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Effect of walking and summer stress on physiological, heamatological and antioxidant profiles in mithun bulls

P PERUMAL[1], M H KHAN[2], SUKKUM CHANG[3] and C T R SANGMA[4]

ICAR-National Research Centre on Mithun, Jharnapani, Nagaland 797 106 India

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ABSTRACT

The study was conducted to evaluate effect of walking stress on physiological, heamatological and antioxidant profiles in mithun bulls. Mithun bulls (12) of adult age of 4 to 6 yr (500–600 kg body weight) with body condition score 5–6 were selected and divided into group 1 (6): control (not exposed to walking and thermal stress) and group 2 (6): treatment (exposed to walking and thermal stress). The treatment group was allowed to walk 15 km to and fro from the farm without allowing grazing. The control animals were kept in the adjacent shed covered with asbestos sheets and surrounded by big trees. The time of walking was from 0900 h to 1400 h. The experiment was conducted during summer (May to July). Immediately after stress, the animals were restrained and physiological parameters such as rectal temperature (RT), respiration rate (RR), pulse rate (PR), heart rate (HR), skin temperature (ST) were measured. Meanwhile, blood samples were collected to study the heamatological such as red blood cells (RBC), haemoglobin (Hb), erythrocyte sedimentation rate (ESR), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and antioxidant profiles such as reduced glutathione (GSH), glutathione reductase (GSHRx), superoxide dismutase (SOD), catalase (CAT), total antioxidant capacity (TAC) and lipid peroxide such as malondialdehyde (MDA). The result revealed that physiological, heamatological profiles and MDA were significantly higher and antioxidant profiles were significantly lowered in stressed animals than in unstressed animal group. It was concluded that the walking stress and hot summer stress has significantly affected the performance of mithun.

Key words: Antioxidants, Heamatological profiles, Mithun, Physiological, Summer stress, Walking stress

Population of mithun (*Bos frontalis*), a mountain cattle and a bovine species available in the north-eastern hill region of India, is decreasing gradually due to lack of suitable breeding bulls, increase in intensive inbreeding practices, declining land area for grazing and lack of suitable breeding and feeding management in mithun rearing region (Livestock Statistics 2007). So it is high time to make efforts to conserve and preserve the mithun population through proper management and enhance the socio-economic and cultural status of the tribal populations.

Mithun is semi-wild hilly mountain bovine species (Simoons 1984) and walks long distance to get adequate feed in the hilly areas especially in summer and winter. Moreover, availability of feed to grazing animals is often of very low quality and also available at low densities per unit area and the time of grazing is limited only in day time exposed to solar radiation with fly and insect irritation (Manteca and Smith 1994). Depending on availability of feed in the livestock farm, the mithun has to walk long distance to get sufficient feed. Restricting or reducing the distance of walking or grazing improved the performance of animals (Gustafson *et al.* 1993) and also stress on the animals is dependent upon the breed difference in livestock species (D'Hour *et al.* 1994). Perusal of literature revealed that no information is available related to the exercise or walking stress in mithun species. Therefore, the present study was designed to evaluate the effect of walking and thermal stress in terms of physiological, heamatological and antioxidant profiles in mithun bulls.

MATERIAL AND METHODS

Location: The experiment was carried out at the mithun research farm, which is located between 25°54'30" North latitude and 93°44'15" East longitude and at an altitude range of 250–300 mean sea level. The average annual minimum and maximum ambient temperature ranges between 6 and 46°C. The present experiment was carried out during May to July. Ambient temperature and relative humidity values were obtained from the meteorology station of ICAR Research Complex, Nagaland Centre located at close proximity for calculation of Temperature Humidity Index. THI was calculated by using the following formula (Kadzere *et al.* 2002). *THI = 0.72 (W +D) + 40.6 (W: Wet bulb temperature (°C) and D: dry bulb temperature (°C).*

Experimental animals: Apparently healthy mithun bulls

Present address: [1–4]Scientist, Animal reproduction Laboratory (perumalponraj@gmail.com).

