J. Animal and Poultry Prod., Mansoura Univ., Vol.9 (12): 515 – 524, 2018

Impact of Total Ammonia on Growth, Physiological Status and Histological Examination of Red Tilapia (*Oreochromis sp.*)

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ABSTRACT

The aim of this study was to evaluate the effect of total ammonia (TAN) on growth, survivability, some blood biochemistry and histological evaluation of the kidney, liver, skin and muscles of red tilapia fingerlings for 56 days. A total of 180 fish were held at a rate of 10 fish fiberglass^{-1} tank with capacity of 100 liters welled water (salinity 27.3 ppt). Treatments of 0.1, 0.9, 1.8, 2.9 and 3.5 mgL^{-1} of TAN were carried out in this study. Fish fed pellets containing 34.7% crude protein. The results showed that fish exposed to 3.5 mg TAN L^{-1} recorded the lowest final body weight and survival rate, these results inversely related with increasing TAN levels. Fish subjected to treatment of 3.5 mg TAN L^{-1} achieved the highest values of serum urea, uric acid and creatinine. The histological investigation of kidney, liver and skin of fish exposed to 3.5 mg TAN L^{-1} showed severe damages in renal tubules, large amount of hemorrhage between renal tubules, vacuolation in hepatic cytoplasm, hepatic congestion, hepatic necrosis, laceration of skin, inflammation in muscle fibers and splitting of large muscle fibers. Based on the results of the present study, it is recommended that increased levels of ammonia should be avoided and eliminating the causes in aquaculture ponds in order to obtain the highest production performance and increase survival rates and maintain fish health and vitality.

Keywords: Ammonia, Red tilapia, Kidney, Liver, Muscles, Physiological changes.

INTRODUCTION

The total global aquaculture production of tilapia is 5,377,000 tons, of which 4,200,000 tons are Nile tilapia and the rest are other tilapia sp. Tilapia is the third most important aquaculture fish (FAO, 2018) because of its rapid growth, good taste and its ability to reproduce under captivity and its resistance to environmental fluctuations. Tilapia has become one of the ecologically attractive fish as a source of animal protein because of its ability to produce high quality protein from lower sources of food. Red tilapia was first produced by hybridization of Mozambique tilapia with Nile tilapia. The expansion of red tilapia popularity among producers in some countries was due to its attractive color, increased marketing and high salinity tolerance (Alceste, 2000). Egypt has a vast coastal area and inland resources; therefore, red tilapia can be a good candidate for aquaculture because it can tolerate high salinity and can also be cultured in sea water (Hassanein *et al*., 2014).

Several problems face fish production in Egypt, most tropical fish species mortality occurs due to, low water quality and pollution including ammonia especially; in intensive fish farm, where fish are held at high densities, gradual accumulation of ammonia in water may occur when water exchange is restricted (Harris *et al.,* 1998 and Le Ruyet *et al*., 2003). Toxicity of ammonia has been intensively investigated in several fish species (Meade, 1985; Stickney, 1994). Most of nitrogenous wastes are excreted by fish gills (Goldstein and Forster, 1961), however, creatine, creatinine and uric acid are being excreted by the kidneys (De Croux *et al*., 2004). Ammonia is permeable to most biological membranes, it can cause physiological stress in fish (Hargreaves and Kucuk, 2001). Ammonia levels in fish plasma and tissues increase as it increases in water. Fish exposed to non-ionizing ammonia accelerates gill ventilation, loses balance, suffers from nervous system disorders and convulsions, leading to high mortality (Foss *et al*., 2003). The higher the ammonia production from amino acids and the adenylate deamination surpasses the excretion rate, the higher the levels of ammonia in the blood. Ammonia levels in fish blood are affected by several exogenous and endogenous factors, especially water temperature and pH level. Elevated water temperature accelerates the deamination rate raising plasma ammonia concentrations. Similarly, increases in exercise lead to excessive production of ammonia over excretion leading to elevated levels of ammonia in the blood (Wood, 1993).

Ammonia is the main excretory product of bony fish (Tomasso, 1994; Cheng *et al*., 2004), comprises the most nitrogen wastes in intensive aquaculture. It is also the final nitrogen product of amino acid and protein oxidation (Smith and Rumsey, 1976). Ammonia is mostly produced in the hepatic cells by transamination of amino acids, but can also be produced in the kidneys, gills and skeletal muscles of fish (Randall and Wright, 1987). Thus, this experiment was aimed to evaluate the effect of different doses of total ammonia nitrogen (TAN mgL^{-1}) on growth, survivability, some blood biochemistry and histological deformation of the kidney, liver, skin and muscles of red tilapia (*Oreochromis sp.*) fingerlings.

MATERIALS AND METHODS

Experimental design

The experiment was carried out at Mariculture Research Center (MRC), Faculty of Environmental Agricultural Sciences, Arish University, North Sinai, Egypt, for a duration of 56 days. Eighteen 100 liters circular conical fiberglass tanks supplied with artesian well water, for red tilapia (*Oreochromis sp.*) fingerlings rearing. Red tilapia was obtained from the kilo 21 hatchery, Alexandria governorate, Egypt. Prior to the experiment, the tanks were treated with 2 mg methylene blue L-1 for one week to avoid disease infection according to the method of Sheriff (1984). A total of 180 fish were stocked at a density of 10 fish/tank with an average initial weight 15.28±0.26 g, and length of 10.53±0.21 cm. Five experimental treatments, (T1, T2, T3, T4 and T5) were designed as 0.1, 0.9, 1.8, 2.9 and 3.5 mg TAN L-1, respectively, besides the control group (without adding of ammonia) and three replicates for each treatment. The levels of TAN were prepared by dissolving the ammonium chloride at the start of the experiment. Fish were observed twice a day to count and remove the dead fish. Each tank was supplied

with air blower, sediments and wastes were removed by siphon and water was partially changed every day and ammonia concentrations were re-adjusted. Fish fed pellets containing 34.7% crude protein at a ratio of 3% of its body weight (Table 1). The ration was introduced twice a day. Every week the ratio of provided feed was adjusted according to fish mortality and fish weight.

