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EFFECT OF SALINITY LEVELS ON SERUM OSMOLARITY AND RELATED PHYSIOLOGICAL PARAMETERS OF NILE TILAPIA (*Oreochromis niloticus*) AND ITS RELATION TO GROWTH PERFORMANCE

Abd El-Ghaffar, H.A.

Spawning and Fish Physiology Department, Central laboratory for aquaculture research, Abbassa, Abu Hammad, Sharkeya.

Dr_Haytham1983@hotmail.com

Abstract

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 distribution. Four treatments were prepared with different salinities (0.15 ppt "control" T1, 3 ppt "T2", 6 ppt "T3" and 9 ppt "T4") for 90 days and use pure NaCl was used for salinity adjustment. At the end of experiment blood samples were collected for blood measurements. The results showed that, water temperature was ranged about 29˚C, the maximum values of Hb and RBCs were recorded in control group (7.32±0.21 g/dl and 2.565±0.023 x 10^6/cmm) respectively. Minimum and maximum ferritin values were (3.77 ± 0.40 ng/ml to 6.62 ±0.35 ng/ml) recoded in T4 and control group respectively. The maximum values of TIBC were recorded in control group (216.9±8.5 µg/dl). Sodium and potassium were significantly increased in T4 (172±2.54 m.mol/l and 6.42±0.55 m.mol/l) respectively. The highest osmolarity value was (378 ± 9.6 m Osmol/kg) in T4. The highest survival rate was noticed in control group (95.8±0.7%).

Key words: salinity, Nile tilapia, osmolarity, ferritin, hematological parameters, growth performances, iron and water quality.

INTRODUCTION

Tilapia culture is one of the fastest growing farming activities, with an average annual growth rate of 13.4%, during 1970–2002. Tilapias are widely cultured in about 100 countries in the tropical and subtropical regions. As a result, the production of farmed tilapia has increased from 383,654 million tons in 1990 to 1,505,804 million tons in 2002, representing about 6% of total

farmed finfish. Nile tilapia is, by far, the most important farmed tilapia species in the world. The production of Nile tilapia reached 3,197,330 tons in 2012 (FAO, 2015). Water quality is the totality of physical, biological and chemical parameters that affect the growth and welfare of cultured organisms. The success of a commercial aquaculture enterprise depends on providing the optimum environment for rapid growth at the minimum cost of resources and capital. Water quality affects the general condition of cultured organism as it determines the health and growth conditions of cultured organism. Quality of water is, therefore, an essential factor to be considered when planning for high aquaculture production (Mallya, 2007). When fish are forced to deal with different salinities, depending on marine or fresh water fish, they spend more energy to hold their homeostasis and grow less. Because osmoregulation break down, fish spends more energy to hold sodium and chloride ions in their bodies or take off them (Küçük *et al*., 2013). The haematological profile and biochemical parameters represents a good indicator of physiological dysfunctions, they are being used as indicators in the measurement of health conditions and toxicological symptoms of organisms (Elahee and Bhagwant, 2007 and Rao, 2006). Küçük *et al.*, 2013 studied the effect of salinity on growth and blood chemistry, they also stated that, when fish are forced to deal with different salinities, depending on marine or fresh water fish, they spend more energy to hold their homeostasis and grow less. Because osmoregulation breaks down, fish spends more energy to hold sodium and chloride ions in their bodies or take off them. Azevedo, *et al.* (2015) showed that the performance and haematological parameters of Nile tilapia, *Oreochromis niloticus* were affected by salinity, where there were differences ($P < 0.05$) on the daily weight gain, feeding conversion rate and survival. The best results were observed for the water salinity levels of 0 and 7 g L-1. Also, the percentage of the haematocrits and the erythrocyte count were influenced ($P < 0.05$) by the water salinity level. Uchida, *et al*, (2000) found that tilapia is adaptable to a wide range of salinity, maintaining the plasma osmolality within physiological levels. Gill Na+, K+-ATPase activity was remarkably increased in response to elevated

environmental salinity. Salinity is one of the important a biotic environmental factors (temperature, dissolved oxygen) that direct fish growth (Mommsen, 1998). Azevedo *et al*. (2015) regarding the haematological parameters evaluated, it was observed that the haematocrite and the erythrocyte count were influenced by the water salinity level with higher values in 0 and 7 g L-1. Joao *et al.* (2009) found that iron is essential for growth and survival, but it is also toxic when in excess. Thus, there is a tight regulation of iron that is accomplished by the interaction of several genes including the iron transporter transferring and iron storage protein ferritin.

