2. The lowest AST activity was found in the T_3 group (Table 2).

Alanine transaminase: Alanine transaminase (ALT) activity in the liver varies significantly ($P<0.05$) among the treatments. T_2 reported significantly ($P<0.005$) the highest ALT activity (Table 2) among the treatment groups.

Superoxide dismutase (SOD): Superoxide dismutase (SOD) activity in the liver of GIFT tilapia varied significantly ($P<0.05$) among the treatment groups (Table 3). Significantly ($p<0.05$), higher SOD was found in the T_3 followed by T_2.

Catalase: Catalase activity in the liver varied significantly ($P<0.05$) among the treatments. Significantly ($P<0.05$), the highest catalase activity was reported in the T_3 group, followed by T_2. The catalase activity was reported lowest in T_1 group (Table 3).

Serum protein profile: The different stocking densities of fishes in the RAS resulted in a significant ($P<0.05$) variation in the serum total protein, albumin and globulin value. However, the A:G ratio did not vary significantly ($P>0.05$) among the treatments. The T_1 group had the highest serum total protein. The serum albumin and globulin were significantly ($P<0.05$) higher in T_1, which was comparable to the T_2 group. The lowest A:G ratio was found in the T_1 followed by the T_2 group (Table 4).

The recirculating aquaculture system is an intensive culture unit which relies mainly on the formulated feed to provide all the nutrient requirements of the fish reared in the system. It needs minimum space and water and operates in a fully controlled environment. In RAS, water is recycled by using waste treatment infrastructures, such as drum filters and biological filters, for controlling and stabilizing the environmental conditions of the fish, reducing the amount of water used, and improving fish welfare (Martins *et al.*, 2010; Bahnasawy, 2009). Feeds increase the waste load to the system, and without appropriate management, they can impair water quality and cause nutrient and organic pollution in water bodies which receive aquaculture effluents (Tucker and Hargreaves 2008). Feed is the main source of nitrogenous waste in the RAS as 32% protein feed of 1kg quantity releases around 30 g ammonia, which needs to be monitored properly (Ebeling *et al*., 2006). In the RAS, the ammonia level needs to be kept below 1mg/l for fish's better growth and survival (Timmons and Ebeling 2007). The current experiment was conducted to know the effects of different stocking density of GIFT fingerlings on the growth, heamato-biochemical and antioxidant activities and release of dissolved wastes in the RAS.

The stocking density and feed quantity are very important in the RAS as they determine the growth rate of fish and also the release of dissolved nutrients such as phosphorus, total ammonia, nitrogen and nitrite. In the current experiment, commercial feed (crude protein: 30%, crude lipid:6%) was used for feeding the fish at different stocking densities in the indoor tanks of RAS. Water quality and filtration capacity are primary determinants of stocking density in the RAS. Overcrowding can lead to the outbreak of diseases in fish reared in intensive farming systems (Van de Nieuwegiessen, 2009). The pH of the water in the indoor tanks varies between 7.20 and 7.60, which is supported by Pompa and Masser (1999), who reported an optimum pH of 5-10 for tilapia growth in the RAS. The temperature recorded during the present experiment was from 25.6±0.33 to 26.6±0.33°C, which falls in the optimum temperature range of 23.5-31.5°C, as reported for tilapia growth in the RAS (Meske, 1985). The dissolved oxygen reported in the experimental tank was in the range of 6.46 to 7.03 ppm, which is supported by Siddiqui *et al*. (1989), who

reported optimum dissolved oxygen between 5.6-8.2 ppm for growth of tilapia in the RAS.

There are different studies on the stocking density of tilapia in the RAS; the highest tolerable stocking density of GIFT was reported as 4000-6000g/m^3 (Menaga and Felix 2017; Wambua, 2021). The growth of fishes at different stocking densities in the current work in the RAS of GIFT tilapia varied significantly. Significantly ($P<0.05$) higher weight gain, weight gain %, SGR, PER and lower FCR were reported in the T_1 (Stocking density of 230g/0.06 m^3), which might be due to the higher feed conversion due to lower energy expenditure required for combating the stress due to ammonia. Arifin *et al.* (2019) reported decreased growth of giant gourami (*Osphronemus goramy)* larvae at higher stocking density (19.2 individuals L^{-1}) reared in a recirculating aquaculture system might be due to a reduction in food intake by the larvae stocked at the highest densities. Higher weight gain % and SGR of tilapia were reported at a stocking density of 480 g/m^3 with 30% crude protein feed (Bahnasawy, 2009). Higher weight gain, weight gain (%), SGR and PER values reported in the T_1 group also might be due to the better feed utilisation by the fishes and ample individual space for their growth. In contrast, Menaga & Felix (2017) reported higher weight gain percent and SGR of GIFT tilapia in the RAS at stocking density 9000g/m^3 fed with 39% crude protein and 8% lipid feed. Also, at high density, the release of nitrogen and phosphorus is high compared to low stocking density in the current experiment, which might have hampered the growth of fish.

