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EFFECT OF AMMONIA STRESS ON GROWTH, HEMATOLOGICAL, BIOCHEMICAL, AND REPRODUCTIVE HORMONES PARAMETERS OF NILE TILAPIA *(Oreochromis niloticus)*

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Abstract

The present study was conducted to evaluate the ammonium hydroxide stress on Nile tilapia *(Oreochromis niloticus)* growth, health, and reproductive hormones. However, fish (70 ± 4 g) were exposed to different levels of ammonium hydroxide (0.0, 150, 250 and 350 pg/L) for 90 days. The results showed that fish growth was retarded as ammonium hydroxide levels increased and lowest growth was observed with fish exposed to 350 pg/L of ammonia. The highest growth was observed with the control group (0.0 ammonium hydroxide). Glucose and cortisol in fish plasma increased significantly, while total protein and total lipids decreased significantly due to ammonia stress. The obtained results show also that ammonium hydroxide stress was harmful to the fish liver and kidney where plasma aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, uric acid, and creatinine values were significantly increased with increasing ammonium hydroxide levels compared to the control one. It was also noticed that lysozyme increased with increasing ammonia levels as compared with the control one. But immunoglobulin M activities of Nile tilapia decreased with increasing ammonia levels as compared with the control one. plasma follicular stimulating hormones (FSH), 17B estradiol (E2) and testosterone (T) were decreased significantly at fish with increasing ammonia levels. On the other hand, the measurements in most parameters of the male fish were larger than the measurements of the female fish that exposed to 150, 250 and 350 pg L-1 ammonium hydroxide for 90 days.

Keywords: Nile tilapia, ammonium hydroxide stress, growth, hematology, immunity, reproductive hormones.

INTRODUCTION

Ammonia is one of the most common stressors to fish health and production. Fish excretion is the main source of ammonia in fish body and its

excretion is directly related to the feeding rate and the protein levels. Also, organic matter produced by algae or added to ponds as feed is a good source of ammonia. The ammonia toxicity to fish depends on many factors including water temperature, pH, fish species, fish size, the exposure period and so on. In intensive fish culture, the most common pollutant is ammonia, which is produced via decomposing of the unconsumed fish food and/or the fecal matter and constitutes a big hazard for fish culture industry as it causes severe respiratory problems as gasping, flaring opercula, asphyxia and the death of fish (Lin and Chen, 2003). In a high density of aquaculture system nitrogen compounds have been identified as major metabolic products in fish culture (Erol *et al.,* 2010). Symptoms of ammonia poisoning in fish includes; purple, red or bleeding gills (inflamed), inflamed eyes or anus. Also, fish may clamp and appeared darker in color, red stricken on fins or body, fish may gasp for at the surface of the water tank exhibiting torn and jagged fins (Fernandez and Mazon, 2003). Ammonia accumulation may reduce growth, increases oxygen consumption and ammonia-N excretion, altering concentrations of haemolymph protein and free amino acids levels and causes mortality increment in fish (Lin and Chen, 2003 and Barbieri and Bondioli, 2015). Recently, Barbieri and Bondioli (2015) found that exposure of Pacu fish to different concentrations of ammonia-N caused an elevation in total hemoglobin and blood glucose. The sub-lethal ammonia effects induced a decreased growth rate and poor food conversion. Decreases in final body weight, average daily gain and specific growth rate as compared to controls Hematological parameters revealed increases in total leukocyte counts in both males and females exposed to stressors. Cortisol level increased in both sexes of fish exposed to ammonia (Kuttchantran, 2013). Also, it negatively affected the health wellbeing. Alexander *et al.* (2017) regarding the role of cortisol as stress factor in teleost sex change and may lead to new tools to control fish sex ratios in aquaculture. Sydney and Helene, 2019 found larger eggs relative to body weight, compared to those with fewer or smaller eggs, suggesting that more mature females are more affected by fasting. Ryo *et al.,* 2018. confirmed that the variations in sex

steroid hormone levels correlated with reproductive status in mature female fish. Strongly suggest that E2 is an indicator for ovarian follicle development, and that T is a useful indicator for both the onset and end of the egg-laying period in fish. Lucas *et al.,* 2019 showed is the first investigation of concurrent changes in reproductive, thyroid and adrenal hormone concentrations in this endemic and physiologically unique South American lizard. These findings set the stage for future investigations to determine the extent to which these hormones influence activity and thermoregulation in *S. merianae.* Steroid hormones were extracted from blubber and testosterone and oestradiol are associated reproductive patterns in fish. Sydney and Helene, 2019 stated that the average body length, body weight and the reproductive hormones in the fish affected by stress factors.

Aim of this work:

Nile tilapia *(O. niloticus)* is one of the most important fish species and its culture increased progressively worldwide due to its high growth and high market value. During its intensive culture, nitrogenous compounds especially ammonia may be highly produced via excretion, decomposition of excess feeds, fish feces and so on. Therefore, the current study was conducted to investigate the toxic effect of ammonia on the growth performance, hematological, and biochemical parameters as well as innate immunity and reproductive hormones of Nile tilapia *(O. niloticus).*

Materials and methods Experimental design:

Healthy male and female Nile tilapia (O. *niloticus)* were collected from nursery ponds of Central Laboratory for Aquaculture Research (CLAR), Abbassa, Abo-Hammad, Sharqia, Egypt. Fish were acclimated for two weeks to laboratory conditions. However, fish were kept in indoor fiberglass tanks contained well-aerated tap water through air pump via air stones during which fish were fed twice a day on a commercial diet (30 % crude protein). After the acclimation period, fish (70± 4 g) were distributed into 24 aquaria 90-L at density of 6 fish per aquarium. Fish were exposed to 0.0, 150, 250, and 350 pg

L-1 ammonia as ammonium hydroxide for 90 days Alkobaby and Hassanien (2007). Each aquarium was continuously aerated with air pumps via air stone. Fish were fed on a commercial diet (30% CP) up to apparent satiation twice a day at 9:00 and 13:00 h. Settled fish wastes were siphoned every day with a three-quarter of aquarium's water, which was replaced by well-aerated de-chlorinated tap water from a storage tank. After that, ammonia concentrations were readjusted by using ammonium hydroxide. At the end of the experiment, fish of each aquarium were collected and group-weighed. Parameters of fish growth were calculated as follows:

Weight gain (g) = final weight (g) - initial weight (g).

