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Effect of Ammonia Toxicity on Carbohydrate Metabolism In Nile Tilapia (*Oreochromis niloticus*)

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Abstract: This study was conducted to evaluate the effects of ammonia on carbohydrate metabolism in Nile tilapia (*O. niloticus*) (50.55±3.10 g) body weight, the temperature was 23±1°C during the experimental period. Tilapia exposed to different concentrations of ammonium chloride (0.0, 2.0, 4.0, 8.0, 10.0 mg/L) for 14 days. The obtained results showed that serum blood glucose, lactic acid and pyruvic acid were significantly increased ($p<0.05$) after exposure to ammonium chloride compared to control group. Also serum, liver and muscle (α) amylase (1,4-α- D glucanglucan hydrolase, EC:3.2.1.1), Glucose 6- phosphate dehydrogenase (G6PDH, E.C.1.1.1.49) and lactate dehydrogenase (LDH,EC 3.4.11.4) were significantly increased ($p<0.05$) compared to untreated control group. Some blood serum hormones like, Cortisol, T3 and T4 were significantly increased after ammonium chloride treatment ($p<0.05$). But glycogen content in liver and muscles showed dsifnificant decrase after ammonium chloride treatment compared to the control ($p>0.05$). For Nile tilapia (*O. niloticus*), carbohydrate metabolism was more sensitive to high concentration of ammonium chloride.

Key words: Ammonium Chloride (NH_3Cl) · Nile Tilapia *(Oreochromisniloticus*) · Enzymes · Hormones · Lactic Acid · Pyruvic Acid · Glycogen

INTRODUCTION

Ammonia is mainly excreted as the un-ionized form NH_3 (UIA). In seawater, NH_3 ionizes to form NH_4^+. The relative proportion of the two forms depends upon pH, temperature and, to a lesser extent, salinity [1, 2]. In seawater, ammonia is measured as total ammonia nitrogen (TAN), which represents the sum of UIA-N and NH_4^+-N. The NH_3 molecule is non-polar and readily soluble in lipids. It is 300 to 400 times more toxic than NH_4^+ [3, 4].

Under intensive rearing conditions and particularly when effluent water is re-used, ambient ammonia concentrations may reach levels that limit fish survival and growth [4]. However, «safe levels» for growth, usually extrapolated from LC_{50} data, are reported to range from 0.05 to 0.2 mg l^{-1} UIA-N, depending on species, age and environment (oxygen concentration, pH) [5, 6]. Lethal concentration for 50% of the population (96-h LC_{50}) have been reported to be 1.7 mg l^{-1} UIA-N (40.0 mg l^{-1} TAN) in seabass juvenile [7]. Tolerance of Tilapia to ammonia has been documented to be as high as 2.4mg l^{-1} (LC_{50}, 48h) in unacclimated fish and 3.4mg l^{-1} (LC_{50}, 48h) in fish acclimated to a sub-lethal level of ammonia [8]. The lethal concentrations (LC_{50}) of unionized ammonia (NH_3) for the larvae and finger lings of Tilapia (*Oreochromis niloticus* L.) for 48 h LC_{50} values were determined as 1.009±0.02 mg/l for larvae and 7.40±0.01 mg/l for the fingerlings [9]. Ammonia is a killer when present in higher concentrations and many unexplained production losses have likely been caused by ammonia [10].

Ammonia is permeable to most biological membranes and can cause physiological stress in fish [11]. Chronic stress can alter hormone levels [12] and enzyme activities [13]. Changes in hormone levels, blood hematological and biochemical parameters can be used for optimizing culture conditions for fish [14]. Plasma cortisol has therefore been monitored as a general index of stress. Goss and Wood [15] stated that cortisol influences an array of physiological parameters, including carbohydrate and hydromineral balance, mobilization of amino and fatty acids from cellular stores, gluconeogenesis and plasma protein production. An increase in cortisol level was seen

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when goldfish (*Carassius auratus* L.) were exposed to high ammonia [16]. The plasma cortisol level was also increased significantly in *Cyprinus carpio* after exposure to ammonia, as mechanism of coping up with stress or impaired immune function [17]. Failure to suppress activation of the hypothalamo-pituitary-interrenal (HPI) axis during stress results in a release of cortisol which in turn causes various secondary stress responses, including increases in circulating levels of glucose and lactate [18]. Stress induced elevations in plasma cortisol are known to suppress immunological capacity in channel catfish [19]. High NH_3-N concentration in the culture water caused an increase in plasma cortisol, glucose and lactate concentrations in both PC and isoeugenol treated catfish [20]. Plasma corticosteroids were increased four to five-fold as a result of exposing channel catfish to NH_3-N [21].

(α) -amylase are important in the diagnosis of acute pancreatitis [22, 23]. Yang *et al.* [24] demonstrated that the changes of enzyme activity hyperamylasemia and hyperlipasemia might be caused by cancer destructed excretory function, inflammation and gallstone, which lead excretion of enzyme into blood. The elevated α-amylase activity has been reasoned to result from pancreatitis or from damaged amylase secretary cells [25]. Both short and long term contamination of the Indian carp *Cirrhinamrigala* by lead acetate led to increased hepatic α-amylase activity by Mujeeb [26]. Increased hepatic and muscle α-amylase activities were also reported in a freshwater fishcollected, from a polluted river caused by heavy metals load including chromium [27]. Cd, Pb and Cr effecton α- amylase, led to significant increases in enzyme activity; the effect-trend was Pb>Cd>Cr [28]. Mercury chloride and copper chloride are potent inhibitors of amylase activity [29, 30].

