التأثيرات السمية والفسيولوجية للأمونيا على البلطي البلطي النيلي (Oreochromis niloticus) عند مستويات مختلفة من الأس الهيدروجيني

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الملخص :

تـــــم اختبار سمية (قيم LC50 لكلوريد الأمونيوم والأمونيا النقابية على أحجام مختلفة (10،15 جرام) من البلطي Oreochromis niloticus في مستويات مختلفة من الأس الهيدروجيني تم التحكم فيها في نطاق 6.5 - 8.5 أظهرت النتائج التي تم الحصول عليها أنه ضمن نفس الرقم الهيدروجيني ، التأثير السام للأمونيا من حيث NH3مجعد عند قيم أقل من الأس الهيدروجيني. استنتج أن + NH4 يمارس قدرا من السمية و / أو أن زيادة تركيز + H يزيد من سمية .NH3 تعرض البلطي Oreochromis niloticus (5جم) لـ 96 ساعة LC50 من الأمونيا النقابية (0.85 مجم (1 / NH3 عند درجة الحموضة 7.5 (مستوى الأس الهيدروجيني المشترك لممرات المياه الليبية). تغيرات الهيموغلوبين (Hb) ، الهيماتوكريت (Ht) ، الهيماتوكريت (Ht) ، الصوديوم (+ Na) ، البوتاسيوم (+ K) ، الجلوكوز ، الأسبيرناتو غير ناقلة AST) ، (E.2.6.1.1، الأمينين أمينوترانسفيراز ALT) ، إي سي 2.1.2) ، تم تسجيل النتائج التي تم الحصول عليها أظهرت أن نسبة الهيموجلوبين في الدم انخفضت بشكل معنوي بعد 6 و 96 ساعة من التعرض بينما انخفض Ht بشكل ملحوظ بعد 6 ساعات من التعرض. أظهر مصل + Na و + K اتجاه عام للزيادة خلال فترة التجربة بأكملها (96 ساعة). أظهر تركيز الجلوكوز زيادة معنوية أولية ثم عاد إلى أقل من قيمة التحكم بعد 96 ساعة من التعرض. كان الانخفاض معنويًا في AST و ALTوبعد ذلك زاد نشاط الإنزيمات بشكل ملحوظ حتى نهاية فترة التجربة. تمت مناقشة أهمية هذه التغييرات.

الكلمات المفتاحية :
AS السمية ، الأمونيا ، البلطي ، الأس الهيدروجيني ، الجلوكوز ، الهيموغلوبين ،

Toxicigological and physiological effects of ammonia On Tilapia *Oreochromis niloticus* At different pH levels

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ABSTRACT

The toxicity (LC50 values) of ammonium chloride and unionized ammonia was tested on different sizes (10,15g) of Tilapia *Oreochromis niloticus* in wich different pH levels were controlled within the range of 6.5 – 8.5 obtained results showed that, within the same pH, the toxic effect of ammonia in terms of NH_3 in creased at lower pH values. It is concluded that NH_4^+ exerts some measure of toxicity and / or that increased H^+ concentration increases the toxicity of NH_3. Tilapia *Oreochromis niloticus* (5g) were exposed to the 96-h LC_{50} of unionized ammonia (0.85mg NH3/1) at pH 7.5 (common pH level of the Libyan water ways). The changes of hemoglobin (Hb), heamtocrite (Ht) haematocrit (Ht), Sodium (Na^+), Potassium (K^+), Glucose, asperateamnotransferase (AST,E.2.6.1.1), alamnine aminotransferase (ALT,E.C.2.1.2), were recorded The obtained results showed that blood Hb significantly decreased only after 6and 96h of exposure while Ht significantly decreased directly after 6h of exposure. Serum Na^+ and K^+ showed general trend of increase during the entire experimental period (96h). glucose

concentration showed initial significant increase then returned to less than the control value after 96h of exposure. The was only a significant decrease in AST, ALT after which the enzymes activity significantly increased till the end of the experimental period. The significance of these changes are discussed.

Key words: Toxicity, ammonia, Tilapia, pH, glucose, Hb, AS

INTRODUCTION

Stress in fish farming system is associated with a variety of reduced oxygen level abrupt variations in temperature, appearance of contaminants or elevated ammonia.

The accumulation of ammonia in water used for intensive fish culture is a potential problem because of its toxicity to fish. Most of the nitrogen in feeds and fertilizers is not converted to fish flesh enters the water as ammonia, either by direct excretion from fish or by bacterial actions on wastes. Thus, ammonia concentration can increase rapidly when water exchange rats are low (Harry and Body.,1987).