Weight gain % = 100 [final weight (g) - initial weight (g)] / initial weight (g).

Water quality Parameters.

Water samples were collected at 20 cm depth from each aquarium. Dissolved oxygen and temperature were measured daily in site using a portable DO meter (Jenway, London, UK). The pH values were measured using a Digital Mini-pH Meter (model 55, Fisher Scientific, Denver, USA). The electric water conductivity was measured using a Portable Conductivity Meter (Jenway, London, UK). The ammonia concentration was measured using a Multiparameter Ion Analyzer (HANNA Instruments, Rhodes Island, USA). Total alkalinity and total hardness were measured by titration method according to Boyd (1984). In all treatments, water temperature ranged from 30 to 31 °C, pH ranged from 7.5 to 7.6 and conductivity ranged from 51 to 52 uS/cm. Total alkalinity and total hardness ranges were 115-116 and 240-241 mg/L as $CaCO_3$, respectively. All the previous water quality parameters are within the acceptable range for fish growth (Boyd, 1984).

Hematological and biochemical analysis.

At the end of the experiment, blood samples were collected from the fish caudal vein by a sterile syringe containing heparin as an anticoagulant. Blood samples were placed into microtubes (2.0 mL). All samples were collected in

the early morning hours and were processed for hematological analysis according to Soivio and Oikari (1976). The total cell count (erythrocytes and leukocytes) were performed by the diluent/dye direct method outlined by Natt and Herrick (1952) in a Neubauer chamber at a dilution of 1:100. Following the total cell count of nucleated cells (leukocytes) in the Neubauer chamber. Blood was used for erythrocyte (RBCs) count (Dacie and Lewis 1984), hemoglobin (Hb) content (Vankampen, 1961) and hematocrit (Hct) value (Britton, 1963) determination. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentrations (MCHC) were calculated. The remaining samples of blood were centrifugation at 4000 rpm for 15 min at room temperature to obtain the plasma for measuring different biochemical parameters. Plasma glucose concentration was measured according to Trinder (1969). Plasma protein content was determined by the Biuret method described by Wootton (1964). Total lipids were determined calorimetrically according to Knight *et al.* (1972). plasma cortisol levels were determined by radioimmunoassay in serum by kits obtained from Bayer for assay, reagents and protocols which were described by Pankhurst and Sharples (1992) was used. Plasma aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), uric acid, and creatinine were determined calorimetrically according to Reitman and Frankel (1975). Plasma lysozyme activity of fish was determined by turbidometric assays as described by Caruso *et al.* (2002). Reproductive hormones (testosterone, 17B estradiol and follicle-stimulating hormone) measured using Radioimmunoassay procedure by Foster and Dunn (1974) also used for determination of plasma thyroid hormones and immunoglobulin M (IgM) using ELISA Kit. Catalog No. CSB-E12045Fh (Cusabio Biotech Co., Ltd).

Statistical analysis:

The obtained data in the present study were statistically analyzed by SPSS for variance ANOVA, LSD (Least significant difference) according to Snedecor and Cochran (1982). Differences among treatment means were compared using Duncan's multiple range tests (Duncan, 1995). Data expressed as means ± SE, means with the same letter in the columns is not significant at $p<0.05$.

RESULTS

The obtained results in the present study showed significant changes in fish growth where final fish weight, weight gain, and weight gain % in the control fish group were significantly higher than those in fish exposed to different ammonium hydroxide levels (Table 1). Fish growth was significantly retarded by increasing ammonia levels and lowest growth parameters were observed with fish exposed to 350 pg/L ammonium hydroxide. On the other hand, the measurements in growth parameters in the male fish were larger than the measurements in the female fish that exposed to different ammonium hydroxide levels for 90 days.

Table 1. Growth parameters of Nile tilapia *(O. niloticus*) exposed to different ammonium hydroxide levels for 90 days.

Doses /parameters		Initial weight (g)	Final weight (g)	Weight gain (g)	Weight gain %
Male	Control	70 ± o.4	87.9 ± 0.8^{d}	16.9 ± 0.25^{d}	24.14 ± 0.51^{d}
	150 ng/L	70 ± 0.4	82.1 ± 0.5^{c}	12.1 ± 0.22^{b}	17.28 ± 0.66^{c}
	250 ng/L	70 ± 0.4	81.8 ± 0.5^{b}	11.8 ± 0.21^{a}	16.85 ± 0.67^{b}
	350 ng /L	70 ± 0.4	79.7 ± 0.6^{a}	9.7 ± 0.11^{a}	13.85 ± 0.99^{a}
Female	Control	70 ± 0.4	82.9 ± 0.8^{d}	12.9 ± 0.25^{d}	18.42 ± 0.51^{d}
	150 ng/L	70 ± 0.4	80.1 ± 0.5^{c}	10.1 ± 0.22^{b}	14.42 ± 0.66^{bc}
	250 ng/L	70 ± 0.4	79.8 ± 0.6^{b}	9.8 ± 0.21^{b}	14.0 ± 0.67^{bc}
	350 ng /L	70 ± 0.4	77.7 ± 0.6^{a}	7.7 ± 0.11^{a}	11.0 ± 0.99^{a}

Data expressed as means ± SE, means with the same letter in the columns is not significant at $p<0.05$.

Table 2 shows that RBCs counts, Hb content, Hct percentage and MCHC in Nile tilapia *(O. niloticus)* decreased significantly, while MCV and MCH values increased significantly as ammonia levels increased. Similarly, numbers of WBCs of fish decreased significantly as ammonia levels increased showing their lowest values at fish exposed to 350 pg/L of ammonia. The highest values of hematological parameters were obtained with fish of the control group. On the other hand, the measurements of RBCs count, Hb content, Hct percentage, and MCHC in the male fish were larger than the measurements in the female fish that exposed to 150, 250 and 350 pg L-1 ammonium hydroxide for 90 days.

