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# EFFECT OF DIFFERENT CONCENTRATIONS OF AMMONIA ON PHYSIOLOGICAL STATE AND GROWTH PERFORMANCE OF NILE TILAPIA (*Oreochromis niloticus)*

**Mona H. Ahmed [1]; Hyitham A. Abd El-Ghaffar[2];**
**Nourhan H. Ahmed[3] and Amany A. Gharib[2]**

*[1]Department of limnology, Central Laboratory for Aquaculture Research, Agriculture Research Center, Ministry of Agriculture, Egypt.*

*[2]Department of Spawning and Fish Physiology, Central Laboratory for Aquaculture Research, Agriculture Research Center, Ministry of Agriculture, Egypt.*

*[3]Department of Animal production, Faculty of Agriculture, Zagazig university*

***Abstract***

This experiment was conducted at the Central Laboratory for aquaculture research, Ministry of Agriculture, Egypt. This study was devoted to study the effect of different sub lethal concentrations of total ammonia on growth performance and some physiological parameters of Nile tilapia *(Oreochromis niloticus).* One hundred and forty four Nile tilapia fish with average weight 52.3±0.81 g, were reared in twelve aquaria of 60x40x50 cm represented four replicated groups, the first group used as a control group (fresh water with no adding of ammonia) and the other three groups were treated with different concentrations of ammonia determined as 3, 5 and 7 mg/l.

The experimental period was two months. Dissolved oxygen, temperature, salinity, pH, total alkalinity and total hardness were constantly measured twice weekly. Under these treatments, fish growth performance, Hemoglobin concentration (Hb), erythrocyte counts (RBC), hematocrite (Htc) percentage, other blood indices, total iron, total iron binding capacity (TIBC), insulin, amylase and lipase were measured. The results showed that there was significant decrease in growth performance, blood indices, serum total iron, total iron binding capacity (TIBC), amylase and lipase by increasing ammonia concentration. It could be concluded that increasing concentrations of total ammonia in water ponds more than permissible limits decreases the levels of digestive enzymes and TIBC which reflecting on reducing growth performance of Nile tilapia.

**Key words:** Ammonia, Nile tilapia, water quality, iron, digestive enzymes, growth.

## INTRODUCTION

Aquaculture has been growing steadily in recent times as an excellent source of high-quality protein (Krishen, *et al.,* 2009). Researchers focused on Nile tilapia because of its 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). As size increasing, fish growth rate and feed efficiency gradually decrease (Liu *et al.*, 2016). Ammonia is the principal nitrogenous waste product of fishes and is normally oxidized first to nitrite then to nitrate. It is also, the main nitrogenous waste material excreted by gills beside urea and amines. Moreover, creatine, creatinine, and uric acid are being excreted through the kidneys (De Croux *et al.*, 2004). Chronic un-ionized ammonia exposure may affect fish and other organisms in several ways, e.g. gill hyperplasia, muscle depolarization, hyper excitability, convulsions and finally death (Ip *et. al.,* 2001). Un- ionized form of ammonia is the most toxic form to aquatic organisms as it can readily diffuse through cell membranes and is highly soluble in liquids. It can cause impairment of cerebral energy metabolism, damage to gill, liver, kidney, spleen and thyroid tissue in fish (Smart, 1978). Moreover, it is a common aquatic pollutant. It enters natural waters with municipal, agricultural, fish-cultural, industrial wastes, and from energy- development processes such as oil-shale retorting, coal gasification, and coal liquefaction, it is also, a natural degradation product of nitrogenous organic matter (Randall and Tsui, 2002). Thangam *et al*. (2014) reported that the reduction in RBC and WBC count of common carp, *C. carpio,* after exposed to ammonia. The ammonia exposure also caused a notable reduction in MCV, MCH, and MCHC of Giant Sharkminnow *"S. schlegelii"*. Fish growth is an outcome of feeding, digestion and metabolism of food material, the previous studies indicate that the digestive, absorptive, and metabolic capabilities of nutrients are related to fish size (Luo *et al.*, 2013).

The aim of the present study was to investigate the effect of different sub lethal concentrations of total ammonia on growth performance, and some physiological parameters of Nile tilapia (*Oreochromis niloticus*).

## MATERIALS AND METHODS

This study was carried out at the indoor wet lab of spawning and fish physiology department, Central Laboratory for Aquaculture Research, Agriculture Research Center, Egypt. This experiment was devoted to study the effect of different sub lethal concentrations of total ammonia on growth performance, some physiological parameters of Nile tilapia *(Oreochromis niloticus*). The experimental period was two months.

