ELBA BIOFLUX
Extreme Life, Biospeology & Astrobiology
International Journal of the Bioflux Society

Extreme ambient temperature associated variations in enzyme markers of carbohydrate metabolism in Rathi calves from arid tracts

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Abstract. Present investigation was envisaged to find out extreme ambient temperature associated variations in enzyme markers of carbohydrate metabolism in male and female calves of Rathi breed from arid tracts in India. The enzyme markers selected were glucose-6-phosphate dehydrogenase (G-6-PDH), sorbitol dehydrogenase (SDH), malate dehydrogenase (MDH) and glucose-6-phosphatase (G-6-Pase). Blood samples were collected to harvest sera during extreme hot, extreme cold and moderate ambient temperature periods. The mean values of enzyme markers in UL^{-1} during moderate ambient temperature were 8.90±0.002, 8.50±0.002, 42.00±0.40 and 8.03±0.002, respectively. The mean value of G-6-PDH was significantly ($p \leq 0.05$) higher during cold ambient temperature whereas significantly ($p \leq 0.05$) lower during hot ambient temperature in comparison to moderate period. It was 3.04 times higher in cold than hot period. The mean values of SDH, MDH and G-6-Pase were significantly ($p \leq 0.05$) higher during hot and cold ambient temperatures in comparison to respective moderate mean value. However, the increase was more in cold than hot ambient temperature for each marker (1.33, 1.31 and 1.42 times, respectively). In each period the gender and age effects were significant ($p \leq 0.05$). It could be concluded that variations in serum enzyme markers were associated with changes in ambient temperatures. Probably extreme temperatures were able to produce profound effect on carbohydrate metabolism in calves. On the basis of result it can be suggested that during the periods of extreme ambient temperatures balanced nutrition must be provided along with proper management to decrease the severity of temperature impact.
Key words: Ambient temperature, cold, enzyme regulators, hot, serum, Rathi calves.

Introduction. Calf raising with proper scientific inputs is always coupled with profitability. Under natural husbandry conditions it is inevitable to prevent the exposure of the animals to extreme ambient temperatures like hot and cold. Modulations are observed in the pathways of carbohydrate metabolism due to abiotic stressors. Higher ambient temperature is one of the factors producing negative impact on the growth rate of calves. Prolonged elevation in the ambient temperature can reduce the feed intake of the animals tremendously thus diverting their metabolic pathways in an attempt to provide greater energy for maintenance. Extreme variations in the ambient temperatures can influence the growth of calves to a greater extent and prolonged exposure may lead to stunting of the calves along with a compromised immune status.

Heavy economical loss due to abiotic stressors has prompted the scientific community to think regarding various measurement aspects which can be instrumental in rearing of calves in a better way. Assessment of the extent of metabolic modulations due

to extreme ambient temperatures can help a clinician in diagnosing pathological conditions, as many a times stressed animals show a wide range of changes in the enzyme levels (Kataria & Kataria 2006). The common stressors faced by the ruminants include heat, cold, dehydration, infection, drought etc. (Kataria & Kataria 2005a). Exposure of the animals to extreme 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 & Kataria 2005b). Carbohydrate metabolism is important in calves beginning from growth to attainment of adulthood. A study of pathways of carbohydrate metabolism can help in scientific management of the calves. Rate of metabolic pathways can be assessed indirectly by quantifying the regulatory enzymes serving as markers of carbohydrate metabolism. Enzyme markers can be taken as scaffolding in understanding the essential aspects of the reproduction and production.

Rathi is an important indigenous milch breed of cattle in arid tract. Despite of immense quality characteristics, very little scientific attention has been paid to understand regulatory aspect of various metabolic processes. First step in understanding the implications of metabolism in formulating strategies for health maintenance, establishment of a particular breed's reference values is necessary. Therefore, the present investigation was planned to determine some of the enzyme markers of the carbohydrate metabolism during extreme ambient temperatures in the serum of Rathi calves with a goal to set their physiological reference values for the use in diagnostics.

Materials and Methods. One hundred and eighty apparently healthy Rathi calves of either sex, from one month to one year of age, were screened to determine enzyme markers of carbohydrate metabolism in serum during extreme ambient temperature periods. Calves were kept in similar conditions of management by private dairies. In each ambient temperature period, 60 blood samples were collected and the animals were grouped into male (30) and female (30). Further each group was divided according to age as below 6 months (15 male and 15 female) and 6 months – one year of age (15 male and 15 female). The mean maximum ambient temperatures during moderate and hot periods were 27.99± 0.02 and 45.82 ± 0.001 ^{o}C, respectively, whereas mean minimum temperature during cold ambience was 1.01 ± 0.001 ^{o}C.

