RESEARCH ARTICLE Indian Journal of Animal Research, Volume 57 Issue 11: 1469-1473 (November 2023)

Impact of Extreme Hot Environmental Temperature Period on Marker Enzymes of Carbohydrate Metabolism in Non-descript Sheep from Arid Tracts of Rajasthan

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10.18805/IJAR.B-4396

ABSTRACT

Background: The present investigation was envisaged to find out the impact of extreme hot environmental temperature period (ETP) on marker enzymes of carbohydrate metabolism in male and female non-descript sheep of various age groups *i.e.* 4 to 13 months from arid tracts of Rajasthan. Glucose-6-phosphate dehydrogenase (G-6-PDH) and malate dehydrogenase (MDH) marker enzymes of carbohydrate metabolism were considered for study.

Methods: During the period October 2016-June 2017 blood samples were collected to harvest sera for spectrophotometric method from 240 healthy animals selected from private slaughter house during moderate and extreme hot environmental temperature periods (ETPs). The mean values of markers attained during moderate ETP were reckoned as the control. It was 10.00±0.10 UL-1 and 42.00±1.00 respectively.

Conclusion: The mean value of MDH was significantly ($p \leq 0.05$) higher while G-6-PDH significantly ($p \leq 0.05$) lowers during extreme hot temperature in comparison to moderate period. Therefore, it could be concluded that variations in enzyme markers were associated with changes in environmental temperatures. Probably ETP were able to produce a profound effect on carbohydrate metabolism in sheep. Therfore it can be suggested that during the period of extreme temperature balanced ration must be provided to the animal along with proper management to decrease the severity of temperature impact.

Key words: Enzyme, Hot, Metabolism, Non-descript sheep, Sera.

INTRODUCTION

Environment and animal interaction are an imperious argument in scientific deliberations to formulate ploy for the comfort and security of animals and simultaneously to function as an armour of environment (Srivastava *et al*., 2018a). The non-living stressors encourage the environmental elements further to disturb the animal performance or physiology in a notable mode (Thori, 2015). The most prevalent stressor is off-putting environmental temperature period (Behera *et al*., 2005).

Animals managed under natural situations are supple targets of the threat of extreme alterations in ambient temperatures (Yatoo, 2016). These animals belong basically to unorganized areas. Higher environmental temperature period is one of the factors producing a negative impact on the growth rate of sheep (Srivastava and Kataria, 2018). Prolonged elevation in the environmental temperature can alter the feed intake of the animals tremendously thus diverting their metabolic pathways in an attempt to provide greater energy for maintenance (Srivastava *et al*., 2018b).

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 non-descript sheep in a better way (Wang *et al*., 2016). Assessment of the extent of metabolic modulations due to extreme environmental temperature periods can help a clinician in diagnosing pathological conditions, as many times stressed animals show a wide range of changes in the enzyme levels (Varley,1988). Carbohydrate metabolism is important in sheep beginning from growth to the attainment of adulthood (Sharma, 2011).

A study of pathways of carbohydrate metabolism can help in scientific management of the non-descript sheep. Rate of metabolic pathways can be assessed indirectly by quantifying the regulatory enzymes serving as markers of carbohydrate metabolism (Janero *et al*., 1994). Enzyme markers can be taken as scaffolding in understanding the

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How to cite this article: Srivastava, A. and Kataria, N. (2023). Impact of Extreme Hot Environmental Temperature Period on Marker Enzymes of Carbohydrate Metabolism in Non-descript Sheep from Arid Tracts of Rajasthan. Indian Journal of Animal Research. 57(11): 1469-1473. doi: 10.18805/IJAR.B-4396.

Submitted: 06-01-2021 **Accepted:** 16-06-2021 **Online:** 04-08-2021

essential aspects of reproduction and production (Kataria *et al*., 2011). Despite immense quality characteristics, verylittle scientific attention has been paid to understand the regulatory aspect of various metabolic processes. 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 non-descript sheep to set the physiological reference values for the use in diagnostics.