A number of 144 Nile tilapia (*Oreochromis niloticus*) with average 52.3±0.81g, body weight were reared in twelve aquaria of 60x40x50 cm, the experimental aquaria divided into four groups, the first group used as a control group "TC" (fresh water with no adding of ammonia), and the other three groups were treated with different concentrations of ammonia as follows: 3 mg/l "T1", 5 mg/l "T2" and 7 mg/l "T3" with three replicates for each. 36 fish were chosen randomly from the stocked and were used in each treatment (divided into 12 fish in each aquarium). The fish were acclimated to the test conditions for two weeks before treatment started and they were fed with fish pellets at ratio of 3% of total body weight daily. The water in the aquaria was replaced daily then readjustment of ammonia concentration carried out and aerated continuously experimental period. Ammonia stress was induces by adding ammonium chloride to each aquarium (Xu *et al.*, 2005). Total ammonia concentrations were determined by nesslerization method (APHA, 2000), and then unionized ammonia ($NH_3$) values were calculated through a coefficient related to water pH and temperature values measured at the time of sampling, according to Boyd and Tucker (1992).

Water samples 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 by APHA

(2000). Also, growth performances can be measured at the end of the experiment, fish from each aquarium were weighed to evaluate weight gain and specific growth rate as follows, Weight gain (g/fish) = W2 – W1. daily weight gained = weight gained/ time of experiment. Specific growth rate (SGR) = 100 [Ln $wt_1$– Ln wTc] / T Where: Ln = Normal logb. $Wt_1$ = The final weight (g). WTc = The initial weight (g). T = the time of experiment (days).

Blood samples were collected from the caudal veins at the end of experimental period (2 months), and the following parameters were measured: 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 and 2001). 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 (%) = 100. Hb / PCV. The total iron content (TI, mg/ml) and unsaturated iron binding capacity (UIBC, mg /ml) of serum were 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). Total iron binding capacity (TIBC mg/ml) was calculated as the sum of TI and UIBC. 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 of fish were subjected to one-way ANOVA. Differences between means were tested at the 5% probability level using Duncan test (Duncun, 1955). All the statistical analysis was done using SPSS version 10 (SPSS, Richmond, USA) as described by Dytham (1999).

## RESULTS

Water quality parameters were measured biweekly then tabulated as one average in Table (1). It was observed that temperature was around (29.3±0.03 ˚C) for all treatments. The dissolve oxygen ranged between (4.7±0.07 mg/l) in the third treatment and (5.4±0.03 mg/l) in control group "TC", pH concentration ranged from 7.8 (T2 and T3) and 8.2 in TC. Salinity averaged from 0.12±0.003ppt at T3 to 0.14ppt in first and second treatments. Higher alkalinity was obtained (362.3±1.86 mg/l) in the highest ammonia concentration group (T3), and the lowest alkalinity (256.3±1.86 mg/l) was determined in the control group. The highest and lowest total hardness were determined in the first treatment "T1" and control one "TC" (295.3±2.27 mg/l and 234.0±2.31mg/l) respectively.

**Table 1.** Average values (means±SD) of some physico-chemical parameters during the experimental period.

| Treatment | Temperature (˚ C) | Dissolved oxygen (Mg/l) | pH | Salinity (ppt) | Total alkalinity (Mg/l) | Total hardness (Mg/l) |
|---|---|---|---|---|---|---|
| Control group "TC" | 28.2±0.03[a] | 5.4±0.03[a] | 7.7±0.03[a] | 0.13±0.015[a] | 256.3±1.86[d] | 234.0±2.31[d] |
| 3 mg/l ammonia "T1" | 28.2±0.03[a] | 5.1±0.03[b] | 7.7±0.03[a] | 0.14±0.015[a] | 263.0±1.53[c] | 295.3±2.27[a] |
| 5 mg/l ammonia "T2" | 28.3±0.001[a] | 5.0±0.03[c] | 7.6±0.03[b] | 0.14±0.007[a] | 309.0±2.08[b] | 272.7±2.19[b] |
| 7mg/l ammonia "T3" | 28.3±0.03[a] | 4.7±0.07[d] | 7.5±0.03[b] | 0.12±0.003[a] | 362.3±1.86[a] | 254.3±2.33[c] |

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

The growth performance was showed in Table (2), weight gained was significantly decreased with ammonia concentration where value of it was 28.7±0.15g in the control group TC, while the minimum value was obtained in T3 (15.67±0.67g). There were significant differences in the daily weight gain among the four treatments. The highest feed conversion ratio (FCR) was observed in the third treatment (1.85±0.02%), while the control group TC had the lowest value (1.34±0.02%). The highest specific growth rate (SGR) was obtained in the control group (0.75±0.01), while the lowest one (0.44±0.02) was observed in the third treatment.

