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Serum cytokine concentration in native Nicobari fowl of Andaman and Nicobar Islands

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The fundamental key role in immune and inflammatory responses is mediated by cytokines through activation and regulation of other cells and tissues. They are proteins or peptides in nature being secreted by a wide variety of cells (Wigley and Kaiser, 2003). Cytokines control infectious diseases of poultry in a massive way. Receptors presenting on the surface of the target cells mediate the action of cytokines which modulate the cell's activity. There are varieties of cytokines and receptors depending on the cell's function producing them. In birds, leukocytes and fibroblasts are secretary cells for type I interferons (IFN-α and IFN-β) and T-lymphocytes and natural killer cells produce type II (IFN-γ). Interleukins (IL), the important components of the immune system, regulate inflammation and systemic inflammatory status. Deregulation of cytokine process results in pathological disorders (Tayal and Kalra 2007). IL-6 has the most endocrine activity (Gabler and Spurlock 2008), pro-inflammatory role (Xing *et al.* 1998) and anti-inflammatory effects (Scheller *et al.* 2011). Among the Toll-like receptors (TLRs), TLR4 has key role in the immune response recognizing lipopolysaccharide (LPS) of gram negative bacteria (Kenzel and Henneke, 2006). Major histocompatibility complex (MHC) attributes to genetic resistance to diseases (Kaufman 2000). Relative resistance or susceptibility to common poultry diseases is determined by breeds and lines of chicken (Leon and Michael 2019) including genes within the major histocompatibility complex (MHC) region of the genome (Kim *et al.* 2009). Screening of these cytokines in chicken is necessary in the field of immunocompetence to poultry diseases. Detection of cytokine profiles and their appearance serves as a tool to determine the type of preferentially activated immune response (Mucksova *et al.* 2018). Understanding the subtle differences in the normal physiological status of immunity among various breeds of chicken could be fundamental to exploiting immune genes to improve the health of chicken and thereby enhancing the overall efficiency of poultry industry. Most of these standard haplotypes are almost studied from the white leghorn (Livant *et al.* 2001). Preliminary work on indigenous chicken and wild birds reveals the presence of wide range of these cytokines (Livant *et al.* 2001). Indigenous poultry farming is of great significance in nutritional security of rural farmers, however cytokine profile of native poultry has been studied a little (Baelmans *et al.* 2005). Nicobari fowl, a native chicken of Andaman and Nicobar Islands are never vaccinated. However, this population continues to survive in harsh environments for generation to generation. Thus is worthwhile to examine the distribution of cytokines and subsequently the contribution for disease resistance in this native poultry. Studies on the cytokine profile of native birds and their similarities with other commercial poultry are essential for understanding its immuno-competence. Hence, in the present study, serological cytokine profile was studied in native Nicobari fowl of A&N Islands and its comparative evaluation was carried out with other local poultry population.

In the present study, blood was collected in clot activator vials from 150 poultry comprising thirty samples each of native Nicobari fowl and commercial layer at 45 weeks of age, broilers (35 days old), desi bird (laying stage) and Vanaraja poultry (16 weeks). Serum was separated by centrifugation at 3,000 rpm for 15 min and the concentration of cytokines was determined by immunoenzymatic assay (ELISA) as described by Gaça *et al.* (1999). ELISA kits of Arsh Biotech Pvt. Ltd. India were used for estimation of chicken interferon alpha and beta (IFN-α and IFN-β ng/L) (LT71008EAYQ and LT20008EAYQ), chicken interleukin-6 (IL-6 ng/L) (LThC4000EA), chicken interleukin-12 (IL-12 pg/ml) (LThC5000EA), chicken toll like receptor (TLR 4 ng/ml) (LThC5310EA) and chicken major histo-compatibility complex MHC (ng/ml) (LT23008EAYQ). Absorptions were measured at a specific wavelength as described in the manual. Cytokine concentrations were calculated from the standard curve by means of a software product. The results are presented as means±SE. All the mean values were compared using the Duncan's new multiple range test at $P \leq 0.05$.

