Egypt. J. Chem. **Vol. 63,** No. 4, pp. 1575- 1584 (2020)

Egyptian Journal of Chemistry
http://ejchem.journals.ekb.eg/

Effect of Density on Growth Hormone and Some Physiological Parameters and its Relation to Growth Performance

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THE EXPERIMENT was performed in the central laboratory for aquaculture research, Egypt. Experimental fish about 10.2 g Nile tilapia were divided into four different treatments in different ratios, and fed on 25% protein pellets for 120 days at 3% of body weight. Some parameters like some digestive enzymes (amylase and lipase), glucose, cortisol, insulin, growth hormone, iron and total binding iron capacity were measured in blood serum; the 4th treatment showed the minimum value for theses measurements except for glucose and cortisol 153.6±9.2 mg/dl and 176.3±5.2 ng/ml, respectively. Haemoglobin showed the highest and minimum value (7.63±0.03 g/dl & 6.27±0.03 g/dl) in treatments (1 and 4) respectively. Erythrocytes count showed the highest value 2.52±0.01(X10^6/Cmm) in the first treatment, while the lowest value 2.09±0.03 (X10^6/Cmm) was recorded among treatment (4). Water samples for physico-chemical analysis were monitored bi-weekly during the study period then collected and tabulated in one mean. The growth performance of experimental fishes was significantly decreased with increasing fish density.

Keywords: Nile tilapia, Insulin, Lipase, Amylase, Haemoglobin, Water quality, Growth performance.

Introduction

Carp is first most important cultured world fish, then Tilapia. Tilapia farm is being practiced in most of the tropical, subtropical and temperate regions [1]. In 2012, tilapias aquaculture production attained 75.54% of the total aquaculture [2]**.** Stocking density is important character in culture of fish systems, since it has direct effects on the expansion and survival, thence on production. it's a longtime indisputable fact that growth rate of fishes were be increasingly increase because the stocking density had be decreased and vice-versa. But to obtain more economic returns might be necessary to stock the ponds at optimum stocking density for optimum growth in relevancy inputs and productivity of the water body [3]. The use of the suitable density is a commercially beneficial operation, focusing on maximizing the utilization of the rearing system, water and financial resources [4].

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Received 17/12/2019; Accepted 29/1/2020
DOI: 10.21608/ejchem.2020.21105.2258

The activity of amylase was determined in different organs (liver pseudostomach, upper and lower intestines) and showed that fishes weight 5.7 g was highest at pH 6, but fish weight 35.8 g was recorded amylase activity from these organs high at pH 7, 8, 6 and 7, respectively and at pH 2 for fish weight 92.1 g, another meaning, amylase activities were increased with increasing size fish [5], showed that amylase activities were increased in salivary glands of *Lygus hesperus* and *L. lineolaris* at pH 6.5 reported that Nile tilapia (55-255g body weight) amylase activities were being reached maximum value at pH level of 7.5 [6].

Environmental stress effect on physiological response are mainly divided into three types; first (neuroendocrine, corticosteroid–catecholamine responses), second type (metabolic changes, hematological, osmoregulatory and immunological responses) while third kind (responses to whole organism physiological and behavioral changes) [7, 8]. Dietary proteins, lipids and carbohydrates are essential for development (anabolism) and energy source to run body catabolism. Proteins were consisted of chains of amino acids; it has conclusively been shown in our previous studies that novel amino acid candidates are promising as biologically activate [9-29]. Amino acids were be used for growth and catabolic functions [30]. Levels of growth hormone and muscle protein ratio may be the good indicators of herbicide contamination in tilapia species [31].

Effect of both protein and stocking density on some growth performance parameters was studied [32] and they noticed stocking ratio significantly affected the body weight, specific growth rate and feed conversion ratio. In Nile tilapia [1] growth and survival in bamboo net cages trial were depended on stocking density, and found no significant differences in daily weight gain, specific growth rate, final weight, relative growth rate, feed conversion ratio (FCR) and survival rate, whereas there were significant differences in fish harvest, profit index and chemical composition of fish carcass [1] .