Table 1. Chemical composition of ingredients and fish diet (% on dry matter basis)

Ingredients composition	(%)	Chemical analysis	(%)
Fish meal (70% CP) *	21	Crude protein (CP)	34.73
Soybean meal (44% CP)	34	Ether extract (EE)	13.22
Wheat bran	9	Ash	6.35
Rice bran	25	Crude fiber (CF)	5.98
Linseed oil	2	Nitrogen free extract (NFE)	39.72
Fish oil	2	Gross Energy (GE) Kcal/100 g DM (3)	484.23
Sun flower oil	2		
Vitamins premix (1)	2		
Minerals premix (2)	2		
Binders	1	Carboxy Methyl Cellulose (CMC)	

***: Herring fish meal, Revision, Co. Denmark.**

(1): Each kilogram vitamin premix contained 10.000.000 / IU, 2.000.000 / IU, 10.000 mg, 1000 mg, 1000 mg, 5000 mg, 2000 mg, 10 mg, 50 mg, 10.000 mg, 30.000 mg, 1000 mg, 500 mg, 20.000 mg, 10.000 mg, 1000 mg, 1000 mg and 50 mg of Vitamin A, D3, E, K3, B1, B2, B6, B12, Biotin, C, Nicotinic acid, Pantothenic acid, Folic acid, D. L. methionine, L. lysine, L. threonine, L. tryptophan and propylene glycol.

(2): Each kilogram minerals premix contained 3000 mg, 100 mg, 5000 mg, 4000 mg, 100 mg, 1000 mg, 1000 mg, 76500 mg and 36000 mg of minerals Fe, Co, Mg, Zn, Se, Cu, I, P and Ca.

(3): Gross energy (GE) Kcal/100 g DM= CP×5.64+EE×9.44+ NFE×4.11 according to McDonald *et al.* (1973).

Physical and chemical analysis of water

Water temperature, salinity, pH, dissolved oxygen (DO) and TAN were measured twice daily. Water salinity and temperature were recorded using conductivity-temperature meter (SET model 315i, Weilheim, WTW GmbH, Germany). DO was measured by oximeter (SET model 315i, Weilheim, WTW GmbH, Germany). The pH was measured using a pH-meter (SET model 315i, Weilheim, WTW GmbH, Germany). TAN was measured by using ammonia nesslerization method (Eaton *et al.*, 1992). The wasted TAN produced from fish diet was 0.011 mgL^{-1}, which was calculated according to Soderberg (1995).

Growth performance measurements

The growth performance parameters were calculated using the following formulas:

Total weight gain (TWG, g $fish^{-1}$) = W_f - W_i

Where, W_f = the final weight (g), W_i= the initial weight (g).

Average daily gain in weight (ADGW, g $fish^{-1}$ day^{-1}) = (W_f - W_i) / Days of feeding in the experimental period.

Relative gain in weight (RGW, %) = WG by fish (g) / W_i (g)×100

Specific growth rate (SGR, % day^{-1}) = 100 (ln W_f - ln W_i / period (days).

Condition factor (K, %) = (W_f / L^3) ×100.

Where, W_f = the final weight and L is the final length.

Biochemical parameters

At the end of the trial, five fish from each tank were anesthetized by dipping in 40L tank containing 0.1% Quinaldine (2-4 methyl chinolin) for morphology and anatomy measurements. The blood samples were collected from the caudal vessels and taken in dry clean centrifuge tubes to obtain the serum. Serum samples were separated at 3000 rpm for 15 minutes using the centrifuge and stored in plastic vials well stoppered at – 20 °C until the biochemical analyses were done. Serum urea enzymatic was measured according to Patton and Crouch (1977), uric acid was determined according to Young (1995), creatinine was estimated according to the method described by Henry (1974), total protein was determined according to Doumas (1975), and glucose was analyzed by the enzymatic colorimetric method (Tietz, 1986).

Histological examination

Fish liver, kidneys, skin and muscles specimens were dissected and placed in isotonic saline solution, then they were fixed in Bouin's solution for about 24 h. The specimens were preserved in 70% ethyl alcohol, dehydrated in ascending series of ethanol, cleared in xylene and embedded in paraffin wax as the ordinary histological procedures. Sections of 4-6 µ thickness were cut and mounted on chemically cleaned glass slides. The sections were prepared and then stained with Harri's Hematoxylin and Eosin (H&E) according to Pearse (1972).

Statistical analysis

All numerical data were analyzed by analysis of variance (ANOVA) using SAS program (SAS, 1988). Duncan's multiple range tests was used according to Zar (1996) to verify significance of mean differences among treatments at $P \leq 0.05$.

RESULTS

Water quality parameters

Water temperature (°C), salinity (gL-1), DO (mgL-1) and pH of red tilapia (*Oreochromis sp.*) fingerlings rearing in tanks and TAN (mgL-1) concentrations are expressed in Table 2. Results indicated that no significantly ($P \geq 0.05$) differences were observed between treatments, except for TAN which significantly ($P \leq 0.05$) increased by increasing the tested ammonia levels.

Table 2. Physical and chemical characteristics of the fish rearing water

Items	Control	T1	T2	T3	T4	T5
Temperature (°C)	30.55±0.92	30.51±1.03	30.78±0.98	30.80±1.02	30.58±0.96	30.45±0.97
Salinity (gL^{-1})	27.46±0.83	27.33±0.72	27.31±0.71	27.30±0.78	27.39±0.73	27.47±0.86
pH	7.91±0.13	7.84±0.21	7.90±0.19	7.93±0.21	7.91±0.15	7.94±0.10
DO (mgL^{-1})	5.35±1.75	4.96±1.70	5.03±1.45	5.49±1.06	5.22±1.45	5.99±1.63
Total ammonia nitrogen (mgL^{-1})	0.003^{t}±0.004	0.12^{e}±0.02	0.92^{d}±0.02	1.83^{c}±0.02	2.79^{b}±0.52	3.52^{a}±0.05

Values in each row with different superscripts are significantly different ($P \leq 0.05$).

Survival rate

Figure 1 shows the survival rate of red tilapia fingerlings during the experimental periods. By increasing ammonia concentrations to 0.1, 0.9, 1.8, 2.9 and 3.5 mgL^{-1}, survival rates were decreased by the end of the experiment recording values of 85%, 80%, 60%, 55% and 50% for treatments T1, T2, T3, T4 and T5, respectively compared to 95 % for the control group.

