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Effect of cold stress on growth, physiological responses, blood metabolites and hormonal profile of native Malpura lambs under hot semi-arid tropics of India

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ABSTRACT

The study was conducted to determine the effect of cold stress on growth, physiological responses, blood metabolites and hormones. Growing native Malpura lambs (18), 45 to 60 days of age, 14.8 kg body-weight, were used in this study. The lambs were randomly divided into 2 groups as group 1 (9) and group 2 (9). The experiment was conducted from mid-January to mid-February when the environmental temperature was at the lowest. The lambs of G_1 were kept under the asbestos shed open from the sides while the lambs of G_2 were provided protection against the cold weather. The body weight (kg), physiological responses i.e. respiration rate (RR, breath/min), pulse rate (PR, beat/min) and rectal temperature (RT,°C) were recorded at fortnightly interval. The blood samples were also collected at fortnightly interval and analyzed for packed cell volume (PCV,%), haemoglobin (Hb, g/dl) and glucose (mg/dl). Besides the T_3 (nmol/l), T_4 (nmol/l) and cortisol (nmol/l) were also estimated. The lambs of G_1 had higher RR, PR and RT than the G_2 lambs. A significant increase in the PCV, Hb and glucose was observed in G_1 lambs as compared to G_2. The ADG was significantly higher in G_2 lambs as compared to G_1 lambs.

Key words: Blood metabolites, Body weight, Hormones, Malpura lambs, Physiological responses

In the arid and semi-arid region of India, sheep are generally exposed to various climatic extremes like elevated temperature, feed and water scarcity during grazing (Maurya *et al.* 2004, Sejian *et al.* 2010). In these regions of India, most of the lambs are born in January and February, and were exposed to cold stress. New born lambs are more likely to be at risk from cold exposure and the lamb mortality by hypothermia is the main cause of economic loss in most of the sheep production systems (Slee *et al.* 1987). During early post-natal life, homeotherms undergo marked developmental changes to control their body temperature (Symonds *et al.* 1992). Apart from fluctuations in the physiological and biochemical profiles, T_3 and T_4 also play a major role in thermogenesis (Doubek *et al.* 2003). As energy intake is used mainly for thermoregulation, it is possible to observe a depression in body weight gain and an increase in mortality (Slee *et al.* 1980, Duddy *et al.* 2007).

Low survival rate because of cold stress is one of the main factors that adversely affect lamb production and resulting into sizeable economic losses in sheep farming. The degree to which lambs survive to marketable age is one of the key indicators of efficiency of sheep production. The objectives of this study are: (i) to examine the effect of cold stress on physiological adaptability, (ii) to assess the magnitude of blood and hormonal profile variation due to cold stress, and (iii) to assess the consequence of these factors with respect to lamb growth.

MATERIALS AND METHODS

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 75º 28´E and the latitude of 26º 26´N and at altitude of 320 m above mean sea level. The mean relative humidity ranged between 21 and 86%. The maximum and minimum ambient temperature ranged between 4° and 46.5ºC. The annual rainfall in this area ranges from 250 to 450 mm with an erratic distribution throughout the year. The experiment was initiated during January to February. During study period mean environmental temperature and relative humidity ranged between 12.9 and 22.4°C and 48.1 and 67.7%, respectively.

Eighteen growing Malpura lambs (45 to 60 days old) with mean body weight of 14.8 kg were randomly divided into group 1 (9) and group 2 (9). The experiment was conducted from mid-January to mid-February when the environmental

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temperature was at the lowest. The lambs of G_1 were kept under the asbestos shed open from the sides while the lambs of G_2 were provided protection against the cold weather. The lambs kept under enclosure having a roof and side enclosure of gunny bags and cotton tarpaulin. The floor was covered by wheat straw and dry leaves bedding. The bedding of lambs was changed daily by new wheat straw and dry leaves. Uninterrupted light arrangement was made in the closed shed.

