~~The survival,~~ Investigation on the ~~impact~~ effects of different water treatments on the growth, and health of ornamental fishes raised under ~~the~~ freshwater culture conditions.

Abstract

In f~~F~~reshwater fish culture, w~~W~~ater quality poses a serious hazard to survival and growth.~~; it may cause serious issues~~. To ~~avoid~~ understand this high-risk factor associated with poor water quality in the aquarium system, the hematological, histological, immunological, antioxidant, and growth performance of *C. auratus*, *C. carpio*, and *P. hypophthalmus* in the aquarium with different water treatment efficiencies and one without waiter filtration were compared. ~~An~~ The experiment was conducted for ~~roughly~~ approximately 120 days using water treatments: 1. Pot filter, 2. Bamboo filter, 3. Pot and Bamboo filter 4. Pot and bamboo filtration with UV irradiation, 5. without water treatment. ~~The present study focuses on the water treatment role for aquarium water quality.~~ ~~and~~ Results showed that F~~f~~ish health concerning turbidity (98 %) was significantly reduced during post-filtration (PF, BF, PBF, and PB-UV-F) ($p<0.05$). Water treatments could observe significantly better growth of fish and attained a 90% survival rate in experimental tanks ($p<0.05$). In pot and bamboo filters, the hematological parameters such as RBC (93%, 94%, and 93%), WBC (79%, 18%, and 91%), and MCHC (93%, 45%, and 90%) we~~a~~re in the optimum levels during post filtration experiment. The immune response (IgM, C3, C4, NOS, and LSZ) of enzyme activities in the gut and liver of aquarium fishes and antioxidant parameters in the gut and liver (ABTS, CAT, GR, SOD, and LPO) were also the low-risk level where the water treatments in the aquarium tanks. In conclusion, our findings show that installing filtration systems in freshwater fish culture has helped to successfully regulate the fish health and water quality of aquariums and ensure their proper maintenance.

Keywords: Water turbidity, Ornamental fishes, Fish health and growth, Filtration system, Removal efficiency,

Introduction

A recent assessment found that iridescent shark catfish (*Pangasianodon hypophthalmus*) ~~pangas~~ produce 2% of the 112 million tonnes of aquaculture products made worldwide (Naylor *et al.*, 2021). The yearly harvests of the species are typically between 10 and 15 T/Ha, and they can grow up to~~1 to~~ 1.5 kg in a single year. According to the National

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Fisheries Development Board, pangasius was thought to be cultivated in an area of about 40,000 ha by 2008. Over time, this species' culture in India has developed and spread to many states' fish producers. Currently, production is thought to be between 400,000 and 425,000 metric tons per year (GAA Goal 2016 statistics).

~~The physiology of fish can be negatively impacted by intensification, which can lead to long-term stress and decreased susceptibility to disease~~. Insufficient amounts of clean water frequently result in increased pond stocking rates and levels, which in turn cause a decline in water quality, including turbidity, dissolved oxygen, pH, and ammonia (Sundh *et al.*, 2019). This negatively affects fish growth and health, leads to long-term stress and makes them more vulnerable to disease. According to Bondad-Reantoso *et al.*, (2005), regularly restocking ponds with fish of questionable health condition to offset mortality rates may ultimately lead to a repeat of sub-clinical infections.

Also, increased suspended sediment levels can harm fish physiologically by causing damage to their tissues and organs or by reducing light penetration and visual clarity in the water. These consequences can range from behavioral abnormalities to fish death (Collins *et al.*, 2011). The majority of direct consequences result from the scouring and abrasive action of suspended particles, which harms gill tissues or clogs gills to impede breathing, decreasing growth or mortality or reducing resistance to infection or disease.

Fish kept in aquariums can get both infectious and non-infectious diseases. Various biological entities, including bacteria, viruses, fungi, and protozoa are ~~the~~ sources of infectious diseases. These organisms proliferate and spread to other fish in the tanks. Non-infectious diseases are not spread and can be caused ~~on~~ by several issues, like inadequate nutrition and contaminated water. In addition to fin rot, white spots, and local infections from traumatic injuries, bacterial illnesses were also frequently detected in the ornamental fish species kept in aquariums.

In ponds, tanks, cages, etc., catfish are produced intensively with high yield owing to their rapid growth, omnivorous feeding, and high feed conversion efficiency (Anh Ngoc *et al.*, 2018). Still, pangs culture is threatened by disease, harsh feeding, and declining water quality because of heavy stocking, ~~and~~ rigorous feeding, and declining water quality (Faruk *et al.*, 2017). Although this species is very productive and has significant economic value, little is known about its physiology and biochemistry, ~~and~~ as standards for health monitoring are currently being developed. Physiological and metabolic conditions of animals, including fish, can be diagnosed using biochemistry and blood components. ~~Due to a lack of physiological and biochemical data, it is difficult to assess a species' health and manage its diseases.~~ In

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pangas species, ~~few works~~previous researches have examined the p~~P~~robiotics~~,~~ different dietary proteins and nucleotides sourced (Datta *et al.*, 2018)~~,~~ hematological and clinical parameters of stress study (Shahjahan *et al.*, 2018)~~,~~ infection (Kumar and Ramulu, 2013)~~,~~ and use of immunostimulants (Daniel *et al.*, 2018). Due to a lack of physiological and biochemical data, it is difficult to assess a species' health and manage its diseases. Therefore, ~~,~~t~~T~~his investigation was carried out to investigate the culture of Goldfish (*C. auratus*), Koi Carp (*C. carpio*), and Iridescent Shark Catfish (*P. hypophthalmus*) in different water treatments (PF, BF, PBF, PB-UVF, and WoF) and its effects on growth performance, hematological parameters, and histological changes as well as~~.~~ t~~T~~o analyze the antioxidant and immunological parameters of fish tissue samples.

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Materials and methods

Experimental setup and tank installations

A 120-day experiment was done at the Aquarium Trade Centre in Coimbatore, Tamil Nadu, India, for the Kongunadu Arts and Science College. The study involved different water treatment systems including pot, bamboo, and ultra-violet (UV) filters for Goldfish, Koi carp, and iridescent shark catfish~~.~~ ~~including pot, bamboo, and UV filters.~~ The water was discharged to biofilters, which adjusted the flow with motors. The water percolated down to an inlet funnel with an electrically driven aerator, and filtered water was pumped out through the outlet. The water was then recycled at a rate of 3m/3 hours. To increase the organic content, 3 grams of pelleted commercial fish feed was added to the rearing tank. The results were compared with and without a filtration system for 16 weeks. The experimental setup detailed explanation in the previous journal (Navaneethan and Raja, 2024).

