Adult Zebrafish Model for Potential Probiotics Tissue-Specific Oxidative Stress and Antioxidant Assessment

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The importance of identifying and studying new antioxidant additives is evident in all areas of animal husbandry, including aquaculture. The use of Danio rerio to select new antioxidants seems relevant due to its genetic tractability and established use in toxicological studies[1]. This work introduces a model designed to investigate tissue-specific responses to oxidative stress, and subsequently, the effects of antioxidants and probiotics, using adult zebrafish, instead of a common practice of using zebrafish embryos [2]. Such a model will have applications in the development of probiotics and feed additives for aquaculture and beyond.
A model of oxidative stress was established in adult zebrafish D.rerio to evaluate the antioxidant effect of feed additives. Paraquat, at a final concentration of 10 ?M was used as an inducer of oxidative stress, generating free radicals and providing detectable levels of oxidative stress. Two groups of D. rerio were used: wild type (WT) and genetically modified line «GloFish». From each group were formed a control group kept under normal conditions and an experimental group kept in water with paraquat at a concentration of 10 µM. Oxidative stress was assessed in three tissue types (muscle, intestine, and gills) using two methods: (1) a bioluminescent test on E. coli MG1655 pSoxS-lux biosensor to measure antioxidant activity, and (2) quantification of malondialdehyde (MDA), a marker of lipid peroxidation (LPO). Measurements were taken over a 35-day period.
The highest level of antioxidant activity was observed in the intestine of wild-type fish. Exposure to paraquat resulted in increased antioxidant activity in the intestine by the end of the experiment, while it gradually decreased in the absence of paraquat. Conversely, in muscle tissue antioxidant activity gradually decreased by the end of the experiment.
Similar trends were observed in the GloFish group. Specifically, in the presence of paraquat, intestinal antioxidant activity in GloFish decreased to 5% by day 14, then recovered to 25% by day 35. In contrast, muscle tissue showed an increase in protective effect by day 14, followed by a decrease to 35% on day 35. MDA levels were elevated in the muscle and intestine of wild-type D. rerio on day 35 of the experiment, indicating increased LPO. The study revealed that wild type Danio rerio were more resistant to the effects of paraquat than «GloFish».
A negative correlation was observed between changes in antioxidant activity and the levels of oxidation products, supporting the validity of the model.
To prove the applicability of this model to testing probiotics for aquaculture, we added preparation of two probiotic Bacillus strain with confirmed in vitro antioxidant activity. Although probiotic itself did not enhance the survival of fish in normal conditions, it increased survival rate under conditions of oxidative stress by 46.16%.
This study demonstrates the efficacy of combining biochemical assays and biosensor technologies to comprehensively assess the dynamic interplay between antioxidant and pro-oxidant factors in zebrafish. The findings highlight the tissue-specific and strain-dependent differences in the response to oxidative stress and the negative correlation between antioxidant activity and lipid peroxidation.

References

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