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Research Article

Effect of Polypropylene Microplastic and Florfenicol Antibiotic on Some Hormonal and Haematological Biomarkers in Yellowfin Seabream (*Acanthopagrus latus*)

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ABSTRACT

Microplastics (MPs) and antibiotics, such as florfenicol (FFC), are emerging pollutants affecting aquatic ecosystems. This research examined the separate and joint impacts of MPs and FFC on hematological and hormonal indicators in yellowfin seabream *(Acanthopagrus latus)*. The fish were categorized into four groups according to their dietary treatments: a control group with a standard diet and three experimental groups receiving diets containing 15 mg/kg of FFC, 100 mg/kg of MPs, or both for 10-day. The ELISA method was used to measure hormones in plasma. Blood collection occurred on the first, fourth, seventh, and fourteenth days after feeding stopped to evaluate FFC concentration in the plasma and various hematological and hormonal parameters. Exposure to FFC and MPs, alone or together, considerably decreased red blood cell, hemoglobin levels, hematocrit values, mean corpuscular hemoglobin, mean corpuscular hemoglobin, white blood cell, lymphocyte, and erythropoietin concentrations. Neutrophil and cortisol levels increased. Mean corpuscular volume was elevated only in the group receiving both FFC and MPs. After a 14-day recovery period, all measured parameters returned to baseline levels in the FFC-only group. Co-exposed group showed the highest concentration of FFC in the plasma on the first day. The groups administered MPs, individually or collectively, exhibited a reduction in thyroid hormones. These findings indicate that both MPs and FFC induce anemia and stress in yellowfin seabream, with co-exposure exacerbating these effects. Although the toxic effects of FFC were temporary, the lasting presence of MPs indicates potential long-term risks.

Keywords: Yellowfin seabream, *Acanthopagrus latus*, Florfenicol, Haematological/ Hormonal indices, Microplastics

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INTRODUCTION

Microplastics (MPs), artificial polymers smaller than 5mm in size, are predominantly found in the pharmaceutical and cosmetics industry (Athira et al., 2024). They enter marine environments through wastewater treatment plant leaks and surface water runoff (Patil et al., 2024). MPs are classified into various types based on the compounds used, with polypropylene and polyethylene and their by-products being commonly found in marine environments (Maghsodian et al., 2021). At present, MPs are a significant pollution risk in coastal ecosystems, leading to growing public concern (Athira et al., 2024). MPs can be transferred through the food chain when aquatic organisms consume them, resulting in their accumulation in different fish species. This accumulation in the essential tissues of fish can cause harmful effects on their hematological indices, immune system function, oxidative stress, and DNA integrity (Li et

al., 2025; Sankar et al., 2025). Aquatic environments are heavily influenced by anthropogenic, resulting in the presence of multiple types of pollutants, such as antibiotics, cyclic aromatic hydrocarbons, heavy metals, MPs, and pesticides. MPs have the capacity to absorb various pollutants, particularly antibiotics, which enhances their bioavailability and intensifies the toxic effects on aquatic organisms (Banaee et al., 2023).

Antibiotics such as florfenicol (FFC) are designed for application in veterinary medicine and aquaculture and enter ecosystems through wastewater from animal and aquatic farming (Bardhan et al., 2022). Antibiotics can easily penetrate the cell membrane due to their high lipophilic properties and generate reactive oxygen species that may harm cells (Zheng et al., 2025). Previous researchers have observed a toxic effect of FFC on blood indices and hematopoietic tissues of Nile tilapia (*Oreochromis niloticus)* (Bardhan et al., 2024).

Although there is growing awareness of the individual hazards posed by MPs and antibiotics (Akbari & Jaafari, 2025; Yi et al., 2025), there remains a considerable lack of understanding regarding their combined effects on aquatic. Most research has concentrated on the impact of single pollutants; however, in natural settings, organisms frequently encounter multiple stressors at once. Therefore, additional studies are essential to enhance our comprehension of how various pollutants interact and their effects on marine ecosystems. This understanding could facilitate the creation of effective strategies to tackle this issue.

