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Differential Expression of IL-6 and IGF-II in Guinea Fowl and Chicken

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Abstract: Differential expression of IL-6 and IGF-II genes were studied in guinea fowl and broiler chicken using semi-quantitative analysis. A 219 bp fragment of IL-6 and 215 bp fragment of IGF-II were amplified in guinea fowl and broiler chicken using chicken specific primers. Semi-quantitative analysis revealed the adjusted Integral Density of 0.853 and 0.051 for IL-6 band in guinea fowl and broiler chicken respectively, revealing 16.62 fold higher IL-6 mRNA expression in LPS induced PBMCs from guinea fowl as compared to that from broiler. However, adjusted Integral Density of IGF-II band was 0.082 and 1.106 for IGF-II band in guinea fowl and broiler chicken respectively, which revealed 13.43 fold increase in IGF-II mRNA expression in LPS induced PBMCs in broiler chicken as compared to that in guinea fowl. Hence, guinea fowl showed higher expression of pro-inflammatory cytokine (IL-6) and lower expression of IGF-II in comparison to broiler chicken. These findings were as per expectation in view of much higher immuno-competence and lower growth rate in guinea fowl in comparison to chicken.

Key words: Differential expression, IL-6, IGF-II, guinea fowl

INTRODUCTION

Chicken has undergone under tremendous changes at genetic level through intense selection for improving production potential and as a result of the rigorous selective breeding, the improved egg type chicken lays about 330 eggs/year (~ 1 egg/day) and meat type chicken attain about 1.5 kg in one month time. Selection for production traits results in the loss of many rare alleles influencing livability, which may reduce the ability to encounter the infections (Muir *et al*., 2008). Selection to production traits in chickens has been shown to have negative effects on parameters of immune response (Lamont *et al*., 2003; Cheng and Lamont, 2008). It calls the need of exploring the factors influencing disease resistance as well as production traits simultaneously, not in isolation. Such strategies require suitable models. Use of diverse poultry species for such studies may be an alternate to the existing models like divergent lines within same species. Guinea fowl is an important poultry species and have the resistance to most of common chicken diseases (Aitken *et al*., 1977).

Among the factors influencing disease resistance, cytokines are important immune modulator as they are essential effector molecules of innate and acquired immunity that initiate and coordinate cellular and humoral responses aimed at eradicating pathogens. In the midst of different cytokines, Interleukin-6 (IL-6) is a multi functional Pro-inflammatory cytokine that plays a major role in regulating immune responses, acute phase reactions and haematopoiesis. IL-6 is produced by many different cell types and acts on B cells, T cells, hepatocytes, haematopoietic progenitor cells and cells of the central nervous system (Schneider *et al*., 2001). An increase in IL-6 mRNA expression is associated with increased resistance to *Salmonella enteritidis* (SE) infection in chicken (Ferro *et al*., 2004; Swaggerty *et al*., 2004).

Amongst the genes influencing growth, insulin like growth factor-I (IGF-I) and insulin like growth factor-II (IGF-II) have been demonstrated as an indicator of growth rate in chicken by several authors (Jones and Clemmons, 1995; Beccavin *et al*., 2001). The IGF-I has been well studied and showed consistent association with growth traits (Kajimoto and Rotwein, 1989; Kikuchi *et al*., 1991; Beccavin *et al*., 2001; Duclos, 2005), while the IGF-II is not explored so well, however it also showed positive association with growth (Beccavin *et al*., 2001). Hence we have made an attempt to study the differential expression of IL-6 and IGF-II in guinea fowl and chicken.

MATERIALS AND METHODS

Experimental populations: The chicken and Guinea fowl populations maintained at the Central Avian research institute, Izatnagar were used. The chicken population was white-plumaged Synthetic Broiler Dam Line (SDL), which has been undergone long term selection for economic traits, mainly, juvenile body weight and egg number. The guinea fowl population was an indigenous population, which has not undergone artificial selection. Both the populations were reared under scientific

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feeding and managemental conditions. Six birds (8 weeks of age) were randomly selected from each population for differential expression analysis.

