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Research Article

Assessment of Acute Ammonia Stress on Growth Performance, Hematological, Serological, and Histopathological Changes in Grass Carp (*Ctenopharyngodon Idella*)

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Abstract

Background: Fish are a vital source of high-energy nutrition, especially for economically disadvantaged populations. Grass carp (*Ctenopharyngodon idella*), prized for their quality meat, are widely consumed worldwide including in Pakistan. However, environmental ammonia pollution from aquaculture poses significant risks to their health, impacting physiological functions, growth, and survival. Understanding ammonia toxicity is crucial for sustainable aquaculture and environmental protection. *Objectives:* This research aimed to investigate the toxicity of ammonium hydroxide on the growth, hematological parameters, and histopathological changes in gills of grass carp. *Methods:* Grass carp were exposed to various concentrations of ammonium hydroxide (0, 1, 1.5, and 2 mg/L) for a duration of 14 days in glass aquaria. Growth performance, hematological parameters (including Hb, RBC, MCHC, WBC, MCV, MCH, and cholesterol levels), and serological parameters (such as bilirubin, creatinine, uric acid, SGPT, and alkaline phosphate) were assessed. Histopathological analysis of the gills was conducted to observe any abnormalities. *Results:* The results revealed that increased concentrations of ammonium hydroxide led to retarded growth in grass carp, with the lowest growth rate observed in the experimental group exposed to 2 mg/L of ammonium hydroxide and the highest growth rate in the control group (0 mg/L). Hematological parameters indicated a significant decrease in Hb, RBC, MCHC, and WBC counts with increasing levels of ammonium hydroxide, while MCV, MCH, and cholesterol levels increased. Serological parameters showed elevated levels of bilirubin, creatinine, uric acid, SGPT (ALT), and alkaline phosphate with increasing ammonium hydroxide concentrations. Histopathological analysis of the gills revealed significant effects, including chloride cell hyperplasia, sideway swimming, hyperemia, epithelial lifting, curling of secondary lamellae, lamellar fusion, and shortening of secondary lamellae.

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Keywords

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1. Introduction

Fish are one of the best sources of food for both humans and animals due to their high energy value. Their consumption plays an essential role in controlling cardiovascular diseases [9]. Fish are often referred to as a rich source of nutrients for economically disadvantaged populations because they provide nutrients with high biological value, especially protein and fat. The nutritional value of fish can be determined by their major nutrients, protein, and fats [23].

Grass carp (*Ctenopharyngodon idella*) is one of the major carp species commonly consumed in Pakistan. Due to its high-quality meat and taste, grass carp has gained attention from fish culturists worldwide [19]. Grass carp are not only herbivores but also feed on animal origin food when bred under laboratory conditions and provided with both plants and animals. They consume 76% animal food and 24% plant-based food [27].

Ammonia is a toxic compound present in two forms in the environment: ionized ammonia (NH_4^+) and unionized ammonia (NH_3^0). It is one of the most toxic compounds for fish health and production. Its toxicity can be influenced by various factors, including water temperature, pH, exposure times, fish species, fish mass, and fish life stages [1]. Ammonia and urea are the two main excretory products of teleost fish, but ammonia is the major excretory product, accounting for about 75 to 90% [20]. It is produced naturally by the decomposition of organic matter and is also eliminated by fish during excretion. In fish, ammonia is produced as a waste product during protein digestion and is primarily removed from the body through the gills, with a small amount excreted in urine [21].

Ammonia produced by fish can be converted by bacteria into nitrate and nitrite, which are harmless to fish survival in natural aquatic environments and are utilized by algae and plants [16]. The toxic effects are primarily due to unionized ammonia (NH_3^0), as it is more soluble in lipids and can easily penetrate lipid bilayer membranes, such as gill membranes, and enter tissues [3]. This compound is toxic to all aquatic vertebrates, causing coma, convulsions, and even death due to elevated levels of NH_4^+ displacing potassium, depolarizing neurons, and leading to calcium influx and cell death in the central nervous system [32].

