

## **Epigenetic gene expression regulation in chickens after prebiotic and synbiotic *in ovo* delivery**

Epigenetic regulation of gene expression is a form of interaction of the external environment on reading and transcription of genetic information encoded in nucleic acids. It does include inherited changes which result from DNA sequence modification. This regulation plays a key role in the development and differentiation of cells.

Under the influence of various external factors such as nutrition, sanitation, stress or climate, the multi-stage process of transcription and translation of genetic information to the protein is changing, resulting in changes of the individual phenotype. **The most important epigenetic mechanisms are: microRNA and DNA methylation.** MicroRNA (miRNA) is a fraction of small RNA molecules encoded in the genome that have a fundamental impact on gene expression. DNA methylation involves the addition of methyl residues to the cytosine present in within the CpG islands. This modification blocks the enzyme access (including transcriptase) to DNA what inhibits the transcription of DNA into mRNA.

The research hypothesis of the proposed project assumes that **in chickens stimulated with bioactive substances - prebiotic or synbiotic *in ovo* in the 12<sup>th</sup> day of embryonic development, an epigenetic mechanism for the silencing of gene expression associated with the immune system via methylation of gene promoters and / or microRNA activity has developed.**

Prebiotics are non-digestible nutrients that selectively stimulate the growth or activity of bacteria present in the host gastrointestinal tract (in this case - chickens).

The synbiotic is a term referring to the simultaneous administration of prebiotics and probiotics in a single dose and their synergistic effect. The use of prebiotics and probiotics in a single product, provides the opportunity to improve the viability of probiotic bacteria by delivery of prebiotic which is used as a substrate for fermentation. Our research team demonstrated the significant effect of *in ovo* application of prebiotics and synbiotics on the microbial composition and changes in gene expression, also on many physiological and production traits of broiler chickens.

The proposed project aims to:

1. Bioinformatics analysis (meta-analysis) of gene expression dataset from other projects using prebiotic/synbiotic *in ovo*. The result of these analyzes is selection of candidate genes for analysis of miRNA and DNA methylation
2. Expression analysis of candidate miRNA and estimation the level of CpG islands methylation of candidate genes in chicken tissues stimulated by prebiotic/synbiotic *in ovo*
3. Proposing the epigenetic model of gene regulation in chickens where microorganisms inhabiting the digestive tract (microbes) were stimulated by prebiotic and synbiotic during embryonic development (*in ovo*).

The proposed project assumes the verification of the epigenetic character of gene expression modulation via mechanisms of cytosine methylation in CpG sequence of silenced genes promoters and action of molecule regulating the miRNA. Biological material is a unique collection of tissues from adult broiler chickens which were given prebiotic and synbiotic *in ovo* at 12<sup>th</sup> day of embryo development. Synbiotics were based on *Lactobacillus* and *Lactococcus* probiotic bacteria. Tissues selected for this project were analyzed at the gene expression on whole genome level.. **This analysis showed a significant effect of gene expression silencing associated with inter alia the functioning of the immune system.** The available data will be used for meta-analysis to select immune-related genes expressed at DNA level (DNA methylation analysis) and / or post-translational (miRNA activity) as a result of prebiotic/synbiotic stimulation of the chicken microbiome in early stages of embryo development. It will allow for *in silico* selection of promoter gene sequence and microRNA.

**The research proposed in this project will provide new insights into gene silencing mechanisms which regulation is stimulated by delivery of bioactive substances (prebiotic, synbiotic) *in ovo*.** The subject of the project is in line with interactions and relationships between the host (in this case - the chicken) and the intestinal microflora. **The results of the proposed experiments will greatly expand the knowledge of microbiome reprogramming in poultry and it will verify its epigenetic character. Such studies have not been carried out so far.**