MATERIALS AND METHODS

The experiment was performed in wet laboratory of Spawning and Fish Physiology Department of 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 distribution. Ninety six Nile of fingerless tilapia (*Oreochromis niloticus*) of mean weight 68.5±0.28 g were chosen and randomly distributed in twelve aquaria of size 60X40X50 cm for each aquarium, representing three treatments and control in three replicates for each one. Four treatments were prepared with different salinities (0.15 ppt "control" T1, 3 ppt "T2", 6 ppt "T3" and 9 ppt "T4") for 90 days and use pure NaCl was used for salinity adjustment. Fish were fed on 25% protein diets with 2.5% of fish weight daily "6 days a week" during the experimental period that lasted for three months. Water in aquaria was replaced each three days with dechlorated water, and then readjusts salinity levels. At the end of experiment blood samples were collected in two parts first part on EDTA solution for measuring haemoglobin, erythrocytes count, haematocrite and calculated blood indices, second part in sterile tube and centrifuged to isolate serum to measure ferritin (ng/ml), TIBC (ug/dl), Na (m mol/L), K (m mol/L) and osmolarity (m Osmol/kg).

Water parameters were measured biweekly as follows, Temperature and dissolved oxygen using oxygen-meter AQUA LYTIC (model OX 24) instrument. Portable pH electronic paper apparatus "Hanna instruments. Salinity by portable salinity-conductivity meter Lovibond Sensodirect "model, Con 200" UK, Total alkalinity. Total hardness and ammonium concentration was measured by HACH comparison apparatus following the method reported by (APHA, 2000).

At the end of the experiment hemoglobin concentration (Hb), erythrocyte counts (RBC) 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 and expressed as number of cells per cubic ml. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were also calculated according to standard formulas, MCV (fl) = 10. PCV / RBCs, MCH (μμg) =10. Hb / RBCs, MCHC (g/dl) = 100. Hb / PCV.

Plasma TIBC was measured using Darman Kav Co. kit (Isfahan, Iran). In this method, iron ions are added to plasma sample making plasma transferrin fully saturated. Then, excess iron ions are precipitated by adding magnesium carbonate. Transferrin-bound iron was released in acidic pH and measured photometrically (Ramsay, 1975). Plasma osmolality was measured using a freezing point osmometer (μOsmette, Advanced Instruments, Norwood, MA USA).

Plasma [Na+] and [K+] were determined by flame photometry (Instrumentation Laboratory, Inc., Model 343 Lexington, MA USA), zeroed with a Lithium diluents blank and calibrated with Na+/K+ standards, using plasma diluted in Lithium diluents (1:200) measured by positive displacement pipette. 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 [Ln wt_1– 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).

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, physiological, hematological parameters and growth performances of fish were measured during the experimental period then collected and analysis then tabulated in four tables. In Table (1), water temperature was ranged around 29˚C, dissolved oxygen was not significantly different at all treatments and ranged from 5.17 ± 0.06 to 5.27 ± 0.06 mg/l. pH value had significantly decrease in T4 in compared with the other treatments. Salinities were adjusted to 3, 6 and 9 ppt for 2nd, 3rd and 4th treatments, while the control group it was 0.15 ± 0.02 ppt. The values of alkalinity and hardness were significantly decreased in control group with other treatments. Ammonia had no significant difference in all treatments with salinity, ranged from 0.12 ± 0.03 to 0.14 ± 0.02 mg/l in T1 and T3.

The hematological parameters are illustrated in Table (2). Haemoglobin (Hb) and erythrocytes (RBCs) were significantly affected by changes in salinity, and there were significant differences between their treatments. The maximum values of Hb and RBCs were recorded in T1 (7.32±0.21 g/dl and 2.565±0.023 x 10^6/cmm) respectively. While, the minimum values of Hb and RBCs were recoded in T4 (5.68±0.22 g/dl and 1.986±0.008 x 10^6/cmm) respectively. The packed cell volume (PCV) was non significant between the 2nd and 3rd treatments, while the other treatments were significant different. The

mean corpuscular volume (MCV), showed significant differences between all treatments, and was significantly affected by salinity. The control group "T1" showed the highest MCV value (84.6±0.6 fl), while the lowest value was recorded in "T4" (77.5±0.2 fl). The mean corpuscular haemoglobin (MCH) values had no significant, its values were ranged from 28.0±0.3 to 28.7±0.2 μμg**.** The mean corpuscular hemoglobin concentration (MCHC) values had significant among the four groups during the experiment. The minimum MCHC values were 33.6±0.05g/dl in the control group "T1", while its maximum value recorded in T4 37.0±0.02g/dl.