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Turbidity

The water samples were collected once every 15 days ~~once~~ for 16 weeks of the experiment at three different treatment (TI, TII, and T~~-~~III) samples. The turbidity helps to determine the EPA guidelines and is measured using a spectrophotometer. Results were expressed as Nephelometric Turbidity Unit (NTU) and turbidity removal percentage as follows equation (1):

$$Turbidity\ removal\ efficiency = \frac{TBF-TAF}{TBF} \times 100 \qquad \ldots\ldots\ldots\ldots (1)$$

Growth performances

~~They~~ Growth performances were collected at the end of the experiment, and the total weights were calculated for the control and experimental tanks. The following equations (2)

were used to compute weight gain (WG), specific growth rate (SGR), and feed conversion ratio (FCR) (Maynard *et al.*, 1979).

WG = final weight (g) -initial weight (g) / initial weight (g) × 100;

FCR = feed intake/weight gain and consumption (g);

SGR (%) = [(final weight) - (initial weight)/120 days] × 100.

SR = Final number of fish in the tank/Initial number of fish in the tank × 100 ……. (2)

Fish sampling

Each initial tank's fish was chosen at random and sedated with MS-222 (60 mg/L). Using syringes, 1 mL of whole blood was taken from the caudal vein and collected in an EDTA tube. Following that, the kidney, liver, and gut of each fish were surgically removed through vivisection, promptly flash-frozen, and preserved at 80 °C. A total of 10 times of distilled water was added to the tissues to dilute them, before centrifugation for 5 minutes at 4°C (5000 r/min) and storage at 4°C for the supernatants.

Fish tests were carried out according to the guidelines set forth by the Animal Ethics Council for the use of animals in research in India. Every effort was made to minimize the number of fish used and their agony.

Histological examination

After a 24-hour fixation in 10% buffered formalin, the intestinal samples were cleaned with 0.9 % NaCl solution. After being cleaned in Xylene, the fixed samples were put in paraffin wax. Iwashita *et al.*, (2008) stated that after making thin cross-sectional slices (5 µm), the samples were stained with hematoxylin and eosin (H&E). An Olympus light microscope, a Zeiss Cyber-Shot on-board camera, and Adobe Photoshop were used for the analysis and photography of the histology slides. In the end, the degree of histological alterations (degeneration, necrosis, and mucosal abscission) brought on by experimental diets was assessed using a semi-quantitative scoring method.

Hematological analysis

Hematology characteristics were determined using whole blood samples from fish. A Neubauer counting chamber (Maule and Schreck, 1990) was used to count red blood cells (RBC) and white blood cells (WBC). A Sahli's haemoglobinometer (Dethloff *et al.*, 1999) was used to determine the hemoglobin (Hb) content. Hematocrit (Ht) levels were determined using the Wintrobe tube method (Wentrobe, 1967). MCV. MCH, and MCHC

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Include methods used in flash-freezing (dry-ice? Liquid nitrogen?) Cite reference used for this method

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Immunological activities

The immunological characteristics were measured using homogenized liver and intestinal supernatant samples. Complement C3, C4, Nitric Oxide Synthase (NOS), and immunoglobulin M (IgM); Lysozyme (LSZ) activity was determined using the ELISA test kit according to the protocol of Yu *et al*., (2020)

Antioxidant activities

Antioxidant ability - Superoxide dismutase (SOD) activity, Total Antioxidant Capacity (T-AOC) activity, Catalase (CAT) activity, Glutathione Reductases (GR) activity, and Lipid peroxidation (LPO) wWere determined using the method according to the protocol of Feng *et al.,* (2016).

Statistical analysis

SPSS statistics (IBM – SPSS software 2020) was used to analyze the experimental data using one-way ANOVA followed by the Duncan test. The data visualization graphics were created by Origin Pro 8.5 software (Origin Lab Corporation). Statistical analyses were conducted using three replicates and expressed as means ± standard errors.

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Results

Removal efficiency of turbidity

Table 1 displays the descriptive statistics of turbidity metrics in the research groups throughout 120 days using the filter system. The turbidity was significantly higher in the WoF system when compared to the filtration system ($p<0.05$). On the 120th day, the turbidity was TI: 9.15±0.14; TII: 6.15±0.05; TIII: 7.00±0.05 in the without filtration system but with filtration treatment, PF - TI: 0.14±0.00; TII: 0.15±0; TIII: 0.15±0.00, BF - TI: 0.22±0.00; TII: 0.20±0.00: TIII: 0.21±0.00, PBF - TI: 0.12±0.00; TII: 0.12±0.00; T III: 0.12±0.00, and the PB-UVF - TI: 0.12±0.00; TII: 0.12±0.00; T III: 0.12±0.00. The filtration system maintains the turbidity level in the < 5 NTU as per the EPA guidelines.

The efficacy of turbidity removal was also found to be adversely affected by the combination of the bamboo and pot filtering system. The highest turbidity removal percentage (98%) and lowest turbidity value (0.12 NTU) were achieved while utilizing the PBF and

PBUVF filtering systems. The turbidity in the water was efficiently eliminated by the PBF and PBUV F filters, compared to the PF and BF filters (Figure 1).

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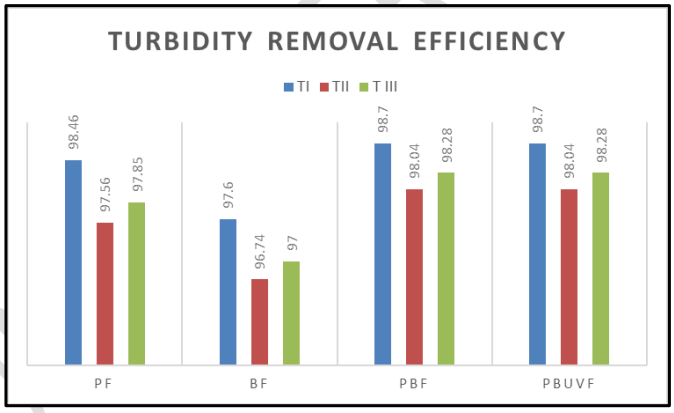
Growth performance and survival

Results of the overall production of three different species of ornamental fishes in both WoF and with filtration treatments are summarized in Table 2. The average size of the three different species harvested in the Without filtration system. The average growth was 0.12g/day and the final weight gain of the three different treatments PBF shows significantly higher when compared to the other filters. The survival rate was numerically lower than that of fish without a filtration system. The feed conservation ratio increased significantly when fishes were cultured in the PBF system. Between the five experimental groups, when fish were with a filtration system the growth, Feed conservation ratio, and survival rate were significantly higher than without a filtration system in the experimental setup.

