



Impact of short term exposure to different environmental temperature on the blood biochemical and endocrine responses of Malpura ewes under semi-arid tropical environment

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ABSTRACT

The study was conducted to establish the impact of short term exposure to different environmental temperature on adaptive capability of Malpura ewes in terms of changes in blood biochemical and endocrine responses. The experiment was conducted for 21 days in 28 Malpura ewes, which were randomly divided into 4 groups of 7 animals each (G1-control, G 2–23°C, G 3–40°C, G 4–42°C). The ewes were exposed to different temperature for a week and blood collection was carried out on day 0, day 1, day 4 and day 7 for each group. The effect of different temperature exposure was studied on blood biochemical and endocrine parameters. Haemoglobin (Hb), packed cell volume (PCV), plasma glucose, total cholesterol, total protein and albumin also showed highly significant variation for the different temperature exposure. All endocrine parameters showed highly significant variation for the treatment. The highest cortisol concentration was recorded in group 4 (34.73 nmol/L) while the lowest being in group 2 (8.35 nmol/L). Plasma thyroid hormones showed reverse trend as that of cortisol in that the highest concentration was recorded in group 2 while the lowest being in group 4. The data indicated that Malpura ewes have the capability to alter their adaptive capability on exposure to different environmental temperatures. Further, the study proved that heat stress effect was established both at 40°C and 42°C in these ewes but the effect was very severe on exposure to 42°C. This shows that each degree increase in upper critical temperature might be very detrimental to the survival of these animals.

Key words: Adaptation, Cortisol, Malpura ewes, Sheep, Thermal stress, Thyroid hormones

Climatic factors or seasonal changes greatly influence the behaviour of animals due to neuroendocrine response to climatic elements, consequently affecting production and health of animals (Shelton 2000, Sejian *et al.* 2010a, Baumgard *et al.* 2012). In Indian subcontinent/peninsula, heat stress is the most important climatic stress which adversely affects the livestock and sometimes even threatens the survival of the animals (Sejian *et al.* 2012). In India sheep are very important farm animals in semi-arid zone, especially the Malpura breed (Sejian *et al.* 2010b) and their exposure to elevated ambient temperature negatively affects biological functions as reflected in impairment of their production and reproduction traits (Marai *et al.* 2007). Study involving simultaneous exposure to low and high temperature under natural environmental condition is practically not possible as the temperature variation depends on seasonality. The

Malpura sheep is an indigenous breed generally adapted to semi-arid tropical environment (Sejian *et al.* 2010a). Studies involving adaptation of Malpura breed generally considered only constant temperature exposure and information are very meagre pertaining to their adaptive capability to different temperature exposure. Further, in an era where climate change has become a challenge, there is a need to better understand the adaptive mechanisms of livestock genetic resources (how they cope it up) under different temperature variability and design pertinent strategy to mitigate the challenge. From this perspective, the current research is an attempt to establish the impact of short term exposure to different environmental temperatures on blood biochemical and endocrine changes in Malpura ewes under semi-arid tropical environment.

MATERIALS AND METHODS

Location: The experiment was carried out at the Central Sheep and Wool Research Institute farm, which is located in the semi-arid region of the country at longitude 75° 28'E and the latitude of 26° 26'N and at altitude of 320 m above

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mean sea level. The average annual maximum and minimum ambient temperature ranges between 6 to 46°C. The mean annual relative humidity ranges between from 20 to 85%. The annual rainfall in this area ranges from 200 to 400 mm with an erratic distribution throughout the year. The experiment was carried out during July. The mean environmental temperatures, relative humidity, and wind velocity during the study period (21 days) are depicted in Table 1.

Animals: Malpura is a triple purpose hardy sheep breed, which originated in the arid and semi-arid areas of Western tropical India. The study was conducted in 28 Malpura non-pregnant ewes (2-year-old) weighing between 30 and 35 kg. The animals were housed in well-ventilated sheds made up of asbestos roofing at the height 2.4 m and open from side and maintained under proper hygienic conditions. The animals had *ad lib.* access to good quality drinking water. Prophylactic measures against sheep diseases like sheep pox, peste des petits ruminants, enterotoxaemia, endo- and ectoparasitic infestations were carried out as prescribed by the health calendar of the institute to ensure that the animals were in healthy condition throughout the study.

