TOXIC EFFECTS OF AMMONIA EXPOSURE ON GROWTH AND HEMATOLOGICAL RESPONSE OF *CLARIAS BATRACHUS* (LINNEAEUS, 1758)

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Abstract. In aquatic environments, ammonia nitrogen is the primary pollutant that depletes oxygen. In this study effects of ammonia exposure on growth and hematological parameters of *Clarias batrachus* was investigated. Fish with a mean initial total length of 13.33 ±1.08, 13.46±1.80, 13.53±0.94, 13.40±1.94, and 14.11±0.69 cm, and with a body weight of 22.36±5.84, 24.36±9.93, 23.38±5.17, 23.11±9.36 and 25.66±6.00 g were exposed for 3 months with ammonia concentrations (0, 0.25, 0.50, 0.75 and 1.00 mg/L), in T-o, T-I, T-II, T-III, and T-IV, respectively. A significant decrease was observed in mean values of growth, condition factor and various hematological parameters such as White Blood Cell (WBC), Hemoglobin (Hb), Red Blood Cell (RBC), Hematocrit (HCT), Platelets (PLT), Monocyte (Mon), Lymphocyte (LYM), and Mean Platelet Volume (MPV), while other hematological parameters including Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Mean Corpuscular Volume (MCV) and Red Cell Distribution Width Standard Deviation (RDW-SD) were significantly increased ($P< 0.05$) as determined by Duncan's multiple range test.
Keywords: *toxicity, fish, growth performance, hematological parameters, condition factor*

Introduction

In aquatic ecosystems, nitrogenous compounds have been increasing globally in recent years. These nitrogenous compounds significantly affect the health of aquatic organisms (Molayemraftar et al., 2022). Ammonia is the most toxic substance for aquatic animals, found in water as ammonia (NH_3) and ammonium ions (NH^{4+}) (Mirghaed et al., 2020). Its quantity depends relative to pH, salinity, and temperature. The NH_3 and NH^{4+} in combined form constitute the total concentration of ammonia (Kır et al., 2019). Due to high-fat solubility rate, ammonia passes through the cell membrane easily and affects the metabolic system (Foss et al., 2009), rate of growth (Li et al., 2014), blood circulatory system (Zhao et al., 2021), nervous system (Ching et al., 2009), respiratory system, immune system (Lia et al., 2014) and excretory system (Sinha et al., 2015).

In aquatic ecosystems, ammonia is the primary pollutant (Xia et al., 2018). Industrial waste, biological waste decomposition, agricultural run-off and industrial waste have toxic effects on aquatic organisms (Attah et al., 2021). The most important and common biomarkers are hematological parameters used for analyzing the functional and structural conditions of fish exposed to ammonia and pollutants (Burgos Aceves et al., 2019).

Hematological parameters analysis is considered reliable in the valuation of toxicity of various chemicals like ammonia in fish (Hoseini et al., 2019). Hematological parameters

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depend on the concentration and duration of ammonia exposure (Alimba et al., 2019). Prolonged ammonia exposure in high concentration induces quick changes in fish physiology and behavior and produces visible lesions on the fish body (Brucka-Jastrzebska and Protasowicki, 2005). Variations in the number of WBCs regarded as predictive tool or early indication in fish hematology disturbance (Oladokun et al., 2020).

The concentration of ammonia affects fish physiology depending on the feeding strategy and frequency of water exchange in the aquaculture system (Vaage and Myrick, 2022). Commonly, acute, and chronic exposure to ammonia causes an increase in blood ammonia concentration in fish rapidly (Gao et al., 2017).

The metabolism of protein produces ammonia as an excretory product in fish. This excretory waste (ammonia) is excreted in water through urine and gills (Wood et al., 2017). Environments that have high ammonia can cause physiological damage to aquatic animals, specifically fish. This high exposure can result oxidative stress by increasing the ammonia concentration (Guo et al., 2022).

A high level of ammonia stresses fish health and growth. Fish excreta contain ammonia, and its concentration in excretion directly depends on the feeding rate and protein levels. Factors like the water temperature, fish size, the duration of exposure and pH are responsible for increasing ammonia toxicity in fish. In the fish aquaculture system, ammonia is produced through decomposition of unconsumed fish food and faecal matter, which is a great risk for the fish culture industry. It results respiratory illness such as flaring opercula, gasping, asphyxia, and fish death (Chen et al., 2019).