Months	Dry bulb temperature	Wet bulb temperature	Maximum temperature (°C)	Minimum temperature (°C)	Relative humidity (%)	THI value
May	23.81	20.78	32.43	21.88	76	72.70
June	26.23	23.75	32.99	24.75	81	76.59
July	26.27	24.12	32.02	25.20	83	76.88

(12) of approximately 4 to 6 yr of age (500–600 kg body weight) with good body condition (score 5–6) were selected from the herd derived from various hilly tracts of the NEH region of India and maintained under uniform feeding, housing and lighting conditions. The experimental animals were maintained under proper hygienic conditions. The prophylactic measures like deworming and vaccination were undertaken properly as per the farm schedule. The experiment was divided group 1, control (not exposed to walking stress); and group 2, treatment (exposed to walking stress).

Housing management: The animal house was made up of asbestos-roof, and half of the wall made up of wire mesh and half was by the bricks wall in all four sides of the shed which is surrounded by the trees. The experimental animals were kept under uniform feeding, watering and managemental conditions. At 0900 h, both groups of mithun bulls were removed from the shed. At 1400 h, the animals were restrained in their original positions in the shed. This procedure was followed throughout the study period.

Experimental procedure: The present study was conducted for 7 weeks during the summer (May –July). The bulls were randomly selected into 2 groups of 6 animals each: group 1 (6; control), and group 2 (6; walking stress). The animals were maintained under a semi-intensive system of management. Both groups were stall-fed at 0700 h and allowed access to feed and water up to 0900 h. The walking stress group (group 2) bulls were made to walk 15 km. The bulls were subjected to walking stress (group 2) were prevented from grazing by applying a face mask made of cotton thread. Blood samples were collected from the jugular vein at weekly intervals to study the effects of walking stress on the physiological, heamatological and antioxidant profiles.

Blood collection and plasma separation: Blood samples (7 ml) were collected at weekly intervals from both the groups simultaneously from the external jugular vein in tubes with heparin anticoagulant. Blood samples were collected at 1400 h immediately after completing the walking. Plasma was separated from blood by centrifugation at 3500g at room temperature for 20 min. The plasma was divided into aliquots in microcentrifuge tubes and kept frozen at “80°C until further analysis. Plasma samples were used to estimate the antioxidant profiles.

Parameters studied: Physiological responses such as RT, RR, PR, HR and ST (measured by keeping the thermometer between the skin fold in the body surface, pelvic and pectoral cradle and make average) were recorded twice daily at 0800 and 1400 h. Blood parameters such as RBC, Hb, ESR, PCV, MCV, MCH and MCHC were estimated using whole blood samples using standard methods (Sastry 1982). Antioxidant profiles such as GSH, GSHRx, SOD, CAT and TAC and lipid peroxide such as MDA were estimated using commercially available diagnostic kits.

Statistical analysis: The results were analysed statistically and expressed as the mean±SEM. Means were analyzed by one way analysis of variance (ANOVA), followed by the Tukey’s post hoc test to determine significant differences among the weeks and student “t” test between the treatment and control groups in different weeks of experiment using the SPSS/PC computer program. Differences with values of P< 0.05 were considered to be statistically significant by using SPSS 15.

RESULTS AND DISCUSSION

The effects of walking stress on physiological (Table 1) and heamatological (Table 2) profiles for different weeks between the groups are presented in tables and correlation coefficient between the heamatological and physiological parameters were presented in both stressed (Table 3) and unstressed (Table 4) animal groups.

Physiological profiles

The physiological profiles such as RT, RR, PR, HR and ST revealed that there was a significant (P<0.05) difference between the experimental groups in different weeks of the experimental periods and also a significant (P<0.05) difference was observed among the experimental weeks. Between the experimental groups, stressed animals suffered significantly (P<0.05) than in stressed animal groups. The present study helps understand the effect of walking stress on production performance and to maintain the mithun in efficient management condition.

There is paucity of information on effect of walking stress on the physiological, haematological and antioxidant profiles in the mountain cattle species.

