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STUDIES ON THE EFFECT OF MICROPLASTICS (POLYETHYLENE GLYCOL) ON THE GROWTH PERFORMANCE AND HAEMATOLOGY OF LABEO ROHITA

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Abstract
The present study aimed to assess the physical effects of polyethylene glycol microplastics on growth performance and haematological parameters in freshwater *Labeo rohita*. Fish exposed to different microplastics concentrations T0 was treated as a control group while the T1, T2 and T3 were exposed to 1, 10 and 100 mg/L of microplastics, respectively, for six weeks. Ten individuals were stocked in each experimental tank. Growth performance was measured weekly regarding weight gain, specific growth rate, condition factor, total length and survival rate. In contrast, haematological parameters i.e., RBCs, WBCs, differential WBCs, results showed significant effects of polyethene glycol on growth performance and haematology of fish. Maximum weight, length gain, specific growth rate and condition factor were observed in T0 while the minimum values were observed in T3. RBCs, haemoglobin, haematocrit, MCV, MCH and MCHC were significantly increased in treatment T3 as compared to control group T0. Platelets numbers in *Labeo rohita* weredecreased and WBCs were significantly increased in treatment T3 as compare to control group T0. Differential cells showed fluctuations in all treated groups (T1, T2 and T3). Resultssuggested that exposure of fish to high concentrations of polyethylene glycol microplastics have negative impacts on the fish physiology.

Keywords: Labeo rohita, Growth performance, Haematology, Weight gain

INTRODUCTION

Plastics are the most common and wide spread water pollutants producing by the degradation of plastic bottles, bags and the cosmetic industries (Napper *et al*., 2015). The contamination of freshwater ecosystems and oceans with plastic waste is a matter of great concern (Andrady *et al*., 2011). Microplastics are synthetic polymers with diameters less than5 mm in their longest dimension (Cole *et al*., 2011). They have several different shapes, colours, sizes and chemical composition (Rochman *et al*., 2019). Microplastics do not have severe lethal effects on living organisms. Microplastics can cause chronic toxicity which is considered a major problem (Li *et al*., 2018).
Primary microplastics possess a large number of applications and can enter the environment through

the disintegration of c polymer products (Wang *et al*., 2018). The secondary microplastics are produced by the breakdown of large plastic waste over time. Plastic fragments are exposed to environmental factors in water bodies and put pressure on the material which in turn accelerate the process of aging and rotting of the material (Hintersteiner *et al*., 2015). Freshwater may accumulate numerous microplastics i.e., Polystyrene (Sussarellu *et al*., 2016) polyester and nylon (Browne, 2015) polyethylene, and
polyvinylchloride (Andrady, 2011). Polyethylene glycol is one of the most common types of plastics (Zhang et al., 2018). Polyethylene glycol are used as humectants because theypossess nontoxic and nonimmunogenic characteristics (Bertuglia *et al*., 2006).
Aquatic organisms are very sensitive to any change in the environment and respond quickly in various ways (Bassem, 2020). Numerous active and passive routes of microplasticsuptake by aquatic organisms. They have been proposed leads to accidental uptake of microplastics and their transmission through the food chain (Roch *et al*., 2020). Several fishesand invertebrates have been reported to carry microplastics in their gut. Microplastics can stuck there and stop the digestion that may cause malnutrition and starvation (Rosenkranz *et al*., 2009). Fish is considered to be an ideal model for studying the effects of toxic substances in aquatic environment (Scott and Sloman, 2007).

Labeo rohita is an important species in carp polyculture systems as it grows very fast and reaches marketable size in less than a year (Khan *et al*., 2003). Ingestion of these plastics by fish can cause physical damage to the gastrointestinal tract and neural dysfuction (Oliveira et al., 2013). Hematological parameters red blood cells, hematocrit, hemoglobin, blood glucose, cholesterol, total protein, aspartate aminotransferase and alkaline phosphatase can beaffected due to microplastics ingestion (Kim and Kang, 2014). Polyethylene glycol and its derivatives are nontoxic compounds (Shi, 2013). Blood is the best indicator for determining the health of an organism. Changes in hematological parameters depends on fish age, sexual maturity cycle, species and health status (Hrubec *et al*., 2001).