Fish showed the symptoms of ammonia toxicity as; gills color turning red or purple, bleeding from gills, gill inflammation and eyes and anus inflammation. The fish body may appear darker in color and clamped, reddish stricken on fins and surface gasping (Fernandes and Mazon, 2003).

In aquatic animals, a high mortality rate, decreased growth performance, tissue damage and degeneration, and immune suppression in immunity can all result from excessive ammonia due to its toxic effects on blood and tissues. In addition to neurotoxicity, oxidative stress, and impairments in oxygen delivery, the ammonia exposure also results in hyperactivity, convulsions, and coma (Lemarie et al., 2004).

Ammonia exposure can cause a fast increase in ammonia concentration in blood (Egnew et al., 2019). Continuous exposure can accumulate ammonia in the body of fish to a toxic level. Ammonia directly affects the growth of aquatic animals (Hoseini et al., 2019). It decreases the disease resistance capacity of the fish body (Romano and Zeng, 2013) and affects animal physiology (Rajabiesterabadi et al., 2020). The high ammonia concentration can result oxidative damage, leading to fish mortality (Yousefi et al., 2020). Fishes are used as target organisms to study ammonia toxicity (Kim et al., 2019). Ammonia toxicity indicated synergistic or antagonistic effects in different species (Zhang et al., 2015). The high level of ammonia toxicity results severe problems such as gasping, asphyxia, flaring opercula, and fish mortality (Lin and Chen, 2009). It also affects the growth rate and reduces fish's disease resistance capability and food conversion ratio. The accumulation of ammonia may change the hemolymph protein concentrations and increase the mortality rate of fish (Barbieri and Bondioli, 2015). Hematological parameters are analyzed to observe physiological changes in aquatic animals. Factors that affect the hematology of animals are nutrition, gender, age, pollutants, ammonia, and aquatic environment. These hematological parameters have been used to evaluate fish's level of ammonia toxicity (Fazio, 2019; Singh et al., 2019).

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Clarias batrachus (walking catfish) is well-known freshwater fish species (Abdel-Tawwab et al., 2020). *C. batrachus* is used as a bioindicator species in many studies (Chen et al., 2019). Status of water quality, particularly nitrogenous compounds tolerance, has become an important issue for the culture of *C. batrachus*. It is necessary to enlarge our understanding of pollutant toxicity as exposure to ammonia in aquatic animals. This increases our knowledge and will result in improved aquaculture management. There is no data available about the effects of ammonia exposure on the growth and hematology of *Clarias batrachus*. Therefore, basic objective of the current study was to analyze the effects of ammonia exposure toxicity on the growth and hematology of *Clarias batrachus*.

Materials and Methods

Experimental Site and Design

Clarias batrachus fish with a mean initial total length of 13.57±1.45 cm and body weight of 23.77±7.97 g was obtained from Al-Raheem Fish Hatchery, Muradabad, Muzaffargarh, Pakistan (Lat. 30°20'0"N; Lon. 71°05'0"E) and transported to Animal House, BZU, Multan (Lat. 30°16'02.19"N; Lon. 71°30'05.76"E), Pakistan. Fish were held in a hauler for 2 weeks to acclimatize at room temperature. The fish were fed a diet comprising 35% crude protein with 4% of body weight of the fish, twice a day at optimum water quality parameters (*Table 1*). Ten fish from each treatment group were exposed to ammonia in a 50-L plastic hauler (T-o, T-I, T-II, T-III, and T-IV) with the concentrations 0, 0.25, 0.5, 0.75, and 1mgL^{-1}, respectively, following Shin et al. (2016) and Kim et al. (2017), for 3 months (March to May, 2021). Ammonium chloride (NH_4Cl) (Merck Germany) was dissolved in the plastic Hauler to make the respective concentrations. The plastic hauler water was thoroughly changed, after 15 days and the concentration maintained at set level.

Growth Performance

Fish samples body weight (g) and total length (cm) were measured after every 15 days throughout the experiment. The following equation calculated condition factors:

$$\text{Condition factor} = (\text{Weight}/\text{Lenght}^3) \times 100 \qquad \text{(Eq.1)}$$

Blood Sampling and Hematological Assay

A total of 50 fish samples (10 from each treatment) were selected to study blood sampling and hematological assay. Blood samples of experimental fish were collected from the caudal vein by 1mL disposable syringes and stored in EDTA vials at 4°C till the analysis of hematological parameters such as WBC, Hb, RBC, HCT, PLT, Mon, LYM, MPV, MCH, MCHC, MCV, and RDW-SD by Medonic Hematology analyzer (M-series M32).

