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Toxicity of Dietary Non-biodegadable Microplastics on Growth Performance, Carcass Composition, Nutrient Digestibility and Hematology of *Labeo rohita*

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ER conducted the study and wrote the manuscript. SMH acquired funds, administered and supervised the project. SS and MA helped in analyzing the data. MH edited and reviewed the manuscript.

Key words
***L. rohita*, Non-biodegradable microplastics, Growth performance, Nutrients utilization, Carcass position**

ABSTRACT

Microplastics (MPs) pollution is one of the major environmental problems facing the world today. Concerns over the harmful effects of MPs on aquatic life has grown. The current study was carried out to assess the toxic effects of non-biodegradable MPs on growth, carcass composition, nutrient digestibility and hematology of *Labeo rohita* fingerlings. Six sunflower meal (SFM) based test diets having different MPs levels such as test diet I (control, without MPs), test diet II (0.5% MPs), test diet III (1% MPs), test diet IV (1.5% MPs), test diet V (2% MPs) and test diet VI (2.5% MPs) were prepared and fed to triplicate groups of 15 fingerlings at 5% of their live wet body weight for 90 days. The results showed that dietary exposure of MPs significantly reduced growth rate and feed utilization in *L. rohita* fingerlings. Lowest weight gain (5.29g) was recorded in fingerlings fed test diet VI (2.5% MPs) with highest feed conversion ratio (3.86) when compared to control diet (without MPs). Apparent digestibility of all dietary nutrients of SFM based diet also decreased directly with increase in MPs level in SFM based diet. By being exposed to non-biodegradable MPs, carcass composition altered significantly, with an increase in body fat and moisture content and a decrease in body protein and ash content. The hematological parameters (RBCs, Hb, PLT and PCV) showed a significant decline after exposure to MPs, while WBCs, MCHC, MCH and MCV increase significantly by MPs ingestion. Conclusively, non-biodegradable MPs are toxic agents showing adverse impacts on health of fish.

INTRODUCTION

Plastic polymers are being used for various purposes in paint, textile packaging, pipe and fabric industry that has resulted in remarkable amount of plastic waste being dumped into the ecosystem. Most of the plastic trash makes its way into aquatic systems in various ways. According to statistics, aquatic environments get roughly 14 million tons of plastic trash each year (IUCN, 2023). In aquatic ecosystems, plastic waste can decompose into smaller pieces by various chemical, biological and physical processes (Buwono *et al*., 2022). The term microplastics (MPs) refers to plastic particles having a diameter smaller than 5 mm. According to source of plastic waste, MPs fall into two categories: Primary MPs and secondary MPs. Primary MPs come from cosmetics and personal care items, while secondary MPs result from dissociation of large plastic via environmental conditions (Jiang *et al*., 2022). Owing to their smaller size and light weight, MPs are transported over great distances and are thus present in a variety of environments, including the land, sea, and air (Zhang *et al*., 2021). Being aquatic pollutants, MPs can accumulate in the body of aquatic animals having detrimental physiological and physical impacts on them (Miller *et al*., 2020).

Non-biodegradable MPs may enter and absorb into the body of aquatic animals via ingestion and respiration because of their diminutive size as animals take MPs as food particles (Li *et al*., 2021). MPs and their adverse impacts have been documented in algae, fish, zooplanktons,

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aquatic reptiles and mammals (Botterell *et al*., 2019; Wu *et al*., 2019; Kim *et al*., 2021). Fish is a significant predator among these and is a rich source of protein for humans. They are therefore regarded as the best species to access the hazardous effects of contaminants, particularly MPs. Additionally; both freshwater and marine fish have been reported to consume MPs. Previous research studies have confirmed the negative impacts of MPs on fish growth, feed intake, survival, metabolism, immunity and other toxic responses (Anand *et al*., 2018; Miller *et al*., 2020; Lu *et al*., 2022; Hao *et al*., 2023). The most important conduits for MPs from the land into marine environments are freshwater wetlands (Miller *et al*., 2017). That's why, the current study focused the edible freshwater fish species *Labeo rohita* to study the toxic effects of dietary MPs for 90 days.

L. rohita (rohu) is an important omnivorous fish species of Indian major carps (IMCs). Rohu is most popular cultivable species because of its fast growth rate, high market value, better disease resistance, and delicious flesh quality (Anand *et al*., 2018). Around 87% of all freshwater aquaculture production comes from IMCs, with rohu claiming 35% of that total productivity (Mir *et al*., 2017). Therefore, it is necessary to protect and preserve freshwater fish species *L. rohita* from toxic impacts of MPs. So, this research designed to evaluate the negative impacts of degradable MPs on growth, nutrient digestibility, hematology and carcass composition of *L. rohita* exposed via SFM based test diets. According to our knowledge, it's the first research study on dietary exposure of MPs and their adverse impacts on *L. rohita* fingerlings under controlled laboratory conditions.

MATERIALS AND METHODS

The goal of the current research was to address the negative effects of non-biodegradable MPs on the *L. rohita* fingerlings growth performance, carcass composition, nutrient digestibility and the hematology. The trial was carried out in the Fish Nutrition Laboratory located in Department of Zoology at Government College University, Faisalabad, Pakistan.