MATERIALS AND METHODS

Blood samples were taken to yield the serum from apparently healthy 240 non-descript male and female sheep of 4-13 months of age groups selected on basis of dentition belonging to owners of private slaughter houses reared under standard management provisions. The study was carried out during the period October 2016 to June 2017, in the Department of Veterinary Physiology, College of Veterinary and Animal Science, Bikaner. Blood samples were obtained during slaughtering from the private slaughter houses under the permission of Institutional Animal Ethics Committee (IAEC), College of Veterinary and Animal Science, Bikaner, Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan. Clear, non-haemolysed sera were used for the investigation.

Outline of experiment

Experiment is based upon the division of the whole experimental period into moderate and extreme hot ETPs. October and November months were taken as moderate where minimum and maximum environmental temperature (°C) ranges from 20.12±0.10 to 30.33±0.10 and relative humidity (%) ranges from 61.54±3.11 to 33.11±1.10. May and June months were considered as extreme hot ETP when the minimum and maximum environmental temperature (°C) range from 29.51± 0.09 to 45.33±0.07 and relative humidity (%) ranges from 28.22±0.70 to 11.01±0.05. In each ETP, 120 blood samples were obtained (60 males and 60 females). In both ETP, the male and female non-descript sheep were grouped as 4-7 months (20 male and 20 female); 7-10 months (20 male and 20 female) and 10-13 months (20 male and 20 female) of age groups. Moderate period was taken as control period.

Glucose-6-phosphate dehydrogenase (G-6-PDH)

Spectrophotometric method as illustrated by King (1965) with modifications (Anonymous, 2010) was employed for the determination of activity. G-6-PDH catalyses the initial step of the oxidative pathway of glucose also named as the pentose phosphate shunt. Assay for the estimation employs the rate of variation of absorbance at 340 mμ.

Reagents

1. Tris buffer (0.1M, pH 7.6).
2. Substrate (0.025 M) solution.

Procedure

A clean dried spectrophotometer cuvette was taken. To this, 1.5 ml buffer, 0.2 ml activator solution, 0.2 ml coenzyme solution and 1 ml serum were introduced and mixed gently with a thin glass rod. To start the reaction, 0.2 ml of substrate solution was added and the enhancement in optical density was measured at 340 mμ in a spectrophotometer (Shimadzu UV-1800) at 3 minute interval for 12 minutes. Four readings were taken. Then change in OD (DOD) per minute was computed.

Calculation

$$\text{Activity } (UL^{-1}) = \Delta OD \text{ per min.} \times Tf \text{ m-I.U.} \times 1000 \times 0.5$$

Tf (Temperature correction factor) = 1 at 25°C;
1000 = conversion factor.
0.5= Correction value.

Malate dehydrogenase (MDH)

Spectrophotometric method as illustrated by King (1965) with modifications (Anonymous, 2010) was employed. MDH is an enzyme of Kreb's citric acid cycle. In the last reaction of citric acid cycle, NAD-linked L-malate dehydrogenase catalyses the oxidation of L-malate to oxaloacetate. It is also present in the system forming urea. It utilises Coenzyme I (NAD). It is one of the smaller enzymes. The assay uses oxaloacetate substrate and reduced coenzyme. The procedure measures the rate of decrease in optical density at 340 mμ.

Reagents

1. Sorensen M/15 phosphate buffer, pH 7.4.
2. Substrate solution.
3. Coenzyme solution.

Procedure

A spectrophotometer cuvette was taken. To this 0.2 ml serum, 2.5 ml buffer and 0.2 ml coenzyme were introduced in a sequential manner. Then it was permitted to stand for 20 minutes at 25°C to allow the reduction of substrates of endogenous origin. Recording of optical density was done by using spectrophotometer (Shimadzu UV-1800) at 340 mμ at one minute interval till achievement of a steady reading. It was discerned that 2nd and 3rd readings were became steady at 2nd and 3rd minute, respectively after the initiation of recording. Then addition of 0.2 ml of freshly prepared substrate was done. Then it was well mixed using a thin glass rod. Again optical density was observed after one minute at 340 mμ (Shimadzu UV-1800) and then at interval of every one minute for 5 minutes (5 recordings were made).