The yellowfin seabream (*Acanthopagrus latus*) is a marine species known for its resilience to challenging environmental conditions. It is widely fished and farmed, especially in Iran, underscoring its importance for both ecological and economic research (Shirmohammadi et al., 2017). Changes in hematological and hormonal parameters, which serve as biological indicators, are often influenced by physiological and environmental and hormonal factors. Therefore, when fish are exposed to stressors, it is expected that certain hematological and hormonal parameters will undergo changes. In fact, the hematological and hormonal characteristics of fishes are crucial evidence of their physiological stages and reflect the relationship between the aquatic ecosystem and their health (Harikrishnan et al., 2024; Azam, 2025). The enzyme-linked immunosorbent assay (ELISA) is a highly sensitive, specific, and reproducible method for measuring hormones (Azam et al., 2025), making it the preferred technique for this study. The aim of this study was to assess the hypothesis that simultaneous exposure to MPs and FFC enhances hematological and hormonal stress responses in yellowfin seabream more than exposure to either substance individually. Also, in this study, thyroid hormones (T3 and T4) were evaluated as critical biomarkers to assess endocrine disruption potential of MPs and FFC in fish.

MATERIALS AND METHODS

Chemicals

The Jampilen Petrochemical Company in Iran supplied the polypropylene powder (less than 44 µm). The Rooyan Darou Company in Iran provided the FFC. Kits for Hemoglobin (Hb; Code: THB-Z-100) are sourced from ZiestChem in Iran, while erythropoietin (EPO; Code: RK02771) is obtained from ZellBio in Germany. Additionally, triiodothyronine (T3; Code: E-T3), thyroxine (T4; Code: T4-192-10), and cortisol (Code: E-S-2601) kits are supplied by IDEAL in Iran. All additional chemical substances needed for the analyses were purchased from Merck in Germany. Florfenicol ($C_{12}H_{14}Cl_2FNO_4S$; MW 358.2 g/mol) is a broad-spectrum bacteriostatic antibiotic that inhibits protein synthesis by binding to the 50S ribosomal subunit. It exhibits moderate lipophilicity (log Kow = 3.2), water solubility of 1.1 g/L at 25°C, and pKa of 8.7. Polypropylene microplastics are hydrophobic polymers (water contact angle ~100°) with low density (0.85-0.92 g/cm³) with a typical melting point of 160–170°C.

Fish maintenance

A total of 144 young yellowfin seabream that were healthy had an average weight of 41.12 ± 10.2 g. These fish were obtained from a private company located in Khuzestan province, Iran. For a period of 10 days, the fish were adjusted to their new environment in twelve 300 L tanks. During this adaptation phase, they were fed a commercial pelleted diet provided by Beyza 21 Manufacturing Company in Iran. The water conditions and the nutritional content of their basic diet followed the standards outlined by Shirmohammadi et al. (2024) for yellowfin seabream. The fish received two feedings daily, which totaled 2% of their body weight. To maintain good water quality, approximately 80% of the water in the tanks was replaced each day to remove waste. Throughout the study, the water's physicochemical properties were regularly checked and kept at ideal levels.

Preparation of trial diet

Initially, the primary diets were pulverized. Subsequently, the FFC and MPs were incorporated into the mixture in the preferred ratios, along with water to create a thick paste. A meat grinder was then used to shape this paste into noodles. After that, the noodles were dried in the air and cut into suitable lengths. They were then frozen at -20 °C until ready for use (Banaee et al., 2023).

Experimental design and sampling

After the adjustment period, the fit fish were randomly split into four groups, with three replications in each group, totaling 12 fish per group. The fish involved in the experiment were grouped as follows:

Control: received a standard commercial diet without any additives.

FFC: was given a diet containing 15 mg/kg of FFC.

MPs: had a diet including 100 mg/kg of polypropylene.

FFC+MPs: consumed a diet that included 15 mg/kg of FFC and 100 mg/kg of polypropylene.

Throughout the 10-day trial, the fish were provided with 2% of their body weight in food two times a day. They were served the experimental diet in the morning and the standard diet in the afternoon (Del Piano et al., 2023). The amounts of polypropylene utilized in this study were determined based on earlier toxicity studies conducted on fish (Jeyavani et al., 2023; Yedier et al., 2023). The chosen therapeutic dose of FFC aimed to assess any potential harmful effects of the treatment on yellowfin seabream.

This evaluation aimed to determine the safety and tolerability of the administered dose concerning aquatic health (Shirmohammadi et al., 2024). Any observable changes in fish behavior were monitored daily.