Acclimatization of fish and trial conditions

L. rohita fingerlings were bought from Government Fish Hatchery located on Satiana Road, Faisalabad. For a period of two weeks, these were exposed to laboratory environment for acclimatization. The fingerlings were treated with 5% saline solution to get rid of any ecto-parasites and prevent any further parasitic infections (Rowland, 1991). Temperature, pH, and other indicators of water quality, such as dissolved oxygen, were regularly checked. In order to maintain constant aeration in each of the fish tanks, the capillary method was used throughout the trial period.

Feed ingredients and experimental diets

Ingredients composition of test diets is presented in Table I. All ingredients of feed were purchased from the commercial feed mill and chemical composition was assessed prior to test diets formulation by using standard methods (AOAC, 2005). Non-biodegradable MPs were obtained from the Department of Environmental Sciences, Government College University, Faisalabad. Six SFM (sunflower meal) based experimental diets were prepared by supplementing MPs at the levels of 0%, 0.5%, 1%, 1.5%, 2%, and 2.5%. The feed ingredients were ground fine enough to pass via a sieve of 0.5 mm diameter. All elements for the feed were mixed in an electric mixer for 5 min before adding the fish oil. The gradual fish oil and water inclusion ultimately resulted a dough with the suitable texture for pellet formation via a pelleting machine. Then, feed pellets were dried and stored to use in trial.

Table I. Composition (%) of test diets.

Experimental ingredients	TD-I (control)	TD-II	TD-III	TD-IV	TD-V	TD-VI
Non-biodegradable-MPs (%)	0	0.5	1	1.5	2	2.5
Sunflower meal	54	54	54	54	54	54
Wheat flour	17	16.5	16	15.5	15	14.5
Fish meal	10	10	10	10	10	10
Rice polish	8	8	8	8	8	8
Fish oil	7	7	7	7	7	7
Minerals premix	1	1	1	1	1	1
Vitamins premix	1	1	1	1	1	1
Chromic oxide	1	1	1	1	1	1
Ascorbic acid	1	1	1	1	1	1

Experimental layout

A 90-day trial was performed to assess the effects of MPs on the *L. rohita* fingerlings. Total 270 *L. rohita* fingerlings were divided into triplicates for each test diet with fifteen fingerlings. These were fed on test diets @ 5% of the live wet body mass twice a day. Then, tanks were washed properly to clean unconsumed feed. Fecal matter was collected through the fecal collecting pipes of tanks, 2 h later of feeding session. Then, fecal material was oven dried and stored for estimation of nutrient digestibility.

Growth studies

At end of trial, the fish from every tank were bulk weighed to assess growth. Standardized formulae were utilized to determine the weight gain percentage (WG%), specific growth rate (SGR) and feed conversion ratio

(FCR), of fish (NRC, 2003).

$$WG\% = \frac{(\text{Final weight} - \text{initial weight})}{\text{Intial weight}} \times 100$$

$$FCR = \frac{\text{Total dry feed intake (g)}}{\text{wet weight gain (g)}}$$

$$SGR = \frac{[\text{In (final weight)} - \text{In(initial weight)}]}{\text{Duration of trial in days}} \times 100$$

Digestibility calculations

The apparent nutrient digestibility coefficients (ADC%) for test diets with MPs were estimated by this formula (NRC, 2003).

$$ADC\% = \left[100 - 100 \times \left(\frac{\%\text{ maker in diet} \times \%\text{ nutrients in feces}}{\%\text{ maker in feces} \times \%\text{ nutients in diet}}\right)\right]$$

Chemical assay of feed, feces and fish

Test diets, feces, and fish body samples taken from each tank at the end of trial were evaluated using the standardized method (AOAC, 2005), after homogenization by mortar and pestle. A microkjeldahl apparatus was utilized to find out crude protein (CP) (N×6.25). Crude fat (CF) was extracted by Soxtex HT2 1045 system with the petroleum ether extraction technique. Ash was calculated by heating samples in electric furnace for 12 h at 650°C (Eyela-TMF 3100). Gross energy (GE) was estimated by bomb calorimeter.

Chromic oxide estimation

The amount of chromic oxide in diet and feces samples was determined with UV-VIS 2001 spectrophotometer, standardized to absorbance at 370 nm after the oxidation with per chloric-reagent (Divakaran *et al*., 2002).

Hematological assessments

After the 90-day experimental trial, the blood samples were taken from three fish from every replicated tank and anaesthetizing them with 150 mg^{-1} tricane methane sulfonate solution (Wagner *et al*., 1997). Blood samples were collected from caudal vein of fingerlings with a heparinized syringe. Hematocrit or packed cell volume (PCV) was determined by capillary tubes and micro-hematocrit method (Brown, 1988). White blood cells (WBCs) and red blood cells (RBCs) were counted with a Neubauer counting chamber and hemocytometer equipment (Blaxhall and Daisley, 1973). The method of Wedemeyer and Yastuke (1977) was considered to determine the hemoglobin (Hb). Mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH) and mean corpuscular volume (MCV) were calculated by following formulae:

$$MCHC = Hb/PCV \times 100$$

$$MCH = Hb/RBC \times 10$$

$$MCV = PCV/RBC \times 10$$

Statistical analysis

Data on the growth parameters, carcass composition, apparent nutrient digestibility and hematological parameters of fingerlings was evaluated by using one-way ANOVA (Steel and Torrie, 1996). Mean differences were determined by Tukey's Honest Significant test (Snedecor and Cochran, 1991). Computer program Co-Stat was utilized for the statistical analysis. The significance level was taken as $p < 0.05$.

