**Prebiotics and probiotics are bioactive substances that beneficially modulate chicken host microbiome.** Prebiotics are natural, not digested by the host, nutritional sources for beneficial intestinal bacteria, e.g. lactic acid bacteria. Probiotics are beneficial bacterial strains that exert pro-health effects in the host organism. The chicken performance is reflected in health status of internal organs and in the physiological condition of a bird. The microbiota species that colonize the immature organism, together with a transfer of maternal antibodies (from the laying hen), have a major impact on shaping the immunological system of unhatched and newly hatched chickens. There is a considerable gap in knowledge on how the intestinal cells interact with probiotic bacteria and what are the effects of such interaction, e.g. probiotics are capable to produce postbiotics, lantibiotics or neurotransmitters upon interaction with the host's cells. **Our aim is to contribute novel knowledge as to how probiotics and prebiotics function in the chicken intestine biology.**

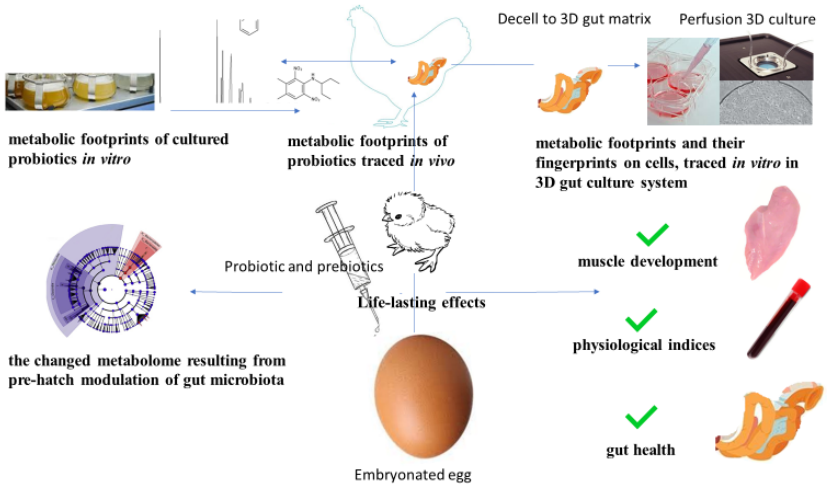


**Figure. Schematic overview of the major assumptions, that will be verified in this project**

To our knowledge, there are **two possible time-points** to modulate chicken microbiome in the embryonated egg (*in ovo*): on day 12$^{th}$ or in between 18-19,5$^{th}$ days of egg incubation. The genetics of a broiler chicken will guarantee a consistent genetic material throughout our study and full breeding control. This would not be possible in any other higher organism. We will use our in house developed *in ovo* techniques to precisely administer the bioactive substances to the embryo as early as possible in the developmental phases. Following that, we propose an interdesciplinary networking within the research team, to study the interactions of probiotics and prebiotics with gut cells. To fully characterize and understand the effects, we will perform assessment of the embryos, of the hatched chickens and of the matured birds.

Firstly, the signatures of the probiotic metabolic activity will be identified *in vitro*, in the culture of bacteria on their 'feeding' substrate (prebiotic). Those signatures are so called metabolic footprints. We assume, that those footprints can be traced in the chicken tissue after administration of the same probiotic *in ovo*. The image of those interactions will be completed by applying a novel 3D *in vitro* model that will mimic, in a very simplified way, the chicken intestine. A 'tissue laundry' technique will allow us to obtain the chicken gut scaffold to be seeded with intestinal cell lines. The cells will be co-cultured with probiotic bacteria and we expect to identify characteristic metabolic signatures of probiotic activities in those cells, referred to as the metabolomic fingerprints.

Secondly, we propose to identify a unique microbiome profile in the gut of the chickens, that are stimulated with probiotics and prebiotics early, prior to hatching (*in ovo*). The specific microbial populations in gut of those animals will be analysed using the next generation sequencing of the microbial genetic material (metagenomics).

Thirdly, to increase understanding, we propose to study the holistic effect of the probiotics and prebiotics on the chicken organism, through its whole life-span, by the complex examination of: zootechnical parameters, gut microstructure, muscle micro vascularisation, regulation of gene expression in the gut-brain axis, probiotic metabolic footprint in the gut, and biochemical indices.

This project involves advanced metagenomic, transcriptomic and proteomic analyses. Thus, to provide useful biological contextualization, we will simultaneously integrate all that multi-omic data using the in-house developed pipeline for statistical analysis and a powerful GeneSpring software. The findings of this project will contribute to scientific basis of **zootechnics (animal science), microbiology, chemical, biochemical, meat science, poultry science and avian genetics.** New knowledge about probiotic function in the host can also contribute to applied sciences, eg. recent evidences on the role of a microbiome in modulating patient's immunity, have accelerated the oncological research towards using probiotics and prebiotics to improve response of the patients to immunotherapies.