Table 2. Hematological parameters of Nile tilapia (*O. niloticus*) exposed to different ammonium hydroxide levels for 90 days.

Doses / parameters		RBCs (10^6/mm^3)	WBCs (10^3/mm^3)	Hb	PCV	MCV	MCH	MCHC (%)
Male	Control	2.24±0.2d	3.7 ±0. 3^b	7.8±0.6^d	25.9±0.1	112.9±8^a	35.1±4^a	31.1±1
	150 ng/L	2.22±0.3^b	3.2 ± 0.7^{ab}	6.6±0.5^c	24.2±0.4	115±1^b	35.5±2^{ab}	31.1±6
	250 ng/L	1.74±0.1^a	3.2 ±0. 1^{ab}	5.8±0.6^b	23.9±0.1	120±6^c	36±4^{ba}	29±2^b
	350 ng /L	1.54±0.3^a	3.1 ± 0.3^a	5.1±0.3^a	21±0.4^a	132±8^d	37±2^b	28±6^a
Female	Control	2.11±0.3^c	3.5 ±0. 4^b	6.7±0.5^d	24.1±0.3	112±9^a	33±5^a	30±7^d
	150 ng/L	1.81±0.3^b	3 ± 0.4^{ab}	5.2±0.3^c	23.2±0.1	120±7^b	34±2^b	28±3^c
	250 ng/L	1.51±0.1^a	3.1 ±0.7^{ab}	4.8±0.7^b	22.2±0.3	129±09^c	35±5^c	27±7^b
	350 ng /L	1.11±0.3^a	3 ± 0.5^{ab}	4.7±0.5^a	21±0.1^a	139±9^d	37±5^d	25±4^a

Data were presented as mean ± SE and significant difference was declared at $P < 0.05$.

Significant increases of plasma glucose, cortisol and lysozyme accompanied with significant decreases of plasma total immunoglobin M, total protein and total lipids were observed in Nile tilapia with increasing ammonium hydroxide levels (Table 3). The lowest values of plasma Lysozyme, glucose and cortisol accompanied with highest values of plasma IgM, total protein and total lipids were observed in the control fish group. On the other hand, the measurements of innate immunity and lysozyme and glucose, cortisol, total protein and total lipids in the male fish were larger than the measurements in the female fish that exposed to different ammonium hydroxide levels for 90 days.

Table 3. Changes in plasma glucose, cortisol, Lysozyme, IgM, total protein, and total lipids of Nile tilapia *(O. niloticus)* exposed to different ammonium hydroxide levels for 90 days.

Doses / parameters		Glucose (mg/dl)	Cortisol (g/dl)	Lysozyme (ng /ml)	IgM value (ng /mi)	Protein	Lipids (g/dl)
Male	Control	58.1 ±1.1^a	13±0.23^a	22.5±0.5^a	3.5±0.2^d	5.5 ± 0.5^d	8.3 ± 0.1^d
	150 ng/L	69.3 ±	13.5±0.3^{bc}	22±0.5^b	3 ±0.4^c	3.3± 0.4^c	7.4 ± 0.2^c
	250 ng/L	79.5 ± 2^c	13.8±0.4^{bc}	25.1±0.3^c	2.7±0.2^b	2.5 ± 0.5^b	6.7 ± 0.3^b
	350 ng /L	82 ± 2.1^d	19.6±0.2^c	27.4±0.1^d	2.2±0.1^a	2.0 ± 0.1^a	5 ± 0.2^a
Female	Control	58.2 ±	12.2±0.3^a	22.4±0.3^a	3.1±0.1^b	5.0 ± 0.1^d	7.3 ±0.1^d
	150 ng/L	65.3 ±	16.1±0.2^b	23.5±0.6^b	3.0±0.2^b	3.9 ± 0.2^c	6.1 ±0.1^c
	250 ng/L	77.1 ± 3^c	20.3±2^c	25.7±0.8^c	2.5±0.1^{ab}	2.8 ± 0.1^b	5±0.3^b
	350 ng /L	79.8 ± 2^d	24.4±2^d	27±0.9^d	2.5±0.1^{ab}	1.7 ± 0.2^a	4.5 ± 0.1^a

Data expressed as means ± SE, means with the same letter in the columns is not significant at $p<0.05$.

Table 4 shows that AST, ALT, and ALP activities and levels of uric acid and creatinine in fish plasma increased significantly with increasing levels of ammonium hydroxide and their lowest values were obtained in the control group. On the other hand, the measurements of AST, ALT, and ALP activities and levels of uric acid and creatinine in the male fish were larger than the measurements in the female fish that exposed to different ammonium hydroxide levels for 90 days. Whereas a significant reduction was recorded in triiodothyroxine (T3) and thyroid stimulating hormone (TSH) of fish that exposed to ammonium hydroxide than that control group. But, there were significantly increased in tetriodothyroxine (T4) in plasma fish that exposed to ammonium hydroxide for 90 days than that control group.

Table 4. Changes in liver, kidney and thyroid functions of Nile tilapia *(O. niloticus)* exposed to different ammonium hydroxide levels for 90 days.