RESULTS

Growth performance

Growth parameters of the fingerlings exposed to non-biodegradable MPs were recorded and compared with that of control (Table II). WG of fingerlings fed diet with MPs decreased linearly with increase in MPs level in test diets. WG (g) of fingerlings (5.29g) fed test diet VI (2.5%MPs) decreased significantly ($p < 0.05$) in comparison to control diet without MPs (15.37g). By ingestion of MPs based diet, FCR also increased significantly with highest values (3.86) by 2.5% level of MPs (Fig. 1A). Moreover, fingerlings fed test diet VI with highest MPs level (2.5%) gave the lowest SGR (0.62%) than that in control (1.28%) (Fig. 2).

Table II. Growth performance of *L. rohita* fed on test diets with different levels of non-biodegradable microplastics.

Experimental diets	Non-biodegradable-MPs (%)	Initial Weight (g)	Final Weight (g)	Weight Gain (g)	Weight gain/90
Test diet- I	0%	7.12±0.01^{a}	22.49±1.10^{a}	15.37±1.10^{a}	0.17±0.01^{a}
Test diet-II	0.5%	7.13±0.01^{a}	19.55±0.48^{b}	12.4±0.47^{b}	0.14±0.01^{b}
Test diet-III	1%	7.15±0.02^{a}	16.71±0.19^{c}	9.56±0.21^{c}	0.11±0.00^{c}
Test diet-IV	1.5%	7.15±0.02^{a}	14.54±0.16^{d}	7.39±0.17^{d}	0.08±0.00^{d}
Test diet-V	2%	7.14±0.01^{a}	13.27±0.02^{de}	6.13±0.01^{de}	0.07±0.00d^{e}
Test diet-VI	2.5%	7.15±0.02^{a}	12.43±0.19^{e}	5.29±0.20^{e}	0.06±0.00^{e}

Values are means of triplicates. Values along the columns varies significantly ($p<0.05$) if superscripts are different.

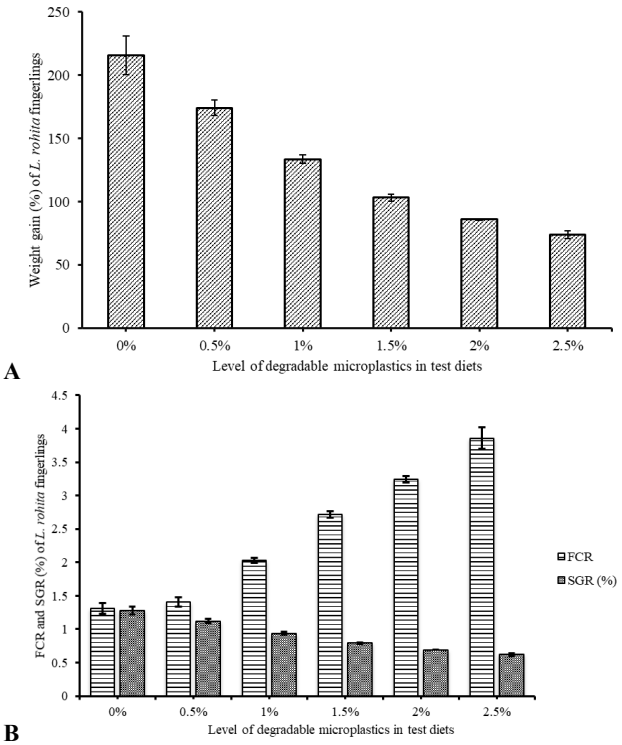

Fig. 2. Effect of non-biodegradable MPs with sunflower meal based diet on the weight gain (%) (A) and FCR and SGR (%) (B) of *L. rohita* fingerlings.

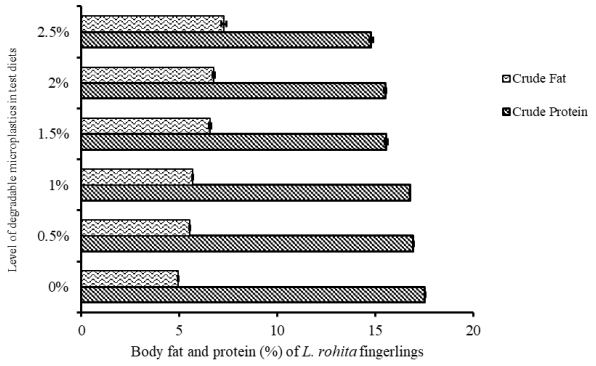

Fig. 2. Effect of non-biodegadable MPs on crude protein and crude fat (%) of *L. rohita* fingerlings.

Carcass composition

The carcass composition of *L. rohita* fingerlings affected significantly ($p < 0.05$) by the dietary exposure of MPs (Table III). Body fat increase with increase in MPs level (Fig. 2). Highest body fat (7.27%) was observed in fingerlings fed test diet VI (2.5% MPs) than that fed control diet (0% MPs), While carcass protein decreased linearly with increase in MPs level and was lowest (14.79 %) by test diet VI (2.5% MPs). Similarly, the highest value of moisture (75.74%) was seen by test diet VI with 2.5% MPs. The ash content also decreased significantly from 4.74% in control to 2.60% in fingerlings fed test diet VI (2.5% MPs).

