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Cold stress elevates HSP70, TLR2 and TLR4 of indigenous chicken

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Mizoram, located at 1020 m altitude and 23°44′12 N latitude enjoys a pleasant climate throughout the year except for a very short period of winter towards the end of December to beginning of January. As perceived from the local farmers, poultry farming in winter in Mizoram suffers from great loss due to cold associated diseases and complications. Stress markers, viz. HSP60 and HSP70 expression have been indicated to associate cold stress in broiler and indigenous chicken (Zhao *et al.* 2013, Chen *et al.* 2014, Tarkhan *et al.* 2020). Cold stress had also been reported to affect immune organs and compromise the immune status of chicken (Zhao *et al.* 2014, Wei *et al.* 2018). The present study reveals elevation of HSP70, TLR2 and TLR4 of indigenous chicken of Mizoram during cold stress.

Indigenous chicken (12) of Mizoram in the age group of 5 weeks were reared in the backyard rearing system. The birds were divided into control and cold stress groups where each group had both sexes in equal ratio. The birds in cold stress group were acclimatized in a locally made bamboo cage for night stay for 15 days. After acclimatization, the birds in control were kept in the shed for night stay while the birds in cold stress group were subjected to natural cold condition by keeping them in bamboo cage outside the shed for 8 h, i.e. from 9:00 PM to 5:00 AM of the next morning for a period of 21 successive days, i.e. from 15 January 2016 to 4 February 2016.

Ambient temperature (maximum ambient temperature Tmax, minimum ambient temperature Tmin and average ambient temperature Tav) was recorded by using room thermometer at the interval of every 2 h during the periods of acclimatization (record of 8 h at night inside the shed from 9:00 PM to 5:00 AM of the next morning for 15 successive days), cold control (record of 8 h at night inside the shed from 9:00 PM to 5:00 AM of the next morning for 21 successive days) and cold exposure (record of 8 h at night outside the shed from 9:00 PM to 5:00 AM of the next morning for 21 successive days).

Blood samples were collected from jugular veins of each bird on last day of acclimatization and 21st day of cold exposure from all the birds in two groups. HSP60, HSP70, TLR2, TLR3 and TLR4 were estimated in plasma by ELISA by following the standard protocols as prescribed in the commercial kits, viz. Chicken Heat Shock Protein 60 (Hsp-60) ELISA Kit (Cusabio Biotech Co. Ltd), Chicken Heat Shock Protein 70 (Hsp-70) ELISA Kit (Cusabio Biotech Co. Ltd), Ch TLR-2 ELISA Kit (Shanghai BlueGene Biotech Co. Ltd), Ch TLR-3 (Shanghai BlueGene Biotech Co. Ltd) and Ch TLR-4 ELISA Kit (Shanghai BlueGene Biotech Co. Ltd) respectively. Data were subjected to one way ANOVA for statistical significance followed by Duncan's post-hoc multiple comparisons to evaluate the differences between different groups on all the parameters under the study and $P<0.05$ was accepted as statistically significant.

The records of average Tmax, Tmin and Tav are presented in Table 1. Effect of cold exposure on plasma concentration of HSP60, HSP70, TLR2, TLR3 and TLR4 of indigenous chicken is presented in Table 2. There was no significant change in HSP60 of indigenous chicken due to cold exposure in the present study unlike previous reports of increased HSP60 in heart in response to acute and chronic cold stress (Zhao *et al.* 2013). The cold exposure increased plasma HSP70 in the present study (Table 2). Increase in expression of HSP70 mRNA and protein in immune tissues of chicken in response to acute and chronic cold stress had been reported (Chen *et al.* 2014, Zhao *et al.* 2014). Increase in HSP60 and HSP70 had been indicated to have cryoprotective roles in the oxidative and inflammatory stress induced by cold stress (Zhao *et al.* 2013, Chen *et al.* 2014, Zhao *et al.* 2014).

Several studies reported that cold exposure influenced the function of the immune system (Fleshner *et al.* 1998, Onderci *et al.* 2003, Hangalapura *et al.* 2004, Helmreich *et al.* 2005, Hangalapura *et al.* 2006, Wei *et al.* 2018). In the present study, concentration of TLR2 during the acclimatization period and control group was below the detectable level of the ELISA kit (0.1 ng/ml) which rose to 2.31±0.11 ng/ml after cold exposure (Table 2). TLR3

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Table 1. Tmax, Tmin and Tav during acclimatization, control and cold exposure

Parameter	Acclimatization period	Control	Cold exposure
Tmax	16.92±0.6	18.31±0.62	14.22±0.37
Tmin	10.99±0.2	12.22±0.36	11.65±0.71
Tav	13.95±0.3	15.02±0.65	13.20±0.37

Table 2. Effect of cold exposure on plasma HSP60, HSP70, TLR2, TLR3 and TLR4 of indigenous chicken

Parameter	Acclimatization period	Control	Cold exposure
HSP60 (ng/ml)	0.78±0.11	0.67±0.15	0.95±0.09
HSP70 (ng/ml)	$0.10±0.02^{b}$	$0.08±0.01^{b}$	$0.56±0.10^{a}$
TLR2 (ng/ml)	< 0.1	< 0.1	2.31±0.11
TLR3 (ng/ml)	< 0.1	< 0.1	< 0.1
TLR4 (ng/ml)	$2.52±0.11^{b}$	$2.42±0.08^{b}$	$3.73±0.23^{a}$

Values are mean±SE. Means with different superscripts in the same row differ significantly from each other at P<0.05.

concentration in plasma of indigenous chicken in the present study could not be detected by ELSA whose minimum detection level was 0.1 ng/ml as reported earlier in indigenous chicken, Sikhar (Mayengbam *et al.* 2018). The possible failure to estimate TRL3 could be due to lower expression of TLR3 in indigenous chicken as expression of TLR3 mRNA in indigenous chicken was lower as compared to other exotic chicken (Ramasamy *et al*. 2010, 2011). Cold exposure however caused significant increase in TLR4 concentration (Table 2). It was apparent that there was activation of the immune status of indigenous chicken by elevating expression of TLRs. Increase in TLR4 expression was in association with increase in HSP70 in response to cold stress. The cryoprotective role of HSP70 (Zhao *et al.* 2013, 2014) could possibly cause upliftment of the immune status by elevating the innate immune status. It also suggested that increase in HSP70 was an important protective protein to activate immune function of chicken in cold stress.

SUMMARY

The study revealed that cold stress elevates the expression of HSP70, TLR2 and TL4 of indigenous chicken. Increase in expression of HSP70 was most likely to have cryoprotective properties by elevating the TLR2 and TLR4 of indigenous chicken during cold stress.

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