

EFFECTS OF pH ON GROWTH INDEX, BLOOD PHYSIOLOGICAL PARAMETERS AND SOME OF DIGESTIVE ENZYMES OF THE NILE TILAPIA FISH, *Oreochromis niloticus* (LINNAEUS, 1758)

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Abstract

A total of 90, *Oreochromis niloticus* (L.) of a mean initial weight (22.53 ± 0.09 g) were collected from Abbassa fish farm, Sharkia, Egypt and randomly distributed in nine aquaria representing three treatments with three replicates, pH (6, 7 and 8.5), during the experimental period 45 days. The examined fishes were fed on 25% protein diets with 2 % of fish weight daily "5 days a week". Sulfuric acid and Sodium hydroxide were used to maintain the acidic and alkaline media in aquaria respectively. The physico-chemical measurements were tested. The results showed that the daily fish weight gained ranged from (0.25 ± 0.0001 to 0.41 ± 0.003 g). The best specific growth rate (SGR) was (1.34 ± 0.006) recorded in the third treatment. Total hardness was significantly decreased by increasing pH values. pH had significant effects on all blood and Physiological parameters measured. The digestive enzymes, amylase and lipase were highly significant in the alkaline treatment. Lowest value of insulin was (0.17 ± 0.03 μ IU/mL) recorded in the alkaline treatment while the lowest value of iron was (27.37 ± 1.46 μ g/dL) obtained in the acidic treatment.

Keywords: Physiological parameters, pH values, Nile Tilapia, RBCs, Hb, amylase, lipase, and Abbassa Egypt.

INTRODUCTION

Nile tilapia culture has a great importance since it has quick reproduction rate, tolerance to hard environments endurance to disease and possibility to be cultured under diverse farming systems (Yosef, 2009 and Soto-Zarazua *et al.*, 2010).

The hydrogen-ion concentration (pH) of natural waters is an important environmental factor for the growth of fishes. Increases in the concentration of H^+ ions results lower pH value and low H^+ ions concentration brings about higher pH value. The pH of water is the logarithm of the reciprocal of hydrogen ion concentration (Sagar *et al.*, 2012). The physical and chemical qualities of water are critical to successful aquaculture. To a great extent water determines the success or failure of an aquaculture operation. Very high (greater than 9.5) or very low (less than 4.5) pH values are unsuitable for most aquatic organisms. Young fish and immature stages of aquatic insects are extremely sensitive to pH levels below 5 and may die at these low pH values. High pH levels (9-14) can harm fish by denaturing cellular membranes and most ammonium in water is converted to toxic ammonia (NH_3) which can kill fish. Through biological processes, toxic ammonia can be degraded to harmless nitrates. Moreover, cyanobacterial toxins can also significantly influence fish populations (Yokogawa, 2014). Klahan *et al.* (2009) indicated that different sizes of Tilapia fish had different levels of enzymatic activities and Tilapia modifies their secretion of digestive enzyme when their diet is valid. The animal physiology works within certain species-specific environmental conditions. The water pH variations that deviate from the ideal range for the species may affect fish survival and performance (White *et al.*, 2014). Fish try to adapt its behavior and physiology when subjected to stressful pH conditions. Some groups of fish exhibit greater body growth in waters with pH values away from neutrality. These species of fish adapted to live in acidic (Duarte *et al.*, 2013), and alkaline (Saha *et al.*, 2002) waters are called acidophilic and basophilic, respectively.

There was a significant improvement in feed conversion ratio (FCR) and protein efficiency ratio (PER) due to water acidification (Rebouças *et al.*, 2015). The final average body weight after 60 days of stocking showed great differences among different pH levels with a decrease at low pH (El-Sherif and El-Feky, 2009).