Experimental

The experiment was performed in the central laboratory for aquaculture research, Agriculture Research Center (CLAR), Egypt. Experimental fish about 10.2 g were collected and acclimated for 15 days before the experiment start. The experiment was divided into four groups containing 2, 4, 6 and 8 fish per aquarium each aquarium (40 X 50 X 60 Cm), representing the densities 20, 40, 60 and 80 fish per cubic meter, and each group containing four aquaria representing four replicates. Fish were fed on 25% protein pellets daily of a 3% of fish body weight six days in week. Water exchange was carried out every two days during the experiment time (120 days).

Blood samples were collected from the caudal veins at the end of experimental period (120 days) and divided into two portions. The first part of blood was collected within clean and dry tubes then centrifuged for 15 minutes at 5000 rpm. The blood serum was separated, labeled and freezing for blood biochemical analysis. The second part was collected in heparanized tubes for haemotological parameters determination. The total iron content (TI, mg/ml) and unsaturated iron binding capacity (UIBC, mg /ml) of serum were measured using a kit (Sigma no. 565) based on the method described by [33]. Total iron binding capacity (TIBC mg/ml) was measured as the sum of TI and UIBC. Activity of amylase was measured by the starch-hydrolysis method of [34]. Activity of lipase was determined according to the method of [35]. Cortisol levels in serum were measured by immunological method [36]. Serum glucose was analyzed by using a blood sugar determination kit Boehringer Mannheim as described by [37]**.** Plasma insulin was determined by RIA (radioimmunoassay) using a guinea pig anti-procine- insulin antiserum as an antibody, labeled insulin as a tracer, and PEG (polyethylene glycol) as separation method [38, 39]. The standard curve was calculated using the logit-log method and was linearized by an on-line Hewlett Packard 9815A calculator. The sensitivity, recovery and parallelism of insulin assay were checked as described by [40]. Tilapia growth hormone was measured by double antibody technique [41].

Hemoglobin was estimated by the colorimetric method using Boehringer Mannheim Kit as described by [42]. The count of RBC was determined by taking 0.5 ml of blood containing the anticoagulant EDTA and counted using a double hemocytometer under the microscope as described [43] and expressed in million per cubic ml. Hematocrite (Hct) value was measured by centrifuging the heparinized blood in the micro-haematocrit tube, at 5000 rpm for five minutes until the blood corpuscles were separated from the plasma [44]. Blood indices also were calculated [45] as follows :

Mean corpuscular volume (MCV) (fl) = 10X Hct / RBCs ($X10^6/\mu l$).

Mean corpuscular hemoglobin (MCH) (Pg) = 10X Hb / RBCs ($X10^6$/µl).

Mean corpuscular hemoglobin concentration (MCHC) (g/dl) = 100X Hb / Hct.

Samples of water for physico-chemical analysis were monitored bi-weekly during the study period then collected and tabulated in one mean. Dissolved oxygen, temperature, salinity and pH were measured as described [46] and Total ammonia concentrations were determined by nesslerization method [46], Also, growth performances parameters were measured and calculated at the end of the experiment. Weight gain (g/fish) = W2 – W1. Daily weight gained = weight gained/ time of experiment. Specific growth rate (SGR) = 100 [Ln wt1– Ln wTc] / T Where: Ln = Normal logb. Wt1 = final weight (g). WTc = initial weight (g). T = the time of experiment (days).

1. Statistical Analysis:

Obtained data was subjected to one-way ANOVA. Differences among means were tested at the 5% probability level using Duncan test [47]. All the statistical analysis was done using SPSS version 10 (SPSS, Richmond, USA) as described by [48].

Results

Some biochemical analysis was done on serum and their data are shown in Table (1). Amylase enzyme was measured in blood serum and was significantly different, its highest value 72.67 ± 0.88 u/mg (T1), and its lowest value 50.67±0.88 (T4). Serum lipase was significant and ranged from 36.37±0.15 (T4) to 48.43±0.09 u/mg (T1).

Serum glucose was increased significantly with lower density, showing its highest value 153.6±9.2 mg/dl in treatment (4), while its lowest value 76.2±4.6 mg/dl was recorded in treatment (1). Serum cortisol was increased significantly by decreasing densities, showing its maximum value 176.3±5.2 ng/ml in (T4), while its minimum value 102.8±6.9 ng/ml was observed among (T1).

