

ENGLISH ABSTRACT

***In-ovo* stimulation as a tool to improve gut health of broiler chickens.**

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In-ovo stimulation refers to the administration of bioactive substances into eggs during the 12th day of incubation and aims to modulate the gut microbiome and immune system of chickens thus improving their gut health. The effects of this approach largely depends on the bioactive substances used. Therefore, this PhD thesis was conducted to optimize an *in-ovo* stimulation protocol using anti-pathogenic bioactive substances to improve the gut health of broiler chickens. *In-vitro* growth kinetics assays were performed to identify compatible synbiotic (probiotic + prebiotic) and prophybiotic (probiotic + phytobiotic) pairs to be used in this protocol. The most potent anti-pathogenic probiotic was identified by performing anti-*Salmonella* (spot overlay, well diffusion, co-culture and co-aggregation assays) and anti-*Campylobacter* (well diffusion assay) assays. *In-ovo* stimulation of ROSS308 chickens was performed to validate the *in-vivo* effects of the protocol on the gut microbiome (feces and cecal content), expression of immune-related genes (in the cecal mucosa), histomorphology (in ceca) together with various production parameters (hatchability, hatch quality, body weight, feed conversion ratio, carcass and meat quality). The results show that prebiotics selectively promoted the growth of probiotics studied. Turmeric and garlic extracts did not inhibit the growth of all the probiotics studied indicating their broad potential for use in prophybiotic combinations. *Leuconostoc mesenteroides* B/00288 (LM) strain was selected as the most potent anti-pathogenic probiotic based on its overall anti-*Salmonella* and anti-*Campylobacter* activity. Accordingly, *in-ovo* stimulation with 10⁶ CFU/egg LM alone and in combination with 0.5% garlic aqueous extract (the dose at which garlic was non-inhibitory to LM) was performed in ROSS308 broiler chickens. The treatments resulted in beneficial changes on the gut microbiome, gene expression in the cecal mucosa and histomorphology in the ceca. These changes indicated a possible prophylactic effect without compromising the production parameters. In conclusion, this *in-ovo* stimulation protocol can be used as a tool to improve the gut health of broiler chickens.