

## EFFECT OF DIFFERENT SALINITY LEVELS ON GROWTH PERFORMANCE, HEMATOLOGICAL PARAMETERS AND PROXIMATE COMPOSITION OF *CYPRINUS CARPIO*

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#### Key words:

*Cyprinus carpio*, Salinity levels Growth performance, Hematological parameters, Proximate composition.

### ABSTRACT

The current study was conducted to evaluate the effect of different salinity levels on the growth performance, proximate composition, and hematological parameters of Common carp (*Cyprinus carpio*). The experiment was designed under Laboratory conditions with twice replica as five treatments: T0: 0-ppt, (control); T1: 2-ppt, T2: 4-ppt, T3: 6-ppt and T4: 8-ppt and fish were randomly stocked (5 fish /aquarium). The *C. carpio* were fed 5% (commercial diet CP-30%) of their body weight and water was replaced regularly after every alternate day. The present study describes the significant changes ( $p < 0.05$ ) in growth parameters (viz; final weight, weight gain, feed intake and feed conversion ratio) with poor feeding behavior and stress were observed with further increase in salinity. Furthermore, body composition (Protein, fat, moisture, and ash contents) also showed significant changes ( $p < 0.05$ ). The crude protein and moisture contents were significantly decreased while crude fat and ash contents were increased with the increase of salinity respectively. The results of hematological parameters also decrease with the increase of salinity and found significant changes ( $p < 0.05$ ). The results of this study indicate that *C. carpio* exhibits a great degree of adaptability and resistance to salinity stress. This study serves as a basis for developing strategies to optimize the rearing conditions and welfare of common carp in different salinity regimes. Further research is warranted to elucidate the molecular and cellular mechanisms involved in the observed responses, enabling more precise management practices for the sustainable cultivation of *C. carpio*.

## 1. INTRODUCTION

Salinity is an abiotic factor that affects body composition, growth performance and hematological parameters of aquatic organisms (Moffett *et al.*, 2023). It is considered as an important factor that impact on osmosis, physiological functions, hormones, enzymes, immune metabolism and survival of fish (Wang & Zhu, 2002; Akhtar *et al.*, 2010).

High salinity causes mortality, low reproductive rates and in some freshwater species reproductive failure is also reported (Hintz & Relyea, 2019). In the worldwide, natural waters are exposing to continuous rise in salinity levels due to human activities such as the use of salt or desalination of plants and uneven rainfall (Al-Faiz *et al.*, 2009; Fazio *et al.*, 2013; Hintz & Relyea, 2019). Therefore, climatic conditions of Pakistan do not sustain and changed into underground brackish and saline water (Jarwar, 2014; Khan *et al.*, 2016).

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Aquaculture plays an important role in solving worldwide crises such as salinity, famine and malnutrition by the production of fish in intensive and non-intensive culture systems. In aquaculture practices some organisms have evolved to fully adapt to tolerate a wide range of high salinity levels, such as the common carp (*Cyprinus carpio*) (Triantaphyllopoulos et al., 2020). *C. carpio* belongs to Family Cyprinidae considered as potential candidate live in freshwater habitat, although it can tolerate to some salinity and has been testified to improves growth and survival, but higher levels of salinity seem to be injurious (Barus et al., 2001; Mangat & Hundal, 2014). It is known as a potential candidate for profitable aquaculture worldwide (Eurasia) due to its high adaptive capabilities (Parkos & Wahl, 2014; Tessema et al., 2020). The common carp is stenohaline fish that can tolerate various environmental conditions such as extreme salinity and resistance to pathogens. It can adjust the hematological characteristics, allied with immune response display physiological status which effects on growth and physical appearance (Akinrotimi et al., 2012). In aquaculture, common carp is a familiar fish due to its fabulous taste and its excellent market demand (Nedoluzhko et al., 2021).

Salinity also can impact on hematological parameters which are assessed as indicators, play vital roles in maintaining physiological functions and overall health of organisms (Kim & Kang, 2016; Ahmed et al., 2020). The alterations in blood parameters are thought to be adaptive responses to changes in the osmotic balance caused by variations in salinity. The increase in salinity levels inspiring to decrease in red blood cell counts, suppress immune responses, leading to decrease in the number of white blood cells and a compromised immune system (Soegianto et al., 2017; Ramesh et al., 2018). Such immunosuppression can render the fish more susceptible to various diseases and infections in brackish or seawater environments (Jackson et al., 2020).