Table 1. Average values (means±Sd) of water temperature "˚C", dissolved oxygen "mg/L", pH, salinity "ppt", total alkalinity " mg/L", total hardness "mg/L" and ammonia "mg/l" in aquaria during the experimental period of Nile tilapia.

Treatment	Temp	Dissolved oxygen (mg/l)	pH	Total alkalinity (mg/l)	Total hardness (mg/l)	Ammonia (mg/l)
T1 "control"	29± 0.10 ^{a}	5.27±0.06^{a}	8.13±0.06 a	163.7±7.57^{d}	211±5.57^{c}	0.12 ± 0.03 ^{a}
T2 "3ppt"	29.1±0.20 ^{a}	5.27±0.06^{a}	8.13±0.06^{a}	187.3±6.7^{c}	271±5.57^{b}	0.14 ± 0.01 ^{a}
T3 "6ppt"	28.9±0.10 ^{a}	5.17±0.06^{a}	8.23±0.06^{a}	209±5.3^{b}	288.7±8.5^{b}	0.14 ± 0.02 ^{a}
T4 "9ppt"	29.1±0.15 ^{a}	5.20±0.10^{a}	7.93±0.06^{b}	234.3±4.04^{a}	314.7±4.5^{a}	0.13 ± 0.02 ^{a}

The means have the same letter in the same column are not significant P<0.05

Table 2. Average values (mean±Sd) of haemoglobin concentration (g/dl), erythroctic count (cmm), haematocrite percentage (%) and blood indices were measured in Nile tilapia at the end of exposure to different levels of salinities.

Treatment	Hb (g/dl)	RBCs (x 10^{6}/cmm)	PCV %	MCV (fl)	MCH (μμg)	MCHC (g/dl)
T1 "control"	7.32±0.21^{a}	2.565±0.02^{a}	21.7±1.1^{a}	84.6±0.6^{a}	28.5±0.4^{a}	33.6±0.05^{d}
T2 "3ppt"	6.6±0.13^{b}	2.312±0.01^{b}	18.4±0.4^{b}	79.6±0.3^{c}	28.5±0.2^{a}	35.9±0.03^{b}
T3 "6ppt"	6.32±0.15^{c}	2.254±0.02^{c}	18.3±0.7^{b}	81.2±0.5^{b}	28.0±0.3^{a}	34.4±0.08^{c}
T4 "9ppt"	5.68±0.22^{d}	1.986±0.01^{d}	15.4±0.3^{c}	77.5±0.2^{d}	28.7±0.2^{a}	37.0±0.02^{a}

The means have the same letter in the same column have no significant difference.

Ferritin had highly significant in treatment (1 and 4), while treatment (2 and 3) had no significant. The Ferritin values were ranged from (3.77 ± 0.40 ng/ml to 6.62 ±0.35 ng/ml). In T1 and T4 the total iron binding capacity (TIBC) had significance differences by salinity levels. The maximum TIBC values were recorded in control group (216.9±8.5 ug/dl), while the minimum one was (149.9 ± 6.58 ug/dl) in T4. Sodium was significantly increased in the fourth treatment 172±2.54 m.mol/l, while its concentration in the control group was significantly low 143±3.8m.mol/l. potassium had a significant in the last two treatments, while it was no significant in first and second treatments. The potassium recorded the minimum value 3.56±0.35 m.mol/l in the control group, while its maximum value 6.42±0.55 m.mol/l was in the last treatment. All treatments are significant in osmolarity except in the second and third treatments. The highest osmolarity value was 378 ± 9.6 m Osmol/kg in treatment (4), while the lowest one 312±8.5 Osmol/kg obtained in the control group.

Table 3. Serum ferritin (ng/ml), total binding iron capacity (ug/dl), sodium (m mol/L), potassium (m mol/L) and osmolarity (m Osmol/kg) in Nile tilapia (*Oreochromis niloticus*) at the end of the experimental period.