Nowadays, there has been a significant increase in the aquaculture industry, which provides us with more food but is indirectly contributing to the pollution of the aquaculture environment. For instance, due to the high levels of fish farming and the overuse of synthetic feed, there has been a rise in the total ammonia nitrogen levels in water, leading to severe ecological problems [7]. The grass carp industry ranks as the second-largest fisheries plant in the world, trailing only behind the silver carp industry, accounting for 14.7% of the world's fish culture income, with an average yearly increase of 14% in China [17]. Nitrogen compounds have been identified as major metabolic waste products in high-density aquaculture systems [13].

Continuous exposure of fish to high levels of ammonia affects their normal physiological activities. Ammonia can elevate the concentrations of glucose (GLU), cortisol (COR), and cholesterol (TC), thereby increasing the body's chemical reactions and protein production speed in fish to resist the oxidative harm caused by ammonia nitrogen to tissues [25]. Ammonia accumulation may also alter growth, increase oxygen consumption, negatively impact normal levels of hemolymph proteins and free amino acids, and increase fish lethality [4]. It is considered a toxic compound because it disrupts the brain and spinal cord, causing ammonia intoxication, which includes convulsions and death [26].

Previous research on common carp has shown that ammonia toxicity negatively affects the normal serum cortisol and malondialdehyde levels while reducing thyroid hormone levels, superoxide dismutase (SOD), and catalase [7]. Acute ammonia toxicity can significantly reduce serum antioxidant enzyme activities and increase malondialdehyde concentrations in grass carp. Further diagnosis of grass carp liver has shown that antioxidant and apoptosis pathways are greatly activated by ammonia [7, 15]. The negative effects of ammonia toxicity include nervous disorders, increased mucus production in both gills, sideway swimming, convulsions, loss of equilibrium, gill bleeding, necrosis, kidney injury, circling, spiraling movements, and ultimately death [36].

High levels of ammonia can alter the hematology of carp fish, leading to decreases in erythrocyte quantity and increases in leukocyte and hematocrit amounts. Furthermore, oxyhemoglobin levels decrease, while general hemoglobin, deoxyhemoglobin, and methemoglobin levels increase. Ammonia can also cause denaturation effects of acids and alkalis [18, 31]. Additionally, ammonia toxicity can lead to respiratory diseases such as gasping, flaring opercula, and asphyxia, ultimately resulting in the death of the fish [24]. Infected ammonia fish exhibit symptoms such as inflamed eyes or anus, purple, red, or bleeding gills, red streaks on fins or body, compressed appearance, and darkening of the body [30].

2. Materials and Methods

2.1. Sample Collection

Healthy grass carp (*Ctenopharyngodon idella*) specimens were procured from a hatchery located in district Mardan, Khyber Pakhtunkhwa, Pakistan. The fish were then transferred to well-aerated glass aquaria and allowed to acclimate to laboratory conditions for a period of one week. Following the acclimatization period, all male fish with an average weight of 82±2 g were randomly allocated into eight separate aquaria, each containing 60 liters of water, with a stocking density of 6 fish per aquarium. Subsequently, the fish were exposed to varying concentrations of ammonia, administered as ammonium hydroxide, at levels of 0, 0.5, 1, and 1.5 mg, over a duration of 14 days. Continuous aeration of all aquaria was ensured using air pumps, and regular removal of fish waste was conducted throughout the experimental period [36].

2.2. Water Quality Parameters

For monitoring water quality parameters, water samples were obtained from the depth of each aquarium. Temperature and dissolved oxygen concentration were monitored regularly using a DO meter. Additionally, the pH of the water was measured daily using a digital pH meter. Throughout the experimental duration, the temperature ranged from 20 to 21 ºC, while the pH ranged from 7 to 8. Ammonia concentration was determined using a Multiparameter ion analyzer. It is worth noting that all water quality parameters remained within favorable ranges for the survival of the fish throughout the experiment [36].

2.3. Hematological and Biochemical Analysis

At the conclusion of the experiment, blood samples were collected from the fish and divided into two types of tubes: one containing heparin as an anticoagulant agent, and the other without heparin. The non-heparin blood tubes were centrifuged at 4000 rpm for 10 minutes at room temperature to separate the plasma for the calculation of serological parameters. The obtained plasma was used for the measurement of HDL and LDL cholesterol, triglycerides, urea, creatinine, ALT, bilirubin, and ALP levels.