The aim of this scientific study is to investigate the impact of varying salinity levels on growth performance, hematological parameters and proximate composition of *Cyprinus carpio* to provide valuable insights into the species' adaptability and optimize their husbandry in diverse aquatic environments.

## 2. MATERIALS AND METHODS

The Common carp (*Cyprinus carpio*) were collected from pond of fish hatchery, Saline Water Aquaculture Research Centre (SWARC) Muzaffargarh and transported to Laboratory. Fish were acclimatized for 7 days in freshwater glass aquaria before the experiment began. The experiment was designed into five treatments (T1: 2-ppt, T2: 4-ppt, T3: 6-ppt and T4: 8-ppt) respectively and control group (T0: 0-ppt). Each treatment had two replicas and 5 fish/aquarium were randomly stocked in each treatment. Fish were fed 5% of their body weight with Commercial diet CP-30% and water was exchanged regularly after every alternate day. The aquariums were properly equipped, and continuous aeration was provided to maintain dissolved oxygen. All of the physicochemical parameters were maintained by using Apera 8500 EC meter, Apera 8500 pH meter and P-512 dissolved oxygen meter on daily basis. The salinity of the treatments were gradually maintained by increases of salt and tested by Salinity meter on daily basis. Detritus and uneaten feed were removed on daily basis by siphoning out.

### Growth performance

Growth parameters viz; Final weight, weight gain, feed intake, growth rate and FCR were recorded by following formulas:

$$\begin{aligned}\text{Weight gain} &= \text{Final weight} - \text{Initial weight} \\ \text{FCR} &= \text{feed given (g)} / \text{Weight gain (g)} \\ \text{Growth Rate (\%)} &= \text{WG (g)} / \text{WI (g)} \times 100 \\ \text{Feed Intake; FCR} &= \text{FCR} \times \text{Weight gain (g)}\end{aligned}$$

The following measurements were taken (Owais et al., 2023)

### Proximate composition

The proximate inspection of desiccated fish meat was supported by procedures of Association of Analytical Chemist (AOAC, 1984). Samples were weighted at 105°C to determine moisture. Protein content was determined by measuring nitrogen (N×6.25) using the Kjeldahl method. Fat was confirmed by ether extraction using Soxhlet method. Crude ash was calculated following combustion at 550 °C for 6h (Owais et al., 2023).

### Blood sampling

The blood samples of experimental and control group fish were taken at the end of the experiment. It was collected by using a 5 ml disposal syringe. From each fish 1 ml of blood was collected into blood count test tubes that contain anticoagulant EDTA solution. The hematological parameters (Hemoglobin: Hb, Platelet count: PLT, Red blood cell: RBC, mean corpuscular volume: MCV, Hematocrit: HCT and Mean corpuscular hemoglobin: MCH) were tested by using a fully automated blood cell counter machine.

### Statistical Analysis

SPPS (ver. 22, USA) was used for statistical analysis of the data. Data were subjected to one-way ANOVA and Duncan's multiple range tests to determine the significant differences between the means.

## 3. RESULTS AND DISCUSSION

The results of growth performance revealed that decrease in Final weight (g), Weight gain (g), Feed intake (g), Growth rate (%) while feed conversion ratio increased when salt concentrations gradually rose from 0-ppt to 8-ppt. The statistical analysis shows significant differences ( $P \geq 0.05$ ) in treatments. Results of growth parameters show weight gain (g); T0: 0-ppt ( $11.28 \pm 0.28$ ), T1: 2-ppt ( $10.34 \pm 0.38$ ), T2: 4-ppt ( $9.02 \pm 0.17$ ), T3: 6-ppt ( $8.52 \pm 0.27$ ) and T4: 8-ppt ( $7.64 \pm 0.26$ ) respectively. Feed intake (g); T0: 0-ppt ( $14.58 \pm 0.37$ ), T1: 2-ppt ( $14.47 \pm 0.42$ ), T2: 4-ppt ( $12.95 \pm 0.12$ ), T3: 6-ppt ( $12.26 \pm 0.36$ ) and T4: 8-ppt ( $12.38 \pm 0.51$ ) respectively. Growth Rate (%); T0: 0-ppt ( $95.72 \pm 0.93$ ), T1: 2-ppt ( $87.39 \pm 1.75$ ), T2: 4-ppt ( $81.52 \pm 2.95$ ), T3: 6-ppt ( $73.54 \pm 2.51$ ) and T4: 8-ppt ( $69.73 \pm 3.33$ ) respectively. Feed conversion ratio; T0: 0-ppt ( $1.27 \pm 0.01$ ), T1: 2-ppt ( $1.39 \pm 0.01$ ), T2: 4-ppt ( $1.40 \pm 0.03$ ), T3: 6-ppt ( $1.41 \pm 0.02$ ) and T4: 8-ppt ( $1.65 \pm 0.05$ ) respectively (Table 1, Figure 1).

