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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}, biochemical trait of l^{th} animal belonging to i^{th} genotype in j^{th} season of k^{th} breed; μ, 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, $\sigma^2 e$).

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

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Table 1. Position of SNPs and their nucleotide changes in HSP90AA1 and HSP70 genes

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 breed 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 as 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 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 fat-tailed sheep. This is also in consonance with the heat stress response for cortisol, T3 and T4 observed by Sejian *et al*. (2013) in Malpura sheep breed. In another study on the influence of seasons on thyroid glands, lower level of T4 was recorded in summer in healthy dogs of various breeds in the different weight groups. However, T3 concentration was not influenced by varying the ambient temperature (Fialkovicova *et al*. 2012). Analogous results were also obtained by other workers in goat (Sivakumar *et al.* 2010, Helal *et al*. 2010). When an animal is subjected to heat stress, secretion of the thyroid hormones (T3 and T4) is inhibited to avoid thermogenesis and adrenal gland hormones (cortisol) increases to restore the compromised energy homeostasis by stimulating gluconeogenesis, lipolysis and proteolysis in stressed animals (Bhimte *et al*. 2018). 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).

Reference baseline values for thyroid (T3 and T4) and adrenal gland (cortisol) hormones in four native sheep breeds (Chokla, Marwari, Magra and Madras Red) were generated to assess their physiological status under normal and heat stressed conditions. In addition to the markers identified in the present study, more SNP markers of HSP genes and their association with thermos-tolerance may be 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. Heat stress affects the productivity of the livestock species, which causes severe economic losses to the animal keepers. The present study was undertaken to establish reference baseline values for thyroid (T3 and T4) and adrenal gland (cortisol) hormones in four indigenous 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 were observed in the winter with a low temperature humidity index (THI) than in summer with high THI. Cortisol was non-significantly higher in the summer than in winter season. Overall, the influence of the breeds on these three parameters was non-significant. Genotypes of SNP1 (HSP90 gene) and SNP2 (HSP70 gene) had significant effect on T3 level. These SNP markers may be useful for identifying animals that are more adaptable to heat stress following a thorough association analysis over a large sample size.

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