USING BIOMARKERS TO IDENTIFY OXIDATIVE STRESS RESPONSE IN ZEBRAFISH *(Danio rerio)* FED WITH MICROPLASTICS (FRAGMENTS AND VIRGIN PELLETS)

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Introduction

Fish can serve as bioindicators of environmental pollution, playing a significant role in understanding a potential risk related to environmental conditions since they are directly exposed to chemicals and pollutants such as microplastics, which represent an increasing problem today. Oxidative stress is one of the possible consequences that those animals face and enzymes as catalase (CAT), glutamate S-transferase (GST) and lipid peroxidation (LPO) could be important biomarkers that enable us to understand any possible imbalance between pro-oxidant and anti-oxidant ratio which leads to the generation of ROS (reactive oxygen species). Thus, analyzing how the enzymatic defense mechanisms vary along the exposure period to contaminants leads us to a better understanding of a possible interaction between microplastic and fishes. In the study was taken into consideration also a possible interaction with animals' longitude and weight.

Methods

Statistical Analysis
Data were elaborated with SPSS (vers. 26). One-way ANOVA procedure was used for statistical analysis.

A Commercial flakes (control)
B Commercial flakes + 10% virgin MPs
C Commercial flakes + 10% Lambra's MPs collected
D Commercial flakes + 10% Poris' collected MPs

4 treatments → 30 days exposure time → Sampling at T0, T7 and T30 → Results' analysis

Catalase (CAT)
Aebi (1984); Demarchi et al. (2020)

- Sample + substrate solution + buffer
- Reading absorbance at 240nm during 3 minutes

Lipid Peroxidation (LPO)
Barboza et al. (2020)

- Sample + TCA (12%) - vortex
- 240μl TRIS-DTPA + 300μl TBA (0.73%);
- 100°C – during 1 hour
- Centrifugation at 11500rpm during 5' at 4°C
- Reading absorbance at 535nm

Glutathione S-transferase (GST)
(Frasco & Guilhermino, 2002; Habig et al., 1974)

- Sample + substrate solution + buffer
- Reading absorbance at 340 nm during 5 minutes

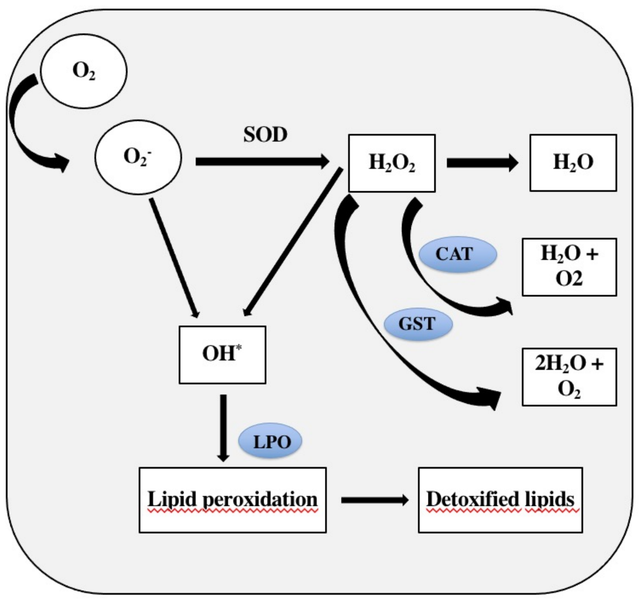

Fig. 1 Illustration representing the antioxidative ROS remotion promoted in cells by the studied enzymes (CAT, GST); and LPO indicating oxidative damage.

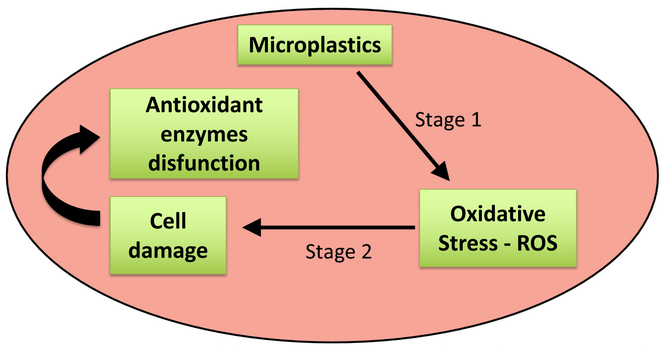

Fig. 2 Representation of the experimental process from the introduction of MPs in the diet to the consequences of oxidative stress.

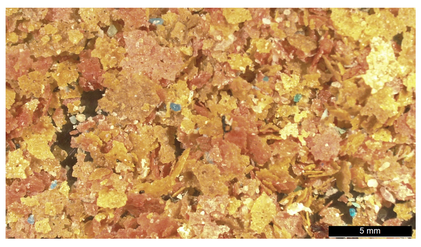

Fig. 3 Example of treatment

Fig. 4 Example of length measurement

Fig. 5 Example of treatment: for the picture was used treatment C

Results

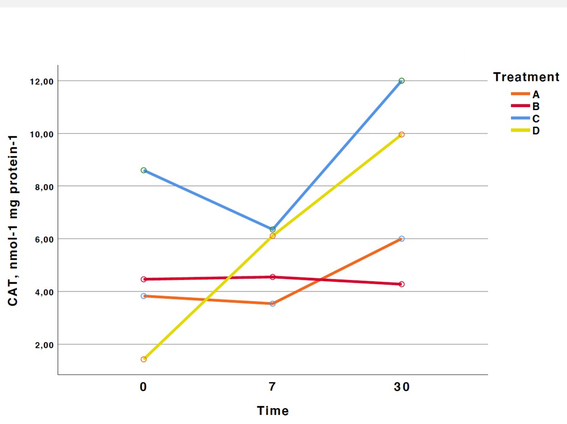

Fig. 6 Catalase activity (CAT)

