

EXPRESSION PROFILE OF TLR7 GENE IN LYMPHOID TISSUES OF THE NIGERIAN INDIGENOUS AND ISA BROWN COMMERCIAL LAYER CHICKENS

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ABSTRACT

This work was designed to evaluate the expression profile of toll-like receptor 7 (TLR7) gene in the liver and thymus tissues from the Nigerian indigenous chickens and ISA Brown commercial layer chicken. The study population comprises naked neck, normal and frizzled-feathered chickens in rain forest and Guinea Savannah regions respectively; Fulani ecotype chicken from Guinea Savannah region, and ISA brown (exotic) commercial layer chicken. Tissues from the thymus and the liver were aseptically collected from two (2) clinically healthy chickens from each of the eight (8) groups and were immediately transferred into separate 1.5 ml Eppendorf tubes containing 1 ml of RNALater solution. Total RNA was isolated using ISOLATE II RNA Mini kit (BIOLINE, USA). Complementary DNA (cDNA) was synthesized using SensiFAST™ cDNA synthesis kit (BIOLINE, USA), following manufacturer's instructions. The expression of chTLR7 RNA was determined by qPCR assay; β -actin was used as the reference gene. Constitutive expressions of TLR7 in the liver and thymus were significantly different ($P < 0.01$) among the eight chicken groups; the Nigerian indigenous chickens expressed more TLR7 gene than ISA brown commercial layer chicken. Rain forest naked neck chicken had significantly ($P < 0.01$) the highest TLR7 expression of 2.07 ± 0.07 fold. However, expression of TLR7 gene in the liver of rain forest frizzle-feathered and normal chicken, Guinea Savannah naked neck, frizzle-feathered and normal chicken, and Fulani ecotype chicken were similar ($P > 0.05$). Gene expression analysis of TLR7 mRNA suggests that the Nigerian indigenous chicken genotypes could have similar antiviral response, but comparatively more antiviral immune response than ISA brown (exotic) commercial layer chicken.

Keywords: Chicken, expression, immune response, Toll-like receptor, lymphoid tissues.

INTRODUCTION

The toll-like receptor 7 (TLR7) is an immunity related gene. It is a member of the multi-gene family, which perform a vital role in the innate immune system of their host organism through the recognition of foreign invaders called pathogens. The influence of breed, age, sex and rearing system of birds on constitutive expression of immunity related genes has been reported (Abasht *et al.*, 2009; Ramasamy *et al.*, 2010; Gautham *et al.*, 2013; Annamalai *et al.*, 2014; Kolluri *et al.*, 2014; Xiong *et al.*, 2015). However, there is dearth of information on the effect of chicken genotype and ecotype on the expression profile of chTLR7 gene in the Nigerian indigenous chicken populations and ISA brown commercial layer chicken. We hypothesized that Nigerian indigenous chicken genotypes found in different ecological locations could vary in the expression of TLR7 gene in their immunity related tissues such as the liver and the thymus as compared to ISA brown commercial layer chicken. This could be as a result of adaptation of the Nigerian indigenous chickens to their tropical ecological environment.

This research, therefore, was designed to evaluate the expression pattern of TLR7 gene in the liver and thymus tissues from the Nigerian indigenous chicken populations found in different ecological locations, and ISA brown (exotic) commercial layer chicken.

MATERIALS AND METHODS

Tissue collection, RNA extraction and Real-Time Polymerase Chain Reaction (qPCR)