On the other hand, the heparin blood tubes were utilized for the determination of mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count, neutrophil count, lymphocyte count, monocyte count, eosinophil count, erythrocyte count, hemoglobin concentration, hematocrit, and mean corpuscular volume [30].

2.4. Histological Analysis

For histological analysis, the fish were dissected, and the gills were collected at the conclusion of the experiment. All collected gills were immersed in 10% formalin, a widely used fixative for tissue preservation. The organs were left in the fixative solution for a period of 15 hours. Subsequently, the specimens underwent dehydration by immersion in a series of ethanol solutions. To remove excess fats from the specimens, xylene was employed as a clearing agent.

For the preparation of microtome sections, tissue blocks were embedded in paraffin wax. Thin sections of tissue were then obtained using fully automatic microtomes for subsequent light microscopic analysis [22].

2.5. Statistical Analysis

All data from the research were presented as means and standard deviations (SD). For the analysis of group comparisons, one-way ANOVA was performed using SPSS software, version 2.5. The results are displayed in tabular form, with all values expressed as means and standard deviations.

3. Results

3.1. Growth Rate of Grass Carp When Exposed to Ammonium Hydroxide for 14 Days

Table 1 described the significant changes in the growth rate of fish when exposed to various concentrations of ammonium hydroxide for consecutive 14 days. Final growth rate of control group was upper than those fish which were exposed to various levels of ammonium hydroxide for 14 days. Fish growth rate was retarded to the concentration of ammonium hydroxide and lowest growth were observed at highest (2 mg) concentration of ammonium hydroxide in experimental group 3.

Table 1. *Growth rate of Grass Carp when exposed to various concentration of ammonium hydroxide for 14 days.*

	Doses/ Parameters	Initial weight (g)	Final weight (g)	Weight gain (g)	Weight gain%
Male	Control	82.2 ± 19.7	85.5 ± 20.8	3.3 ± 28.65	4.01 ± 34.86
	Group 1	82.2 ± 19.7	79.5 ± 18.46	−2.7 ± 26.99	−3.28 ± 32.83
	Group 2	82.2 ± 19.7	76.7 ± 20	−5.5 ± 28.07	−6.69 ± 34.15

Doses/ Parameters	Initial weight (g)	Final weight (g)	Weight gain (g)	Weight gain%
Group 3	82.2 ± 19.7	73.66 ± 20.1	−8.54 ± 28.14	−10.39 ± 34.23

3.2. Hematological Parameters of Grass Carp When Exposed to Ammonium Hydroxide for 14 Days

The hematological parameters presented in *Table 2* indicate that Hb, RBC, MCHC, TLC, neutrophils, monocytes, lymphocytes, and eosinophils significantly decreased as the ammonium hydroxide concentration increased. Conversely, MCV and MCH increased with rising ammonium hydroxide levels, with the highest values observed at a concentration of 2 mg/L in experimental group 3.

Table 2. *Hematological parameters of Grass Carp when exposed to various concentration of ammonium hydroxide for 14 days.*

Groups	Hb (g/dl)	RBC (mg/dl)	MCV (FL)	MCH (pg)	MCHC (g/dl)	TLC $\times 10^3/\mu l$	Neutrophils (%)	Monocytes (%)	Lymphocytes (%)
Control	4.5 ± 0.5	3.5 ± 0.4	131.5 ± 2.1	40.5 ± 1.8	48.1 ± 1.3	5.8 ± 0.6	3 ± 1	2.9 ± 0.6	90.2 ± 2.2
Group 1	3.2*** ± 0.4	2.5*** ± 0.4	132.9 ± 2.1	42.1 ± 1.63	44*** ± 0.9	5.3*** ± 0.4	2.1 ± 1	2.3**± 0.6	86.7 *± 2.9
Group 2	2.4*** ± 0.5	1.6 *** ± 0.3	134 ± 2.1	42.9 ± 2.4	41.8*** ± 0.9	4.6*** ± 0.5	1.3 ± 0.8	1.3 **± 0.6	85* ± 2
Group 3	1.8*** ± 0.5	1*** ± 0.2	134.5 ± 2	43.3 ± 0.7	39*** ± 0.6	3.9*** ± 0.6	1 ± 0.4	1** ± 0.6	83.3* ±1

