EFFECT OF AMBIENT STRESSORS ON METABOLISM OF MURRAH BUFFALOES OF VARIOUS PHYSIOLOGICAL STATES[#]

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ABSTRACT

Effect of ambient stressors on metabolism of Murrah buffaloes of various physiological states belonging to arid tracts were investigated. Healthy adult female Murrah buffaloes were grouped according to physiological states into group A (non-pregnant milch, pregnant milch and pregnant dry) and group B (primipara and multipara) and blood samples were collected during moderate, hot and cold ambiences to obtain sera. Metabolic responses were assessed by analyzing serum sorbitol dehydrogenase (SDH), malate dehydrogenase (MDH), glucose-6-phosphatase (G-6-Pase) and glucose-6-phosphate dehydrogenase (G-6-PDH) enzymes. Mean values of the enzymes during moderate ambience were considered as control and they were 6.00±0.04 U L^{-1}, 40.00±0.40 U L^{-1}, 6.98±0.10 U L^{-1} and 6.00±0.05 U L^{-1}, respectively. The mean value 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 whereas 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 as compared to respective moderate mean value. The mean values of non pregnant milch, pregnant milch and pregnant dry animals differed significantly ($P \leq 0.05$) from each other in all the ambiences. It was concluded that extreme ambiences produced metabolic changes in the Murrah buffaloes of all physiological states, which was reflected in the form of altered status of the metabolic enzymes determined in the serum.

Key words: Ambience, cold, hot, metabolism, Murrah buffalo

Introduction

Physiological states of the animals are governed by many internal and external factors and ambience is one of them. Adverse ambience can modulate the physiological mechanisms of the animals affecting metabolism. Direct effects are related to heat and cold stress. Negative impact can occur during pregnancy influencing maternal and foetal metabolism (Collier *et al*., 1982). Enzymes are important to run metabolic reactions within the cell. Metabolic enzymes are produced in the body as and when required. Therefore assessment of their levels is crucial in deducing metabolic pathways of animals. Changes in ambient temperature may bring out variations in metabolism of animal. Heat loss pathways infer a different thermoregulatory strategy, suggesting a different adaptation to semi-arid environment and strong association with metabolism (Pereira *et al*., 2014).

There are several enzymes like glucose-6-phosphate dehydrogenase (G-6-PDH), malate dehydrogenase (MDH), sorbitol dehydrogenase (SDH) and glucose-6-phosphatase (G-6-Pase) playing an important role in regulating metabolic pathways. Glucose 6 phosphate dehydrogenase is an enzyme of the pentose phosphate pathway that supplies reducing energy to cells by maintaining the level of the co-enzyme nicotinamide adenine dinucleotide phosphate (NADPH). Malate dehydrogenase is an enzyme of Kreb's citric acid cycle and belong to the NAD-dependent dehydrogenases (Minrik *et al*., 2002). The activities of serum malate dehydrogenase proved to be more useful than those of aspartate aminotransferase (AST) in diagnosis of liver diseases (Kawai and Hosaki, 1990). Sorbitol dehydrogenase 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. Scientists have used its values clinically to diagnose liver problems (Wiesner *et al*., 1965). Glucose 6-phosphatase is an enzyme that hydrolyzes glucose-6-phosphate, resulting in the formation of a phosphate group and free glucose. Glucose is then exported from the cell. This enzyme plays a key role in the homeostatic regulation of blood glucose levels. Glucose-6-phosphatase is a phosphomonoesterase which, apart from hydrolysing glucosamine 6-phosphate, is specific for glucose 6-phosphate. It catalyses the final step in the mobilisation of glycogen to glucose and is mainly present in liver and kidney.

The Murrah is the most important Indian breed of buffalo and is the premier milking buffalo. Extreme ambient heat and cold bring about changes in enzymes necessary for metabolism. Exposure of buffaloes to solar radiation evokes a series of drastic changes in physiological functions including reduced feed intake, decreased efficiency of feed utilization, disturbances in metabolism, enzyme reactions, blood metabolites etc. Such changes result in impairment of growth, production and reproduction performance (Marai and Haeeb, 2010). The inevitability of exposure of these animals to extreme temperatures of arid and semiarid tracts makes determination of enzymes of metabolic pathway an appropriate field of investigation to explore adaptive physiological measures of the body and their use in health management and clinical diagnosis. Therefore the present investigation was planned to determine serum enzymes of metabolic significance in Murrah buffaloes of various physiological states during extreme hot and cold ambiences.

