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Effect of Ammonia on the Growth and Haematological Response of *Catla catla*

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Abstract

This study investigated the impact of varying ammonia or Total Ammonia Nitrogen (TAN) levels on the growth performance and haematological parameters of *Catla* juveniles over a 30-day period. Before the experiment, an acute toxicity study was conducted to determine the appropriate TAN treatment levels. The experimental fish, measuring 12.6 ± 0.13cm in length and weighing 18.26 ± 0.07g, were exposed to TAN concentrations of 10, 15, and 20ppm, with a 0ppm control group. Haematological parameters, including Total Erythrocyte Count (TEC), Total Leukocyte Count (TLC), Haemoglobin Content (Hb), Haematocrit Value (Ht), Mean Corpuscular Volume (MCV), and Mean Corpuscular Haemoglobin Concentration (MCHC), were monitored weekly. In contrast, water quality parameters were assessed daily. The results indicated a negative correlation between TAN levels and growth performance. As ammonia concentration increased, weight gain, length, Specific Growth Rate (SGR), and Protein Efficiency Ratio (PER) declined, while the Food Conversion Ratio (FCR) increased, signifying reduced feed efficiency. Haematological analysis revealed that TEC, Hb, Ht, and MCHC were lowest at 20ppm, which suggests physiological stress and potential impairment of oxygen transport. Conversely, TLC levels were elevated at 20ppm, likely indicating an immune response to ammonia-induced stress. MCV values varied across treatments, peaking at 10 ppm before decreasing in the order of 0ppm, 20ppm, and 15ppm. In conclusion, elevated TAN levels significantly affected both growth performance and haematological parameters, with higher concentrations leading to stress-induced physiological changes. These findings highlight the importance of maintaining optimal water quality in aquaculture systems, as prolonged exposure to high ammonia levels can negatively impact the health and growth of *Catla* juveniles.

Keywords: *Catla*, Haematological Parameters, Length, TAN Level, Weight

1. Introduction

As the global human population grows, so does the demand for food, including more extravagant culinary choices. India ranks second in total aquaculture production among all countries, with a production of 10.23 million tonnes[1]. In global aquaculture, cyprinid fish species dominate, with carp species such as *Catla*, grass carp, common carp, bighead carp, and crucian carp being the most widely cultivated[2]. Due to its rapid growth rate, high meat content, desirable taste, high protein levels, and strong market demand, *C. catla* has become a preferred

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species for aquaculture. Environmental changes induce stress responses in fish, which can disrupt physiological functions and overall health, leading to secondary responses such as alterations in blood constituents.

Ammonia is a prevalent pollutant in aquatic environments, accumulating through natural biological and chemical processes such as ammonification, biological nitrogen fixation, and eutrophication in water bodies and their surrounding ecosystems[3,4]. Ammonia is the second most widely produced chemical on Earth by weight[5]. With advancements in ammonia synthesis technologies, global production is expected to double within the next decade and could expand more than 100-fold in the future as energy demands continue to rise[6]. The increasing production of ammonia will lead to greater emissions into the air, water, sediments, and soils[7].

Fish growth and survival are significantly impacted by ammonia, the second most important water quality parameter after oxygen[8]. In aquatic environments, ammonia exists in two forms: the ionised form (NH_4^+ or NH_4-N) and the unionised form (NH_3 or NH_3-N), collectively referred to as TAN, commonly known as 'ammonia'. Fluctuations in ammonia concentration can cause lethargy in fish, and at higher levels, it can be lethal. The ionic composition of water plays a crucial role in influencing the growth, survival, and haematological parameters of farmed fish[9-11]. Several studies have documented the accumulation of amino acids in various fish tissues due to ammonia stress[12-16], which exerts harmful effects by poisoning the central nervous system[17,18]. Understanding haematological parameters is essential, as they serve as reliable indicators for monitoring physiological and pathological changes in fish[19-21]. These parameters provide crucial insights into blood composition, enabling the assessment of a fish's physiological state. By analysing haematological responses, researchers and aquaculturists can better manage and prevent diseases caused by environmental stressors, ensuring the overall health and productivity of cultured fish[22].

It is hypothesised that increasing levels of TAN in the aquatic environment will have a significant negative impact on the growth performance, survival, and haematological parameters of *Catla* juveniles. Higher TAN concentrations are expected to induce physiological stress, leading to alterations in blood composition and reduced overall fitness. Additionally, the adaptability of *C. catla* to varying ammonia levels will provide insights into its resilience in aquaculture settings, influencing the sustainability of its culture under different environmental conditions.