Data are presented in Mean ± Standard deviation and *P* value (**p<0.05, **p<0.01, ***p<0.001)*

3.3. Serological Parameters of Grass Carp When Exposed to Ammonium Hydroxide for 14 Days

The data presented in *Table 3* show that the cholesterol levels in grass carp decrease as the concentration of ammonium hydroxide increases, with the lowest values observed in experimental group 3, which was exposed to the highest concentration. Conversely, bilirubin levels increase with higher concentrations of ammonium hydroxide.

Table 3. *Serological parameters of Grass Carp when exposed to various concentration of ammonium hydroxide for 14 days.*

Parameters	Creatinine (mg/dl)	Uric acid (mg/dl)	SGPT (µ/l)	ALP (µ/l)
Control	38.9 ± 0.8	38.3 ± 0.8	29.7 ± 6.8	183.3 ± 23.7
Group 1	39.6 ± 10.5	38.9 ± 10.6	31 ± 6.3	185 ± 19.8
Group 2	39.8 ± 10.6	39.8 ± 10	31.8 ±6.9	186.7 ± 20
Group 3	40.4 ± 10.6	40.3 ± 10.5	32.8 ± 6.7	187.8 ± 19.7

3.4. Histopathological (gills) Effects When Exposed to Various Level of Ammonia Hydroxide for 14 Days

The data presented in figure 1 shows significant changes in gills when exposed to different concentrations of ammonium hydroxide for 14 days. Severity of the changes are in group 3 while lowest changes are in group 1. These changes are hyperemia, lamellar fusion, shortening of secondary lamella and epithelial lifting.

4. Discussion

Grass carp is a major carp species commonly consumed in Pakistan due to its high-quality meat and taste. These fish typically feed on small aquatic weeds and grasses and produce ammonia as a secondary metabolite. In intensive fish farming, ammonia is a common toxic compound for fish health and production, largely originating from fish excretory products linked to their feeding behavior. In this study, grass carp were exposed to 0 mg, 0.5 mg, 1.5 mg, and 2 mg concentrations of ammonium hydroxide for 14 days. The results (*Table 1*) showed a reduction in growth rate in experimental groups, with the lowest growth observed in group 3, which had the highest ammonium hydroxide concentration. In contrast, the control group exhibited the highest growth performance, consistent with previous research findings.

Frances et al. reported a reduction in the final mass of silver perch when exposed to elevated ammonia levels (Frances *et al.*, 2000). Similar results were observed in juvenile spotted wolfish [14], European sea bass [11, 20], and Nile tilapia [2]. Yang *et al.*, (2011) and Sakala & Musuka (2014) also noted reduced growth in juvenile crucian carp and *Tilapia rendalli* due to ammonia exposure [35, 28]. Shin *et al.*, (2016) reported decreased growth in rockfish exposed to various ammonia concentrations [29].

The observed weight loss in ammonia-exposed fish is due to decreased appetite and disrupted normal body metabolism. Hematological parameters (*Table 2*) showed significant decreases ($P<0.05$) in RBC count, Hb, and MCHC in grass carp exposed to ammonium hydroxide. This may be due to damage to vital organs (gills, liver, spleen, and kidney) caused by ammonia toxicity, which impairs hematopoietic tissue in these organs [10].

