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THE PHYSIOLOGICAL PROFILES AND GROWTH PERFORMANCE ON NILE TILAPIA (*OREOCHROMIS NILOTICUS*) AFTER EXPOSURE TO DIFFERENT LEVELS OF TEMPERATURE

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ABSTRACT

The present study aims to evaluat the possible effect of different levels of temperature in *Tilapia Nilotica*. Fishes of Nile tilapia were divided into four groups. The1st group (lefet as control group (23 ºC) without adjustment. The 2nd, 3rd, 4th groups exposed to (26, 29 and 32 ºC adjustment by using thermostats in water treatment). High temperature (32ºC) caused reduction significantly (P<0.05) in erythrocyte count (RBCs), haemoglobin content (Hb), haematocrit value (Hct) values when compared to the control groups (2.03 ±0.05 x106, 7.81±0.21g/100ml and 17. 9±0.2%). On the other hand, all another treatments of temperature were non-significant changes in hematological parameter (RBCS, HB and HV).

The total protein (TP) in plasma of fish exposed to two high temperature was decreased significantly as compared to the control group While, the values of glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT) alkaline phosphatase (ALP) and Uric acid were increased significantly in fish exposed to high doses of temperature.

As well as significant differences in growth parameters during different temperatures between them compared to the control groups, (Final weight (FW), weight gain (WG), Average Daily Gain (ADG) and (SGR). It value of all parameter growth were increased significantly in fish rearing in high level of temperature. (This study showed that the Nile tilapia fish was positively affected by the high temperature.

Key words: Temperature, *Nile tilapia, blood* parameters, biochemical analysis and growth performance.

INTRODUCTION

Aquaculture remains one of the fastest growing food-producing sectors and acts 46.8 per cent from global fish production (Food and Agriculture Organization (FAO) 2018). In recent years, fish farming in Egypt has developed at a rapid rate, and tilapia have increased in intensive systems, and Egypt producing 1,935 million tons, 80.1 percent from farming (General Authority for Fish Resources Development (GAFRD, 2018). Fish is of high quality animal protein, having essential fatty acids, especially long-chain polyunsaturated ones, which are much greater in fishes than in terrestrial animal food source (Weaver *et al.*, 2008). Despite the fact that Egypt has all these plentiful water bodies, the Egyptian fish production is not enough to cover local consumption of Egyptians.

Many factors have contributed to the rapid global growth of tilapia. Tilapia culture has been practiced in more than 100 countries around the globe, yet most tilapia farmers, farm owners, farm managers, researchers, and graduate students in developing countries have little or lack accurate and critical information on tilapia culture (El-Sayed, 2006). Aquaculture is the most developed sector in terms of the supply of high-quality food products that meet human nutritional requirements (Ottinger *et al.*, 2016). The expansion in aquaculture practices accompanied by intensification of aquaculture caused pollution that is directly linked to the elevated levels of fish stressors, both environmental and physiological. Tilapia are cultured and highly adaptable to a wide range of environmental conditions. (Green, 2006).This publication provides a brief overview of environmental and nutritional requirements of tilapia. (reproduction and growth) are attained at temperature and salinities up to 19 parts per thousand (El-Sayed, 2006). Generally, tilapia are warm water fish, they require thermal environment of 25–28 C that is optimum for their reproduction and feeding (Wohlfarth and Hulata, 1983). They cannot survive temperatures less than 10–12 C for more than few days (Chervinski, 1982). Temperature is a major metabolic modifier in these fish. Optimal growing temperatures are typically be-tween 22° C (72° F) and 29° C (84° F); spawning normally occurs at temperatures greater than 22° C (72° F). Most tilapia species are unable to survive at temperatures

below 10° C (50° F), and growth is poor below 20° C (68° F). Blue tilapia are the most cold tolerant, surviving at temperatures as low as 8° C (46° F), while other species can tolerate temperatures as high as 42° C (108° F; (Mires, 1995).

The aim of the present study was to examine the effects of different levels of temperature (23, 26, 29 and 32 °C) on physiological parameters and growth performance of cultured Nile tilapia (*Oreochromis niloticus*).

