8. SUMMARY

Modern poultry production pursuits high productivity while ensuring broiler health and limiting the use of antibiotics in breeding. However, achieving these goals is associated with compromised bird immunity, increased chick morbidity and mortality, and the development of microbial drug resistance. These phenomena prompt the search for alternative methods of prophylaxis and growth stimulation, among which *in ovo* supplementation of bioactive additives is especially noteworthy. Studies have shown that supplying these compounds at an early stage of embryonic development increases chick survival, accelerates their development, and strengthens immunity, reducing the need for antibiotic use during rearing. The research that is the subject of this doctoral dissertation assessed the impact of *in ovo* administration of a multistrain probiotic containing effective microorganisms (EM) and zinc glycine chelate (Zn- Gly) on the health status, immune potential, and production parameters of broiler chickens. It hypothesized that the coupled administration of the probiotic and organic zinc at the embryonic stage would contribute to the stimulation of the chicks' immune system, diminishing susceptibility to infections in the first days of life and reducing the mortality rate. *In ovo* supplementation of selected feed additives was also expected to affect the quality of broiler meat and other production parameters, which would translate into economic effects in broiler farming. Furthermore, it was speculated that the supplementation of a probiotic containing Effective Microorganisms (EM) and zinc glycine chelate on the 17th day of incubation would produce a synergistic effect, expressed by strengthened humoral and cellular immunity, increased antioxidant potential of chicks, and intensified metabolic transformation, thereby ultimately contributing to improved production performance.

The study was conducted with a total of 1,400 fertilized eggs of broiler chickens (Ross 308), divided into four groups according to the preparation administered *in ovo*. On day 17 of incubation, the eggs were injected with 500 μL/egg of: Group I (control) – 0.9% NaCl; Group II – probiotic containing Effective Microorganisms (EM); Group III – probiotic containing Effective Microorganisms (EM) + zinc glycine chelate (Zn-Gly); and Group IV – zinc glycine chelate (Zn-Gly). The eggs were incubated until hatching, and chicks were reared until 42 days of age under standard conditions. Hatchability, mortality, and production rates were recorded throughout the experiment, and samples were collected for laboratory analyses on day 0 (12 hours post hatching) and on day 7 of chick life. The study involved phenotypic assessment of T and B lymphocyte subpopulations in blood and spleen using flow cytometry, determination of pro- and anti-inflammatory cytokine levels, determination of immunoglobulins and acute

phase proteins using ELISA and qPCR for Ig mRNA in the ileum, and assessment of the oxidative status in serum and tissues. Muscle fatty acid profiles and basic blood biochemical markers were analyzed as well. In addition, proteomic analysis was performed using SDS-PAGE and MALDI-TOF MS, and zinc concentration was determined in tissues. The analysis and evaluation of the indicated parameters was expected to explain the mechanisms behind the effects of the analyzed supplements on the body at the molecular level.

The combined *in ovo* supplementation with the probiotic (EM) and zinc glycine chelate was well tolerated by the embryos, although it caused a slight decrease in hatchability. The lowest hatchability rate was determined in the probiotic (EM) + zinc glycine chelate group chelate, whereas the probiotic alone had a negligible effect on hatchability. Over the rearing period, the chicks from the probiotic group showed lower feed intake and slower growth, and their final body weights were significantly lower compared to those from the control group. The combined probiotic + zinc glycine chelate supplementation made their final body weights comparable to or higher than those of the control chicks, and improved their feed conversion ratio. The group administered only the zinc glycine chelate achieved results similar to those noted in the control group. The mortality rate of chicks was low and comparable across all groups, proving that the *in ovo* supplementation had no adverse effect on bird survival rate. In turn, it had a tangible influence upon the development of the birds' immunity. A higher percentage of mature T and B lymphocytes in the blood and spleen, compared to the control birds, was demonstrated in the chicks supplemented with the probiotic + zinc glycine chelate as early as at hatching, and this trend persisted until day 7 of life. Furthermore, positive changes in the immune mediator profile were observed in this group, including lower concentrations of proinflammatory cytokines and higher concentrations of anti-inflammatory cytokines, as well as enhanced antibody synthesis in the intestinal mucosa. The study also demonstrated a positive effect of the *in ovo* supplementation with probiotics + zinc glycine chelate on the oxidative homeostasis in chicks. The birds from this group also exhibited a higher antioxidant potential and the lowest MDA index in tissues, which indicates oxidative stress mitigation. However, the chicks administered only the zinc glycine chelate showed signs of oxidative stress in the intestines, despite the overall reduction in MDA compared to the control birds. Analysis of the fatty acid profile in muscles of the chickens from the combined supplementation group demonstrated intensive muscle tissue development. The increased proportion of saturated fatty acids, along with a higher PUFA/SFA ratio and a lower MUFA proportion compared to the control group, were indicative of the improved fat quality indicators. Meat of birds from this group had lower atherogenicity and thrombogenicity indices and a higher hypo- to hypercholesterolemic fatty

acid ratio (h/H). Additional molecular analysis revealed increased expression of proteins related to chick metabolism and immunity, while the assessment of biochemical markers indicated effective zinc absorption.

The study findings enable concluding that the combined *in ovo* supplementation with the probiotic (EM) and Zn-Gly chelate exerts a multifaceted, beneficial effect on broiler growth and development. It enhances the immune system activity already at the embryonic stage, which is reflected in increased percentages of mature T and B lymphocytes and boosted immunoglobulin synthesis, under preserved balance of cellular and humoral responses and simultaneous counteracting excessive inflammatory reactions. Furthermore, the *in ovo* supplementation increases the body's antioxidant potential and reduces the severity of oxidative stress in young chickens. Its beneficial effects are also evidenced by a modified metabolic profile, an improved feed conversion ratio, and a health-promoting lipid profile of meat, expressed by reduced atherogenic and thrombogenic fat indices, which indicates increased dietary value of meat. Zinc glycine chelate administered *in ovo* is a valuable source of this element for the developing embryo, while the probiotic provides beneficial microorganisms that stimulate the immune system. The combined administration of both supplements has a synergistic effect, noticeable in the positive impact on the growth and development of chicks. The application of the *in ovo* technology for probiotic (EM) and zinc glycine chelate administration should be considered a prospective strategy for increasing immunity and improving the health status of broiler chickens from the first days of life. Under production conditions, the effects of the analyzed supplements translate into increased growth rates and feed conversion ratio, as well as improved meat quality, which altogether impact economic viability and indirectly reduce the use of antibiotics in poultry farming.

Keywords: chicken, *in ovo*, chickens' immune system, multi-strain probiotics, Effective Microorganisms (EM), zinc glycine chelate (Zn- Gly)