Serum insulin was significant and ranged from 0.2±0.0001 µIU/mL (T4) to 0.4± 0.0001 µIU/mL (T1). Total iron binding capacity (TIBC) was significant and ranged from 245.43±0.75 µg/dL (T4) to 288.67±0.27 µg/dL (T1). Serum iron was significant and showed highest value 31.27±0.12 µg/dL in treatment (2) and lowest value 23.3±0.36 µg/dL in treatment (4). Growth hormone was measured in serum and was significant, it ranged from 4.07±0,03 mu/l (T4) to 6.23±0.03 mu/l (T1).

Hematological parameters and blood indices are illustrated in Table (2). Haemoglobin (Hb) and erythrocyte count (RBCs) were non significant in the first and second treatments, while they were significant different in third and fourth treatments. The highest Hb 7.63±0.03g/dl was recorded in the first treatment, the lowest one 6.27±0.03g/dl was in the fourth treatment. RBCs varied from 2.09±0.03 x 10^6/cmm (T4) to 2.52±0.01 x 10^6/cmm (T1). Packed cell volume (PCV) showed lowest value 17.55±0.1% in treatment four, and showest highest one 21.37±0.09 % in treatment one. Mean corpuscular volume (MCV) was non significant, it ranged from 83.86±0.89 fl (T4) to 84.7±0.13 (T1). The mean corpuscular Haemoglobin (MCH) was non significant, its highest value 30.25±0.05 Pg was recorded in treatment (1), while its lowest value 29.03±1.3Pg was recorded in treatment (3). The mean corpuscular Haemoglobin concentration (MCHC) was non significant and its value was about 35.7±0.007g/dl.

The physico- chemical properties of water are illustrated in Table (3), by seeing this table it is clear that water temperature was in the range of 29.2˚C, water temperature was not significantly different among all treatments. Dissolved oxygen was significantly different in the control group, while T2 and T3 were non significant, also T3 and T4 were non significant, the dissolved oxygen concentration ranged from 5.0±0.03 to 5.4±0.03 mg/l. pH values were non significantly different and ranged between 8.1±0.03 and 8.2±0.06. Salinity values were ranged between 0.11±0.01 and 0.16±0.02 ppt, salinity values are non significant. Total alkalinity values were significantly different, except T1 and T4 were non significant. Total hardness values were non significant, while T2 was significantly lower than the other treatments. The highest total alkalinity and total hardness were 291.3±2.4 and 314±2.08 mg/l respectively, their lowest values were 274.7±1.45 and 302±1.76 mg/l. ammonia concentration ranged from 012±0.003 to 014±0.003 mg/l, the first treatment was significantly higher than the other treatments.

Growth performance parameters data are shown in Table (4). Growth performance parameters were significantly different among the four treatments. The initial weight for different treatments was 10.2g. Final weight was significantly high in the first treatment (65.8±0.36 g). The highest weight gained (55.6±0.2g) was

TABLE 1. Serum means values (mean±SD) of some biochemical parameters (amylase (u/mg), lipase (u/mg), glucose (mg/dl), insulin (µIU/mL), total iron binding capacity TIBC (µg/dL), total Iron (µg/dL) and growth hormone (mu/l)) in Nile tilapia at the end of experimental period at different treatments.

Items Treatments	Amylase (u/mg)	Lipase (u/mg)	Glucose (mg/dl)	Cortisol (ng/ml)	Insulin (µIU/mL)	TIBC (µg/dL)	T. Iron (TI) (µg/dL)	Growth hormone (mu/l)
T1	72.67±0.88^{a}	48.43±0.09^{a}	76.2±4.6d	102.8±6.9d	0.40±0.0001^{a}	288.67±0.27^{a}	30.13±0.29^{b}	6.23±0.03^{a}
T2	68.33±1.2^{b}	48.07±0.09^{b}	89.4±6.7c	125.8±8.5c	0.350±0.03^{b}	248.23±0.20^{c}	31.27±0.12^{a}	5.9±0.06^{b}
T3	60.67±0.88^{c}	42.23±0.12^{c}	123.1±8.2b	159.5±9.6b	0.30±0.0001^{c}	261.47±0.79^{b}	26.67±0.45^{c}	4.37±0.03^{c}
T4	50.67±0.88^{d}	36.37±0.15^{d}	153.6±9.2a	176.3±5.2a	0.20±0.0001^{d}	245.43±0.75^{d}	23.30±0.36^{d}	4.07±0.03^{d}

Means have the same letter in the same column are non-significant (P<0.05).