MATERIALS AND METHODS

Experimental animal:

Healthy *Oreochromis niloticu*s of 35-40,gram were collected from the ponds of the Central Laboratory for Aquaculture Research at Abbassa, Abo-Hammad, Sharkia, Egypt. They were collected locally and confined to large plastic aquaria bearing tap water for up to 15 days in the laboratory for acclimation. The present study aims to evaluate the effect of temperature in *Tilapia Nilotica*. Fishes of Nile tilapia were divided into four groups. Fish were distributed in twelve glass aquaria of about 100-litre capacity each and stocked at a rate of 10-fish /aquarium. The glass aquaria were supplied with dechlorinated tap water and air stones average water temperature maintained according at each treatment. The aquaria were divided into 4 groups with three replicates per group. The 1st group was left as control (23^{0}c).The 2nd, 3rd, and 4th groups exposed to (26, 29 and 32°C, respectively of temperature adjustment by using thermostats in water treatment groups (Table, 1). Fish of the experimental groups were fed on a pelleted fish diet containing 32% CP, and the diet was fed at a rate of 3% of live body weight twice daily for 90 days. Semi-dynamic method for removal of excreta was used every day by siphoning a portion of water from the aquarium and replacing it by an equal volume of water sampling.

Table 1. Design of experiment.

T1 (Control)	23°C
T2	26°C
T3	29 °C
T4	32 °C

Temperature:

According to Boyd and Tucker (1998), water temperature was measured by oxygen-thermometer apparatus YSI model 58 (Yellow Spring Instrument Co. Yellow prings, Ohio, USA).Water temperature were measured daily at 12.00 O'clock at the site of the aquarium and temperature adjustment by using thermostats in water treatment all each group.

Physiological Analyses:

After 60 days of the experiment, fish were not fed for 24 h before blood sampling. Blood was collected from the caudal vein of 5 fish from each group, anaesthetized with buffered MS222 (50 mg /L) by sterile syringe using EDTA solution as anticoagulant. These blood samples were used for determining, red blood cells (RBCs according (Dacie and lewis, 1991), hemoglobin content (Vankampen, 1961) and. Heamatocrit value (Hct) were calculated according to the formulae mentioned by Britton (1963).

Plasma was obtained by centrifugation of blood at 3000 rpm for 15 min and non-haemolyzed plasma was stored in deep freezer for further biochemical analyses. Total protein content and creatinine concentration were determined colorimetrically according to Henry (1974). Plasma glucose was determined, using glucose kits supplied by Boehring Mannheium kit, according to Trinder (1969). Activities of aspartate amninotransferase (AST) and alanine aminotransferase (ALT) were determined colorimetrically according to Reitman and Frankel (1957), while alkaline phosphatase (ALP) was measured by using Diamond diagnostics kits according to the method of Bergmeyer (1972). Also urea concentration was determined according to Patton and Crouch (1977).

Fish growth performance:

Fish Performance parameters as follow:

Weight gain (WG): g/fish weight gain (WG) = final body weight (g) – initial body weight (g)

Average daily gain (ADG, g / fish / day): ADG = (weight gain (g) / Experimental period (days)

Specific growth rate (SGR): calculated according to Jauncey and Rose (1982). SGR = (Ln final mean body weight – Ln initial mean body weight/ Experimental period (days) x100.

Survival rate (SR %): (%) = Number of fish survived at the end of the experiment / Number of fish stocked at the start of the experiment X 100

Statistical analysis:

The obtained data were subjected to analysis of variance according to Snedecor and Cochran (1982). Differences between means were done at the 5% probability level, using Duncan's new multiple range test (Duncan, 1955).

Results and Discussion

The results of erythrocyte count (RBCs), hemoglobin content (Hb) and hematocrit value (Hct) obtained from fish exposed to levels of temperature are given in Table (2). The high level of temperature (32°c) induced a reduction of all examined blood parameter. RBCs were significantly different (1.91±0.02 10^3/ul) when compared to the control group (2.03±0.05 10^3/ul), while it was changed insignificantly in the other treatments when compared to the control group. It was clear that the fish group exposed to high level of temperature showed significant decrease in the hemoglobin level (7.62± 0.31 g/100ml) compared to 7.81± 0.21, g/100 ml in the control group. While these levels were significantly increased in fish exposed to 26 and 28°C of temperature (8.51± 0.03 and 8.11± 0.21 g/ 100 ml respectively), when compared to the control groups. The hematocrit level of Nile tilapia were decreased significantly **(**16.8 ± 0.2%) in

fish exposed to high temperature when compared to the control group (17.9 ± 0.2%).