Table III. Body composition (%) of *L. rohita* fed on test diets with different levels of non-biodegradable microplastics.

Experimental diets	Non-bio-degrada-ble-MPs (%)	Ash	Moisture
Test diet-I	0%	4.74±0.01^{a}	72.80±0.03^{a}
Test diet-II	0.5%	3.82±0.01^{b}	73.71±0.01^{b}
Test diet-III	1%	3.74±0.02^{bc}	73.83±0.01^{c}
Test diet-IV	1.5%	3.59±0.06^{c}	74.28±0.035^{d}
Test diet -V	2%	2.84±0.06^{d}	74.91±0.06^{d}
Test diet-VI	2.5%	2.59±0.13^{e}	75.74±0.10^{e}

Values are means of triplicates. Values along the columns varies significantly ($p<0.05$) if superscripts are different

Nutrient digestibility

Data on nutrient digestibility of CP, CF and GE is presented in Table IV. Dietary exposure of MPs to *L. rohita* fingerlings significantly ($p < 0.05$) decreased the digestibility of nutrients of SFM based diet. Apparent digestibility coefficient (ADC%) of CP, CF and GE were lowest in fingerlings exposed to 2.5% MPs with values (18.40%), (4.34%), and (2.17%), respectively. The decrease in ADC% was directly dependent on level of MPs in diet.

Hematological indices

Hematological indices of fingerlings exposed to non-biodegradable MPs are shown in Table V. RBCs, Hb, PLT and PCV values decreased with increase in MPs level with lowest values (1.16×10^{6}mm^{-3}), (6.95 g/100ml), (290.53), (20.84%), respectively, by test diet VI (2.5% MPs). While in case of WBCs, MCHC, MCV, and MCH fish ingested 2.5% MPs gave highest values (1.76×10^{3}mm^{-3}), (41.38%), (152.56fl) and (54.44fl), as compared to control diet.

DISCUSSION

MPs abundance in aquatic ecosystems has increased due to the influx of plastic trash and the dissociation of pre-existing plastics. This has resulted in accumulation of MPs in fish bodies, showing a number of harmful impacts on fish health, i.e., growth retardation, digestive dysfunction, oxidative stress and behavioral abnormalities (La Daana *et al.*, 2017; Bhuyan, 2022). Therefore, concerns about negative effects of MPs on aquatic organisms have grown

Table IV. The analyzed composition (%) of crude protein (CP), crude fat (CF), and gross energy (GE) in the feed, feces and their apparent digestibility coefficients in *L. rohita* fed on test diets with different levels of non-biodegradable microplastics.

Parameters	TD- I (0% MPs)	TD-II (0.5 % MPs)	TD-III (1% MPs)	TD-IV (1.5%MPs)	TD-V (2% MPs)	TD-VI (2.5%MPs)
Analysis of feed						
CP (%)	30.96±0.03^{a}	30.8±0.11^{a}	30.87±0.05^{a}	30.64±0.09^{a}	30.83±0.17^{a}	30.86±0.05^{a}
CF (%)	8.05±0.02^{a}	8.05±0.07^{a}	8.05±0.01^{a}	8.05±0.04^{a}	8.05±0.01^{a}	8.06±0.02^{a}
GE (kcalg^{-1})	3.33±0.11^{a}	3.40±0.07^{a}	3.40±0.02^{a}	3.45±0.03^{a}	3.44±0.03^{a}	3.42±0.09^{a}
Analysis of feces						
CP (%)	8.82±0.45^{f}	11.34±0.03^{e}	12.34±0.03^{d}	14.05±0.04^{c}	15.62±0.02^{b}	18.40±0.13^{a}
CF (%)	2.14±0.05^{f}	2.65±0.02^{e}	3.25±0.10^{d}	3.49±0.08^{c}	3.93±0.05^{b}	4.34±0.05^{a}
GE (kcalg^{-1})	1.40±0.07^{d}	1.65±0.03^{c}	1.73±0.05^{c}	1.91±0.07^{b}	2.06±0.03^{ab}	2.17±0.09^{a}
Apparent nutrient digestibility						
CP (%)	76.26±0.28^{a}	69.12±0.76^{b}	65.711±1.13^{c}	59.49±0.48^{d}	54.45±0.60^{e}	44.59±0.71^{f}
CF (%)	77.34±0.28^{a}	71.77±0.27^{b}	64.90±0.94^{c}	61.08±1.08^{d}	55.64±0.74^{e}	50.47±0.38^{f}
GE (kcalg^{-1})	64.25±0.65^{a}	58.26±1.22^{a}	55.79±1.38^{a}	50.59±2.63^{a}	45.60±0.68^{a}	40.48±0.32^{a}

Values are means of triplicates. Values along the columns varies significantly (p<0.05) if superscripts are different

Table V. Hematology of the *L. rohita* fingerlings fed non-biodegradable MPs with sunflower meal based test diet.