G6PDH is the first enzyme in the pentose phosphate pathway. The main physiological function of G6PD is to produce NADPH and ribose5-phosphate, which is essential for reductive biosynthesis, nucleic acid and membrane lipids synthesis [31]. G6PDH is the rate limiting enzyme of the pentose phosphate pathway, which is cytosolic enzyme convert glucose6-phosphate to 6- phosphogluconate [32]. NADPH is critical modulator of redox potential of the cell and is the principal intracellular reductant for various biosynthetic reactions and protects the cell against oxidizing agents by producing reduced glutathione. NADPH is also a synthetic coenzymes used in several biomolecules such as in fatty acids, steroids and amino acids [33]. G6PDH is widespread in all tissues and blood cells and is a house keeping enzyme [34]. The deficiency of this enzyme may cause neonatal jaundice, acute hemolysis, kernicterus and even death [35]. These enzymes are important and acting in the protection of cells against oxidative damage [36]. The activity of G6PDH, in liver tissues of fish exposed to different concentrations of NH_3 showed significant increase, the degree of increase in activity was positively related to ammonia concentration [37]. The metal ions Hg, Cd, Pb, Cu and Zn cause inhibition of the G6PDH activity [38]. The stimulation effect of cadmium on the gill, liver and kidney tissues of trout, the G6PDH activity levels were stimulated by approximately 60% in gills, 68% in liver and 67% in kidneys compared to control groups [39].

LDH catalyzes the conversion of pyruvate to lactate. Ammonium ion stimulates glycolysis through the activation of phosphofructokcinase [40]. Ammonia is known to deplete citric acid cycle intermediates along with mitochondrial swelling [41]. Reddy and Rao [42] reported that in prawn a decrease in LDH activity and increase in lactate levels are indicative of reduced mobilization of pyruvate into the TCA cycle. Similar increase in lactate levels and decrease in pyruvate content in fish treated with ammonium sulphate was reported earlier [43]. Ceron *et al.* [44] had earlier reported a reduction in lactate concentration when fish were exposed to Diazinon at acute concentrations and the decrease in LDH activity indicated decrease metabolic activities of the exposed fish. The inhibition of these enzymes would result in the accumulation of metabolic intermediates in the liver therefore causing physiological stress in fish, which eventually lead to mortality if exposure is prolonged as suggested by Das *et al*. [45]. Ammonia elevation leads to a depletion of cerebral α-ketogluterate and thus affects citric acid cycle resulting in decreased energy output [46]. Systemically there will be an increase of blood glucose α-ketogluterate, pyruvate and lactate as the rate of general metabolism is initially stimulated and then ultimately inhibited by progressive ammonia toxicity [47].

Glycogen is a multibranched polysaccharide of glucose that serves as a form of energy storage in animals, represents the main storage form of glucose in the body. Short and long term exposure of *Tilapia mossambica* to sublethal concentration of ammonia reduced glycogen content in fish tissues [43]. Anaerobic conditions caused by stress are known to result in muscle glycogen and lactate breakdown, with some of the lactate being released into circulation [48]. Confinement stress, without sedation, has been observed to increase plasma glucose and lactate, indicative of glycogen mobilization

and breakdown, have been associated with poor quality and rigor development of fish tissues [49]. Obula *et al*. [50] clear that ammonia could significantly decrease pyruvate content while markedly elevate lactate levels in all the exposure periods suggesting a shift in cellular respiratory metabolism towards anaerobiosis as a prelude towards adaptability to cope with the enhanced energy demands. Jabeen [51] reported that in *Tilapia mossambicus* inhibition of pyruvate oxidation by ammonia leading to the accumulation of lactate and that increase in lactate content is useful in neutralizing the effects of ammonia, which would otherwise cause alkalosis besides its involvement in osmoregulation. The glycogen contents in the liver and muscular tissue was significantly decreased, glucose and lactate concentrations in the blood were significantly increased [52].

Thyroid hormones play an important role in the growth and development of larvae and juvenile fish. Earlier studies have shown that, thyroid activity fluctuates in response to various environmental stimuli [53]. According to Silberman *et al*. [12], decreases in serum thyroid hormone levels due to chronic mild stress have been observed to negatively modulate T-cell response; this may have also impacted the expression of T3 and T4 levels. Besides, physiological responses to chronic stress conditions are mediated by stress hormones that could have affected their expression. Reduced food intake has been associated with reduced thyroid hormones concentrations in fish and other vertebrates; besides, thyroid hormones are generally associated with an increase in metabolic rate and are usually reduced during periods of food deprivation as a means to conserve energy [54]. Thyroxin (T4) in fish exposed to 100 mg/L ammonium perchlorate was elevated compared with control fish, but 3,5,3-triiodothyronine (T3) was not significantly affected in any exposure group [55]. The sub-chronic selenium exposure increase plasma T3, T4 of tilapia [56]. The aim of this study to evalute the effect of ammoniuma chlorid on carbohydrate metabolism in nile tilapia (corea chromis, ailoticus).

MATERIALS AND METHODS

This study was carried out at the indoor wet Lab of Aquaculture Research Center, department of reproductive physiology, Agriculture Research Center, Egypt, in order to evaluate the effect of different levels of NH_4Cl on carbohydrate metabolism of Nile tilapia (*O. niloticus*).

Experimental Fish: Nile tilapia (*O. niloticus*) with mean average weight of 50.55±3.10 g body weight were obtained from Fish Central Lab. Fish were homogenous in size, body weights and apparently healthy. They were stoked 2 week, prior to adapt them for the experimental conditions. Fifteen glass aquaria (40×70×60 cm) with capacity of 60 L water were used for rearing the fish. De-chlorinated water in aquaria was aerated by a constant supply of compressed air pump and was exchanged daily [57].

Experimental Design: This experiment was devoted to study the effect of NH_4Cl on carbohydrate metabolism in Nile Tilapia *(O. niloticus)*. The fish were stocked at five different levels of NH_4Cl i.e., 0.0, 2.0, 4.0, 8.0 and 10.0mg/L, with three replicates for each treatment. NH_4Cl solution was added gradually into the water to increase the NH_4Cl concentration [58] and Hcl was used to obtain pH 6 and 7 [57]. Tilapia was stocked for 7 days at a rate of 10 fish per aquarium. Commercial diet containing 26.58% crude protein was used through the experiment period with daily ration rate 3% of fish weight in the morning (10.00 AM).