On the 1st, 4th, 7th, and 14th days after stopping the feeding, three fish from each tank were randomly selected and sedated with a solution of 200 µl/L of 2phenoxyethanol. Blood was collected from the tail vein using a syringe treated with heparin and was split into two parts. One part was used for analyzing hematology parameters, while the other part was spun in a centrifuge at 6000 rpm for 10 min to assess hormone levels and FFC build-up. The plasma obtained was stored at -80°C (Shirmohammadi et al., 2017). The tests were performed following the regulations of the National Ethical Committee for Animal Research in Iran.

FFC accumulation assay

Plasma FFC levels were determined using High-Performance Liquid Chromatography (HPLC) equipped with a UV detector (Model K2500, KNAUER, Germany), following Jangaran Nejad et al. (2017). In summary, 1 mL of plasma was combined with 4 mL of ethyl acetate. The supernatant was then evaporated with nitrogen at 40°C for 45 minutes, and the remaining substances were dissolved again in 1 mL of mobile phase and 0. 5 mL of hexane. After the centrifugation process (16,000 rpm for 20 min), the aqueous phase was filtered (0.45 µm nylon) and analyzed (20 µL injections). Results were expressed as µg/mL.

Haematological assay

Heparinized blood was mixed with NattHerrick's solution at a ratio of 1:30 to measure the white blood cell (WBC; $10^3/mm^3$) and red blood cell (RBC; $10^6/mm^3$) counts using a hemocytometer. Giemsa stained smears were employed for the differential leukocyte counts. A microhematocrit centrifuge (Hettich, Germany) was utilized to find the hematocrit (Ht, %), and Hb (g/dL) was assessed colorimetrically using the cyanmethemoglobin method. RBC indices including the average corpuscular volume (MCV; fl/cell), average corpuscular hemoglobin (MCH; pg/cell), and average corpuscular hemoglobin concentration (MCHC; g/dL) were also calculated (Shirmohammadi et al., 2018).

Hormonal assay

Plasma cortisol, EPO, T3, and T4 levels were quantified via ELISA using a DYNEX DS2 plate reader (USA), following kit protocols. Results were reported as ng/mL (cortisol), mU/mL (EPO), and ng/mL (T3, T4) (Barry et al., 1993; Lai et al., 2006; Azam et al., 2025). Initially, 50 µL of each plasma sample and standard was added to the wells of a 96-well microplate. Next, 100 µL of enzyme-conjugated antibody was dispensed into all wells (except the blank control wells), followed by incubation at room temperature (25°C) for 2 hours in the dark. After incubation, the microplates were washed five times with a wash buffer (saline containing surfactant and the preservative ciprofloxacin hydrochloride). Subsequently, 100 µL of 3,3',5,5'-tetramethylbenzidine (TMB) substrate was added to each well, and the plates were incubated for 35 minutes at 25°C. The reaction was then stopped by adding 1 N sulfuric acid. After 10 minutes, the absorbance was measured at 450 nm (with a reference wavelength of 630 nm) using a microplate reader. The concentration of the samples was determined using the standard curve and the corresponding line equation derived from it.

Statistical analysis

Data were expressed as means ± standard deviation (SD; n=9). Normality was assessed with the Shapiro-Wilk test. One-way ANOVA with Duncan's post hoc test (IBM-SPSS v26.0, Chicago, USA) evaluated inter-group differences across sampling times, with significance set at $p < 0.05$

RESULTS AND DISCUSSION

The results indicated that the group exposed to both FFC and MPs showed the highest concentration of FFC in plasma on the first day after exposure ($p < 0.05$; Figure 1). This suggests that MPs could act as a carrier for adsorbing antibiotic residues, facilitating their uptake into aquatic tissues (Shirmohammadi et al., 2024). The study observed a decreasing trend ($p < 0.05$) over the 14-day recovery period in plasma levels of FFC, likely attributed to its metabolism. This finding aligns with Kverme et al. (2019), who reported similar results in lumpfish (*Cyclopterus lumpus*) treated with FFC for the same duration.

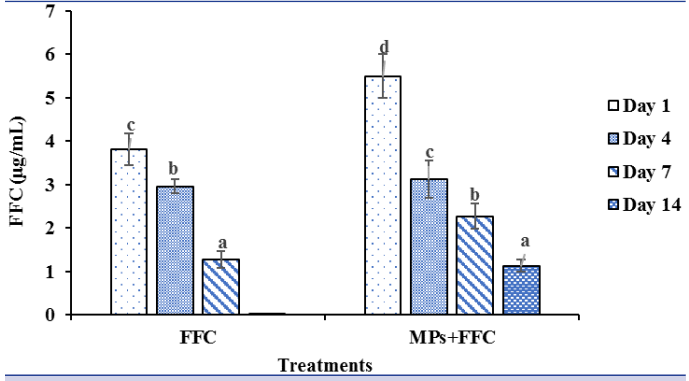

Figure 1. FFC accumulation in plasma at different times (Mean ± SD; n=9). The fish that were tested on various days within the same groups showed notable differences, which were marked by different letters ($p < 0.05$).

