Effect of season and SNPs of HSP90 and HSP70 genes on the biochemical traits in Indian sheep (*Ovis aries*) breeds

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Global warming and climate change have become the major threats to the sustainability of livestock production systems (Gaughan et al. 2016). Most part of India lies in tropical region where temperature even goes up to 46°C during summer hindering the overall productivity of the livestock species. Heat shock proteins (HSPs) are evolutionary conserved family of proteins induced in a living cell in response to various biological stresses, including heat shock, high pressures and toxic compounds (Benjamin and McMillan 1998). Thyroid hormones increase the metabolic rate and oxygen consumption of most tissues and stimulate the generation of heat (Freak and Oppenheimer 1995). The hypothalamus-pituitary-adrenal (HPA) axis is the primary neuroendocrine pathway involved in the stress response that secretes cortisol hormone (Atkinson et al. 2006). Cortisol change is adaptive to the adverse environment and may be used as a stress assessment index (Dong and Liu 2013). In addition, the functions of thyroid gland hormones (T3 and T4) and cortisol are affected by the heat load (Sejian et al. 2013, Rathwa et al. 2017, Bhimte et al. 2018). Sheep is an important livestock species reared for wool, meat, skin and manure which plays an important role in the livelihood of a large percentage of small and marginal farmers. Therefore, the present study was undertaken to establish reference baseline values for thyroid (T3 and T4) and adrenal gland (cortisol) hormones in four different native sheep breeds so as to assess their health and physiological status under heat stress condition. In addition, the effect of seasons and genotypes of HSP90 and HSP70 genes on the functions of thyroid gland and cortisol levels was studied in order to facilitate selection of sheep that are more resistant to heat stress.

MATERIALS AND METHODS

A total of 80 plasma samples (10 animals per breed per season, viz. summer and winter) were analyzed among 4 sheep breeds (Chokla, Marwari, Magra and Madras Red). Collection of the plasma samples was carried out in two seasons (summer and winter) as per the method described earlier (Singh et al. 2017). In brief, female animals aged 1–1.5 years with almost similar body weight were incorporated. Isolation of the plasma was carried out immediately after blood collection. The samples were brought to the laboratory while maintaining the cold chain. Enzyme-linked Immunosorbent Assay (ELISA) kits for sheep tri-iodothyronine, thyroxine and cortisol were used for the estimation of plasma T3, T4 and cortisol level. The concentrations of samples were determined on the basis of the OD values as defined in the operation manual (Bioassay Technology Laboratory, ELISA kit). Already identified 5 SNPs of HSP90 and HSP70 genes (Table 1) were screened for the determination of the genotype of each sample (Singh et al. 2017). The significant effect of genotypes on biochemical traits was analyzed by SPSS software version 22.0.0 using the following model.

\[ Y_{ijkl} = \mu + G_i + S_j + B_k + e_{ijkl} \]

where \( Y_{ijkl} \) is biochemical trait of \( l \) th animal belonging to \( i \) th genotype in \( j \) th season of \( k \) th breed; \( \mu \), overall mean; \( G_i \), effect of \( i \) th genotype (\( i = 1 \) to \( n \)); \( S_j \), effect of \( j \) th season; \( B_k \), effect of \( k \) th breed (\( k = 1 \) to \( 4 \)), and \( e_{ijkl} \), random error associated with \( Y_{ijkl} \) observation and assumed to be NID (0, \( \sigma^2e \)).

RESULTS AND DISCUSSION

In the present study, a total of 80 plasma samples (10 animals per breed and per season) were analyzed for the estimation of T4, T3 and cortisol level. The standard curve was generated for the estimation of each parameter in Chokla, Marwari, Magra, and Madras Red breed of sheep. Least square analysis was carried out to find the effect of season and genotype.

The plasma T4 levels were significantly higher in winter (\( P<0.05 \)) (57.14±11.24) than summer (29.53±10.71). The effect of all the five SNPs of HSP90 and HSP70 gene was non-significant (\( P>0.05 \)). The mean values for the Chokla, Marwari, Magra and Madras Red breed were 50.80±12.08, 40.45±14.30, 44.40±13.36 and 37.68±13.55 (nmol/L), respectively. The plasma T3 level was significantly higher in winter (\( P<0.05 \)) (7.85±1.13) than summer (5.54±1.16). Genotypes of SNP1 (HSP90 gene) and SNP2 (HSP70 gene) had significant (\( P<0.05 \)) effect on the T3 level. Their mean
SNP1 (HSP90 gene) and SNP2 (HSP70 gene) had a significant effect (P<0.05) on the Thyroid hormones (T3 and T4). Genotypes of the HSP90 and HSP70 genes were explored to have a comprehensive picture of animals that are more adaptable to heat stress.