The present study aims to evaluate the physiological and haematological responses of *C. catla* juveniles to varying levels of TAN in controlled aquaculture conditions. Specifically, the research focuses on assessing the effects of ammonia stress on fish growth, survival, and blood parameters, which serve as critical indicators of physiological and pathological changes. By analysing these responses, the study intends to determine the adaptability of *C. catla* to different environmental conditions, providing insights into the potential success or failure of culture systems in natural and controlled aquaculture settings.

This study is unique in its comprehensive approach to examining the effects of ammonia toxicity on growth performance and haematological parameters of *C. catla*, a species of significant economic and nutritional importance. While previous studies have focused on general ammonia toxicity in fish, limited research has specifically explored the physiological adaptability of *C. catla* to varying TAN levels in aquaculture settings. The novelty of this research lies in its integration of growth metrics with detailed haematological profiling, offering a deeper understanding of how ammonia stress influences fish health at both systemic and cellular levels. The findings will contribute to the development of better water quality management strategies, ensuring sustainable and efficient aquaculture production of *C. catla* under different environmental conditions.

2. Materials and Methods

A commercial nursery located in Mollur, SPSR Nellore District, Andhra Pradesh, India, provided healthy juveniles of *C. catla* with an average length of 12.6 ± 0.13cm and an average weight of 18.26 ± 0.07g. The juveniles were then transported to the Department of Fisheries Resource Management's wet laboratory at the College of Fishery Science, Muthukur, SPSR Nellore District. The fish were kept in 500-litre collapsible tarpaulin tanks equipped with aeration facilities for one week to allow for acclimatisation. They were fed a commercial pellet diet containing 24% protein twice daily at a rate of 5% of their body weight. Faeces and uneaten feed were removed daily, and every other day, 25–30% of the water was replaced with freshwater. The experiment

was conducted from December 2019 to January 2020 for a total duration of 30 days.

Laboratory reagent-grade Ammonium Chloride (NH_4Cl) was dissolved to prepare the stock ammonia (TAN) solution, which was then diluted to achieve the desired ammonia (TAN) concentrations. Before the experiment, the fish were exposed to varying ammonia (TAN) concentrations to determine appropriate treatment levels. For approximately 96 hours (h), 10 fish per 270-litre tank were subjected to ammonia (TAN-N) concentrations ranging from 0 to 30ppm (0, 5, 10, 15, 20, 25, and 30ppm, respectively) in triplicate. Observations were recorded every 12 hours during this period. Based on the results, TAN levels of 10, 15, and 20ppm were selected as experimental treatments, while 0 ppm served as the control. Following acclimatisation, 120 fish were randomly selected and stocked in 12 collapsible fibre experimental tanks (10 fish per tank), filled with water to a depth of 50cm, containing ammonia solutions of 10, 15, 20ppm and control (0ppm) in triplicate.

The fish were fed a commercial floating pellet diet containing 24% protein twice daily at a rate equivalent to 5% of their average body weight. The experimental tanks were continuously aerated, and faeces and uneaten feed were removed daily by siphoning. The water level in the tanks was maintained throughout the study by periodically adding fresh water while ensuring the ammonia concentration remained stable.

Each day, various water quality parameters were measured and recorded, including temperature (using a General Purpose Thermometer, Omsons Labs, Cat No. 3004), pH (using an EcoTestr pH 1 digital pH meter), salinity (using a Hanna HI96822 seawater refractometer), ammonia (using the Phenate method), dissolved oxygen (using Winkler's titrimetric method), total alkalinity (using titration), and total hardness (using EDTA titration)[23]. Every week, fish samples were randomly selected from each treatment to evaluate growth and survival metrics like FCR, SGR, PER, and Length-Weight based on standard formulae[24,25]. Additionally, blood samples were collected weekly in the morning to minimise diurnal variations in blood parameters. Using a sterile syringe fitted with a 26 G needle pre-rinsed with anticoagulant (Heparin), blood was drawn from the caudal peduncle region and transferred into 2 ml K_2-EDTA coated tubes. These blood samples were immediately subjected to haematological analysis, including TEC[26], TLC[26], Hb[27], Ht[27], MCV[28], and MCHC[28]. The following standard formulas were used for haematological analysis, which was conducted using conventional procedures.