Two (2) clinically healthy chickens from each of the eight (8) genetic groups were slaughtered for tissue collection. The thymus and the liver were aseptically collected from each bird and were

immediately transferred into separate 1.5 ml Eppendorf tubes containing 1 ml of RNALater solution. The two chickens from each genetic group served as biological replicates. Total RNA was extracted from the liver and the thymus using ISOLATE II RNA Mini kit (BIOLINE, USA). Complementary DNA (cDNA) was synthesized using SensiFAST™ cDNA synthesis kit (BIOLINE, USA), following manufacturer's instructions. The expression pattern of chTLR7 RNA in the liver and thymus were determined by qPCR assay. The EVA Green dye chemistry was used in detection. The PCR primers were designed using Primer3 and blast option at the NCBI website. The primers used for qPCR were TLR7-F: CCTCGATCTCAACCCTACTTCT, and TLR7-R: CAGTATCTTTTCCTCACCACACA. House-keeping gene β -actin was used as the reference gene. Real-Time PCR reactions were performed in triplicates in 25 μ l reaction volume comprising 5 μ l PCR Master mix (FIREPol^R, Solis BioDyne, Estonia), 0.6 μ l each of forward and reverse primers, 4 μ l of template cDNA and 14.8 μ l of nuclease-free water in a smart cycler tube. A 25 μ l non-template reaction mixture was also run alongside as a negative control. The qPCR conditions were as follows: 40 cycles each of initial denaturation at 95°C for 15 minutes; denaturation at 95°C for 15 s; annealing at 65°C for 20 s and extension at 72°C for 20 s.

Assembling of qPCR data and statistical analysis

Data on qPCR of chTLR RNA were assembled using Microsoft Excel (2010). The average C_T values of technical replicates and change in C_T values ($\Delta C_T = C_T$ target gene – C_T reference gene) were also inferred using Microsoft Excel (2010). Fold changes in expression levels of TLR7 gene in the Nigerian indigenous chickens were calculated relative samples of ISA brown layer chickens. Fold changes ($2^{-\Delta\Delta C_T}$) in gene expression were determined using the comparative $\Delta\Delta C_T$ method as described by Livak and Schmittgen (2001). Data on fold changes in gene expression were subjected to One-way Analysis of Variance (ANOVA) and means with significant differences were separated using Duncan's multiple range tests. ANOVA and mean separation were performed using Statistical Package for the Social Sciences (SPSS) software version 21.0.

RESULTS AND DISCUSSION

The constitutive expressions of TLR7 mRNA in the liver and thymus tissues of the Nigerian indigenous chickens relative to ISA brown commercial layer chickens are shown in figures 1 and 2 respectively. TLR7 expression in the liver was significantly different ($P < 0.01$) between the eight chicken genetic groups. Rain forest naked neck chicken (RN) had significantly ($P < 0.01$) the highest TLR7 expression of 2.07 ± 0.07 fold. However, expression of TLR7 gene in the liver of rain forest frizzle-feathered chicken (RF) 1.34 ± 0.02 fold, rain forest normal chicken (Rn) 1.47 ± 0.05 fold, Guinea savannah naked neck chicken (GN) 1.39 ± 0.02 fold, Guinea savannah frizzle-feathered chicken (GF) 1.49 ± 0.03 fold, Guinea savannah normal chicken (Gn) 1.37 ± 0.05 fold and Fulani ecotype chicken (FE) 1.52 ± 0.05 fold were similar ($P > 0.05$), but higher than that of ISA brown commercial layer chicken, 1.01 ± 0.14 fold.

The expression of TLR7 gene in thymus was significantly different ($P < 0.01$) between the eight chicken genetic groups. TLR7 gene expression in thymus of RN (2.18 ± 0.04 fold), Rn (2.14 ± 0.07 fold), GN (2.30 ± 0.08 fold) and Gn (2.34 ± 0.04 fold) were similar but significantly ($P < 0.01$) higher than that of RF (1.39 ± 0.02 fold), FE (1.30 ± 0.02 fold) and GF (1.13 ± 0.02 fold). The expression of TLR7 gene in thymus of GF chicken was not significantly different ($P > 0.01$) from that of ISA brown layer chicken (1.01 ± 0.10 fold).