Treatment	Ferritin (ng/ml)	TIBC (ug/dl)	Na (mmol/L)	K (mmol/L)	Osmolarity (m Osmol/kg)
T1 "control"	6.62±0.35^{a}	216.9±8.5^{a}	143±3.8^{d}	3.56±0.35^{c}	312±8.5^{c}
T2 "3ppt"	5.76±0.47^{b}	179.3±4.97^{b}	154.6±4.16^{c}	3.97±0.32^{c}	349 ± 6.47^{b}
T3 "6ppt"	5.74±0.38^{b}	163.5±4.21^{c}	164.3±3.98^{b}	4.9±0.25^{b}	357 ± 7.68^{ab}
T4 "9ppt"	3.77±0.40^{c}	149.9±6.58^{d}	172±2.54^{a}	6.42±0.55^{a}	378 ± 9.6^{a}

The means have the same letter in the same column have no significant difference for different treatments.

The growth performance parameters of fish during the experimental period are shown in Table (4). There were significantly differences between salinity and growth performance parameters for all groups. The final weigh ranged from 91.2±0.23g in treatment (4) and 115.97±1.53g in the control group, the four treatments had significantly difference. The highest gain weight was recorded in the control group (47.47 ± 1.99 g), while the lowest gain weight values were obtained in T4 (23.0 ± 0.56 g). Daily weight gained had significant

differences in all treatments, and ranged from 0.25±0.01g to 0.53±0.02g in treatments (1 and 4). The lowest feed conversion ratio (FCR) was recorded in the control group (1.38±0.02%). The highest specific growth rate (SGR) was recorded in T1 (0.58±0.08), while the minimum was obtained in T4 (0.32±0.09). The highest survival rate was noticed in control group (95.8±0.7) followed by T2, T3 and T4 which were (91.6±0.8, 83.3±0.7 and 79.2±0.9) respectively.

Table 4. The values (Means±Sd) of growth performance parameters of Nile tilapia (*Oreochromis niloticus*) were measured during the experimental period.

Treatment	Initial weight (g)	Final weight (g)	Total gain weight (g)	Daily weight gained (g)	FCR (%)	SGR	Survival rate (%)
Г1 "control"	68.5±0.28a	115.97±1.53a	47.47±1.99a	0.53±0.02a	1.38±0.02d	0.58±0.08a	95.8±0.7a
T2 "3ppt"	68.5±0.68a	107.57±1.43b	39.0 ±0.74b	0.43±0.01b	1.42±0.04 c	0.5±0.09b	91.6±0.8b
T3 "6ppt"	68.1±0.49a	97.13±0.88c	29.03±0.45c	0.32±0.003c	1.49±0.03b	0.39±0.07c	83.3±0.7c
T4 "9ppt"	68.2±0.40a	91.2±0.23d	23.0±0.56d	0.25±0.01d	1.52±0.06a	0.32±0.09d	79.2±0.9d

The means have the same letter in the same column have no significant difference.

DISCUSSION

Physico-chemical parameters have important role on physiological and growth performance of Nile tilapia *(Oreochromis niloticus*) especially salinity which change disturbance of surrounding media of electrolytes changes in salinity companied by changes in total alkalinity and total hardness these results agree with Küçük *et al.* (2013) who showed that salinity is defined as the sum of all ions in water which comprises mainly of sodium, chloride, calcium, magnesium, potassium, bicarbonate and sulfate ions. Uchida *et al*. (2000) found that the excellent salinity tolerance of tilapia appears to be attributed to their ability to develop chloride cells in response to increased environmental salinity. McCormick (1995) when euryhaline teleosts are transferred from FW to SW, chloride cells are increased in number and size, along with an increase in Na+, K+-ATPase activity.

Hematological parameters were used as indicator of fish health especially when fish was changed its natural water medium, so, increasing salinity levels decrease the hemoglobin, erythrocyte and hematocrit percentage these results hand in hand with Verdegem *et al*. (1997), found that the influence of salinity and dietary' composition on blood parameter values (haematocrit. leucocrit. immature lymphocytes, mature lymphocytes, granulocytes. plasma osmolarity and total plasma protein) of red hybrid tilapia, *Oreochromis niloticus* (Linnaeus) x 0. *mossambicus* (Peters), was studied and concluded that the environmental parameters investigated in the present study should be considered when estimating fish health based on blood parameter values. Farghaly *et al.* (1973) they found that the increase in blood cell volume at higher salinities might be due to a loss of water from the blood to the hypertonic environment or it may be a physiological response to increase the oxygen-carrying capacity of the blood in support of the higher metabolic demands in the saline environment.