Haematological Calculations:

- Total Leukocyte Count (TLC) (cells/cu.mm):

$$TLC = \frac{WBC\ counted \times Dilution\ factor}{Volume\ counted\ in\ cu.mm}$$
$$= \frac{WBC\ counted \times 20}{0.1 \times No.\ of\ large\ squares\ counted}$$
$$= WBC\ counted \times 50\ cells/cu.mm$$

- Total Erythrocyte Count (TEC) (cells/cu.mm):

$$TEC = \frac{No.\ of\ RBCs\ counted \times Dilution\ factor}{Area\ counted \times Depth\ of\ fluid}$$
$$= \frac{No.\ of\ RBCs\ counted \times 200}{0.2 \times 0.1}$$
$$= RBC\ counted \times 10000\ cells/cu.mm$$

- Mean Corpuscular Volume (MCV) (femtoliters, fL):

$$MCW = \frac{Haemotocrit(\%) \times 10}{RBC\ coount\ in\ millions}$$

- Mean Corpuscular Haemoglobin Concentration (MCHC) (g/dL):

$$MCHC = \frac{Haemoglobin(g/dl)}{Haematocrit(\%)}$$

Growth Performance Calculations:

- Specific Growth Rate (SGR) (%/day):

$$SGR = \frac{[In(final\ weight) - In(initial\ weight)] \times 100}{total\ days\ of\ experiment}$$

- Feed Conversion Ratio (FCR) (g/g):

$$FCR = \frac{Dry\ weight\ of\ feed\ given}{Wet\ weight\ of\ fish\ gained}$$

- Protein Efficiency Ratio (PER) (g/g):

$$PER = \frac{Increased\ body\ weight}{Protien\ consumed}$$

The mean values for the three replicate tanks in each treatment were calculated after analysing the blood

samples and growth parameters. A two-way Analysis of Variance (ANOVA, Univariate) was performed using SPSS (version 16.0) to assess the interaction effect of different TAN levels on growth and haematological markers.

3. Results and Discussion

Fish can physiologically adapt to various stressors to maintain homeostasis, osmolality, and haematological balance[29,30]. In the present study, fish growth in terms of length and weight decreased with increasing TAN levels from 10ppm to 20ppm (Figure 1). The highest length (13.32cm) and weight (19.80g) were observed in the control, whereas the lowest length (12.82cm) and weight (18.64g) were observed at a TAN level of 20ppm. Despite noticeable variations between the treatments and the control, no significant difference ($p > 0.05$) was observed in the recorded length between TAN levels of 10ppm and 15ppm. However, a significant difference in length was observed among weeks and in the interaction between treatments and weeks. Growth performance was significantly reduced following ammonia exposure, likely due to the energy required for ammonia detoxification, which, in turn, led to reduced energy availability for growth[31,32]. The concentration of ambient ammonia determines the total amount of nitrogen accumulated in ammonia in plasma[33]. High ammonia levels in fish culture environments act as a toxic factor, causing growth inhibition, immune suppression, tissue erosion, degeneration, and mortality in aquatic organisms. An increase in environmental ammonia automatically raises ammonia levels in the blood and tissues of aquatic animals.

A decreasing trend in SGR and PER was observed from 10ppm to 20ppm, with the highest values recorded in the control (SGR: 0.54, PER: 3.18) and the lowest at 20ppm (SGR: 0.11, PER: 0.64) (Figure 2A). The SGR between the TAN level of 10ppm and the control did not differ significantly, although significant differences were observed between 15ppm, 20ppm, and the control. However, PER values differed significantly ($p < 0.05$) between treatments and across weeks. Similarly, a significant interaction between treatments and weeks was observed. In contrast, SGRs recorded across weeks and the interaction between treatments and weeks did not show any significant differences. The reduction in catla growth rate following exposure to high ammonia levels in this study aligns with findings on *Nile Tilapia*[34]. However, a significant difference was observed between the SGR of *Oreochromis niloticus* exposed to 0.1 and 0.15ppm unionised ammonia[35]. Similarly, SGR was inversely proportional to the concentration of unionised ammonia, which led to decreased food intake[36]. The findings of the present study align with previous research, which demonstrated that weight gain, SGR, and PER in juvenile blunt snout bream significantly decreased as ammonia concentrations increased. This suggests that chronic exposure to toxic ammonia levels negatively impacts fish growth performance by reducing feed intake[37].