Blood Collections and Tissue Preparation: At the end of experiment, blood, liver and muscle tissue samples were collected, blood collected from the caudal vein of fish after exposure to NH_4Cl for 2 weeks as well as from the control group fish. The blood left to clot then centrifuged at 3000 rpm for 15 minutes. The separated serum samples were stored at -20°C for biochemical analysis. Fish were sacrificing freshly after collection of blood samples immediately, liver and muscle tissues were tacked and homogenized, then divided into two parts, the first part was, centrifuged (at 3500 rpm for 30-minutes) and the supernatant stored at -20°C until used for biochemical analysis and the second part was used without centrifugation for lactate, pyruvate and glycogen determination.

Biochemical Analysis: Plasma glucose levels were analyzed by the glucose-oxidase colorimetric method [59]. Lactate and pyruvate were measured enzymatically [60]. Glycogen content was estimated colorimetrically by treating with anthrone reagent [61].

Enzymes Assays: G6PDH activity is determined by spectrophotometric assay [62]. LDH was determined as the formation of NADH during conversion of L-lactate to pyruvate [63]. (α)Amylase activity was measured by estimating the reducing sugars produced due to the action of glucoamylase and α-amylase on carbohydrates [64].

Hormones Estimation: Cortisol was determined by a time resolved fluoroimmuno assay which has been validated for channel catfish [65]. Plasma total T3 and T4 levels were detected using commercial kits according to Plohman *et al*. [66].

Statistical Analysis: Data were analyzed by analysis of variance using the SAS program [67]. Duncan's multiple range tests [68] was used to verify significance of the mean differences among treatments.

RESULTS

Glucose level in serum of Nile tilapia (*O.niloticus*) after treated with different concentration of NH_4Cl showed significant increases ($P<0.05$) (87.99±1.3 mg/dl), in all groups compared to the control group (38.86±2.2 mg/dl).

Lactic and pyruvic acid concentration in serum of Nile tilapia (*O. niloticus*) groups after treated with different concentrations of NH_4Cl showed significant increased ($P<0.05$), compared to the control Table 1. (α)-amylase activity showed no significant increase ($P>0.05$) (5.79± 1.81 µ mol/ml) in serum of Nile tilapia (*O. niloticus*) after treated with NH_4Cl compared to (4.67±1.71 µmol/ml) control group.

LDH and G6PDH concentration in serum of Nile tilapia (*O. niloticus*) groups after treated with different concentrations of NH_4Cl showed significant increased ($P<0.05$), compared to the control Table 1.

Lactic and pyruvic acid level of Nile tilapia (*O.niloticus*) after treated with different concentration of NH_4Cl showed significant increases ($P<0.05$) (0.411±0.03mg/g), (0.596±0.6 mg/g), in all groups compared to the control (0.301±0.03 mg/g), (0.408±0.05 mg/g), respectively Table 2.

Glycogen content in liver tissues of Nile tilapia (*O. niloticus*) groups showed significantly decreased ($P<0.05$) (152.96±0.13mg/g), in fish after treated with different concentrations of NH_4Cl compared to the control (204.91±0.13mg/g) (Table 2).

LDH and G6PDH activity in liver tissues of Nile tilapia (67.63±0.15µmol/g), (79.19±1.14µmol/g) showed significant increase ($P<0.05$) compared to control fish (28.55 ±0.36µmol/g) (32.54±1.92µmol/g) respectively (Table 2).

Lacticand pyruvic acid level of Nile tilapia (*O. niloticus*) after treated with different concentration of NH_4Cl showed significant increases ($P<0.05$) (0.948±0.06 mg/g), (0.489±0.05 mg/g), in all groups compared to the control fish group (0.595±0.08 mg/g) (0.358±0.06mg/g), respectively (Table 3). Glycogen content in muscles of Nile tilapia (*O. niloticus*) groups showed significantly decrease ($P<0.05$) (2.96±0.13 mg/g), in fish after treated with different concentrations of NH_4Cl compared to the control fish group (8.21±0.13 mg/g), as in Table 3. LDH and G6PDH activity in muscle tissues of Nile tilapia (47.73 ±0.55µmol/g), (65.14±1.33µmol/g) after treated with different concentrations of NH_4Clshowed significant increase ($P<0.05$) compared to control (18.95 ±0.26µmol/g), (27.94±1.57µmol/g) (Table 3).

Cortisol level increased significantly ($P<0.05$) (79.06±0.03ng/ml) in blood serum of Nile tilapia (*O. niloticus*) treated with NH_4Cl compared to for control (18.09±0.08 ng/ml). Also T3 and T4 levels was (10.21±0.05 ng/ml), (3.44±0.03ng/ml) increased significantly ($P<0.05$) in blood serum of Nile tilapia (*O. niloticus*) treated with NH_4Cl compared to untreated control fish (2.50±0.19 ng/ml), (6.11±0.09 ng/ml) respectively.

DISCUSSION

Blood glucose level in serum of Nile tilapia (*O.niloticus*) after treated with different concentration of NH_4Cl showed highly significant increases ($P<0.05$) (87.9 6±1.3 mg/dl), in all groups compared to the control fish group (38.86±2.2 mg/dl). Ammonia an effect is known to increase the levels of catecholamines, activating glycogenolysis and glyconeogenesis with a net result of increasing plasma glucose levels. These results confirm the corticosteroid response to high ammonia observed by Tomasso *et al*. [21]. Who that blood glucose increased due to stimulation of glucocorticoids in stressed catfish. Davis *et al.* [19] reported that High NH_3-N concentration caused a significant increase in plasma glucose concentrations in both PC and isoeugenol treated catfish. In red drum *(Sciaenopsocellatus*), hydrochloride has also been shown to prevent stress-related increases of plasma glucose [48]. Confinement stress has been shown to induce increases in plasma glucose levels in several species of fish [18, 49].

