



## Expression of toll like receptors (TLR3 and TLR4) during growth and sexual maturity of indigenous chicken ‘Sikhar’ of Mizoram

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Sikhar bird is desi/local type of chicken found in Mizoram which can thrive and produce with irregular supply of feed and water and with minimum healthcare (Padhi 2016, Mayengbam *et al.* 2017). This chicken is of scavenging type and is being reared purposely for catching Red Jungle Fowl from the forest areas (Mayengbam *et al.* 2017).

Genetically distant chicken lines were found to show different patterns of TLR genes expression with higher expression of toll like receptore (TLR) 3 mRNA in White Leghorns and of TLR4 and TLR5 mRNA in indigenous chicken like Aseel and Kadaknath (Ramasamy *et al.* 2010). Higher expression of TLR4 in heterophils had been indicated to be a marker for higher level of innate immunity in indigenous chicken (Ramasamy *et al.* 2011) and expression of Nicobari chicken TLR2, TLR3, TLR4, TLR5, TLR15 and TLR21 were found to be influenced by immunomodulatory phytochemicals (Sunder *et al.* 2016).

Being an indigenous chicken, Sikhar birds are also expected to possess different expression profiles for different TLRs.

The study was carried out in Sikhar bird of Mizoram reared in the backward rearing system where the climate was tropical monsoon type. Blood was collected from jugular vein of five different groups, viz. group 1 (2 months old), group 2 (4 months old), group 3 (6 months old), group 4 (8 months old) and group 5 (10 months old). Each group comprised 12 birds of either sexes. The body weight recorded were 217.92±7.11, 484.67±21.69, 728.33±27.38, 1010.80±67.21 and 1134.20±69.58 g in groups 1, 2, 3, 4 and 5 respectively as reported earlier (Mayengbam *et al.* 2017). Time to time behavioural changes of each of the birds was recorded in respect of their age and sex.

Plasma concentration of TLR3, TLR4, thyroid stimulating hormone (TSH), tri-iodothyronine (T<sub>3</sub>), thyroxine (T<sub>4</sub>) and follicle stimulating hormone (FSH) were

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estimated by using commercially available enzyme link immunosorbent assay (ELISA) kits from Cusabio Biotech Co. Ltd., China for TLR3, TLR4, TSH, T<sub>3</sub> and T<sub>4</sub> and YH Biosearch Laboratory, China for FSH.

Data were subjected to one way ANOVA for statistical significance followed by Duncan’s post hoc multiple comparisons to evaluate the differences between different age groups on all the parameters under the study and P<0.05 was accepted as statistically significant.

Plasma concentration of TLR3, TLR4, TSH, T<sub>3</sub>, T<sub>4</sub> and FSH of Sikhar birds in different age groups is presented in Table 1.

During young age at 2 months, the plasma concentration of TLR3 could not be estimated with ELISA which could be due to too low concentration of plasma TLR3 which was below the detectable range of the ELISA kit used. There was increase in TLR3 expression with age (Table 1). TLR3 was stable till the age of 8 months and increased significantly (P<0.05) at 10 months of age (Table 1). Previous studies also revealed lower expression of TLR3 mRNA in heterophils of indigenous chicken as compared to White Leghorn chicken (Ramasamy *et al.* 2010). Due to

Table 1. Plasma TLR3, TLR4, TSH, T<sub>3</sub>, T<sub>4</sub> and FSH profile of Sikhar birds (Mean±SE)

Parameter	Group I	Group II	Group III	Group IV	Group V
TLR3 (ng/ml)	–	3.24±0.80 <sup>a</sup>	2.98 <sup>a</sup> ±1.00	2.53±0.70 <sup>a</sup>	7.12±0.69 <sup>b</sup>
TLR4 (ng/ml)	0.99±0.03 <sup>a</sup>	1.84±0.17 <sup>c</sup>	1.32±0.11 <sup>ab</sup>	1.16±0.12 <sup>ab</sup>	1.40±0.08 <sup>b</sup>
TSH (µIU/ml)	5.69±0.49	6.35±0.45	6.80±0.36	5.60±0.57	5.56±0.28
T <sub>3</sub> (ng/ml)	2.38±0.14 <sup>c</sup>	2.04±0.13 <sup>ab</sup>	3.03±0.34 <sup>d</sup>	1.98±0.08 <sup>ab</sup>	1.71±0.05 <sup>a</sup>
T <sub>4</sub> (ng/ml)	214.90±16.32 <sup>b</sup>	157.86±11.45 <sup>a</sup>	159.15±11.26 <sup>a</sup>	138.29±9.92 <sup>a</sup>	170.37±14.48 <sup>a</sup>
FSH (mIU/ml)	36.75±2.56 <sup>a</sup>	44.33±3.19 <sup>ab</sup>	35.58±2.95 <sup>a</sup>	45.33±4.91 <sup>ab</sup>	50.22±3.52 <sup>b</sup>

Values in the same row with different superscripts differ significantly (P<0.05).

unavailability of data on plasma concentration of TLR3, the present findings could not be compared. The study however revealed higher expression of TLR3 in adult birds as compared to young ones.