In contrast, an increasing trend in FCR was observed from 10ppm to 20ppm (Figure 2B). The highest FCR (7.26) was recorded at 20ppm, while the lowest (1.32) was observed in the control, with significant differences ($p < 0.05$) between treatments. Similarly, significant differences were also observed between weeks and in the interaction between treatments and weeks. The present findings are consistent with those observed in tilapia, where the mean FCR increased with unionised ammonia concentration[35]. The increase in FCR may be attributed to stress in the experimental fish under hyperosmotic

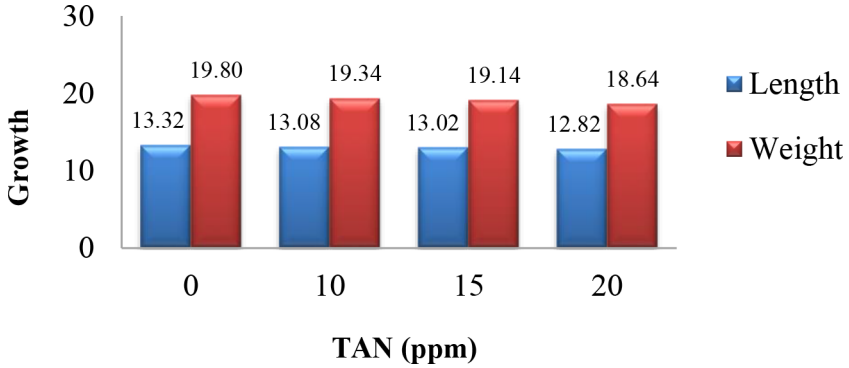


Figure 1. Mean of growth (length and weight) of *C. catla* juveniles at different TAN levels.

conditions, which could have reduced food intake and conversion efficiency[38].

The mean values of haematological parameters are presented in Table 1. Among these, an increasing trend in leukocyte count was observed from 10ppm to 20ppm (Figure 3A). The highest TLC (44.67 × 10^3/µl) was recorded at 20ppm, while the lowest (36.89 × 10^3/µl) was observed in the control. TLC showed an increasing trend from the first to the fourth week across all treatments. A similar observation of increasing leukocyte count was reported in rohu when subjected to different ammonia concentrations over exposure durations of 48, 72 and 96 hours[39]. TLC counts showed significant variation ($p < 0.05$) between treatments (10 ppm, 15ppm and 20ppm) and the control, as well as across weeks. Similarly, a significant interaction between treatments and weeks was observed. The increase in ammonia toxicity in the culture media may have influenced the immunological function of the experimental fish, leading to increased leukocyte production. Furthermore, the increase of leukocytes might be due to enhanced lymphopoiesis, which, in turn, promotes the release of lymphocytes from lymphoid tissue[40,41]. A similar trend of increasing White Blood Cells (WBC) count throughout the study period was reported in juvenile blunt snout bream[42].

A declining trend in erythrocyte count was observed from 10ppm to 20ppm (Figure 3B). The highest TEC count (2.32 × 10^6/µl) was recorded in the control, while the lowest (1.80 × 10^6/µl) was observed at 20ppm. A decreasing trend in TEC was noted from the first to the fourth week across all treatments. No significant difference ($p > 0.05$) was observed in TEC between 10ppm and 15ppm. However, TEC values for all treatments differed significantly ($p < 0.05$) from the control, although no significant differences were observed between weeks or in the interaction between treatments and weeks. These results align with previous research, which reported a decrease in erythrocyte count throughout the study period[43]. Some studies have shown that Red Blood Cells (RBC) count significantly decreases when fish are exposed to unionised ammonia[31,44,45]. In blunt snout bream, a reduction in RBC count was attributed to the inhibition of RBC production and haemoglobin synthesis due to ammonia toxicity at high concentrations[37]. Research indicates that ammonia toxicity inhibits hematopoiesis by damaging the production sites of red blood cells. A decrease in RBC count, along with an increase in surface area and distorted cell shape, is likely a result of tissue hypoxia in fingerlings exposed to higher ammonia concentrations[46]. The lower RBC count observed in the