The physiological parameters such as RT, RR, PR, HR and ST increased significantly (P<0.05) in mithun affected with walking stress. Respiration rate was affected to a greater extent after draught work followed by pulse rate and rectal temperature in bovine species. The RT and RR as the suggestive marker of stress in domestic livestock species (Daramola and Adeloye 2009, Sejian *et al.* 2010b). In caprine and bovine, similar reports that RT and RR increased as the animal was going to exercise (Kasa *et al.* 1995, Coulon and Pradel 1997). Based on the previous literature in other animal species, it was suggested that the mithun undergone walking and grazing suffered severe

Table 1. Mean (±SE) physiological parameters of mithun bulls exposed to walking stress at different weeks

Parameters	Experimental periods							Overall	
	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week		
RT	GI	99.52±1.03$^{a A}$	99.66±1.09$^{a A}$	99.96±0.90$^{ab A}$	99.20±1.06$^{a A}$	100.62±0.96$^{b A}$	99.35±0.91$^{a A}$	100.11±0.88$^{ab A}$	99.77±0.70^{A}
	GII	103.57±1.04$^{c B}$	102.53±1.12$^{ab B}$	102.34±1.11$^{a B}$	102.60±1.00$^{abc B}$	103.60±0.83$^{c B}$	102.10±0.99$^{a B}$	103.39±0.93$^{bc B}$	102.88±0.79^{B}
RR	GI	27.50±1.20$^{ab A}$	27.60±1.04$^{ab A}$	28.10±1.09$^{ab A}$	27.00±1.15$^{a A}$	28.80±1.30$^{b A}$	27.50±1.13$^{ab A}$	28.40±1.36$^{ab A}$	27.84±0.79^{A}
	GII	42.40±1.23$^{c B}$	39.80±1.30$^{b B}$	37.40±1.16$^{a B}$	39.60±1.51$^{b B}$	43.40±1.40$^{c B}$	37.60±1.26$^{a B}$	43.40±1.47$^{c B}$	40.51±1.60^{B}
PR	GI	64.90±1.09$^{ab A}$	65.10±1.17$^{ab A}$	65.20±1.15$^{ab A}$	64.20±1.11$^{a A}$	66.50±1.26$^{c A}$	64.70±0.82$^{ab A}$	65.80±1.02$^{bc A}$	65.20±0.87^{A}
	GII	75.90±1.09$^{b B}$	74.40±1.23$^{a B}$	73.00±1.37$^{a B}$	73.30±1.08$^{a B}$	77.10±1.29$^{b B}$	73.30±0.97$^{a B}$	76.10±1.26$^{b B}$	74.73±1.28^{B}
HR	GI	72.10±0.94$^{a A}$	72.40±0.92$^{ab A}$	72.30±0.97$^{ab A}$	72.50±0.99$^{ab A}$	72.60±1.04$^{ab A}$	72.30±1.08$^{ab A}$	73.20±1.07$^{b A}$	72.49±0.59^{A}
	GII	82.00±1.25$^{bc B}$	80.30±1.22$^{a B}$	80.40±1.20$^{a B}$	80.70±1.25$^{ab B}$	82.50±1.36$^{cd B}$	80.10±1.40$^{a B}$	83.70±1.16$^{d B}$	81.39±1.17^{B}
ST	GI	98.49±1.10$^{a A}$	98.62±1.18$^{ab A}$	99.13±1.08$^{ab A}$	98.05±1.14$^{a A}$	99.77±1.06$^{b A}$	98.68±1.08$^{ab A}$	98.84±1.10$^{ab A}$	98.80±0.74^{A}
	GII	101.60±1.11$^{bc B}$	101.51±1.17$^{abc B}$	101.23±1.13$^{abc B}$	100.36±1.08$^{a B}$	102.10±0.96$^{c B}$	100.70±1.12$^{ab B}$	101.63±1.02$^{bc B}$	101.30±0.77^{b}

RT, Rectal temperature (°F); RR, respiration rate (beats/min); PR, pulse rate (beats/ min); HR, heart rate (beats/ min); and ST, skin temperature (°F). GI, Unstressed animal group; GII, stressed animal group. Within rows, means with different letters (a, b, c, d) differ significantly ($P < 0.05$). Within columns, means with different letters (A, B) differ significantly ($P < 0.05$) between the stressed and unstressed for the particular parameter

Table 2. Mean (±SE) heamatological parameters of mithun bulls exposed to walking stress at different weeks