Experimental design: The experiment was conducted for 21 days in 28 Malpura ewes. The animals were randomly divided into 4 groups of 7 animals each. All groups were subjected to specific temperature exposure separately. G 1 ewes were maintained in shed under thermo-neutral condition (G1- control), G 2 animals were subjected to 23°C (G 2–23°C), G 3 ewes were subjected to 40°C (G 3–40°C), and G 4 ewes were subjected to 42°C (G 4–42°C). G 1 ewes were maintained in the shed where the average temperature and relative humidity (RH) varied between 32–38°C and 50–60% respectively. The different temperature exposure for G 2, G 3 and G 4 ewes were followed only for 6 h between 10:00 h to 16:00 h in the climatic chamber. A constant temperature of 23°C, 40°C and 42°C was maintained in each week between 10:00 h to 16:00 h in climatic chamber for G 2, G 3, and G 4 ewes respectively. In the first week of study, information pertaining to various parameters was recorded for G 1 and G 2 while in second and third week, information was recorded for G 3 and G 4 respectively. The average relative humidity in the chamber for 23°C, 40°C and 42°C were 72%, 30% and 22% respectively. The climatic chamber has the provision to hold 7 animals at a time. The chamber has a programmable temperature regulator to regulate the temperature between 5°C to 60°C. The climatic chamber has a trevice to restrain each animal separately. The animals were acclimated to the climatic chamber and restraining inside the chamber before

the start of the experiment. For rest of the period of day except between 10:00 to 16:00 h GII, G 3 and G 4 ewes were maintained in similar shed like G 1 ewes. The animals were stall fed with a diet consisting of 70% roughage (*Cenchrus ciliaris*) and 30% concentrate (barley 650 g/kg, groundnut cake 320 g/kg, minerals 30 g/kg including 10 g/kg NaCl, 180 g/kg crude protein and 650 g/kg total digestible nutrients). The feeding strategy followed in this experiment is based on standard farm management practices (Sejian *et al.* 2012). Ewes were fed on individual basis and their feed intake was recorded daily. The animals did not have access to feed and water between 10:00 h to 16:00 h in the chamber. This ensures the uniform accessibility of all groups to feed and water. Blood collection was carried out on day 0, day 1, day 4 and day 7 from each group. The study was conducted after obtaining approval from the institute ethical committee for subjecting the animal to different temperature.

Blood collection and plasma separation: Blood samples (7 ml) were collected on day 0, day 1, day 4 and day 7 of all groups at 11:00 h using 20 gauge sterilized needles and plastic syringe from external jugular vein in tubes with heparin anticoagulant. The time of blood collection was fixed at 11:00 h after ensuring that heat stress is established based on heat stress markers like respiration rate, rectal temperature and plasma cortisol. Blood samples were divided into two aliquots. One aliquot was used for estimation of Hb and PCV while the other is subjected for plasma separation. Plasma was separated from blood by centrifugation at 3,500 rpm at room temperature for 20 min. The plasma was divided into aliquots in microcentrifuge tubes, and kept frozen at –20°C till further analysis. Plasma samples were used to estimate biochemical and endocrine parameters.

Parameters studied: Hb and PCV were estimated using whole blood samples according to Balasubramaniam and Malathi (1992) and Jain (1986), respectively. The biochemical parameters viz. plasma glucose Tietz (1976) total plasma protein, albumin and globulin Tietz (1995) and total plasma cholesterol Allain *et al.* (1974) were estimated using diagnostic kits as per standard method using the UV-visible recording spectrophotometer.

Hormonal parameters T₃ (analytical sensitivity 0.1 nmol/L, intra-assay and inter-assay coefficient of variations 3.3% and 8.6% respectively), T₄ (analytical sensitivity 13 nmol/L, intra-assay and inter-assay coefficient of variations 5.1% and 8.6% respectively), and cortisol (analytical sensitivity was 10nM, the intra-assay and inter-assay coefficient of variations were 5.8% and 9.2% respectively),

Table 1. Climatological data during the experimental period

Time of Recording	Minimum temperature (°C)	Maximum temperature (°C)	Dry bulb temperature (°C)	Wet bulb temperature (°C)	RH (%)	Wind velocity (m/sec)
Morning (8:00 h)	31.42±0.36	31.86±0.36	29.37±0.49	25.58±0.20	65.70±2.02	4.5
Afternoon (14:00 h)	34.88±0.38	35.31±0.40	35.52±0.62	26.89±0.24	56.50±1.96	

were estimated by radio immuno assay (RIA) using gamma counter and RIA kits.

Data analysis: The data were analysed by GLM (SPSS 17.0). The linear model used for all the respondent variables using least squares analysis of variance. Effect of fixed factors namely treatment (G1, control, G 2, 23°C; G 3, 40°C; and G 4, 42°C) and days (longitudinal time over which experiment was carried out; day 0, day 1, day 4 and day 7) and also interaction of treatment and days was analyzed on the various parameters studied. Comparison of means of the different subgroups was made by Duncan's multiple range tests as described by (Kramer, 1957).

