

Since the early 1950s the significant growth of plastic production is observed worldwide. In 2021, production exceeded 390 Mt and approximately 26 Mt of plastic waste are produced annually in Europe. Most of plastic products are not biodegradable, and decompose into smaller particles. Products of diameter ≤ 5 mm are called microplastics (MP) and products of diameter ≤ 1 μ m are called nanoplastics (NP). They are characterized by small size, lightweight, high durability, and extended stability, and are easily spread to various environments by the action of wind and waves. MP has great impact on the environment, animal, and human health and is the area of interest of many scientists.

Water and terrestrial birds are exposed to MP contamination of environment. They confuse MP with food, which leads to its accumulation in the gastrointestinal tract and causes many harmful consequences, like malnourishment, dehydration or starvation. Majority of the available data comes from the studies on the influence of MP environmental pollution on free-living birds. The meagre number of studies was conducted in laboratories on different avian models like chickens or quails.

The main aim of this project is to study an impact of MP in diet of Japanese quails on their health and health of their descendants: children and grandchildren, respectively. We presume, that MP will affect composition of microbiota and functioning and integrity of digestive tract. We think, that MP may accumulate in eggs, internal organs and tissues and may influence different metabolic pathways and physiological processes (also fertility). We presume, that this impact will result in differences in metabolomic profiles of serum. As we want to define the exact mechanisms of MP effect in living organisms, we decided to check in one, global study all potential mechanisms of MP action previously suggested by other authors. Hence, we want to perform untargeted metabolomic studies in serum and combine their results with other data regarding hormone levels, lipid profiles and antioxidant status, obtained in this project to find potential new mechanisms and their biomarkers. Moreover, we want to check how exposure to MP of parents transfers into their descendants and whether limitation of MP exposure in progeny will reverse or inhibit the impact of parental exposure to MP, and by what mechanisms. For this purpose we plan to study three generation: parents (F0), children (F1) and grandchildren (F2). To explore the dependencies among all investigated parameters we decided to choose different chemometric tools.

Birds as model animals have been historically important in different biomedical research. Japanese quails are an attractive alternative, very similar to chicken, characterised by small size, early maturity, quick life cycle, rapid reproductive rate, and producing large number of eggs, but with much shorter generation interval. Japanese quails are also particularly sensitive to environmental contaminants and we think they are very good model for MP exposure studies. The whole experiment will be conducted in accordance with **Endocrine Disruptor Screening Program Test Guidelines OCSPP 890.2100: Avian Two-generation Toxicity Test in the Japanese Quail**. Birds from F0 generation will be divided into two experimental groups: control (CON) and orally exposed to microplastic (MP). Eggs will be used to assess i.a. MP accumulation and to establish descendant generation (F1). Progeny of MP will be divided into two groups: MP/CON free of MP and MP/MP receiving MP. Eggs and progeny of F1 will be examined for MP accumulation and used to establish F2 generations. Also in F2 generation, progeny of MP/MP group exposed to MP will be divided into two groups including MP/MP/CON group free of MP and MP/MP/MP group receiving MP.

MP content will be determined in plasma, serum, internal organs, cecum content and faeces. Histopathological examination of tissues will be also performed. We will also study microbiota in cecum, thyroid and steroid hormones, activity of antioxidant enzymes, biomarkers of oxidative stress like oxysterols or malondialdehyde and selected lipid parameters including fatty acids will be determine in material from three generations (F0, F1 and F2) and combine with untargeted metabolomic profiling of serum.

The present project is the first attempt of complete explanation of MP impact in transgenerational metabolomic studies in birds. The main innovation of this project is an examination of descendant generations differing in MP exposure and combining of different parameters regarding eggs, tissues and organs with metabolome studies in serum. Obtained results will permit in future to use this model in further experiments, e.g. regarding developmental programming.