Parameters	Experimental periods							Overall	
	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week		
RT	GI	99.52±1.03$^{a A}$	99.66±1.09$^{a A}$	99.96±0.90$^{ab A}$	99.20±1.06$^{a A}$	100.62±0.96$^{b A}$	99.35±0.91$^{a A}$	100.11±0.88$^{ab A}$	99.77±0.70^{A}
TRBC	GI	5.44±0.77$^{a A}$	5.43±0.79$^{a A}$	5.93±0.76$^{ab A}$	5.91±0.73$^{ab A}$	6.32±0.76$^{b A}$	5.56±0.77$^{a A}$	6.18±0.78$^{b A}$	5.83±0.60^{A}
	GII	7.47±0.86$^{ab B}$	6.95±0.81$^{a B}$	7.29±0.77$^{a B}$	7.06±0.61$^{a B}$	8.14±0.76$^{c B}$	6.89±0.76$^{a B}$	7.94±0.75$^{bc B}$	7.39±0.70^{B}
HB	GI	10.92±0.78$^{a A}$	11.13±0.69$^{b A}$	11.33±0.81$^{ab A}$	11.03±0.91$^{b A}$	11.88±0.80$^{b A}$	10.89±0.80$^{a A}$	11.42±0.76$^{ab A}$	11.23±0.59^{A}
	GII	12.88±0.82$^{bc B}$	12.39±0.73$^{ab B}$	12.13±0.76$^{a B}$	12.12±0.91$^{a B}$	13.45±0.84$^{c B}$	11.85±0.88$^{a B}$	12.98±0.78$^{bc B}$	12.54±0.76^{B}
ESR	GI	4.70±0.59$^{ab A}$	4.73±0.64$^{ab A}$	4.84±0.62$^{ab A}$	4.90±0.67$^{ab A}$	5.35±0.62$^{c A}$	4.55±0.62$^{a A}$	5.01±0.65$^{bc A}$	4.87±1.51^{A}
	GII	5.47±0.61$^{a B}$	5.33±0.57$^{a B}$	5.49±0.66$^{a B}$	5.61±0.68$^{a B}$	6.43±0.65$^{c B}$	5.22±0.59$^{a B}$	5.99±0.67$^{b B}$	5.65±0.65^{B}
PCV	GI	32.20±0.97$^{ab A}$	32.43±1.08$^{ab A}$	32.49±1.11$^{ab A}$	32.85±0.95$^{b A}$	32.85±0.89$^{b A}$	31.65±1.00$^{a A}$	31.82±0.77$^{a A}$	32.33±0.68^{A}
	GII	36.77±1.02$^{c B}$	36.94±1.09$^{c B}$	36.20±1.13$^{bc B}$	33.57±0.94$^{a B}$	36.91±1.21$^{c B}$	35.33±0.91$^{b B}$	36.44±0.94$^{c B}$	36.02±1.10^{B}
MCV	GI	59.9±2.85$^{b A}$	60.63±2.99$^{b A}$	55.40±2.65$^{ab A}$	56.11±2.53$^{ab A}$	52.41±2.35$^{a A}$	57.60±2.72$^{ab A}$	51.99±2.37$^{a A}$	56.30±1.84^{A}
	GII	49.75±2.44$^{abc B}$	53.69±2.50$^{c B}$	50.06±2.33$^{abc B}$	47.68±1.74$^{ab B}$	45.54±1.94$^{a B}$	51.59±2.14$^{bc B}$	46.12±1.98$^{a B}$	50.06±1.99^{B}
MCH	GI	20.37±1.80^{A}	20.79±1.73^{A}	19.30±1.56^{A}	18.88±1.69^{A}	18.96±1.51^{A}	19.82±1.65^{A}	18.68±1.52^{A}	19.54±0.90^{A}
	GII	17.45±1.55^{B}	18.01±1.49^{B}	16.75±1.28^{B}	17.21±1.24^{B}	16.62±1.31^{B}	17.34±1.47^{B}	16.44±1.30^{B}	17.12±0.74^{B}
MCHC	GI	33.89±0.97$^{a A}$	34.33±1.12$^{a A}$	34.88±1.28$^{abc A}$	33.54±1.31$^{a A}$	36.16±1.28^{c}	34.42±1.38$^{ab A}$	35.89±1.28^{bc}	34.73±0.99
	GII	35.01±0.99$^{ab B}$	33.54±1.02$^{a B}$	33.53±1.28$^{a B}$	36.07±1.28$^{b B}$	36.46±1.34^{b}	33.56±1.51$^{a B}$	35.62±1.20^{b}	34.83±1.13

RBC, Red blood cells ($\times10^6$/ mm^3); HB, haemoglobin (g/dl); ESR, erythrocyte sedimentation rate (mm/hr); PCV, packed cell volume (%); MCV, mean corpuscular volume (µ3); MCH, mean corpuscular haemoglobin (µg); MCHC, mean corpuscular haemoglobin concentration (%). G 1, unstressed animal group; G 2, stressed animal group. Within rows means with different letters (a, b, c, d) differ significantly ($P < 0.05$). Within columns means with different letters (A, B) differ significantly ($P < 0.05$) between the stressed and unstressed for the particular parameter.

