BREED EFFECT ON TOLL-LIKE RECEPTOR 2 (TLR2) GENE EXPRESSION LEVEL

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ABSTRACT

Toll-like receptors (TLRs) are a family of transmembrane proteins which belong to the larger group of receptors, called Pathogen-recognizing receptors (PRRs), found in animal cells. They are responsible for the recognition of highly conserved products of microbial metabolism called PAMPs (Pathogen-associated molecular patterns), expressed by pathogens commonly invading the host like: lipopolysaccharide (LPS), lipoprotein, flagellin or double stranded RNA. Signaling through TLRs lead to a widespread immune induction of the cellular components of the innate and adaptive immune systems. The TLR 2 gene expression level was done by using a quantitative Real-Time PCR. The assessments of TLRs gene expression level between breeds (White Leghorn & Green-Legged Partridgelike) and organs (lung, ovary, liver, thymus, duodenum, spleen, large intestine), will let us better understand the innate immune mechanisms in which Toll-like receptors play the key role.

Keywords: Toll-Like Receptor 2, immunity, chicken, breed, White Leghorn, Green-Legged Partridgelike, safe food.

INTRODUCTION

During the first few days of their life chickens are highly susceptible to opportunistic pathogens that are common in the environment of poultry facilities. This observation has generally been attributed to an immaturity of the immune system (**Lowenthal et. al., 1994**), also due to their embryonic development, which takes place outside the organism of the parent (**Ruminska et. al., 2008**).

Immunity system is divided in two main parts: the innate and the adaptive immunity. The skin, mucous membranes and innate immune system are the innate defenses against the diseases. This part is immediately available in case of pathogen invasion and infection (macrophages are the first line of defense). The lymphocytes T, B and immunological memory are the weapon of adaptive immunity responses. Adaptive immunity responses in chickens develop already during embryogenesis. First of all transport of specific antibody IgY from the hen's serum to the offspring occurs. Next the IgY is transported from the serum to the egg yolk, in analogy to the crossplacental transfer of antibodies in mammals (**Hamal et.al., 2006**). There is a three-phase-process of maturation and differentiation of B lymphocytes in the bursa Fabricius, during the embryogenesis. After hatching, bursal follicles consist of B lymphocytes (85-95%) and approximately 4% of T lymphocytes. B lymphocytes are able to generate a wide scale of antibodies of three classes: IgM, IgY and IgA. The thymus gland determines the micro-environment for differentiating T lymphocytes. T lymphocytes colonize the germ of this organ in the form of precursor cells from the marrow during embryonic development. The migration of diverse T cells from the thymus gland to the circuit lasts several weeks after hatching. Although adaptive immunity responses in chickens develop already during embryogenesis, they need to be challenged by invading pathogen to get fully mature and be able to work.

The important part of the innate immunity system are the pattern recognition receptors (PRRs). They are responsible for the recognition of highly conserved products of microbial metabolism called PAMPs (Pathogen-associated molecular patterns), expressed by pathogens commonly invading the host like: lipopolysaccharide (LPS), lipoprotein, flagellin or double stranded RNA (**Akira et al., 2006**). In this manuscript, especially one group of PRRs - the Toll-like Receptors, will be studied. Signaling through TLRs lead to widespread immune induction of the cellular components of the innate and adaptive immune systems as well as directing the host response into particular differentiation pathways (**Iqbal et al., 2005**). The assessments of TLRs gene expression level between breeds and organs, will let us better understand the innate immune mechanisms in which Toll-Like Receptors play the key role. In this manuscript the TLR 2, will be studied. It recognizes the lipoproteins and glycolipids of Gram negative and Gram positive bacteria. The aim of presented study was to estimate the expression of chicken TLR 2 gene in choosen organs: lung, ovary, liver, thymus, duodenum, spleen, large intestine of two chicken breeds: White Leghorn and Green-legged Partridgelike.

MATERIALS & METHODS

Two chicken breeds selected for this experiment differ for their genetic background and phenotypic performances. The White Leghorn is a typical, commercial laying hen, selected for breeding traits and the Green-legged Partridgelike is known by their resistance to the severe climatic conditions, excellent hatching, rearing abilities as well as of their disposition for good utilization of pasture (**Cywa-Benko, 2002; Witkowski et.al, 2009**). All birds were kept in a litter in the same environmental conditions, with ad libitum feed and water access.

From four-week old chickens the organs were collected, then immediately frozen in liquid nitrogen and stored in -80 ° C.

The first step of this experiment was the extraction of the total cellular RNA, followed by the reverse transcription reaction to obtain complementary DNA (cDNA) on the base of mRNA. RNA was isolated using a TRI Reagent (Ambion), according to manufacturer's instruction. The cDNA was obtained by using Reverse Transcriptation Kit (Applied Biosystem N8080234), according to manufacturer's instruction. A quantitative analysis of the nucleic acids was conducted with the spectrophotometer (Eppendorf Biophotometer AG 22331) and gel electrophoresis.