Results of hematological studies revealed that decrease in blood counts like red blood cells (RBC), hemoglobin (Hb), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), hematocrit (HCT) and platelet count (PLT) when the salt concentrations gradually rose to 0-ppt to 8-ppt. The statistical analysis showed significant differences ( $P \geq 0.05$ ) in treatments. The RBC  $10^6 \times \text{mm}^3$  counts as in treatments; T0: 0-ppt ( $0.42 \pm 0.09$ ), T1: 2-ppt ( $0.38 \pm 0.05$ ), T2: 4-ppt

( $0.33 \pm 0.06$ ), T3: 6-ppt ( $0.29 \pm 0.06$ ) and T4: 8-ppt ( $0.23 \pm 0.01$ ) respectively. Hb g/dl; T0: 0-ppt ( $3.33 \pm 0.07$ ), T1: 2-ppt ( $3.28 \pm 0.07$ ), T2: 4-ppt ( $2.62 \pm 0.21$ ), T3: 6-ppt ( $2.22 \pm 0.01$ ) and T4: 8-ppt ( $2.17 \pm 0.01$ ) respectively. MCH pg; T0: 0-ppt ( $78.70 \pm 0.45$ ), T1: 2-ppt ( $75.86 \pm 0.39$ ), T2: 4-ppt ( $73.68 \pm 0.19$ ), T3: 6-ppt ( $70.62 \pm 0.33$ ) and T4: 8-ppt ( $65.00 \pm 0.59$ ) respectively. MCV  $\mu\text{m}^3$ ; T0: 0-ppt ( $76.68 \pm 0.43$ ), T1: 2-ppt ( $73.30 \pm 0.48$ ), T2: 4-ppt ( $69.24 \pm 0.53$ ), T3: 6-ppt ( $65.06 \pm 0.39$ ) and T4: 8-ppt ( $59.14 \pm 1.34$ ) respectively. HCT %; T0: 0-ppt ( $3.34 \pm 0.03$ ), T1: 2-ppt ( $3.25 \pm 0.06$ ), T2: 4-ppt ( $2.83 \pm 0.02$ ), T3: 6-ppt ( $2.68 \pm 0.02$ ) and T4: 8-ppt ( $2.35 \pm 0.01$ ) respectively. PLT  $10^9/\text{l}$ ; T0: 0-ppt ( $717.40 \pm 1.02$ ), T1: 2-ppt ( $710.60 \pm 1.12$ ), T2: 4-ppt ( $670.00 \pm 7.07$ ), T3: 6-ppt ( $654.20 \pm 1.52$ ) and T4: 8-ppt ( $562.00 \pm 2.67$ ) respectively (Table 2, Figure 2).

The results of proximate composition revealed that decrease in Crude Protein and Moisture with the increase of salinity while Crude fat and Ash content increase with the increase of salinity. The significant differences are found in the treatments. The values of Crude Protein %; T0: 0-ppt ( $17.44 \pm 0.16$ ), T1: 2-ppt ( $16.48 \pm 0.08$ ), T2: 4-ppt ( $15.42 \pm 0.15$ ), T3: 6-ppt ( $14.24 \pm 0.10$ ) and T4: 8-ppt ( $13.64 \pm 0.12$ ) respectively. Crude fat %; T0: 0-ppt ( $9.38 \pm 0.08$ ), T1: 2-ppt ( $11.44 \pm 0.23$ ), T2: 4-ppt ( $13.44 \pm 0.16$ ), T3: 6-ppt ( $14.32 \pm 0.11$ ) and T4: 8-ppt ( $15.28 \pm 0.08$ ) respectively. Moisture %; T0: 0-ppt ( $80.44 \pm 0.14$ ), T1: 2-ppt ( $77.74 \pm 0.049$ ), T2: 4-ppt ( $73.92 \pm 0.54$ ), T3: 6-ppt ( $71.18 \pm 0.46$ ) and T4: 8-ppt ( $65.16 \pm 0.98$ ) respectively. Ash %; T0: 0-ppt ( $1.46 \pm 0.02$ ), T1: 2-ppt ( $1.74 \pm 0.05$ ), T2: 4-ppt ( $2.68 \pm 0.08$ ), T3: 6-ppt ( $2.86 \pm 0.09$ ) and T4: 8-ppt ( $3.60 \pm 0.07$ ) respectively (Table 3, Figure 3).