Table 2. The Erythrocyte counts×10^6 the hemoglobin content (g/100ml) and hematocrit (%) in the blood serum of Nile tilapia exposed to different levels of temperature.

Treatment*	Erythrocyte count×10^6	Hemoglobin (g/100ml)	Hematocrit value (%)
T1 (control)	2.03^{ab} ±0.05	7.81^{b} ±0.21	17.9^{c} ±0.2
T2 (26˚C)	2.29^{a} ±0.14	8.51^{a} ±0.03	20.4^{a} ±0.5
T3 (29˚C)	2.08^{ab} ±0.03	8.11^{a} ±0.21	19.4^{b} ±0.6
T4 (32˚C)	1.91^{b} ±0.02	7.62^{b} ±0.31	16.8^{d} ±0.2

Means having different letter in the same column are significantly different at ($P \leq 0.05$).
*T1 (Control), T2, T3 and T4 were 23, 26, 29 and 32˚C, respectively.

Erythrocyte count, hemoglobin and hematocrit values in *O. niloticus* exposed to high temperature, were lower than the control resulting in hypochromic anemia. This type of anemia could attribute to the effect of ammonia on the erythrocytes as a result of preoxidative effect. The decline of RBC count, Hb content and Hct value were observed (high temperature) groups, could be attributed to shrinkage of erythrocytes, decline of erythrocytes production in the hematopoietic tissue and hemodilution and intravascular destruction due to the combined stress and toxicity of ammonia and high temperature. Since, red blood cells of teleost produced from hematopoietic tissue of the kidney, spleen and liver the stress impact of ammonia might damage such organs to the degree that they caused reduction of erythrocytes (Das *et al.*, 2004). Similarly El-Sherif and El-Feky (2008) demonstrated histopathological damage to the kidneys of tilapia fish exposed to high temperature which caused increase of ammonia. Kidney damages were marked hyaline droplet degeneration and swelling of renal tubules, majority of renal tubules showed vacuolations, necrotic epithelia, necrosis in melanomacrophages center and increased thrombus formation and infiltration of melanomacrophage cells between the renal tubules. These tissue damages might contribute to the decreased ability of renal hematopoietic function. Similarly, (Horiguchi *et al.*, 1994) showed that tubular

nephrosis leading to drop in erythropoietin production and attenuation of erythropoiesis. These data are in agreement with Moustafa *et al.*, (2016), they reported significant decreased in RBC, Hb, Hct, in Nile tilapia after exposure to high temperature. This may be attributed to depletion of interstitial hemopoietin tissue in posterior kidney and white pulp depletion in spleen. Also, the decrease in RBCs count may be attributed to hematopathology or acute hemolytic crisis that results in severe anemia in most vertebrates including fish species exposed to different environmental pollutants (khangarot and Tripathi, 1991) or may be the decrease in the RBCs may be attributed to reduction of growth and other food utilization parameters which results in severe anemia (James and Sampath, 1999).

Plasma total protein:

The quantitative determination of tissue total protein content gives an estimate of the rate of protein synthesis in these tissues. Furthermore, its determination in the blood serum gives an idea about the condition of the liver cells and consequently its vulnerable effect in the diagnoses of the toxicity in fishes. The total protein levels in serum is a frequently parameter of ammonia poisoning in fish. However, data available did not allow to assessment of the direction of these changes, since the same ammonia may cause increase or decrease in total protein.