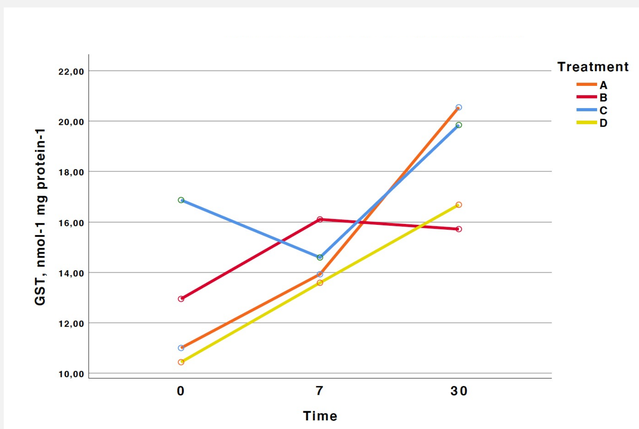

Fig. 7 Glutathione S-transferase activity (GST)

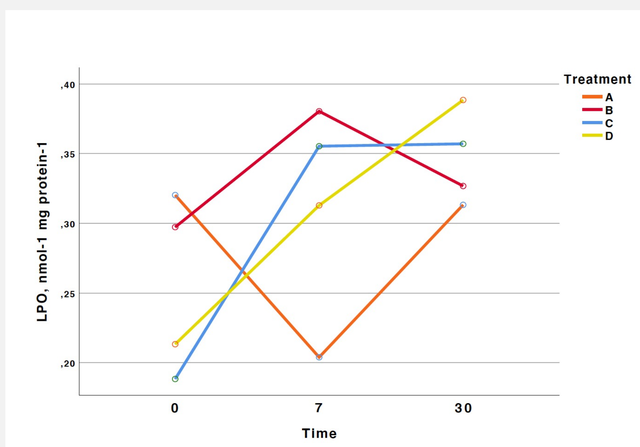

Fig. 8 Lipid peroxidation activity (LPO)

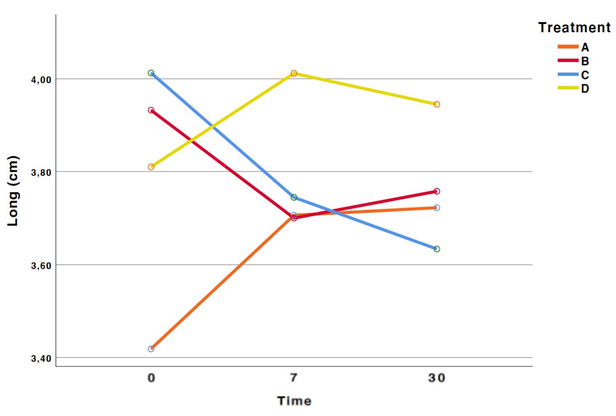

Fig. 9 Longitude (cm)

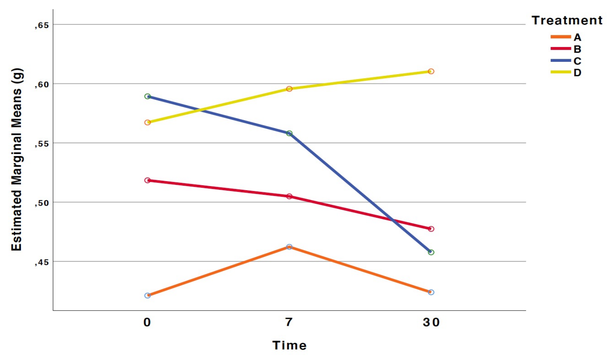

Fig. 10 Weight

- No significant difference ($p > 0.05$) was found for CAT activity (*Fig. 1*) *and* no significant difference ($p > 0.05$) was found for LPO activity (*Fig. 3*);
- No significant difference ($p > 0.05$) was found for GST activity (*Fig. 2*) between treatments (A; B; C; D) but significant different was found among the different exposure times ($p < 0.05$): T_0 - T_7 ($p = 0.001$); $T_7 - T_{30}$ ($p = 0.20$). This indicates a gradual increase of antioxidant activity;
- Significant difference ($p < 0.05$) was found in longitude between treatments and time (*Fig. 4*): significant difference was found among treatment A-B ($p = 0.040$); A-D ($p = 0.000$);
- Significant difference ($p < 0.05$) was found in weight among treatments (*Fig. 5*): A-C (p = 0.023); A-D (p = 0.000); B-D (p = 0.045).

Conclusions

- Treatments with different typology of microplastics does not seem to increase oxidative stress in animals' muscular tissue at enough quantity that detoxifying enzymes reacts, at T_{30};
- Even thought over time data shows that there is a common related increase of antioxidant enzymes. This leads us to a new hypothesis that probably is required higher concentrations of plastic (>10%) included in the food diet and a longer time exposure to contaminants, to begin to see an interaction and a significant difference between treatments;
- The significant difference found in longitude, between treatments A-B and A-D. The delayed growth could be related to MPs injection and its negative interaction with the organism;
- A significant difference was reported for the weight parameter, as was found a significant difference between treatments and time: animals fed with diet B, C and D show a loss of mass along the time. This could either be due to not real satiety caused by MPs injection or to contaminants related to MPs, affecting animals' homeostasis.

Bibliography

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