Lactic acid concentration in blood serum, liver and muscle tissues of Nile tilapia (*O.niloticus*) were [serum (0.285±0.03 mg/ml), liver (0.411±0.03mg/g), muscle (0.948±0.06e mg/g)] after treated with different concentrations of NH_4Cl showed significant increased ($P<0.05$) compared to the control fish group (0.381±0.03 mg/ml), (0.301±0.03 mg/g), (0.595±0.08 mg/g) respectively (Table 1). These results are in agreement with confinement stress, without sedation, has been observed to increase plasma lactate levels in Atlantic salmon (*Salmo salar L.*) [49]. And failure to suppress activation of the hypothalamo-pituitary-interrenal (HPI) axis during stress

Table 1: Effects of different concentration of NH_4Cl on blood serum glucose, lactic acid, pyruvic acid, α-amylase LDH and G6PDH of Nile tilapia (*O. niloticus*) treated with ammonium chloride (Mean±SE)

	Experimental groups				
Measurements parameters	Normal	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Blood glucose mg/dL	38.86± 2.2^{e}	43.36± 1.3^{d}	49.58±2.3^{c}	64.96 ±1.3^{b}	87.99± 1.3^{a}
Lactic acid mg/ml serum	0.285±0.03^{e}	0.348±0.05^{d}	0.368±0.08^{c}	0.372±0.07^{b}	0.381±0.03^{a}
pyruvic acid mg/ml serum	0.260±0.05^{e}	0.338±0.04^{d}	0.427±0.03^{a}	0.316 ±0.6^{c}	0.380±0.04^{b}
(α)-amylase µmol/ml serum	4.67 ± 1.71^{a}	4.87± 1.46^{a}	4.96 ± 1.33^{a}	5.47± 1.25^{a}	5.79± 1.81^{a}
LDH µmol/ml serum	9.65 ±0.29^{d}	25.52 ± 0.33^{c}	44.53 ±0.41^{b}	38.75 ±0.15^{a}	58.60 ±0.16^{a}
G6PDH µmol/ml serum	22.54 ±1.92^{e}	34.54 ±1.68^{d}	64.63 ±1.60^{c}	78.23±1.50^{a}	69.19±1.14^{b}

Means with the same letter at the same row for each parameter is not significantly different.
Highly significant difference among groups ($p<0.01$).

Table 2: Effects of different concentration of NH_4Cl on lactic acid, pyruvic acid, glycogen, LDH, G6PDH in liver tissues of Nile tilapia (*O. niloticus*) (Mean ± SE)

	Experimental groups				
Measurements parameters	Normal	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Lactic acid mg/g liver wet. wt.	0.301±0.03^{e}	0.358±0.05^{d}	0.398±0.08^{c}	0.412±0.07^{b}	0.411±0.03^{a}
Pyruvicacid mg/g liver wet.wt.	0.408±0.05^{d}	0.488±0.04^{b}	0.487±0.03^{b}	0.596±0.6^{a}	0.465±0.04^{c}
Glycogen mg/g liver wet wt.	204.91±0.13^{a}	190.21±0.13^{b}	184.96±0.13^{c}	171.31±0.13^{d}	152.96±0.13^{e}
LDH µmol/g liver wet. wt.	28.55 ±0.36^{e}	34.52 ±0.33^{d}	41.15 ±0.54^{c}	56.72 ±0.18^{b}	67.63 ±0.15^{a}
G6PDH µmol/g liver wet.wt.	32.54±1.92^{e}	54.54±1.68^{d}	76.63±1.60^{b}	66.23±1.50^{c}	79.19±1.14^{a}

Means with the same letter at the same row for each parameter is not significantly different.
Highly significant difference among groups ($p<0.01$).

Table 3: Effects of different concentration of NH_4Cl on lactic acid, pyruvic acid, glycogen, LDH, G6PDH in muscle tissues of Nile tilapia (*O. niloticus*) (Mean ± SE)

	Experimental groups				
Measurements parameters	Normal	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Lactic acid mg/g muscles wet. wt.	0.595±0.08^{a}	0.748±0.04^{b}	0.870±0.03^{c}	0.968±0.08^{d}	0.948±0.06^{e}
Pyruvic acid mg/gmuscles wet.wt.	0.358±0.06^{d}	0.431±0.07^{b}	0.489±0.05^{a}	0.433±0.09^{b}	0.414±0.07^{c}
Glycogen mg/g muscles wet wt.	8.21±0.13^{a}	7.21±0.13^{b}	4.96±0.13^{c}	3.31±0.13^{d}	2.96±0.13^{e}
LDH µmol/g muscles wet. wt.	18.95 ±0.26^{e}	24.32 ± 0.13^{d}	31.35 ±0.24^{c}	46.52 ±0.28^{b}	47.73 ±0.55^{a}
G6PDH µmol/g. muscles wet. wt.	27.94±1.57^{e}	38.39±1.23^{d}	57.63±1.25^{b}	46.47±1.56^{c}	65.14±1.33^{a}

Means with the same letter at the same row for each parameter is not significantly different.
Highly significant difference among groups ($p<0.01$).

Table 4: Effects of different concentration of NH_4Cl on cortisol, T3 and T4 in blood serum of Nile tilapia (*O. niloticus*) (Mean ± SE)

	Experimental groups				
Measurements parameters	Normal	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Cortisol ng/ml serum	18.09± 0.08^{e}	26.07± 0.06^{d}	48.03 ± 0.04^{c}	56.06± 0.05^{b}	79.06± 0.03^{a}
T3 ng/ml serum	2.50 ± 0.19^{e}	2.79 ± 0.04^{d}	2.99 ± 0.02^{c}	3.09 ± 0.04^{b}	3.44 ± 0.03^{a}
T4 ng/ml serum	6.11 ± 0.09^{a}	7.21 ± 0.01^{b}	7.81 ± 0.04^{c}	8.71 ± 0.02^{d}	10.21 ± 0.05^{d}

Means with the same letter at the same row for each parameter is not significantly different.
Highly significant difference among groups ($p<0.01$).

results in a release of cortisol which in turn causes various secondary stress responses, including increases in circulating levels of lactate [18, 49]. Brian [20] showed the Levels of plasma lactate increased significantly ($P<0.05$) followed by acute oxygen depletion in channel catfish.