**Table 2.** Mean values (means± S.D) of initial weight (g), final weight (g), weight gained (g), daily weight gained (g), FCR and SGR% of Nile tilapia during the experimental period.

| Treatment | Initial weight (g) | Final weight (g) | Weight gained (g) | Daily weight gain (g) | FCR | SGR (%) |
|---|---|---|---|---|---|---|
| Control group "TC" | 52.3±0.81[a] | 82.00±0.79[a] | 29.7±0.15[a] | 0.49±0.003[a] | 1.34±0.02[d] | 0.75±0.01[a] |
| 3 mg/l ammonia "T1" | 52.3±0.98[a] | 76.23±0.96[b] | 23.93±0.09[b] | 0.4±0.0001[b] | 1.46±0.03[c] | 0.63±0.0.01[b] |
| 5 mg/l ammonia "T2" | 52.3±0.92[a] | 70.23±0.78[c] | 17.93±0.15[c] | 0.3±0.0001[c] | 1.65±0.0.02[b] | 0.49±0.01[c] |
| 7mg/l ammonia "T3" | 52.3±0.69[a] | 67.97±0.75[d] | 15.67±0.67[d] | 0.26±0.01[d] | 1.85±0.02[a] | 0.44±0.02[d] |

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

The hematological parameters are shown in Table (3). Haemoglobin (Hb) concentrations had significant differences between all treatments. The highest Hb values were observed in control group TC (9.37 ± 0.12g/dl), while the lowest one was determined in T3 (6.4 ± 0.29g/dl). The highest count of RBCs was obtained in the control group (3.23 ± 0.02 x $10^6$/cmm), whereas it was (2.17 ± 0.09 x $10^6$/cmm) in T3. The highest Packed cell volume (PCV) was showed in control group TC (26.23± 0.02%) while the lowest were obtained in T3 (17.92 ± 0.81%) respectively. T2 was showed the lowest MCV value (80.88 ± 0.07fl), whereas the highest one was found in T3 (92.68 ± 0.74fl). The highest MCH value was recorded in T3 (29.53 ± 0.26 µµg), and the lowest one was recorded in T2 (28.89 ± 0.02 µµg). The mean corpuscular hemoglobin concentration (MCHC) had no significant differences among the four groups during the experiment.

From Table (4), it is clear that, when ammonia concentration increased there were significant decreasing differences between total iron, the lowest Fe was determined in T3 (30.67±0.58), while the highest one was obtained in the control group TC (124.4±1.47). Total binding iron capacity (TIBC) was significantly decreased by increasing ammonia, the highest value was recorded in control group (264.13±1.24), whereas, the lowest one was (204.4 ±4.79) in T3. The highest values amylase and lipase was showed in the control group TC (65.67±0.33 and 70.33±1.2) respectively, while the lowest values of them were observed in T3 (43.67±0.33 and 43.67±0.88) respectively. Insulin significantly increased by increasing ammonia concentrations. The highest insulin value was recorded in T3 (0.470.03), while the lowest value was recorded in control group TC (0.2±0.0001).

**Table 3.** Means of blood values (mean±S.D.) of haemoglobin, erythrocytes count, haematocrite, and blood indices.

| Treatment | Hb (g/dl) | RBCs (x $10^6$/cmm) | PCV (%) | MCV (fl) | MCH (µµg) | MCHC (g/dl) |
|---|---|---|---|---|---|---|
| Control group "TC" | 9.37 ± 0.12[a] | 3.23 ± 0.02[a] | 26.23±0.02[a] | 81.97±0.22[a] | 29.28±0.38[a] | 35.71±0.0001[a] |
| 3 mg/l ammonia "T1" | 8.9 ± 0.11[b] | 3.04 ± 0.03[b] | 24.92 ±0.31[b] | 81.9 ±0.26[a] | 29.25±0.29 [a] | 35.71±0.0001[a] |
| 5 mg/l ammonia "T2" | 7.8 ± 0.17[c] | 2.72 ± 0.06[c] | 21.84 ±0.48[c] | 80.88±0.07[b] | 28.89±0.02[b] | 35.71±0.0001[a] |
| 7mg/l ammonia "T3" | 6.4 ± 0.29[d] | 2.17 ± 0.09[d] | 17.92 ±0.81[d] | 92.68±0.74[a] | 29.53±0.26[a] | 35.71±0.0001[a] |