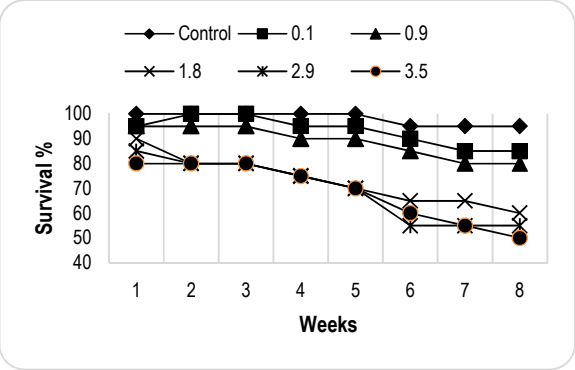

Fig. 1. Survival rate of red tilapia fingerlings exposed to different levels of TAN during the experimental periods.

Growth performance parameters

Results of growth parameters showed that final body weight (g), weight gain (g), relative weight gain (%), average daily gain (g fish^{-1}), specific growth rate (% day^{-1}), final length (cm), gain in length (cm) and condition factor (%) were significantly ($P \leq 0.05$) differed between treatments (Table 3). Fish in the control group followed by T1 achieved the highest values of growth performance parameters, while T5 recorded the lowest values of growth parameters compared with other exposed fish.

Serum biochemical parameters

Results of serum urea (mg dL^{-1}), serum uric acid (mg dL^{-1}) and serum creatinine (mg dL^{-1}) for red tilapia (*Oreochromis sp.*) exposed to different levels of water TAN indicated significantly ($P \leq 0.05$) differences between treatments. Where, the lowest values were recorded for the control group, while the highest values were recorded for T5 treatment (Table 4).

The lowest ($P \leq 0.05$) values of serum total protein (g dL^{-1}), and glucose (g dL^{-1}) were observed in T1, and T2, respectively. While, the highest ($P \leq 0.05$) values of serum total protein, and glucose were observed in T4, and T1, respectively (Table 4).

Table 3. Mean ± SE of growth parameters of red tilapia (*Oreochromis sp.*) fingerlings at different levels of water TAN

Items	Control	T1	T2	T3	T4	T5
IBW (g fish^{-1})	15.10±0.71	15.15±0.91	15.30±2.12	15.45±0.64	15.70±0.70	15.00±0.14
FBW (g fish^{-1})	22.50^{a}±0.42	17.45^{b}±0.21	17.75^{b}±1.06	16.75^{b}±0.35	15.75^{c}±1.06	14.50^{c}±2.12
TWG (g fish^{-1})	7.40^{a}±1.13	2.30^{b}±0.40	2.45^{b}±1.06	1.30^{bc}±0.28	0.05^{bc}±0.35	-0.50^{c}±0.07
RGW %	49.0^{a} ±9.79	15.18^{b}±2.97	16.01^{b}±6.24	8.41^{b}±45	0.32^{c}±0.01	--**
ADGW (g fish^{-1} day^{-1})	0.13^{a}±0.02	0.04^{b}±0.007	0.04^{b}±0.01	0.02^{bc}±0.005	0.0009^{c}±0.006	--**
SGR (% day^{-1})	0.71^{a}±0.08	0.25^{b}±0.03	0.27^{b}±0.10	0.14^{c}±0.03	0.006^{d}±0.003	--**
IBL (cm fish^{-1})	10.90±0.14	10.35±0.21	10.45±2.02	10.40±0.57	10.65±0.21	10.45±0.07
FBL (cm fish^{-1})	12.50^{a} ±0.71	12.07^{ab} ±0.05	11.00^{b}±1.41	10.95^{c}±0.07	10.95^{c}±0.70	10.70^{c}±0.14
GL (cm fish^{-1})	1.60^{ab} ±0.61	1.72^{a}±0.28	0.55^{bc}±0.63	0.55^{bc}±0.45	0.30^{c}±0.14	0.25^{c}±0.07
K (%)	1.15^{ab} ±0.12	0.99^{b}±0.01	1.33^{a}±0.31	1.27^{a}±0.001	1.20^{a}±0.07	1.18^{ab}±0.17

Values in each row having the different superscripts are significantly different ($P \leq 0.05$). ** Growth parameters were decreased

Table 4. Mean of serum biochemical parameters at different levels of water TAN

Items	Control	T1	T2	T3	T4	T5
Urea (mg dL^{-1})	17.63^{c}±1.00	29.01^{c}±1.00	23.24^{d}±1.00	27.57^{c}±1.00	37.34^{b}±2.00	40.87^{a}±1.00
Uric acid (mg dL^{-1})	0.86^{c}±0.10	0.99^{c}±0.10	1.34^{bc}±1.00	1.34^{bc}±1.00	2.97^{b}±1.00	4.72^{a}±1.00
Creatinine (mg dL^{-1})	36.00^{f}±1.00	48.00^{e}±1.00	113.0^{d}±1.00	300.0^{c}±1.00	354.0^{b}±1.00	370.0^{a}±1.00
Total protein (g dL^{-1})	1.76^{bc}±1.00	0.84^{c}±0.10	0.86^{c}±0.10	1.63^{bc}±1.00	3.33^{a}±1.00	3.31^{ab}±1.00
Glucose (mg dL^{-1})	145.8^{b}±1.00	205.5^{a}±1.00	41.30^{f}±1.00	115.4^{c}±1.00	78.61^{d}±1.52	54.44^{e}±1.00

Values in each row having the different superscripts are significantly different ($P \leq 0.05$).

Histological examination

The kidney

Figure 2 shows the normal structure of red tilapia kidney in the control treatment. Examination of kidney tissues from T1 showed marked hyaline droplet, deterioration and abnormal enlargement of kidney tubules. Renal epithelium having basophilic cytoplasm, vacuolation of some cells, and congestion of some blood vessels were observed (Figure 3). Hyaline droplets in the kidney tubule epithelial cells suggested reabsorption of excessive amounts of proteins from glomerular filtrate. Kidney tissues from T2 exhibit degeneration of the endothelium of the blood vessels, necrosis of epithelial cells was observed in most renal tubules, blood clotting between renal tubules and shrinking and aggregation of large number of glomeruli (Figure 4). Histological examination of kidney tissues from T3 and T4 showed thrombus in blood vessels, necrosis of the renal tubule epithelium, acute blood clotting between renal tubules. Moreover, tissue examined show shrinkage and aggregation of large number of glomeruli, the glomerular tuft has some mononuclear cells infiltration, in addition to destroyed brush border of renal tubules and the presence of large spaces between the tubules (Figure 5 and 6). The kidney tissues from fish exposed to the highest level of TAN (T5) showed formation of more thrombus and higher infiltration of cells, majority of destroyed renal tubules, hemorrhage, hemolysis and hemosiderin (a yellowish-brown granular intracellular pigment) between renal tubules and in the renal parenchyma (Figure 7).