Pyruvic acid concentration in blood serum, liver and muscle tissues of Nile tilapia (*O.niloticus*) [serum (0.427±0.03mg/ml), liver (0.596±0.6 mg/g), muscle (0.489±0.05 mg/g)] after treatment with different concentrations of NH_4Cl showed significant increased ($P<0.05$) compared to untreated control fish group

(0.260±0.05 mg/ml), (0.408±0.05 mg/g), (0.358±0.06mg/g) respectivelyas in Tables 1, 3, 4.These results are in agreement with, systemically there will be an increase of blood glucose α-ketogluterate, pyruvate and lactate as the rate of general metabolism is initially stimulated and then ultimately inhibited by progressive ammonia toxicity [47]. Reddy and Rao [42] reported that a decrease in LDH activity and increase in lactate levels in prawn are indicative of reduced mobilization of pyruvate into the TCA cycle. Similar increase in lactate levels and decrease in pyruvate content in fish treated with ammonium sulphate was reported earlier by Santhi [43]. Jabeen[51] also reported an inhibition of pyruvate oxidation by ammonia leading to the accumulation of lactate and that increase in lactate content is useful in neutralizing the effects of ammonia on *Tilapia mossambicus*, which would otherwise cause alkalosis besides its involvement in osmoregulation.

The glycogen content in liver and muscles tissues of Nile tilapia (*O. niloticus*) groups showed significant decrease (P<0.05) (152.96±0.13mg/g), (2.96±0.13 mg/g), respectively in fish after treated with different concentrations of NH_4Cl compared to control groups (204.91±0.13mg/g), (8.21±0.13 mg/g), respectively as in (Tables 3, 4). These results are in agreement with findings of Santhi [43] who reported that the short and long term exposure of *Tilapia mossambica* to sublethal concentration of ammonia reduced glycogen content in fish tissues. Anaerobic conditions caused by stress result in muscle glycogen breakdown [48]. Confinement stress, without sedation, has been observed to increase, indicative of glycogen mobilization and breakdown, have been associated with poor quality and rigor development of fish tissues [49]. Obula *et al*. [50] clear that ammonia could increase significantly in cellular respiratory metabolism towards an aerobiosis as a prelude towards adaptability to cope with the enhanced energy demands. The glycogen contents in the liver and muscular tissue was significantly decreased, but glucose and lactate concentrations in the blood were significantly increased [52].

Blood -α-amylase activity showed no significantly increase (P>0.05) (5.79±1.81 µmol/ml) in serum of Nile tilapia (*O. niloticus*) after treated with NH_4Cl compared to (4.67±1.71 µmol/ml) control group (Table 1). The level of amylase was significantly affected in the ammonium chloride. Amylase was notdifferent significantly among the ammonium chloride treatments and also in comparison with the control having the least concentration. The elevated amylase activity has been reasoned to result from pancreatitis or from damaged amylase secretary cells according to Skjervold *et al*. [49]. Comparable to this study, both short and long term contamination of the Indian carp *Cirrhina mrigala* by lead acetate led to increased hepatic amylase activity [26]. Increased hepatic and muscle amylase activities were also reported in a freshwater fish, *Torputitora,* stemming from a polluted river caused by heavy metals load including chromium [27].

LDH activity in serum, liver and muscles tissues of Nile tilapia after treatment with NH_4Cl showed significant increase (P<0.05) [serum (58.60±0.16µmol/ml) liver (67.63±0.15µmol/g), muscle (47.73±0.55 µmol/g) compared to untreated group (9.65±0.29µmol/ml), (28.55±0.36µmol/g), (18.95±0.26 µmol/g) respectively. These results are in agreement with that of SuGden and Newsholme [40] who stated that ammonium ion stimulate glycolysis through the activation of phosphofructokcinase. In line with this, the LDH activity was increased in all the tissues glycolytic rate was stepped up during ammonia toxicity [23]. Ammonia is known to deplete citric acid cycle intermediates along with mitochondrial swelling [41]. The inhibition of LDH enzymes would result in the accumulation of metabolic intermediates in the liver therefore causing physiological stress in fish, which eventually lead to mortality if exposure is prolonged as suggested by Das *et al*. [45]. Ammonia elevation leads to a depletion of cerebral α-ketogluterate and thus affects citric acid cycle resulting in decreased energy output [46].

G6PDH activity in blood serum, liver and muscle tissues of Nile tilapia was (69.19±1.14µmol/ml), liver (79.19±1.14µmol/g),muscle(65.14±1.33µmol/g)]increased significantly (P<0.05) than the levels of G6PDH in control group (22.54±1.92µmol/ml), (27.54±1.92µmol/g), (32.94±1.57µmol/g) (Tables 1, 2, 3). The result are in agreement with the increase of G6PDH activity in liver tilapia exposed to different concentration of NH3 [37], the degree of increase in activity was positively related to ammonia concentration [37]. The stimulation effect of cadmium on the gill, liver and kidney tissues of trout, the G6PDH activity levels were stimulated by approximately 60% in gills, 68% in liver and 67% in kidneys, over the base-line enzyme activity of the control groups during the 7 day experimental period [39]. In addition, G6PDH is a regulatory enzyme in NADPH-dependent xenobiotic biotransformation and defenses against oxidative damage and is very sensitive to inactivation by chronic exposure to pollutants in highly contaminated marine habitats [36].

Cortisol concentrations in the present study showed significant increase ($P<0.05$) in blood serum of Nile tilapia (*O. niloticus*) treated with NH_4Cl (79.06±0.03ng/ml) compared to control group (18.09±0.08 ng/ml). Plasmacortisol has therefore been monitored as a general index of stress, Goss and Wood [15] stated that cortisol influences an array of physiological parameters, including carbohydrate and hydromineral balance, mobilization of amino and fatty acids from cellular stores, gluconeogenesis and plasma protein production. An increase in cortisol level was seen when goldfish (*Carassius auratus* L.) were exposed to high ammonia (4-10 days) by Sinha *et al*. [16]. Cortisol was also increased significantly in *Cyprinus carpio* after exposure to ammonia; plasma cortisol level might have resulted from the release of cortisol from the internal tissue as amechanism of coping up with stress or impaired immune function [17]. Failure to suppress activation of the hypothalamo-pituitary-interrenal (HPI) axis during stress results in a release of cortisol which in turn causes various secondary stress responses, including increases in circulating levels of glucose and lactate [18]. Stress induced elevations in plasma cortisol are known to suppress immunological capacity in channel catfish [19]. Plasma cortisol, increased significantly ($P<0.05$) followed by acute oxygen depletion and high ammonia in PC and isoeugenol treatment catfish [20]. Tomasso *et al*. [21] found plasma corticosteroids increased four to five-fold as a result of exposing channel catfish to NH_3-N.