Doses / parameters		AST (u/l)	ALT (u/l)	ALP (u/l)	Uric acid (mg/dl)	Creatinine (mg/dl)	Total T3 (ng/ml)	Total T4 (nmol/L))	TSH (ng/ml)
Male	Control	31.5 ± 2.1^{a}	42± 4.4^{a}	40± 1.9^{a}	9±1.4^{a}	0.25±0.1^{a}	1.67±0.2^{c}	20.1±1.1^{a}	3.4±0.9^{c}
	150 pg/L	32.1 ± 3.2^{b}	43 ± 3.2^{b}	41 ± 1.1^{b}	10 ±1.2^{b}	0.27±0.1^{b}	1.66±0.1^{c}	22.3±1.6^{b}	3.1±0.6^{b}
	250 pg /L	33 ± 2.2^{c}	44 ± 2.4^{c}	42 ± 2.1^{c}	11 ±1.4^{c}	0.29±0.1^{c}	1.50±0.2^{b}	24.5±1.1^{c}	3±0.4^{b}
	350 pg /L	35.5±3.1^{d}	52 ± 5.2^{d}	43± 2^{d}	12 ±1.1^{d}	0.34±0.2^{d}	1.35±0.3^{a}	25.7±1.6^{d}	2.7±0.1^{a}
Female	Control	30.8 ± 4.1^{a}	41 ± 3.2^{a}	40± 4.2^{a}	9 ±1.2^{a}	0.25±0.1^{a}	1.62±0.1^{c}	22.3±2.1^{a}	3.2±0. 5^{c}
	150 pg/L	36 ± 3.2^{b}	47 ± 3.4^{b}	43± 2.1^{b}	11 ±1.1^{b}	0.31±0.2^{b}	1.51±0.3^{b}	24.1±1.5^{b}	2.5±0.4^{ab}
	250 pg /L	41 ± 2.2^{c}	49 ± 4.4^{c}	47± 3.2^{c}	15 ±2.1^{c}	0.4±0.4^{c}	1.3±0.2^{a}	24.3±2.2^{b}	2.2±0.1^{ab}
	350 pg /L	43.5 ±1.3^{d}	52 ±2.1^{d}	50± 1.9^{d}	16 ±2.4^{d}	0.42±0.2^{d}	1.3±0.1^{a}	26.2±2.4^{c}	2.3±0. 5^{a}

Data expressed as means ± SE, means with the same letter in the columns is not significant at $p<0.05$.

Plasma levels of follicular stimulating hormone (FSH), 17P-estradiol (E2) and testosterone (T) of Nile tilapia decreased significantly with the increase of ammonium hydroxide levels to be lower than these of the control fish group (Table 5). The highest values of the above mentioned hormones were obtained at the control fish group, whereas their lowest values were obtained with fish exposed to 350 pg/L of ammonium hydroxide. On the other hand, the measurements of plasma levels of testosterone (T) in the male fish were larger than the measurements in the female fish that exposed to different

ammonium hydroxide levels for 90 days. But, plasma levels of follicular stimulating hormone (FSH) and 17P-estradiol (E2) were increased in the female fish than in male one Table 5.

Table 5. plasma levels of follicular stimulating hormone (FSH), 17p-estradiol (E2) and testosterone (T) of Nile tilapia *(O. niloticus)* exposed to different ammonium hydroxide levels for 90 days.

Doses / parameters		FSH ng/ml	E2 ng/ml	T ng/ml
Male	Control	5.9±0. 2^{d}	11.5±0. 8^{c}	0.88±0.5^{d}
	150 pg /L	3.8±0. 7^{c}	11.4±0.2^{c}	0. 43±0.1^{c}
	250 pg /L	3.2±0. 1^{b}	10.5±005^{b}	0.33±0.1^{b}
	350 pg /L	2.9±0.6^{a}	8.9±0.4^{a}	0.31±0.2^{a}
Female	Control	9.7±0. 2^{d}	18.8±0. 7^{d}	0.23±0.1^{d}
	150 pg /L	7.6±0. 7^{c}	17.9±0. 4^{c}	0.20±0. 2^{c}
	250 pg /L	5.2±0. 1^{b}	15.9±0.6^{b}	0.16±0. 2^{b}
	350 pg /L	4.5±0.6^{a}	14.6±0.4^{a}	0.14±0. 1^{a}

Data expressed as means ± SE, means with the same letter in the columns is not significant at $p<0.05$.

DISCUSSION

Ammonia is a metabolite of aquatic organisms which might reach deleterious levels in intensive fish farms. In the present study, the fish exposed to different ammonium hydroxide levels showed a retarded growth and lowest final weight, weight gain, and weight gain % were observed with fish exposed to 350 pg/L of ammonium hydroxide. Meanwhile, the control fish grew faster showing highest growth performance. Evidently, several studies reported similar results. Frances *et al.* (2000) reported a decrease in body weight of silver perch as a consequence to elevated ammonia in water. Also, Foss *et al.* (2003) obtained similar trend in juvenile spotted wolfish. Moreover, Dosdat *et al.* (2003) and Lemarie *et al.* (2004) reported such phenomena in European sea bass. Alkobaby and Hassanien (2007) exposed Nile tilapia to control, 0.098, 0.15, and 0.24 mg/L of unionized ammonia for 60 days. They found a retarded growth as ammonia level increased. Yang *et al.* (2011) investigated the effect of long-term ammonia exposure on feeding, growth, and some blood parameters of juvenile crucian carp *(Carassius auratus),* which was exposed to different concentrations of NH_3-N (0.107, 0.214, 0.321 and 0.428 mg/L) for 45