The results of this study showed that exposure to FFC and MPs, whether separately or together, led to a notable decrease in RBC, Hb, Ht, and WBC ($p < 0.05$; Figures 2a, b, c and 3a). The observed hematological reductions suggest a potential risk of anemia and immune dysfunction, likely attributable to impaired erythropoiesis in hematopoietic tissues, oxidative damage to cellular membranes, and possible internal hemorrhaging (Kondera et al., 2020; Harikrishnan et al., 2024). Comparable hematological disturbances were noted in rainbow trout (*Oncorhynchus mykiss*) subjected to FFC (Shiry et al., 2020) and in zebrafish (*Danio rerio*) exposed to MPs (Ammar et al., 2023).

The research discovered that being exposed to FFC and MPs, whether separately or in combination, led to a notable reduction in MCH and MCHC levels. Conversely, the presence of both substances was linked to an increase in MCV values ($p < 0.05$; Figure

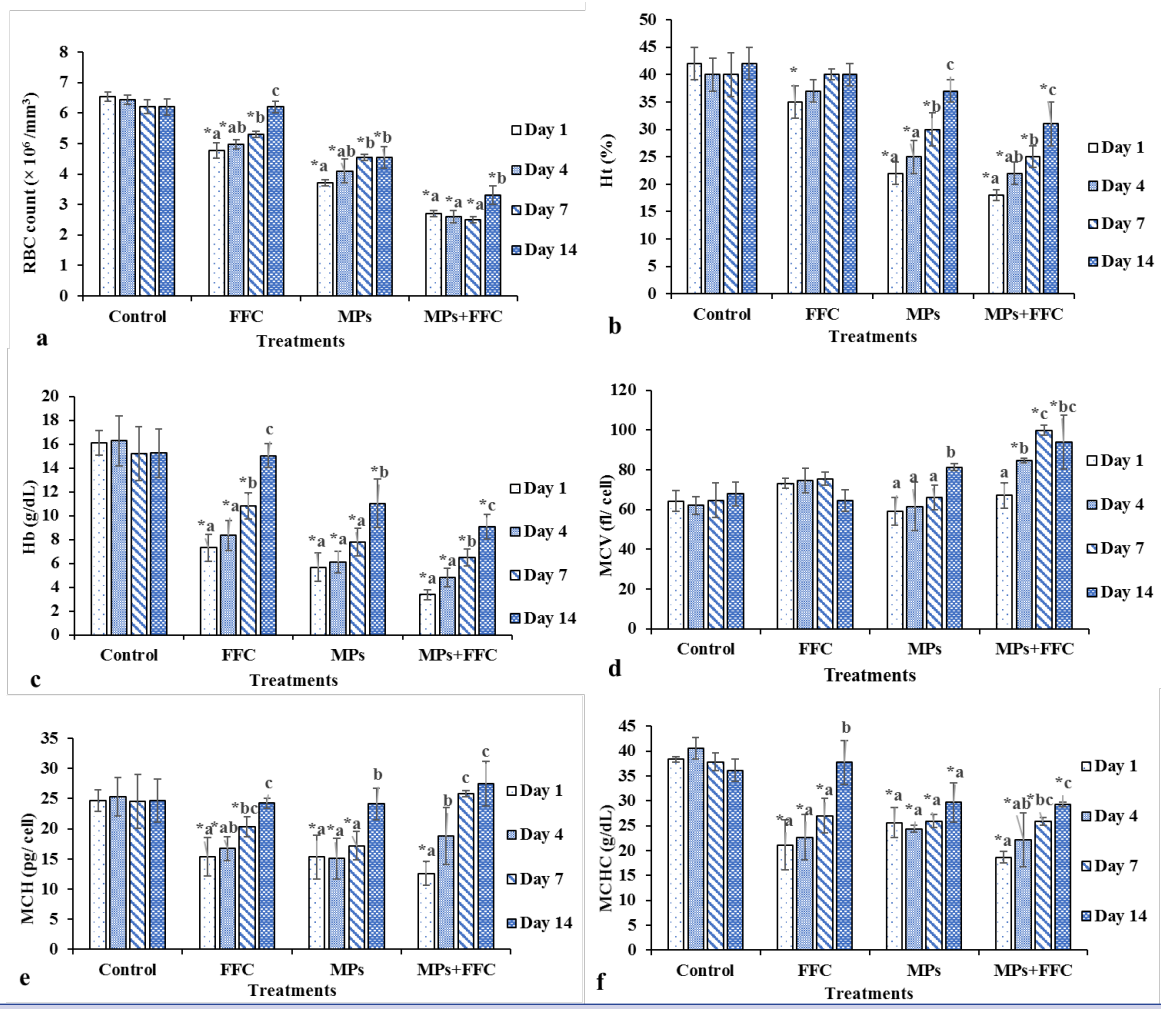

Figure 2. The impact of feeding yellowfin seabream with FFC and MPs, either separately or together, on hematological factors (a) red blood cell (RBC), (b) hematocrit (Ht), (c) Hemoglobin (Hb), (d) Mean corpuscular volume (MCV), (e) Mean corpuscular hemoglobin (MCH) and (f) Mean corpuscular hemoglobin concentration (MCHC) (Mean ± SD; n=9). The fish that were tested on various days within the same groups showed notable differences, which were marked by different letters. An asterisk (*) indicated a meaningful difference between the treated group and the control group ($p < 0.05$).