Table 3. Correlation coefficients among the physiological and heamatological parameters of unstressed mithun bulls

	RT	RR	PR	HR	ST	TRBC	HB	ESR	PCV	MCV	MCH	MCHC
RT	1	0.98**	0.98**	0.46	0.92**	0.72*	0.95**	0.81**	0.22	–0.68	–0.35	0.93**
RR		1	0.97**	0.50	0.92**	0.71*	0.90**	0.73*	0.04	–0.71*	–0.40	0.97**
PR			1	0.48	0.90**	0.66	0.90**	0.77*	0.10	–0.63	–0.30	0.95**
HR				1	0.19	0.69	0.51	0.54	–0.16	–0.75	–0.66	0.65
ST					1	0.59	0.86*	0.66	0.16	–0.56	–0.26	0.86**
TRBC						1	0.84*	0.89**	0.32	–0.97**	–0.90**	0.76*
HB							1	0.93**	0.42	–0.76*	–0.53	0.88**
ESR								1	0.57	–0.78*	–0.65	0.72*
PCV									1	–0.10	–0.18	–0.05
MCV										1	0.92**	–0.78*
MCH											1	–0.49
MCHC												1

RT, rectal temperature; RR, respiration rate; PR, pulse rate; HR, heart rate; ST, skin temperature; TRBC, total red blood cells; HB, heamoglobin; ESR, erythrocyte sedimentation rate; PCV, packed cell volume; MCV, mean corpuscular volume; MCH, mean corpuscular heamoglobin; MCHC, mean corpuscular heamoglobin concentration. * Correlation coefficients were significant, P< 0.05.** Correlation coefficients were highly significant, P< 0.01.

Table 4. Correlation coefficients among the physiological and heamatological parameters of stressed mithun bulls

	RT	RR	PR	HR	ST	TRBC	HB	ESR	PCV	MCV	MCH	MCHC
RT	1	0.96**	0.94**	0.87**	0.73**	0.85**	0.94**	0.72*	0.47	–0.71*	–0.38	0.72*
RR		1	0.95**	0.91**	0.70*	0.83*	0.93**	0.75*	0.43	–0.79*	–0.35	0.75*
PR			1	0.85**	0.84**	0.86**	0.97**	0.76*	0.62	–0.69	–0.34	0.62
HR				1	0.63	0.89**	0.84**	0.75*	0.38	–0.75*	–0.63	0.68
ST					1	0.75*	0.86**	0.61	0.91**	–0.23	0.27	0.22
TRBC						1	0.91**	0.92**	0.47	–0.67	–0.75*	0.69
HB							1	0.84**	0.60	–0.66	–0.43	0.67
ESR								1	0.25	–0.75*	–0.72*	0.81*
PCV									1	0.11	–0.27	–0.17
MCV										1	0.46	–0.92**
MCH											1	–0.50
MCHC												1

RT, rectal temperature; RR, respiration rate; PR, pulse rate; HR, heart rate; ST, skin temperature; TRBC, total red blood cells; HB, heamoglobin; ESR, erythrocyte sedimentation rate; PCV, packed cell volume; MCV, mean corpuscular volume; MCH, mean corpuscular heamoglobin; MCHC, mean corpuscular heamoglobin concentration* Correlation coefficients were significant, P< 0.05.** Correlation coefficients were highly significant, P< 0.01.

stress as evident from the physiological parameters. Moreover, these values in the present study were significantly higher than the value reported in previous study of mithun species (RT: 99.30±0.10°F, PR: 62.11±0.50 beats/min, RR: 27.09±0.54 beats/min, ST: 98.50±0.45, HR; Verma 1994). Measurement of RR indirectly helps to estimate the heat production during the stress condition (Berhan *et al.* 2006) and increased RR in mithuns may have more homeostatic relevance for the dissipation of excessive heat and the maintenance of a lower body temperature (Rahardja *et al.* 2011). The respiration rate was significantly higher in stressed animal as it is needed to maintain the thermoregulation (Sejian *et al.* 2012). Moreover, the experiment was conducted in summer causing enhancement of the detrimental effects of walking stress leads to increase heat load and consequently increased the RR in mithun. Walking stress also imposed severe energy deficiency as the locomotor activity utilizes substantial energy for walking. Further, the energy level was decreased as increased respiratory muscular activity due to higher respiratory movements during stress to dissipate body heat during walking.