Peripheral blood mononuclear cells (PBMCs) isolation and culture: Three millilitres of heparinized venous blood was obtained from each bird under experimental conditions, Blood is layered on 3 ml Histopaque-1077[3] and centrifuged at 800 x g for 30 min. Mononuclear cells at the interphase were collected and washed three times with RPMI 1640 medium[3]. Viability of these cells was assessed using trypan blue staining thereafter cells were counted in a hemocytometer and the concentration adjusted to 2 x 10^6 cells per ml in RPMI-1640 medium. Equal concentration of cells were then dispensed in six-well tissue-culture plates[4] in triplicate containing RPMI 1640 medium supplemented with 10% fetal calf serum, 2 mM L-glutamine, 2 mM L-arginine, penicillin (1000 IU/ml) and incubated for 4 h in 5% CO_2 tension at 41°C in a humidified chamber. Lipopolysaccharide from *Salmonella enteritidis*[3] was added (2 mg/ml of medium) to each well for induction.

Total RNA isolation: Induced PBMCs were harvested and total RNA was extracted from harvested cells by the trizol reagent[5], according to the manufacturer's instructions. Concentration and purity of RNA preparations were determined spectrophotometrically and integrity was confirmed by agarose gel electrophoresis. Possible traces of genomic DNA were removed by RNase-free DNase[6] according to the manufacturer's instructions.

Semi-quantitative RT-PCR: Dnase-treated total RNA sample were reverse-transcribed using the RevertAid First strand cDNA synthesis kit[6], according to the manufacturer's instructions. The resultant cDNA was stored frozen at -20°C until further use. Duplicate parallel PCR reactions were performed in a thermal cycler[7] on equal aliquots of cDNA from both groups, in separate tubes, for the amplification of IL-6, IGF-II and β-actin genes using respective specific primers (Table 1). The PCR was performed in a total volume of 25 µl containing 2 µl cDNA, 10 mM Tris-HCl (pH 8.8), 50 mM KCl, 2.5 mM $MgCl_2$, 2.5 mM deoxynucleotide triphosphate and 2 Unit Taq DNA polymerase[8] and 10 Pico mole of each forward and reverse primer (Table 1). Amplification conditions were: 94°C for 5 min and 35 cycles of 30 s at 94°C, 30s at 59°C and 30s at 72°C and final extension of 5 min at 72°C. The PCR products were analyzed on 2.0% agarose gel followed by ethidium bromide staining (2.0 µg/ml gel solution) and visualized under ultraviolet light and photographed. PCR product sizes were verified by comparison with a 20-bp DNA ladder[9].

To regulate efficiency variations in the RT step among different experimental samples, β-actin mRNA expressions (a 'house-keeping' gene, presumed to be expressed in constant amounts) were also calculated, along with IL-6 and IGF-II mRNA expression by densitometry analysis using imageJ software[10]. Adjusted integrated densities were determined as arbitrary units, defined as the ratio of integrated densities of specific gene to that of corresponding β-actin after subtraction of background intensity [value = (intensity; gene of interest - intensity; background)/ (intensity; β-actin - intensity; background)]. Mean values of three measurements of each bird were taken for analysis. The fold changes in mRNA expression were calculated keeping one population as control. All results were presented as the mean (±SE, n = 6) and analyzed by ANOVA/t-test as appropriate using SPSS 10.0 software.