To assess the expression profile of TLR 2, the quantitative Real-Time PCR (qRT-PCR) method was used. This method enables monitoring the amount of the increasing amount of DNA (**Klein, 2002; Valasek and Repa, 2005**) due to fluorescence-labeled probe. The experiment was done using the Real-Time PCR 7700 Applied Biosystem. On a base of sequence available in Gene Bank (TLR 2 gene ID: 769014, NCBI), the set of primers was designed in Primer Express 3.0 programme (Applied Biosystems). Forward primer: ACTGCCTGCAACGGTCAT, the reverse primer:CATCAGCTTCATTGTTGGTTTCTGT and the probe: CTCAGCTACACCAAAATG.

RESULTS

The statistical analysis of TLR 2 gene expression comparison, didn't show significant difference ($P<0.05$) between breeds in investigated organs: lung, ovary, liver, duodenum, spleen, thymus and large intestine (Tab.1). Each organ was tested individually.

Organ	Variance	T test
Lung	0.911	0.633
Ovary	0.866	0.274
Liver	0.127	0.183
Thymus	0.714	0.575
Duodenum	0.030	0.714
Spleen	0.099	0.190
Large intestine	0.202	0.140

Tab.1: The statistical analysis of TLR 2 gene expression comparison between breeds in single organ, by using STATISTICA programme and Student's T test.

DISCUSSION

The results of the newest research show that differences in the sequence of TLRs genes between breeds occur (**Laveque et al., 2003; Dhinakar Raj et al., 2009**). Authors report also different expression levels of TLR genes within different organs and breeds (**Iqbal et al., 2005; Yilmaz et al, 2005; MacKinnon et al., 2009; Dhinakar Raj et al, 2009**). However other authors claim that there is no significant difference between lines or breeds. According to **Nerren et al., 2009** there is no statistically significant difference in the mRNA expression level of TLR4 and TLR5 between resistant and susceptible commercial chicken lines. The same authors have found a difference in mRNA expression level in TLR 15. In the same year, **Redmond et al.,** reported that TLR 4 gene showed similar level of expression across different genetic lines: broiler, Fayoumi and Leghorn.

The lack of statistically significant difference in TLR2 gene expression between breeds under the study might be explained by the genetic background or/and the environmental conditions which could affect the immunity system. It might be mentioned here that immunity responses could be stimulated by applying probiotics or pathogens (or their parts).

According to **Voltan et al.**, 2007 the oral supplementation with *L. crispatus* M247 in mice, modified the TLRs mRNA and protein levels in the intestinal mucosa and epithelial cells. The level of TLR4 was drastically reduced, whereas TLR2 was up-regulated. Therefore, probiotics like *L. crispatus* M247 and *L. casei*, which increase the level of mucosal TLR2 expression over that of TLR4, might establish a higher level of mucosal sensitivity to commensally nonpathogenic gram-positive bacteria, such as lactic acid bacteria, bifidibacteria, and Enterococcus spp., (**Kelly et al., 2005**). Probiotics have also been found to exert other functions, such as maintenance of health and promotion of growth, in chickens (**Khan et al., 2007**).

It is proved that chickens challenged with bacteria the gene expression profile of TLRs changes. Genetic line effect was significant ($P < 0.05$) for TLR 2, TLR 4 and TLR 5 mRNA expression in the spleen of *Salmonella Enteritidis*-infected birds. The Fayoumi line had higher TLR2 and TLR4 expression than Leghorn, higher TLR2 mRNA expression than broiler, and the broiler line had higher TLR5 expression than Leghorn and Fayoumi (**Abasht et al., 2009**).

To find a definite answer on the bases of differences in TLR genes expression level between breeds or organs the further investigations should be done including genes sequence comparison.

The subject of this research is in top priority for in European Union research projects, what is confirmed in the guidelines of European Technology Platform (ETPs final raport, 2008). the Sustainable Farm Animal Breeding and Reproduction Technology Platform (FABRE TP) is one of the technology platforms created within the EU. (**FABRE TP, 2008**). EU shows a professional interest in producing safe and healthy food; in breeding robust, adapted, healthy animals; in diversity and distinctiveness, genomics, genetics, and reproduction. The main goal of the modern research is focused on decreasing risk of food-borne zoonoses, the use of antibiotics and feed additives and also reduction of the level of pollutants in animals. There is also a strong need to deliver more sustainable breeding strategies that meat consumers demand for safe, high quality and sustainable food production. To obtain such a product we should search for breeds/lines which carry in their genotype a good parameters of innate immunity (**Brzezinska, 2009**).

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