Sampling was carried out in morning hours during moderate, hot and cold ambient temperature periods. Blood was collected directly into the clean, dry test tubes without any anticoagulant to harvest sera. Supernatant clear non-hemolyzed serum was used for the study.

Spectrophotometric assays were used (King,1965) to determine glucose-6-phosphate dehydrogenase (G-6-PDH), sorbitol dehydrogenase (SDH), malate dehydrogenase (MDH) and glucose-6-phosphatase (G-6-Pase) 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; Steel & Torrie 1980). In each case, the moderate mean value served as the control.

Results and Discussion. The mean value of G-6-PDH (Table 1) was significantly ($p \leq 0.05$) higher during cold ambience whereas significantly ($p \leq 0.05$) lower during hot period in comparison to moderate period. It was 3.04 times higher in cold than hot ambient temperature period. The mean values of SDH, MDH and G-6-Pase (Table 1) were significantly ($p \leq 0.05$) higher during hot and cold ambient temperature periods in comparison to respective moderate mean value. However, the increase was more in cold than hot ambient temperature period for each case (1.33, 1.31 and 1.42 times, respectively). In each ambient temperature period, the gender and age effects were significant ($p \leq 0.05$) on each enzyme marker. The mean values were higher significantly ($p \leq 0.05$) in male calves for all the enzyme markers except G-6-PDH in which the activity was significantly ($p \leq 0.05$) higher in female calves. All enzyme markers showed higher activities significantly ($p \leq 0.05$) in the calves of below 6 months of age except G-6-PDH in which the activity was significantly ($p \leq 0.05$) lower in the animals of below 6 months of age.

Table 1

Mean± SEM values of serum glucose-6-phosphate hydrogenase (G-6-PDH), sorbitol dehydrogenase (SDH), malate denydrogenase (MDH) and glucose-6-phosphatase (G-6-pase) in Rathi calves during various ambient temperatures.

Ambient temperature	Serum enzymes markers, U L^{-1}			
	G-6-PDH	SDH	MDH	G-6- Pase
Moderate (60)	8.90±0.002 [a]	8.50±0.002 [a]	42.00±0.40 [a]	8.03±0.002 [a]
Gender				
Male (30)	7.80±0.002 [b]	10.00±0.001 [b]	47.00±0.28 [b]	9.07±0.002 [b]
Female (30)	10.00±0.001[b]	7.00±0.003[b]	37.00±0.34 [b]	7.00±0.001[b]
Age				
Below 6 months (30)	7.30±0.003 [c]	11.00±0.002 [c]	48.00±0.18 [c]	9.23±0.002 [c]
6 months- one year (30)	10.50±0.002[c]	6.00±0.001[c]	36.00±0.20 [c]	7.13±0.001[c]
Hot (60)	5.00±0.001 [a]	12.00±0.002[a]	58.00±0.19 [a]	10.22±0.002[a]
Gender				
Male (30)	3.5±0.002 [d]	14.50±0.002 [d]	62.00±0.20 [d]	11.05±0.001 [d]
Female (30)	6.5±0.002[d]	9.50±0.001 [d]	54.00±0.18[d]	9.40±0.002[d]
Age				
Below 6 months (30)	3.80±0.002 [e]	15.50±0.002 [e]	76.00±0.10 [e]	11.91±0.002 [e]
6 months- one year (30)	6.20±0.001 [e]	8.50±0.002 [e]	40.00±0.15 [e]	9.17±0.001 [e]
Cold (60)	15.22±0.002[a]	16.00±0.002[a]	76.00±0.19 [a]	14.53±0.002[a]
Gender				
Male (30)	11.22±0.001 [f]	20.50±0.001 [f]	82.00±0.14[f]	15.67±0.001[f]
Female (30)	19.22±0.002[f]	11.50±0.002[f]	70.00±0.16 [f]	13.40±0.002[f]
Age				
Below 6 months (30)	13.40±0.002[g]	17.20±0.002[g]	90.00±0.12 [g]	15.45±0.001[g]
6 months- one year (30)	16.60±0.002[g]	14.80±0.002 [g]	62.00±0.11 [g]	13.35±0.002[g]

Figures in the parenthesis indicate number of calves and same superscripts within a column differ significantly ($p \leq 0.05$) from each other.

Glucose-6-phosphate dehydrogenase. The key function of G-6-PDH in carbohydrate metabolism is well understood. However, its association with the mechanisms of oxidative stress has also been recognised (Cramer et al 2006). Decreased activity of this enzyme during hot ambient temperature period specified its antioxidant property. Its activity is known to provide defense against stress (Ercal et al 2002). Oxidative pathway of G-6-PDH yields NADPH for fat synthesis and are used for steroid formation and insulation. Goroshinskaia et al (1984) attributed higher activity to cooling stress. It higher activity in females indicates towards the greater lipogenic activity with a greater need for fatty acid synthesis through generation of NADPH via HMPS. Gender effect is also reported by other workers (Eguinoa et al 2003). Nutritional status of the females can also influence the activity of enzyme (Kelley et al 1986). Scientists have observed that age can change G-6-PDH activity considerably and increased activity can be correlated with lipogenic activity (Eguinoa et al 2003; Pandey et al 2013).