Experimental diets	Non-biodegradableMPs (%)	RBCs (10^{6}mm^{-3})	WBCs (10^{3}mm^{-3})	PLT	Hb (g/100ml)	PCV (%)	MCHC (%)	MCH(f1)	MCV(fl)
TD-1	0%	1.74±0.03^{a}	1.25±0.02^{e}	311.5±0.10^{a}	8.25±0.02^{a}	24.36±0.04^{a}	32.83±0.02^{c}	43.45±0.03^{f}	137.48±0.02^{f}
TD-II	0.5%	1.56±0.02^{b}	1.34±0.04^{d}	306.93±0.49^{b}	7.74±0.03^{b}	23.84±0.03^{b}	35.66±0.02^{bc}	46.24±0.04^{e}	142.87±0.01^{e}
TD-III	1%	1.46±0.03^{c}	1.47±0.01^{c}	304.6±0.2^{c}	7.55±0.03^{c}	23.5±0.06^{c}	36.92±0.01^{bc}	48.36±0.03^{d}	146.34±0.03^{d}
TD-IV	1.5%	1.37±0.03^{d}	1.54±0.02^{c}	298.60±0.26^{d}	7.45±0.03^{d}	22.47±0.02^{d}	38.14±0.03^{abc}	50.36±0.03^{c}	149.82±0.02^{c}
TD-V	2%	1.27±0.01^{e}	1.64±0.03^{b}	295.60±0.17^{e}	7.26±0.03^{e}	21.57±0.03^{e}	40.44±0.03^{ab}	52.46±0.03^{b}	151.36±0.03^{b}
TD-VI	2.5%	1.16±0.02^{f}	1.76±0.03^{a}	290.53±0.15^{f}	6.95±0.02^{f}	20.84±0.02^{f}	41.38±0.01^{a}	54.44±0.03^{a}	152.76±0.02^{a}

TD, test diet; RBC, red blood cells; WBC, white blood cells; PLT, Platelet; Hb, Hemoglobin; PCV, packed cell volume; MCHC, mean corpuscular hemoglobin concentration; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume. Values are means of triplicates. Values along the columns varies significantly (p<0.05) if superscripts are different.

in recent years. Thus, the main aim of this trial was to check the toxicity of non-biodegradable MPs exposed via SFM based diet on the growth, carcass composition, nutrient digestibility, and hematology of *L. rohita* fingerlings, reared in experimental fish tanks. Because fish species are extremely sensitive to the existence of aquatic toxins, these are frequently utilized as bio indicators of environmental contamination (Moyson *et al*., 2016).

MPs ingestion can lead to malabsorption, which has an impact on daily energy consumption and food processing (Bour *et al*., 2018). According to current findings, fingerlings growth rate and feed utilization decreased remarkably by MPs ingestion present in diet. In accordance to results, Hao *et al*. (2023) found prominent reduction in growth rate of grass carps (*Ctenopharyngodon idella*) when exposed to different concentrations of polystyrene MPs (100 µg/L, 500 µg/L) for 7 and 14-days. Ouyang *et al*. (2021) noted negative delayed effect of MPs on growth of fish where they observed no adverse impact of MPs on growth for first 30 days of exposure, while growth reduction occurred during excretion of MPs in next 30 days, indicating a contact between disordered eating and MPs on growth. Similarly, Naidoo and Glassom (2019) discovered that consuming MPs reduced the fish growth rate. In this study, feed utilization decreased due to MPs with increase in FCR. It has been demonstrated that the consumption of MPs causes an increase in energy demand of organisms as well as energy losses via lipid

catalysis (Cedervall *et al*., 2012; Wright *et al*., 2013). Liang *et al*. (2023) observed decreased in daily feed intake in *Carassius auratus* (goldfish) treated to water borne MPs fibers. Once MPs enter the body, they do not leave but accumulate in fish's body and cause functional and anatomical changes in cells. *L. rohita* has an agastric stomach, so MPs accumulation retards growth rate, which might be due to oxidative stress, metabolism disturbance and imbalanced microbiota (Huang *et al*., 2020).

The nutrient composition of a fish offers a sense of its nutritional and physiological status (Ali *et al*., 2005). Current research findings showed that the carcass composition of *L. rohita* fingerlings changed significantly. By dietary exposure of MPs for 90 days, carcass protein and ash content decreased while body fat and moisture increased in *L. rohita* fingerlings. Yin *et al*. (2018) found that after being exposed to polystyrene (PS) MPs, CP and CF levels in fish decreased significantly. Ingestion of MPs particles has been shown to impair lipid digestion and absorption, which has a negative impact on fish growth and nutritional status (Bhagat *et al*., 2020). In contrast to our findings, MPs at different environments do not always cause significant damage to fish body (Liu *et al*., 2021).

MPs consumption causes dysbiosis (microbe imbalance), and this inflammation in the gut impairs nutrient absorption and metabolism in fish (Lu *et al*., 2018). Cedervall *et al*. (2012) studied that fish consumed zooplanktons having PS nanoparticles which led to weight loss and the fat metabolism was also changed. It is also in accordance to our results as fat metabolism of *L. rohita* was changed due to exposure of MPs. The digestibility of crude fat, crude protein and gross energy was reduced significantly in *L. rohita* when exposed to MPs in SFM based diets. Chronic MPs exposure to yellow perch resulted in a decrease in protein metabolism (Lu *et al*., 2022). MPs are indigestible, so when they enter in gut they restrict the digestion and then enter organs and tissues from the intestinal mucosa. Since important nutrients are metabolized by essential organs such as the liver and the gut, MP accumulation causes serious harm to these organs, hinders their smooth functioning, lowers the nutritional contents of fish, impaires protein digestion and disturbs metabolism (Yin *et al*., 2018; Haave *et al*., 2021).