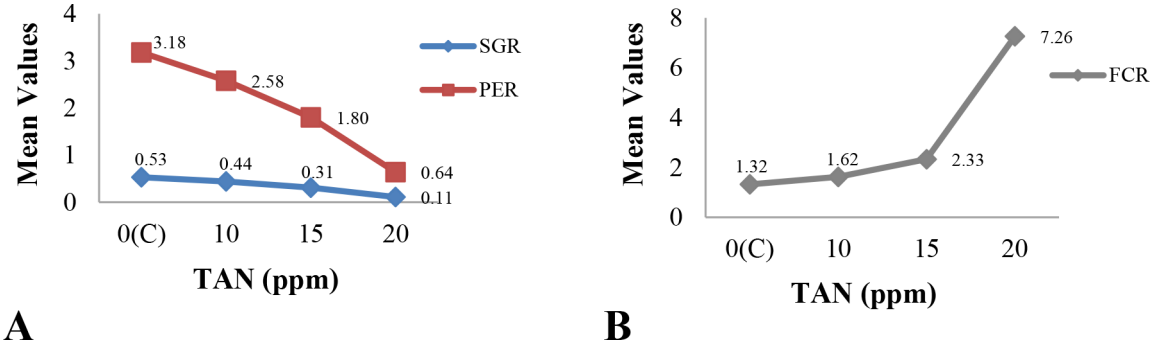


Figure 2. Mean variation of growth parameters of *C. catla* juveniles at different TAN levels: **A)** SGR and PER, **B)** FCR.

Table 1. Mean variations in haematological parameters of *C. catla* juveniles exposed to different TAN levels

TAN Levels/ Parameters	0ppm (C)	10ppm	15ppm	20ppm
TLC (10^3/µl)	$36.89^a \pm 0.60$	$38.57^b \pm 0.84$	$40.02^c \pm 2.14$	$44.67^d \pm 0.78$
TEC (10^6/µl)	$2.32^c \pm 0.15$	$2.02^b \pm 0.16$	$1.93^b \pm 0.15$	$1.80^a \pm 0.10$
Haemoglobin (gdL^{-1})	$10.50^d \pm 0.34$	$08.15^c \pm 0.52$	$07.03^b \pm 0.58$	$06.59^a \pm 1.00$
Haematocrit (%)	$31.44^d \pm 0.98$	$28.44^c \pm 0.78$	$25.23^b \pm 0.70$	$23.94^a \pm 0.84$
MCV (fL)	$135.90^a \pm 10.45$	$141.47^b \pm 09.39$	$131.42^a \pm 08.92$	$133.53^a \pm 06.33$
MCHC (gdL^{-1})	$33.43^c \pm 1.06$	$28.62^b \pm 1.36$	$27.84^a \pm 1.75$	$27.43^a \pm 3.31$

current study may be due to anaemia, which inhibits erythropoietin production.

A decreasing trend in haemoglobin (Hb) and hematocrit (Ht) levels was observed from 10ppm to 20ppm (Figures 4A and 4B). The highest (10.50 g/dL) and Ht (31.44%) values were recorded in the control, whereas the lowest values (6.59 g/dL and 23.94%, respectively) were observed at 20ppm. Similarly, a decreasing trend in Hb and Ht was noted from the first to the fourth week across all treatments. Hb and Ht levels significantly differed ($p < 0.05$) between treatments (10ppm, 15ppm, and 20ppm) and across weeks compared to the control. Additionally, a significant difference was recorded in the interaction between treatments and weeks. As toxicant concentrations increased, fish exhibited a continuous decline in haemoglobin content[40,45]. Similar findings were reported in common carp fingerlings, where reductions in Hb and Ht following acute exposure to ammonia toxicity were attributed to erythrocyte shrinkage, diminished erythrocyte production in hematopoietic tissue, hemodilution, and intravascular destruction[47]. Elevated ammonia levels in the environment enhance haemoglobin's tendency to bind ammonia instead of oxygen, leading to anoxic conditions and elevated blood ammonia levels[46]. In this study, the reduction in haemoglobin levels may be linked to increased oxygen intake and elevated methemoglobin levels, potentially resulting from gill damage caused by elevated external TAN levels. Hematocrit percentage gradually decreased when fish were exposed to subacute and acute ammonia levels[39,48]. Exposure of *Colisa fasciatus* to sublethal concentrations of lead resulted in hemolytic anaemia characterised by erythrocyte lysis, along with decreased haemoglobin content, hematocrit values, and erythrocyte count[49].