The present study was conducted to the Effect of different salinity levels on growth performance, Hematological parameters and proximate composition of *Cyprinus carpio*. The findings of the study revealed that no mortality on different salinity regime between 0 to 8-ppt. The growth parameters revealed that decrease in Final weight (g), Weight gain (g), Feed intake (g), Growth rate (%) while feed conversion ratio increased with the increased of salinity. Mylonas *et al.*, (2009) revealed that regime in salinity 0-ppt to 8-ppt affects the growth rate, feed conversion and feed intake. However, fish was perfectly able to normalize body functioning such as osmoregulatory process and metabolic rate. The

high level of salinity increases metabolic rate because of new osmotic conditions leads to control necessary ions through osmoregulation and to maintain the internal stability (homeostasis) which needs high energy cause reduction in growth rate and effects the feed conversion ratio and feed intake. Abo- Hegab & Hanke, (1982) describe changes in feed conversion rate of common carp with the increase of salinity upto 15-ppt. DeBoeck et al. (2000) studied common carp (*Cyprinus carpio*) at 10-ppt salinity and found negatively impact on feed conversion rate, weight gain and feed intake while Laiz-Carrión et al. (2005) describes the effect of salinity on fish reported poor growth performance depends on species and duration of exposure. Barman et al. (2005) entitled that decline in growth parameters viz; feed conversion and feed intake of grey mullet (*Mugil cephalus*) on high salinity. Luz et al., (2008) studied the goldfish *Carassius auratus* and reported low feed conversion rate at 8-ppt. Furthermore, Xia et al., (2010) studied grass carp *Ctenopharyngodon Idella* at different salinity levels and reported low feed conversion rate at 10-ppt.

The current study results exhibited a significant effect of various salinity levels on hematological parameters decrease in haemoglobin content, RBCs and haematocrit in treated groups. These findings are in similar agreement with another researcher who found a significant effect of salinity on RBCs, HCT and Hb of different fish species and high salinity levels with the osmoregulatory dysfunction (Fazio et al., 2013; Soltanian et al. 2016). McCormick, (2001) explained that blood parameters play a significant role in health status of fish and reported salinity influence on physiological changes which leads to decrease in blood parameters. *Cyprinus carpio* hematological parameters are found significantly decreases in blood parameters at high salinity.

Luz et al. (2008) explained that salinity is used as stress indicators for hematological and physiological parameters of the fish and reported that increase in salinity level affects the ion exchange mechanism. Al-Hilali & Al-Khshali, (2016) examined the impact of high salinity on the blood parameters of common carp and reported that salinity levels did influence certain hematological parameters. Elarabany et al. (2017) reported significant changes in hematological parameters of Nile tilapia (*O. niloticus*) at higher

salinities levels. Mubarik et al. (2019) examined hematological parameters of *Cyprinus carpio* under different critical and chronic salinity regimes. It found significant effects on various blood parameters, including hemoglobin concentration. Murmu et al., (2020) explained the effect of salinities on hematological parameters on Rohu fingerlings and explained the decreased in RBC counts at 6-ppt. Similarly, MCH and haemoglobin content are also drastically reduced in the fishes treated with high salinity. Salati et al. (2021) reported the impact of salinity levels in *C. carpio* and demonstrate variations in hematological parameters.