In this study, total plasma protein decreased significantly in fish exposed to 32 $^{\circ}$C of temperature. This result may be attributed to the great demands and cellular damage that occurred in the tissues of NH3 - toxicated fish and higher temperature may be possible cause protein breakdown or may be attributed to either a stage of hydration and change in water equilibrium and/ or disturbances in the liver protein synthesis resulted due to the toxicity of high temperature. These results agree with those of Shalaby (1997), who attributed these decreases in plasma total protein to the great demands and cellular damage occurred in the tissues of fish exposed to Cd. The data shown in figure (1) revealed that the mean level of plasma total protein of the control group was 3.45±0.15 g/100 ml). This value of total proteins was decreased significantly to (3.14±0.19 and 2.98±0.14,

g/100 ml respectively) in fish exposed to high two level of temperature when compared to the control group.

Blood glucose is a sensitive reliable indicator of environmental stress in fish. From the results, it is clear that the high temperature as shown by elevated blood glucose level affected as a stress or in fish. Hyperglycemia occurred in *O. niloticus* after exposure to NH^3 and with high temperature may be due to changes in liver carbohydrate metabolism (activation of liver glycogenolysis and glycolysis) as well as increased levels of plasma glucose. Similarly, Metwally and Wafeek (2014) showed that different concentration of NH_3Cl induced hyperglycemia with decreased in liver glycogen in Nile tilapia. Figure (1), illustrates the data for the plasma glucose concentration of Nile Tilapia of the control three group was 68.3 ±1.5 mg/L. plasma glucose concentration showed Higher significant value (P <0.05) in all treatments of experimental.

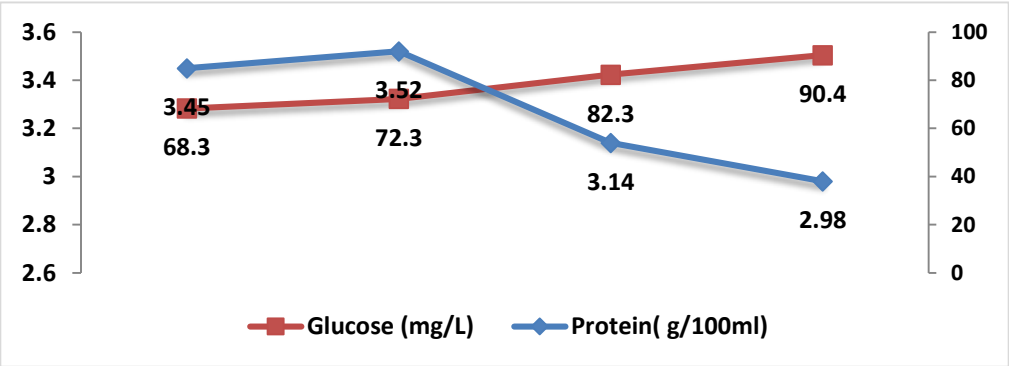

Figure 1. The changes of plasma total protein and glucose concentration in the blood of Nile tilapia exposed to different levels of temperature for 60 days**.**

Enzymatic activities:

Data in Tables (3) showed that the AST and ALT activity in *O. niloticus* in control group were (22.4±2.4, and 14.2 ±0.6 iu/l, respectivel**y**). Its values in AST were increased significantly to (27.2±1.2 and 30.9±2.4 iu/l, respectively) in *O. niloticus* exposed to 29 and 33ºC of temperature when compared to the control group, Also the ALT was significantly increased to (17.3±0.8 and 20.7±1.2 iu/l, respectively) in fish exposed to high temperature.

Enzymatic inhibition or activation by sub lethal concentration of chemical including those having ecological significant has been described in a variety of organism (Webb, 1979). According to Oser (1965) the metabolic role of transaminases is well recognized and AST or ALT are two key enzymes which are clinically important as well. In hepatocellular disorders , The ALT other rises sooner, faster and high than AST and necrosis in tissue like myocardium, skeleton muscles , brain, liver and kidney , which are rich in AST and ALT, results in liberation of large quantities of these enzymes into the blood. Also AST & ALT are often considered to be important in the assessment of astute of the liver as well as some organs (Abo- Hegab, 1991). The enzymes AST and ALT activities are important enzymes that are often increased in liver injury (Hukkeri *et al*., 2002). In this study *O. niloticus* rearing in high temperature, this is a marker for loss of functional integrity of cell membrane of hepatocytes, hepatic damage and damage of liver cell lead to leakage of enzymes.. This study supported by Shalaby *et al*. (2018) found hepatic toxicity of in *O. niloticus* revealed an increase in the levels of liver enzymes. Also, Shalaby *et al*. (2019) reported that feeding of Nile tilapia with diet containing high level (40-160 mg folic acid /kg was exhibited increased plasma AST and ALT which indicate to the probable impairment in liver function and other organs.

