LIÈGE université
Gembloux
Agro-Bio Tech

Le Corps professoral de

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

Monsieur LI Peng,

Titulaire d'un master's degree of agronomy majoring in preventive veterinary science,

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

DOCTEUR EN SCIENCES AGRONOMIQUES ET INGENIERIE BIOLOGIQUE,

le 14 septembre 2018, à 16 heures précises (personne ne sera admis après cette heure),

en l'auditorium ZT1 (Zootechnie, bât. 1),

Passage des Déportés, 2, à 5030 GEMBLOUX.

Cette dissertation originale a pour titre :

« Effects of MicroRNA and fructooligosaccharide on immunomodulatory to *Salmonella Enteritidis* Infection in young chicken ».

Le jury est composé comme suit :

Président : Prof. J. BOGAERT, Professeur ordinaire,
Membres : Prof. N. EVERAERT (Promoteur), Prof. J. WEN (Copromoteur - CAAS, Chine), Prof. L. WILLEMS, Prof. Y. BECKERS, Prof. J. PAESHUYSE (KU Leuven).

UNIVERSITE DE LIEGE – GEMBLOUX AGRO-BIO TECH – SERVICE INSCRIPTIONS ET APPARITORAT
Passage des Déportés, 2 - 5030 Gembloux - Belgique
Téléphone +32(0)81 62 23 45 - Fax +32(0)81 62 21 68 - www.gembloux.ulg.ac.be

Summary

As a foodborne disease, *Salmonella* Enteritidis (SE) not only causes huge economic losses to the poultry industry, but also seriously threatens human public health. Poultry are considered to be important sources and carriers of the disease. An improved understanding of host immunological resistance and response mechanisms in chickens should be a top priority. The aim of research described in this thesis was divided into two parts: (1) To identify the splenic microRNAs and mRNAs that were differentially expressed following infection of chickens with SE using RNA sequencing; (2) to investigate whether fructooligosaccharides (FOS) addition alters the expression of inflammatory genes involved in MyD88-dependent signaling in immune tissues during *Salmonella* infection. To achieve these objectives, the research strategy involved construction of an improved disease model, transcriptome screening, identification of resistance genes and pathways, and evaluating the effect of FOS addition, both by dietary supplementation and by exposure of chicken immune cells *in vitro*.

In the first part, differentially expressed microRNAs and gene transcripts (mRNAs), as well as signaling pathways were investigated in resistant (R, SE challenged-slight clinical symptoms and $< 10^5$ cfu SE / 10 µL blood), susceptible (S, SE challenged-severe clinical symptoms and $> 10^7$ cfu SE / 10 µL blood) and control birds (C, non-challenged, no SE in blood) using the splenic microRNAome and transcriptome. A total of 934 significant differentially expressed (DE) genes and 32 DE miRNAs were identified in comparisons among the C, R and S birds. There was evidence of cross-talk among these pathways, perhaps contributing to susceptibility to *Salmonella* infection, including the FoxO signaling pathway, cytokine-cytokine receptor interaction and Jak-STAT signaling pathway. Importantly, TLR4 signaling was also significantly enriched among C, R and S birds. In addition, two DE miRNAs, gga-miR-101-3p and gga-miR-155, were identified as candidates being potentially associated with SE infection.

The second part of the study investigated whether provision of FOS altered the expression of inflammatory genes involved in TLR4 signaling during *Salmonella* infection. Three days post-hatch, birds from two treatment groups (diets with or without 1% FOS, which is confirmed as the optimum level of adding dietary FOS for effective protection against *Salmonella* infection in this study) were also orally challenged with SE or vehicle PBS. Dietary FOS significantly reduced the gene expression of pro-inflammatory cytokines *IL-6* and *TNF-α*, as well as the transcript abundance of inflammation-related pathway genes *TLR4*, *MyD88*, *TRAF6* and *NF-κB* in spleen and in cecal tonsils during *S.* Enteritidis infection in young chickens. Using HD11 chicken macrophages *in vitro*, exposure to FOS directly increased the expression of *IL-6* and *TNF-α* and reduced the extent of increase in abundance of pro-inflammatory factors, otherwise provoked by added LPS. Taken together, these findings provide novel information that FOS may reduce production of the pro-inflammatory cytokines through TLR4-MyD88-dependent signaling during the early stages after *Salmonella* infection. It is emphasized that further research of this direct immunomodulatory role of FOS on TLR4 signaling is warranted.

In conclusion, this research with chicks has systematically exposed novel information on the immune mechanism of the host in providing some protection against *Salmonella* by use of a high-throughput sequencing combined with an improved experimental design strategy. Several important signaling pathways and miRNAs have been identified and will be the focus of future research. In addition, evidence for FOS having a direct regulatory influence on innate immunity in chickens was obtained. These mechanistic findings will help facilitate the understanding of resistance and susceptibility to *Salmonella* infection in the earliest phases of the host immune response, they will provide new approaches for developing strategies for *Salmonella* prevention and treatment, and they may aid in enhancing innate resistance using genetic selection.