MCV values exhibited an irregular trend across treatments and weeks, except at 10ppm (Figure 5A). The highest MCV value (141.47 fL) was observed at 10ppm, while the lowest (131.42fL) was recorded at 15ppm. MCV values did not differ significantly between treatments at

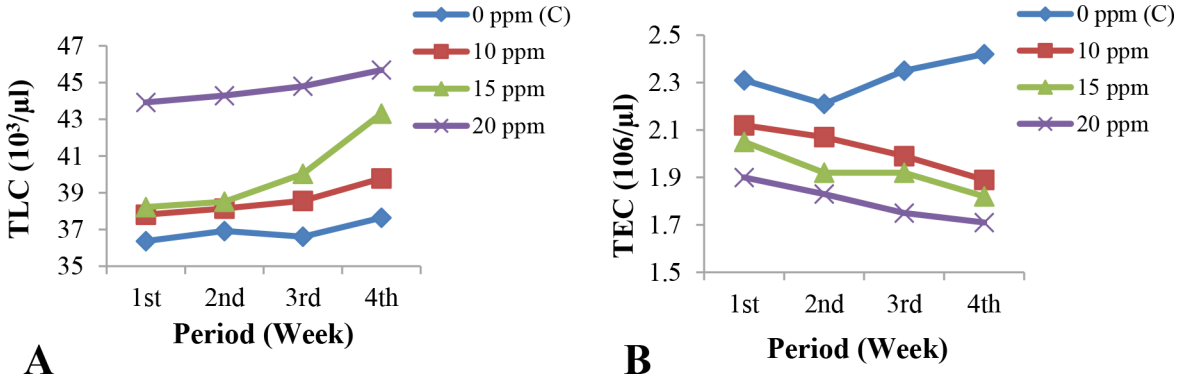


Figure 3. Weekly blood composition analysis of *C. catla* at varying TAN levels: **A)** Total leukocyte count (TLC), **B)** Total erythrocyte count (TEC).

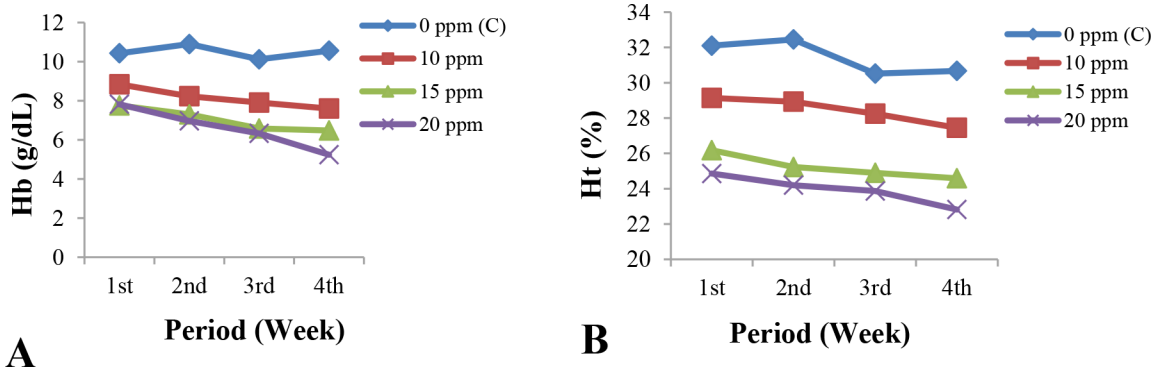


Figure 4. Weekly blood composition analysis of *C. catla* at varying TAN Levels: **A)** Total Haemoglobin (Hb) **B)** Total Haematocrit (Ht).