T3 and T4 concentrations was (10.21±0.05 ng/ml), (3.44±0.03ng/ml) respectively, increased significantly ($P<0.05$) in blood serum of Nile tilapia (*O. niloticus*) treated with NH_4Cl compared to untreated control (2.50±0.19 ng/ml), (6.11±0.09 ng/ml) respectively. Thyroid hormones play an important role in the growth and development of larvae and juvenile fish. Whole-body thyroxin (T4) in fish exposed to 100 mg/L ammonium perchlorate was elevated compared with control fish, but 3,5,3-triiodothyronine (T3) was not significantly affected in any treated group [55]. The sub-chronic selenium exposure increase plasma T3, T4 of tilapia [56]. Earlier studies have shown that, thyroid activity fluctuates in response to various environmental stimuli [53]. According to Silberman *et al*. [12], decreases in serum thyroid hormone levels due to chronic mild stress have been observed to negatively modulate T-cell response. Thyroid hormones are generally associated with an increase in metabolic rate and are usually reduced during periods of food deprivation as a means to conserve energy [54].

CONCLUSION

The Nile tilapia (*Oreaucromis niloticus*) is considered one of the chief edible fishes in all regions. Ammonia is one of the serious problems in fish culture especially in recirculation systems, aquaria and fish ponds. In freshwater fish, some biochemical changes takes place under the effect of ammonia toxicity, cortisol, T3, T4 concentration in serum and the G6PDH, LDH, Amylase activity in serum and tissues. Also lactic acid, pyruvic acid, glycogen content in serum and tissues theses are thought to be involved in the carbohydrate metabolism and since this biochemical is apparently disrupted by ammonia toxicity. The present study, it is concluded that ammonia is highly toxic has a profound influence on the carbohydrate metabolism of fish.

REFERENCES

1. Whitfield, M., 1974. The hydrolysis of ammonium ions in sea water a theoretical study. J. Mar. Biol. U.K., 54: 565-580.
2. Bower, C.E. and J.P. Bidwell, 1978. Ionization of ammonia in seawater: effects of temperature, pH and salinity. Journal of Fish Research Board Can., 35: 1012-1016.
3. Thurston, R.V., C. Chakoumakos and R.C. Russo, 1981. Effect of fluctuating exposures on the acute toxicity of ammonia to rainbow trout (*Salmo gairdneri*) and cutthroat trout (*S. clarki*). Water Res., 15: 911-917.
4. Haywood, G.P., 1983. Ammonia toxicity in teleost fishes: a review. Can. Tech. Rep. Fish. aquat. Sci., No., 1177.
5. Ruffier, P.J., W.C. Boyle and J.K. Kleinschmidt, 1981. Short-term acute bioassays to evaluate ammonia toxicity and effluents standards. J. Water Poll. Contr. Fed., 53: 367-377.
6. Handy, R.D. and M.G. Poxton, 1993. Nitrogen pollution in mariculture: toxicity and excretion of nitrogenous compounds by marine fish. Rev. Fish Biol. Fish., 3: 205-241.
7. Person-Le-Ruyet, J., R. Galland, A. Le Roux and H. Chartois, 1997. Chronic ammonia toxicity in juvenile turbot *Scophthalmus maximus*. Aquaculture, 154: 155-171.
8. Redner, B.D. and R.R. Stickney,1979. Acclimation to ammonia by *Tilapia aurea*. Trans. Am. Fish. Soc., 108: 383-388.