RESULTS AND DISCUSSION

Blood biochemical parameters: The effects of exposure to different temperature variation on blood biochemical parameters are given in Table 2. Both Hb and PCV increased significantly ($P < 0.01$) in G 3 and G 4 as compared to G 1. However, exposure to 23°C did not influence much the level of both Hb and PCV as compared to control group. However, both experimental days ($P < 0.01$) and interaction between treatment and experimental days ($P < 0.05$) had significant influence only on Hb but not on PCV. Plasma glucose also differed significantly ($P < 0.01$) between the groups. Also the experimental days had significant ($P < 0.01$) effect on plasma glucose. Further, the interaction between treatment and experimental days also had significant ($P < 0.05$) influence on plasma glucose level. Total plasma cholesterol differed significantly ($P < 0.01$) for the different temperature treatment. The highest total cholesterol concentration was recorded in G 4 while the lowest being in G 2. Further, experimental days ($P < 0.01$) and interaction between treatment and experimental days ($P < 0.05$) also significantly influenced plasma total cholesterol. Plasma total protein

decreased significantly ($P < 0.05$) in G 3 and G 4 as compared to G 1. However, total protein in G 2 did not differ with G 1. Plasma albumin also showed similar trend to that of total protein. Further, experimental days and interaction between treatment and experimental days did not significantly influence total protein, albumin and globulin.

Endocrine parameters: The effect of exposure to different temperature on plasma T_3 level are given in Fig. 1. Plasma level of T_3 increased significantly ($P < 0.01$) in G 2 while decreased in G 3 and G 4 as compared to G 1. However, both experimental days and interaction between treatment and experimental days did have any significant effect on T_3 . Fig. 2 describes the effect of exposure to different temperature on plasma T_4 level. Plasma T_4 level also showed similar trend

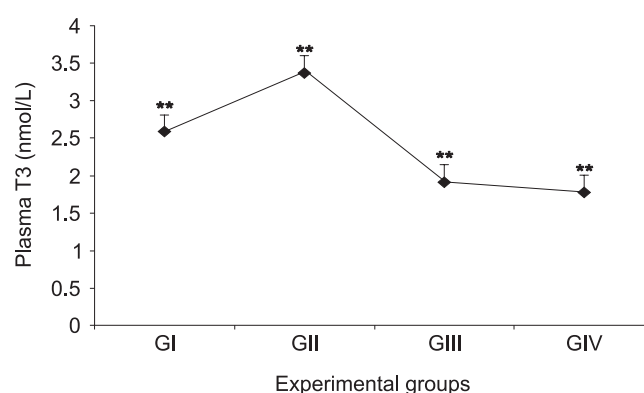


Fig. 1. Effect of short term exposure to different environmental temperatures on Plasma T_3 (nmol/L) concentrations in Malpura ewes. G 1, control; G 2, at 23°C; G 3, at 40°C; G 4, 42°C. ** Indicates level of significance @ $P < 0.01$. Plasma T_3 concentration showed highly significant ($P < 0.01$) effect between the groups. The highest T_3 concentration was recorded in G 2 while the lowest being in G 4.

Table 2. Effect of short term exposure to different environmental temperatures on Hb (g/dL), PCV (%), plasma glucose (mg/dL), total plasma cholesterol (mg/dl), total plasma protein (g/dl), plasma albumin (g/dl) and plasma globulins (g/dl) in Malpura ewes