The SOD and catalase enzymes were evaluated in the liver and gill at the end of the experiment, and they varied significantly ($P<0.05$) among the treatments. The stress created due to higher stocking densities might not have reached the threshold to elicit antioxidant responses. Higher stress, increased peroxidation and higher cell degradation have been found in fish reared at high stocking density (Sahin *et al.*, 2014). Ramy *et al*. (2020) reported an optimum immune response at a lower stocking density of GIFT tilapia (700g/m^3) compared to a higher stocking density (1400g/m^3) in the RAS system. The T_1 group stocked with lower stocking density reported low SOD and catalase activity. Low stress of fishes in the lower stocking density might be the reason for lower SOD and catalase in this group.

Phosphorus, a metabolic waste, is a dissolved nutrient released in the culture unit that creates environmental problems. Feed is the origin of most dissolved phosphorus waste, which results from intensive aquaculture practice. The excess of phosphorus in the effluents of the aquaculture system led to eutrophication and a consequent change in the aquatic ecosystem (Jahan *et al.,* 2003). Significantly (<0.05) lower release of dietary phosphorus was observed at astocking density of 230g/0.06m^3. Menaga and Felix (2017) reported phosphorus release of 4.01±0.34 mg/L in the RAS of GIFT tilapia fed with 39% crude protein and 8.00% lipid feed. Ammonia is the main excretory product of fish, and the protein present in the feed is responsible for its excretion in the water. In the present experiment, 30% protein was used for feeding the fish. The lower release of total ammonia nitrogen, nitrite and nitrate in the T_1 group might be due to the lower stocking density and better utilization of the feed by the fish. However, the higher release of total ammonia nitrite and nitrate in other groups stocked at higher stocking density might be due to more excretion of ammonia due to high metabolic demand due to stress. Menaga and Felix (2017) reported a total ammonia release of 0.07±1.10mg/L in the RAS of GIFT tilapia fed with 39% crude protein and 8.00% lipid feed. However, Maryam *et al*. (2012) reported that the highest ammonia concentrations were observed in the stocking density of 100 fish/m^3 (1.04 mg/L) in their experiment conducted on red tilapia culture in RAS. The release of phosphorus was significantly ($P<0.05$) lower in the T_1, which may be due to the proper utilisation of the feed by the fish.

An increase in the total serum protein, albumin and globulin levels is thought to be associated with a stronger innate response in fishes. Gamma globulin fraction is the source of almost all the immunologically active proteins of the blood (Jha *et al.*, 2007). The T_1 group stocked with lower stocking density manifested significantly ($P<0.05$) higher serum protein, albumin and globulin levels but lower A/G ratio than other groups. At lower stocking density, fishes have utilised the feed properly, and better assimilation takes place. That's why T_1 reported a good serum protein profile. Ramy *et al.* (2020) reported better growth and serum protein at a lower stocking density of GIFT tilapia 700g/m^3 compared to a higher stocking density of 1400g/m^3 in the RAS system.

The survival of fish depends on the quality of feed, which supplies all the required nutrients to the fish. RAS is a totally feed-based system. The T_1 with stocking density (3833g/m^3) in the present experiment showed higher survival than the other stocking densities. These results are also supported by lower release of phosphorus, total ammonia, nitrite and nitrate in the T_1. The high-density group (7166g/ m^3) had lower survival of the fishes as the fishes at higher density might be under stress, resulting in low survival. Klanian and Adame (2013) reported higher survival (%) of GIFT tilapia in the RAS system at the lower stocking density of 800 g/m^3.

Table 1: Water quality parameters of different experimental tanks of RAS.