days. They found that ammonia significantly impaired the feeding and growth of fish. Recently, Sakala and Musuka (2014) reported a toxic effect of increased ammonia on the growth rate of *Tilapia rendalli.* The loss of weight in ammonia-treated fish is more likely due to the inhibition of the fish appetite leading to significant reduction of feed intake and disturbance in fish metabolism. Shin *et al.* (2016) exposed rockfish, *Sebastes schlegelii* to different ammonia levels of 0, 0.1, 0.5, and 1.0 mg/L for 4 weeks. They found that growth performance was significantly reduced by the ammonia exposure. Similar result discussed by Alkobaby and Hassanien (2007) who found decreases in final body weight, average daily gain and specific growth rate as compared to controls. Hematological parameters revealed increases in total leukocyte counts in both males and females exposed to stressors. Cortisol level increased sexes of fish exposed to ammonia. Also, it negatively affected the health wellbeing. The present study revealed that there were significant decreases ($P < 0.05$) of RBCs count, Hb, and Hct values of Nile tilapia exposed to different ammonium hydroxide levels as compared with the control fish group. The decreases in RBCs, Hct and Hb concentration obtained in the present study might be attributed to the ammonia toxicity in the media which might have caused damages to the vital organs (i.e. gills, liver, spleen, kidneys). Since, RBCs count of teleost produced from hematopoietic tissue of the kidney, spleen and liver and the stress impact of ammonia might damage such organs to the degree that they caused reduction of erythrocytes (Das *et al.,* 2004). Additionally, the increase of ammonia in the blood circulation might ruptured high percent of RBCs and/or caused hemodilution resulting in a disturbance of osmoregulation across gill epithelium (Vosyliene *et al.,* 2003). Thangam *et al.* (2014) reported a notable reduction in RBC count of common carp, *Cyprinus carpio,* exposed to ammonia. Similar results were recorded in red tilapia (Bonnie and Liu, 2004) and in mrigal fish (Das *et al.,* 2004). Surprisingly, there found a species differences on the tolerance of different fish to ammonia toxicity. This has been shown by Dosdat *et al.* (2003) who reported no changes in Hct value in European sea bass exposed to 0.014 -

0.493 mg NH_3-N/L for 60 days. Greater reduction (~ 45%) in Hb was recorded in tilapia fish exposed to 0.15 mg NH_3/L for 60 days (El-Sherif and El-Feky 2008). Tilak *et al.* (2007) reported a substantial decrease in Hb of common carp, *C. carpio*, exposed to ammonia, which is caused by the increase in the oxygen intake and elevation in methemoglobin by gill damage. There were no changes ($P>0.05$) in MCH values among different treatments. Contrariwise, percentage of MCHC exhibited significant decreases ($P < 0.05$) in ammonia-exposed fish compared with the control fish. The increase in MCV as a consequence to ammonia exposure might be ascribed to the increased water content in RBCs resulting of chloride shift and the decreased of plasma chloride at the same state of high ammonia in water. Moreover, the decrease of MCHC value might be attributed to the hemodilution and/or the lack of production of Hb in circulation. Toxicity of ammonia was found to impair the oxygen uptake by carp fish resulting in hazardous consequences on red blood cells production and their components (Tilak *et al.*, 2007). Shokr (2015) obtained similar results when he exposed Nile tilapia to ascending levels of ammonium nitrates. Shin *et al.* (2016) found that the ammonia exposure caused a notable reduction in MCV, MCH, and MCHC of rockfish, *S. schlegelii.* Data of the present study revealed that there were significant decreases ($P < 0.05$) of WBC count and its constituents of Nile tilapia exposed to different ammonia levels. These findings agree with other studies, which recorded an increase of WBCs in red tilapia after exposure to 3 mg NH_4Cl/L for 3 days (Bonnie and Liu, 2004). Moreover, Das *et al.* (2004) obtained similar trend concluding that the increase in WBCs as a result of exposure to stresses is involved in the regulation of immunological function of fish. Such an increase in total leukocyte occurs by the increase in lymphopoies is and/or enhanced release of lymphocytes from lymphoid tissues. Thangam *et al.* (2014) reported a notable reduction in RBC and WBC count of common carp, *C. carpio*, exposed to ammonia. The biochemical and physiological alterations in fish blood can be occurred by the toxicants in an aquatic environment, and the blood parameters can be a sensitive and reliable indicator to evaluate the physiological status of fish (Barton, 2002 and Mazon *et al*., 2002). High levels of ammonia cause

stress and produce harmful physiological response such as osmoregulatory disturbances, kidneys and branchial epithelium damages and retarded growth (Meade, 1989 and Soderberg, 1994), inefficient immune response and increase in plasma glucose results from catecholamine mobilizing energy resources supporting responses. (Cheng *et al.*, 2004 and Pinto *et al.*, 2007) and reduced survival (Jobling, 1994). Vosyliene and Kazlauskiene (2004) reported a negative change in blood chemistry of rainbow trout, *Oncorhynchus mykiss,* exposed to ammonia. The present study found a massive increase in serum glucose, cortisol, lysozyme activities and decreased in immunoglobine M in ammonia-exposed fish over than the control fish. In similar studies, Shokr (2015) reported vast increases of glucose and cortisol in fish exposed to ammonia. The increase in glucose may be a consequence of glycogenolytic activity of catecholamines and gluconeogenetic effect of glucocorticoids by the stress response under toxic substance exposure (Dobsikova *et al.*, 2011). Evidently, hypothalamo-pituitary interrenal axis, stimulated by ammonia as a stressor elevated blood levels of cortisol, which in turn leads to lipolysis, glycogenolysis, and gluconeogenesis to provide energy under stress conditions. The hyperglycemic condition observed in many teleosts release condition is mainly mediated by effect of catecholamine on glucose release from the liver which is considered the main carbohydrate store in fish. It is noticed in the present study that serum total protein and total lipids decreased significantly in fish as ammonia level increased. These reductions may indicate to their use as energy source to cope with the ammonia stress. On the other hand, AST, ALT, ALP, uric acid, and creainine values increased significantly in fish as ammonia level increased. These results indicate to the presence of some kind damage of liver and kidney tissues due to ammonia exposure. In similar study, Shin *et al.* (2016) found that the total protein of rockfish, *S. schlegelii* was significantly decreased due to ammonia exposure. The AST and ALT in serum components can be generally used to assess the tissue damage of the liver and kidney (Agrahari *et al.*, 2007). Vedel *et al.* (1998) and Shin *et al.* (2016) also reported a considerable increase in AST and ALT activities of rainbow trout, *O. mykiss,*