Figure 1: This figure shows the removal efficiency of turbidity in different filtration system



PF (Pot filter), BF (Bamboo filter), PBF (Pot and Bamboo filter), PBUV F (Pot, Bamboo and UV filter)

Histological study

In the ~~with~~with ~~-~~filtration system, the general structure of the gills shown in the PB-UV F (Figure 2: e, e_1, e_2) in all three fish samples (TI, TII, T III) and consists of the primary and secondary lamellae. Squamous epithelial cells bound the secondary lamellae. Hyperplasia of

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secondary lamellae is shown in Figure 2a. Figure 2 (a, a_1, a_2,b_1,b_2, c_2, d_1) shows the close view of interlamellar hyperplasia with almost total fusion of secondary lamellae. Telangiectasia of secondary lamellae is shown in figure $2c_1$. Eosinophilic infiltrate (Figure 2c) and edema in gill in primary lamellae was shown in Figure 2 (a_2).

A single layer of mesothelial cells and a thin fibrous capsule envelop the cultured fish liver samples and normal hepatocytes of fish shown in the filtering system (Figure $3d_2$ & e_2). The liver alterations were found in the with and without filtration system cultured fishes. Dilation and congestion in sinusoids are shown in Figure 3 (a, a_1, a_2, b_1). Hepatopancreas damage is characterized by loss of contact between hepatocytes (Figure 3b & c). Melano-macrophage aggregation and cellular degenerations are shown in Figures $3b_2$, b_3.

The normal histological structure of the glomerulus, renal tubules, and bowman's capsules are shown in Figure 4 (e, e_1, & e_2). Detached epithelial cells from basal lamina and dilated tubules are observed in Figure 4 (a, a_1, a_2, b, c, c_2, d, & d_1).

Hematological analysis

The hematological parameters (RBC count, WBC count, Hb, and Ht; MCV, MCH, and MCHC) of *C. auratus*, *C. carpio*, *P. hypophthalmus* in different filtrations are demonstrated in Table 3. PF, BF, PBF, PB-UVF, and WoF treatment groups were analyzed in the filtration treatment group, Hb, MCHC, and MCH significantly differed from the WoF treatment. The RBC content was higher in the fish kept in filtration tanks as compared to the without filtration tanks. The RBC content was significantly higher in the PBF followed by the PF and PBUVF. The group of fish kept in the filters showed significantly different WBC and Hb in all the filtration tanks (PF, BF, PBF, and PBUVF) as compared to those without filtration tanks. The WBC showed insignificant variation among the TI, T2, and T3 and the filters.

Significant Hb content was found in fish kept in a filtration system over 120 days. The entire treatment group showed significantly higher Hb content as compared to the without filtration tanks. The highest no of Hb was recorded in the TI (PF) tank, TII (PBUVF) tank, T III (BF) tank.

Antioxidant Indices

Each experimental group measured antioxidant enzymes (SOD, CAT, ABTS, GR, and LPO) in its liver and gut according to its treatment (WoF, PF, BF, PBF, and PB-UV-F) (Table 4). There was a significant difference between the WoF treatment and the other treatment groups in GR and CAT activities in liver and gut samples ($p > 0.05$). There is a significant

increase in PBF in ABTS compared to the other treatments. WoF, PF, PBF, and PB-UVF are significantly lower when compared to the BF treatment.

Days	Samples	WoF	PF	BF	PBF	PB-UVF
	T-I	0.32±0.00^{a}	0.17±0.01^{c}	0.23±0.00^{b}	0.18±0.00^{c}	0.12±0.00^{d}
	T-II	0.43±0.01^{a}	0.15±0.00^{c}	0.23±0.00^{b}	0.14±0.01^{c}	0.12±0.00^{c}
15	T-III	0.21±0.00^{a}	0.14±0.00^{c}	0.25±0.00^{a}	0.13±0.00^{c}	0.13±0.00^{c}
	T-I	0.59±0.01^{a}	0.16±0.00^{c}	0.23±0.00^{a}	0.13±0.00^{cd}	0.12±0.00^{d}
	T-II	0.24±0.02^{a}	0.15±0.00^{b}	0.21±0.00^{a}	0.13±0.00^{b}	0.12±0.00^{b}
30	T-III	0.25±0.02^{a}	0.14±0.00^{b}	0.23±0.00^{b}	0.13±0.00^{b}	0.12±0.00^{b}
	T-I	0.90±0.03^{a}	0.15±0.00^{bc}	0.23±0.00^{b}	0.12±0.00^{c}	0.13±0.00^{c}
	T-II	3.85±0.06^{a}	0.15±0.00^{b}	0.21±0.00^{b}	0.13±0.00^{b}	0.12±0.00^{d}
45	T-III	5.71±0.05^{a}	0.15±0.00^{b}	0.23±0.00^{b}	0.13±0.00^{b}	0.13±0.00^{b}
	T-I	4.68±0.11^{a}	0.15±0.00^{b}	0.21±0.00^{b}	0.11±0.00^{b}	0.13±0.01^{b}
	T-II	4.52±0.20^{a}	0.15±0.00^{b}	0.21±0^{b}	0.12±0.00^{b}	0.13±0.00^{b}
60	T-III	5.89±0.09^{a}	0.14±0.00^{b}	0.22±0.00^{b}	0.12±0.00^{b}	0.12±0.00^{b}
	T-I	6.46±0.02^{a}	0.15±0.00^{c}	0.21±0.00^{b}	0.13±0.00^{c}	0.13±0.01^{c}
	T-II	4.67±0.23^{a}	0.15±0.00^{b}	0.22±0.00^{b}	0.12±0.00^{b}	0.13±0.00^{b}
75	T-III	6.24±0.06^{a}	0.15±0.00^{b}	0.21±0.00^{b}	0.12±0.00^{b}	0.12±0.00^{b}
	T-I	7.53±0.12^{a}	0.15±0.00^{b}	0.21±0.00^{b}	0.12±0.00^{b}	0.11±0.00^{b}
	T-II	5.47±0.90^{a}	0.15±0.00^{b}	0.22±0.00^{b}	0.12±0.01^{b}	0.12±0.00^{b}
90	T-III	6.40±0.06^{a}	0.15±0.00^{b}	0.22±0.00^{b}	0.13±0.01^{b}	0.12±0.00^{b}
	T-I	8.68±0.13^{a}	0.15±0.00^{b}	0.2±0.00^{b}	0.13±0.00^{b}	0.13±0.00^{b}
	T-II	5.53±0.01^{a}	0.14±0.00^{c}	0.21±0.00^{b}	0.13±0.00^{c}	0.12±0.00^{c}
105	T-III	6.62±0.05^{a}	0.15±0.00^{b}	0.22±0.00^{b}	0.13±0.01^{b}	0.11±0.00^{b}
	T-I	9.15±0.14^{a}	0.14±0.00^{b}	0.22±0.00^{b}	0.12±0.00^{b}	0.12±0.00^{b}
	T-II	6.15±0.05^{a}	0.15±0^{b}	0.20±0.00^{b}	0.12±0.00^{b}	0.12±0.00^{b}
120	T-III	7.00±0.05^{a}	0.15±0.00^{b}	0.21±0.00^{b}	0.12±0.00^{b}	0.12±0.00^{b}