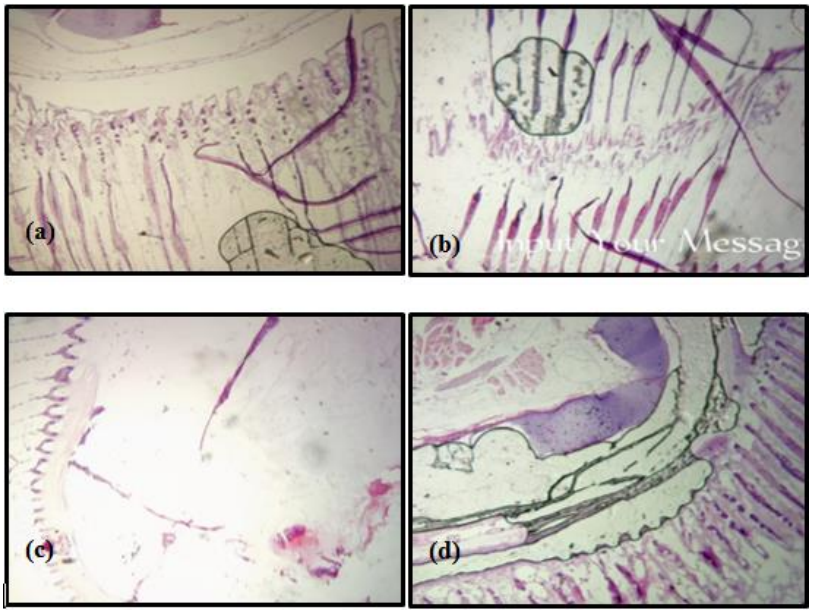

Figure 1. *Histopathological effects of different concentrations of ammonium hydroxide on fish gills (a) Control, (b) Group 1, (c) Group 2 and (d) Group 3.*

Previous studies have reported similar findings, including significant reductions in RBC count in *Cyprinus carpio* [32] and tilapia exposed to ammonia [6, 10]. El-Sherif & El-Feky (2008) observed reduced Hb levels in tilapia exposed to 0.15 mg NH3/L for 60 days, and Tilak *et al.*, (2007) reported decreased Hb concentration in *Cyprinus carpio* due to increased oxygen intake and methemoglobin levels from gill damage [12, 33].

In this study, MCV and MCH concentrations increased due to ammonia exposure (*Table 2*), likely from increased water levels in RBCs and reduced plasma chloride. A notable reduction in WBC count and its components was observed in grass carp exposed to ammonium hydroxide (*Table 2*), consistent with previous research [6, 10].

The study also found a significant reduction in total cholesterol levels, indicating its use for energy to combat am-

monia toxicity. Conversely, bilirubin, uric acid, creatinine, ALP, and ALT (SGPT) levels increased (*Table 3*), suggesting liver and kidney damage due to ammonia exposure. Similar results were reported by Shin *et al.*, (2016) and Vedel *et al.*, (1998) in rainbow trout and rockfish [29, 34].

Ammonia enters fish within 15 minutes of exposure, with gills being the first organ to respond due to their roles in respiration, osmoregulation, nitrogenous waste elimination, and acid-base balance [5, 8]. Histopathological analysis showed significant gill damage, including chloride cell hyperplasia, hyperaemia, epithelial lifting, curling of secondary lamella, lamellar fusion, and shortening of secondary lamella, with the severity increasing with higher ammonia concentrations. These findings are consistent with previous studies on Nile tilapia and immature blunt snout bream [5, 37].

5. Conclusion

It was concluded that increase in aquatic ammonia from their optimum level have negative effects on fish. In the present research work due to acute toxicity of ionic ammonia the main adverse effects of ammonium hydroxide on grass carp fish were the significant decrease in body growth rate, negatively change in blood parameters and damage of the normal histology of gills.

Abbreviations

GLU	Glucose
COR	Cortisol
TC	Total Cholesterol
SOD	Superoxide Dismutase
HDL	High-Density Lipoprotein
LDL	Low-Density Lipoprotein
ALT	Alanine Aminotransferase
ALP	Alkaline Phosphatase
SD	Standard Deviation
Hb	Hemoglobin
RBC	Red Blood Cells
WBCs	White Blood Cells
MCHC	Mean Corpuscular Hemoglobin Concentration
TLC	Total Leukocyte Count
MCV	Mean Corpuscular Volume
SGPT	Serum Glutamate Pyruvate Transaminase (Another Name for ALT)

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Ethical Approval and Consent: All procedures were in agreement with the declaration of Helsinki. The Advance Study and Research Board at University of Education, Lahore approved the protocol of the present study. Permission from Ethical Committee, University of Education, Lahore, was also taken for the research work.

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Conflicts of Interests

The authors declare no conflicts of interest.

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