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ORIGINAL ARTICLE

Ambient Stress vis-a-vis Enzyme Regulators of Carbohydrate Metabolism in *Marwari* goat from arid tracts in India

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The study was designed to determine modulation of enzyme regulators of carbohydrate metabolism in *Marwari* breed of goat of both sexes and varying age groups during moderate, extreme hot and cold ambiences. The enzyme regulators included sorbitol dehydrogenase (SDH), malate dehydrogenase (MDH), glucose-6-phosphatase (G-6-Pase) and glucose-6-phosphate dehydrogenase (G-6-PDH) which were determined in the serum. The moderate ambience was considered as control for each enzyme regulator and the mean values in UL^{-1} were 8.67±0.005, 40.87±0.32, 8.04±0.003 and 7.53±0.005, respectively. The mean values of SDH, MDH and G-6-Pase were significantly ($p \leq 0.05$) higher during hot and cold ambiences in comparison to respective moderate mean value. However, the increase was more in cold than hot ambience for each case. The mean value of G-6-PDH was significantly ($p \leq 0.05$) higher during cold ambience while significantly ($p \leq 0.05$) lower during hot ambience in comparison to moderate mean value. In each ambience the sex and age effects were significant ($p \leq 0.05$) on each enzyme regulators. It could be concluded that extreme ambiences produced modulations in the metabolic reactions reflected on the basis of pattern of variations of enzyme regulators in the serum.

Key words: Ambience, cold, enzyme regulators, goat, hot, Marwari, serum

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Animals living in natural environment are exposed to drastic variations in ambient temperatures. Various physiological reactions are highly sensitive to the variations in the ambient temperatures since exposure of animal to extreme ambiences affect the *milieu interior*. Enzymatic

reactions in health are greatly influenced by metabolic status and environmental temperatures. Establishment of these variations become essential to explore the metabolic modulations and to compare the levels with other pathological conditions, as many a times stressed animals show a wide range of changes in the enzyme levels (Kataria and Kataria, 2006). The common stressors faced by the ruminants include heat, cold, dehydration, infection, drought etc. (Kataria and Kataria, 2005a). Several investigators have studied ruminant glucose kinetics, glucose absorption, and contributions of various substrates or precursors to ruminant gluconeogenesis, however, an approach to correlate them with extreme environmental temperatures is rare. Consequently, considerable confusion and conjecture exist about quantitative details of regulators of the metabolism. Carbohydrate metabolism in ruminants is highly significant for various physiological mechanisms required for maintaining the animal body like reproduction and production. Much of the carbon to support gluconeogenesis is derived from either propionate or glucogenic aminoacids (Bergman, 1973).

Besides performing metabolic role, many enzymes of carbohydrate metabolism actively participate in the other physiological processes of the body. The reactions catalysed by glucose-6-phosphate dehydrogenase help to generate NADPH required for synthesis. An understanding of these pathways is essential to monitor the physiology of the animals particularly ruminants, who require great metabolic power to meet environmental challenges in addition to support their physiological production machinery. Exposure of the animals to varying environmental temperatures may impose stress, which can produce changes at cellular levels. Temperature variations can affect productivity and resistance to infectious diseases and produce economical losses to animal owners (Kataria and Kataria, 2005b).

Marwari breed of goat constitutes a major portion of the goat population in western part of Rajasthan and plays an important role in the economy of arid and semi arid tract. Oppressive heat and cold during extreme ambiences bring about changes in enzymes necessary for metabolic adjustment. It is a melancholy that despite of immense quality characteristics of *Marwari* breed of goat very little attention has been paid to understand regulatory aspect of these metabolic processes. To understand the real worth of these animals, establishment of their own norms becomes very important in the field of veterinary clinical medicine. Therefore, the present investigation was planned to determine some of the enzyme regulators of the carbohydrate metabolism during extreme ambiences in the serum of *Marwari* goat and to set their physiological reference values for the use in veterinary medicine and future research.

MATERIALS AND METHODS

To carry out the investigation, six hundred and thirty apparently healthy *Marwari* goat of either sex, between 6 months to 4.5 years of age were screened to determine enzyme regulators of carbohydrate metabolism in serum during extreme ambiences. All the animals were kept in similar conditions of management. In each ambience 210 blood samples were collected and the animals were grouped into male (105) and non pregnant goat (105). Further each group was divided according to age as below 1 year (35 male and 35 female); 1-2 years (35 male and 35 female) and 2-4.5 years (35 male and 35 female).The mean maximum ambient

temperatures during moderate and hot periods were 28.60± 0.32 and 45.5 ± 0.08 °C, respectively, whereas mean minimum temperature during cold ambience was 2.08 ± 0.10 °C.