Statistical Analysis

Pearson Correlation was used to study the effect of ammonia concentration on body weight of the fish. While ANOVA and Duncan's Multiple Range Test was used to assess the significant differences of hematological parameters among various studied treatments at the level of $P<0.05$ by using SPSS (20.0).

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Table 1. *Mean ± S.D values of various physico-chemical parameters of water during experiment*

Groups	Temp. (ºC)	Color	Odor and Ammonia	Dissolved Oxygen (mg/l)	pH	Chlorides (mg/l)	Light Penetration (cm)	Electrical Conductivity	Total Alkalinity (mg/l)	Total Hardness (mg/l)	Free Carbon Dioxide (ppm)	Total Dissolve Solid (ppm)
T-o	27.7±4.11	Muddy	Odorless /0	5.1±0.19	7.1±0.29	110±8.13	7±0.45	774±33.22	260±14.3	216±13.11	8.0±0.21	385±23.98
T-I	27.7±4.33	Muddy	Ammonia smell /1	5.8±0.47	7.0±0.26	108±8.04	8±0.46	665±31.11	260±14.4	216±13.17	6.0±0.23	332±23.72
T-II	27.7±3.32	Muddy	Ammonia smell /1.5	5.4±0.42	7.15±0.33	100±7.49	6±0.34	648±30.74	260±14.2	220±13.24	7.0±0.12	324±23.24
T-III	27.6±3.52	Muddy	Ammonia smell /2	5.7±0.32	7.2±0.35	74±6.22	7±0.46	691.8±32,12	270±15.2	215±13.33	6.0±0.18	346±23.65
T-IV	28±4.09	Muddy	Ammonia smell /3.0	4.9±0.18	7.2±0.34	80±7.11	No penetration	661±31.01	280±14.9	210±13.01	7.0±0.19	330.5±23.22

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Results and Analysis

The study was conducted to analyze the ammonia exposure effect for 90 days on the growth and hematological parameters of *Clarias batrachus.* It was observed that significant decreasing trend in growth pattern was observed in all treatment groups (T-I, T-II, T-III, and T-IV) with the concentrations (0.25, 0.5, 0.75, and 1 mgL^{-1}, respectively) for 3 months (March to May 2021) while a significant increasing growth pattern was observed in the fish specimens, reared in control group (T-o). Maximum growth decreased in 1 mgL^{-1} (T-IV) with an increase in the ammonia concentration 25.66±6.00 to 17.59±2.00 g in (T-IV), While increase growth in control group at 0.0 mgL^{-1} in T-o, 22.36±5.84 to 32.7±4.94 g (*Table 2*). Analysis of variance (ANOVA) among body weight in all the studied groups showed a significant positive correlation among T-o with T-I and T-II; T-III with T-o, T-II, and T-III; T-II with T-o, T-I, and T-III; T-III with T-I, and T-II. A significant negative correlation was observed among T-o with T-IV; T-I with T-IV; T-II with T-IV; T-III with T-IV, while no significant correlation was observed among T-o with T-III (*Table 3*). At the same time, the average condition factor in different treatments varied from 0.985 to 0.660. Values of condition factor were found to be maximum in the control group (T-o), while minimum with the highest ammonia concentration in T-IV (*Table 4*).

Hematological parameters (RBC, WBC, Hb, Ht, MCV, MCH, and MCHC) of *Clarias batrachus* are showed in *Table 5*. Results of ANOVA showed that all the studied hematological parameters were significantly ($P<0.05$) affected by the ammonia exposure, except for MPV which showed non-significance ($P>0.05$) among the studied treatment. Mean values of WBC ($10^3/\mu L$), HGB(g/dl), RBC ($10^6/\mu L$), HCT (%), PLT ($10^3/\mu L$), MON and LYM ($10^3/\mu L$) were found significantly highest ($P<0.05$) in the control group (T-o). While the mean value of MCH (pg), MCHC (g/dl), MCV (μm^3) and RDW-SD (%) in the blood of *C. batrachus* found higher in T-IV than other ammonia treated groups and control group. In WBC, RBC, HCT, PLT, Mon, LYM and HGB count, a notable decline was shown in the ammonia concentration exposure increasing from 0 to 1.0 mg/L, while the remaining hematological parameters significantly increased with an increase ammonia concentration. During the experiment no mortality was recorded, hence the survival rate was remained 100%, as shown in *Table 6*.

