Epigenetics is driven by environmental factors that activate or deactivate various mechanisms that control gene expression at the level of transcription, post-transcription, and translation. If epigenetic mechanisms influence gene expression, the major research question is: **what controls epigenetic mechanisms?** Under the influence of various environmental factors, such as: nutrition, housing and hygiene, stress or climate the multi-stage process of transcription of genetic material to protein undergoes changes, which may result in modulation of an individual's phenotypic features. One of the most specific and intimate relationships an animal develops throughout its life is with its **gut microbiota.** The gut microbiota plays a key role in keeping poultry healthy. It affects the host's organism by regulating the immune response, metabolic and digestive processes as well as the absorption of nutrients.

In the project entitled „**ActEpi: Activation of the epigenetic mechanisms in poultry through a programming of the gut microbiota"** we address the **crosstalk between intestinal microbiota and poultry epigenome.** The motivation of this project proposal is based on the results of the our previous study showing that regulation of the gene expression might be done by prebiotic or synbiotic delivered during embryo development. The main observable result is methyl-dependent silencing of gene expression. **To better understand this relationship, it is necessary to analyze the molecular and biochemical mechanisms that underlie communication between the gut and the host.** The current project proposal is based on the evidence that the *in ovo* administration of bioactives on day 12 of egg incubation affects the health status and welfare of the host throughout the rearing period. Following this initial concept, the research **hypothesis was formulated that the directed activation of epigenetic mechanisms regulating gene expression is possible through early programming of the gut microbiota by prebiotic and bacteria metabolites. In this project we aim to present the proof of concept that the microbial programming by bioactives is responsible for epigenetic-depended gene expression.**

Scientific data indicate that **modulation of the intestinal environment has a significant impact on the regulation of epigenetic mechanisms** in humans and animals. A thorough analysis of the mechanisms of epigenetic regulation of gene expression, unrelated to the DNA sequence, would be the key to understanding the molecular basis of the influence of the environment on the phenotype. **Despite the well-known gut microbiome and the growing knowledge of epigenetic regulation, through DNA methylation, histone modification or miRNA activity, there is little research linking both issues. Therefore, the research proposal will focus on the analysis of the effects of a prebiotic (galactooligosaccharide with already confirmed epi-modulating properties) and postbiotic (butyric acid - with potential epi-modulating properties) delivered during embryonic development on the profile of intestinal bacteria.**

The specific objectives of the project are:

1/ What is the importance of early stimulation of the gut microbiota in the epigenome-microbiome axis?

2/ What is the direct influence of prebiotic and postbiotic on molecular and biochemical mechanisms, and indirect influence on the host phenotype?

3/ How does the profile of intestinal bacteria change after administration of a single *in ovo* dose of a bioactive substance?

4/ What epigenetic mechanism drives gene expression silencing?

5/ Are the changes in the expression at the mRNA level reflected at the protein level?

The proposed research project has been divided into **two research tasks**: **Task 1** that is a **pilot study** for an optimization of postbiotic *in ovo* and **Task 2** that includes the **main experiment. Task 1** is planned as a **preliminary screening of postbiotic dose (sodium butyrate)** and to **characterize main properties in intestines.** At this point, the main goal **is to provide as optimal dose of postbiotic as possible**. We also hypothesize that the fermentation product of the prebiotic, butyrate, which is a short-chain fatty acid, might have a significant impact on the host organism. **Butyric acid is a histone deacetylase inhibitor**. Hence, it might be assumed that upon his action significant epigenetic changes will occur. **Butyric acid is also a stimulator of mucin secretion in the intestine.** The selected dose of postbiotic will be used in **Task 2 – to stimulate the intestinal microbiota by postbiotic and prebiotic and create the most pronounced epigenetic response corelated with phenotypic and molecular traits.** The analysis of microbiome-mediated epigenetic gene regulation will be analyzed on both, microbial and host sides. On microbial side, we are going to characterize basic metabolome and microbiota activity and composition. In the host, we are going to study performance of the broiler chickens, histology of intestine and liver, wide range of biochemical analysis. We also will perform a detailed multiomic analysis (transcriptomics, epigenomics, proteomics) in the intestines and liver, focusing on explaining epigenetic regulation of gene expression.