TABLE 2. Blood mean values (mean±SD) of some haematological parameters (haemoglobin (g/dl), erythrocytes count (x 10^{6}/cmm), haematocrite (%), and blood indices in Nile tilapia at the end of experimental period at different treatments.

Items treatments	Hb (g/dl)	RBCs (x 10^{6}/cmm)	PCV (%)	MCV (fl)	MCH ((Pg)	MCHC (g/dl)
T1	7.63±0.03a	2.52±0.01a	21.37±0.09a	84.70±0.13a	30.25±0.05a	35.71±0.001^{a}
T2	7.47±0.03a	2.46±0.01a	20.91±0.09a	84.99±0.2a	30.35±0.07a	35.71±0.001^{a}
T3	6.67±0.09b	2.20±0.03b	18.67±0.25b	84.85±0.1a	29.03±1.3a	35.71±0.001^{a}
T4	6.27±0.03c	2.09±0.03c	17.55±0.1c	83.86±0.89a	29.94±0.32a	35.70±0.007^{a}

Means have the same letter in the same column are non-significant (P<0.05).

TABLE 3. Average of some physico-chemical parameters of water (Temperature, dissolved oxygen (mg/l), pH, salinity (ppt), total alkalinity (mg/l), total hardness (mg/l) and ammonia (mg/l)) in aquaria during the experiment.

Items / Treatments	Temp (°C)	Dissolved oxygen (mg/l)	pH	Salinity ppt	T. alkalinity (mg/l)	T. hardness (mg/l)	T. ammonia (mg/l)
T1	29.2±0.03^{a}	5.0±0.03^{c}	8.2±0.06^{a}	0.16±0.02^{a}	288.0±1.73^{a}	312.0±1.53^{a}	0.14±0.003^{a}
T2	29.3±0.03^{a}	5.4±0.03^{a}	8.1±0.03^{a}	0.15±0.03^{a}	281.3±1.86^{b}	302.3±1.76^{b}	0.12±0.003^{c}
T3	29.3±0.03^{a}	5.3±0.07^{ab}	8.1±0.03^{a}	0.16±0.01^{a}	274.7±1.45^{c}	314.0±2.08^{a}	0.12±0.003^{c}
T4	29.4±0.03^{a}	5.2±0.07^{b}	8.1±0.12^{a}	0.11±0.01^{a}	291.3±2.40^{a}	310.7±0.67^{a}	0.13±0.003^{b}

Means have the same letter in the same column are non-significant ($P<0.05$).

TABLE 4. Mean values of some growth performance parameters (initial weight (g), final weight (g), weight gained (g), daily weight gain (g), FCR (%) and SGR (%)) of Nile tilapia at different densities in aquaria during experimental period.

Items / Treatments	Initial weight (g)	Final weight (g)	Weight gained (g)	Daily weight gain (g)	FCR (%)	SGR (%)
T1	10.2±0.17^{a}	65.8±0.36^{a}	55.6±0.2^{a}	0.62±0.003^{a}	1.3±0.02^{d}	3.11±0.02^{a}
T2	10.2±0.29^{a}	53.2±0.55^{b}	43.0±0.26^{b}	0.48±0.003^{b}	1.45±0.03^{c}	2.75±0.03^{b}
T3	10.3±0.3^{a}	45.2±0.25^{c}	34.87±0.23^{c}	0.39±0.003^{c}	1.59±0.01^{b}	2.46±0.04^{c}
T4	10.2±0.23^{a}	36.07±0.19^{d}	25.87±0.15^{d}	0.27±0.003^{d}	1.64±0.01^{a}	2.11±0.03^{d}

Means have the same letter in the same column are non-significant ($P<0.05$).

recorded in the first treatment, while the lowest one (25.87±0.15g) was in the fourth treatment. Daily weight gained was significantly different, and it was in between 0.27±0.003g and 0.62±0.003g in the fourth and first treatments respectively. The lowest feed conversion ratio (FCR) 1.3±0.02 was obtained from the first treatment, while the highest one 1.64±0.01 was in the fourth treatment. Specific growth rate (SGR) was significant different and its highest and lowest values (3.11±0.02 % and 2.11±0.03%) were recorded in treatments (1 and 4) respectively.