Discussion

Toxic substances like ammonia (NH_3) exposure in aquatic environments can negatively affect growth performance and hematology of fish (Kim and Kang, 2015; Molayemraftar et al., 2022). In aquatic animals, nitrite (NO_2) exposure produces oxidative stress, which induces free radical accumulation resulting in multiple oxidative stress-induced toxic effects (Tomasso, 2012). In the present study, fish were subjected to an acute NH_3 stress (T-I to T-IV), and we reported a high level of malfunction in fish body weight, growth and hematology, as reported by Francis-Floyd (2009) Kır et al. (2019). *Table 2* of the present study showed that the body weight of *Clarias batrachus* decreased with exposure to ammonia in T-I to T-IV, as reported by Kim and Kang (2015), and Mirghaed et al. (2020). The fish Body weight showed the highest observable effect with a value of 13.2 g in T4 at ammonia exposure of 1 mg/L (*Table 2*). This result is like other investigators reported by El-Shafai et al. (2004), Foss et al. (2003, 2009), Thrane et al. (2013), Burgos Aceves et al. (2019) and Alimba et al. (2019).

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Table 2. *Effect of ammonia exposure on body weight (g) of Clarias batrachus*

No. of days	Control (0 mg/L)		Treatment-I Ammonia exposure (0.25mg/L)		Treatment-II Ammonia exposure (0.5mg/L)		Treatment-III Ammonia exposure (0.75mg/L)		Treatment-IV Ammonia exposure (1mg/L)	
	Initial weight (g)	Final weight (g)	Initial weight (g)	Final weight (g)	Initial weight (g)	Final weight (g)	Initial weight (g)	Final weight (g)	Initial weight (g)	Final weight (g)
15	22.36±5.84	23.73±5.44	24.356±9.67	25.26±9.59	23.38±5.17	24.06±5.19	23.11±13.17	22.34±13.94	25.66±6.00	24.29±3.97
30	23.73±5.44	25.23±4.58	25.26±9.59	26.04±9.76	24.06±5.19	25.04±4.96	22.34±13.94	21.015±13.61	24.29±3.97	23.09±2.97
45	25.23±4.58	27.18±4.90	26.04±9.76	27.03±10.42	25.04±4.96	26.04±4.87	21.015±13.61	20.23±13.41	23.09±2.97	21.69±2.57
60	27.18±4.90	30.06±4.58	27.03±10.42	28.07±10.60	26.04±4.87	27.07±4.78	20.23±13.41	19.51±13.40	21.69±2.57	19.99±4.33
75	30.06±4.58	31.7±4.40	28.07±10.60	30.09±11.80	27.07±4.78	28.13±4.94	19.51±13.40	18.8±13.45	19.99±4.33	18.19±2.47
90	31.7±4.40	32.7±4.94	30.09±11.80	32.01±11.92	28.13±4.94	29.17±5.14	18.8±13.45	18.28±12.91	18.19±2.47	17.59±2.00

Table 3. *Statistical analysis of ammonia exposure on body weight of fish Clarias batrachus*

		T-o	**T-I**	**T-II**	**T-III**	**T-IV**
T-o	Pearson Correlation	1	.538**	.801**	.221	-.678**
	Sig. (2-tailed)		**.000**	**.000**	**.066**	**.000**
	N	50	50	50	50	50
T-I	Pearson Correlation	.538**	1	.252*	.569**	-.658**
	Sig. (2-tailed)	**.000**		**.035**	**.000**	**.000**
	N	50	50	50	50	50
T-II	Pearson Correlation	.801**	.252*	1	.239*	-.538**
	Sig. (2-tailed)	**.000**	**.035**		**.046**	**.000**
	N	50	50	50	50	50
T-III	Pearson Correlation	.221	.569**	.239*	1	-.320**
	Sig. (2-tailed)	**.066**	**.000**	**.046**		**.007**
	N	50	50	50	50	50
T-IV	Pearson Correlation	-.678**	-.658**	-.538**	-.320**	1
	Sig. (2-tailed)	**.000**	**.000**	**.000**	**.007**	
	N	50	50	50	50	50

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Table 4. *Effect of ammonia exposure on condition factor (K) of Clarias batrachus*

Control (0 mg/L)	Treatment-I Ammonia exposure (0.25mg/L)	Treatment-II Ammonia exposure (0.5mg/L)	Treatment-III Ammonia exposure (0.75mg/L)	Treatment-IV Ammonia exposure (1mg/L)
0.985±0.04	0.900±0.14	0.897±0.004	0.835±0.10	0.660±0.15