MATERIALS AND METHODS

The experiment was performed in the central laboratory for aquaculture researches, Abbassa, Abu Hammad, Sharkeya, Egypt. Experimental fish were collected from Abbassa fish farm and stocked in a fiberglass inside the laboratory for about two weeks for acclimatization before the experiment started. Ninety Nile tilapia fish, (*Oreochromis niloticus*) of a mean initial weight ($22.53 \pm 0.09\text{g}$) were chosen and randomly distributed in nine aquaria representing three treatments, whereas each treatment represented as three replicates. The three treatments were prepared with different pH values as following treatment 1 (pH 6) {T1}, treatment 2 (pH 7) {T2} and treatment 3 (pH 8.5) {T3}. Hydrochloric acid and sodium hydroxide were used to adjust the acidic and alkaline media in aquaria respectively. The experiment time was 45 days, during the experimental period; fish were fed on 25% protein diets with 2 % of fish weight daily "6 days a week". Water in aquaria changed every three days and then readjusted to the required pH value by Portable pH electronic paper apparatus "Hanna instruments. The water parameters were measured twice a week as follows; temperature and dissolved oxygen using oxygen-meter AQUA LYTIC (model OX 24) instrument. Salinity was measured twice a week using portable salinity-conductivity meter Lovibond Sensodirect "model, Con 200" UK, ammonium concentration was measured by HACH comparison apparatus but total alkalinity and total hardness by the method reported by (APHA, 2000). Growth parameters would be measured as initial weight (g), final weight (g), food intake (g), total gain weight (g), daily gain weight (g), feed conversion ratio (FCR) and specific growth rate (%) from the beginning, during and at the end of the experiment as following, Feed Conversion Ratio (FCR) = Total feed consumption(g)/weight gain(g). Specific growth rate (SGR) = $100 [\text{Ln wt}_1 - \text{Ln wt}_0] / T$ Where Ln = Normal logb Wt_1 = The final weight (g) Wt_0 = The initial weight (g) T = The time of experiment (days).

Blood samples were collected in two parts first part on EDTA solution for measuring haemoglobin, erythrocytes count, haematocrite and other blood indices, whereas the second part were taken on clean sterile and dry tube to

measure lipase, amylase, insulin and total iron after centrifugation for 15 minutes then isolate serum and put in deep freeze until measuring. Hemoglobin concentration (Hb g/dl), erythrocyte counts (RBC $\times 10^6/\text{cmm}$) and hematocrite (Ht %) percentage were measured based on unified methods for hematological examination of fish (Svobodova *et al.* 1991). The count of RBC was determined by counted using the hemocytometer under the microscope. Mean corpuscular volume (MCV fl), mean corpuscular hemoglobin (MCH μg), and mean corpuscular hemoglobin concentration (MCHC g/dl) were also calculated according to standard formulas, $\text{MCV (fl)} = 10 \times \text{PCV} / \text{RBCs}$, $\text{MCH } (\mu\text{g}) = 10 \times \text{Hb} / \text{RBCs}$, $\text{MCHC (g/dl)} = 100 \times \text{Hb} / \text{PCV}$. The total iron content (TI, mg/ml) of serum was determined using a kit (Sigma no. 565) based on the method described by Persijn *et al.* (1971). The manufacturer's procedure was followed with the modifications that all volumes were reduced by a factor of 10 (langston *et al.* 1998). Amylase activity was determined by the starch-hydrolysis method of Bernfeld (1955). Lipase activity was determined according to the method of Furne *et al.* (2005).

Statistical Analysis:

The obtained data were subjected to one-way analysis of variance (ANOVA) to test the effect of ammonia simultaneously tested (Duncun, 1955). Duncan's Multiple Range test was used as a post-hoc test to compare between means at $P \leq 0.05$. The software SPSS, version 10 (SPSS, Richmond, VA, USA) was used as described by Dytham (1999).

RESULTS

The physico-chemical measurements were measured and illustrated in table (1). Water temperature was around 28.37 ± 0.03 ° C, and there were no significance between treatments. The three treatments were significantly different in dissolved oxygen ratio which was around 5.3 ± 0.01 mg/l. The pH values were adjusted to 6, 7 and 8.5 for the treatments respectively. There were no significant differences between the three treatments in salinity. The maximum alkalinity was found in (treat. 3) which was $(274.67 \pm 2.6 \text{ mg/l})$, whereas the minimum alkalinity was clear in (treat. 1) that recorded $(238.67$

± 3.93 mg/l). Total water hardness was significantly affected by pH, and all treatments were significantly different. The highest hardness value (334.33 ± 2.6) was found in treatment (1), while the lowest one (302.33 ± 1.45) was in the third treatment. Ammonia was significantly affected by pH value, and ranged from (0.34 ± 0.01 to 0.38 ± 0.003) mg/l in treatment (1) and treatment (3) respectively.