When fish are stressed, stress hormones released (primary response), the composition of blood and tissues is altered and variations in ventilation and heart rate frequency occur (secondary response), (Mazeaud et al., 1977).

The toxicity of ammonia to different fish species regarding its lethal effects, physiological detoxification mechanism (s) as well as histological changes. Has been extensively investigated (Thurston et al 1978; Chetty at al., 1980; Russo, 1985; Bader., 1990; Newman., 1995; Abel., 1998 and Salah El-Deen., 1999).

It was demonstrated that, the toxicity of ammonia depends principally upon the presence of NH_3, which can readily diffuse across the gill membrane due to its from with charged entities which cannot readily pass through the hydrophobic micro pores in the gill membrane due to its lipid solubility and lack of charge whereas the from occurs as larger hydrated from with charged entities which cannot readily pass through the hydrophobic micro pores in the gill membrane (Hasan and Macintoch., 1986).However NH_4^+ is excreted across the gill only via a carrier mediated process in exchange for Na^+ and may also show considerable toxicity under low pH conditions (Yamgata and Niwa., 1982 and Hasan and Macintosh., 1986).

Information on ammonia toxicity and its physiological effects on such species the present study was undertaken to measure the acute toxicity of ammonia to Tilapia (*O.niloticus*) over the entire range recommended by U.S. (EPA., 1977). Criterion for pH. Another goal of the study is to evaluate some physiological alterations in Tilapia. *Oreochrmis niloticus* fingerlings of stackable size and at normal pH value of Libyan water ways (pH-7.5), in response to acute exposure to the lethal concentrations of ammonia.

MATERIAL AND METHODS

Three different size of Tilapia *Oreochromis nilotcus* (Table,2) were collected from Ain keam (El- khoms – Libya) fish farm. and acclimatized to laboratory conditions for one week prior to the experiment. Mortality was less than 5% during the acclimation period. Fish were stocked in test aquaria with dechlorinated aerated water. Feeding was done once daily using a polluted diet at rate 3% of body weight of the fish. Feeding was discontinued 48h prior to and during and the study. The experimental set-up consists of thirty, 112 liter aquaria. The water was changed daily to avoid fish metabolite accumulations in glass aquaria. The water aquaria was aerated 24h before use and then aeration ceased while experimentation. Water characteristics used were measured according to the method of (APHA. 1950). And presented in table (1). Ammonia chloride (NH_4 Cl). The toxicant was obtained from Merck Company (Reagent grade) and was mixed with water to obtain the required concentration. Dilute solutions of reagent-grade sodium hydroxide or hydrochloric acid were used to maintain the desired pH in test; fish were placed in the test aquaria in water of pH 7.5. Adjustments in pH in the desired test pH conditions were made gradually over 24h period and the fish were than maintained at the test pH for 48h period to starting the ammonia exposure test. The concentrations of unionized ammonia (NH_3 mg/l) were calculated by means of the dissociation constants of (body, 1990). After measuring water temperature and pH.

1- In the first experiment (toxicity test): fish were divided into two groups according to different body weights (10 and 15 gm). Each group of fish was placed in two aquaria (12 fish/aquarium) and exposed to different concentrations of NH_4 Cl mg/lat different pH levels. Tests no. 1 to 3,4 to 6,8 _and 9 were challenged to different pH values of approximate 6.5, 7.5 and 8.5

respectively response relationship (LC_{50} value) and 95% confidence limits (Cl) were determined according to the method of (Litchfield and wilcoxon., 1949).

2- In the second experiment (physiological effect): *O. niloticus* fingerlings with average weight of 15.5±0.5 were placed in water containing the 96 LC_{50} NH_3 (mg/1) determined from the first experiment. The pH of the solution was adjusted to be 7.5 every 6h. A freshly repaired solution was replaced every 24 hours of exposure. A control group with no toxicant was also in- clouded in the experiment and treated similarly.Group of fish were randomly selected after 6,12,24,48 and 96h of exposure to the toxicant. Blood samples were withdrawn from the arterial caudal. The needle (heparin zed glass pipette) was insertedquite deep through the middle line just behind the anal fine in the dorso- cranial direction Hemoglobin content (Hb), heamatocrit level (Ht), serum glucose, Sodium, Potassium, Aspartate aminotransferase (AST,E.C.2.6.1.2). activities were measured according to methods described in (Brgmyer., 1974 and APHA., 1995).

* Statistical analysis's:- Data are expressed as the mean ± SD. The data analysis using t- test according to (Hill., 1960).

