

1. PHD PROJECT DESCRIPTION (4000 characters max., including the aims and work plan)



Project title: MicroEpiQ: contact with adult as a major factor in microbiome-associated epigenetic modifications in Japanese quail (*Coturnix japonica*)
<https://microepiq.umk.pl>

1.1. Project goals

In the proposed project we address the crosstalk between the microbiome and the epigenome using quail as a biological model. The motivation of the project is based on the concept that the perinatal contact of the newly hatched quails with the adult mimics the natural transfer of the maternal microbiota to the offspring. This way, the droppings left by the adult initiate the inoculation of the microbiota of the neonates.

We hypothesize that the intestinal microbiota stimulated by the contact with the adult provides sufficient environmental cue to trigger epigenetic modifications. To test this hypothesis we plan to reconstruct a three-generational family of quails stimulated vs. non-stimulated by the contact with adult and analyze epigenome-metabolome-microbiome axis. Hereby, **the primary goal of this project is to present the proof of concept that the contact with adult is the major factor in microbiome-associated epigenetic modifications in Japanese quail (*Coturnix japonica*).**

The detailed research goals have been formulated as follows:

1. To characterize intestinal microbiota of quails stimulated vs. non-stimulated by contact with adult over three generations;
2. To identify the intestinal and serum metabolites in the quails, whose intestinal microbiota has been stimulated vs. non stimulated by contact with adult over three generations;
3. To characterize epigenetic activity of miRNA in intestine of the quails stimulated vs. non-stimulated by contact with adult over three generations;
4. To determine methylation status of the host DNA in the intestine by methylSeq;
5. To model microbiome-metabolome-epigenome crosstalk in quails stimulated by contact with adult.

1.2. Outline

The experimental outline is presented in Fig. 1. Newly hatched chicks are kept with a contact (adult) quail or without. The contact lasts for 1-2 days and is supposed to facilitate the transfer of the microbiota and colonization of the neonatal chicks. The chicks are kept under reaching sexual maturity and the eggs are collected to generate F1 offspring. We do not sex chicks and mating is done within each cage. The new generation is stimulated by contact with adult hen and reproduced in the same manner to obtain F2.

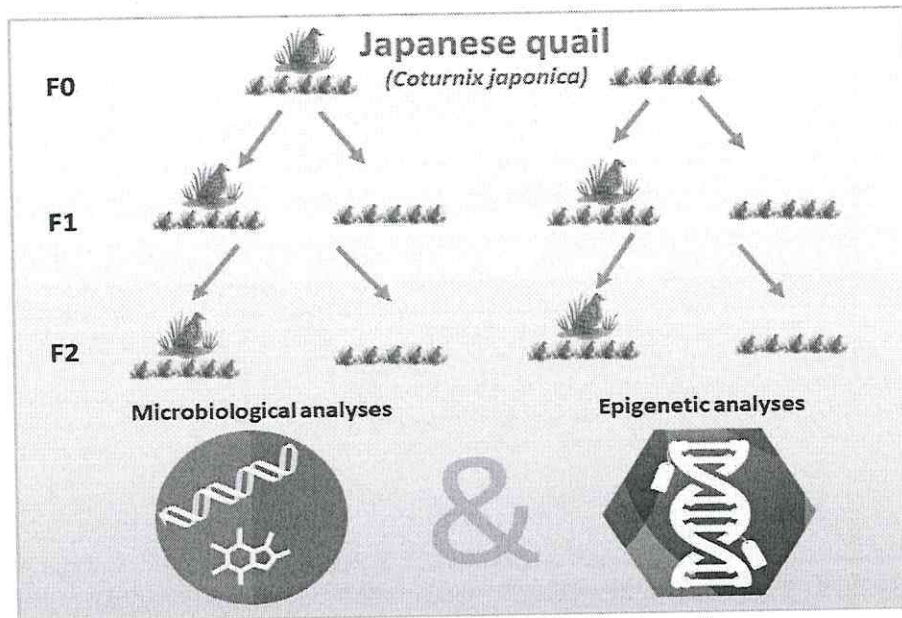


Fig 1. Experimental design of the MicroEpiQ project

The birds will be housed in isolated units in biosafety facility equipped with a HEPA filtering system and an automated system for pressure control in corridors, bird units and hygiene stations to prevent contamination of experimental premises. Every group of quails will be housed in a separate unit. The biological material sampled for analytical procedure will include: caecal digesta (for 16s rRNA sequencing of microbiota and metabolites), blood serum (for metabolites), caecal tonsils (for miRNA sequencing and methylSeq). Samples will be taken from all animals (F0, F1 and F2 generations), on week 6 of rearing.