and rockfish, *S. schlegelii*, respectively, exposed to ammonia indicating to some degree of tissue necrosis. The immune responses can be affected by the stress in aquatic animals where the innate immune system in fish is regarded as the first line of defense against toxicants (Saurabh and Sahoo, 2008). The present study declared that there was a decrease in the innate immunity of fish exposed to high levels of ammonia. Yue *et al.* (2010) reported the ammonia exposure induced the reduced lysozyme activity of swimming crab (*Portunus trituberculatus)* by ammonia stress. Kim *et al.* (2015) reported that the lysozyme and phagocytosis activity of Rockfish, *S. schlegelii* were considerably decreased due to the ammonia exposure. Cheng *et al.* (2003) reported the inhibited phagocytosis of giant freshwater prawn *(Macrobrachium rosenbergii)* by the ammonia exposure. Therefore, the ammonia exposure inhibited immune responses such as lysozyme activity and IgM of Nile tilapia which may reflect the immunosuppression of ammonia toxicity. In the present study, levels of reproductive hormones and thyroid hormones decreased significantly as ammonia level increased. Gonadal development and fecundity of fish are affected by certain endocrine disrupting chemicals. According to Casanova *et al.* (2011), endocrine disrupting compounds exert their biological activity either by interacting with endogenous hormone receptors or by disturbing endogenous hormone metabolism. Accordingly, ammonia stress may cause fish sterility reducing their capability to reproduce. similar results described by Alexander *et al.* (2017) regarding the role of cortisol as stress factor in teleost sex change and may lead to new tools to control fish sex ratios in aquaculture. Sydney and Helene, 2019 found larger eggs relative to body weight, compared to those with fewer or smaller eggs, suggesting that more mature females are more affected by fasting. RyoNozua *et al.*, 2018. confirmed that the variations in sex steroid hormone levels correlated with reproductive status in mature female fish. Strongly suggest that E2 is an indicator for ovarian follicle development, and that T is a useful indicator for both the onset and end of the egg-laying period in fish. Also, Lucas *et al.*, 2019 showed is the first investigation of concurrent changes in reproductive, thyroid and adrenal hormone concentrations in this endemic and physiologically unique South

American lizard. Findings set the stage for future investigations to determine the extent to which these hormones influence activity and thermoregulation in *S. merianae.* Steroid hormones were extracted from blubber and testosterone and oestradiol are associated reproductive patterns in fish. The present result showed decreased in FSH, estradiol and testosterone and thyroid hormones agreement with Sydney and Helene, 2019 who showed that the average body length, body weight and the reproductive hormones in the fish had the trend of annual variation. the reproductive hormone levels and the migratory reproductive activities are synchronized. This due to metabolic effect of thyroid hormones.

CONCLUSIONS

Ammonia is one of the most risky stressors to freshwater fish, which may be elevated in the aquaculture environment. The main negative consequences of the elevated ammonia in tilapia culture are the sharp decrease in body growth rate, change in hematological traits, increased cortisol and glucose, decreased the reproductive hormones in the blood to cope with ammonia toxic effects.

REFERENCES

Agrahari, S.; K.C. Pandey and K. Gopal, 2007. Biochemical alteration induced by monocrotophos in the blood plasma of fish, *Channapunctatus* (Bloch). Pestic Biochem Physiol., 88: 268-72.

Alexander, G.; E.V. Todd and N.J. Gemmell, 2017. Stress and sex: does cortisol mediate sex change in fish. Reproduction., 154: R149-R160.

Alkobaby, A.I. and H.A. Hassanien, 2007. Acute and chronic ammonia toxicity to Nile tilapia *(Oreochromis niloticus)* fingerlings. Egyptian Journal of Aquatic Research, 33(1): 455- 465.

Barbieri, E. and A.C.V. Bondioli, 2015. Acute toxicity of ammonia in Pacu fish *(Piaractus mesopotamicus* Holmberg, 1887) at different temperatures levels. Aquaculture Research, 46: 565-571.

Barton, A.B., 2002. Stress in fish: a diversity of response with particular reference to changes in circulating corticosteroids. Integrated and Comparative Biology, 42: 517-525.

Bonnie, S.P. and D.I. Liu, 2004. Effect of ammonium on blood characteristics and lipoxygenase activities in cultured tilapia. The hotel tropical conference center. Manaus, Amazonas, Brazil, 149-161.

Boyd, B.S., 1984. Ethanol-Induced Inhibition of Chick Brain Growth Gerhard W. Kalmus PhD Sam N. Pennington PhD.

Britton C.J., 1963. Disorders of The Blood. 9.ed. London: J&A Churchill. 332p.

Caruso D.; C. Schlumberger and J-P D. Proteau, 2002. Plasma lysozyme levels in sheatfish Silurus glanis (L.) subjected to stress and experimental infection with Edwarsiella tarda. Aquacul. Res., 33:999-1008.

Casanova-Nakayama, A.; M. Wenger; R. Burki; E. Eppler; A. Krasnov and H. Segner, 2011. Endocrine disrupting compounds: Can they target the immune system of fish? Marine Pollution Bulletin., 63(5-12): 412-416.

Cheng, W.; S.M. Chen; F.I. Wang; P.I. Hsu; C.H. Liu; J.C. Chen, 2003. Effects of temperature, pH, salinity and ammonia on the phagocytic activity and clearance efficiency of giant freshwater prawn *Macrobrachium rosenbergii* to *Lactococcus garvieae.* Aquaculture, 219: 111-121.

Cheng, W.; I.S. Hsiao and J.C. Chen. 2004. Effect of ammonia on the immune response of Taiwan abalone Haliotis diverscolor supertexta and its susceptibility to vibrio parahaemolyticus. Fish Shellfish Immunology, 17: 193-202.

Dacie, J.V. and S.M. Lewis, 1984. Practical Hematology. 6.Ed. New York: Churchill. 22p.

Das, P.C.; S. Ayyappan; J.K. Jena and B.K. Das, 2004. Acute toxicity of ammonia and its sublethal effects on selected haematological and enzymatic parameters of mrigal, *Cirrhinus mrigala* (Hamilton). Aquaculture Research, 35: 134-143.