Table 5. *Effect of ammonia exposure on hematological parameters of Clarias batrachus*

Blood parameters	Control	Ammonia exposure treatment-1 (0.25mg/L)	Ammonia exposure treatment-II (0.5mg/L)	Ammonia exposure treatment-III (0.75mg/L)	Ammonia exposure treatment-IV (1mg/L)	Sig.
WBC ($10^3/\mu L$)	33.1±11.68^a	15±5.02^b	7.4±1.66^c	5.6±1.28^{cd}	0.8±0.31^d	.000
HGB(g/dl)	9.2±3.60^a	8.8±0.83^a	6.9±0.81^b	2.9±0.39^c	2.7±0.65^c	.000
MCH (pg)	48.9±9.60^c	46.3±5.71^c	30±7.28^d	93.1±13.81^b	104.8±16.17^a	.000
MCHC(g/dl)	35.8±8.36^c	38.5±7.04^c	41.4±7.32^c	65.9±6.41^b	94.6±9.10^a	.000
RBC ($10^6/\mu L$)	1.88±0.71^a	1.49±0.09^b	0.84±0.48^c	0.58±0.32^{cd}	0.29±0.04^d	.001
MCV(μm^3)	118.7±19.90^c	128.1±5.62^{bc}	128.7±5.71^{bc}	136.7±9.36^{ab}	141.4±13.14^a	.000
HCT (%)	25.2±11.62^a	12.9±0.79^b	9.3±0.57^b	4.1±0.78^c	2.0±0.46^c	.000
PLT ($10^3/\mu L$)	176±33.54^a	162±11.07^a	75±4.88^b	38±3.54^c	28±9.37^c	.000
MON	4.9±1.00^a	2.5±0.37^b	0.9±0.25^c	0.3±0.16^d	0.1±0.09^d	.000
LYM ($10^3/\mu L$)	98.6±15.75^a	88.7±3.33^b	33.3±5.14^c	13.8±4.49^d	9.2±1.20^d	.000
RDW-SD (%)	75.1±24.83^b	78.2±1.91^b	88.2±16.13^b	198.2±11.69^a	212.8±51.94^a	.000
MPV(μm^3)	6.5±0.85^{ns}	6.1±1.15 ^{ns}	5.8±0.99 ^{ns}	5.3±0.88 ^{ns}	5.3±0.46 ^{ns}	.069

Table 6. *Impacts of ammonia exposure on mortality rate of Clarias batrachus*

Treatments	No. of Fish	Ammonia Conc.	Mortality at						Total Mortality	Mortality (%)	Alive (%)
			15 days	30 days	45 days	60 days	75 days	90 days			
Control	10	0	0	0	0	0	0	0	0	0	100%
Treatment-I	10	0.25	0	0	0	0	0	0	0	0	100%
Treatment-II	10	0.50	0	0	0	0	0	0	0	0	100%
Treatment-III	10	0.75	0	0	0	0	0	0	0	0	100%
Treatment-IV	10	1.0	0	0	0	0	0	0	0	0	100%

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In present study growth of fishes showed decreasing trend with increasing ammonia exposure which may be due to fact that exposure to ammonia reduces the intake of and nutrient digestibility (Mueller-Harvey, 2006; Azaza et al., 2009; Omnes et al., 2017). Moreover, a significant decreasing trend in growth pattern with an increase in ammonia concentration exposure is in general agreement with the results of those reported by Uchenna et al. (2022).

In the present study, the growth rate of *Clarias batrachus* observed decreased with an increase in exposure with ammonia concentration. Fish exposed to ammonia are critical to the environment and act as a limiting factor for inhibition of growth with a decrease in feed intake (Foss et al., 2003). Several investigators report that higher ammonia concentrations could significantly induce the growth pattern, Silver perch (Frances et al., 2000); European sea bass (Lemarie et al., 2004); turbot (Foss et al., 2009); and Atlantic halibut (Paust et al., 2011). Reduction of growth performance may result in a deficiency of energy to detoxify the ammonia toxicity (Clearwater et al., 2002). Sakala and Musuka (2014) demonstrated the toxic effects of an increase in ammonia on tilapia growth rate. Exposure of ammonia can induce growth in aquatic animals. The exposure to NH_3 caused a decrease in growth. This decrease in growth may outcome from the energy demand to ammonia detoxication (Clearwater et al., 2002).