MPs are toxic agents and change the normal hematological indices. The current findings revealed that hematological parameters including RBCs, Hb, PLT and PCV in fingerlings showed significant reduction after being exposed to 0.5%, 1%, 1.5%, 2% and 2.5% non-biodegradable MPs for 90 days. While, other blood indices, WBCs, MCHC, MCH, and MCV, showed a significant increase. When MPs interact with RBCs, they lose their oxygen carrying capacity and become flaccid, while WBCs increase which might be due to inflammation or infection (Hwang *et al*., 2020). According to Raza *et al*. (2023), hematological indices i.e. Hb, RBCs, hematocrite and PLT decreased significantly, while MCV and MCH concentration increased in *Oreochromis niloticus* exposed to polyacrylamide for about 28 days. Digka *et al*. (2018) explained that MPs with particle sizes less than 5 µm can enter to the bloodstream and then directly affects the hematological indices such as PCV, Hb and RBCs, causing metabolic problems, inflammatory responses and lipid peroxidation. MPs adhere to the walls of RBCs and cause their aggregation. Chemical or physical damage to RBCs results in hemolysis and decrease in Hb and hematocrit levels (Barshtein *et al*., 2016). In accordance to current findings, Yu *et al*. (2023) also found significant reduction in RBCs, Hb and hematocrit in crucian carp, *Carassius carassius* exposed to waterborne polyethylene MPs for 2 weeks. On the whole, MPs impaired the growth, altered nutritional quality, and reduced digestibility of the fish. Furthermore, MPs have a negative impact on market production, resulting in massive economic losses.

CONCLUSION

This research study confirmed that dietary non-biodegradable MPs exposure in a level-dependent manner resulted in reduced growth performance, feed utilization and nutrient digestibility in *L. rohita* fingerlings. MPs exposure also significantly alters the carcass composition of fingerlings by increasing their body fat and decreasing the protein content. Blood parameters of *L. rohita* fingerlings also affected remarkably by non-biodegradable MPs exposure. Results showed that higher levels of non-biodegradable MPs resulted more damage to health of fish as compared to low levels.

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IRB approval

All applicable institutional, national and international guidelines for the care and use of animals were followed.

Ethical statement

All the procedures and methods used in this study followed the ethical guidelines provided by Government College University Faisalabad.

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Ali, M.Z. and Jauncey, K., 2005. Approaches to optimizing dietary protein to energy ratio for African catfish *Clarias gariepinus* (Burchell, 1822). *Aquacult. Nutr.*, **11**: 95-101. https://doi.org/10.1111/j.1365-2095.2004.00325.x

Anand, G., Bhat, I.A., Varghese, T., Dar, S.A., Sahu, N.P., Aklakur, M.D. and Sahoo, S., 2018. Alterations in non-specific immune responses, antioxidant capacities and expression levels of immunity genes in *Labeo rohita* fed with graded level of carbohydrates. *Aquaculture*, **483**: 76-83. https://doi.org/10.1016/j.aquaculture.2017.10.014

AOAC (Association of Official Analytical Chemists), 2005. *Official methods of analysis*. 15th ED. Association of Official Analytical Chemists, Washington, DC. USA., pp. 1994.

Barshtein, G., Livshits, L., Shvartsman, L.D., Shlomai, N.O., Yedgar, S. and Arbell, D., 2016. Polystyrene nanoparticles activate erythrocyte aggregation and adhesion to endothelial cells. *Cell Biochem. Biophys*., **74**: 19–27. https://doi.org/10.1007/s12013-015-0705-6

Bhagat, J., Zang, L., Nishimura, N. and Shimada, Y., 2020. Zebrafish: An emerging model to study microplastic and nanoplastic toxicity. *Sci. Total Environ.,* **728**: 138707. https://doi.org/10.1016/j.scitotenv.2020.138707

Bhuyan, M.S., 2022. Effects of microplastics on fish and in human health. *Front. environ. Sci*., 10: 250. https://doi.org/10.3389/fenvs.2022.827289

Blaxhall, P.C. and Daisley, K.W., 1973. Routine hematological methods for use with fish blood. *J. Fish Biol.*, **5**: 771-781. https://doi.org/10.1111/j.1095-8649.1973.tb04510.x

Botterell, Z.L., Beaumont, N., Dorrington, T., Steinke, M., Thompson, R.C. and Lindeque, P.K., 2019. Bioavailability and effects of microplastics on marine zooplankton: A review. *Environ. Pollut*., **245**: 98-110. https://doi.org/10.1016/j.envpol.2018.10.065

Bour, A., Avio, C.G., Gorbi, S., Regoli, F. and Hylland, K., 2018. Presence of microplastics in benthic and epibenthic organisms: Influence of habitat, feeding mode and trophic level. *Environ. Pollut.*, **243**: 1217-1225. https://doi.org/10.1016/j.envpol.2018.09.115

Brown, B.A., 1988. *Routine hematology procedures*. Hematology: Principle and Procedures, pp. 7-122.