Treatments	Water Flow Rate (L/min)	DO (ppm)	pH	Temp(°C)	TAN (Mg/l)	Nitrite (Mg/l)	Nitrate (Mg/l)	Phosphorus (Mg/l)
T_1	1.93±0.06	7.03^{b}±0.60	7.23^{a} ±0.05	25.6±0.33	0.06±0.009	0.006^{a} ±0.005	1.612^{a}±0.002	4.29^{a} ±0.47
T_2	1.90±0.10	6.76^{b} ±0.03	7.36^{a} ±0.08	25.6±0.33	0.09±0.027	0.009^{b} ±0.005	1.616^{a}±0.001	6.64^{b} ±0.28
T_3	1.90±0.10	6.46^{a} ±0.20	7.60^{b} ±0.01	26.6±0.33	0.84±0.070	0.012^{c} ±0.001	1.618^{b} ±0.001	7.49^{d} ±0.15

Stocking density T_1 (3833g/ m^3, 250 fishes/m^3), T_2 (5500g/m^3, 366 fishes/m^3) and T_3 (7166g/ m^3, 466 fishes/m^3).

Table 2: Protein metabolic enzymes activities in liver of GIFT fingerlings fed with 30% protein diet for the period 6 weeks.

Treatments[1]	AST[2]	ALT[3]
	Liver	Liver
T1	20.5 ± 9.68	3.54^{a} ± 0.38
T2	13.7 ± 2.00	5.44^{b} ± 0.26
T3	12.6 ± 1.75	3.05^{a} ± 0.57

Data are presented as Mean ± SE (n=3) Mean values in the same column with different superscripts differ non-significantly (p>0.05) [2]AST, Aspartate aminotransferase, the activity is expressed as micromoles of oxaloacetate formed/min/mg protein at 37°C [3]ALT, Alanine aminotransferase, the activity is expressed as micromoles of sodium pyruvate /min/mg protein at 37°C.

Table 3: Activities of oxidative stress enzymes in liver and gill of GIFT fingerling fed with 30% protein diet for the period of 6 weeks.

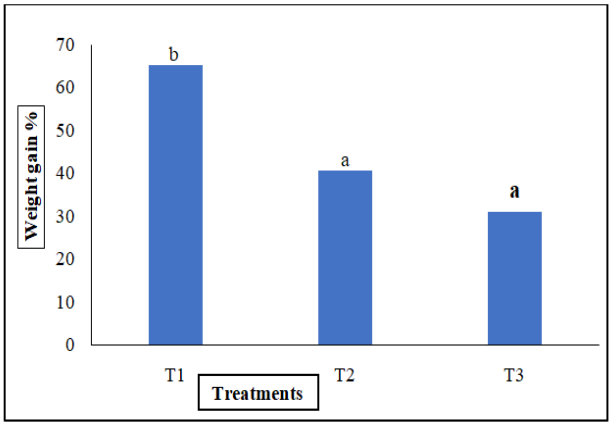
Treatments	[1]Catalase	[1]SOD
T_1	0.78^{a} ± 0.06	14.88^{a}±0.42
T_2	1.09^{b} ± 0.01	22.30^{b}±0.48
T_3	1.32^{c} ± 0.04	28.26^{c}±0.32

Data are presented as Mean ± SE (n=3) Data are presented as Mean ± SE (n=3) Mean values in the same column with different superscripts differ significantly (p<0.05); T_1 (3833g/ m^3, 250 fishes/m^3), T_2 (5500g/ m^3, 366 fishes/m^3) and T_3 (7166g/ m^3, 466 fishes/ m^3). [1]SOD, Superoxide dismutase, oxidation/min/mg protein; [2]CAT, Catalase, the activity is expressed as nanomoles H_2O_2 decomposed/min/mg protein.

Table 4: Serum profile of GIFT fingerlings fed 30% protein diet for the period of 6 weeks.

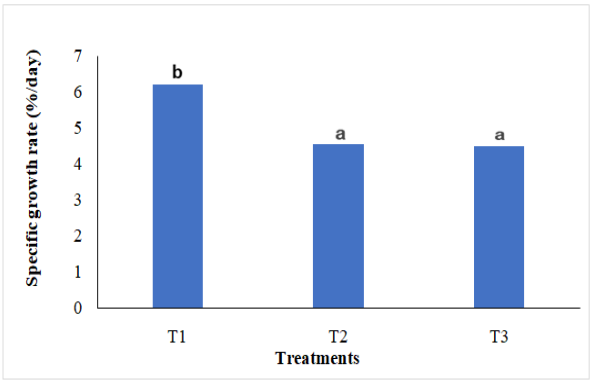
Treatments[1]	Serum total protein (g/dl)	Serum albumin (g/dl)	Serum globulin (g/dl)	Serum A:G[2]
T_1	11.05^{b} ± 1.02	4.21^{b} ± 0.05	6.83^{b} ± 0.97	0.64 ± 0.08
T_2	9.38^{a} ± 0.30	3.93^{b} ± 0.07	5.44$^{a\,b}$ ± 0.48	0.72 ± 0.03
T_3	7.80^{a} ± 0.10	3.28^{a} ± 0.51	4.52^{a} ± 0.32	0.73 ± 0.09