Barbieri and Bondioli (2015) reported that exposure of various concentrations of NH_3 affected the total hematological parameters. This elevation in ammonia decreased growth rate, disease resistance, and poor efficiency of food conversion (Kuttchantran, 2013). Ammonia exposure and excretion of fish are the main factors for fish that reduce growth, and body weight and affect blood chemistry. The fish excreta are directly related to feed (Masautso et al., 2014). The fish body weight decreases in ammonia-treated fish because of loss of fish appetite. This reduces the urge to feed leads to a significant reduction in growth rate, body weight, and hematology, and affect the metabolism (Das et al., 2004). Ammonia higher level can cause stress and produces damaging physiological-based responses like disturbance in osmoregulatory mechanism, branchial epithelium, kidney damage, growth retardation (Soderberg, 1994), and inefficient immune responses (Pinto et al., 2007), and decrease survival (Jobling, 1994). *Table 5* of the present study revealed that continuous NH_3 exposure significantly reduced the hemoglobin level in *Clarias batrachus* as previously reported by Yang et al. (2011) in *C. gariepinus*. Accumulation of NH_3 increases oxygen consumption and reduces growth, increasing hemolymph (Barbieri and Bondioli, 2015). Nitrate in ammonia affects hematology (El-Sayed, 2015).

Study data to analyze the ammonia effect on WBCs, RBCs and hemoglobin are shown in *Table 5*. Complete blood cells in the fish indicated the physiological-based effect of stress responses (Das et al., 2004). There is a significant reduce in the level of red blood cells, and hemoglobin in fish as it is exposed to ammonia (Knoph and Thorud, 1996). The present finding agrees with these findings, with a reduce in RBC, HCT, and Hb levels with an increase in exposure to the ammonia concentration. A similar trend was found with a concentration of hemoglobin lower in ammonia-exposed fish than in control conditions as a reduction of approximately 45% in tilapia exposed to 0.15 mg ammonia (El-Sherif and El-Feky, 2008). There is a significant increase in mean corpuscular volume values recorded in *C. batrachus* with the increase in the ammonia concentration. This is because an increase in water content in RBC results in a decrease in plasma chloride and their shift with high ammonia levels in the water (Tilak et al., 2007; Fazio, 2019; Singh et al., 2019).

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A significant reduce in RBCs and WBCs count of *Cyprinus carpio* was reported by Thangam et al. (2014) as exposed to ammonia. The physiological and biochemical alterations in fish hematology can be induced by the toxicants like ammonia (Barton, 2002). *Table 6* of this study revealed that NH_3 concentration at various levels T-I, T-II, T-III and T-IV in *C. batrachus* showed no mortality after 15, 30, 45, 60, 75 and 90 days as reported by Kim et al. (2017) in *Anoplopoma fimbria*. In comparison, Uchenna et al. (2022) reported mortality in *Clarias gariepinus* in these concentrations. The present study indicated that an increased ammonia concentration affects the growth performance, hematology, and the health of *Clarias batrachus*. In four different treatments, ammonia with a concentration higher than 0.5 mg/L can significantly reduce the growth performance with a prominent physiological effect on *C. batrachus*. The study is important for various environmental-based studies and also the stocking of various fish for the farming system.

Conclusion

The elevated levels of ammonia and its derivatives in the aquaculture environment are one of the threats to freshwater fish that can cause stress. The response to *C. batrachus* growth and mortality in the present study provided the basis for predicting the overall impact of NH_3. The ammonia exposure to *C. batrachus* produced a notable decrease in growth performance (weight gain and condition factor), While hematological parameters showed a significant decrease in White Blood Cells (WBCs), Hemoglobin (Hb), Red Blood Cells (RBCs), Hematocrit (HCT), Platelets (PLT), Monocyte (Mon), Lymphocyte (LYM), and Mean Platelet Volume (MPV). Therefore, it is recommended from this study that the level of ammonia (NH_3) is necessary to manage in aquaculture systems can help reduce the dangers posed by NH_3 buildup from unutilized foods and significantly increase fish food intake. To the best of our knowledge, there is no report in national and international literature on effect of various concentrations of ammonia on the growth and hematology of *Clarias batrachus*, hence this is the first attempt on the evaluation of effect of studied concentration (0.25, 0.50, 0.75 and 1.00 mg/L) on growth and hematology of *C. batrachus* from Pakistan.

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