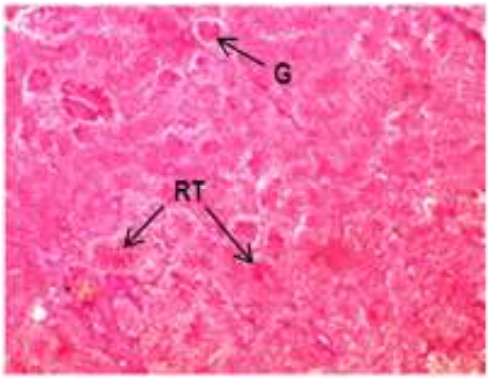

Fig. 2. Photomicrograph of normal kidney of red tilapia in the control treatment stained by H&E stains 100 X; Glomerulus (G); Renal tubules (RT).

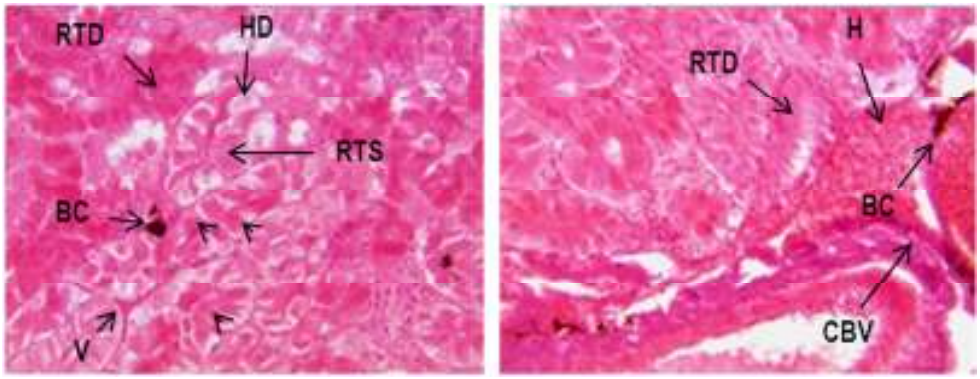

Fig. 3. Photomicrograph of T1 kidney stained by H&E stains 400 X; shows hyaline droplet (HD), renal tubules degeneration (RTD) and swelling (RTS), hemorrhage (H) between renal tubules and blood clotting (BC). Basophilic cytoplasm in renal epithelial cells (arrowheads), vacuolated cells (V), and congestion of blood vessel (CBV) are observed.

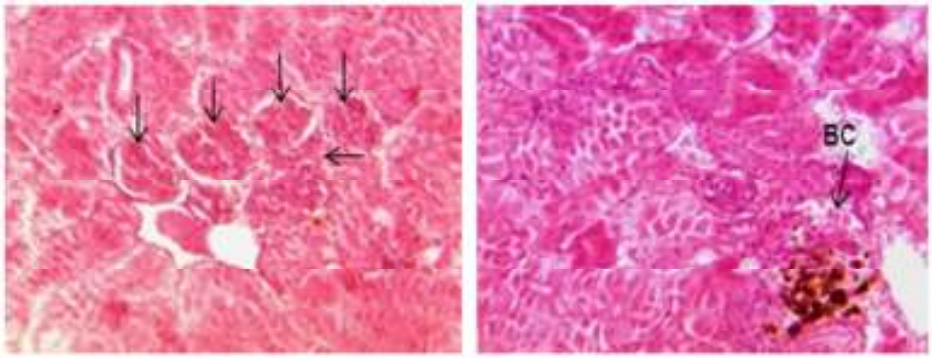

Fig. 4. Photomicrograph of T2 kidney stained by H&E stains 400 X; shows blood clotting (BC) between renal tubules and shrinking and aggregation of glomeruli (arrows)

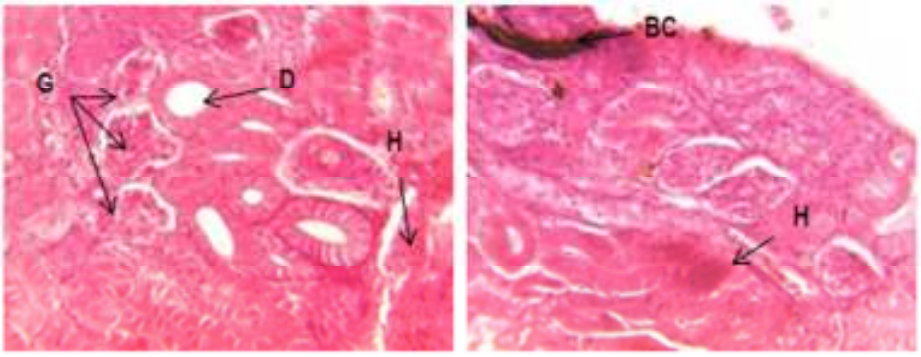

Fig. 5. Photomicrograph of T3 kidney stained by H&E stains 400 X; shows hemorrhage (H) between tubules, blood clotting (BC) between renal tubules, dilation (D) in lumen of renal tubules, shrinking and aggregation of glomeruli (G).

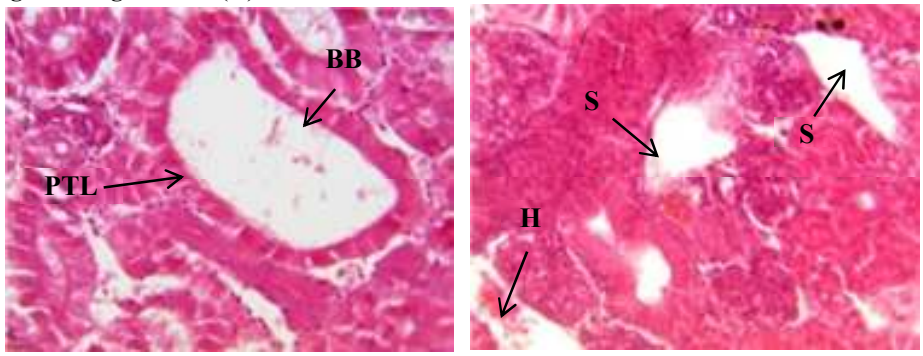

Fig. 6. Photomicrograph of T4 kidney stained by H&E stains 400 X; shows hemorrhage (H) between tubules and large spaces (S) between renal tubules, brush border (BB) and dilation in proximal tubule lumen (PTL).