The expression of TLR4 significantly increased from group 1 to group 2 and declined thereafter in group 5 ( $P < 0.05$ ). TLR4 was highest in group 2 ( $P < 0.05$ ) as compared to rest of the groups. TLR4 expression however declined significantly in group 3, 4 and 5 ( $P < 0.05$ ). The adult birds in group 5 had higher ( $P < 0.05$ ) TLR4 expression than young birds in group 1 (Table 1).

The TLR4 expression estimated in plasma in the present study was in close resemblance to TLR4 expression of 5 weeks old indigenous chicken (Ralte 2016). Sikhar birds used in the present study had not been vaccinated against any of the diseases. The increase in expression of TLR4 in group 2 was indicative of increase in the acquired immune status of birds with age as reported earlier (Ramasay *et al.* 2010, Ramasamy *et al.* 2011). It was apparent that there was activation of TLR4 expression in response to natural exposures initially with age and as the birds grew up and became adults, a stable immune status was maintained.

Plasma concentration of TSH of Sikhar was stable from 2 months of age till 10 months of age (Table 1). TSH of Sikhar birds was in higher range as compared to TSH of broiler chicken (Chotinsky and Mihaylov 2013). Mutations in TSHR affected typical domestication traits and mutations in TSHR had been indicated to contribute significantly to the evolution of the modern domestic chicken (Karlsson *et al.* 2015). Maintenance of higher plasma TSH of Sikhar could be due their closeness to their wild ancestor (Red Jungle fowl) which however could not be explained with the present investigation.

The level of  $T_3$  declined initially from group 1 to group 2 ( $P < 0.05$ ) and increased significantly to group 3 ( $P < 0.05$ ) and declined ( $P < 0.05$ ) to a stable level in groups 4 and 5.  $T_3$  was highest in 6 months old birds (Table 1).  $T_4$  of Sikhar birds was highest in group 1 which declined significantly ( $P < 0.05$ ) with growth in group 2 and thereafter maintained a relatively stable  $T_4$  in groups 3, 4 and 5 (Table 1). Similar to present finding,  $T_3$  and  $T_4$  had been found to be higher in young age group birds (Biswas *et al.* 2010). Higher level of  $T_3$  in group 3 at the age of 6 months could be due to activation of the thyroid axis during the pre-pubertal period of Sikhar birds as thyroid hormones act via nuclear receptors in regulation of the pituitary-gonadal axis (Sechman 2013).

The plasma concentration of  $T_3$  of Sikhar birds detected in the present study were in close resemblance with  $T_3$  of Kadaknath and Aseel peela (Biswas *et al.* 2010).  $T_4$  level of Sikhar birds were in much higher ranges as compared to  $T_4$  of Kadaknath and Aseel peela (Biswas *et al.* 2010).  $T_4$  concentration was also different between different Indian native breeds while breed wise difference did not occur in respect of  $T_3$  (Biswas *et al.* 2010). Karlsson *et al.* (2015) observed that domestic chicken homozygous for the “domestic” allele (d/d) at the TSHR locus showed decreased levels of plasma  $T_4$  as compared to wild type homozygotes

(w/w) or heterozygotes (d/w). The finding of higher level of  $T_4$  of Sikhar as compared to other indigenous chicken could indicate its closeness to wild origin.

Estimate of FSH indicated fluctuation in different age groups with highest values at 8 months and 10 months of age. These two were the stages when the birds started laying eggs and were at the peak laying stages respectively. Previous studies in broiler breeder hens indicated fluctuating FSH with age with peaks before and after puberty (Onagbesan *et al.* 2006) while FSH was found to rise significantly at 25 weeks of age and a slight decline thereafter in broiler males (Kirby and Vizcara 2015). Presence of higher FSH at 8 months and highest at 10 months indicated the involvement of FSH at active reproductive stage among the different groups.

#### SUMMARY

The study revealed age wise variation in expression of TLR3 and TLR4 of indigenous chicken, Sikhar of Mizoram. The Sikhar birds apparently had higher expression of TLR4 than TLR3 which could easily be estimated in plasma. The study also indicated presence of higher  $T_3$  and  $T_4$  in young age. Sikhar had higher concentration of TSH and  $T_4$  in plasma unlike other breeds and lines of domestic chicken indicating the closeness of Sikhar chicken to its wild ancestor, Red Jungle Fowl. Study indicated attainment of sexual maturity of Sikhar chicken comparatively at later age than other indigenous chicken.

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