Table 1: This table shows the mean±SE of the water turbidity analysis with the different treatments of WoF, PF, BF, PBF, and PB-UV-F for 120 days during the culture period of *Crassius auratus*, *Cyprinus carpio*, *Pangasianodon hypophthalmus*.

Each value is represented in Mean ± SE (n=3). Different superscripts are significant differences between the filtration system ($p<0.05$).

Table 2: shows the growth performance of *C. auratus*, *C. carpio*, and *P. hypophthalmus* with the different treatments of WoF, PF, BF, PBF, and PB-UV-F.

Growth Performance	Treatments	WoF	WF			
			PF	BF	PBF	PB-UV-F
IW	T1	2.73±0.21	2.16±0.02	2.17±0.01	2.20±0.01	2.08±0.01
	T2	5.32±0.77	2.18±0.01	2.19±0.02	2.46±0.06	2.14±0.01
	T3	19.2±1.52	19±0.06	18.85±0.02	19.27±0.04	19.02±0.01
FW	T1	2.91±0.29	3.02±0.02	3.21±0.07	3.13±0.09	2.99±0.03
	T2	6.22±0.77	3.08±0.04	2.89±0.03	3.28±0.11	2.70±0.04
	T3	20±1.51	20.01±0.04	19.8±0.05	20.43±0.04	19.89±0.04
WG	T1	4.79±0.67^{d}	39.2±1.09^{c}	44.8±0.86^{a}	42.09±3.95^{b}	43.7±1.06^{a}
	T2	3.79±0.81^{e}	41.5±2.19^{a}	31.75±1.08^{c}	33.09±2.43^{b}	26.3±1.48^{d}
	T3	2.09±0.42^{e}	4.88±0.27^{c}	4.93±0.19^{b}	5.93±0.10^{a}	4.54±0.12^{d}
SGR	T1	0.09±0.01^{d}	0.70±0.01^{c}	0.80±0.01^{a}	0.09±0.01^{b}	0.75±0.02^{b}
	T2	0.16±0.18^{e}	0.75±0.03^{a}	0.57±0.02^{c}	0.16±0.18^{b}	0.46±0.02^{d}
	T3	0.32±0.05^{c}	0.77±0.04^{a}	0.77±0.02^{a}	0.32±0.05^{c}	0.71±0.01^{b}
FCR	T1	0.12±0.01^{a}	0.009±0.00^{b}	0.009±0.00^{b}	0.013±0.00^{b}	0.01±0.00^{b}
	T2	0.17±0.03^{c}	0.011±0.00^{a}	0.015±0.00^{a}	0.01±0.001^{c}	0.01±.0.00^{b}
	T3	0.32±0.07^{c}	0.12±0.00^{d}	0.11±0.00^{d}	1.10±0.32^{a}	0.34±0.21^{b}

Each value is represented in Mean ± SE (n=3). Different superscripts are significant differences between the filtration system ($p<0.05$).

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Immunological indices

In immune enzyme activities of fish liver and gut samples are shown in Table 5, LSZ: The with-filtration (PF, BF, PBF, and PBUVF) fish gut and liver samples were significantly different when compared to the without-filtration fish samples ($p > 0.05$). C3: Gut was increased dramatically in PBF compared to the other treatment groups; Liver was significantly reduced in WoF, PF, BF, and PB-UVF ($p > 0.05$). C4: Gut and liver samples of TI, TII, and T III showed significantly higher PF when compared to the other treatments. Compared to the other treatments, PBF significantly increased IgM and Nos ($P > 0.05$).

Discussion

Effect of different filtration treatments on growth performance

Among the fish treated with WoF, the survival rate was significantly higher for 120 days of the experimental period. As reported by Attramadal et al., (2012a, 2012b, 2014, 2016), marine fish larvae treated with FTS had lower survival rates than larvae treated with RAS, which supported the hypothesis that *C. auratus*, *C. carpio*, *P. hypophthalmus* juveniles reared

in PF, BF, PBF, PB-UV-F will show a higher weight gain and survival compared to the WoF treatment. In rainbow trout culture, the recommended nitrate level was 75 mg l^{-1} (Davidson *et al.*, 2014) where there are no growth effects, however some harmful effects on overall wellness.

Figure 2: Histological observation of fish gill in WoF, PF, BF, PBF, and PB-UV-F treatments.