Table 1 represents the average serum cytokine concentrations in experimental chickens. The results revealed that the concentrations of IFN-α and IFN-β in serum is significantly higher in commercial layers and significantly

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Table 1. Serum cytokine concentration (mean±SE) of various poultry population of Andaman and Nicobar

Poultry	Interferon alpha* (IFN-α ng/L)	Interferon Beta* (IFN-β ng/L)	Interleukin-6* (IL-6 ng/L)	Interleukin-12* (IL-12 pg/ml)	Toll Like Receptor* (*TLR*4 ng/ml)	Major Histo-Compatability* (MHC ng/ml)
Commercial Layer	20.18^{a}±3.11	26.44^{a}±11.03	11.02^{b}±5.52	789.67^{b}±10.23	2.468^{b}±0.48	3.212^{a}±0.66
Nicobari fowl	16.79^{ab}±2.60	24.82^{ab}±17.50	6.83^{c}±2.90	1005.06^{a}±12.10	2.374^{b}±0.41	2.944^{ab}±0.37
Desi bird	3.05^{c}±0.54	19.63^{c}±17.50	5.92^{c}±1.54	865.47^{ab}±41.54	1.201^{c}±0.39	0.905^{c}±0.21
Vanaraja bird	11.03^{b}±3.13	21.24^{b}±10.81	14.52^{a}±4.85	373.26^{b}±11.49	1.111^{c}±0.87	2.854^{b}±2.10
Commercial Broiler	10.86^{b}±1.40	20.24^{bc}±9.11	16.22^{a}±0.42	884.56^{ab}±15.55	3.435^{a}±1.14	2.504^{b}±1.10

*Significant (P<0.05); NS,Not Significant; Values bearing different superscript in a column differ significantly.

lower in desi birds while broilers and vanaraja birds are statistically comparable. The native Nicobari fowl is statistically comparable in sero concentration of IFN-α and IFN-β with commercial layers. In the present study the concentration of IFN-α in broiler is lesser than the concentration in broilers (@ 14.58 ng/L) reported by Deng-sheng *et al.* 2018 and 15.0 (ng/l) by Zhou *et al.* (2007) and more than 8.05±0.43 ng/L as reported by Karakolev *et al.* (2015). IFN is a pleiotropic molecule that influences all stages of the immune response to a specific extent. Comparatively higher levels of IFN-α and IFN-β in Nicobari fowls as reported in the present study attribute to the scientific evidence for higher immunity of these native poultry towards Ranikhet and Marek's disease (Rai and Ahlawat, 1995) and IBD (Jai Sunder *et al.* 2004) as these cytokines are mainly involved in antiviral immunity (Schroder *et al.* 2004). Similarly, the higher levels of IFNs in commercial layers found in present study opined with the reports of Arun *et al.* (2014) that white leghorn had significantly (P<0.01) higher fold, i.e. 1.61 than that of other breeds in expression of IFN-β indicating that White Leghorn has comparatively more antiviral immune response. The cytokine, IL-6 was significantly lowest in native Nicobari fowl and desi birds and highest in broilers and vanaraja birds. Lowest serum concentration of IL-6 in Nicobari fowl and desi birds might be attributed to their low genetic potential for higher production performance (Zulkifli *et al.* 2014, Deng-sheng *et al.* 2018). The present observations are opined with Wu *et al.* (2017) who also reported the normal level of IL-6 in broiler at around 20 pg/ml. Broiler, Vanaraja and layers are under intensive system of management and are constantly exposed to bacterial invasions of serovars of *Salmonella enteric* and induces the production of interferons in the body (Bailey *et al.* 2007, Kaiser *et al.* 2012) that in-turn might have resulted in an 8-fold increase of IL-6 mRNA to induce a strong inflammatory and immune response preventing development of systemic disease. Further, enhanced levels of IFNs might have induced the pro-inflammatory cytokines of IL-6 through regulated interferon stimulating genes (Scheller *et al.* 2011, Garrido *et al.* 2018) in breeds of broilers, vanaraja and commercial layers. Significantly higher serum concentration of IL-12 was recorded in Nicobari fowl and it was statistically comparable with desi birds and broilers. Vanaraja birds were having significantly lower serum concentration of IL-12. The higher level of cytokine IL-12 circulating normally in the Nicobari fowl confirms that these native birds have higher innate immunity to most of the poultry diseases (Rai and Ahlawat 1995, Sunder *et al.* 2004). Recently it has also been acknowledged by Leon and Michael (2019) that commercial broilers with relatively up-regulated IL-12 and down regulated IFN-α were more resistant to Necrotic enteritis and coccidiosis. Hence, vaccination against commonly occurring poultry disease is although not provided to these indigenous fowl under farm and field conditions, they have better immunity as because it is reported that the cytokine levels of IL-12 were significantly increased in the immunized poultry (Kim *et al.* 2008).