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

**Table 4.** Serum values (mean±SD) measured of total Iron, total binding iron capacity, amylase, lipase and insulin in Nile tilapia blood at the end of experimental period.

| Treatment | Fe (total) (µg/dL) | TIBC (µg/dL) | Amylase (u/mg) | Lipase (u/mg) | Insulin (µIU/mL) |
|---|---|---|---|---|---|
| **Control group "TC"** | 124.4±1.47[a] | 264.13±1.24a | 65.67±0.33[a] | 70.33±1.2[a] | 0.2±0.0001[d] |
| **3 mg/l ammonia "T1"** | 105±1.2[b] | 251.8±2.48[b] | 61.67±0.33[b] | 60.33±0.88[b] | 0.27±0.03[c] |
| **5 mg/l ammonia "T2"** | 57.7±0.9[c] | 235.27±4.25[c] | 54.33±0.88[c] | 53.33±1.2[c] | 0.37±0.03[b] |
| **7mg/l ammonia "T3"** | 30.67±0.58[d] | 204.4±4.79[d] | 43.67±0.33[d] | 43.67±0.88[d] | 0.470.03[a] |

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

## DISCUSSION

The toxicant exposure can induce the inhibition of growth performance in aquatic animals (Shin *et al*., 2016). In this study, the ammonia exposure caused a notable decrease in growth performance of *O. niloticus*. Several investigators reported similar results, Foss *et al.* (2002) who recorded similar results in juvenile wolfish. Moreover, Dosdat *et al.* (2003) and Lemarie *et al.* (2004) reported such phenomena in European sea bass. Recently, Sakala and Musuka (2014) reported a toxic effect of increased ammonia on tilapia growth rate.

In this study, mean body weight gain, average daily gain (ADG) and specific growth rate (SGR) were significant decrease ($P<0.05$) by increasing ammonia concentrations compared with control. This was attributed to a decrease in daily food consumption. Similar results were obtained by Atle *et al*. (2004) and Saber *et al.* (2004). Whereas Wang and Walsh (2000) reported that the reduction in average daily body weight gain was attributed to physiological disturbances, High levels of ammonia cause stress and produce harmful physiological response such as osmoregulatory disturbances, kidneys epithelium damages and retarded growth (Soderberg, 1994). The reduction of growth performance may result from the demand for energy to detoxicate the ammonia which affects the drop in the energy for growth (Clearwater *et al.*, 2002), or due to the inhibition of the fish appetite leading to significant reduction of feed intake and disturbance in metabolism (Zeitoun *et al.*, 2016). Mean feed conversion ratio was significant decreased by increasing ammonia concentrations, these results were agreement with Atle *et al.* (2004) and Saber *et al.* (2004) .While John and Semra (2001) found that there was no effect on growth or feed conversion ratio of channel catfish and blue tilapia.

The blood parameters could be sensitive and reliable indicator to evaluate the physiological status of fish. In this study, the RBC count, hemoglobin, and hematocrit of *O. niloticus* were significant decreased by increasing ammonia concentrations. Our results agreement with, Tilak *et al*. (2007) who reported a substantial decrease in hemoglobin of common carp exposed to ammonia, Thangam *et al.* (2014) reported a notable reduction in RBCs count of common carp exposed to ammonia, Shin *et al.* (2016) recorded that ammonia exposure of rockfish induced a significant decrease in red blood cell (RBC) count , hemoglobin (Hb), and hematocrit (Ht) and finally Zeitoun *et al.* (2016) recorded significant decreases in RBCs, PCV and Hb concentration of Nile tilapia after exposed to ammonia. 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). As it has been well established that liver and kidneys are the sites of erythrocyte production, the damage caused by ammonia might suppressed this process. Additionally, the increase of NH3 in the blood circulation might ruptured high percent of RBCs and caused hemodilution resulting in a disturbance of osmoregulation across gill epithelium (Vosyliene *et al.*, 2003). In the opposite, there found a species differences on the tolerance of different fish to ammonia level. This has been shown by Hrubinko *et al.* (1996) found that Hb increase when exposed to high concentrations of ammonia and also, Dosdat *et al*. (2003) reported no changes in hematocrit value in European sea bass exposed to ammonia. The ammonia exposure also caused a notable reduction in MCV, MCH and MCHC of *O. niloticus* but this decrease was non significantly in MCHC. These results agreement with Saravanan *et al.* (2011) where they suggested that the diazinon pesticide exposure to European catfish, *Cyprinus carpio* resulted in a considerable decrease in MCV, MCH, and MCHC, which is due to the increase of immature red blood cells by the toxicant exposure. In the opposite, Zeitoun *et al.*(2016) recorded increasing in MCV and MCH values of *O. niloticus* than control as a consequence to ammonia exposure might be ascribed to the increased water content in red blood cells resulting of chloride shift and the decreased of plasma chloride at the same state of high ammonia in water. Moreover, decrease in MCHC this decreasing might be attributed to the hemodilution and/or the lack of production of hemoglobin in circulation.