Calculation

The activity of the enzyme was computed from the readings which showed identical decrease in equal time intervals and adjusted to 25°C by means of the Tf (1 at 25°C); serum 0.2 ml and correction factor 0.5.

$$\text{Activity } UL^{-1} = \Delta OD_{340}/\text{minute} \times 1000 \times \frac{1}{0.2} \times 0.5 \times Tf$$

Statistical analysis

Various computer programmes were used to calculate means and standard error (http://www.miniwebtool.com) and analysis of variance (www.danielsoper.com) to test thesignificance of the effects of environmental temperature, sex and age groups (Kaps and Lamberson, 2004). The variations in the means were assessed by Duncan's new multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

The mean value of serum G-6-PDH attained during moderate environmental temperature period was 10.00±0.10 UL^{-1}. In the calculation of G-6-PDH control value the range obtained was 8.65-11.30 UL^{-1} (Table 1).

Influence of extreme hot environmental temperature period on serum G-6-PDH

A significant ($p \leq 0.05$) decrease was found in the mean value at the time of extreme hot ETP when compared to moderate ETP mean value of serum G-6-PDH. Influence of extreme ETP was established to be highly significant ($p \leq 0.01$) on serum G-6-PDH as expressed by analysis of variance test. Per cent change in the serum G-6-PDH mean value was evaluated to be -30.00, when ETP was extreme hot as compared to moderate.

Influence of sex and age on serum G-6-PDH

Mean values of G-6-PDH were recorded to be significantly ($p \leq 0.05$) higher in male sheep as compared to female sheep in both the ETPs. However, magnitude of per cent lowering due to extreme hot ETP was more in male than female sheep. Mean values of serum G-6-PDH were observed to be highest in sheep of 10-13 months age group and lowest in 4-7 months of age group. All the alterations were found to be significant ($p \leq 0.05$). Age group of 10-13 months in the investigation revealed lowest percent alteration during extreme hot ETP and 4-7 months group exhibited maximum per cent alteration during extreme hot ETP. Sex and age effects were also reported by Joshi (2012) in buffaloes and Pandey (2012) in goat for serum G-6-PDH values. Effect of sex and age was observed on the mean values of G-6-PDH in the erythrocytes of goat by Chaturvedi (2011) and erythrocytes of sheep by Deeksha (2015).

Table 1: Mean±SEM values and Per cent alterations of serum glucose-6-phosphate dehydrogenase (G-6-PDH, UL^{-1}) in the non-descript sheep during moderate and extreme hot environmental temperature periods (ETPs).

Effects	Moderate ETP mean±SEM (120)	Extreme hot ETP mean ±SEM (120)	Per cent alterations in hot ETP
Environmental temperature periods ETP (120)	10.00^{b}±0.10	7.00^{b}±0.10	-30.00
Male (60)	11.00^{bd}±0.07	8.00^{bd}±0.06	-27.27
Female (60)	9.00^{bd}±0.07	6.00^{bd}±0.06	-33.33
4-7 Month (40)	8.75^{bf}±0.03	5.10^{bf}±0.04	-41.71
7-10 Months (40)	10.05^{bf}±0.05	7.20^{bf}±0.03	-28.35
10-13 Months (40)	11.20^{bf}±0.03	8.70^{bf}±0.03	-22.32

i. Figures in the parenthesis indicate number of non-descript sheep.
ii. 'b' marks significant ($p \leq 0.05$) differences between moderate and hot ETPs for a row.
iii. 'd' marks significant ($p \leq 0.05$) differences between male and female mean values within an ETP.
iv. 'f' marks significant ($p \leq 0.05$) differences among mean values of the age groups within an ETP.
v. Per cent alterations have been calculated from respective moderate mean value.

Table 2: Mean±SEM values and Per cent alterations of serum malate dehydrogenase (MDH, UL^{-1}) in the non-descript sheep during moderate and extreme hot environmental temperature periods (ETPs).