RESULTS AND DISCUSSION

IL 6 mRNA expression: Presence of a single band of approximately 219 bp in all lanes (Fig. 1) suggesting the specific amplification of IL6 fragment in both the populations. Intensity of the 219 bp IL-6 fragment in guinea fowl was much higher as compared to that in broiler chicken. Semi-quantitative analysis by using ImageJ software revealed that the adjusted Integral Density of IL-6 band was 0.853 and 0.051 in guinea fowl and broiler chicken respectively (Fig. 2). Semi-quantitative analysis based on adjusted Integral Density revealed that the IL-6 mRNA expression was 16.62 fold higher in LPS induced PBMCs in guinea fowl as compared to that in broiler chicken (Fig. 3). These results are in alignment with the general consensus that the guinea fowl is more resistant to diseases as compared to chicken (Aitken *et al*., 1977). Several workers have reported higher expression of the pro-inflammatory cytokines including IL-6 in the chicken resistant to *Salmonella enteritidis* (SE) infection in comparison to the susceptible ones (Kogut *et al*., 1995;

Table 1: Details of the primers used

Gene	Type	Sequence (5'-3')	Product size (bp)
IGF-II	Forward	GGCGGCAGGCACCATCA	215
	Reverse	CCCGGCAGCAAAAAGTTCAAG	
IL-6	Forward	GAAATCCCTCCTCGCCAATCTGA	219
	Reverse	TGAAACGGAACAACACTGCCATCT	
β-actin	Forward	CAGGGTGTGATGGTTGGTATGG	273
	Reverse	GGCTGGGGTGTTGAAGGTCTC	

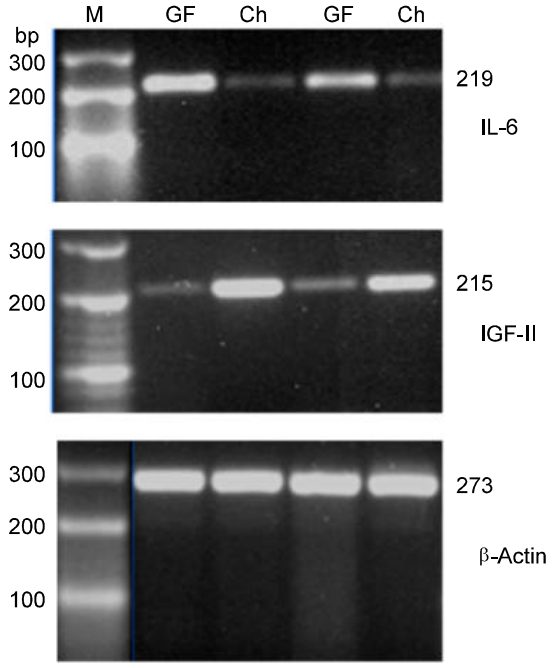

Fig. 1: Resolution of amplified 219 bp IL-6 fragment, 215 bp IGF-II fragment and 273 bp β-Actin fragment in Guinea Fowl (GF) and broiler Chicken (Ch). M: 20-bp DNA ladder, GeneRular; Fermentas

Swaggerty *et al*., 2004; Chiang *et al*., 2008). Very recently, Redmond *et al*. (2009) measured mRNA expression of IL-6 in more diverse chicken lines i.e. commercially selected (broiler, Leghorn) as well as native chicken (Fayoumi) and found that heterophils stimulation with SE *in vitro*, increased the expression of IL-6 in the Fayoumi line, while the broiler and Leghorn line heterophils had decreased or no changes in the cytokine gene expression levels. The unique response of the Fayoumi line is in contrast to the lines with a history of genetic selection to increase growth or reproduction, a process which may favor reduced or suppressed inflammatory responses.

IGF-II mRNA expression: Presence of a single band of approximately 215 bp in all lanes (Fig. 1) suggesting the specific amplification of IGF-II fragment in both the populations. Intensity of the 215 bp IGF-II fragment in broiler chicken was much higher as compared to that in guinea fowl. Semi-quantitative analysis by using ImageJ software revealed that the adjusted Integral Density of IGF-II band was 0.082 and 1.106 in guinea fowl and broiler chicken respectively (Fig. 2). Semi-quantitative analysis based on adjusted Integral Density revealed that the IGF-II mRNA expression was 13.43 fold higher in LPS induced PBMCs in broiler chicken as compared