RESULTS AND DISCUSSION

1- Toxicity test:

In the first experiment, the lethal toxicity (LC_{50}) values and 95% confidence limits for ammonia acute toxicity on Tilapia (*O. reochromis nilotcus*) terms of unionized ammonia and ammonium chloride at different pH levels and fish size are presented in Table (2). Ranges of temperature values in all testes are also reported because of the importance of this variable in the aqueous ammonia equilibrium. The range reported for different variables are the extreme low and high measured values from all aquaria for each test.

The obtained toxicity results at the specified exposed exposure time showed insignificant difference between different fish size, because there was overlap between confidence limits (APHA. 1995).

Such finding was reproducible at the three different tested pH values. This may indicate that the toxic effect of ammonia is obviously independent of fish size.

In addition, the present results showed that, when pH increases toxicity increases and the effective concentration of ammonia chloride. Meanwhile, when comparing the toxicities at different pH values, if the unionized forms of ammonia are solely responsible for the toxic action of the test fish, then one would expect that, the unionized ammonia LC_{50} values would be reasonably constant for all tests regardless of the solution pH and total ammonia present.

The maximum LC_{50} values in terms of unionized within the pH of about 8.5, while the values at pH 6.5 are only 42.76% of these or these or less (after 96h of exposure). A lower LC_{50} value indicates greater toxicity of the toxicant being tested. It is not readily apparent from the data available whether the unionized ammonia LC_{50} values above pH8 reach a peak and then decrease, or whether they plateau. It is apparent however, that the unionized ammonia LC_{50} values are markedly less at low pH, than they are at high values. The simplest possible explanation for this would appear to be that the toxicity of NH_3 is not constant over the pH range tested..i.e. increased h^+ concentration increases the toxicity of NH_3.This explanation is highly supported by the work of (Thurston et al., 1981 and Salah El-Deen., 1999). Another explanation is that NH_4^+ exerts toxic a toxic effect at low concentration of ammonia chloride and the effect of NH_4^+ is masked by the effect of the much toxic NH_3 fraction though at higher concentrations of ammonium chloride. The toxic NH_4^+ can be noticed. A similar explanation has been proposed by (Thurston et al., 1981 and russo., 1985), (Hassa and Macintosh., 1986).

In conclusion, it has been demonstrated by the present study and other studies cited earlier that the toxicity of ammonia to fishes in terms of unionized ammonia alone does not remain constant over the pH range considered acceptable of freshwater aquatic life. It would therefore seem advisable that water quality criteria on to protect aquatic life bi based either on unionized ammonia.

2- Physiological effect:

The results obtained from the second experiment are presented in table 3. Hemoglobin content (Hb) of Tilapia (*O. niloticus*) exposed to the 96h LC_{50} of ammonia showed a significant change (P.001) when compared to the control value. However, only after 6 and 96h exposure a significant decrease

(p.05) in Hb content was recorded compared to the control value. On the other hand, Ht level significantly increased (P001) directly after 6h of exposure and continued till the end of experiment period (96h). Serum Na^+ showed a significant increase during the entire experimental period except after 24h of exposure. Whereas serum k^+ showed a general trend to increase at all the exposure time and was pronounced (P.05) after 6, 24 and 48h of exposure.

Serum glucose levels significantly increased (P0.001) directly after 6h of exposure to ammonia (Table, 3).

However, serum glucose level showed a significant decrease after 24h of exposure till the end of exposure time (96h). The results of Tilapia Nilotica showe that, there was only a significant decreased till the end of the experimental period (Table, 3).

Investigation of the flux NH_3across gill of fish (Smith. 1982). Have indicated that movement between blood and environment is determined by the partial pressure (pNH_3) gradient across the respiratory epithelium. The pNH_3, in turn, is a function of the pH and ionic-strength-dependent equilibrium between NH_4^+ and NH_3 and the concentration of the total ammonium. Therefore, efflux of NH_3 occure when the internal pNH_3 is greater than the external pNH_3, and influx when the gradient between reversed description of pNH_3 gradient between blood and water.