Blood samples were collected from jugular vein during slaughtering from private slaughter houses (Bikaner, Rajasthan, India). Sampling was carried out in morning hours during moderate, hot and cold ambiences. Blood was collected directly into the clean, dry test tubes without any anticoagulant in duplicate. After collection of the blood, test tubes were kept in the slanting position for 30 minutes and blood was allowed to clot. Then the clot was separated from the walls of the each test tube with the help of sterilised stainless steel wire and then each test tube was centrifuged at 3000 rpm for 20 minutes. Supernatant clear serum (non-haemolysed) was pipetted out into sterilised plastic vials. All the enzymes were stored at 0°C- 4 °C in the refrigerator for a very short time period for analysis as freezing of serum was reported to cause partial inactivation (King, 1965).

Spectrophotometric assay were used (King, 1965) to determine sorbitol dehydrogenase (SDH), malate dehydrogenase (MDH), glucose-6-phosphatase (G-6-Pase) and glucose-6-phosphate dehydrogenase (G-6-PDH) with slight modifications (Kataria et al., 2010). To test the significance, the changes in the means were measured by using multiple mean comparison procedures (Duncan, 1955 and Steel and Torrie, 1980). In each case, the moderate mean value served as the control.

RESULTS AND DISCUSSION

The mean values of SDH, MDH and G-6-Pase (Table1) were significantly ($p \leq 0.05$) higher during hot and cold ambiences in comparison to respective moderate mean value. However, the increase was more in cold than hot ambience for each case. The mean value of G-6-PDH (Table 1) was significantly ($p \leq 0.05$) higher during cold ambience while significantly ($p \leq 0.05$) lower during hot ambience in comparison to moderate mean value.

Ambient stress can stimulate metabolic activity of liver thereby increasing serum SDH levels, probably through its increased synthesis in the cell (Alemu *et al.,* 1977), and simultaneous leakage into the plasma due to enhanced permeability of cell membrane (Keyse, 2000). Increased serum SDH activity during cold ambience (Kataria *et al.,* 2010) showed the regulation of carbohydrate metabolism in a way to generate more glucose (Wolf and Williams,1973). Extreme ambience associated increase in serum SDH was explicitly related with the increase in enzyme regulators of metabolism providing a new insight to understand the activity of SDH. Significant increase in serum MDH activities during hot and cold ambiences was probably enough to provide evidences regarding strategies of the animal to modulate the metabolic pathways for energy generation. It is an enzyme of immense significance in citric acid and urea cycles and its higher activity shows increased rate of gluconeogenesis. Higher activity of serum MDH in cold than hot ambience unequivocally suggested the role of increased thyroid activity during cold condition as MDH synthesis in hepatocytes is stimulated by insulin and thyroid hormones (Goodridge *et al*., 1984).

In ruminants gluconeogenesis is an important pathway in which in the final step, glucose-6-phosphate is converted to glucose, catalysed by G-6-Pase (Kaneko *et al*., 1999). This step is considered as the site of metabolic control for glucose. The fact that G-6-Pase is inactivated during lipid peroxidation (Koster, 1986), indicates the association of this enzyme with the oxidative stress.

Hot and cold ambiences probably served as stressors and in order to maintain the blood glucose the activity of enzyme was higher. This helped in maintaining the blood glucose level. In heat stressed animals increased G-6-Pase activity is related with low glucose and increased concentration of intermediate substrates (Miova *et al*.,2008). The major portion of carbohydrates available to the ruminants is supplied by gluconeogenesis, and there must be a continuous and rapid flux through this pathway even in the fed state (Annison and Lewis, 1962). Hot and cold ambiences probably worked as stressors which initiated adaptive responses (Kataria and Kataria, 2005a). The pivotal role of G-6-PDH in metabolism has been identified by the research workers with the hypothesis that its expression is modulated by free radicals during oxidative stress (Cramer *et al*., 2006). The lower concentration of this enzyme in hot ambience indicated its antioxidant type role which showed the depletion in an attempt to fight with free radicals. Defense against stress is also dependent upon G-6-PDH activity (Ercal *et al*., 2002), as stress can depress red blood corpuscles and leucocyte functions (Aslan *et al.,* 2005). Oxidative pathway of G-6-PDH is as an adaptive mechanism, yielding NADPH for fat synthesis, used for steroid formation and insulation. Higher levels of G-6-PDH are important for glucose oxidation through the hexose mono phosphate shunt, essential for synthesis of fat and the major source of NADPH, which maintains the reductive environment for all biosynthetic processes using NADPH as a cofactor (Kaneko *et al.,* 1999). Goroshinskaia *et al.* (1984) attributed higher activity to cooling stress.