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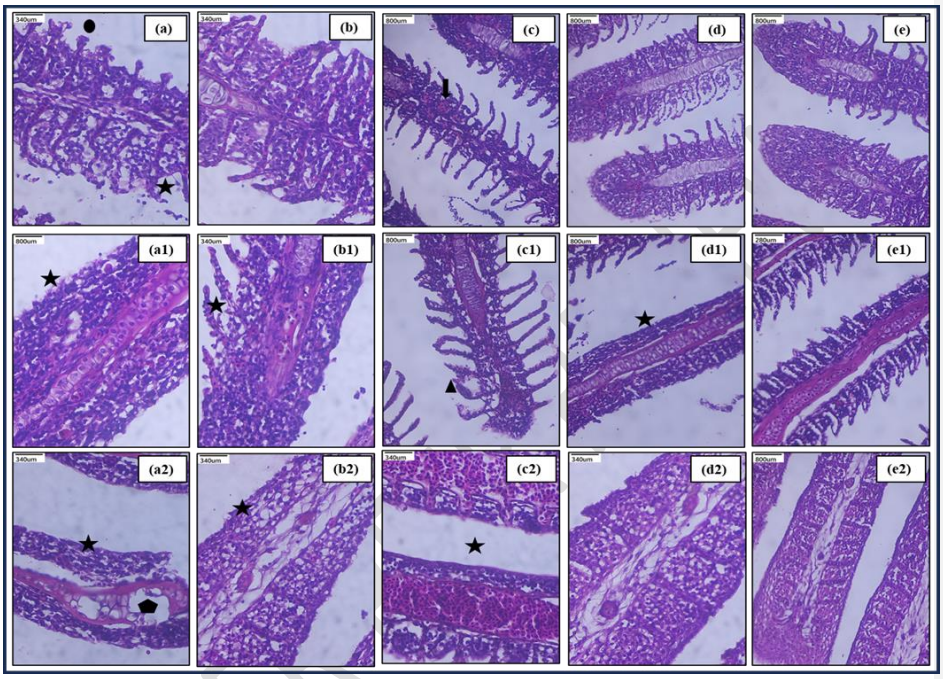
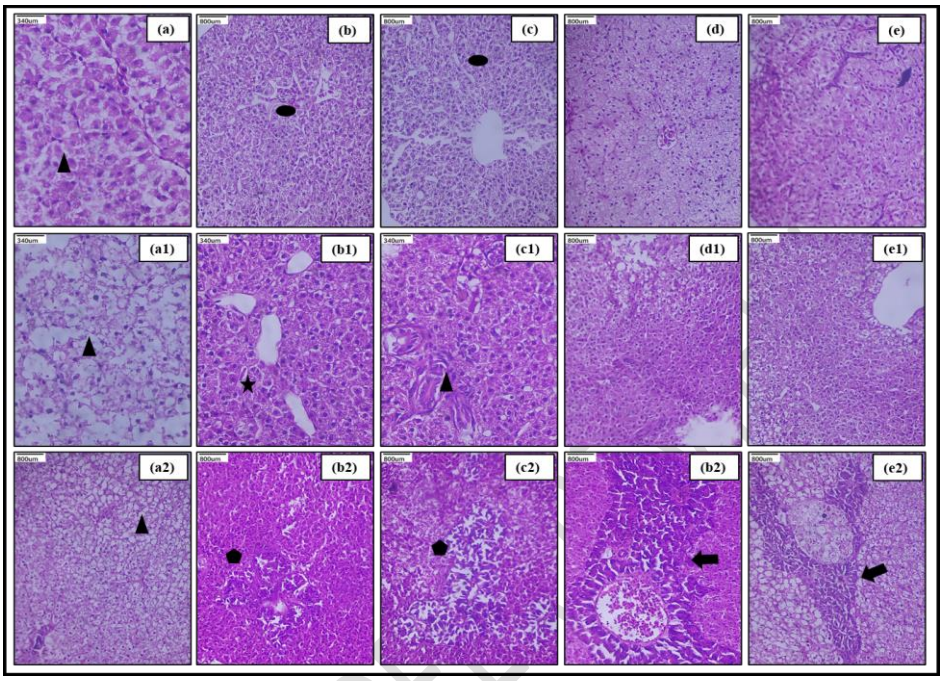


This figure shows (a) Histological study of treatment I fish gill in WoF, (b) PF, (c) BF, (d) PBF, (e) PB-UVF; (a1), Treatment II WoF, (b1) PF, (c1) BF, (d1) PBF, (e1) PB-UVF; (a2) Treatment III WoF, (b2) PF, (c2) BF, (d2) PBF, (e2) PB-UVF.

Figure 3: Histological observation of fish liver in WoF, PF, BF, PBF, and PB-UV-F treatments



This figure shows (a) Histological study of treatment I fish liver in WoF, (b) PF, (c) BF, (d) PBF, (e) PB-UVF; (a1), Treatment II WoF, (b1) PF, (c1) BF, (d1) PBF, (e1) PB-UVF; (a2) Treatment III WoF, (b2) PF, (c2) BF, (d2) PBF, (e2) PB-UVF.

~~Here, we found~~This research noted that higher turbidity caused the growth effects on the culture of *C. auratus*, *C. rubrofuscus*, *P. hypophthalmus*. Growth in ~~our~~ this study has fallen under low water changes, which an increase in organic material and excessive sediment loads may have caused.

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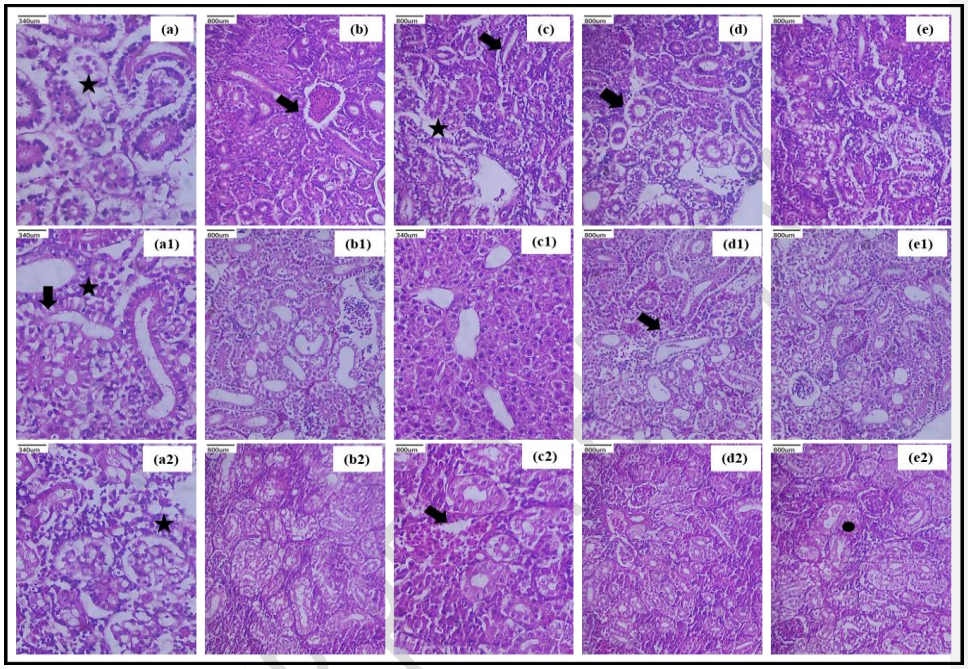
Effect of different filtration treatments on hematological parameters

A pathologic reflector of the entire body is the blood. The various biological and hematological responses of aquatic animals directly reflect the various stressors like transport, excess crowding, and pollution. Therefore, research on changes in fish's hematological characteristics can offer useful information in identifying stress, environmental contamination, and pathology (Bhawna S *et al.*, 2020). In the present study, h~~H~~ematological parameters are notably increased when compared to the WoF treatment. The existence of anemia is due to the reduction of hemoglobin percentage and the total erythrocyte count. In environmental stress,

Hb seems to be the best blood indicator. De Almeida-val (2005) reports the low oxygen levels by altering several physiological and biochemical parameters.