9. Aysel, C.K.B. and K. Gulten, 2005. The acute toxicity of ammonia on tilapia (*Oreochromis niloticus* L.) larvae and fingerlings. Turk. J. Vet. Anim. Sci., 29: 339-344.
10. Francis-Floyd, R. and C. Waston, 1996. Ammonia. UF/IUFAS, pp: 1-6.
11. Hargreaves, J.A. and S. Kucuk, 2001. Effects of unionized ammonia fluctuation on juvenile hybrid striped bass, channel catfish and blue tilapia. Aquaculture, 195: 163-181.
12. Silberman, D.M., M. Wald and A.M. Genaro, 2002. Effects of chronic mild stress on lymphocyte proliferative response. Participation of serum thyroid hormones and corticosterone. J. Int. Immunoipharmacol., 2: 487-497.
13. Romi, D., S. Indraneel, P. Suman, B. Arindam and S. Gaurisankar, 2006. Immunosuppression, hepatotoxicity and depression of antioxidant status by arecoline in albino mice. Toxicol., 227: 94-104.
14. Zarejabad, A.M., M. Sudagar, S. Pouralimotlagh and K.D. Bastami, 2009. Effects of rearing temperature on hematological and biochemical parameters of great sturgeon (*Husohuso linnaeus*, 1758) juvenile. Comp.Clin Path., 19: 367-371.
15. Goss, G.G. and C.M. Wood, 1988. The effects of acid and acid/aluminum exposure on circulating plasma cortisol level and other blood parameter in the rainbow trout, *Salmo gairdneri*. J. Fish Biol., 32: 63-76.
16. Sinha, A.K. Liew, Hon Jung Diricx, Marjan Kumar, Vikas Darras, Veerle M. Blust and Ronny De Boeck, Gudrun, 2012. Combined effects of high environmental ammonia, starvation and exercise on hormonal and ion-regulatory response in goldfish (*Carassiusauratus* L.). Aquatic Toxicol., 109: 33- 46.
17. Chezhian, A. and D. Senthamil Selvan, 2012. Hormonal responses in fresh water fish *Cyprinus carpio* var. Communis (Linnaeus, 1758) exposed to ammonia toxicity. International Journal of Environmental Biology, 2(3): 142-145.
18. Rotllant, J., P.H. Balm, J. Perez-Sanchez, S.E. Wendelaar-Bonga and L. Tort, 2001. Pituitary and interrenal function in Gilthead Sea bream (*Sparus aurata* L., Teleostei) after handling and confinement stress. Gen. Comp. Endocrinol., 121: 333-342.
19. Davis, K.B., B.R. Griffin and W.L. Gray,2003. Effect of dietary cortisol on resistance of channel catfish to infection by *Ichthyophthirius multifiliis* and channel catfish virus disease. Aquaculture, 218: 121-131.
20. Brian C. Small, 2004. Glucose and lactate dynamics in channel catfish *Ictalurus punctatus* exposed to three stressors. Aquaculture, 238: 469-481.
21. Tomasso, J.R., K.B. Davis and B.A. Simco, 1981. Plasma corticosteroid dynamics in channel catfish (*Ictalurus punctatus*) exposed to ammonia and nitrite. Can. J. Fish. Aquat. Sci., 38: 1106-1112.
22. Lott, J.A., S.T. Patel, A.K.Sawhney, S.C. Kazmierczak, J.E. Love and Jr.,1986. Assays of serum lipase analytical and chemical considerations. Clin. Chem., 32: 1290-1302.
23. Lott, J.A. and C.J. LU, 1991.Lipase isoforms and amylase isoenzymes: assays and application in the diagnosis of acute pancreatitis. Clin. Chem., 37: 361-368.
24. Yang, R.W., Z.X. Shso, Z.Y.Y. Chen, Yin and W.J. Wang, 2005. Lipase and pancreatic amylase activities in diagnosis of acute pancreatitis in patients with hyperamylasemia. Hepatobiliary Pancreat Dis Int., 4: 600-603.
25. Yousafzai, A.M. and A.R. Shakoori, 2011. Hepatic responses of a freshwater fish against aquatic pollution. Pakistan Journal of Zoology, 43: 209-221.
26. Mujeeb, K.A., 1985. M.Sc. Thesis. Toxic effects of lead on the liver and muscle of a freshwater fish. Department of Zoology, University of the Punjab, Lahore, Pakistan.
27. Yousafzai, A.M. and A.R. Shakoori, 2007. Toxic effects of aquatic pollution on the white muscle of mahaseer, *Torputitora* (Hamilton) from River Kabul. NWFP, Pakistan. Pakistan Journal of Zoology, 39: 215-227.
28. Zeynab Abedi, Fatemeh Hasantabar, Mohammad, K. Khalesi and Sedigheh Babaei, 2013. Effect of Sublethal Concentrations of Cadmium, Lead and Chromium on Some Enzymatic Activities of Common Carp; *Cyprinus carpio*. World Journal of Zoology, 8(1): 98-105, ISSN 1817-3098.
29. Sultana M. and V.S. Lomte, 1997. Environment and Ecology, 15(4): 803-809.
30. Mukke, V.K., 2012. Mercury chloride and copper sulphate induced alterations in enzyme secretory activity of freshwater crab, *Barytelphusa guerini*. Journal of Chemical and Pharmaceutical Research, 4(2): 1308-1311.
31. Lehninger, A.L., D.L. Nelson and M.M. Cox, 2000. Principles of Biochemistry. 2nd ed., Worth Publishers Inc. New York, pp: 558-560.