Table 3. The changes of aspartate amino transferees (AST) and alanine amino transferees (ALT) in the blood of Nile tilapia exposed to different levels of temperature for 60 days.

Treatment*	AST (iu/l)	ALT (iu/l)
T1 (control)	$22.4^{c} \pm 2.4$	$14.2^{c} \pm 0.6$
T2 (26˚C)	$23.2^{c} \pm 3.5$	$14.8^{c} \pm 0.7$
T3 (29˚C)	$27.2^{b} \pm 1.2$	$17.3^{b} \pm 0.8$
T4 (32˚C)	$30.9^{a} \pm 2.4$	$20.7^{a} \pm 1.2$

Means having different letter in the same column are significantly different at ($P \leq 0.05$).
*T1 (control), T2, T3 and T4 were 23, 26, 29 and 32˚**C**, respectively.

The alkaline phosphates and Uric acid:

Concerning, the activity of alkaline phosphates in the blood plasma of *O. niloticus* (Table, 4), was increased significantly to 15.2 ±0.3 and 16.3±0.5 iu/l in fish exposed to high levels of temperature, respectively as compared to the control group (11.5±0.5 iu/l). These results are in agreement with those of Shalaby (1997) and (2001) who found that a significant increase in alkaline phosphatase in the common carp and Nile tilapia after toxicities with copper or mercury, respectively. These increased in alkaline phosphatase may be due to damage and dysfunction of liver. Similarly the uric acid as the same trained increased significantly to (0.41±0.02, 0.40±0.01mg/L respectively in fish reared in high temperature when compared to the control group (0.35 ±0.02mg/L) (Table 4).

Table 4. Changes of alkaline phosphatase and uric acid in the blood of Nile tilapia exposed to different levels of temperature for 60 days**.**

Treatment*	Alkaline phosphates	Uric acid
T1 (control)	11.5^{c} ±0.5	0.35^{b} ±0.02
T2 (26˚C)	12.1^{c} ±0.4	0.39^{b} ±0.01
T3 (29˚C)	15.2^{b} ±0.3	0.41^{a} ±0.02
T4 (32˚C)	16.3^{a} ±0.5	0.40^{a} ±0.01

Means having different letter in the same column are significantly different at ($P \leq 0.05$).
*T1 (control), T2, T3 and T4 were 23, 26, 29 and 32˚**C**, respectively.

The blood uric acid in Nile tilapia (*O. nilotucus*) was increases significantly after exposed to two high levels of temperature they may be due to the fact that, the kidney is target organ of ammonia accumulation which induces renal failure. This study is supported by El gaml *et al.* (2015) and Shalaby *et al* (2017) who found renal toxicity of lead acetate in *O. niloticus* revealed by an increase in the levels of urea and creatinine. An increase in the urea and creatinine levels might be due to the glomerular insufficiency, also the increase in the production of reactive oxygen species and kidney injury as a result of Pb induced nephrotoxicity. The same results were obtained by Robert *et al*. (1984). Renal epithelium showed more basophilic cytoplasm, some cells are vacuolated,

congestion of some blood vessels and melanomacrophages center exhibited some necrotic changes.

The growth performance:

The data presented in Table (5). The initial weight in Nile tilapia was non-significant variation. The Final weight was an increased significantly to (82.77±2.51, 84.82±3.36 and 83.69±4.61 g/fish after raised with all treatment of temperature when compared to the control group (71.99±2.52 g/fish, Table 5). Also, the weight gain was increased significantly in Nile tilapia exposed to all treatment of temperature when compared to the control group (34.52±1.13 g/ fish, Table 5). Similarly, The specific growth rate and daily growth rate was increased significantly with increased temperature as compared to the control group. The present study concludes that, growth parameter is strongly correlated with fluctuations in the temperature of the system. These results agree with those of Mustafa *et al*. (2006). It suggests that careful regulation of pH, ammonia and temperature can lead to avoidance of excessive stress and better growth and survival of farmed fish. Beaumont *et al*. (2003) attributed the impairment of swimming performance to serum ammonia accumulation because there was no evidence of major disruptions to cardiorespiratory metabolism, particularly such as problems with O2 uptake and cardiovascular convection.