**SUMMARY**

Heat shock proteins (HSPs) are evolutionary conserved family of proteins produced by living cells in response to various biological stresses, including heat shock. The authors are thankful to the Director, ICAR-NBAGR; Incharge ARC, CSWRI, Bikaner; Incharge, PGRIS, Kattupakkam, TANUVAS, Chennai for providing the necessary facilities for the research work and sample collection.

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| Gene       | SNP No. | Amplicon size (bp) | Nucleotide changes* |
|------------|---------|--------------------|---------------------|
| HSP90AA1   | SNP1    | 490                | Exon 6–7; 390 T>G    |
|            | SNP3    | 354                | Exon 9; 180 C>T      |
|            | SNP4    | 499                | Promoter; 112 G>C    |
| HSP70      | SNP2    | 609                | Exon1; 459 A>G       |
|            | SNP5    | 594                | Exon1; 222 G>A       |

*w.r.t amplified fragments.*

values for the Chokla, Marwari, Magra and Madras Red were 7.11±1.24, 7.04±1.44, 7.44±1.32 and 5.17±1.35 (nmol/L), respectively. Plasma cortisol level were significantly higher in summer (P>0.05) (7.85±0.98) than winter (6.07±0.97). Their mean values for the Chokla, Marwari, Magra and Madras Red breeds were 4.78±1.35, 6.74±1.35, 9.14±1.46 and 7.19±1.35 (ng/ml), respectively. Thus, there was non-significant difference (P>0.05) of cortisol level among breed, season and five identified genotypes of HSP90AA1 and HSP70 genes. However, the effect of breed on all three parameters was non-significant (P>0.05).

Overall analysis indicated that the plasma hormone concentration of T3 and T4 were 1.41 and 1.93 times reduced (P<0.05) while the concentration of cortisol was 1.29 times increased (P>0.05) during the summer compared to winter. Thus, the plasma thyroid hormones (T3 and T4) showed reverse trend to that of cortisol for the seasonal variation, i.e. higher level of cortisol and lower level of T3 and T4 in the summer. Similar results were obtained for the cortisol, T3 and T4 levels by Rathwa et al. (2017) in Indian sheep breeds and Nazifi et al. (2003) in Iranian fatted sheep. This is also in consonance with the heat stress following a thorough association analysis over a large sample size.

In the present study was undertaken to establish reference baseline values for thyroid (T3 and T4) and adrenal gland (cortisol) hormones in four native sheep breeds (Chokla, Marwari, Magra and Madras Red). The effect of the seasons and genotypes of the HSP90 and HSP70 genes on the functions of the thyroid gland as well as cortisol levels were examined in plasma samples of these sheep breeds. A total of 80 plasma samples (10 animals per breed for the summer and winter season) were analyzed. The least square analysis revealed significant seasonal effect on Triiodothyronine (T3) and Thyroxine (T4). Significantly higher T3 and T4 levels observed in the winter with low temperature humidity index (THI) than in summer with high THI. Cortisol was non-significantly higher in the summer than with high THI. The effect of breed on all three parameters was non-significant (P>0.05).

The reduction in the thyroid gland function and increased cortisol level in summer might be due to an acclimatization response of these sheep breeds to cope up with the stressful condition due to heat. Least square analysis revealed significant effect (P<0.05) of season on Triiodothyronine (T3) and Thyroxine (T4). Genotypes of SNP1 (HSP90 gene) and SNP2 (HSP70 gene) had significant effect only on T3 level. Significantly higher levels of T3 and T4 were observed in winter with low THI than summer with high THI. Cortisol level was non-significantly higher in summer than winter (P>0.05). However, the effect of breeds on these three parameters was non-significant (P>0.05).

| Gene       | SNP No. | Amplicon size (bp) | Nucleotide changes* |
|------------|---------|--------------------|---------------------|
| HSP90AA1   | SNP1    | 490                | Exon 6–7; 390 T>G    |
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| Genes      | No. (bp) | Values for the Chokla, Marwari, Magra and Madras Red | Significance |
|------------|----------|-------------------------------------------------------|--------------|
| HSP90AA1   | SNP1     | 490                                                   |              |
|            | SNP3     | 354                                                   |              |
|            | SNP4     | 499                                                   |              |
| HSP70      | SNP2     | 609                                                   |              |
|            | SNP5     | 594                                                   |              |

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