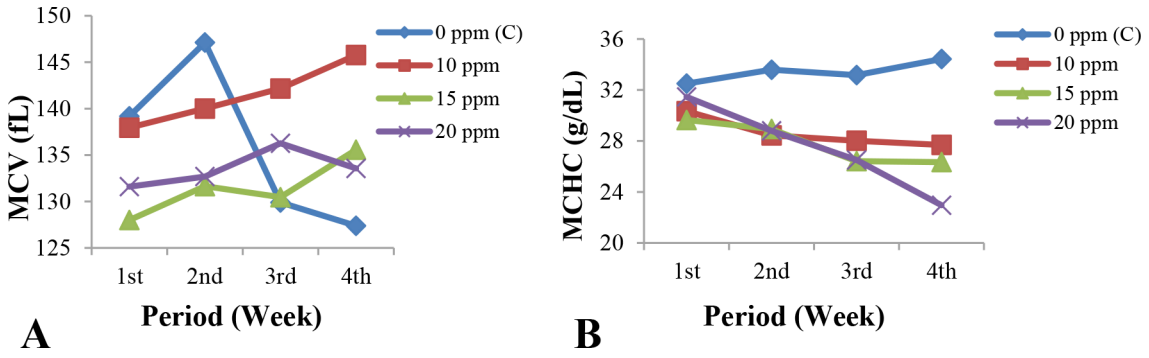


Figure 5. Weekly blood composition analysis of *C. catla* at varying TAN Levels: **A)** Mean corpuscular volume (MCV), **B)** Mean corpuscular haemogolobin concentration (MCHC).

15ppm and 20ppm, except for 10ppm, which showed a significant difference from the control. However, no significant differences were observed between weeks or in the interaction between treatments and weeks. A decreasing trend in MCHC was observed from 10ppm to 20ppm (Figure 5B). The highest MCHC value (33.43 g/dL) was recorded in the control, while the lowest (27.43 g/dL) was observed at 20ppm. Similarly, MCHC values showed a declining trend from the first to the fourth week across all treatments. A significant difference ($p < 0.05$) was observed in MCHC values between treatments and the control, although no significant difference was noted between 15ppm and 20ppm. Additionally, significant differences were observed in MCHC values between weeks and in the interaction between treatments and weeks. Studies on *Cyprinus carpio* exposed to diazinon pesticides reported a significant drop in MCV and MCHC values, suggesting that toxicant exposure may contribute to an increase in immature red blood cells[50]. A significant decrease in MCV values at different ammonia concentrations was also observed by some researchers[31,51]. Exposure to ammonia may cause a drop in plasma chloride levels and an increase in MCV, possibly due to increased water content in red blood cells resulting from chloride shifting. Similarly, the decrease in mean corpuscular haemoglobin concentration (MCHC) might be attributed to hemodilution and reduced haemoglobin production in circulation.

4. Conclusion

The present study provides valuable insights into the physiological and haematological responses of *C. catla* juveniles to varying levels of TAN. The findings indicate that exposure to elevated ammonia concentrations significantly affected fish growth, metabolism, and overall health. The decline in SGR and PER at higher TAN levels suggests that the fish expended considerable energy to cope with ammonia-induced stress, thereby reducing nutrient assimilation and growth efficiency. Simultaneously, the increase in FCR at elevated TAN levels highlights poor feed utilisation, likely due to reduced appetite and increased feed wastage. Behavioural observations revealed that *C. catla* maintained normal activity up to 10 ppm TAN, while at 15 and 20ppm, the fish exhibited reduced swimming activity, possibly as a strategy to minimise energy loss under stress.

The haematological alterations observed in this study further confirm the physiological distress caused by ammonia toxicity. A significant increase in TLC at higher TAN levels suggests an immune response to chronic stress, indicating leukocytosis due to prolonged ammonia exposure. Conversely, the reduction in TEC at elevated TAN levels indicates anaemia, likely a consequence of erythropoietin inhibition and impaired red blood cell production. The decline in haemoglobin concentration and hematocrit percentage at higher ammonia levels may be attributed to erythrocyte shrinkage and reduced hematopoietic activity. Alterations in MCV were associated with changes in hematocrit levels, while the decrease in MCHC suggests impaired haemoglobin synthesis, further compromising oxygen transport efficiency.

Overall, while *C. catla* exhibited the ability to tolerate TAN concentrations up to 20ppm. Its physiological health and growth performance were significantly compromised. This study underscores the importance of maintaining optimal water quality in aquaculture systems, as prolonged exposure to elevated ammonia levels can lead to metabolic stress, immune suppression,

and reduced productivity. These findings highlight the need for effective ammonia management strategies in aquaculture to ensure sustainable growth, improved feed efficiency, and overall health of *C. catla* in culture systems.

5. Author Contributions

Concept and methodology - M.S.N., T.V.R., A.B. and T.N.; research work assistance - M.S.N., R.S.S., L.V.N., K.B. and C.H.L.; analysis - M.S.N., R.S.S. and K.B.; draft corrections - A.B.

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