2d, e, f). The alterations in these indicators are typically associated with variations in the quantity and dimension of RBCs, as well as the synthesis of Hb in fish exposed to pollutants (Ammar et al., 2023). Elevated MCV and decreased MCHC indicate macrocytic and hypochromic anemia, resulting from impaired erythropoiesis and immature RBCs in circulation (Clauss et al., 2008). Similarly, exposure to chloramphenicol led to decreased levels of MCH and MCHC in African catfish (*Clarias gariepinus*; Nwani et al., 2014). In a related finding, exposure to polypropylene resulted in an increase in MCV while simultaneously lowering MCH and MCHC in Nile tilapia (Nair & Perumal, 2024), suggesting disruptions in hematological parameters.

In this research, every treatment (FCC, MPs and FFC+MPs) led to a noticeable rise in the neutrophil count ($p < 0.05$; Figure 3b), probably as a result of heightened phagocytic activity in reaction to cellular damage (Shirmohammadi et al., 2018). In contrast, there was a significant decline in lymphocyte counts ($p < 0.05$; Figure 3c), suggesting possible immunosuppression in fish (Shirmohammadi et al., 2017). Also, the substances had no effect on eosinophils and monocytes (Figure 3d, e). The findings are consistent with earlier research on rainbow trout exposed to FFC, as noted by Shiry et al. (2020). Additionally, Harikrishnan et al. (2024) found that after 20 days of exposure to MPs, zebrafish (*Danio albolineatus*) exhibited a significant decrease in lymphocyte counts in both male and female fish, accompanied by an increase in neutrophils and monocytes.

The study found that after one day of exposure, cortisol levels peaked in all treatment groups ($p < 0.05$; Figure 4a and Figure 5a). While cortisol levels decreased over time in the treated groups, they did not return to control levels, except for the FFC-treated group, which normalized after 14 days. Increased cortisol levels during stress lead to higher energy demands, which can weaken the immune response (Lemos et al., 2023). The continued presence of high cortisol levels in the MPs and FF-