Commercial refection of Nile tilapia aquaculture is related by increasing digestive enzymes actively and subsequently increasing rate of absorption and growth, so, increasing ammonia is reverse for the previous rule as cleared from our results this agreement with Radhakrishnan *et al.* (2015), who stated that the amylase and lipase activity levels showed the same tendency with that of protease activity. Jiang *et al.* (2015) stated that plasma lipase and amylase activities in Jian carp were linear relationships between natural logarithms and body weight. Enzymes activities showed negative allometry against body weight of Jian carp which were partial reasons to explain fish growth rate decreasing. The digestive capacity of fish is governed by the activities of digestive enzymes and brush border enzymes in the intestine (Wu *et al*., 2011).

## CONCLUSION

This study included that increasing concentrations of ammonia in water ponds more than permissible limits decreases the levels of digestive enzymes and TIBC which reflecting on reducing growth performance of Nile tilapia.

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# تأثير تركيزات مختلفه من الأمونيا علي الحالة الفسيولوجية وأداء النمو لسمكة البلطي النيلي

**مني حامد أحمد[1]، هيثم أحمد عبد الغفار[2]،**
**نورهان حامد أحمد[3]، أماني عبد العزيز غريب[2]**

[1] *قسم الليمنولوجي، المعمل المركزي لبحوث الثروة السمكية، مركز البحوث الزراعية، وزارة الزراعه، مصر.*

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

[3] *قسم الانتاج الحيواني، كلية الزراعه، جامعة الزقازيق، مصر.*

## الملخـص العربـي

أجريت هذه التجربة بالمعمل المركزي لبحوث الثروة السمكية. وتمت هذه الدراسة لدراسة تأثير تركيزات مختلفة من الأمونيا علي أداء النمو وبعض القياسات الفسيولوجية بالدم لسمكة البلطي النيلي. استخدم لهذه التجربة ١٤٤ سمكة بمتوسط وزن ٥٢.٣ جم. تكونت التجربة من ٣ معاملات مختلفة التركيزات من الأمونيا (٣، ٥ و ٧ مجم/لتر) بالإضافة لمجموعة كنترول تركت بدون تغيير لخواص الماء، وكل معاملة اشتملت علي ثلاث مكررات، استمرت التجربة لمدة شهرين، تم خلالها قياس خواص الماء مثل الأكسجين الذائب، درجة حرارة الماء، الملوحة، ودرجة الأس الهيدروجيني باستمرار مرتين أسبوعيا طوال مدة التجربة. وتم حساب معلات النمو وأيضا قياس بعض العناصر بالدم بعد التجربة مثل الهيموجلوبين وعدد كرات الدم ونسبة الحديد والحديد الكلي المرتبط وإنزيمي الليبيز والأميليز والأنسولين. أوضحت النتائج أن هناك نقص نوعي في أداء النمو، مكونات الدم، الحديد الكلي، سعة الحديد الكلي المرتبط وإنزيمي الليبيز والأميليز وذلك بزيادة الأتركيز الأمونيا الكلية. ويستخلص من هذه التجربة أن زيادة تركيز الأمونيا في مياه المزارع عن المستويات المسموح بها يؤدي لنقص معدلات الانزيمات الهاضمة وسعة الحديد الكلي المرتبط والذي ينعكس بدوره علي قلة أداء النمو لسمكة البلطي النيلي.