However, under conditions of elevated ambient ammonia, movement of NH_3 will be along the pNH_3 gradient, whereas excretion of NH_4^+ for external Na^+ across the brachial cells (Matez., 1973 and Smith., 1982). On the basis of model of excretion of NH_4^+or H^+ in model of exchange for Na^+ and HCO_3 for CI^- (Matez and Garcia Romeu., 1964 and Smith., 1982). Across the gills. The significant elevation of NH_4^+ to maintain blood ammonia at normal level. However, the slight reduction in Na^+ after 24h of acute exposure to ammonia is constant with the observation of competition between external NH_4^+ and N^+ for common entry sites through the gills when excretion occurs against a concentration gradient (Matez and Garcia Romeu., 1964), (Matez., 1973 and Salah El-Deen.,1999 and Wang Walsh., 2000). On other hand, the elevated K^+ concentration in the serum of Tilapia niloticus in the present experiment may have resulted from intravascular damage to erythrocyte membrane and subsequent leaking of K^+ into serum, reduced inward transport, or some

combination of tense factors. This assumption is in agreement with the work on Coho Salmon, *Onchohyncus kisutch* by ammonia, Health. 1987).

Hematological studies are promising tools from investigating physiological strain, age maturity (Mc Cathy et al., 1973).

The changes were observed in the number of erythrocytes number, Hb or Ht in the fresh water *Labeo capensis* uponshort exposure to sub lethal (Hattingh., 1976). And lethal (Smart. 1978). Levels of ammonia. An initial increase in erythrocytes, Hb and Ht levels of *Oreochromis niloticus* subjected for 24h exposure time (96h/days). The authors attributed the initial increase to the coexisting process of renovation of erythrocytes, while the decrease may reflect a hemolytic anemia. Another explanation was reported by (Miels et al., 1998). The authors attributed the increase of Hb and Ht in rainbow trout *Oncorhynchus mykiss* after 1 day of ammonia exposure to the haemo concentration via spleen concentration or increased dieresis.

In the present study, the decrease in Hb and Ht after acute exposure to ammonia could be attributed to shrinkage of erythrocytes, decrease of erythrocytes production in the hematopoietic tissue, heamadilution and/or intravascular distraction. Similar results were previously reported for the African catfish *clarias gariepinus* and blue Tilapia; *Oreochromis aureus*, *Onchorhyncus kisutch* and *Oreochrmis niloticus* subjected to ammonia toxicity (Buckely et ai., 1979).and (Ahmed et al., 1992, respectivelty).

In fact, the increase in mean K^+ concentration and the frequency of the hematologic change is relationship that support the hemolytic explanation (assumption) described earlier (Salah El-Deen., 1999).

Analysis of serum constituents have been proved to be useful in the detection and diagnosis of metabolic disturbance and disease process (Shreck., 1990).

Blood glucose measurements are known to be sensitive indicator of environmental stress in fish (Nemcsoc and Bross., 1982). Exposure to toxic agents causes stress hormones such as catecholamines are through glycogenolysis (Shreck., 1990).

Corticosteroids, however, maintain a hyperglycemic condition by stimulating protein gluconeogenesis (sharck., 1990). This generalized endocrine response, which results in rapid mobilization, enables the organism to meet

increased energy demands during exposure and resistance to stress (Thomas., 1990).

In the present study, ammonia intoxication caused initial significant increase in glucose concentrations indicating that Tilapia *nailotica* experiencing in glucose concentration near to the control value which indicates that the fish attempted to reestablish (Selys. 1973).

Similar observation was previously reported by (Thurston et al., 1978). When rainbow trout; *Onchorhyncus mykiss* was exposed to 0.34mg NH_3/1 and by (Salah El-Deen., 1999). When Tilapia Nilotica *Onchorhyncus kisutch* was exposed to 0.7mg NH_3/1 at pH7.5.

Ionic balance is the process by which the total electrolytes content and water volume in an organism are held relatively constant. Strict ion regulation is necessary for aquatic organism in order to maintain water and ion homeostasis stress inducted disturbances in ion regulation which is manifested by alerted plasma ion concentrations (Heath., 1987), (Abbas., 1994) and (Abu El-Ella., 1996 and Salah El-EDeen., 1999).

The increased levels of sodium, and potassium might be due to the renal dysfunction (Lauren and McDonal., 1985). And /or may be due to the alteration in the active transport of ions (Tulasi et al., 1990) the ionic Disturbances could also be attributed to the outward leakage of intracellular ion, especially potassium, caused by stress (Haux and Larsson., 1982).