Figure 4: Histological observation of fish kidney in WoF, PF, BF, PBF, and PB-UV-F treatments

Commented [OJ22]: What do the arrows and the stars in the plate point to?



This figure shows (a) Histological study of treatment I fish gill in WoF, (b) PF, (c) BF, (d) PBF, (e) PB-UVF; (a1), Treatment II WoF, (b1) PF, (c1) BF, (d1) PBF, (e1) PB-UVF; (a2) Treatment III WoF, (b2) PF, (c2) BF, (d2) PBF, (e2) PB-UVF.

Effect of different filtration treatments on histology

Gill's health is a crucial sign of the well-being and health of fish in farming practices (Marshall and Bellamy, 2010). Fish gills are an ideal indicator of the interaction between them and their environment because of the thin, highly sensitive respiratory epithelium that covers the lamellae of the gills (Strzyzewska *et al.*, 2016). Along with being an essential immune tissue, the gills also regulate processes like gas exchange, acid-base balance, nitrogenous waste excretion, ion and osmoregulation, and home hormone metabolism (Evans *et al.*, 2005). Better gill and liver health in this experiment was seen in the PF, BF, PBF, and PB-UVF compared to the WoF treatment.

Table 3: This table shows, the effects of hematological parameters on the culture of *P. hypophthalmus* in different filtration treatments.

Commented [OJ23]: Why is only this spp. Listed here

Blood parameters	Treatments	WoF	WF			
			PF	BF	PBF	PB-UV-F
RBC	T1	1.09±0.00^{b}	1.15±0.01^{a}	1.12±0.01^{ab}	1.19±0.02^{a}	1.14±0.00^{ab}
	T2	1.06±0.01^{b}	1.16±0.01^{ab}	1.08±0.00^{ab}	1.12±0.02^{a}	1.12±0.01^{a}
	T3	1.06±0.02^{b}	1.08±0.01^{ab}	1.10±0.01^{ab}	1.13±0.01^{a}	1.12±0.01^{a}
WBC	T1	6.82±0.01^{c}	8.52±0.01^{a}	8.51±0.05^{a}	8.58±0.02^{a}	8.40±0.02^{b}
	T2	3.03±0.01^{d}	10.78±0.37^{c}	10.88±0.29^{c}	16.1±0.11^{a}	11.95±0.25^{b}
	T3	7.52±0.01^{d}	8.17±0.00^{b}	8.04±0.02^{c}	8.24±0.02^{a}	8.15±0.02^{b}
Hb	T1	7.11±0.00^{c}	11.88±0.02^{a}	10.52±0.17^{b}	11.7±0.00^{a}	11.72±0.11^{a}
	T2	5.26±0.14^{d}	11.46±0.15^{b}	11.31±0.09^{b}	11.46±0.20^{c}	11.98±0.24^{a}
	T3	17.1±1.14^{b}	16.09±0.15^{c}	18.40±0.19^{a}	18.3±0.11^{a}	15.43±0.18^{d}
Ht	T1	10.8±0.2^{c}	29.13±0.31^{a}	22.2±0.63^{b}	30.7±0.37^{a}	22.3±1.24^{b}
	T2	10.13±0.07^{c}	16.23±0.37^{ab}	17±0.25^{b}	14.6±0.25^{ab}	18.7±0.40^{a}
	T3	17.3±0.11^{b}	17.19±0.55^{b}	17.1±0.35^{b}	18.6±0.12^{a}	18.3±0.41^{ab}
MCH	T1	39.2±0.17^{c}	57.5±1.01^{b}	63.1±0.75^{a}	62.5±0.65^{a}	62.6±0.55^{a}
	T2	53.16±0.12^{b}	82.8±0.52^{a}	84.2±2.49^{a}	83.8±0.33^{a}	86.7±3.87^{a}
	T3	11.16±0.14^{d}	66.6±1.45^{a}	64±1.73^{a}	16.4±0.14^{c}	56±1.51^{b}
MCV	T1	101.36±0.31^{c}	115.1±2.6^{b}	101.6±5.23^{c}	151.2±0.52^{a}	114±3.21^{b}
	T2	90.6±0.2 ^{d}	151.6±6.96 ^{a}	132±7.23 ^{b}	127.4±0.20 ^{b}	112.3±1.20 ^{c}
	T3	62.26±0.23^{b}	85.6±0.88^{a}	85±2.30^{a}	71.53±0.08^{ab}	76±13.5^{ab}
MCHC	T1	59.63±0.14^{b}	63±0.7^{a}	55.23±0.29^{c}	63.7±0.37^{a}	57.3±1.23^{c}
	T2	48.26±0.12^{d}	112.63±2.24^{a}	114.3±1.7^{a}	107±0.40^{b}	88.2±0.02^{c}
	T3	68.26±0.12^{d}	83±3.05^{ab}	85.33±2.18^{a}	75.6±0.25^{c}	79.15±0.25^{bc}

Each value is represented in Mean ± SE (n=3). Different superscripts are mentioned as the significant differences between the filtration system ($p<0.05$).

Table 4: This table shows, the effects of antioxidant parameters on the culture of *C. auratus*, *C. carpio*,and *P. hypophthalmus* in different treatments WoF, PF, BF, PBF, and PB-UV-F.