The body weight (kg), physiological responses i.e. respiration rate (RR, breath/min), pulse rate (PR, beat/min) and rectal temperature (RT,°C) were recorded at fortnightly interval. The blood samples were also collected at fortnightly interval and analyzed for packed cell volume (PCV,%), haemoglobin (Hb, g/dl) and glucose (mg/dl). T_3 (nmol/l), T_4 (nmol/l) and cortisol (nmol/l) were also estimated. Hormonal parameters such as cortisol (analytical sensitivity 10 nM; the intra-assay and inter-assay coefficient of variations 5.8% and 9.2%, respectively), T_4 (analytical sensitivity 13 nmol/l; intra-assay and inter-assay coefficient of variations 5.1 and 8.6%, respectively), T_3 (analytical sensitivity 0.1 nmol/l; intra-assay and interassay coefficient of variations 3.3% and 8.6%, respectively), were estimated by RIA using the gamma counter, employing RIA kits. All hormonal parameters were estimated by radio immuno assay (RIA) using a gamma counter.

Data were presented as mean±SEM and statistical analysis were carried out using SPSS software programme, version 10.0. Level of statistical significance was set at $P < 0.05$. *Post hoc* analyses were performed using Duncan's multiple range tests for pair-wise comparisons.

RESULTS AND DISCUSSION

The meteorological data of experimental period are given in Table 1. The open shed temperature was significantly (P<0.05) lower at 4.00 h than 8: 00 h and 14: 00 h respectively. The temperature during 8: 00 h and 14: 00 h differed significantly (P<0.05) in open shed. The dry bulb and wet bulb in open shed were significantly (P<0.05) lower at 4.00 h as followed by to 8: 00 h and 14: 00 h. The relative humidity in open shed was significantly (P<0.05) higher at 4.00 h as compared to 8: 00 h and 14: 00 h. In protected shed the environmental temperature, wet bulb and dry bulb temperature and relative humidity did not differ significantly at 4.00 and 8.00 h however, at 14.00 h these values differed significantly (P<0.05). The values presented in Table 1 showed that the lambs in open were in extreme cold stress during night.

The effect of cold stress on body weight and average daily gain is presented in Table 2. The initial and final body weight showed a significant (P<0.05) difference. A significant (P<0.05) increase in the average daily gain of G2 lambs was obtained as compared to G_1 lambs. The above result depicts a positive average daily gain in the G_2 lambs as a result of their protection against the cold stress. In agreement with our finding Symonds *et al.* (1995) also reported higher daily weight gain in lambs maintained in comfortable temperature than the lambs exposed to cold.

The mean values of physiological responses (RR, PR and RT) of lambs in G_1 and G_2 are presented in Table 3. A significant increase (P<0.05) in RR from morning to afternoon in all the groups of lambs were observed but the rate of increase was greater for G_1 as compared to G_2 lambs. The PR also used to increase significantly (P<0.05) from morning to afternoon. But among the group of lambs PR did not differ significantly. Same trend was also obtained for the RT from morning to evening fluctuations. The increase in ambient temperature from morning to afternoon was in agreement with the finding of Maurya *et al.* (2007). The above physiological responses were consistently higher in the lambs exposed to cold stress. The reason for this could be that the homoeothermic animals initially react to cold stress by enhancing the thermoregulatory mechanism, such as increase in RR and PR, to avoid undesirable increase in RT. Slee *et al.* (1991) and Muller and McCutcheon (1991) also reported same finding while working on new born Merino lambs.