Data are presented as Mean ± SE (n=3) Mean values in the same column with different superscripts differ significantly (p<0.05) [2]A:G, Albumin to globulin ratio



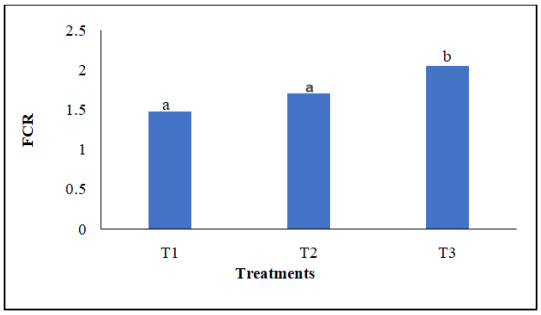
Data are presented as Mean ± SE (n=3); Bars with different superscripts differ significantly (p<0.05)

Fig. 1. Weight gain (%) of GIFT fingerlings fed with 30% protein diet for the period of 6 weeks.



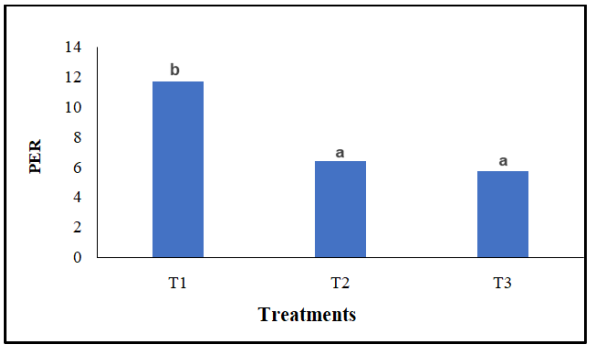
Data are presented as Mean ± SE (n=3); Bars with different superscripts differ significantly (p<0.05)

Fig. 2. Specific growth rate (%/day) of GIFT fingerlings fed with 30% protein diet for the period of 6 weeks.



Data are presented as Mean ± SE (n=3); Bars with different superscripts differ significantly (p<0.05)

Fig. 3. Feed conversion ratio (FCR) of *GIFT* fingerlings fed with 30% protein diet for the period of 6 weeks**.**



Data are presented as Mean ± SE (n=3); Bars with different superscripts differ significantly (p<0.05)

Fig. 4. Protein efficiency ratio (PER) of *GIFT* fingerlings fed with 30% protein diet for the period of 6 weeks**.**

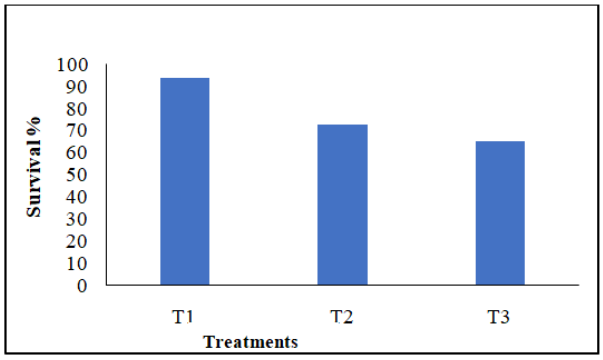


Fig. 5. Survival % of *GIFT* fingerlings fed with 30% protein diet for the period of 6 weeks.

CONCLUSIONS

In the present experiment, the stocking density 3833g/ m^3 (250 fishes /m3) showed better growth performance of GIFT tilapia fingerlings, elevated antioxidant and immune responses with lower release of phosphorus, total ammonia, nitrite and nitrate in the RAS.

FUTURE SCOPE

The present study will be very helpful for recirculating aquaculture of genetically improved farmed tilapia (GIFT). The effects of dietary intervention on the release of dissolved wastes such as nitrogen and phosphorus and solid wastes are required to be studied in the RAS of GIFT tilapia.

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Conflict of Interest. None.

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