Table 1. Physico-chemical parameters of water that measured during the experiment.

Treatment	Temperature (° C)	Dissolved oxygen (mg/l)	Salinity (ppt)	Total alkalinity (mg/l)	Total hardness (mg/l)	Ammonia (mg/l)
T1 "pH 6"	28.23 \pm 0.03a	5.19 \pm 0.04c	0.2 \pm 0.0001a	238.67 \pm 3.93c	334.33 \pm 2.6a	0.34 \pm 0.01c
T2 "pH 7"	28.37 \pm 0.03a	5.3 \pm 0.01 b	0.2 \pm 0.0001a	266.33 \pm 1.45b	311.33 \pm 1.86b	0.37 \pm 0.01b
T3 "pH 8.5"	28.6 \pm 0.3a	5.44 \pm 0.02a	0.2 \pm 0.0001a	274.67 \pm 2.6a	302.33 \pm 1.45c	0.38 \pm 0.003a

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

Growth index parameters were summarized in Table (2), by observing this table, it is clear that the pH value had significantly differences on growth performance parameters. The daily weight gained ranged from (0.25 ± 0.0001 to 0.41 ± 0.003 g) in the first and last treatments respectively. And was significantly increased in (T3) (0.41 ± 0.003). The highest FCR value was (1.86 ± 0.025) recorded in the first treatment, while the lowest value was (1.3 ± 0.01), found in treatment (3). The best specific growth rate (SGR) was 1.34 ± 0.006 recorded in the third treatment. But the lowest (SGR) was (0.89 ± 0.003) in the first treatment.

Table 2. Growth parameters for Nile Tilapia exposed to different pH levels.

Treatment	Initial Weight(g)	Final Weight(g)	Gain Weight(g)	Daily Gain Weight(g)	FCR	SGR (%)
T1 "pH 6"	22.63 \pm 0.09a	33.85 \pm 0.10c	11.22 \pm 0.02c	0.25 \pm 0.0001c	1.86 \pm 0.025a	0.89 \pm 0.003 c
T2 "pH 7"	22.53 \pm 0.09a	38.97 \pm 0.22b	16.43 \pm 0.15b	0.36 \pm 0.003b	1.42 \pm 0.02b	1.22 \pm 0.006 b
T3 "pH 8.5"	22.53 \pm 0.09a	41.17 \pm 0.23a	18.63 \pm 0.15a	0.41 \pm 0.003a	1.3 \pm 0.01c	1.34 \pm 0.006 a

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

Some parameters were measured in the whole blood of experimental fish, and results are illustrated in Table (3). From this table it is observed that pH had significant effects on all parameters measured. There were significant differences between the three treatments; the highest (Hb) value was recorded in treatment (T3) that reached 7.36 ± 0.04 g/dl, while the lowest value was 5.83 ± 0.06 g/dl in treatment (T1). The red blood cells count was significantly high ($2.28 \pm 0.01 \times 10^6/\text{cmm}$) in treatment (3), while it was significantly low ($1.81 \pm 0.02 \times 10^6/\text{cmm}$) in the first treatment. There was a significant effect for the pH on RBCs count. The packed cell volume (PCV) ranged from ($16.03 \pm 0.17\%$ to $20.23 \pm 0.10\%$), and there were significant differences between treatments. The mean corpuscular volume (MCV) values were non significant among the three treatments, its values ranged around 88.73fl. There were no significant in mean corpuscular haemoglobin (MCH) between treatments and ranged around 32.26 μg . while the mean corpuscular hemoglobin concentration (MCHC) had significantly different among (T1 and T2), while (T3) recorded 36.37 ± 0.003 g/dl.

Table 3. Hb, RBCs, PCV, and blood indices in hall blood of Nile tilapia exposed to different pH degrees.