The pulse rate reflects primarily the homeostasis of circulation along with the general metabolic status (Sejian *et al.* 2010a). Pulse rate in group 2 mithun increased significantly from the control after exposure to stress in the present study. This view was supported by the fact that there is a correlation between heart rate and metabolic heat production (Marai *et al.* 2007). The ST was significantly higher in the stressed group than unstressed group, but is lower than the RT because the sweating leads to reduce the ST. Sweating has also been used to evaluate the response to heat stress in some mammalian species such as cattle, sheep and horses (Kumar *et al.* 2011). This indicated that apart from relieving the heat through respiratory evaporative cooling, the animal also needs cutaneous evaporation to eliminate heat from the body indicating the severity of stresses on these animals in physiological parameters. Further, it is also fact that RT acts as an indicator of walking stress in the sheep (Sejian *et al.* 2011). The present study was comparable with the findings of Yadav and Dhaka (2001) in Hariana bullocks and Behera *et al.* (2008) in Surungi bullocks. Similar findings were also reported by Tomar and Joshi (2008) in Kenkatha bullock, Atakare and Siddiqui (2009) in Deoni bullocks, Shelke and Siddiqui (2009) in Red Kandhari bullocks and Singh and Nanavati (2013) in crossbred bullocks.

Heamatological profiles

Heamatological profiles such as TRBC, HB, ESR, PCV, MCV, MCH and MCHC differed significantly (P<0.05) between the experimental groups. Similarly, these parameters differed significantly (P<0.05) among the experimental weeks. Group 2 has significantly (P<0.05) higher TRBC, HB, ESR, PCV and MCHC than group 1 and MCV, MCH were significantly (P<0.05) lower in group 2. Strenuous exercise or work or walk results in severe haematological, metabolic, acid-base and ionic changes in animals, which are specie-specific and are influenced by the magnitude of the cumulative effects of physiological and physical trauma related to the stressful events (Hassanein 2010). Acidosis and physical stress result in disturbances in haematological parameters (Moyes *et al.* 2006). Its profiles in animals provide means of assessing the internal environment and understanding the causes of impairment in homeostasis as evidenced by marked fluctuations in physiogical indices under different internal and external environmental conditions (Sattar and Mirza 2009). Blood constituents reflect the influence of normal physiological and pathological states of the animals including stress (Satue *et al.* 2009).

Haematological parameters such as PCV, RBC, MCV, MCHC and MCH are used to evaluate adaptability of animals to adverse environmental conditions (Koubkova *et al.* 2002). The blood parameters were significantly affected in stress induced mithun as indicated that these animals were suffered with severe haemoconcentration. The normal values reported in mithun were lower than the present study (Borah *et al*. 1999). The higher value in stress affected animal was due to adaptive mechanism exhibited by the mithun. But this mountain cattle is cold loving and are mostly be seen in the deep and thick forest area. When the temperature was lowered down, the animal is moving towards the plain or lower hilly areas. In general, the oxygen consumption rate is increased when the animal walking or running. Moreover, the mithun is living in the semi-wild and moving long distance in the forest to search the feed especially in winter and summer seasons. This leads to increase the oxygen demand to meet the muscular activity. That may leads to increase the RBC production and Hb concentration in mithun. Moreover, as the mean sea level of the experimental area is approximately 300 MSL leads to the concentration of oxygen is less than in lower mean sea level. Further, in the experiment, the animals were not allowed to drink water or feed the fodder that leads to haemo-concentration leads to increase of the blood parameters particularly RBC, HB, PCV and ESR (Sejian *et al.* 2012). Similar observation was reported in other species (cattle: Garcia-Belenguer *et al.* 1996), sheep: Sejian *et al.* 2012). Generally during thermal stress, severe dehydration has been reported in livestock which ultimately leads to increased level of Hb and PCV (Marai *et al.* 2007, McManus *et al.* 2009). Further, severe water deprivation in these mithun during walking stress could have aggravated the condition. Moreover, the study was conducted in summer season, leads to more severe effects on these parameters. It also revealed that walking in summer season was a highly energy spending activity for mithun and also more sweating during the walking leads to high haemoconcentration. In addition, PCV and Hb are considered to be the indices of the organic response to exercise stress (Sejian *et al.* 2010b).