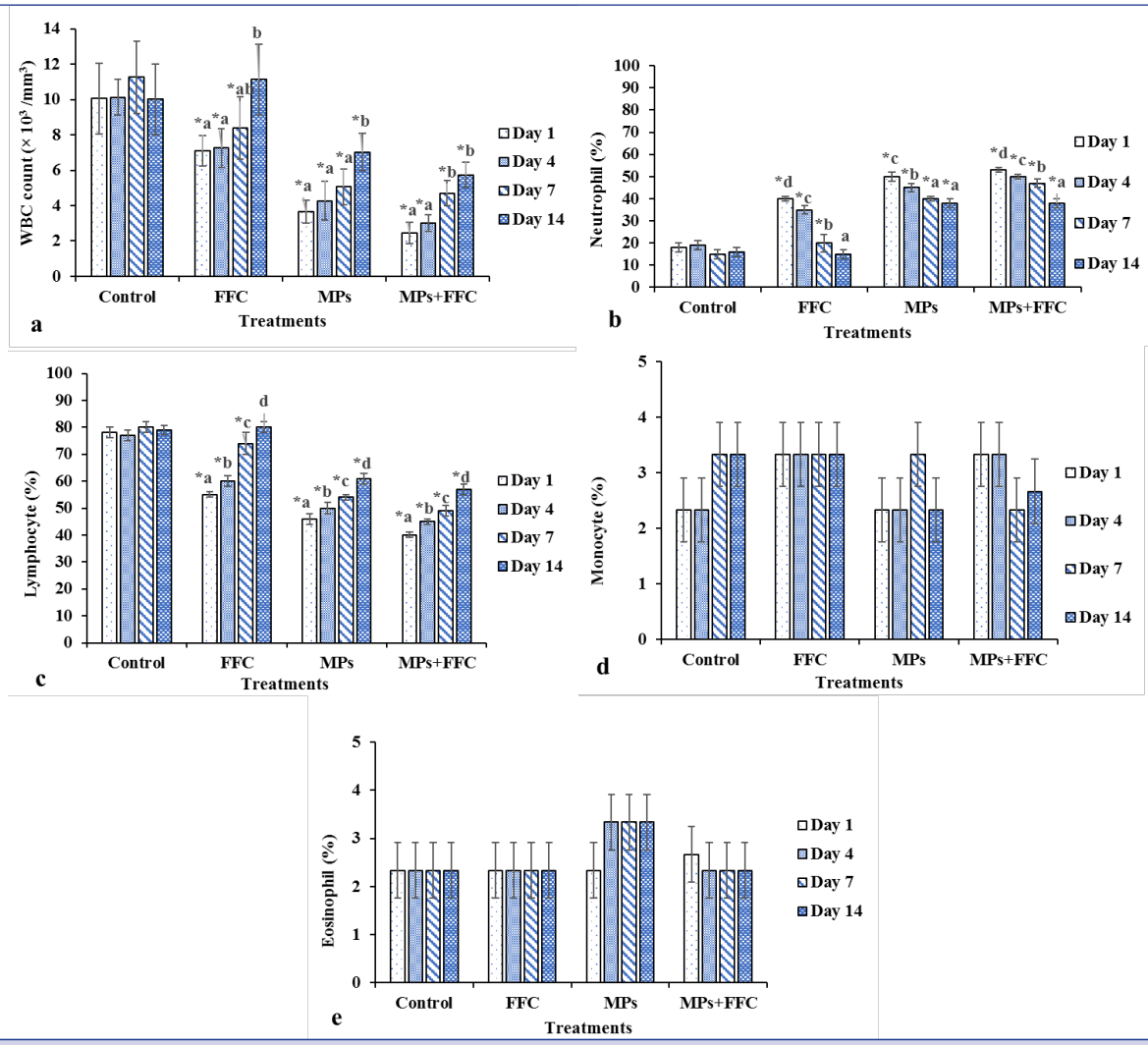

Figure 3. The impact of feeding yellowfin seabream with FFC and MPs, either separately or together, on total and differential white blood cell count (a) white blood cell (WBC), (b) neutrophil, (c) lymphocyte, (d) monocyte and (e) eosinophil (Mean ± SD; n=9). The fish that were tested on various days within the same groups showed notable differences, which were marked by different letters. An asterisk (*) indicated a meaningful difference between the treated group and the control group ($p < 0.05$).

C+MPs groups indicates that MPs might disrupt the hypothalamic-pituitary-interrenal axis, which plays a crucial role in managing stress responses. Such disruption could extend the stress response, persisting even after the stressor has been eliminated (Ding et al., 2025). The findings align with previous research by Harikrishnan et al. (2024) on zebrafish and Hoseini and Yousefi (2019) on rainbow trout, both of which noted increased cortisol levels in response to exposure to MPs and medicinal compounds like oxytetracycline, respectively.

The study found that EPO levels fell in all groups that received treatment on the initial sampling day but slowly rose over the course of the study, though they still remained much lower compared to those in the control ($p < 0.05$; Figure 4b and Figure 5b). Stressors may have damaged the fish's kidney tissue, which is responsible for producing EPO, leading to decreased hormone levels due to impaired hormone secretion (Sadeghi et al., 2015). Our results are consistent with previous research on stressed rohu (*Labeo rohita*; Datta et al., 2022). The FFC group was the only one to show a full recovery of EPO levels after 14 days, further emphasizing the transient nature of the toxicity caused by FFC.