Treatment	Hb (g/dl)	RBCs ($\times 10^6/\text{cmm}$)	PCV (%)	MCV (fl)	MCH (μg)	MCHC (g/dl)
T1 "pH 6"	$5.83 \pm 0.06\text{c}$	$1.81 \pm 0.02\text{c}$	$16.03 \pm 0.17\text{c}$	$88.54 \pm 0.23\text{a}$	$32.19 \pm 0.09\text{a}$	$36.35 \pm 0.003\text{b}$
T2 "pH 7"	$6.83 \pm 0.04\text{b}$	$2.12 \pm 0.01\text{b}$	$18.79 \pm 0.10\text{b}$	$88.76 \pm 0.04\text{a}$	$32.27 \pm 0.03\text{a}$	$36.35 \pm 0.003\text{b}$
T3 "pH 8.5"	$7.36 \pm 0.04\text{a}$	$2.28 \pm 0.01\text{a}$	$20.23 \pm 0.10\text{a}$	$88.73 \pm 0.09\text{a}$	$32.26 \pm 0.03\text{a}$	$36.37 \pm 0.003\text{a}$

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

Other parameters were measured in serum of experimental fish after the experiment, like insulin, amylase, lipase and total iron; these parameters are illustrated in Table (4).

The digestive enzymes amylase and lipase measured in serum, showed significant differences between all treatments for these enzymes. The highest values for amylase and lipase were obtained in T (3), that were (73.03 ± 0.79

u/mg) and (55.33 ± 0.49 u/mg) respectively, while the lowest values were recorded from (T1), which were (51.8 ± 0.32 u/mg) and (27.9 ± 2.17 u/mg) for amylase and lipase respectively. Insulin was significantly increased in the first treatment (0.53 ± 0.05 μ IU/mL), while its value was significantly decreased in the alkaline treatment (T3) and recorded (0.17 ± 0.03 μ IU/mL). Total iron measured in serum had significantly different among the three treatments, its lowest value was obtained in the acidic treatment (T1) (27.37 ± 1.46 μ g/dL), while the lowest value was observed in the alkaline treatment, (54.96 ± 1.7 μ g/dL).

Table 4. Amylase, Lipase, Insulin and total iron in serum of Nile tilapia after exposed to different pH.

Treatment	Amylase (u/mg)	Lipase (u/mg)	Insulin (μ IU/mL)	Total iron (μ g/dL)
T1 "pH 6"	51.8 \pm 0.32c	27.9 \pm 2.17 c	0.53 \pm 0.05a	27.37 \pm 1.46c
T2 "pH 7"	61.1 \pm 1.59b	48.00 \pm 1.5 b	0.33 \pm 0.04b	44.02 \pm 2.03b
T3 "pH 8.5"	73.03 \pm 0.79a	55.33 \pm 0.49 a	0.17 \pm 0.03c	54.96 \pm 1.7a

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

DISCUSSION

The aim of this study is describing the effect of pH, some of blood physiological parameters and some of digestive enzymes on growth index of the most famous farmed fish, *Oreochromis niloticus* (Linnaeus, 1758) in Egypt. Knowledge of specific enzyme activity, along with animal habits and digestive capacity is essential in formulating an appropriate diet for any species. So, the effects of pH and temperature on enzyme activity were also evaluated via the use of specific substrates (Candiotto *et al.*, 2017).

It was demonstrated that the effect of various modifications as well as pH-function on growth of fishes may change the digestive enzyme activities in different degrees depends on its species (Kuz'mina and Nevalenny, 1983 and Golovanova *et al.*, 1994). The pH values had significantly affected the total alkalinity; this was shown in table (1). The increase of pH from 6 to 8, 5 cause