Antioxidant profiles

Antioxidant profiles such as SOD, CAT, GSH, GSHPx, GSHRx and TAC were significantly (P<0.05) affected between the stress and stress free animals. In stressed animals, the level of antioxidants was significantly (P<0.05) lowered than in unstressed animal group. Similarly malondialdehyde (MDA) production was significantly (P<0.05) higher in stressed animal than unstressed animal group. GSH, SOD, CAT and TAC were significantly decreased in the stressed animals and MDA production was significantly higher in stressed mithuns (Fig. 1). Deficiency of antioxidants may occur due to different kinds of stress. These free radical oxidations are activated in animals under various types of stresses and lipid peroxidation products accumulate in various organs (Yarovan 2008). In the present study, the free radical production was significantly higher in stressed animals as similar report was observed that heat stress/stress stimulates excessive production of free radicals (Bernabucchi *et al.* 2002, Sivakumar *et al.* 2010). Moreover, exercise/walking is postulated to generate free radicals by a) increasing epinephrine and other catecholamines that can produce oxygen radicals when they are metabolically

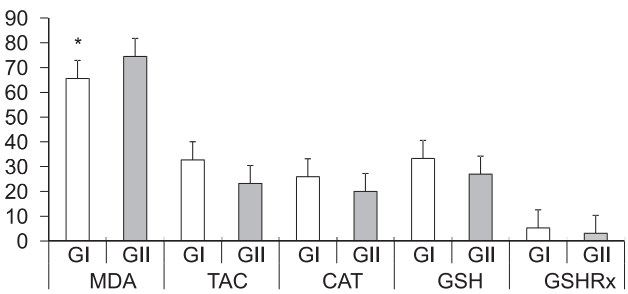

Fig. 1. Effect of walking stress on antioxidants activity and malondialdehyde production in mithun (*P<0.05). MDA, malondialdehyde (nmol/L); TAC, total antioxidant capacity (nmol/μL); CAT, catalase (nmol/min/L); GSH, Glutathione (nmol/L); GSHRx, glutathione reductase (nmol/min/L). GI, unstressed animal group; GII, stressed animal group.

inactivated, b) producing lactic acid that can convert a weakly damaging free radical (superoxide) into a strongly damaging one (hydroxyl), and c) inflammatory responses to secondary muscle damage incurred with over exertion (Sen 1995). The stress can be counteracted by supplementation of antioxidants which are very helpful in case of animal species (Sejian *et al.* 2012) because, the antioxidants are compounds or systems that delay autoxidation by inhibiting the formation of free radicals or by interrupting propagation of the free radical by several mechanisms (Brewer 2011). This intern helps to protect cellular damage during any stressful condition.

Correlation study

The correlation coefficient study revealed that there was a positive significant ($P<0.05$) correlation between the RT and RR, PR, ST, TRBC, HB, ESR and MCHC. Respiration rate has positive significant ($P<0.05$) correlation with PR, ST, TRBC, HB, ESR and MCHC and negative correlation with MCV. PR has positive and significant ($P<0.05$) correlation with ST, HB, ESR and MCHC. TRBC has positive and significant ($P<0.05$) correlation with HB, ESR and MCHC and negative correlation with MCV and MCH. This similar pattern was observed in both unstressed (Table 3) and stressed (Table 4) groups.

Our findings indicated that there should be a suitable feeding and grazing policy for mithun to counteract the stress caused by walking as well as heat in summer and to identify the suitable time for grazing to minimize the thermal and walking stress to mithun. Detailed study needs to be carried out in different seasons by using different age group of animals to measure hormonal and biochemical profiles besides physiological and heamatological profiles in mithun. Moreover, suitable research needs to be conducted regarding feeding and supplementation of electrolytes and antioxidant to counteract the stress caused by walking as well as heat.

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