During the sampling period, the concentration of T3 and T4 hormones exhibited an increasing trend in the groups that received MPs either separately or in combination and were not normal after 14 days. In contrast, FFC did not have a significant impact on either of the thyroid hormones ($p > 0.05$; Figure 4c, d and Figure 5c, d). These alterations can be linked to the presence of MPs in the tissue, leading to increased damage to the thyroid. This theory is supported by previous studies conducted by Wang et al. (2022), which investigated the impact of MPs and biphenyls on juvenile Japanese flounder (*Paralichthys olivaceus*).

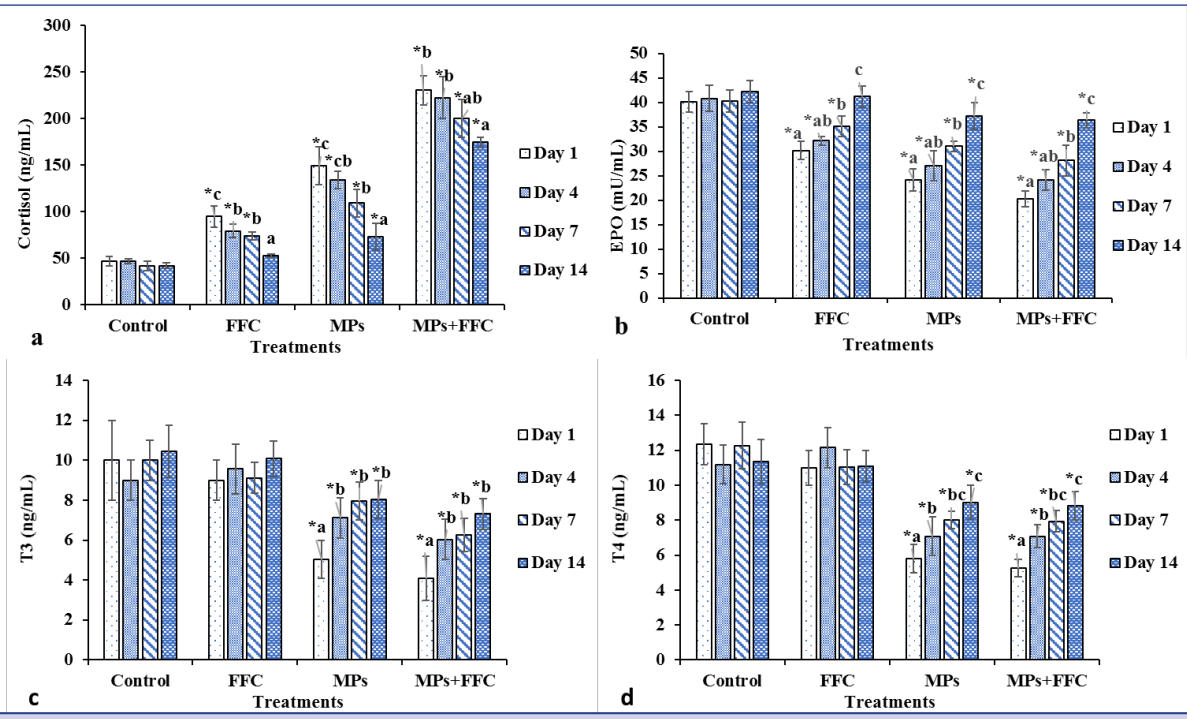

Figure 4. The impact of feeding yellowfin seabream with FFC and MPs, either separately or together, on cortisol (a), erythropoietin (EPO; b), triiodothyronine (T3; c), and thyroxine (T4; d) (Mean ± SD; n=9). The fish that were tested on various days within the same groups showed notable differences, which were marked by different letters. An asterisk (*) indicated a meaningful difference between the treated group and the control group ($p < 0.05$).