MATERIALS AND METHODS

The experimental trail was conducted to check the effects of microplastics consumption on the growth performance and haematology of *Labeo rohita* under controlled conditions. The trail was conducted at University of Agriculture Faisalabad PARS Campus.

Experimental Conditions

Labeo rohita was taken from Govt. fish seed hatchery Faisalabad. Fish was acclimatized at room temperature for ten days. Fish was fed once a day on the basal diet. Water samples were collected weekly from aquarium to check the effects of PH, dissolved oxygen and temperature. Water quality parameters were measured by using electronic meter. Polyethylene glycol microplastics were purchased from Faisalabad chemical market.

Fish exposure

The acclimatized individuals were selected randomly and divided into 4 groups and stocked in glass aquaria. T0 was control group and remaining three treatmentsT1, T2 and T3 were treated with different concentrations of micropastics. A photoperiod 12hour dark and 12hour light was maintained.

Growth performance

Growth performance was maintained by having weekly gross weights of each treatment. Growth rate was determined in terms of weight gain, specific growth rate, and survival rate.

Weight gain:

Weight gain calculated by using formula:

Weight gain(g)= Final weight (g) – Initial weight (g)

Specific growth rate:
Specific growth rate can be calculated by using formula:
SGR=In [Final weight (g) – Initial weight (g)] × 100 Total no. of days

Blood samples:
Blood was taken from caudal part and transfer to the EDTA tube. Blood samples werestored over crushed ice.

Hematological parametersRed blood cells count
Blood taken in erythrocyte pipette and then blood was diluted with hymen solution.
Red blood cells were seen under magnification of 250X.
Total RBC $10^6/mm^3$= No. of erythrocytes in 80 small squares
80X 1/400 X 1/10 X /200

White blood cells count
Blood was taken into pipette for the calculation of white blood cells and then solutionwas diluted with diluted fluid.
Total WRBC (10^3 /mm^3)= No. of leucocytes in four squares of $1mm^3$ 4 X 1/10 X 1/200

Hemoglobin
Blood was taken into drabkin reagent tube for the counting of hemoglobin.
Hb (g/ dl^4)= Unknown sample × Conc. of Hb standard (g/dl) 4 known hemoglobin content

Hematocrit %
Blood was taken in heparinized hematocrit pipette and allowed pipette to centrifuge for some intervals of time. Hematocrit capillary tubes volume were recorded by placing them on reading device.

Statistical analysis
Data was statistically analyzed by one way ANOVA to check the effects of differentconcentrations of microplastics.

RESULTS

Growth Performance
Table 1 show the effect of microplastic (polyethylene glycol) under different concentrations of polyethylene microplastics on weight gain, total length gain, specific growth rate, and condition factor and survival rate. The results of our study showed that weight gain was significantly higher ($p<0.05$) in T1 group (0.612±1.042) during 3^{rd} week.
The similar results of our research showed that length gain (0.02±0.933), specific growth rate (0.503±0.892), condition factor (2.785±1.045) and survival rate were significantly higher ($p<0.05$) in *Labeo rohita* fed with different concentration of polyethylene microplastics in T1 group during 4^{th}, $3^{rd}$and 6^{th} week.

Hematological Parameters
Analysis of variance of hematological parameters i.e. red blood cells, white blood cells, platelets, different white blood cells, hematocrit and hemoglobin concentrations under different concentrations of polyethylene microplastic (polyethylene glycol) were observed in *Labeo rohita*.
The results of our study showed that red blood cells (4.233±0.643), hemoglobin concentration (8.727±0.843), hematocrit concentration (33.81±0.593), mean corpuscular volume

(135.003±0.583), mean corpuscular hemoglobin (48.033±0.583), platelets (290.93±0.643), white blood cells (45.83±0.643), lymphocytes, monocytes, neutrophils, and eosinophils were significantly higher ($p<0.05$) in T3 group as compare with other treatments.