Due to vital role of salinity and its effect in changes of electrolyte concentrations and subsequently rise of osmolarity also, results noticed that increasing salinity levels were be decreased ferritin and total iron binding capacity these obtained results were agreement with Mommsen (1998) who found that salinity is a vital water quality parameter for fish growth. Küçük *et al.* (2013) reported on retarded fish growth at different saline conditions. Fish in marine or freshwater environments use energy to hold ions in or off their bodies respectively through osmoregulation. Whole body lipid of Nile tilapia decreased with the increase of salinity, showing the potentially important roles of lipids and fatty acids in the osmoregulation of fish (Jarvis and Ballantyne, 2003). Azevedo *et al.* (2015) showed that osmoregulation, the effect of water salinity on the performance of fish can be explained by its action upon digestive enzymes, where the exposure to different salinities modifies the water ingestion, altering the salinity of the intestinal content and affecting the activity of digestive enzymes (Moutou *et al*., 2004).

The target value of aquaculture of Nile tilapia is growth performance and survival rate to return an economic return so, the negative effect of increasing salinity levels of growth were be noticed from our data these notices hand with hand with Joa *et al.* (2009) they found that iron is essential for growth and survival, but it is also toxic when in excess. Thus, there is a tight regulation of iron that is accomplished by the interaction of several genes including the iron transporter transferrin and iron storage protein ferritin. These findings demonstrate the evolutionary conservation of transferrin and ferritin dual functions in vertebrates, being involved in both the immune response and iron metabolism. This process can explain the worsening in the alimentary conversion and consequent worsening of the weight gain of tilapias in the highest salinities in this experiment. Ferritin is a 450-kDa protein and has a major role in iron metabolism. Being the main iron storage protein in both eukaryotes and prokaryotes, it keeps iron in a soluble and nontoxic form (Chasteen, 1998; Harrison, 1996 and Theil, 1990).

CONCLUSION

Salinity plays an essential role in disturbance of electrolytes (especially sodium and potassium) in water subsequently related with changes in serum osmolarity of fish and hematological parameters in Nile tilapia.

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تأثير درجات مختلفة من الملوحة علي المصل الأوزمولوزي والعوامل الفسيولوجيه المرتبطه لسمكة البلطي النيلي وعلاقتها بأداء النمو

هيثم أحمد عبد الغفار

قسم التفريخ وفسيولوجيا الأسماك، المعمل المركزي لبحوث الثروة السمكية، مركز البحوث الزراعية، وزارة الزراعه، مصر.

الملخص العربي

أجريت هذه التجربة بالمعمل المركزي لبحوث الثروة السمكية بالعباسه– أبو حماد– شرقيه، لدراسة تأثير تركيزات مختلفة من الملوحة علي الأسموزية وبعض القياسات الفسيولوجية بالدم، وتأثيرها علي أداء النمو لسمكة البلطي النيلي. تكونت التجربة من معاملة كنترول وثلاثة معاملات أخري مختلفة الملوحة (٣، ٦ و٩ جزء في الألف) وكل معاملة تكونت من ثلاث مكررات، استمرت التجربة لتسعين يوما. استخدم ملح الطعام كلوريد الصوديوم للحصول علي تركيزات الملوحة المطلوبة. تم عمل تحاليل المياه مرتين اسبوعيا طوال مدة التجربة. في نهاية التجربة تم قياس الأوزان وسحب عينات الدم لعمل التحليلات المختلفه. أشارت النتائج لأن أعلي قياس للهيموجلوبين وأعلي عدد لكرات الدم الحمراء تم تسجيلهما في مجموعة الكنترول (٧.٣٢ ±٠.٢١ جم/ ديسيلتر و٢.٥٦٥ ± ٠.٠٢٣ مليون خلية / مم ^{٣}) بالترتيب. وتراوحت قياسات الفريتين مابين (٣.٧٧ ± ٠.٤ و ٦.٦٢ ± ٠.٣٥ نانوجم/ملي لتر) في المجموعة الرابعه ومجموعة الكنترول بالترتيب. أعلي قيمه لسعة الحديد الكلي المرتبط تم تسجيلها في مجموعة الكنترول (٢١٦.٩ ± ٨.٥ ميكروجم /ديسي لتر). ولوحظ زياده معنوية للصوديوم والبوتاسيوم في المعامله الرابعه حيث وجدا (١٧٢± ٢.٥٤ و ٦.٤٢ ± ٠.٥٥ ملي مول / لتر) علي الترتيب. أعلي قياس للأسموزية سجل ٣٧٨±٩.٦ ملي أسمول /كجم من خلال المعامله الرابعة. أعلي معدل للإعاشة وجدت في المعاملة الكنترول حيث كانت ٩٥.٨ ± ٠.٧ %).