Items	Hb (g/dl)	PCV (%)	Plasma glucose	Total cholesterol	Total protein	Albumin	Globulins
$\mu \pm SE$	9.065 $\pm$ 0.117	34.633 $\pm$ 0.483	55.16 $\pm$ 0.516	59.979 $\pm$.804	6.442 $\pm$.146	3.840 $\pm$.044	2.742 $\pm$ 0.155
Treatment	**	**	**	**	*	*	NS
G 1	8.175 ^c	32.951 ^b	54.864 ^b	57.300 ^b	6.850 ^a	3.976 ^a	2.879 ^a
G 2	8.785 ^{bc}	32.605 ^b	54.590 ^b	55.348 ^b	6.977 ^a	4.059 ^a	3.077 ^a
G 3	9.159 ^b	34.781 ^b	52.799 ^b	57.126 ^b	6.012 ^b	3.678 ^b	2.334 ^a
G 4	10.141 ^a	38.194 ^a	58.410 ^a	70.142 ^a	5.930 ^b	3.647 ^b	2.676 ^a
Pooled SE for treatment	$\pm$ 0.234	$\pm$ 0.966	$\pm$ 1.033	$\pm$ 1.607	$\pm$.293	$\pm$.089	$\pm$.311
Day	**	NS	**	**	NS	NS	NS
0	7.981 ^c	33.304 ^a	44.469 ^d	54.708 ^b	7.031 ^a	.887 ^{ab}	3.144 ^a
1	9.070 ^b	35.282 ^a	63.353 ^a	59.736 ^a	6.202 ^{ab}	3.687 ^b	2.729 ^{ab}
4	9.399 ^{ab}	34.832 ^a	54.820 ^c	63.111 ^a	5.997 ^b	3.962 ^a	2.151 ^b
7	9.810 ^a	35.114 ^a	58.021 ^b	62.361 ^a	6.541 ^{ab}	.824 ^{ab}	2.943 ^{ab}
Pooled SE for day	$\pm$ 0.234	$\pm$ 0.966	$\pm$ 1.033	$\pm$ 1.607	$\pm$.293	$\pm$.089	$\pm$.311
Treatment * Day	*	NS	*	*	NS	NS	NS

Hb, Hemoglobin ; PCV, packed cell volume; G 1-control, G 2, at 23°C, G 3, at 40°C and G 4 at 42°C; μ , overall mean for the parameter. * ($P < 0.05$), ** ($P < 0.01$); NS, nonsignificant. Means with similar superscripts do not differ significantly ($P > 0.05$) from each other.

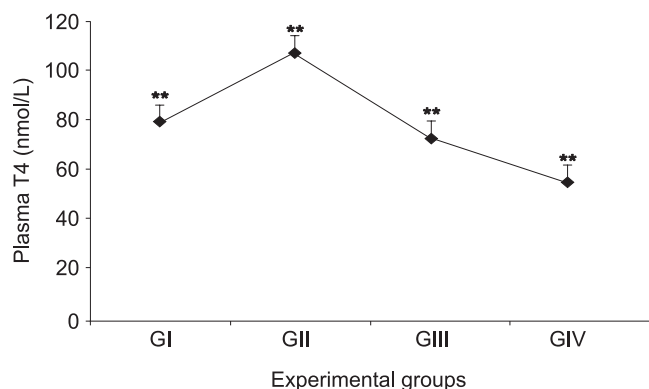


Fig. 2. Effect of short term exposure to different environmental temperatures on Plasma T₄ (nmol/L) concentrations in Malpura ewes. G 1, control; G 2, at 23°C; G 3, at 40°C; G 4, 42°C. ** Indicates level of significance @ P<0.01. Plasma T₄ concentration showed highly significant (P<0.01) effect between the groups. The highest T₄ concentration was recorded in G 2 while the lowest being in G 4.

as that of T₃ for the treatment with the significant (P < 0.01) increase in G 2 and significant (P < 0.01) decrease in G 3 and G 4. In addition, both experimental days and interaction between treatment and experimental days had highly significant (P < 0.01) influence on plasma T₄. Fig. 3 describes the effect of exposure to different temperature on plasma cortisol level. Plasma cortisol showed reverse trend to that of thyroid hormones level. Cortisol level in G 2 significantly (P < 0.01) decreased while significantly (P < 0.01) increased in G 3 and G 4 as compared to G 1. In addition, both experimental days and interaction between treatment and experimental days had highly significant (P < 0.01) influence on plasma cortisol level.

The present study established the effect of exposure to both lower and higher temperature stress in sheep. Heat stress is the major limiting factor affecting livestock production throughout the world (Sejian 2012). The ewes in the present study were able to cope up better to low temperature exposure. However, as the upper critical temperature increased, the animals suffered severely due to heat stress. This is evident from the significant alteration in blood metabolites and endocrine parameters after exposure to heat stress.

Both Hb and PCV increased significantly after exposure to higher temperatures in the present study. But the magnitude of difference was more in G 4 group as compared to G 3 group. The reason for the significant increase in both G 3 and G 4 ewes as compared G 1 and G 2 could be due to severe dehydration as a result of both respiratory and cutaneous evaporation in these ewes. Mc Manus *et al.* (2009) reported a strong positive correlation between Hb and PCV concentration indicating the significance of these parameters for heat tolerance in Brazilian sheep. The higher PCV value was reported to be an adaptive mechanism to provide water necessary for evaporative cooling process (Al-Haidary 2004). Plasma glucose differed significantly between the groups for