Buwono, N.R., Risjani, Y. and Soegianto, A., 2022. Spatio-temporal patterns of occurrence of microplastics in the freshwater fish *Gambusia affinis* from the Brantas River, Indonesia. *Environ. Pollut.*, **311**: 119958. https://doi.org/10.1016/j.envpol.2022.119958

Cedervall, T., Hansson, L.A., Lard, M., Frohm, B. and Linse, S., 2012. Food chain transport of nanoparticles affects behaviour and fat metabolism in fish. *PLoS One*, **7**: e32254. https://doi.org/10.1371/journal.pone.0032254

Digka, N., Tsangaris, C., Torre, M., Anastasopoulou, A. and Zeri, C., 2018. Microplastics in mussels and fish from the Northern Ionian Sea. *Mar. Pollut. Bull.*, **135**: 30-40. https://doi.org/10.1016/j.marpolbul.2018.06.063

Divakaran, S., Leonard, G.O. and Lan, P.F., 2002. Note on the methods for determination of chromic oxide in shrimp feeds. *J. Agric. Fd. Chem.*, **50:** 464-467. https://doi.org/10.1021/jf011112s

Haave, M., Gomiero, A., Schönheit, J., Nilsen, H. and Olsen, A.B., 2021. Documentation of microplastics in tissues of wild coastal animals. *Front. environ. Sci*., **31**. https://doi.org/10.3389/fenvs.2021.575058

Hao, Y., Sun, Y., Li, M., Fang, X., Wang, Z., Zuo, J. and Zhang, C., 2023. Adverse effects of polystyrene microplastics in the freshwater commercial fish, grass carp (*Ctenopharyngodon idella*): Emphasis on physiological response and intestinal microbiome. *Sci. Total Environ.,* **856**: 159270. https://doi.org/10.1016/j.scitotenv.2022.159270

Huang, J.N., Wen, B., Zhu, J.G., Zhang, Y.S., Gao, J.Z. and Chen, Z.Z., 2020. Exposure to microplastics impairs digestive performance, stimulates immune response and induces microbiota dysbiosis in the gut of juvenile guppy (*Poecilia reticulata*). *Sci. Total Environ.*, **733**: 138929. https://doi.org/10.1016/j.scitotenv.2020.138929

Hwang, J., Choi, D., Han, S., Jung, S.Y., Choi, J. and Hong, J., 2020. Potential toxicity of polystyrene microplastic particles. *Sci. Rep.,* **10**: 1-12. https://doi.org/10.1038/s41598-020-64464-9

IUCN, 2023. *Marine plastic pollution*. Available online: https://www.iucn.org/resources/issues-brief/marine-plastic-pollution (accessed on 25 May

2023).

Jiang, Y., Yang, F., Kazmi, S.S.U.H., Zhao, Y., Chen, M. and Wang, J., 2022. A review of microplastic pollution in seawater, sediments and organisms of the Chinese coastal and marginal seas. *Chemosphere*, **286**: 131677. https://doi.org/10.1016/j.chemosphere.2021.131677

Kim, J.H., Yu, Y.B. and Choi, J.H., 2021. Toxic effects on bioaccumulation, hematological parameters, oxidative stress, immune responses and neurotoxicity in fish exposed to microplastics: A review. *J. Hazard. Mater.*, **413**: 125423. https://doi.org/10.1016/j.jhazmat.2021.125423

La Daana, K.K., Officer, R., Lyashevska, O., Thompson, R.C. and O'Connor, I., 2017. Microplastic abundance, distribution and composition along a latitudinal gradient in the Atlantic Ocean. *Mar. Pollut. Bull.,* **115**: 307-314. https://doi.org/10.1016/j.marpolbul.2016.12.025

Li, J., Ouyang, Z., Liu, P., Zhao, X., Wu, R., Zhang, C. and Guo, X., 2021. Distribution and characteristics of microplastics in the basin of Chishui River in Renhuai, China. *Sci. Total Environ.,* **773**: 145591. https://doi.org/10.1016/j.scitotenv.2021.145591

Liang, W., Li, B., Jong, M.C., Ma, C., Zuo, C., Chen, Q. and Shi, H., 2023. Process-oriented impacts of microplastic fibers on behavior and histology of fish. *J. Hazard. Mater.*, **448**: 130856. https://doi.org/10.1016/j.jhazmat.2023.130856

Liu, P., Shi, Y., Wu, X., Wang, H., Huang, H., Guo, X. and Gao, S., 2021. Review of the artificially-accelerated aging technology and ecological risk of microplastics. *Sci. Total Environ.*, **768**: 144969. https://doi.org/10.1016/j.scitotenv.2021.144969

Lu, L., Wan, Z., Luo, T., Fu, Z. and Jin, Y., 2018. Polystyrene microplastics induce gut microbiota dysbiosis and hepatic lipid metabolism disorder in mice. *Sci. Total. Environ*., **631**: 449-458. https://doi.org/10.1016/j.scitotenv.2018.03.051