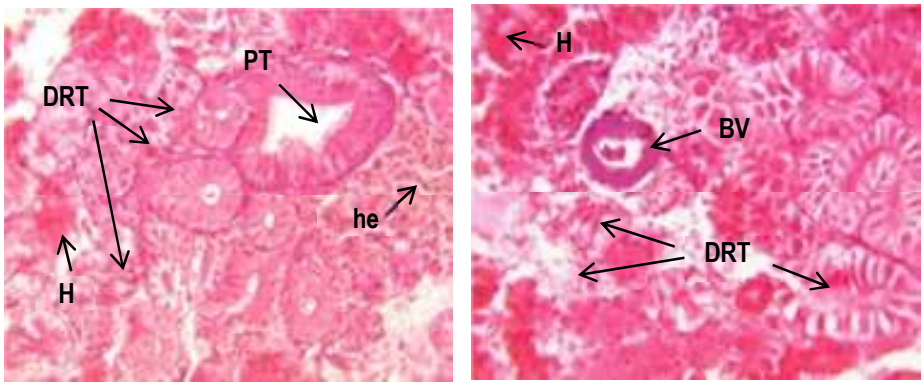

Fig. 7. Photomicrograph of T5 kidney stained by H&E stains 400 X; shows large number of damaged of renal tubules (DRT), large proximal tubule (PT), Blood vessel (BV, renal artery), hemosiderin (he) and large amount of hemorrhage (H) between renal tubules.

The liver

Figure (8) showed the normal red tilapia liver in the control treatment. Examination of liver tissues from T1 after 56 days showed that some hepatic cells were vacuolated, and coagulative blood vessel (Figure. 9) are exist. Examining liver tissues from T2 revealed marked vacuolated cytoplasm with large spaces areas and large areas of hepatic necrosis (Figure 10). When examining liver tissues from T3, the results showed vacuolation, hepatic congestion, coagulative blood vessels, degeneration of some cells and hepatic necrosis were observed (Figure 11). Examining liver tissues from T4, the results showed large areas of vacuolation, degeneration of some hepatic cells and infiltration of melanomacrophage cells and hepatic and pancreatic degeneration (Figure 12). The liver tissues exposed to the highest TAN concentration (T5) resulted in diffuse vacuolar degeneration and large number of hepatic congestions and some hepatic necrosis were observed (Figure 13).

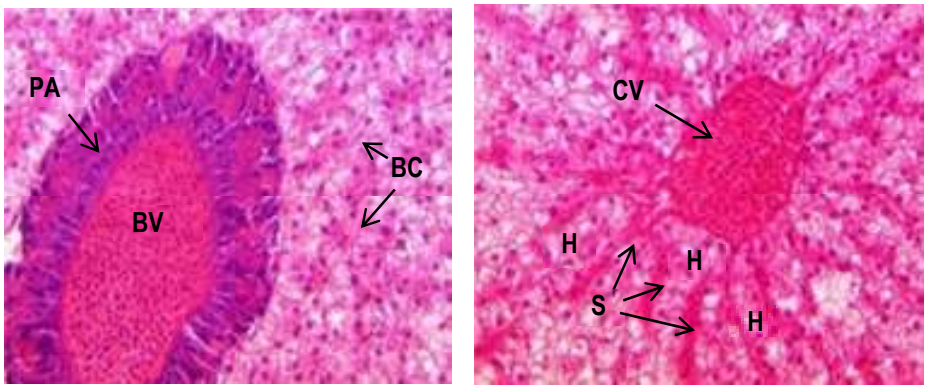

Fig. 8. Photomicrograph of normal red tilapia liver in the control treatment (right) and hepatopancreas (left) stained by H&E stains 400 X; shows central vein (CV), hepatocytes (H), hepatic sinusoids (S), bile canaliculi (BC), pancreatic area (PA, exocrine and endocrine components) inclosing blood vein (BV).

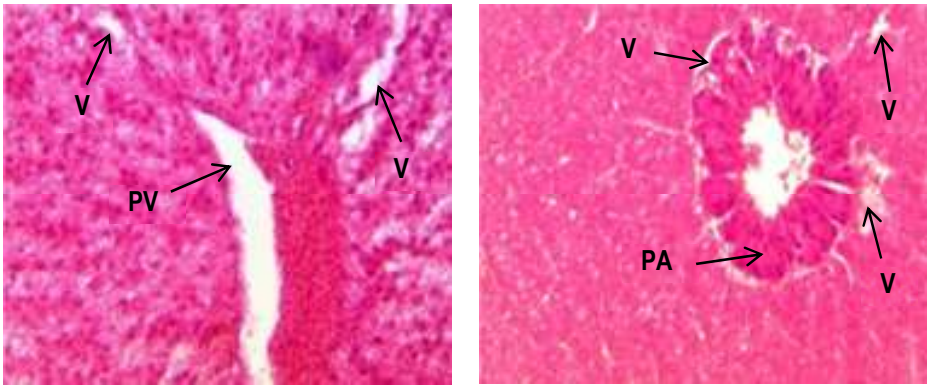

Fig. 9. Photomicrograph of T1 liver stained by H&E stains 100 X; shows vacuolations (V) around the pancreatic area (PA), vacuolations between hepatic sinusoids and coagulation of the blood and dilation of the portal vein (PV).

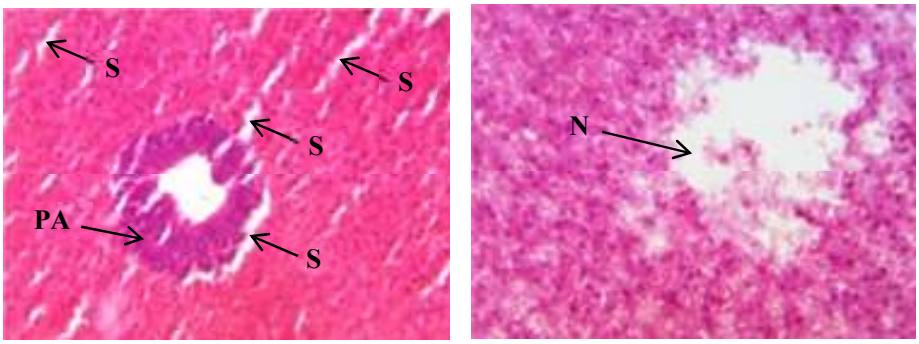

Fig. 10. Photomicrograph of T2 liver stained by H&E stains 100 X; shows hepatic necrosis (N), large numbers of spaces (S) between hepatic sinusoids and around degenerating pancreatic area (PA).