In each ambience the sex and age effects were significant (p≤0.05) on each enzyme regulators. The mean values were higher significantly (p≤0.05) in male animals for all the enzyme regulators except G-6-PDH in which the activity was significantly (p≤ 0.05) higher in female animals in each ambience. All enzyme regulators showed higher activities significantly (p≤0.05) in the animals of below 1 year of age except G-6-PDH in which the activity was significantly (p≤ 0.05) lower in the animals of below 1 year of age.

Higher serum SDH activity in males probably indicated its paracrine regulatory role for opioids in testicular metabolism (Sreenivasan and Vijayan, 1996). Higher SDH activity in males and younger lot probably helped in increasing blood glucose reiterating its metabolic role. Sharma and Patnaik (2008) related higher glucocorticoid levels in males with higher MDH activities. Higher serum glucose levels in the males than females and in the animals of below 1 year of age indicated higher gluconeogenesis. Rumen development and volatile fatty acid production probably influenced the G-6-Pase activity (Purser and Bergen, 1968). Eguinoa et al. (2003) also suggested higher G-6-PDH activity in heifers than bull. Since G-6-PDH is a lipogenic enzyme and related with the oxidation of glucose, its high activity in females probably resulted in lowest concentration of glucose. It has also pointed out towards greater need for fatty acid synthesis through generation of NADPH via HMPS. Nutritional status of the females can also influence the activity of enzyme (Kelley et al., 1986). Age may change G-6-PDH activity (Aslan et al., 2005). As the enzyme is related with the oxidation of glucose, low activity of this enzyme probably resulted in highest concentration of glucose in lower age group. Higher activity in adults reflected towards greater lipogenic activity (Eguinoa et al., 2003).

Table 1. Mean±SEM values of serum sorbitol dehydrogenase (SDH), malate denydrogenase (MDH), G-6-Pase and G-6-PDH in *Marwari* goat.

Ambiences	Serum enzymes regulators, U L^{-1}			
	SDH	MDH	G-6- Pase	G-6-PDH
Moderate (210)	8.67±0.005 [a]	40.87±0.32 [a]	8.04±0.003 [a]	7.53±0.005 [a]
Sex				
Male (105)	9.91±0.005 [b]	42.33±0.33 [b]	9.08±0.003 [b]	6.93±0.005 [b]
Female (105)	7.43±0.009[b]	39.40±0.34 [b]	7.01±0.003[b]	8.11±0.004[b]
Age				
Below 1 Year (70)	10.60±0.005 [c]	44.51±0.22 [c]	9.24±0.006 [c]	6.86±0.005 [c]
1-2 Years (70)	8.66±0.008[c]	41.47±0.33 [c]	8.77±0.003[c]	7.41±0.004[c]
2-4.5 Years (70)	6.75±0.006[c]	36.62±0.21 [c]	7.14±0.009[c]	8.30±0.009[c]
Hot (210)	9.88±0.005[a]	56.26±0.27 [a]	10.22±0.003[a]	4.76±0.004 [a]
Sex				
Male (105)	11.62±0.006 [d]	58.95±0.40 [d]	11.05±0.003 [d]	4.1±0.004 [d]
Female (105)	8.14±0.004 [d]	53.58±0.23 [d]	9.40±0.003[d]	5.4±0.005[d]
Age				
Below 1 Year (70)	12.37±0.010 [e]	80.37±0.32 [e]	11.91±0.004 [e]	4.01±0.004 [e]
1-2 Years (70)	9.08±0.009[e]	50.49±0.22 [e]	10.60±0.003[e]	4.71±0.004 [e]
2-4.5 Years (70)	8.20±0.007 [e]	37.92±0.21 [e]	9.17±0.003 [e]	5.59±0.005 [e]
Cold (210)	15.88±0.006[a]	74.70±0.24 [a]	14.53±0.005[a]	13.05±0.004[a]
Sex				
Male (105)	17.97±0.005 [f]	78.45±0.25 [f]	15.67±0.004 [f]	12.00±0.004 [f]
Female (105)	13.80±0.007[f]	70.95±0.33 [f]	13.40±0.006[f]	14.08±0.006 [f]
Age				
Below 1 Year (70)	17.90±0.005 [g]	79.59±0.30 [g]	15.45±0.006[g]	12.05±0.006[g]
1-2 Years (70)	15.30±0.004[g]	83.6±0.33 [g]	14.80±0.005[g]	13.03±0.003[g]
2-4.5 Years (70)	14.46±0.009 [g]	60.90±0.30 [g]	13.35±0.009[g]	14.07±0.005[g]

Figures in the parenthesis indicate number of animals and same superscripts within a column differ significantly (p≤0.05) from each other.

It could be concluded that extreme ambiences produced modulations in the metabolic reactions reflected on the basis of pattern of variations of enzyme regulators in the serum. The data generated will help in future studies to understand metabolic changes associated with environmental temperature and necessary corrections in the nutrition for health management.

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