Lu, X., Deng, D.F., Huang, F., Casu, F., Kraco, E., Newton, R.J. and Mendoza, L.M.R., 2022. Chronic exposure to high-density polyethylene microplastic through feeding alters the nutrient metabolism of juvenile yellow perch (*Perca flavescens*). *Anim. Nutr.*, **9**: 143-158. https://doi.org/10.1016/j.aninu.2022.01.007

Miller, M.E., Hamann, M. and Kroon, F.J., 2020. Bioaccumulation and biomagnification of microplastics in marine organisms: A review and meta-analysis of current data. *PLoS One*, **15**: e0240792. https://doi.org/10.1371/journal.pone.0240792

Miller, R.Z., Watts, A.J., Winslow, B.O., Galloway, T.S. and Barrows, A.P., 2017. Mountains to the sea: river study of plastic and non-plastic microfiber pollution in the northeast USA. *Mar. Pollut. Bull.*, **124**: 245-251. https://doi.org/10.1016/j.marpolbul.2017.07.028

Mir, I.N., Sahu, N.P., Pal, A.K. and Makesh, M., 2017. Synergistic effect of l-methionine and fucoidan rich extract in eliciting growth and non-specific immune response of *Labeo rohita* fingerlings against *Aeromonas hydrophila*. *Aquacultre*, **479**: 396-403. https://doi.org/10.1016/j.aquaculture.2017.06.001

Moyson, S., Liew, H.J., Fazio, A., Van Dooren, N., Delcroix, A., Faggio, C. and De Boeck, G., 2016. Kidney activity increases in copper exposed goldfish (*Carassius auratus auratus*). *Comp. Biochem. Physiol. C Toxicol. Pharmacol.*, **190**: 32-37. https://doi.org/10.1016/j.cbpc.2016.08.003

Naidoo, T. and Glassom, D., 2019. Decreased growth and survival in small juvenile fish, after chronic exposure to environmentally relevant concentrations of microplastic. *Mar. Pollut. Bull.*, **145**: 254-259. https://doi.org/10.1016/j.marpolbul.2019.02.037

NRC (National Research Council), 2003. *Nutrient requirements of fish*. National Academy Press, Washington, DC. pp. 114.

Ouyang, M.Y., Feng, X.S., Li, X.X., Wen, B., Liu, J.H., Huang, J.N. and Chen, Z.Z., 2021. Microplastics intake and excretion: Resilience of the intestinal microbiota but residual growth inhibition in common carp. *Chemosphere*, **276**: 130144. https://doi.org/10.1016/j.chemosphere.2021.130144

Raza, T., Rasool, B., Asrar, M., Manzoor, M., Javed, Z., Jabeen, F. and Younis, T., 2023. Exploration of polyacrylamide microplastics and evaluation of their toxicity on multiple parameters of *Oreochromis niloticus*. *Saudi J. biol. Sci.,* **30**: 103518. https://doi.org/10.1016/j.sjbs.2022.103518

Rowland, S.J., 1991. *Diseases of Australian native freshwater fishes with particular emphasis on the ectoparasitic and fungal diseases of Murray cod* (Maccullochella peeli), *golden perch* (Macquaria ambigua) *and silver perch* (Bidyanus bidyanus). Dept. of Agriculture, New South Wales.

Snedecor, G.W. and Cochran, W.G., 1991. *Statistical methods*. 8th Ed. Iowa State University Press, USA, pp. 503.

Steel, R. and Torrie, J., 1996. *Principles and procedures of statistics.* McGraw-Hill Book Co, London.

Wedemeyer, G.A. and Yasutake, W.T., 1977. Clinical methods for the assessment of the effects of environmental stress on fish health. *Dep. Inter. Fish*

Wildl. Ser., **89.**

Wright, S.L., Rowe, D., Thompson, R.C. and Galloway, T.S., 2013. Microplastic ingestion decreases energy reserves in marine worms. *Curr. Biol.*, **23**: R1031-R1033. https://doi.org/10.1016/j.cub.2013.10.068

Wu, Y., Guo, P., Zhang, X., Zhang, Y., Xie, S. and Deng, J., 2019. Effect of microplastics exposure on the photosynthesis system of freshwater algae. *J. Hazard. Mater.,* **374:** 219-227. https://doi.org/10.1016/j.jhazmat.2019.04.039

Yin, L., Chen, B., Xia, B., Shi, X. and Qu, K., 2018. Polystyrene microplastics alter the behavior, energy reserve and nutritional composition of marine jacopever (*Sebastes schlegelii*). *J. Hazard. Mater.*, **360**: 97-105. https://doi.org/10.1016/j.jhazmat.2018.07.110

Yu, Y.B., Choi, J.H., Choi, C.Y., Kang, J.C. and Kim, J.H., 2023. Toxic effects of microplastic (polyethylene) exposure: Bioaccumulation, hematological parameters and antioxidant responses in crucian carp, *Carassius carassius*. *Chemosphere*, pp. 138801. https://doi.org/10.1016/j.chemosphere.2023.138801

Zhang, X., Wen, K., Ding, D., Liu, J., Lei, Z., Chen, X. and Lin, Y., 2021. Size-dependent adverse effects of microplastics on intestinal microbiota and metabolic homeostasis in the marine medaka (*Oryzias melastigma*). *Environ. Int.*, **151**: 106452. https://doi.org/10.1016/j.envint.2021.106452