The normal circulating level of *TLR4* in serum was significantly highest in broilers followed by commercial layers and Nicobari fowl and it was lowest in desi and vanaraja birds. The molecules of *TLR4* receptor varied in quantities among chicken strains, thereby varying in strain response to LPS (Dil and Qureshi 2002) might be the reason why less occurrence of Salmonellosis in Nicobari fowl (Rai and Ahlawat, 1995). This finding is in accordance with the concentrations of IFN in the respective breeds which in turn has acted in an autocrine manner and have positive feedback for expression of *TLR4* (Tanabe *et al.* 2003, Tohyama *et al.* 2005). In the present study, commercial broilers, layers and vanaraja birds have significantly higher level of MHC followed by Nicobari fowl while it was significantly lower in desi birds. Based on reports in mammals by De Maeyer and De Maeyer-Guignard (1998), the type I interferon, IFN-α and IFN-β, having anti-viral activity might have led to increased expression of MHC class I molecules in the respective breeds of poultry.

This study concluded that the higher concentrations of serum IFN-α and β, IL-12, *TLR4* and MHC in native Nicobari fowl demonstrated an apparent proof of relatively superior immunocompetence in these indigenous breeds which might attribute for their disease resistance trait as reported by earlier works in other indigenous breeds (Fayeye *et al.* 2006). The sero-profiling of cytokines of native Nicobari fowl is the first report and will be the base reference for any further cytokine studies in these indigenous breed of Andaman and Nicobar Islands and is an important validation of its higher immunity for its further commercial production even in the face of widespread diseases under changing climatic scenario.

SUMMARY

Serological cytokine profile was studied in native Nicobari fowl of A&N Islands and its comparative evaluation was carried out in other local poultry population. Blood was collected from 150 poultry comprising 30 samples each of Nicobari fowl, commercial layer and broilers, desi bird and Vanaraja poultry. The concentration of cytokines viz. chicken interferon alpha and beta (IFN-α and IFN-β) (ng/L), chicken interleukin-6 (IL-6) (ng/L), chicken interleukin-12 (IL-12) (pg/ml), chicken toll like receptor (TLR 4) (ng/ml) and chicken major histo-compatibility complex MHC (ng/ml) were determined by immunoenzymatic assay (ELISA). Results revealed that the concentrations IFN-α and IFN-β in serum is significantly higher in commercial layers that was statistically at par with Nicobari fowl. The cytokine, IL-6 was significantly lowest in native Nicobari fowl and desi birds and highest in broilers and vanaraja birds. Significantly higher serum concentration of IL-12 was recorded in Nicobari fowl and it was statistically comparable with desi birds and broilers. Level of *TLR4* in serum was significantly highest in broilers followed by commercial layers and Nicobari fowl. Commercial broilers, layers and vanaraja birds have significantly higher level of MHC followed by Nicobari fowl. Hence it can be concluded that the higher concentrations of serum IFN-α and β, IL-12, *TLR4* and MHC in native Nicobari fowl indicates their innate immunological balance that maintain immune homeostasis and enhances immune function and resistance to disease by stimulating T and B lymphocyte formation modulating cytokine secretion profiles.

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