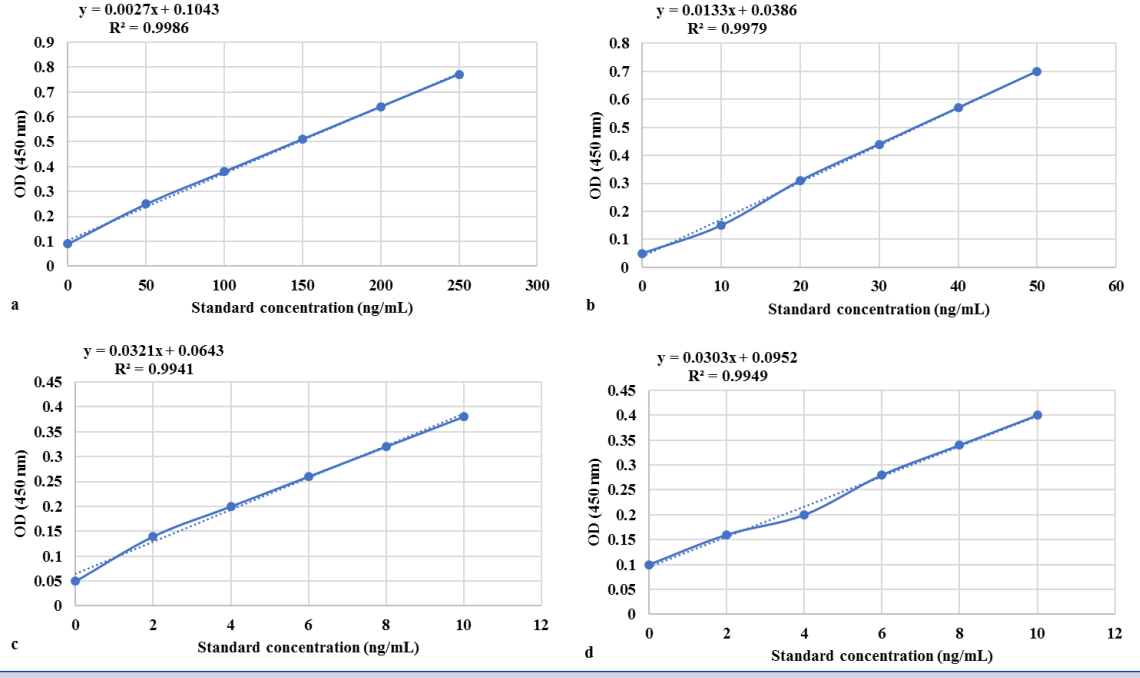

Figure 5. Standard curve of ELISA method for hormones including cortisol (a), erythropoietin (EPO; b), triiodothyronine (T3; c), and thyroxine (T4; d)

Following a two-week period, all hematological and hormonal parameters in the FFC-only group reverted to their baseline levels, likely attributed to the metabolism of FFC (Bardhan et al., 2023). Conversely, the groups exposed to MPs and FFC+MPs exhibited only partial recovery, with Ht and MCH levels returning to normal in the MPs group and MCH levels stabilizing in the mixed group. This indicates that MPs may induce lasting damage, possibly due to their accumulation in various tissues and their function as carriers for antibiotics (Hossain et al., 2023). Our findings align with earlier studies on juvenile common carps exposed to MPs (Ammar et al., 2023). The group that experienced both MPs and FFC exhibited the most significant changes in blood and hormone levels, highlighting the synergistic effects of these factors.

The lack of notable improvement in most hematological and hormonal indicators among the MPs and mixed groups highlights the persistent ecological effects of MP pollution in aquaculture systems. These findings emphasize the need to improve waste disposal methods to minimize MP contamination in the environment. While this study provides important insights into the synergistic effects of MPs and antibiotics, there are limitations that future research should explore. Specifically, it is important to examine the toxicity of MPs and FFC in relation to genomic abnormalities in blood cells of commercially significant species

CONCLUSION

The current study focuses on the impact of FFC and MPs on A. latus over a 14-day period, both individually and in conjunction. Significant alterations in hematological markers, EPO, cortisol, T3, and T4 hormones, along with FFC accumulation were observed in the plasma of the fish. The changes were more noticeable following exposure to a combination of MPs and FFC. The groups that received MPs, either alone or with FFC, did not show normalization in most parameters after 14 days, unlike the group exposed only to FFC, indicating persistent toxicity from MPs. Although the toxic effects of FFC were observed in the present study, these changes were temporary, indicating that this antibiotic is safe for use for this species. However, the continued presence of MPs and their role as carriers of antibiotics highlights the need for improved contamination management strategies. The ELISA data confirmed our hypothesis and underscored its importance in hormone research.

Conflict of interests: The authors report there are no competing interests to declare

Ethics committee approval: The experiments were conducted in accordance with the guidelines of the National Ethical Committee for Animal Research in Iran

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