Antioxidant parameters	Sample	Treatment	WoF	WF			
				PF	BF	PB-F	PB-UV-F
ABTS	Gut	T1	7.43±2.17^{b}	7.13±0.02^{c}	7.11±0.03^{c}	10.63±3.59^{a}	6.89±0.05^{d}
		T2	17.1±2.72^{a}	5.3±0.12^{c}	5.22±0.13^{c}	13.54±2.59^{b}	5.14±0.02^{c}
		T3	21.19±1.94^{b}	11.52±0.01^{e}	11.9±0.02^{d}	22.91±1.94^{a}	12.6±0.18^{c}
	Liver	T1	6.23±1.84^{b}	6.15±0.02^{cd}	6.10±0.00^{d}	9.32±2.35^{a}	6.17±0.01^{c}
		T2	17.4±4.78^{a}	6.17±0.01^{c}	6.12±0.02^{c}	11.54±2.3^{b}	5.47±0.00^{d}
		T3	24.24±2.76^{b}	15.71±0.14^{c}	16.0±0.49^{c}	28.71±2.89^{c}	16.2±0.27^{a}
CAT	Gut	T1	0.24±0.02^{a}	0.15±0.02^{b}	0.23±0.01^{a}	0.22±0.01^{a}	0.25±0.02^{a}
		T2	0.2±.0.01^{a}	0.17±0.01^{b}	0.17±0.01^{a}	0.2±0.01^{ab}	0.21±0.01^{a}
		T3	0.25±0.00^{b}	0.12±0.01^{c}	0.17±0.01^{b}	0.19±0.00^{b}	0.26±0.01^{a}
	Liver	T1	0.24±0.01^{ab}	0.17±0.0^{bc}	0.24±0.02^{a}	0.17±0.01^{c}	0.26±0.01^{a}
		T2	0.17±0.01^{b}	0.16±0.00^{b}	0.22±0.00^{a}	0.2±0.01^{a}	0.24±0.01^{a}
		T3	0.31±0.00^{a}	0.14±0.01^{d}	0.16±0.02^{cd}	0.22±0.03^{bc}	0.22±0.00^{b}
GR	Gut	T1	60.2±9.35^{a}	49±0.20^{b}	43.5±1^{c}	42.6±9.62^{c}	38.8±0.17^{d}
		T2	68.3±8.49^{b}	50.87±0.20^{c}	50.4±0.28^{c}	81.2±15.6^{a}	44.4±0.15^{d}
		T3	31.2±3.49^{a}	22.3±0.15^{c}	22.5±0.2^{bc}	16.9±2.79^{c}	22.8±0.05^{b}
	Liver	T1	64.7±10.23^{b}	40.5±0.23^{c}	39.08±0.04^{a}	93.8±6.07^{c}	35.5±0.23^{d}
		T2	38.1±6.68^{b}	31.1±0.02^{c}	30.75±0.29^{c}	21.3±2.94^{d}	40.2±0.20^{a}
		T3	34.9±10.23^{a}	29.2±0.13^{b}	22.43±0.29^{c}	12.2±3.4^{d}	22.3±0.09^{c}
LPO	Gut	T1	1.01±0.37^{c}	1.13±0.01^{b}	1.25±0.02^{a}	0.65±0.2^{d}	1.16±0.02
		T2	0.66±0.22^{b}	0.47±0.01^{d}	0.51±0.02^{cd}	0.97±0.26^{a}	0.55±0.02^{c}
		T3	0.6±0.12^{a}	0.61±0.00^{a}	0.58±0.00^{a}	0.21±0.05^{b}	0.61±0.01^{a}
	Liver	T1	0.87±0.24^{ab}	0.75±0.02^{b}	0.88±0.05^{a}	0.42±0.08^{c}	0.77±0.01^{b}
		T2	1.07±0.37^{a}	0.95±0.03^{a}	0.81±0.01^{b}	0.31±.00^{c}	0.76±0.01^{b}
		T3	0.51±0.15^{a}	0.46±0.01^{b}	0.47±0.01^{b}	0.41±0.00^{c}	0.46±0.01^{b}
SOD	Gut	T1	59.4±11.7^{a}	46.07±0.49^{b}	44.18±0.05^{c}	58.8±12.5^{a}	41.13±0.03^{d}
		T2	49.15±3.65^{a}	48.5±0.23^{a}	48.9±0.09^{a}	41.7±5.39^{b}	47.7±0.86^{a}
		T3	70.08±11.1^{d}	75.6±0.15^{a}	71.8±0.18^{b}	67.4±7.09^{e}	71.05±0.03^{c}
	Liver	T1	79.9±6.06^{a}	71.03±0.46^{b}	67.4±1.33^{c}	26.8±3.45^{e}	61.7±0.38^{d}
		T2	59.6±5.26^{a}	49.3±0.13^{c}	46.4±0.32^{d}	57.7±6.4^{b}	47±0^{d}
		T3	57.14±8.08^{ab}	67.4±1.33^{ab}	54.3±0.08^{b}	57.7±8.96^{a}	56.1±0.02^{ab}

Each value is represented in Mean ± SE (n=3). Different superscripts are mentioned as the significant differences between the filtration system ($p<0.05$).

Table 5: This table shows, the effects of immunological parameters on the culture of *C. auratus, C. carpio,*and *P. hypophthalmus* in different treatments WoF, PF, BF, PBF and PB-UV-F.