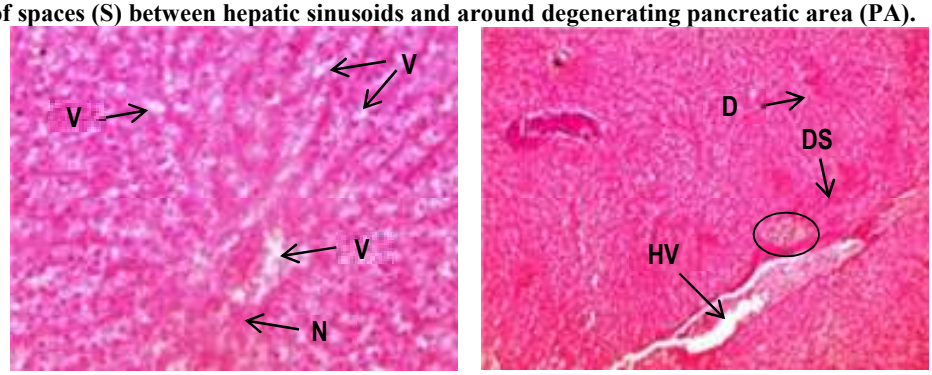

Fig. 11. Photomicrograph of T3 liver stained by H&E stains 100 X; shows hepatic congestion (circle) hepatic vein (HV), degenerating hepatic sinusoid (DS), large numbers of vacuolations (V) between hepatic sinusoids and hepatic necrosis (N).

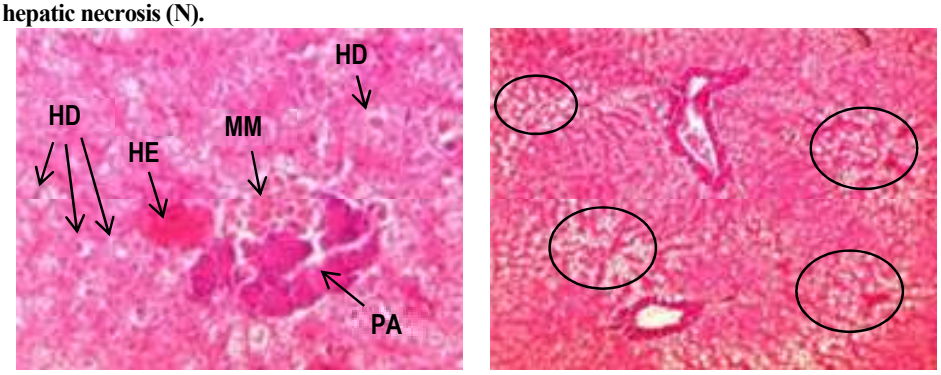

Fig. 12. Photomicrograph of T4 liver stained by H&E stains 100 X; shows increase vacuolation (circles) between hepatic sinusoids, large numbers of hepatic degenerations (HD), hemolysis (HE) and melanomacrophages into degenerating pancreatic area (PA).

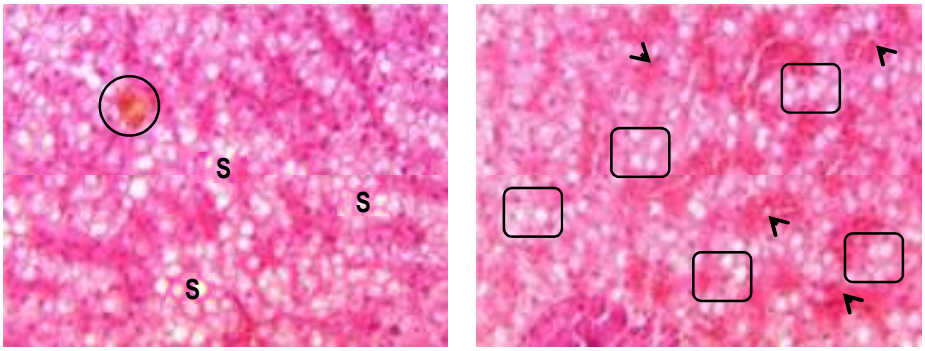

Fig. 13. Photomicrograph of T5 liver stained by H&E stains 100 X; shows large numbers of vacuoles (squares) in hepatic cytoplasm, large numbers of hepatic congestion (arrowheads), large numbers of vacuolations (white spots) between hepatic sinusoids (S) and hepatic necrosis (circle).

The skin and the muscles

Figure (14) shows the normal structures of the red tilapia skin and red (dorsal) muscles without scales in the control treatment. Examination of skin and red muscles from T1 showed that laceration of the skin and inflammations in muscle fibers (Figure 15). Examining

skin and red muscles from T2 revealed muscle bundles edema, splitting in muscle fibers and focal area necrosis under the skin were observed (Figure 16). When examining skin and red muscles from T3, the results showed laceration of the skin and more muscle fibers splitting (Figure 17). When examining skin and red muscles from T4, the results showed laceration of the skin, inflammation and splitting in muscle fibers (Figure 18). The skin and red muscles exposed to the highest TAN concentration (T5) resulted in an increase of skin laceration and more muscle fibers splitting at the cycloid scales structures were observed (Figure 19).

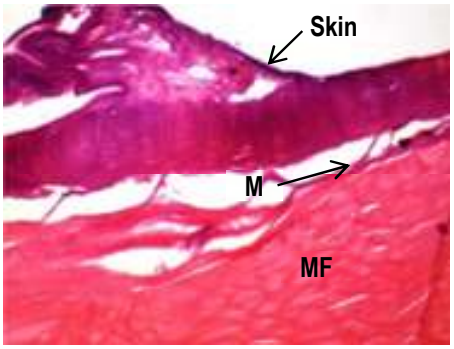

Fig. 14. Photomicrograph of normal red tilapia skin and red muscles in the control treatment stained by Hx and E stains 100 X; shows melanophores (M) and muscle fibers (MF).