The proximate composition such as moisture, protein, lipid, and ash of common carp are affected by salinity. The result revealed significant differences among all of the treatments. The protein and moisture contents decreased with increase of salinity while minimum protein and moisture content were found at 8-ppt. Fat and ash contents increased with increase of salinity, maximum contents were found at 8-ppt respectively. Barman, (2012) studied milk fish proximate composition which shows significant reduction in level of moisture with increase in salinity from 0 to 15-ppt. Ljubojević et al. (2015) reported decreasing in protein content and the increasing in lipid contents in the *Lutjanus guttatus* and carp. Daudpota et al. (2016) studied body composition of *C. carpio* at higher salinities and reported changes in the protein, moisture, lipid, or ash contents because osmoregulation expenditure is required high energy contents. Rahim et al. (2017) studied the body composition of the common carp at higher salinity and found significantly higher fat content because fish store more energy to cope with the osmotic stress of salinity. Mandal et al. (2020) studied the proximate composition of *Pangasius* at different salinity levels, found protein and moisture contents decreased with the increase of salinity while Fat and ash contents increased with the increase of salinity.

#### 4. CONCLUSION

This study summarized that growth performance, hematological parameters and proximate composition of common carp (*Cyprinus carpio*) are influenced by different salinity levels. *C. carpio* exhibits a great degree of adaptability and resistance to salinity stress, which can impact its overall health and physiological



responses. These findings contribute to a profound understanding about ecological significance of salinity in aquaculture and natural aquatic environments. This study may serve as a basis for developing strategies to optimize the rearing conditions and welfare of common carp. Further research is warranted to elucidate the molecular and cellular mechanisms involved in the observed responses, enabling more precise management practices for the sustainable cultivation of this economically important fish. Overall, this scientific investigation sheds light on the intricate relationship between salinity levels, growth and physiological well-being of aquaculture industry and contributes to understanding of aquatic ecosystems.

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## 6. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interests regarding the publication of this article.

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Table 1. Showing growth performance (Mean  $\pm$  SEM) of *Cyprinus carpio* at different salinity levels (0-ppt, 2-ppt, 4-ppt, 6-ppt and 8-ppt and) during the 60-days experiment.

| Treatments | Weight gain (g)   | Feed intake (g)   | Growth Rate (%)   | FCR              |
|------------|-------------------|-------------------|-------------------|------------------|
| 0-ppt      | 11.28 $\pm$ 0.28d | 14.58 $\pm$ 0.37b | 95.72 $\pm$ 0.93a | 1.27 $\pm$ 0.01a |
| 2-ppt      | 10.34 $\pm$ 0.38c | 14.47 $\pm$ 0.42b | 87.39 $\pm$ 1.75b | 1.39 $\pm$ 0.01b |
| 4-ppt      | 9.02 $\pm$ 0.17b  | 12.95 $\pm$ 0.12a | 81.52 $\pm$ 2.95b | 1.40 $\pm$ 0.03b |
| 6-ppt      | 8.52 $\pm$ 0.27b  | 12.26 $\pm$ 0.36a | 73.54 $\pm$ 2.51c | 1.41 $\pm$ 0.02b |
| 8-ppt      | 7.64 $\pm$ 0.26a  | 12.38 $\pm$ 0.51a | 69.73 $\pm$ 3.33c | 1.57 $\pm$ 0.05c |

Means of the same raw with different letters are significantly different ( $p < 0.05$ ). Data was presented as (mean  $\pm$  standard error).

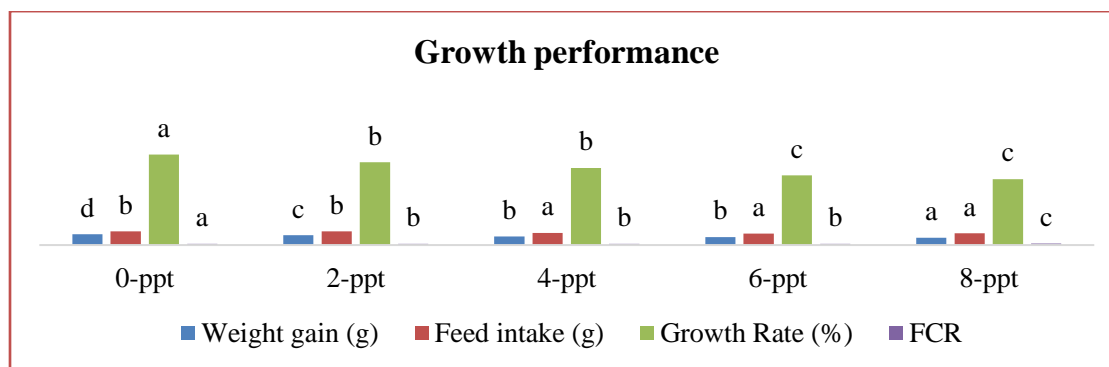


Figure 1. Clustered column bar graph showing growth performance of *Cyprinus carpio* at different salinity levels (0-ppt, 2-ppt, 4-ppt, 6-ppt and 8-ppt) during the 60-days experiment.