Discussion

In the present study, digestive enzymes (amylase and lipase) have an essential role in digestion process to give high benefit of food diet and subsequently growth and insulin hormone were regulated the growth performance of Nile tilapia fish also these results affected by fish density and fish growth.

Lipases and colipases were enzymes of lipid digestion involves in the extracellular hydrolysis in stomach, intestines and cecal lumen [49]. However, in the pyloric ceca and anterior intestines were appeared first sites of hydrolysis lipid for most species [50], noticed that in both the upper and lower intestines and pancreas *Scleropages formosus* (251.5g) were high activity of lipase but in another fishes the pyloric ceca and upper intestine were be appeared lipid hydrolysis [50]. In the pancreatic juice, stomach, intestines and bile amylase has been identified however; the main producers were pancreas and liver [51].

On the other hand amylase activity was directly increasing with increasing fish size. The results are in hand with hand [52], where they were found amylase activity of common carp high in larger fish. In our experiment noticed that growth rate decreased with increasing stocking density that agree with [53] they showed that growth rate is a very important parameter in aquaculture that determines time required to produce marketable size of fish, diet composition [54]. Plasma growth hormone was decreased within one hour of stress but returned to near control levels after 4h [55]. Increase in plasma cortisol was decreased in serum growth hormone and studies in mammals glucocorticoids was inhibited growth and growth hormone secretion predominantly through inhibition by somatostatin [56]. In trout liver somatostatin significantly decreased growth hormone (GH) binding [57]. Changes on blood glucose are measured as physiological effects of stress condition in fish [58]. Also, total serum iron with total binding iron capacity were be decreased with increasing fish density according to obtain results in table 4 whereas, glucose and cortisol have directly effect with fish density increased these informations were been noticed in our results.

Due to blood hematology and blood induces were considered as health indication of fish these results showed that they have low significant decrease with increasing fish density these in hand with hand [59] they showed that some hematological parameters of rainbow trout were be influenced due to crowded density as chronic stress. Significant increase of RBC and Hb concentration observed in the present study could be attributed to the increased stress and oxygen demand. Erythrocytes count and hemoglobin concentration are responsible for elevation blood oxygen-carrying capacity [60]. Also, gilthead sea bream (*Sparus aurata*) was achieved reared at conditions of high density [61,62] they noticed that erythrocytes count, hematocrit, hemoglobin, MCV, MCH and MCHC were changed significantly, also, temperature was reduced as a result oxygen more dissolve in water which resulted in the experimental fish preserved their energy by reducing a hematopoietic process. Metabolism was suppressed causing reduced RBC, hematocrit and Hb concentration [63].

Quality of water were be adjusted at suitable condition for growth with maintenance different stocking densities of fish in our experiment, these results agreement with [64] they showed that quantity and quality of nutrients used in food may be different enzymatic profile and activity of digestive tract in animals. Thus, feed composition could be enhanced biological adaptations and food absorption [65].

Compete cycle of good fish yield was depended on good water quality, good hormonal levels, good physiological state, fish density and subsequently good growth performance. The value of diet is based on digest and absorb through physiological fish (eating habits) and independent mainly on its chemical composition. Throughout digestion, proteins are broken into small compounds (peptides and free amino acids) then absorbed by specific membrane, proteins specialized in peptide transport and useful by the body [66-67].

Nutritional studies of tilapia aquaculture must be concentrated on decrease high costs of generated by feeding and to food utilization (optimize) for reach making fish growth performance, weight gain, survival and growth [58, 59] also, they found growth performance are depended on food source, biochemical composition of ingredients, content and diets. Fish growth is mainly depended on interacting environmental factors (degree of competition; the amount and quality of food ingested; water temperature and age and state of maturity) of fish [19].

Conclusion

From obtained results we concluded that Nile tilapia culture in a suitable density improved physiological state. Subsequently, thus it was reflected on growth performance. If high stocking density was used without controlling environmental conditions, physiological state and growth performance will be declined.

Acknowledgements

This Research was funded by The Deanship of Scientific Research at Princess Nourah Bint Abdulrahman University through the Fast-Track Research Funding Program.

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