The effect of cold stress on the changes in blood profile of lambs is presented in Table 4. The PCV, Hb and glucose concentration were significantly (P<0.05) higher in cold stressed lambs as compared to lambs protected against cold stress. The reason for the increase in PCV and Hb in the G_1 lambs may be due to increase in the synthesis of RBC and

Table 1. Climatological data during the cold stress experimental period

	Time of recording	Temperature (ºC)	Dry bulb temperature (ºC)	Wet bulb temperature (ºC)	RH (%)
Open	Night (4.00 h)	3.2±0.12^{c}	2.8±0.09^{c}	2.3±0.08^{c}	92.5±4.12^{a}
	Morning (8: 00 h)	12.9±0.92^{b}	10.6±0.91^{b}	9.6±0.86^{b}	67.7±3.53^{b}
	Afternoon (14: 00 h)	21.9±0.89^{a}	20.9±1.55^{a}	15.1±0.80^{b}	48.1±3.6^{c}
Closed	Night (4.00 h)	10.2±0.62^{b}	9.5±0.75^{b}	11.8±1.02^{b}	75.12±3.16^{b}
	Morning (8: 00 h)	14.4±0.54^{b}	13.2±0.62^{b}	15.3±0.72^{b}	69.5±2.84^{b}
	Afternoon (14: 00 h)	24.5±1.17^{a}	21.7±1.26^{a}	22.9±0.22^{a}	52.7±4.02^{c}

$^{a\ bc}$ means with different superscript in same column differ significantly (P<0.05).

Hb to maintain the homeostasis. The increase in glucose levels in cold stressed newborn lambs is to produce heat for the neonate (summit metabolism). This increase in glucose comes at the price of depleting glycogen stores. The increase in glucose level in the cold stressed lambs may be due to requirement of more energy to fight against the cold stress. The mobilization of lipid and glycogen to provide energy precursors needed for thermogenesis (Himms-Hagen 1990) which inturn elevates glucose level in the hypothermic calves and is associated with an increase in rectal temperature in calves (Godfrey *et al.* 1991) as also reported in our experiment.

The mean value of plasma T_3 and T_4 profile is given in Table 5. At day 15 and day 30 of the experiment, the mean plasma concentration of T_3 was higher (P<0.05) in the cold stressed lambs as compared to protected lambs. Changes in T_3 were also accompanied by similar alterations in plasma T_4 concentration in both the group of lambs. Tilders *et al.* (1982) also reported higher level of T_3 and T_4 in lambs

Table 2. Effect of cold stress on body weight of growing lambs

Parameter		Initial (kg)	Final (kg)
Body weight	Open	14.89±0.62^{Ba}	17.12±0.58^{Ba}
	Closed	14.70±0.48^{Ba}	18.76±0.36^{Aa}
ADG	Open	-	74.3±5.69 ^{b}
	Closed	-	135.3±7.12 ^{a}

$^{a\ b}$means with different superscript in same column differs significantly (P<0.05). ^{AB} means with different superscript in same row differs significantly (P<0.05).

Table 3. Effect of cold stress on physiological responses of growing lambs

Parameter		Day 0		Day15		Day 30	
		Morning	Afternoon	Morning	Afternoon	Morning	Afternoon
RR	Open	35.33±2.00^{Ba}	62.44±3.60^{Aa}	41.56±1.09^{Ba}	60.00±1.20^{Aa}	39.33±2.13^{Ba}	64.33±3.72^{Aa}
	Closed	32.00±1.00^{Ba}	57.78±1.96^{Aa}	37.56±1.04^{Ba}	56.22±3.19^{Aa}	36.22±1.43^{Ba}	61.78±2.61^{Aa}
PR	Open	92.33±5.03^{Ba}	101.33±2.75^{Aa}	91.33±2.54^{Ba}	102.89±1.67^{Aa}	94.00±1.41^{Ba}	102.67±1.57^{Ab}
	Closed	85.56±2.98^{Ba}	98.44±2.42^{ABa}	84.78±2.11^{Ca}	96.89±3.25^{ABa}	86.44±3.71^{Bb}	99.44±4.99^{Aa}
RT	Open	101.76±0.18^{Ca}	103.18±0.15^{Aa}	102.84±0.18^{Ba}	103.56±0.21^{Aa}	102.51±0.12^{Aa}	103.09±0.09^{Aa}
	Closed	101.36±0.22^{Ca}	102.71±0.08^{Bb}	101.99±0.20^{Cb}	103.04±0.07^{Aa}	101.69±0.12^{Bb}	102.64±0.13^{Ab}

$^{a\ b}$means with different superscript in same column differs significantly (P<0.05) for the individual response. $^{A\ B}$means with different superscript in same row differs significantly (P<0.05).