Table 2. Showing growth concentrations of hematological parameters (Mean  $\pm$  SEM) of *Cyprinus carpio* at different salinity levels (0-ppt, 2-ppt, 4-ppt, 6-ppt and 8-ppt and) during the 60-days experiment.

| Groups | RBC10 <sup>6</sup> ×mm <sup>3</sup> | Hb g/dl          | MCH pg            | MCV $\mu$ m <sup>3</sup> | HCT %            | PLT 10 <sup>9</sup> /l |
|--------|-------------------------------------|------------------|-------------------|--------------------------|------------------|------------------------|
| 0-ppt  | 0.42 $\pm$ 0.09e                    | 3.33 $\pm$ 0.07a | 78.70 $\pm$ 0.45e | 76.68 $\pm$ 0.43e        | 3.34 $\pm$ 0.03d | 717.40 $\pm$ 1.02a     |
| 2-ppt  | 0.38 $\pm$ 0.05d                    | 3.28 $\pm$ 0.07a | 75.86 $\pm$ 0.39d | 73.30 $\pm$ 0.48d        | 3.25 $\pm$ 0.06d | 710.60 $\pm$ 1.12a     |
| 4-ppt  | 0.33 $\pm$ 0.06c                    | 2.62 $\pm$ 0.21b | 73.68 $\pm$ 0.19c | 69.24 $\pm$ 0.53c        | 2.83 $\pm$ 0.02c | 670.00 $\pm$ 7.07b     |
| 6-ppt  | 0.29 $\pm$ 0.06b                    | 2.22 $\pm$ 0.01c | 70.62 $\pm$ 0.33b | 65.06 $\pm$ 0.39b        | 2.68 $\pm$ 0.02b | 654.20 $\pm$ 1.52b     |
| 8-ppt  | 0.23 $\pm$ 0.01a                    | 2.17 $\pm$ 0.01c | 65.00 $\pm$ 0.59a | 59.14 $\pm$ 1.34a        | 2.35 $\pm$ 0.01a | 562.00 $\pm$ 2.67c     |

Means of the same raw with different letters are significantly different ( $p < 0.05$ ). Data was presented as (mean  $\pm$  standard error).

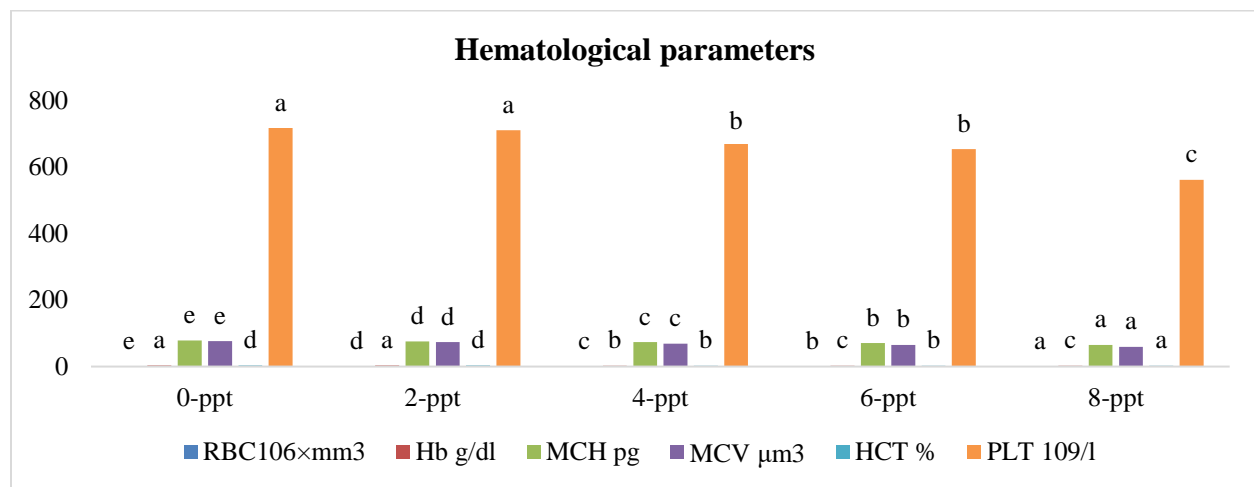


Figure 2. Clustered column bar graph showing hematological parameters of *Cyprinus carpio* at different salinity levels (0-ppt, 2-ppt, 4-ppt, 6-ppt and 8-ppt) during the 60-days experiment.