32. Kletzien, R.F., P.K.W. Harris and L.A. Foellmi, 1994. Glucose- 6- phosphate dehydrogenase: A housekeeping enzyme subject to tissue- specific regulation by hormones, nutrients and oxidant stress. FASEB J., 8: 174.181.
33. Tian, W.N., I.D. Braunstein, J.D. Pang, Q.C.K.M. La Stuhlmeier, X. Tian and R.C. Stanton, 1998. Importance of glucose-6-phosphate dehydrogenase activity for cell growth. J. Biol. Chem., 273: 10609-10617.
34. Beutler, E., 1978. Hemolytic anemia in disorders of red cell metabolism. Plenum medical book company, New York, USA, ISBN, 13: 23-169.
35. Yuregir, G.T., K. Aksoy, A. Arpaci, I. Unlukurt and A. Tuli, 1994. Studies on red cell glucose 6-phosphate dehydrogenase: Evaluation of reference values. Ann.Clin. Biochem., 31: 50-55.
36. Winzer, K., C.J.F. Van Noorden and A. Angela, 2002. Glucose-6-phosphate dehydrogenase: the key to sex-related xenobiotic toxicity in hepatocytes of European flounder (*Platichthy sflesus*L.) Aquatic Toxicol., 56(4): 275-288.
37. Hegazi, M.M., 2011. Effect if chronic exposure to sublethal of ammonia concentrations on $NADP^+$-dependent dehydrogenases of Nile tilapia liver. Egypt J. Aquat. Biol. & Fish., 15(1): 15-28.
38. Cankaya, M., M. Sisecioglu, M. Ciftci and H. Ozdemir, 2011. Effects of some metals ions on trout liver glucose -6- phosphate dehydrogase. Research J. Environ. Toxicol., 5(6): 385-391.
39. Olcay Hisar, Adem Yavuz Sönmez, ŞükrüBeydemir, Şükriye Aras Hisar, Telat Yanik and Tom Cronin, 2009. Kinetic Behavior of Glucose 6-Phosphate Dehydrogenase and 6-Phosphogluconate Dehydrogenase in Different Tissues of Rainbow Trout (*Oncorhynchus mykiss*) Exposed to Non-Lethal Concentrations of Cadmium. ACTA VET. BRNO, 78: 179-185.
40. Su Gden, P.H. and E.A. Newsholme, 1975. Effects of ammonium, inorganic phosphate and potassium ions on the activity of phosphofructokinases from muscle and nervous tissues of vertebrates and invertebrates Biochem., 150: 133-142.
41. Chow, K.W. and V.G. Pond, 1972. Biochemical and morphologicalswelling of mitocihondria in ammonia toxicity. Soc. Exptl. Biol. Med., 139: 150-156.
42. Reddy, M.S. and K.V. Rao, 1991. Phosphamidon, methol parathion and dichlorves impact on tissue oxidative metabolism in panaeid prawn, metapenaeus monocerus. Biochem. Int., 23: 439-446.
43. Santhi, K., 1991. Hitological and metabolic changes in selected tissues of fish, *Oreochromis mossambicus* under chronic ammonia stress. PhD Thesis, S.V. University, Tirupati andhra Pr., India.
44. Ceron, J.J., E. Sancho, M.D. Ferrando, C. Gutierrez and E. Andreu, 1997. Changes in carbohydrate metabolism in the eel, *Anguilla anguilla* during short-term exposure to diazinon. Toxicol. Environ. Chem., 60: 201-210.
45. Das, P.C., S. Ayyappan and J.K. Jena, 2004. Acute toxicity of ammonia and its sublethal effect on selected hematological and enzymatic parameters of mrigal, *Cirrhinus mrigala*. Aquaculture Research, 35: 134-143.
46. Bessman, S.P., J. Fazeks and F.A.N. Brssman, 1954. Uptake of ammonia by thebrain in hepatic coma. Proc. Soc. Exptl. Biol. Med., 85: 66-67.
47. Prior, R.L. and W.J. Visek, 1972. Effects of urea hydrolysis on tissue metabolites concentrations in rats. Am. J. Physiol., 223: 1143-1149.
48. Thomas, P.M., N.W. Pankhurst and H.A. Bremner, 1999. The effect of stress and exercise on post-mortem biochemistry of Atlantic salmon and rainbow trout. J. Fish Biol., 54: 1177-1196.
49. Skjervold, P.O., S.O. Fjaera, P.B. Oestby and O. Einen, 2001. Live-chilling and crowding stress before slaughter of Atlantic salmon (*Salmo salar*). Aquaculture, 192: 265- 280.
50. Obula K.P. Reddy, E. SheshaLatha, D. Muthuraj and P. Neeraja, 1996. Certain metabolic modulation in carbohydrate metabolism of fry, *Cyprinus carpio* on ammonia stress. J. Indian Fish Soc. India, 28(2): 42-45.
51. Jabeen, B.S., 1985. Computation of tissue specific modulation of fresh water teleost *Tilapia mossambicus* under ambient ammonia stress. University, Tirupati andhra Pradesh, India.
52. Lusková, V.M., J. Svoboda and Kolářiová, 2002. The Effect of Diazinonon Blood Plasma Biochemistry in Carp (*Cyprinus carpio* L.). Acta Vet. Brno., 71: 117-123.
53. Leatherland, J.F. and C.Y. Cho, 1985. Effect of rearing density on thyroid and interrenal gland activity and plasma hepatic metabolite levels in rainbow trout, *Salmo gairdneri*. J. Fish Biol., 27: 583-592.
54. Suchiang, P. and B.B.P. Gupta, 2011. Effects of Partial and Full Feed Restriction on the Plasma Levels of Thyroid Hormones and Testicular Activity in the Male Air-breathing Catfish, *Clarias gariepinus* during different Phases of the Breeding Cycle. Int. J. Biol., 3(2): 32-42.

55. Crane, H.M., D.B. Pickford, T.H. Hutchinson and J.A. Brown, 2005. Effects of ammonium perchlorate on thyroid function in developing fathead minnows, *Pimephales promelas*. Environ Health Perspect., 113(4): 396-401.
56. Hossam, H.H. Abbas and Mahmoud M.N. Authman, 2009. Effect of accumulated selenium on some physiological parameters and oxidative stress indicators in tilapia fish *Oreaochromis* spp. American-Eurasian J. Agric. and Environ. Sci., 5(2): 219-225.
57. Randall, D.J. and T.K.N. Tsui, 2002. Ammonia toxicity in fish. Marine Poll. Bull., 45: 17-23.
58. Emerson, K.R., R.C. Russo, R.E. Lund and R.V. Thurston, 1975. Aqueous ammonia equilibrium calculations: effect of pH and temperature. J. Fish. Res. Board Can., 32: 2377-2383.
59. Huggett, A.S. and D.A. Nixon, 1957. Use of glucose oxidase, 1 peroxidase and O-dianisidine in determination of blood and urinary glucose. Lancet, 24(73): 368-370.
60. Bergmeyer, H.U., 1974. Methods of Enzymatic Analysis, 3rd. Weinheim: Springer-Verlag.
61. Hassid, W..J. and S. Abraham, 1957. Chemical procedure for analysis of polysaccharides. In: Methods in E nzymology (Ed. S.P. Colowick and N.O. Kalpan). Vol. III, Academic Press Inc., New York, USA, pp: 35-36.
62. WHO (World Health Organization), 1967. Standardization of procedures for the study of glucose-6-phosphate dehydrogenase. WHO Tech. Rep. Ser., pp: 366-370.
63. Hajzer, S. and J. Jagelkova, 1988. Proposition of standard method for catalytic concentration of lactate dehydrogenase in plasma diagnosis. Biochem. Clin. Bohemoslov., 17: 371-379 (Slovak).
64. Rick, W. and H.P. Stegbauer, 1974. Amylase measurement of reducing groups. In: Methods of Enzymatic Analysis (Ed. H.V. Bergmeyer), 2nd Ed., Vol. II. Academic Press, New York, USA, pp: 885-889.
65. Small, B.C. and K.B. Davis, 2002. Validation of a time-resolved fluoroimmunoassay for measuring plasma cortisol in channel cat fish *Ictalurus punctatus*. J. W orld Aquacult. Soc., 33: 184-187.
66. Plohman, J.C., T.A. Dick and J.G. Eales, 2002. Thyroid of lake sturgeon, *Acipenser fulvescens*: I. Hormone levels in blood and tissues. Gen. Comp. Endocrinol., 125: 47-55.
67. SAS Institute, 1989. SAS/SAT User's guide-version 6. Cary.
68. Duncan, D.B., 1955. Multiple range and multiple F tests. Biometrics, 11: 42-49.