Table 5. Changes of the final weight, weight gain, average daily gain (ADG) and specific growth rate (SGR) and survival rate of Nile tilapia after exposed to different levels of temperature for 60 day.

Treatment*	Initial Weight	Final Weight	Weight Gain	ADG	SGR	Survival rates%
T1 (Control)	37.47 ±2.69	٧1.99[b] ±2.52	34.52[b] ±1.13	0.57 [b] ±0.02	1.09[b] ±0.04	94.1 [b] ±1.51
T2 (26˚C)	37.42 ±2.69	82.77 [a]±2.51	45.31[a] ±1.82	0.75 [a] ±0.03	1.32[a] ±0.03	97.2 [a] ±1.78
T3 (29˚C)	37.52 ±2.13	84.82 [a]±3.36	47.3[a] ±1.25	0.78 [a] ±0.02	1.36[a] ±0.03	95.8 [a] ±1.39
T4 (32˚C)	37.49 ±2.09	83.69[a] ±4.61	4٦.24[a] ±1.78	0.7٧ [a] ±0.01	1.34[a] ±0.01	94.3 [b] ±1.72

Means having different letter in the same column are significantly different at ($P \leq 0.05$).

*T1 (control), T2, T3 and T4 were 23, 26, 29 and 32˚C, respectively.

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التغيرات الفسيولوجية واداء النمو لأسماك البلطى النيلى بعد التعرض لمستويات مختلفة لدرجات الحرارة

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الملخـــــص العربـــــي

نظرا للتغيرات المناخية التي ادت الي اثار سلبية علي البيئة المائية وخاصة الاسماك لذلك أجريت هذه الدراسة لتقييم تأثير درجات الحرارة المختلفة علي معدلات النمو والتغيرات الفسيولوجية لأسماك البلطي النيلي التي تتراوح اوزانها ٣٥-٤٠ جرام حيث تم توزيع الاسماك علي اربع مجموعات وتركت المجموعة الاولي كمجموعة ضابطة عند درجة حرار٢٣ مئوية بينما تعرضت المجموعة الثانية والثالثة والرابعة لدرجات حرارة مختلفة تتراوح بين كلا من (٣٢،٢٩،٢٦) درجة مئوية بحيث استمرت التجربة لمدة ٦٠ يوم واوضحت النتائج الاتي:

١- لوحظ انخفاض معنوي في معدلات مكونات الدم مثل كرات الدم الحمراء والمحتوى الهيموجلوبينى للدم ونسبة الهيماتوكريت والبروتين الكلي في بلازما الدم عند تعرض الاسماك لأعلي مستوي من درجة الحرارة المختلفة.

٢- لوحظ ارتفاع كلا من مستوي السكر في بلازما الدم والنشاط الانزيمي للكبد مثل اسبارتيت امينو ترانس فيريز (AST) والاليلينين امينو ترانسفيريز (ALT) عند تعرض الاسماك لدرجتي الحرارة المرتفعة. بمقارنتها بالمجموعة الضابطة.

٣- كذلك لوحظ ارتفاع مستوي الفوسفاتيز القاعدي (ALP) وحمض اليوريك في بلازما الدم للأسماك التي تعرضت لأعلى درجات الحرارة المختلفة.

٤- كما لوحظ ارتفاع معنوي وايجابي في معدلات النمو لأسماك البلطي النيلى بالمجموعات المختلفة من درجات الحرارة المرتفعة مقارنة بالمجموعة الضابطة.

لذلك يوصي بان درجات الحرارة المثلي لاستزراع اسماك البلطي تتراوح من ٢٩-٣٢ درجة حرارة مئوية.