the growth of enzyme activity in *Tilapia* at all measured temperature values. These results indicated that there was lipase activity in the whole digestive tract. Similar results were evidenced by (Chioma *et al.*, 2005) for stomach importance organ of fish (*Mormyrus rume*, *M. anguilloides* and *G. cyprinoides*) where lipid digestion occurs unlike the higher vertebrates. Also, Achionye-Nzeh *et al.*, (2005) mentioned the same results on *Hemichromis fasciatus*, *Oreochromis niloticus*, *Sarotherodon galilaeus* and *Tilapia zillii* from a small lake in Ilorin, Nigeria. In alimentary tract of *Whitmania pigra*, (Shi *et al.*, 2012) showed that the responses of lipase, and amylase activities were determined over a wide range of temperatures (7-52 degrees C) and pH gradient (2.2-11.2). They concluded that the optimal temperature in alimentary tract of *W. pigra* for lipase and amylase was 37 degrees C, and the optimal pH value was 8.2 and 5.2, respectively. This study showed that the optimum pH for the activity of amylase was 8.5, at 28, 6 ± 0.3 degrees C. It was in accordance with the results obtained by (Candiotta *et al.*, 2017). Their optimum pH for the activity of amylase was 8.0, while optimum temperature was 40 °C of Brazilian flounder *Paralichthys orbignyanus*. In this respect of both the same study and species, (El--Sherif and El-Fekyd, 2009), showed that the growth performance was significantly ($P \leq 0.05$) decreased at pH 6 and pH9, while the differences between pH 7 and pH8 were not significant ($P \geq 0.05$). They concluded that water pH (from 7 to 8) could be more suitable to tilapia culture for optimum growth performance and survival rate. From table (2) the daily gain weight was nearly double times higher at the first to the third treatments in pH of 6 and 8.5, respectively. In alkaline zone of pH, this influence was approximately two times as less. So, the result from this study was the same line with the significant effect of temperature and pH on digestive enzymes which shown in Table (3). But the enzymes activities to a larger extend are influenced by a complex effect of these factors in all studied fish. At the end of experiment, the results showed that the growth index was significant different ($P > 0.05$). The current results corroborate those by Cavalcante *et al.* (2010) who stated that total ammonia, calcium hardness, pH and total alkalinity in the green water aquaria were

significantly higher than rates in the clear water aquaria. They added that lime rearing water with sodium carbonate had no significant effect on tilapia growth performance if the initial total alkalinity of water was higher than (20) mg calcium carbonate per one liter. The work by (Reboucas *et al.*, 2016) aimed at reassessing the suitable range of water pH for culture of Nile tilapia, *Oreochromis niloticus* L. fingerlings. Acidification of water, regardless the degree, i.e., slight or moderate, was not able to significantly affect final body weight, specific growth rate and yield of fish. They concluded that the acidification of water up to pH 5.5 has no negative influence on growth of Nile tilapia juveniles in eutrophic water. Accordingly, the suitable range of water pH for rearing Nile tilapia should be set at 5.5 – 9.0. These results provide additional information regarding the biology of the Nile *Tilapia* fish, *O. niloticus* (L., 1758) and can be used as an attempt for further studies regarding fish feeding physiology.

CONCLUSION

Some digestive enzymes were increased its activity within permissible alkalinity media in water, and reverse in acidic media. In the same direction; haematological parameters and subsequently growth performance of Nile tilapia

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دور الأس الهيدروجيني علي معامل النمو، بعض القياسات الفسيولوجيه بالدم، وبعض الإنزيمات الهاضمة في سمكة البلطي النيلي

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الملخص العربي

تم تجميع عدد ٩٠ سمكة من البلطي النيلي بمتوسط وزن ٢٢.٥٣ جم وتم توزيعهم عشوائيا في ٩ أحواض زجاجية تمثل ثلاثة معاملات مختلفة من الأس الهيدروجيني (٦،٧ و ٨.٥) علي مدار ٤٥ يوم وهي مدة التجربة. تم استخدام علف ٢٥% بروتين للتغذية بنسبة ٢% من الوزن الكلي للأسماك يوميا. وتم تطبيق درجة الأس الهيدروجيني باستخدام حمض الكبريتيك وكرساتلات هيدروكسيد الصوديوم لجعل الوسط حامضي أو قاعدي علي الترتيب. وتم قياس الخواص الفيزيوكيميائية للمياه علي مدار التجربة. الوزن اليومي المكتسب تراوح بين ٠.٢٥ و ٠.٤١ جم. أفضل معدل نمو نوعي كان ١.٣٤% وتم تسجيله خلال المعاملة القاعدية. كان هناك تأثير لدرجة الأس الهيدروجيني علي كل من عسر المياه وتركيز الأمونيا وأيضا علي الخواص الفسيولوجية للدم. وكان هناك تأثير كبير علي إنزيمي الليبيز والأميليز خلال المعاملة القاعدية. أقل نسبة أنسولين في الدم كانت ٠.١٧ ميكرو وحده دوليه/ ملي وتم قياسها في المعاملة الثالثة، أقل نسبة حديد في الدم كانت ٢٧.٣٧ ميكروجم/ديسيلتر وتم قياسها في المعاملة الأولى.