Enhanced activity of the pentose phosphate pathway has been observed in cells under oxidative stress conditions (Janero et al 1994). Enzymes of the PPP are crucial for maintaining the cytoplasmic NADPH concentration, which provides the redox power for known antioxidant systems. The results advocated the fact that modulations in the carbohydrate metabolism are of immense significance in cellular protection against free radicals. Stress is able to reroute the carbohydrate flux from glycolysis to the pentose phosphate pathway to neutralize disturbance in the redox state. Increased activities of

G6PDH indicated a synchronized response to energy intake changes, rendering this enzyme as potential biochemical marker (Laliotis et al 2012).

Sorbitol dehydrogenase. It is an enzyme in carbohydrate metabolism converting sorbitol, the sugar alcohol form of glucose into fructose. Together with aldose reductase, it provides a way for the body to produce fructose from glucose without using ATP. Ambient stress can stimulate metabolic activity by increasing serum SDH levels (Pandey et al 2013). Kataria & Kataria (2012) reported 2.86 times increase in serum SDH value during hot ambience in donkeys. Stress probably stimulates synthesis of this enzyme. Cold ambient temperature associated higher serum SDH activity was most likely to fulfill glucose demand Kataria et al 2010). Variation in SDH activity insisted to understand the probable modulation in carbohydrate pathways. Higher serum SDH activity in males probably indicated its paracrine regulatory role for opioids in testicular metabolism (Sreenivasan & Vijayan 1996). Increased activity was probably to raise blood glucose reiterating its metabolic role.

Malate dehydrogenase. It is considered an enzyme of immense significance in citric acid cycle. Citric acid and urea cycles are linked, therefore its metabolic implications increase tremendously. Fumarate enters mitochondrion where the combined activities of fumarase and malate dehydrogenase transform fumarate into oxaloacetate. It is also important in gluconeogenesis where the oxaloacetate formed from pyruvate in the mitochondrion is reversibly reduced to malate by mitochondrial malate dehydrogenase (Lehninger et al 1993).

Increased activity of serum MDH in cold than hot ambient temperature unambiguously pointed towards cold stimulated thyroid activation since MDH synthesis in hepatocytes is stimulated by insulin and thyroid hormones (Goodridge et al 1984). Hot and cold ambient temperature associated increase was possibly sufficient to substantiate the fact that pathways of carbohydrate metabolism were diverted for energy production. Pandey et al (2013) in goats and Kataria & Kataria (2012) in donkeys also observed increased activity of serum MDH during extreme hot ambience. Increased MDH activity indicated the strategies of the animal to modulate the metabolic pathways for energy generation (Kataria & Kataria 2012) and higher rate of gluconeogenetic process. In ruminants the major portion of carbohydrate available is supplied by gluconeogenesis (Abdel-Fattah et al 2002). Increased activities can also be related to higher glucocorticoid levels (Sharma & Patnaik 2008).

Glucose-6-phosphatase. It is an important enzyme of gluconeogenesis catalysing the final step where glucose-6-phosphate is converted to glucose. Scientists have correlated the activity of this enzyme to the oxidative stress (Pandey et al 2013). Stress is known to modulate the pathways of carbohydrate metabolism and higher glucose production is considered as a stress response. Hot and cold ambient temperatures in present study most likely served as stressors (Kataria & Kataria 2005a) and in order to maintain the blood glucose the activity of this enzyme was higher. The major portion of carbohydrates available to the ruminants is supplied by gluconeogenesis, a process which is known to operate continuously irrespective of feeding stage.

Conclusions. It could be concluded that extreme ambient temperatures produced changes in the markers of carbohydrate metabolism. Probably metabolic pathways were modulated to help the calves in combating ambient stress. Pattern of variation will help in understanding metabolic adjustments required to develop strategies for better health management of the calves. On the basis of result it can be suggested that during the periods of extreme ambient temperatures balanced nutrition must be provided alongwith proper management to decrease the severity of temperature impact.

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Received: 16 February 2013. Accepted: 01 March 2013. Published online: 20 April 2013.
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How to cite this article:
Kataria N., Sharma S., Arora S., Mohammad N., Maan R., Abhimanu S., Kataria A. K., 2013 Extreme ambient temperature associated variations in enzyme markers of carbohydrate metabolism in Rathi calves from arid tracts. ELBA Bioflux 5(1):55-60.