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Materials and Methods

To carry out the study, four hundred and fifty blood samples of apparently healthy adult female Murrah buffaloes between 4 and 12 years of age were collected to harvest the serum during moderate (mean maximum ambient temperature 30.33 ± 0.20), hot (mean maximum ambient temperature 45.5 ± 0.08) and cold (mean minimum ambient temperature 4.88 ± 0.20) ambiences. All the animals belonged to private dairy farms in Rajasthan state, India and were managed in similar conditions of feeding and watering. In each ambience 150 blood samples were collected during morning hours. On the basis of physiological states, animals were broadly divided into group A (non- pregnant milch, pregnant milch and pregnant dry) and group B (primipara and multipara). Each category consisted of 30 animals in each ambience.

Glucose-6-phosphate dehydrogenase, malate dehydrogenase, sorbitol dehydrogenase and glucose-6-phosphatase were determined by standard spectrophotometric method (King,1965) with modifications (Joshi, 2012). The changes in the means were measured by using multiple mean comparison procedures (Duncan, 1955 and Steel and Torrie, 1980).

Results and Discussion

The mean ± SEM values of SDH, MDH, G-6-Pase and G-6-PDH In Murrah buffaloes during moderate, hot and cold ambiences are presented in Table 1.

Effects of hot and cold ambiences

The mean value of SDH, MDH and G-6-Pase were significantly ($p \leq 0.05$) higher during hot and cold ambiences in comparison to moderate mean value whereas 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 overall moderate mean value. Mean values of all the enzymes were significantly ($P \leq 0.05$) higher during cold ambience in comparison to respective hot 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). Various recent experiments have related the higher activity of SDH with oxidative stress and glutathione (Stephen *et al*., 2003) because it is involved in the mechanism underlying oxidative injury (Obrosova *et al*., 1999). Extreme ambience associated increase in serum SDH was explicitly related with the increase in enzyme markers of oxidative stress. It can be concluded that serum SDH activity can be used to understand allied mechanisms of oxidative stress.

Earlier researchers have shown the antioxidant role of MDH (Oh *et al*., 2002), therefore higher activity of serum MDH during extreme ambiences indicated towards generation of reactive oxygen species (Vincent *et al*., 2003). It was concluded that during assessing oxidative stress, serum MDH levels can also be monitored as an associated analyte of oxidative stress. The variations in the serum MDH activities clearly showed the strategies of the animal to change the metabolic pathways for energy generation (Kataria *et al*., 2000) and to combat oxidative stress. Final step of gluconeogenesis, catalysed by G-6-Pase, is considered as the site of metabolic control for glucose. Strong association of this enzyme with the oxidative stress is based upon the fact that G-6-Pase is inactivated during lipid peroxidation (Koster, 1986).

Extreme ambiences can elevate the enzyme levels in order to maintain the blood glucose. 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). It can be reiterated that extreme ambiences modulated the activities of the enzyme to support the homeostasis.

Glucose-6-phosphate dehydrogenase is considered to play a pivotal role in protection from oxidative stress. The expression of G-6-PDH is hypothesised to be 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. Climate effects on blood G-6-PDH levels were discussed by Aslan *et al*. (2005). Extreme ambiences probably worked as stressors for buffaloes and initiated adaptive responses (Kataria and Kataria, 2005). Goroshinskaia *et al*. (1984) attributed higher activity to cooling stress. Defence against stress is also dependent upon G-6-PDH activity and oxidative pathway of G-6-PDH is also considered 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). Several studies have shown that stress can increase incorporation of glucose into fatty acids with increased activity of glucose-6-phosphate dehydrogenase enzyme (Chimin *et al*., 2014). Buffaloes experience a variety of stressors *viz*. heat, cold, drought, dehydration, infection, trauma, transportation, regrouping, crowding, that modify their behaviour, production and performances. To combat the stress, physiological changes occur according to the priorities of the body.

Effects of physiological states

In group A, serum SDH, MDH G-6Pase and G-6-PDH mean values of non pregnant milch, pregnant milch and pregnant dry animals differed significantly ($p<0.05$) from each other in all the ambiences. In each ambience the mean value of serum SDH, MDH and G-6-PDH of non pregnant milch animals were highest whereas it was lowest in pregnant dry animals and in the hot ambience the mean value of serum G-6-Pase of non pregnant milch animals was highest whereas it was lowest in pregnant dry animals and in cold ambience the mean value of serum G-6-Pase of dry pregnant animal is highest and lowest in non pregnant milch animal.

Higher serum SDH activity probably indicated its paracrine regulatory role for opioids in metabolism (Sreenivasan and Vijayan, 1996). Higher SDH activity in also

resulted in increased serum glucose. In group B, the mean values of serum SDH, MDH and G-6-Pase were significantly ($P \leq 0.05$) lower in multipara animals than primipara and the mean values of serum G-6-PDH was significantly ($P \leq 0.05$) higher in multipara animals than primipara in each ambience. Higher activity of SDH in younger lot coincided with the increased serum glucose in present study, reiterating its metabolic role. The activity of G-6-Pase can be correlated with the serum glucose levels in the present study indicating higher gluconeogenesis. These findings corroborated the earlier recordings (Purser and Bergen, 1968).