Dobsikova, R.B.; J. Modra; H. Skoric and M.Z. Svobodova, 2011. The effect of

acute exposure to herbicide Gardoprim Plus Gold 500 SC on haematological and biochemical indicators and histopathological changes in common carp *(Cyprinus carpio* L.). Journal of the University of Veterinary and Pharmaceutical Sciences in Brno., 80: 359-63.

Dosdat, A.; J.P. Ruyet; D. Coves; G. Dutto; E. Gasset; A. Le Roux and L. Gilles, 2003. Effect of chronic exposure to ammonia on growth, food utilization and metabolism of the European sea bass (Dicentrarchus labrax). Aquatic Living Resources, 16: 509-520.

Duncan, D.B., 1995. Multiple range and multiple F-tests. Biometrics, 11: 1-42.

El-Sherif, M.S. and Amal M. El-Feky, 2008. Effect of ammonia on the Nile Tilapia (O. niloticus) performance and some hematological and histological measures. Eighth International Symposium on Tilapia in Aquaculture, 513-530.

Erol, C.I.; K. Sevki; B. Halis and A. Ilhan, 2010. Acute toxicity of some agriculture fertilizers to rainbow trout. Turkish Journal of Fisheries and Aquatic Sciences, 10: 19-25.

Fernandes, M.N. and A.F. Mazon, 2003. Environmental pollution and gill morphology. In: Val, A.L., B.G. Kapoor (Eds.). Fish adaptations. Enfield, Science publishers, 203-231.

Foss, A.; T.H. Evensen; T. Vollen and Q. Victor, 2003. Effect of chronic ammonia exposure on growth and food conservation efficiency in juvenile spotted wolfish. Aquaculture, 238: 215224.

Foster, L.B. and R.T. Dunn, 1974. Single-antibody technique for radioimmunoassay of cortisol in un-extracted serum or plasma. Chemistry, 20: 365-368.

Frances, J.; B.F. Nowak and L.A. Geoff, 2000. Effects of ammonia on juvenile silver perch (Bidyanus bidyanus). Aquaculture, 183: 95-103.

Jobling, M., 1994. Fish bioenergetics. Chapman and Hall, London.

Kim, S.H.; J.H. Kim; M.A. Park; S.D. Hwang and J.C. Kang, 2015. The toxic

effects of ammonia exposure on antioxidant and immune responses in Rockfish, *Sebastes schlegelii* during thermal stress. Environmental Toxicology and Pharmacology, 40: 954-959.

Knight, J.A.; S. Anderson and J.M. Rawle, 1972. Chemical Basis Of The Sulfo-Phospho-Vanillin Reaction For Estimating Total Serum Lipids. Clin. Chem., 18: 199-202.

Kuttchantran, M., 2013. Managing ammonia in fish ponds. Aquatic animal health unit, Universiti Putra Malaysia, Malaysia.

Lemarie, G.; A. Dostat; D. Coves; G. Dutto; E. Gasset and P.R. Jeanine, 2004. Effect of chronic ammonia exposure on growth of European sea bass (*Dicentrarchus labrax*) juveniles. Aquaculture, 229: 479-491.

Lin, C.Y. and J.C. Chen, 2003. Acute toxicity of nitrite on Litopenaeus vannamei (Boone) juveniles at different salinity levels. Aquaculture, 224: 193-201.

Lucas, A.Z.; D. Kathleen; E. HuntdCarlos; A. Navasa and C. LorenBuckd, 2019. Seasonal changes in plasma concentrations of the thyroid, glucocorticoid and reproductive hormones in the tegu lizard Salvator merianae. General and Comparative Endocrinology, 273 (1): 134-143.

Meade, J.W., 1989. Allowable ammonia in fish culture. Progress Fish Culture, 47: 135-145.

Natt, M.P. and CA. Herrick, 1952. A new blood diluent for counting the red and white blood cells of chickens. Poultry Science, 31: 335.

Pankhurst, N.W. and D.F. Sharples, 1992. Effects of the capture and confinement onplasma cortisol concentrations in the snapper(Pagrusauratus), Australian Journalof Marine and Freshwater Research, 43: 345-356.

Pinto, C.; C. Aragao; F. Soares; M.T. Dinis and L.E.C. Concei, 2007. Growth, stress response and free amino acid levels in Senegalese sole (Solea senegalensis Kaup 1858) chronically exposed to exogenous ammonia. Aquaculture Research, 38: 1198-1204.

Reitman, S. and S. Frankel, 1975. Colorimetric Determination of Glutamic Oxaloacetic and Glutamic Pyruvic Transaminase. J. Clin. Pathol., 28: 56-63.

Ryo, N.A.; N. Yanob; R. Furuyamab; R. Matsumotoab and Y. KeiichiSatoab, 2018. General and Comparative Endocrinology, 265 (1): 174-179.

Sakala, M.E. and C.G. Musuka, 2014. The effect of ammonia on growth and survival rate of Tilapia rendalli in quail manured tanks. International Journal of Aquaculture, 4 (22): 1-6.

Saurabh, S. and P.K. Sahoo, 2008. Lysozyme: an important defence molecule of fish innate immune system. Aquacult. Res., 39: 223-239.

Shin, K.W., S.-H. Kim, J.-H. Kim, S.D. Hwang and J.C. Kang, 2016. Toxic effects of ammonia exposure on growth performance, hematological parameters, and plasma components in rockfish, *Sebastes schlegelii*, during thermal stress. Fisheries and Aquatic Sciences, 19: 44-46.

Shokr, E.A.M., 2015. Effects of ammonium on blood contents of *Oreochromus niloticus.* Journal of Chemical and Pharmaceutical Research, 7(3): 1963-1968.

Snedecor, G.W. and W.G. Cochran, 1982. Statistical methods. 8th Ed., Ames. Lowa state university.

Soderberg, R.W., 1994. Flowing water fish culture. CRC Press, Boca Raton, 147.

Soivio, A. and A. Oikari, 1976. Haematological Effects Of Stress On A Teleost, Esox Lucis L. J. Fish Biol., 8: 397- 411.