Determination of enzyme activity in plasma or serum and tissue has proven to be diagnostic in fish health studies (Bouk et al., 1987). Stress has been shown to act specifically by inhibiting certain enzymes, thus interfering with metabolic processes in development (Weis et al., 1981). In the AST and ALT activities showed a significant increase is serum aminotransferase is indicative of some degree of tissue necrosis (Niels, et al., 1998). Or indicative of liver and kidney dysfunction and might be due to leakage of these enzymes from the injured tissue intothe blood (Sala El-Deen., 1999). In addition, increase in ALT activity might also due to increased availability of pyruvate formed due to the increased LDH activity (chetty et al., 1980). The increase level of AST and ALT in Tilapia nilotica after exposure to ammonia may also be due to the loss of kreb's cycle, with the result that these enzymes compensate through providing a- ketoglutarate as previously reported by

(Chetty et al., 1980). After exposing Tilapia *Mosambica* to different concentration of ammonia, (Salah El-Deen., 1999). After exposing Tilapia *O. nilotica* to ammonia at different levels of pH However, the physiological and bio chemical changes of fish in relation to ammonia toxicity and/or water toxfication are a serious problem facing the researches who are working in the field of aquaculture and it needs more studies and furthers investigation

Table (1): Characteristics of the test water used in the experiment

Parameters	Value	Unit
Dissolved oxygen (Do)	6.38±0.21	mg/L
Total hardness	146.6±0.321	mg/L as $CaCo_3$
Total alkalinity	221.3±3.12	mg/L as $CaCo_3$
Electric conductivity	0.423±0.042	Mmohs/cm
Salinity	0.11±0.006	mg/L
Ammonium (NH_4)	0.56±0.063	mg/L
Ammonia(NH_3)	0.05±0.003	mg/L
Nitrite (NO_2)	0.021±0.002	mg/L
Nitrate(NO_3)	1.73±0.94	mg/L
Total dissolved solids	246.2±5.22	mg/L
Sodium $(Na)^+$	37.22±3.1	mg/L
Potassium $(K)^+$	4.9±0.89	mg/L
Calcium $(Ca)^{++}$	42.7±4.3	mg/L
Magnesium $(Mg)^{++}$	10.61±1.98	mg/L
Chloride $(Cl)^-$	23.88±4.53	mg/L

Table (2) Acute toxicities of un- ionized ammonia (NH_3,.mg/1L) and ammonium chloride (NH_4Cl, mg/L) to Tilapia *O.reochroumis niloticus* at different pH (6.5, 7.5 and 8.5) an different fish weight (gm).

Test number	Fishes Weight (gm) fingerlings	Temp c°	pH	LC_{50} NH_3mg/L				LC_{s0}/NH4CI			
				24h	48h	72h	96h	24h	48h	72h	96h
1	10mg	24.1±1	6.50±0.10	0.69	0.49	0.50	0.45	310	240	221	201
2	15mg	22.9±0.1	6.50±0.10	0.78	0.65	0.53	0.40	301	230	199	192
3	10mg	23.9±0.7	7.50±0.20	1.9	1.50	1.00	0.80	107	9.0	65	50
4	15mg	24.1±.1	7.50±0.10	1.98	1.60	1.20	0.85	110	8.2	60	43
5	10mg	24±0.9	8.50±0.20	2.90	2.90	1.30	1.01	20	1.3	9	7.5
6	15mg	24±.2	8.50±0.030	2.99	2.99	1.50	0.99	17	15	10	6.4

*** Data reported for fish weight, temperature and pH.**

*** Toxicity tests were determined from total of three replicates pretrial's.**

Table(3): Changes in levels of some physiological characteristic in Tilapia fish *Oreochromis niloticus* (15g body weight) to the 96h LC_{50} 0f NH_3 (0.85mg/L) at PH = 7.50.

Exposure time	Hb(g/dl) M±SD	Ht (%) M±SD	glucose (g/dl)	Na^+ (mg/L)	K^+ Mg/L)	AST (U/I)	ALT (U/I)
Control	9.1±.25	3.35±0.30	90.35±0.70	135±0.7	6.5±1.2	170.3±3.60	9.9±1.2
6hrs	7.2±.120***	24.10±0.11***	105.2±2.60***	150±0.90***	7.52±1.1	120.5±3.10***	5.1±1.0***
12hrs	8.2±.125***	23±0.80***	101.2±0.92***	148±0.3***	7.26±1.0	155.22±3.60***	8.3±0.11*
24hrs	8.2±.30***	28.1±0.30***	80.2±1.42***	133.8±1.50	8.01±0.8*	220.6±3.40***	9.60±0.13
48hrs	7.2±.40***	21.50±0.24***	79.8±1.60***	142±1.60***	8.13±0.06*	240.7±3.50***	10.50±1.0
96hrs	6.8±.35***	22.90±0.30***	74.3±0.81***	152.74±.82***	7.3±0.5	245.3±2.50***	11.6±0.15*

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