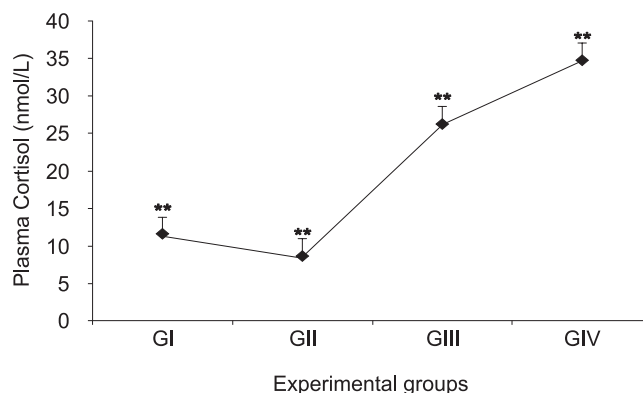


Fig. 3. Effect of short term exposure to different environmental temperatures on plasma cortisol (nmol/L) concentrations in Malpura ewes. G 1, control; G 2, at 23°C; G 3, at 40°C; G 4, 42°C. ** Indicates level of significance @ P<0.01. Plasma cortisol concentration showed highly significant (P<0.01) effect between the groups. The highest cortisol concentration was recorded in G 4 while the lowest being in G 2

the treatment, experimental days and their interaction. The highest plasma glucose concentration was established in G 4. The reason for this could be the higher requirement of energy source in the form of glucose to support effort of physiological mechanisms for thermoregulation. Further, cortisol levels in these ewes are very high which favours hepatic gluconeogenesis to supply more glucose for the respiratory muscular activities to dissipate more heat (Sejian and Srivastava 2010, Sejian *et al.* 2010a).

Plasma total cholesterol significantly increased only in G 4 ewes while there was negligible difference among G 1, G 2 and G 4 ewes. This shows the severity of heat stress at 42°C. The reduced blood glucose level initiates the hepatic gluconeogenesis as a adaptive mechanism for regular supply of energy (Sejian *et al.* 2010a). The highly significant increase in circulating cholesterol might be to support the hepatic gluconeogenesis to supply glucose for adaptive mechanisms. The very high cortisol concentration in these ewes supports this justification. Total plasma protein, albumin and globulin showed similar trend of decreased level in G 3 and G 4. However, this effect is only significant for plasma protein and albumin. The significantly reduced total plasma protein in the heat stressed groups is to support gluconeogenesis to maintain the energy for thermoregulatory process (Sejian *et al.* 2010a). Further, one of the principal functions of cortisol in ruminant species is to favour protein catabolism to supply regular energy for vital body functions (Sejian and Srivastava 2010).

The major exogenous regulator of thyroid gland activity is the environmental temperature (Dickson 1993), so an inverse relationship between ambient temperature and blood thyroid hormone concentrations has been found in sheep (Starling *et al.* 2005; Todini, 2007). Similarly in the present study also plasma thyroid hormones reduced significantly in both the heat stress groups. But the magnitude of reduction

is higher in G 4 as compared to G 3. This shows the difference in adaptive capability of sheep based on the environmental temperature they are exposed to. The significantly lower level of thyroid hormone in heat stress groups indicates that this effect is mediated to reduce the metabolic heat production to prevent additional heat load on these animals. Further, the significant increase in thyroid hormone concentration in G 2 as compared to all other groups indicates the higher metabolic rates in G 2 ewes to produce more heat to cope up to low temperature. Plasma cortisol showed highly significant changes in the heat stress groups as compared to G 1 and G 2. Although both heat stress groups showed significant increase in cortisol level, the level was significantly higher in G 4 as compared to G 3. This shows the severity of heat stress at 42°C as compared to 40°C. The association between heat stress and increased secretion of cortisol, the principal glucocorticoid hormone in small ruminants, is well documented (Ali and Hayder 2008, Sejian *et al.* 2008, Sejian *et al.* 2010a). The significantly lower cortisol concentration in G 2 as compared to G 3 and G 4 indicates the significance of heat stress and this proves that cortisol is paramount in mediating the heat stress relieving effects in sheep.

The data obtained from the study indicated that Malpura ewes have the capability to alter their adaptive capability on exposure to different environmental temperatures. This is evident from the significant alteration in blood metabolites and endocrine response between the groups. Further, on comparison between the two heat stress groups, the effects were more severe when exposed to 42°C than at 40°C. This is evident from the significantly higher level of cortisol and significantly lower thyroid hormone concentrations in G 4 as compared to G 3 ewes. This shows that each degree increase in upper critical temperature might be very detrimental to the survival of these animals.

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