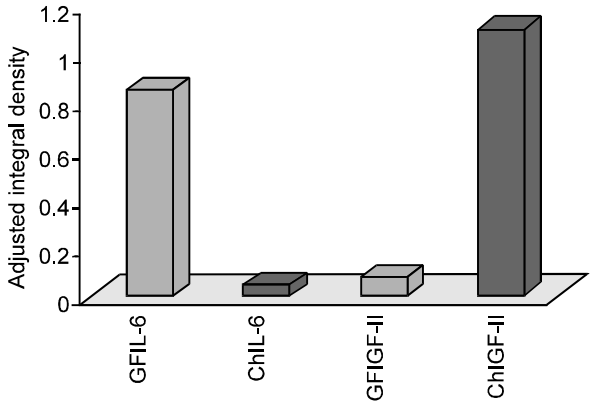

Fig. 2: Integral density of bands for IL-6 and IGF-II adjusted relative to that of β-actin in Guinea Fowl (GF) and broiler Chicken (Ch)

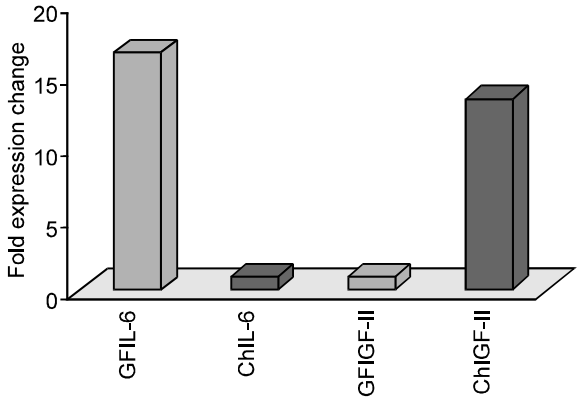

Fig. 3: Fold increase in IL-6 mRNA expression level in Guinea Fowl (GF) relative to that in broiler Chicken (Ch) and fold increase in IGF-II mRNA expression level in broiler Chicken (Ch) relative to that in Guinea Fowl (GF)

to that in guinea fowl (Fig. 3). These findings were also in line with the well established difference in growth rate in guinea fowl and broiler chicken, as broiler chicken have 2-3 times higher growth rate than guinea fowl. Insulin-like Growth Factors (IGF-1 and IGF-2) exert a general effect on overall body growth (Jones and Clemmons, 1995). In chicken, between the divergent lines for growth i.e. High Growth Line (HG) and Low Growth Line (LG), significant differences were observed in circulating IGF-I concentrations and liver IGF-I mRNA, exhibiting high or low levels respectively (Scanes *et al*., 1989; Burnside and Cogburn, 1992; Beccavin *et al*., 2001). Similar, although less clear-cut, differences were observed for IGF-II concentrations and liver IGF-II mRNA levels (Beccavin *et al*., 2001).

Hence, it is clearly evident that guinea fowl showed higher expression of pro-inflammatory cytokine (IL-6) and lower expression of IGF-II in comparison to broiler

chicken. It suggests a negative correlation between growth rate and immune status which has been established in chicken and turkey (Shook, 1989, Nestor *et al*., 1996; Bayyari *et al*., 1997). Further the guinea fowl revealed distinctly higher response to infectious organisms, as assessed by cytokine expression, in comparison to chicken population with a history of intensive selection. Selection is reported to result in the loss of many rare alleles from the majority of the selected populations (Muir *et al*., 2008), which may reduce their ability to effectively meet disease challenges. By assessing the responses of genetically distinct lines/germplasm, future decisions can be better made to enhance innate disease resistance by genetic selection. Presently, research is still in the stage of investigating specific diseases and single defense characters. Increased effort in this area will produce correlations between general disease resistance and production traits. Finally, the eminent effect of genetic interactions of the immune physiology with certain non-immune related (production) traits and the environment on disease resistance needs to be taken into account in a global strategy and guinea fowl may be a suitable model for such studies.

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