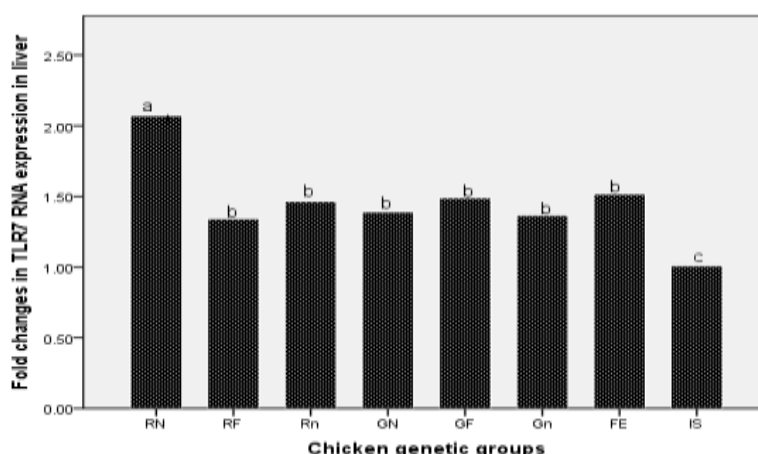


Figure 1: Expression profile of TLR7 mRNA in liver

*RN= Rain forest naked neck chicken, RF= Rain forest frizzle-feathered chicken, Rn= Rain forest normal chicken, GN= Guinea savannah naked neck, GF= Guinea savannah frizzle-feathered chicken, Gn= Guinea savannah normal chicken, FE= Fulani ecotype chicken, IS= ISA brown layer chicken

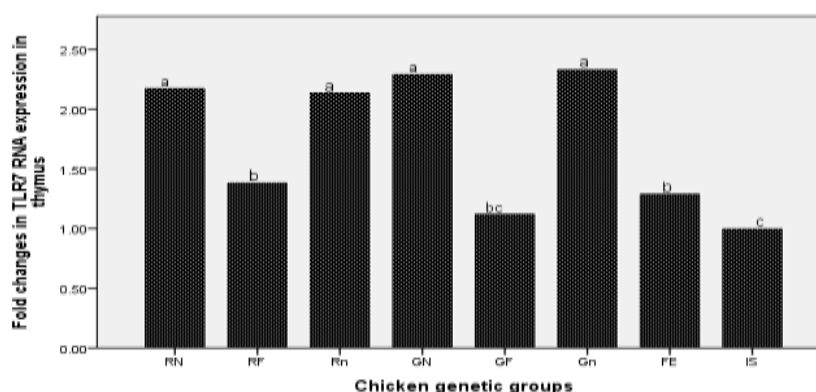


Figure 2: Expression profile of TLR7 mRNA in thymus tissues

It has been reported that constitutive expressions of immunity related genes vary with different breeds and could be responsible for differences in disease resistance reported among chicken breeds (Ruan *et al.*, 2012a, 2012b; Annamalai *et al.*, 2014). Constitutive expression of chTLR7 plays a vital role in the innate immune response of chickens through detection of invading viral pathogens. Our comparative analysis of TLR7 gene expression in the liver and the thymus of the Nigerian indigenous chickens and ISA brown commercial layer chicken showed that the Nigerian indigenous chickens expressed higher TLR7 mRNA than ISA brown commercial layer chickens in the lymphoid organs studied. This suggests that the indigenous chickens could have comparatively more antiviral immune response than ISA brown commercial layer chickens. This observation is an indication of breed differences in TLR7 gene expression, which has been earlier reported (Abasht *et al.*, 2009; Ramasamy *et al.*, 2010).

CONCLUSION AND RECOMMENDATION

Gene expression analysis of TLR7 mRNA suggests that the Nigerian indigenous chickens could have comparatively more antiviral immune response than ISA brown commercial layer chicken. Therefore, the Nigerian indigenous chickens and ISA brown commercial layer chicken should be challenged with viral pathogen in order to evaluate their response to viral disease.

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