1.3. Work plan

Fig 2. shows the MicroEpiQ project implementation plan. The MicroEpiQ project will be carried out in the period 2023-2026 by five collaborating research units. In the first year of the project implementation (2023), experiments on animals will be carried out, which will constitute the experimental basis for testing the research hypothesis. The second year of the project (2024) will be devoted to metagenomic taxonomy and metabolite identification, and the next two years (2024-2025) - epigenetic analyzes (functional genomics and methylome sequencing). The project will be completed in early 2026.

MicroEpiQ Project Timeline

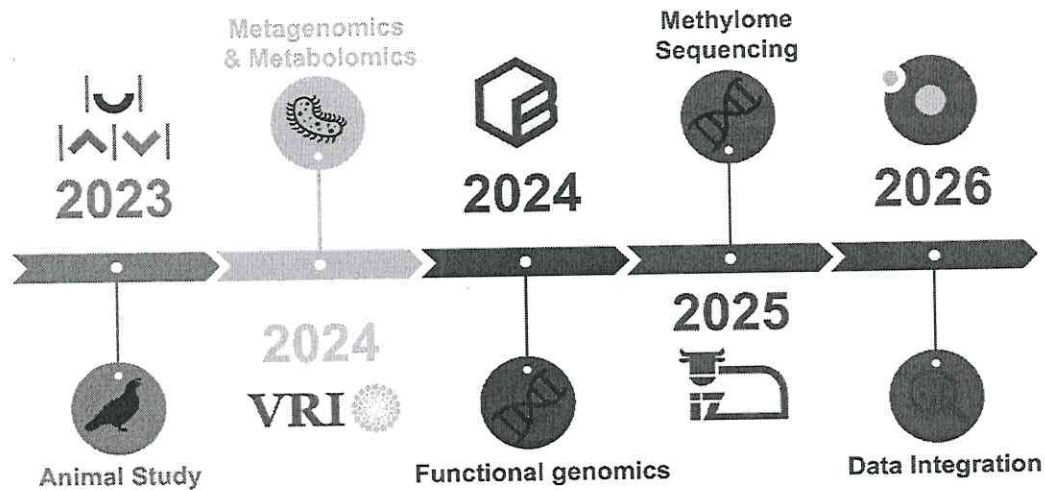


Fig 2. Work plan of MicroEpiQ project

1.4. Literature (max. 10 listed, as a suggestion for a PhD candidate)

- Kubasova T, Kollarcikova M, Crhanova M, Karasova D, Cejkova D, Sebkova A, et al. Contact with adult hen affects development of caecal microbiota in newly hatched chicks. *PLoS One*. 2019;14(3):e0212446.
- Yadav S, Jha R. Strategies to modulate the intestinal microbiota and their effects on nutrient utilization, performance, and health of poultry. *Journal of animal science and biotechnology*. 2019;10(1):1-11.
- Zheng D, Liwinski T, Elinav E. Interaction between microbiota and immunity in health and disease. *Cell research*. 2020;30(6):492-506.
- Miro-Blanch J, Yanes O. Epigenetic regulation at the interplay between gut microbiota and host metabolism. *Frontiers in genetics*. 2019;10:638
- Paul B, Barnes S, Demark-Wahnefried W, Morrow C, Salvador C, Skibola C, et al. Influences of diet and the gut microbiome on epigenetic modulation in cancer and other diseases. *Clinical epigenetics*. 2015;7(1):1-11.

1.5. Required initial knowledge and skills of the PhD candidate

The PhD candidate is required to have the prior knowledge in the area of basic biology, genetics and/or microbiology. The project integrates many aspects of biology with microbial and epigenetic responses to treatment. The candidate should be able to work in the molecular lab (manual precision is a must). The knowledge of English is mandatory. We look for a candidate with high enthusiasm for science.

1.6. Expected development of the PhD candidate's knowledge and skills

Upon having completed the MicroEpiQ project, the candidate will be acquire knowledge and practical skills in many aspects of animal and veterinary sciences, molecular biology, microbiology and data analysis. First,

the PhD candidate will learn quail biology and hands-on management, which can be translated to other species of both farmed and exotic poultry. Second, the sampling methods and various samples processing (tissue, intestinal digesta, blood, and serum). The training will include DNA and RNA isolation from different sources. The training in the following analytical methods will be available in the project partners: DNA and RNA libraries preparations, LC-MS, analysis of miRNA, methyl DNA and RNA-seq data, identification of microbial profile based on 16s rRNA sequencing. The soft skills trained during the PhD period is scientific writing and presentation techniques, team work and grant proposal writing.