Table 3. Showing proximate composition (Mean  $\pm$  SEM) of *Cyprinus carpio* at different salinity levels (0-ppt, 2-ppt, 4-ppt, 6-ppt and 8-ppt and) during the 60-days experiment.

| Parameters      | Treatments        |                    |                   |                   |                   |
|-----------------|-------------------|--------------------|-------------------|-------------------|-------------------|
|                 | 0-ppt             | 2-ppt              | 4-ppt             | 6-ppt             | 8-ppt             |
| Crude Protein % | 17.44 $\pm$ 0.16e | 16.48 $\pm$ 0.08d  | 15.42 $\pm$ 0.15c | 14.24 $\pm$ 0.10b | 13.64 $\pm$ 0.12a |
| Crude fat %     | 9.38 $\pm$ 0.08a  | 11.44 $\pm$ 0.23b  | 13.44 $\pm$ 0.16c | 14.32 $\pm$ 0.11d | 15.28 $\pm$ 0.08e |
| Moisture %      | 80.44 $\pm$ 0.14e | 77.74 $\pm$ 0.049d | 73.92 $\pm$ 0.54b | 71.18 $\pm$ 0.46b | 65.16 $\pm$ 0.98a |
| Ash %           | 1.46 $\pm$ 0.02a  | 1.74 $\pm$ 0.05b   | 2.68 $\pm$ 0.08a  | 2.86 $\pm$ 0.09c  | 3.60 $\pm$ 0.07d  |

Means of the same row with different letters are significantly different ( $p < 0.05$ ). Data was presented as (mean  $\pm$  standard error).

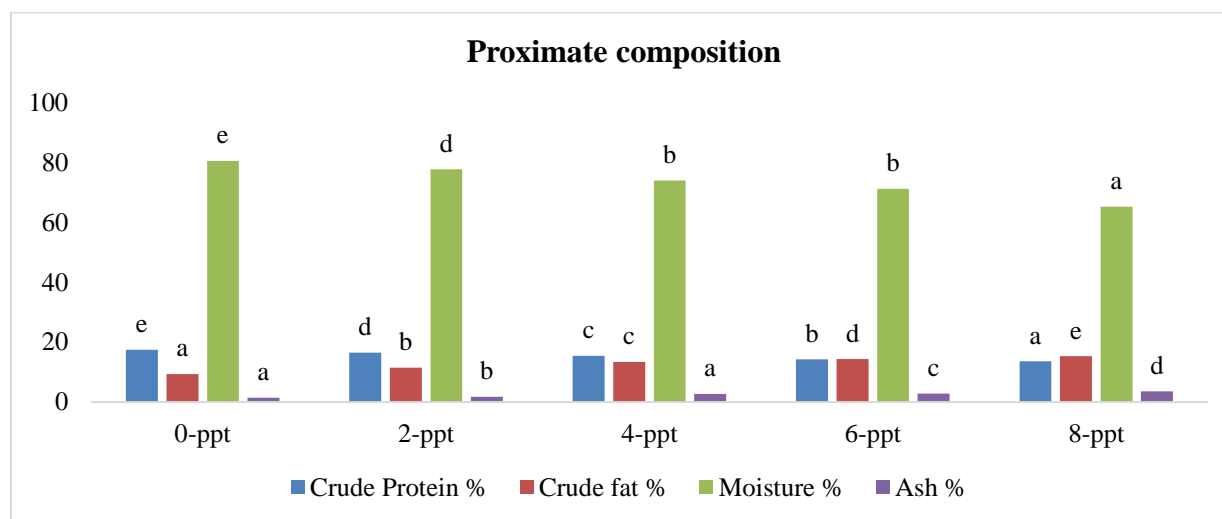


Figure 3. Clustered column bar graph showing proximate composition of *Cyprinus carpio* at different salinity levels (0-ppt, 2-ppt, 4-ppt, 6-ppt and 8-ppt) during the 60-days experiment.