Table 4. Effect of cold stress on blood metabolites of growing lambs

Parameter		Day 0	Day15	Day 30	Overall
PCV%	Open	38.26±0.71^{Ba}	42.80±0.87^{Aa}	41.94±0.98^{Aa}	41.00±0.23^{Aa}
	Closed	37.01±1.35^{Aa}	38.12±1.29^{Ab}	37.46±1.26^{Ab}	37.53±0.08^{Ab}
Hb (g/dl)	Open	10.85±0.30^{Ba}	12.70±0.19^{Aa}	13.12±0.38^{Aa}	12.23±0.16^{Aa}
	Closed	10.79±0.21^{Aa}	11.27±0.32^{Ab}	10.94±0.41^{Ab}	11.00±0.17^{Ab}
Glucose (mg/dl)	Open	54.89±1.66^{Ba}	60.64±1.13^{Aa}	62.16±1.11^{Aa}	59.23±0.54^{Aa}
	Closed	55.44±1.06^{Aa}	54.37±1.04^{Ab}	55.22±1.15^{Ab}	55.01±0.10^{Ab}

$^{a\ b}$means with different superscript in same column differs significantly (P<0.05) column for individual traits. ^{AB}means with different superscript in same row differs significantly (P<0.05).

Table 5. Effect of cold stress on hormonal profile of growing lambs

Parameter		Day 0	Day15	Day 30	Overall
T3 (nmol/l)	Open	2.24±0.10^{Ba}	3.60±0.19^{Aa}	3.45±0.25^{Aa}	3.10±0.13^{Aa}
	Closed	2.15±0.12^{Aa}	1.98±0.11^{Ab}	2.11±0.15^{Ab}	2.08±0.04^{Ab}
T4(nmol/l)	Open	89.72±4.15^{Ba}	112.67±2.52^{Aa}	109.41±2.87^{Aa}	103.93±1.48^{Aa}
	Closed	86.45±2.55^{Aa}	91.08±3.86^{Ab}	88.91±2.13^{Aa}	88.82±1.56^{Ab}
Cortisole(nmol/l)	Open	21.63±0.53^{Ba}	37.40±0.86^{Aa}	39.33±1.03^{Aa}	32.79±0.44^{Aa}
	Closed	20.86±0.30^{Aa}	21.10±0.42^{Ab}	19.83±0.75^{Ab}	20.60±0.40^{Ab}

$^{a\ b}$ means with different superscript in same column significantly (P<0.05) for individual traits differs.^{AB} means with different superscript in same row differs significantly (P<0.05).

exposed to cold stress. A significant ($P<0.05$) increase in cortisol concentration was also obtained in G_1 lambs as compared to G_2 lambs which were kept protected against the cold stress. The increased level of cortisol in lambs exposed to cold stress may be due to increase in lipolysis and utilization of brown adipose tissue for heat production. Our findings also support the finding of Bassett and Alexander (1971) and Steffens and de Boer (1999). They reported that when lambs are exposed to short duration of stress glucose level in the blood of lambs increases. But in chronic cold stress, they did not found any significant increase in the glucose concentration in the lambs.

Our results indicated that the cold stress influences the physiological adaptability, blood metabolites and hormonal levels in the growing lambs. The cold stress reduces the average daily weight gain in the growing lambs which in turn decreases the body weight of lambs. Further long-term study is needed to provide greater insight into the adaptive changes in the lambs as consequence of exposure to cold stress especially in relation to physiological, blood metabolites and hormonal function. The alteration in these vital functions in the lambs affects growth and reproductive efficiency of animals in later stage of life.

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