Immunological parameters	Sample	Treatment	WoF	WF			
				PF	BF	PB-F	PB-UV-F
LSZ	Gut	T1	122±1.85^{a}	86±1.52^{b}	75.6±2.40^{c}	85±2.30^{b}	84.6±2.02^{b}
		T2	209±5.50^{a}	125.6±2.84^{c}	117±1.52^{c}	160±2.88^{b}	123±1^{c}
		T3	228±4.05^{a}	153.6±1.45^{c}	136±2.51^{d}	182±4.04^{b}	126±1.15^{e}
	Liver	T1	91±3.05^{a}	73.6±1.45^{b}	73.3±1.20^{b}	71±3.78^{b}	87±0^{a}
		T2	91.6±1.76^{a}	86±2.51^{b}	74.6±0.66^{c}	72.3±1.85^{c}	71.3±0.33^{c}
		T3	122±2.02^{a}	93.3±1.85^{b}	90.6±3.75^{b}	93.3±2.33^{b}	86±2.51^{b}
C3	Gut	T1	0.52±0.02^{c}	0.46±0.01^{d}	0.36±0.01^{e}	0.72±0.01^{b}	1.22±0.00^{a}
		T2	0.68±0.05^{b}	0.73±0.01^{b}	0.70±0.00^{b}	0.84±0.02^{a}	0.42±0.00^{c}
		T3	1.05±0.02^{c}	1.12±0.02^{b}	0.97±0.01^{d}	1.22±0.01^{a}	0.85±0.02^{e}
	Liver	T1	0.29±0.02^{d}	0.54±0.02^{b}	0.51±0.01^{c}	0.54±0.02^{b}	0.61±0.01^{a}
		T2	0.47±0.01^{c}	0.65±0.03^{a}	0.63±0.01^{ab}	0.58±0.01^{b}	0.64±0.00^{a}
		T3	0.84±0.01^{c}	1.38±0.05^{b}	0.65±0.00^{d}	1.60±0.01^{a}	0.56±0.01^{e}
C4	Gut	T1	0.41±0.01^{d}	0.46±0.01^{c}	0.48±0.01^{c}	0.64±0.02^{a}	0.53±0.01^{b}
		T2	0.44±0.02^{d}	0.83±0.02^{b}	0.88±0.00^{b}	0.93±0.01^{a}	0.71±0.00^{c}
		T3	0.63±0.01^{c}	1.14±0.02^{ab}	0.92±0.00^{abc}	1.05±0.02^{a}	0.72±0.01^{bc}
	Liver	T1	0.37±0.02^{b}	0.52±.02^{a}	0.56±0.00^{a}	0.51±0.01^{a}	0.56±0.01^{a}
		T2	0.39±0.04^{d}	0.62±.01^{b}	0.55±0.00^{bc}	0.64±0.01^{a}	0.52±0.00^{c}
		T3	0.54±0.02^{c}	1.05±0.02^{a}	0.88±0.00^{b}	1.04±0.01^{a}	0.93±0.01^{b}
IgM	Gut	T1	0.18±0.01^{b}	0.69±0.10^{a}	0.64±0.02^{a}	0.68±0.01^{a}	0.74±0.01^{a}
		T2	0.32±0.00^{d}	0.63±0.02^{b}	0.55±0.00^{c}	0.74±0.02^{a}	0.53±0.03^{c}
		T3	0.32±0.00^{e}	1.04±0.00^{c}	0.92±0.02^{d}	1.26±0.02^{a}	1.10±0.00^{b}
	Liver	T1	0.22±0.01^{d}	0.66±0.00^{b}	0.56±0.01^{c}	0.74±0.01^{a}	0.65±0.00^{b}
		T2	0.24±0.01^{d}	0.59±.02^{b}	0.46±0.01^{c}	0.78±0.00^{a}	0.48±0.02^{c}
		T3	0.34±0.01^{e}	1.13±0.02^{b}	0.95±0.02^{d}	1.33±0.02^{a}	1.04±0.03^{c}
Nos	Gut	T1	0.55±0.02^{d}	1.17±0.01^{bc}	1.22±0.00^{b}	1.85±0.03^{a}	1.14±0.02^{c}
		T2	0.36±.01^{c}	1.25±0.01^{a}	1.15±0.01^{b}	1.25±0.01^{a}	1.16±0.02^{b}
		T3	1.82±0.02^{c}	1.96±0.02^{b}	1.45±0^{e}	2.33±0.02^{a}	1.64±0.01^{d}
	Liver	T1	0.57±0.01^{c}	1.22±0.00^{b}	1.24±0.01^{b}	1.31±0.02^{a}	1.25±0.01^{b}
		T2	0.45±0.01^{d}	1.33±0.00^{a}	1.13±0.01^{b}	1.33±0.02^{a}	1.07±0.01^{c}
		T3	1.32±0.01^{b}	2.18±0.05^{a}	1.22±0.00^{bc}	2.25±0.02^{a}	1.16±0.02^{c}

Each value is represented in Mean ± SE (n=3). Different superscripts are mentioned as the significant differences between the filtration system ($p<0.05$).

Effect of different filtration treatments on antioxidant parameters

SOD, T-AOC, CAT, GR, and MDA are among the enzymes and antioxidants that prevent oxygen toxicity (Feng *et al.*, 2016). The antioxidant power of SOD protects active cells from damage and aging, lowers inflammation, and boosts immunity (Campa-Cordova *et al.*, 2002). It is possible to measure T-AOC effectiveness by the body's antioxidant defense system. GR plays a role in preserving the equilibrium of the cellular redox status since it is the most prevalent intracellular non-protein thiol (Feng *et al.*, 2016). The CAT enzyme eliminates free radicals by breaking down H_2O_2 into H_2O and O_2 (Yin *et al.*, 2018). Animals produce MDA as a byproduct of unbalanced lipid peroxidation, an abnormal antioxidant system. MDA damages organisms and hurts cells (Storey, 1996). In the current study, compared with the WoF; CAT, LPO, and GR levels are significantly raised in the liver. While SOD and ABTS were attenuated in the gut and liver. After different water treatments (PF, BF, PBF, and PB-UVF), the levels of antioxidant parameters of fish tissue samples were seen significantly better to compare the WoF treatment, and the results are shown in Table 4.

Effect of different filtration treatments on immunological parameters

Sakai (2010) reported that fish mostly utilize generic and innate immunity when harmful organisms enter or alter their living environment. Several immune components are widely accepted in fish immunity, including C3, C4, IgM, NOS, LSZ, AKP, GOP, GTP, TNF-IL-1IL IL-2, and IL-6, which might indicate a fish's response to environment stress (Li *et al.*, 2019a, b, c). Ekdahl *et al.*, (2019) stated, a humoral immune response can be aided by the macromolecule complement C3 and C4. They are primarily made by hepatocytes and can engage in the immune response through a variety of routes when triggered. They can destroy bacteria and viruses by dissolving immunoglobulin complexes and promoting inflammatory responses, in addition to their direct role in dissolving and neutralizing viruses. Considering that complement activity is down-regulated under some stress conditions, complement activity can be used to measure fish immunological capacity (Ichiki *et al.*, 2012). A fish's health and immunological condition can also be determined by IgM and NOS (Ludwig *et al.*, 2002). C3, C4, IgM, and NOS levels in PF, BF, PBF, and PB-UVF were significantly higher, and liver and gut LSZ levels were significantly lower than the WoF. Following ammonia stress, C4, IgM, and NOS levels decreased in all experimental groups, although immune enzyme levels were significantly greater in the C/N 20 treatments than in the control groups (Zhe Yu *et al.*, 2020).

Conclusion

In conclusion, our results suggest that the different filtration treatments have positive effects on water quality, growth performance, immune response, antioxidant capacity, hematological parameters, and histological observation in aquarium management of *C. auratus*, *C. carpio*, *P. hyphophthalomus* digestion and absorption performance of cultured animals with pot, bamboo, and UV-based filtration system.

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