Table1: Effect of microplastic (polyethylene glycol) under different concentrations ofpolyethylene microplastics on growth performance of *Labeo rohita*.

Parameter	**Treatments**			
	T0 Control	**T1 1mg/L**	**T2 10mg/L**	**T3 100mg/L**
Avg. weight (g)	17.113±0.943	17.077±0.753	17.048±0.292	16.682±0.854
Weight gain (g)	0.632±0.744	0.612±1.042	0.6±1.103	0.328±0.933
Avg. length (cm)	8.578±0.892	8.573±1.042	8.573±1.954	8.55±0.833
Length gain (cm)	0.023±1.022	0.02±0.933	0.017±0.853	0.008±0.984
SGR	0.517±0.843	0.503±0.892	0.495±1.042	0.283±1.954
Critical factor	2.782±0.742	2.785±1.045	2.78±1.053	2.707±0.492
Survival rate (%)	100	100	100	100

Table2: Effect of microplastic (polyethylene glycol) under different concentrations ofpolyethylene microplastics on hematological parameters of *Labeo rohita*.

Parameter	**Treatments**			
	T0 Control	**T1 1mg/L**	**T2 10mg/L**	**T3 100mg/L**
Red Blood Cells (10^6/mm^3)	3.603±0.743	3.587±0.742	3.61±0.853	4.233±0.643
Hemoglobin(Hb)(g/dl)	7.233±1.322	7.3±0.743	7.353±0.492	8.727±0.843
Hematocrit %	31.78±0.733	30.767±1.322	31.5537±0.854	33.81±0.593
MCV (mm^3)	132.703±1.422	132.84±0.733	132.84±0.733	135.003±0.583
MCH (pg)	46.74±0.722	46.513±1.001	45.79±0.073	48.033±0.583
MCHC (g/dl)	37.56±0.983	37.127±1.032	37.023±1.322	39.69±1.492
Platelets (10^3/mm^3)	314.21±0.843	313.76±0.922	310.93±0.742	290.93±0.643
White Blood Cells (10^3/mm^3)	42.87±1.032	42.74±1.302	43±1.021	45.83±0.643
Lymphocytes (%)	87.87±1.492	90.67±1.032	87.76±1.302	94.81±0.722
Monocytes (%)	3.07±0.854	3.08±0.893	3.04±0.833	4.52±0.853
Neutrophils (%)	7.19±0.583	7.06±0.633	7.29±0.873	7.53±0.783
Eosinophils (%)	2.43±0.393	2.15±0.482	2.2±0.872	2.81±0.042

DISCUSSION

Microplastics pollution are commonly found pollutants in the aquatic environment produced by the fragmentation of larger plastic objects (Rochman *et al*., 2019). Different microplastics polyethylene glycol are widely used in the world. Microplastics are commonly used in medicinal industry, cleansers and deodorants as its polymers are soluble in water (Bertuglia *et al.*, 2006). Fish and other aquatic organisms can ingest these microplastics which can cause poisonous diseases and even death to them (Gajdos *et al.*, 2007). The presentstudy investigated the physical effects of microplastics on the growth performance andhaematology of the *Labeo rohita*. Length gain was significantly higher ($p<0.05$) in *Labeo rohita* fed with different concentration of polyethylene microplastics in T1 group during 4^{th} week. Maximum length gain was observed in control group T0 with mean value of 0.023±1.022 while the minimum length gain was observed in treatment T3 with the mean value of 0.08±0.984. These results are corroborating with the results of Mazuris *et al*. (2014) who documented similar results in a dietary trial with polyethylene microplastics.