Effects	Moderate ETP mean±SEM (120)	Extreme hot ETP mean±SEM (120)	Per cent alterations in hot ETP
Environmental temperature periods ETP (120)	42.00^{b} ±1.00	67.00^{b} ±1.00	59.52
Male (60)	46.00^{bd}±0.81	69.00^{bd} ±0.88	50.00
Female (60)	38.00^{bd}±0.82	65.00^{bd} ±0.87	71.05
4-7 Month (40)	36.50^{bf}±0.50	64.00^{bf}±0.52	77.77
7-10 Months (40)	43.00^{bf}±0.51	66.00^{bf}±0.51	53.48
10-13 Month (40)	47.00^{bf}±0.50	71.00^{bf}±0.50	51.06

i. Figures in the parenthesis indicate number of non-descript sheep.
ii. 'b' marks significant ($p \leq 0.05$) differences between moderate and hot ETPs for a row.
iii. 'd' marks significant ($p \leq 0.05$) differences between male and female mean values within an ETP.
iv. 'f' marks significant ($p \leq 0.05$) differences among mean values of the age groups within an ETP.
v. Per cent alterations have been calculated from respective moderate mean value.

Malate dehydrogenase (MDH)

The mean value of serum MDH was 42.00±1.00 UL^{-1}. In the calculation of MDH control value, findings from 120 non-descript sheep, irrespective of sex and age were used. The range obtained was 35.50-47.50 UL^{-1}. Mean values were also calculated to find the alterations taking into account the sex and age in each ETP for serum MDH (Table 2).

Influence of extreme hot environmental temperature period on serum malate dehydrogenase enzyme

A significant ($p \leq 0.05$) increase was found in the mean value at the time of extreme hot ETP when compared to moderate ETP. Influence of extreme ETP was established to be highly significant ($p \leq 0.01$) on serum MDH. It was expressed by analysis of variance test. Per cent change in the serum MDH mean value was evaluated to be 59.52, when ETP was extreme hot as compared to the condition when ETP was moderate.

Influence of sex and age on serum malate dehydrogenase enzyme

Mean values of MDH were recorded to be significantly ($p \leq 0.05$) higher in male sheep in both the ETPs. However, magnitude of per cent lowering due to extreme hot ETP was more in female sheep than male sheep. Mean values of serum MDH were observed to be highest in sheep of 10-13 months and lowest in 4-7 months of age group. All the alterations were found to be significant ($p \leq 0.05$). Age group of 4-7 months in the investigation revealed highest per cent alterations during extreme hot ETP and 10-13 months group exhibited minimum per cent alteration during extreme hot ETP. The trend of changes in the value of serum MDH due to sex and age corroborated the earlier research in different animals (Kour, 2010; Joshi, 2012 and Pandey, 2012). It was observed that animals of both the genders and age groups were affected due to hot ETP.

Glucose-6-phosphate dehydrogenase is an important enzyme of pentose phosphate pathway and supplies reductive energy required for synthetic purposes (Cramer *et al*., 2006). Variation in the levels of this enzyme was clearly an indication of change in metabolomics of the animals owing to hot ambient temperatures (Ballard *et al*., 1972, Sharma and Kataria, 2007 and Sharma and Kataria, 2008). Low levels of this enzyme can reduce supply of NADPH needed for many important synthetic processes (Goroshinskaia *et al*., 1984). Scientists believe that these changes, if persist for a longer period can affect the physiology by influencing growth, production and reproduction (Pandey *et al*., 2012; Khan *et al*., 2015). Higher ETP is associated with the development of oxidative stress in animals (Kataria *et al*., 2010).

Enzyme MDH catalyzes the oxidation of malate to oxaloacetate using the reduction of NAD^+ to NADH (Sharma and Patnaik, 2008). This reaction is an important part of citric acid cycle (Goodridge *et al*., 1984). Alterations in the value of this enzyme during extreme hot ETP indicated that peripheral utilization of glucose was increased in the animals to meet the crisis and to provide more energy in terms of ATPs for the conduction of many vital reactions in the body (Roper, 2017).

CONCLUSION

It could be concluded that extreme ambient temperatures produced changes in the markers of carbohydrate metabolism. Probably metabolic pathways were modulated to help the non-descript sheep in combating extreme hot ETP stress. Pattern of variation will help in understanding metabolic adjustments required to develop strategies for better health management of the non-descript sheep. Based on result comparison between moderate and extreme environmental temperatures, it can be suggested that during the periods of extreme hot ETP balanced nutrition must be provided along with proper management to decrease the severity of temperature impact.

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