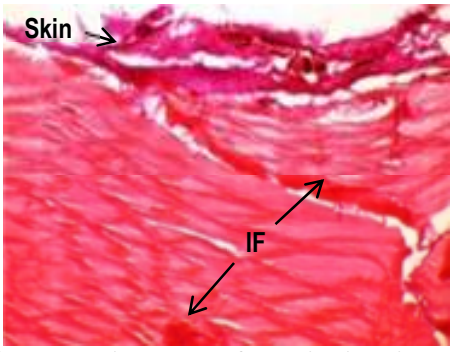

Fig. 15. Photomicrograph of red tilapia skin and red muscles (T1) stained by H&E stains 100 X; shows laceration of skin and inflammation (IF) in muscle fibers.

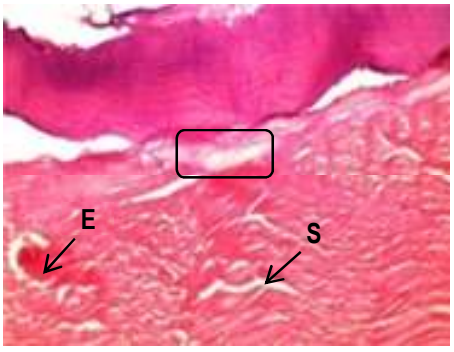

Fig. 16. Photomicrograph of red tilapia skin and red muscles (T2) stained by H&E stains 100 X; shows focal area necrosis (rectangle), muscle fibers edema (E) and splitting (S).

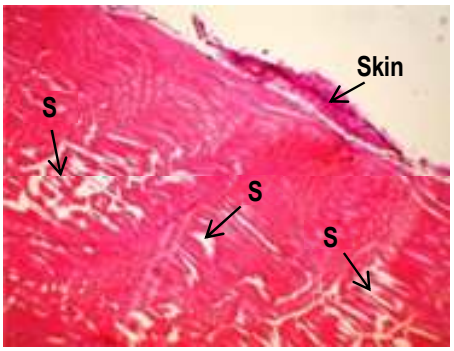

Fig. 17. Photomicrograph of red tilapia skin and red muscles (T3) stained by H&E stains 100 X; shows laceration of skin and more muscle fibers splitting (S).

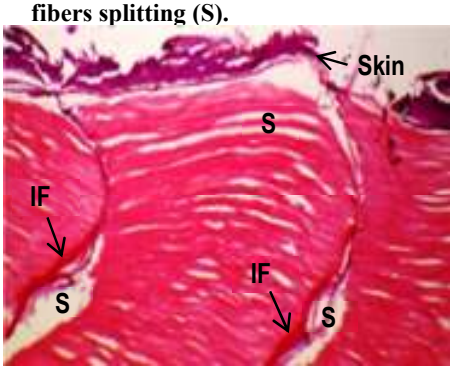

Fig. 18. Photomicrograph of red tilapia skin and red muscles (T4) stained by H&E stains 100 X; shows laceration of skin, inflammation (IF) in muscle fibers and more muscle fibers splitting (S).

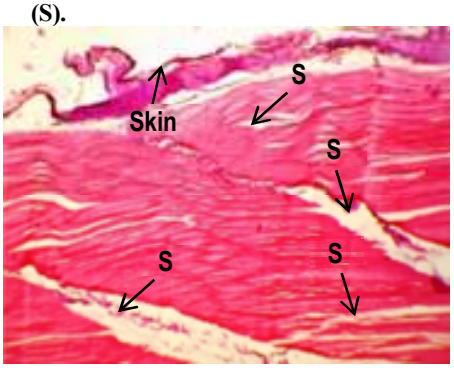

Fig. 19. Photomicrograph of red tilapia skin and red muscles (T5) stained by H&E stains 100 X; shows laceration of skin and splitting (S) in muscle fibers.

DISCUSSION

Water quality parameters including DO, pH and temperature did not differed significantly in all exposed fish groups except for total ammonia-nitrogen. These parameters remained within the acceptable range for red tilapia growth as mentioned by Boyd (1984). Essentially, there are two limiting water quality criteria for aquaculture practices, DO and ammonia.

Data mentioned above, showed there were differences in growth parameters of all exposed fish groups. These differences in growth and survival could be mainly due to the various levels of ammonia. Higher mortalities

detected in T5 could be attributed to the higher exposure dose of TAN. Fish exposed to lower doses (T1, T2 and T3), showed steady growth up to the end of the of exposure period without significant differences between them, similar results were obtained by Zeitoun *et al.* (2016) who reported reduced values of weight gain, ADG and SGR of Nile tilapia in the presence of ammonia (3 mg ammonium chloride per liter). Increased ammonia reduced growth rate in tilapia Sakala and Musuka (2014); in common carp Kawamoto (1961) and in channel catfish Robinette (1976). Inversely with the obtained findings herein, El-Sherif and El-Feky (2008) stated that no mortalities were detected in fish exposed to 0.01, 0.05, 0.1 and 0.15 mgL^{-1} of unionized ammonia (UIA-N). The differences between our findings and those obtained by others may be related with the ammonia levels, source of ammonia, exposure time, fish species, and experimental management.

To verify if there were any relationship between fish physiological changes and environmental stress, some biochemical parameters such as urea, uric acid, creatinine, total protein and glucose levels were assessed. Changes of fish blood biochemistry are indicative of unsuitable environmental conditions or the presence of stress factors (Yang and Chen, 2003; Barcellos *et al.,* 2004; Kamal and Omar, 2011). Therefore, the measurement of serum biochemical parameters is useful as biomarkers in toxicological studies (McDonald and Grosell, 2006).

The results of the present study exhibited higher levels of serum creatinine, urea and uric acid of fish exposure to high levels of TAN. The data suggested that increasing TAN cause glomerular impaired functioning rather than deficiency of kidney tubules. These results are consistent with the fact that the highest elevation of nitrogen compounds in serum of these fish was recorded for uric acid. It can be suggested that the excretion of uric acid and the other nitrogen compounds through gill membranes was inhibited resulting in an accumulation of uric acid in the blood. Similar results have been reported by Zaki *et al.* (2009) who recorded a significant increase in these parameters in Nile tilapia due to cadmium exposure. The concentrations of creatinine, uric acid and urea are often used in fish as an indicator of liver and kidney functions (Adham *et al.,* 2002; Yang and Chen, 2003). Since there is a relationship between the activity of the kidney and concentration of ammonia, data refer to renal failure and catabolism of muscle tissues

Serum total protein concentration of red tilapia was significantly increased, this may be due to exposure to high levels of TAN, such increase in total protein reflects liver dysfunction (Zaki *et al.,* 2010). Protein synthesis of the organism has important diagnostic significance because of its involvement in enzymes, hormones and antibodies. Thus, the effect of toxic substances on the total protein concentration of fish is considered to assess the response to stress and increase energy demand (Hadid *et al*., 2009). In this regard, Das *et al*. (2004) reported that low serum protein in the fingerlings of *Cirrhinus cirrhosus* and *Gibelion catla* may be attributed to protein degradation, the process of converting blood and structural protein to energy, to meet the increasing demand for energy during stress. Hemolysis and deflation of red blood cells may also increase the volume of plasma thus contributing to some extent in lowering the protein content in the blood.