The outcomes of our research showed that specific growth rate was significantly higher ($p<0.05$) in

Labeo rohita. T1 and T2 showed similar specific growth rates as compare so control group with mean values of 0.53±0.892 and 0.495±1.042 respectively. This might be due to stress. Ouyang *et al*. (2121) suggested the similar results when conducted an experiment on common carp. The results of present study are also supported by Karami et al. (2016) who investigated variation of microplastics among different Bangladeshi commercial freshwater fish species and suggested that the ingestion of plastics in fish can be associated tofeeding habits. The results of Mazurais *et al*. (2014) also support the present study as the similar results were reported when exposed eight *Dicentrarchus labrax* larvae. The present obtained results of conditions factor are in agreement with the report of Mizra *et al.* (2017) who conducted an experiment to compare microplastics content with different feeding types. Results showed that omnivorous fish showed more microplastics fibers than in herbivores and carnivores and omnivores specimens of a higher content of microplactic were found to have low conditions factors (K). Huang *et al*. (2020) results and experiment was conducted on Poecilia reticulate. It was found that microplastics showed non significance effects on growth but inhibited the condition factor significantly. The present results are also in accordance with the results of Muller *et al.* (2020) who investigated that no significant impacton growth and condition factor was found except for high levels of microplastics.

The results of our study showed that red blood cells were significantly higher ($p<0.05$) in T3 group as compare with other treatments. Red blood cell counts were significantly increased in treatment T3 with mean value of 4.233±0.643 as compare to controlgroup T0. Similar results as of present study when treated *Labro rohita* with sub lethal dose of diafenthiuron under short and long term experimental conditions. Increase in the numberof red blood cells is in fact a mechanism through which fish might compensate for poor oxygen uptake in prevailing hypoxic conditions (Varadarajan *et al.,* 2013). Hemoglobin concentrations were significantly increased in treatment T3 with mean value of 8.727±0.843 as compare to control group T0. This can be supported by the results of Hamed *et al*. (2019) who suggested that exposure of fish to different concentrations of microplactics affects their circulatory systems particularly hematological parameters such as RBCs, Ht and Hb and caused significant increase in these indices. Hou *et al.* (2021) also reported similar findings and suggested that microplastics can enter the circulatory system and directly impact hematological properties. Hematocrit % was significantly increased in treatment T3 with mean value of 33.81± 0.59 as compare to control group T0. The increase in MCV, MCH and MCHC values is may be due to the increased level of RBCs, Hb, and Hct in the blood of fish another possible reason of the increased values may be due to the presence of a larger amountof older or larger red blood cells as explained by Thangam and Ramesh (2013). The raise in MCV, MCH and MCHC values was previously recorded by Younus *et al.* (2015) in H. fossilis after exposure to heavy metals which is in agreement with the present results. The results of present study expressed that platelets was significantly higher ($p<0.05$) in *Labeo rohita* fed with different concentration of polyethylene microplastics in T1 group as compare with other treatments.The present results are in agreement the results of Babu *et al.* (2016) who observe continuous decrease in platelets during the exposure of freshwater fish *Anabas Testudineus* to different concentrations of microplastics.

The outcomes of present study showed that white blood cells, lymphocytes, monocytes, neutrophils and eosinophils were significantly higher ($p<0.05$) in *Labeo rohita* fed with different concentration of polyethylene microplastics in T3 group as compare with other treatments.The fluctuations in total white blood cells counts may be due to the negative impact of polyethylene glycol microplastics on lymphoid tissues of the exposed fish as explained by Alkuladi *et al*. (2015). Furthermore, the uptake of microplastics may altered the immune system with an impact in animal health and defense. The present results are with agreement to the results of Prusty *et al*. (2011) who observed similar trends in *Labeo rohita* when exposed to fenvalerate as compared to the control group which suggested that theimmune system has been compromised.

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