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Gembloux Agro-Bio Tech - Université de Liège vous prie

de lui faire l'honneur d'assister à la défense publique de la dissertation originale que

Madame SONG Jiao,

Titulaire d'un *master's degree on agricultural extension*,

présentera en vue de l'obtention du grade et du diplôme de

DOCTEUR EN SCIENCES AGRONOMIQUES ET INGENIERIE BIOLOGIQUE,

le 8 septembre 2020, à 13h00 précises, en visioconférence :

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Cette dissertation originale a pour titre :

« Working mechanisms of inulin concerning intestinal mucosal immunity and microbiota in chickens infected with *Salmonella* Enteritidis ».

Le jury est composé comme suit :

Président : Prof. J. BINDELLE, Professeur ordinaire,
Membres : Prof. N. EVERAERT (Promotrice), Prof. J. WEN (Promoteur – CAAS, Chine), Prof. M. SCHROYEN, Prof. D. MARLIER, Prof. J. PAESHUYSE (KU Leuven).

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Abstract

Salmonella, one of the most common foodborne pathogens, is a concern for human health, contaminates poultry and egg products, and causes huge economic losses to the poultry industry. The gut of chicken contains mechanical, immune, chemical, and microbial barriers, and is an important digestive, immune, and endocrine organ. *Salmonella* Enteritidis (SE) damages the intestinal epithelial cells structure, mucosal immunity, and microbial composition. Because of the emergence of antibiotic resistance, the development of dietary alternative strategies to reduce the susceptibility of SE colonization should be a priority. It has been previously concluded that prebiotics, such as inulin, can improve the overall health by providing an intestinal environment that is unfavourable for foodborne pathogens such as Salmonella. The aims of the research described in this thesis are to (1) select an optimal inulin supplementation dosage that optimizes the intestinal mucosal morphology and immune status of specific pathogen-free (SPF) chickens; (2) investigate whether optimal inulin supplementation dosage improve the mechanical, immune, and chemical barriers in JAK–STAT signalling in intestinal mucosal tissue at 1 and 3 days post-infection (dpi) in SE-infected SPF chickens; and (3) investigate whether inulin alter the microbial composition and function of SE-infected SPF chickens.

In the first trial, we determined the optimum level of inulin supplementation for effectiveness in the gut. The mucosal morphology and immune indexes of SPF chickens that fed a basal diet supplemented with 0, 0.25, 0.5, 1.0, or 2.0% inulin were analyzed on days 7, 14, and 21, respectively. The results showed that the SCFA concentrations in the cecum and serum were increased by dietary inulin supplementation at 21 d. The mucosal morphology was improved in the duodenum and ileum of SPF chickens fed inulin. Also, inulin at a low concentration (0.25% or 0.5%) significantly decreased the gene expression of NF-κB and LITAF at 7, 14, and 21 d, and of IL-6 and iNOS at 7 and 14 d; and increased that of MUC2 and claudin-1 in the ileum of SPF chickens at 7, 14, and 21 d. The results indicated that the effects of inulin on mucosal immune function occurred in a dose-dependent manner. A low inulin concentration (0.25% or 0.5%) may be beneficial in promoting intestinal immune function.

In the second trial, we investigated whether and how inulin administration influenced the intestinal barrier function and mucosal immunity with SE infection. A control group (CON) chicken fed a basal diet and three SE-infected groups fed a basal diet supplemented with inulin 0% (SE), 0.5% (0.5% InSE), and 1% (1% InSE), respectively. Until 28 d of addition, chickens in the four groups were orally infected with SE or vehicle (phosphate-buffered saline). The SE challenge significantly increased the mucosal gene expression of proinflammatory cytokines and serum concentrations of antibodies, but significantly decreased the gene expression of MUC2 and claudin-1 compared with the CON group. However, Inulin supplementation reversed these gene expression and relieved the injury of duodenum, jejunum, and ileum at 1 and 3 dpi within the SE-challenged groups. The SE challenge significantly increased ileal protein expression of p-STAT3 and JAK1 at 3 dpi compared with the CON group, while inulin supplementation suppressed p-STAT3 and JAK1 protein expression at 3 dpi compared with the SE group. The results showed that inulin alleviated SE-induced gut injury by decreasing the pro-inflammatory response and enhancing mucosal immunity in chickens.

In the third trial, the impact of inulin on SCFAs, microbial composition, and microbiome functions of SPF chickens infected with SE were investigated. The SE infection led to significantly decreased cecal butyrate concentrations compared with the CON group, and inulin supplementation reversed these changes. SE infection significantly decreased the alpha diversity, while inulin supplementation significantly increased the alpha diversity and richness. At the phylum level, Inulin addition at 1% increased abundance of Firmicutes and decreased abundance of Bacteroidetes compared with the SE group according to the Student's t-test. The correlations of the microbiota genera with metabolite SCFA contents showed that SCFA-producing bacteria had positive correlations with acetate and butyrate contents. Analysis of the functional capacity in cecal microbiome at pathway level 2 showed that inulin supplementation at 0.5% and 1% reversed the abundance of KEGG pathways and KOs in SE infected chickens. In short, SE infection decreased the alpha diversity of cecum microbiota and SCFA contents, and accordingly increased the abundance of KEGG pathways and KOs related to nutritional and energy metabolism, whereas inulin addition reversed these changes and had inhibitory effects on SE infection.

The results of the above three experiments indicate that SE caused the rapid induction of pro-inflammatory cytokines and changes in gut morphology, gut microbiome, microbial functional activities, and SCFA production, which resulted in activation and normalization of the immune system in the bird's early life. However, the intestinal parameters and SCFA concentration were altered by inulin in a dose-dependent manner. The 0.5% inulin relieved the intestinal inflammatory environment and injury induced by SE infection through the JAK–STAT signaling pathway and the changes of SCFA-producing bacteria composition and function. As a whole, these observations may lead to the development of novel prevention and control strategies of Gram-positive bacterial infections with prebiotic administration in chickens and may even be applicable to other animal species. Moreover, determining the role played by inulin in gut microbiota-driven pathways using metagenomics approaches further elucidated how prebiotics contribute to the gut microbiota combating pathogen infection.