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MASTER THESIS PROJECT

Pilot study evaluating microRNAs as a potential biomarker of microplastic exposure in Japanese quails *(Coturnix japonica)*

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Abstract

Microplastics (MPs), plastics < 5 mm, are considered emerging contaminants due to their ubiquitous nature and potentially adverse impact on living organisms. MPs can even be found in remote areas ranging from the Arctic and the Antarctic to Mount Everest and the Mariana trench. A variety of organisms ingest MPs including many species of birds that are widely distributed in various habitats worldwide and can mistake plastic for prey which serves as one of the ways of MPs deposition in their bodies.

MicroRNAs (miRNAs) are a class of non-coding RNAs that play intergral roles in regulating gene expression. Cell-free circulating miRNAs exist in remarkably stable forms in biofluids where their association with diverse disease states highlights their potential to be used as biomarkers. There is no prior study available where the effects of MPs on circulating microRNAs (miRNAs) have been investigated in birds in a controlled environment. Hence, this thesis aimed to assess the influence of MPs exposure on circulating miRNAs in Japanese quail (*Coturnix japonica*) to indicate the potential of miRNAs to be used as a biomarker for MP exposure.

To address this aim, serum samples were obtained from Japanese quails (n=50), that had been fed environmentally realistic doses of a mixture of plastic items with two different polymer types i.e., polyethylene (PE) and polypropylene (PP), in the size ranges of 3mm, <125µm and a mixture of both size ranges for a period of five weeks. miRNA was extracted to prepare the complimentary DNA (cDNA) and run the Real-Time Quantitative Reverse Transcription PCR (qRT-PCR). Our results indicated that a mixture of MP of different polymers collected from the environment in the size of 3mm are negatively correlated to circulating miRNA-221 ($p = 0.022$) showing lower levels of circulating miR-221 in males compared to the control. However, no MPs showed any significant effect on miRNAs when sexes were combined. Also, considering the effect of hemolysis on miRNA abundance in the serum, hemolysis quantification of serum samples was performed by measuring the absorption at 414nm via NanoDrop UV-Vis Spectrophotometer. Results indicated the value of gene count increasing positively with hemolysis thus showing somehow a direct relationship between hemolysis and gene count though a significant correlation was not found for any of the miRNAs. Taken together, we found a correlation between MPs exposure and reduced abundance of circulating miRNA-221 highlighting the potential of miRNAs to be used as a biomarker of MPs exposure in birds. However, further research is required to study the relationship between MPs exposure and changes in miRNAs as this study is preliminary in nature and did not show any causal relationship between miRNA and MPs. Also, the effect of hemolysis on different miRNAs in birds should be evaluated in the future.