Serum glucose level in red tilapia (*Oreochromis sp.*) showed highly significant increases after exposure to different concentration of TAN in all doses compared with the control. Ammonia is known to increase the levels of catecholamines, activate glycogen degradation and gluconeogenesis and thus, increase plasma glucose levels. These results confirm the response of corticosteroids detected by Tomaso *et al*. (1981) against the rise of ammonia. They reported that blood glucose increased due to stimulation of glucocorticoids in stressed catfish. Similarly, with current results, Davis *et al*. (2003) reported that the concentration of high ammonia caused the plasma glucose to rise significantly in the channel catfish. In the red drum, hydrochloride was also shown to prevent increases associated with stress in plasma glucose (Thomas *et al*., 1999). Captivity stress has also been shown to lead to an increase in plasma glucose levels in seabream species (Rotllant *et al*., 2001) or Atlantic salmon (Skjervold *et al*., 2001).

When red tilapia fingerlings are exposed to 0.1 mg TAN L-1 or higher, marked hyaline droplet and renal tubules degeneration and swelling were observed in the tissues of kidney. Similar observations were recorded by Robert and Rosemarie (1983); Robert *et al*. (1984) who mentioned that hyaline droplets in the epithelium of kidney tubule indicated reabsorption of excessive amounts of proteins from glomerular filtrate when fish are exposed to high levels of ammonia. In this regard, Smith and Pepper (1975) and Thurston *et al*. (1984) also mentioned that hyaline droplets, renal tubular epithelial degeneration, and in some cases, partially occluded tubule lumens invariably lead to impaired glomerular blood flow and filtration and may eventually lead to kidney failure.

In the present study, examination of the liver tissues of red tilapia after exposure to 0.1 mgL^{-1} of TAN, resulted vacuolation in some hepatocytes and coagulative blood vessel. This result agreed with those obtained by El-Sherif and El-Feky (2008). Thurston *et al*. (1984) and Saber *et al.* (2004) who revealed marked vacuolated cytoplasm with large spaces areas and large areas of hepatic necrosis when fish were exposed to high ammonia concentrations. Liver tissues from fish exposed to different levels of TAN exhibit vacuolation, hepatic congestion, vascular coagulation, degeneration of hepatocytes and hepatic necrosis, which closely agreed with El-Sherif and El-Feky (2008). The red tilapia liver tissues exposed to 3.5 mgL^{-1} of TAN resulted in diffuse vacuolar degeneration and large number of hepatic congestions and some hepatic necrosis, these findings agreed with those reported by Saber *et al.* (2004). Wajsbrot *et al*. (1993) also referred to liver histopathological defects that may contribute to reducing fish growth by causing hypoxia in tissues.

In the present study, several histological alterations of the skin and muscles of red tilapia fingerlings exposed to different TAN levels were detected. These histological alterations included laceration of the skin and inflammations in muscle fibers. Muscle bundles edema, splitting in muscle fibers and focal area necrosis under the skin were also observed. It is possible that histological defects observed in the experimental fish could be a direct result of the exposure to ammonia.

CONCLUSION

From the data obtained, we can conclude that red tilapia as other fish species can adapt to lower levels of ammonia when its rate is raised gradually and avoid additional stressors. It is also recommended to avoid the causes of rising ammonia levels in water. This is a more important process to increase growth and productivity, enhance the survival and quality of fish, as well as good environmental impacts, which consequently led to higher profitability.

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تأثير الأمونيا الكلية على النمو والحالة الفسيولوجية والفحص النسيجي لإصبعيات البلطي الأحمر

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تهدف الدراسة الحالية إلى تقييم تأثير التركيزات المختلفة من الأمونيا الكلية على أداء النمو ومعدل البقاء والعديد من التغيرات الفسيولوجية و الهستولوجية لكبد وكلية وعضلات إصبعيات أسماك البلطى الأحمر خلال 56 يوم. خزنت 180 سمكه بمعدل 10 سمكات/ حوض فيبرجلاس سعته 100 لتر من مياه بئر جوفى ملوحته 27,3 جزء فى الألف. تم تجريب 5 تركيزات مختلفة للأمونيا الكلية هى 0,1، 0,9، 1,8، 2,9، 3,5 مليجرام/ لتر بجانب المجموعة الضابطة. غذيت الأسماك على عليقة تحتوى على 34,7% بروتين. أظهرت النتائج أن الأسماك المعرضة لأمونيا 3,5 مليجرام/ لتر سجلت أقل وزن نهائى وكذلك أقل معدل بقاء، هذه النتائج ارتبطت بزيادة تركيز الأمونيا حيث فى نهاية التجربة لوحظ أنه بزياده تركيزات الأمونيا يقل الوزن النهائي للأسماك. الأسماك المعرضة 3,5 مليجرام أمونيا / لتر سجلت أعلى معدلات لليوريا وحامض اليوريك والكرياتنين فى سيرم الدم. الفحص النسيجي لكبد وكلية وجلد وعضلات الأسماك المعرضة لتركيزات مختلفة من الأمونيا وخاصة التركيز 3,5 مليجرام/ لتر أوضح أن زيادة تركيزات الأمونيا أدى إلى تحطم الأنيبيبات الكلوية وكمية كبيرة من الأنيبيبات الكلوية المتحللة. كذلك فجوات فى السيتوبلازم الكبدى وإحتقان وتتكرز للخلايا الكبدية. انفصال الجلد عن العضلات والتهاب الليفات العضلية وتمزق كبير للعضلات. بناء على ما سبق يمكن التوصية بضرورة مراعاة تجنب زيادة مستويات الأمونيا وتلاشى مسبباتها في أحواض الاستزراع السمكي من أجل الحصول على أعلي أداء إنتاجى وزيادة معدلات البقاء والمحافظة على صحة وحيوية الأسماك.