Eguinoa *et al.* (2003) also suggested higher G-6-PDH activity in heifers. Since G-6-PDH is a lipogenic enzyme and related with the oxidation of glucose, its high activity 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 female animals can also influence the activity of enzyme (Kelley *et al.,* 1986). Age may change G-6-PDH activity (Nesse and Williams, 1998). 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).

It can be concluded that extreme ambiences produced metabolic changes in the Murrah buffaloes of all physiological states, which was reflected in the form of altered status of the metabolic enzymes determined in the serum. These findings indicated towards the modulation of physiological mechanisms in response to ambient stress. The degree of the extent of changes in the form of values, can be useful to define the role of changes in metabolic activities to combat environmental stress and can be used for clinical diagnosis and in health management of these animals.

Table 1: Mean±SEM values of serum SDH, MDH, G-6-Pase and G-6-PDH in Murrah buffalo

Effects	Serum enzymes (enzymes, U L^{-1})			
	SDH	MDH	G-6- Pase	G-6-PDH
Ambiences				
Moderate (150)	6.00±0.04 [b]	40.00±0.40 [b]	6.98±0.10 [b]	6.00±0.05 [b]
Group A				
Non-pregnant milch (30)	7.40±0.04 [d]	46.00±0.30 [d]	8.48±0.12 [d]	7.10±0.05 [d]
Pregnant milch (30)	6.10±0.03 [d]	41.00±0.40 [d]	7.29±0.10 [d]	6.10±0.04 [d]
Pregnant dry(30)	4.50±0.04 [d]	33.00±0.40 [d]	5.17±0.10 [d]	4.80±0.05 [f]
Group B				
Primipara (30)	7.90±0.03 [f]	42.90±0.40 [f]	8.39±0.10 [f]	5.00±0.04 [f]
Multipara (30)	4.10±0.04 [f]	37.10±0.50 [f]	5.57±0.10 [f]	7.00±0.04 [f]
Hot (150)	9.00±0.04 [b]	60.00±0.50 [b]	10.00±0.11 [b]	3.00±0.02 [b]
Group A				
Non-pregnant milch (30)	12.00±0.04 [d]	79.00±0.43 [d]	12.50±0.10 [d]	4.00±0.02 [d]
Pregnant milch (30)	8.50±0.03 [d]	61.00±0.50 [d]	10.20±0.12 [d]	3.50±0.02 [d]
Pregnant dry(30)	6.50±0.04 [d]	40.00±0.45 [d]	7.30±0.11 [d]	1.50±0.02 [d]
Group B				
Primipara (30)	11.00±0.03 [f]	80.00±0.40 [f]	12.10±0.10 [f]	2.20±0.02 [f]
Multipara (30)	7.00±0.04 [f]	40.00±0.60 [f]	7.90±0.11 [f]	3.80±0.02 [f]
Cold (150)	15.00±0.05 [b]	80.00±0.44 [b]	17.00±0.11 [b]	13.00±0.04 [b]
Group A				
Non-pregnant milch (30)	18.00±0.04 [d]	101.00±0.40 [d]	14.50±0.12 [d]	15.00±0.03 [d]
Pregnant milch (30)	15.50±0.05 [d]	82.00±0.40 [d]	16.50±0.11 [d]	13.50±0.03 [d]
Pregnant dry(30)	11.50±0.06 [d]	57.00±0.45 [d]	20.00±0.10 [d]	10.50±0.03 [d]
Group B				
Primipara (30)	17.00±0.04 [f]	100.00±0.40 [f]	20.60±0.12 [f]	11.40±0.03 [f]
Multipara (30)	13.00±0.06 [f]	60.00±0.44 [f]	13.40±0.10 [f]	14.60±0.04 [f]

Figures in the parenthesis indicate number of animals.
'b' marks significant ($P \leq 0.05$) differences among ambience mean values of a parameter.
'd' marks significant ($P \leq 0.05$) differences among non-pregnant milch, pregnant milch and pregnant dry mean values of a parameter within an ambience.
'f' marks significant ($P \leq 0.05$) differences between mean values of primipara and multipara of a parameter within an ambience
SDH = Sorbitol dehydrogenase
MDH = Malate dehydrogenase
G-6-Pase= Glucose-6-phosphatase
G-6-PDH= Glucose-6-phosphate dehydrogenase

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