Sydney, L. and H. Volkoff, 2019. Effects of fasting on the central expression of appetite-regulating and reproductive hormones in wild-type and Casper zebrafish (*Danio rerio*). General and Comparative Endocrinology, 282: 13-20.

Thangam, Y.; M. Perumayee; S. Jayaprakash; S. Umavathi; S.K. Basheer, 2014. Studies of ammonia toxicity on haematological parameters to

freshwater fish *Cyprinus carpio* (common carp). International Journal of Current Microbiology and Applied Sciences, 3 (12): 535-42.

Tilak, K.S.; K. Veeraiah and J.M.P. Raju, 2007. Effects of ammonia, nitrite and nitrate o Hemoglobin content and oxygen consumption of freshwater fish, Cyprinus carpio (Linnaeus). Journal of Environmental Biology, 28 (1): 45-47.

Trinder, P., 1969. Determination of Glucose in Blood Using Glucose Oxidase With An Alternative Oxygen Acceptor. Ann. Clin. Biochem., 6: 24-7.

Vankampen, E.J., 1961. Determination of Haemoglobin. Clin. Chem. Acta., 6: 538- 44.

Vedel, NE.; B. Korsgaard and F.B. Jensen, 1998. Isolated and combined exposure to ammonia and nitrite in rainbow trout *(Oncorhynchus mykiss*): effects on electrolyte status, blood respiratory properties and brain glutamine:glutamate concentrations. Aquat Toxicol., 41: 32- 42.

Vosyliene, M.Z. and N. Kazlauskiene, 2004. Comparative studies of sublethal effects of ammonia on rainbow trout *(Oncorhynchus mykiss)* at different stages of its development. Acta Zoologica Lituanica, 14 (1): 13-8.

Vosyliene, M.Z.; G. Svecevicius and N. Kazlauskiene, 2003. Toxic effects of ammonia on rainbow trout (*Oncorhynchus mykiss*) in all stages of development. Proceedings of the Seventh International Symposium, Tallinn, Estonia May 12-15.Washington Square, Philadelphia, Usa.

Wootton, L.I., 1964. Micro-Analysis. In: Medical Biochemistry In Micrometer 4.Ed. London: J&A Churchill, 264p.

Yang, W.; H. Sun; F. Xiang; Z. Yang and Y. Chen, 2011. Response of juvenile crucian carp *(CarassiusUauratus)* to long-term ammonia exposure: feeding, growth, and antioxidant defenses. Journal of Freshwater Ecology, 26 (4): 563-570.

Yue, F.; L. Pan; P. Xie; D. Zheng; J. Li, 2010. Immune responses and expression of immune- related genes in swimming crab Portunus trituberculatus exposed to elevated ambient ammonia-N stress. Comp. Biochem. Physiol. A, 157: 246- 251.

تاثير الامونيا على امراض الدم والتغيرات البيوكيميائية وهرمونات التكاثر للبلطى النيلى

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الملخــص العربــى

في هذه الدراسة تم استخدام هيدروكسيد الأمونيوم كمصدر للامونيا لتقييم أثرها السام على صحة أسماك البلطي النيلي ونموها وبعض التغيرات الفسيولوجية والبيوكيمياية وهرمونات التكاثر حيث تعرضت الأسماك (٧٠ ± ٤ جم) لمستويات مختلفة من الامونيا وهي صفر (كنترول)،١٥٠، ٢٥٠، ٣٥٠ ميكروجرام لكل لتر لمدة ٩٠ يومًا وبعدها تم اخذ العينات لدراسة مكونات الدم والمناعة وهرمونات التكاثر ونمو البلطي النيلي. تم تغذية أسماك البلطي النيلي خلال فترة التجربة على عليقة تجارية ٣٠ % بروتين حتى حد الشبع مرتين يوميا. أظهرت النتائج أن نمو الأسماك قد انخفض مع زيادة تركيز الامونيوم وكان اقل نمو فى الاسماك التى تعرضت الي ٣٥٠ ميكروغرام لكل لتر من الامونيا و كان أقل بكثير من نمو اسماك مجموعة الكنترول. وقد أظهرت النتائج وجود زيادة كبيرة في مستويات الجلوكوز والكورتيزول في أسماك البلطي النيلي بينما انخفضت مستويات البروتين الكلي والدهون الكلية بشكل كبير عند تعرضها لتركيزات مختلفة من الامونيا. وقد لوحظ ايضا زيادة أنشطة انزيمات أمينو ترانسفيز، ألانين ترانسفيز، فوسفاتاز قلوي (AST ، ALT ، ALP) وتركيزات حمض اليوريك و الكرياتنين في أسماك البلطي النيلي التي تعرضت لتركيزات مختلفة من الامونيا زيادة كبيرة مقارنةً بمجموعة الكنترول. وتدل هذه النتائج على وجود ضرر بالغ فى انسجة الكبد والكلى نتيجة سمية الامونيا. كذلك أظهرت النتائج ارتفاع معنوى فى نشاط الليزوزيم وانخفاض الغلوبولين المناعي (IgM) فى الاسماك التى تعرضت لتركيزات مختلفة من الامونيا مقارنة بأسماك مجموعة الكنترول. ايضا انخفاض معنوى فى هرمونات الثيروكسين T3 and TSH في المصل و ارتفاع هرموني T4 بشكل ملحوظ في أسماك البلطي النيلي التي تعرضت لتركيزات مختلفة من الامونيا مقارنة بأسماك مجموعه الكونترول و لوحظ ايضا انخفاض معنوى فى هرموني ١٧ استراد يول E2 وتستوستيرون T بشكل ملحوظ في أسماك البلطي النيلي التي تعرضت لتركيزات مختلفة من الامونيا مقارنة بأسماك مجموعه الكونترول. ومن هذه الدراسة نوصي بالاحتراس من زيادة الامونيا في مياه المزارع السمكية حتي لا تتسبب في تدهور صحة ومناعة اسماك البلطى النيلى مما يؤدى الى نفوقها.