Veterinary Research 6 (2): 32-38, 2013
ISSN: 1993-5412

Assessment of Modulations in Gluconeogenesis During Extreme Environmental Temperatures in Marwari Goat from Arid Tracts

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Abstract: A study was conducted to assess modulations in gluconeogenesis in Marwari goat of both sexes ageing 6 months to 4.5 years during moderate, hot and cold environmental temperature periods. Liver samples were collected to determine Phosphoenolpyruvate Carboxykinase (PEPCK), Glucose-6-Phosphatase (Glc-6-Pase), Aspartate amino Transferase (AST) and Alanine Aminotransferase (ALT) enzymes. Moderate mean values of liver tissue PEPCK, Glc-6-Pase, AST and ALT were 562.00±7.25 U g^{-1}, 160.00±4.25 mU g^{-1}, 3.6±0.02 U g^{-1} and 2.6±0.01 U g^{-1}, respectively. The mean values were significantly ($p \leq 0.05$) higher during hot and cold environmental temperatures in comparison to moderate mean value in each case. The sex and age effects were significant ($p \leq 0.05$) in all the environmental temperatures for all the four enzymes and pattern of variation was similar. The mean values were significantly ($p \leq 0.05$) higher in female animals than male animals. Age effect showed a significant ($p \leq 0.05$) increase in the mean values being highest in the animals of 2-4.5 years of age. Variation in the activities of all the enzymes studied clearly indicated the greater effect of hot environmental temperature on the modulation of gluconeogenesis. Hot period related change was maximum in the activity of PEPCK (4.29 fold) followed by Glc-6-Pase (3.5 fold), ALT (2.92 fold) and AST (2.38 fold). PEPCK and Glc-6-Pase are important enzymes of gluconeogenesis. Enhanced activities of these two enzymes showed adaptive response to combat the stress of environmental temperature through increased glucose production.

Key words: ALT, AST, environmental temperature, Glc-6-Pase, Marwari goat, PEPCK

INTRODUCTION

Carbohydrate metabolism in the animals have several pathways and gluconeogenesis is one of them. The main feature of carbohydrate metabolism is to generate energy power but it also functions to support the body in terms of immunity, prevention of oxidative stress and help in biosynthesis of important molecules. Modulations in metabolic pathways can be best assessed by quantitation of governing enzymes. Ruminants require glucose for several important metabolic processes. Glucose must be provided from either glucose absorption or gluconeogenesis from substrates such as propionate, lactate or amino acids. In both the pre ruminant and ruminant states, the need for active gluconeogenesis to maintain glucose homeostasis is apparent. Pyruvate is a common entry point in the gluconeogenic pathway for lactate, alanine and other gluconeogenic amino acids.

Pyruvate formed from lactate and amino acids is transported into the mitochondria and carboxylated to oxaloacetate by pyruvate carboxylase (Donkina and Hammon, 2005).

Liver governs the carbohydrate metabolism efficiently to meet the demand of the animal. When hepatic glycogen reserves become exhausted, hepatocytes activate additional groups of enzymes that begin synthesising glucose from amino acids and non-hexose carbohydrates by gluconeogenesis. The ability of the liver to synthesise this new glucose is of monumental importance in ruminants where end products of digestion are volatile fatty acids and not the glucose. Hepatocytes house many different metabolic pathways by employing several enzymes and damage of these cells due to toxic agents, injury or diseases has been detected in domestic animals by measuring the activity of liver specific enzymes (Reichling and Kaplan, 1988).

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Animals are drastically affected at all levels of their organisation by any change in their thermal surroundings and the process of adaptation of organisms to seasonal changes involve variation in every aspect of physiology (Al-Bassam *et al*., 2007). 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, 2005). Measurement of the activities of various enzymes related with carbohydrate metabolism will help in understanding the modulation of metabolism under harsh conditions in these animals.

Much attention has been paid on the determination of activities of these enzymes in the plasma or serum. There is paucity of literature regarding the activities of enzyme regulators of carbohydrate metabolism in liver tissue. Such studies can be useful in future experiments and in computer modeling analyses of ruminant liver and whole animal metabolism (Looney *et al*., 1987). Marwari goat constitutes a major portion of the goat population in western part of Rajasthan (Kataria and Kataria, 2007). From research and diagnostic point of view, many aspects are yet to be unveiled in Marwari breed of goat. Despite of its immense significance as financial supporter of marginal farmers and animal owners, studies on tissue enzymes have not been well documented. Therefore obtaining of base line data will contribute towards generating reference values which can be used in diagnosis of diseases for criteria of adaptability as well as to elucidate some physiological mechanisms related to energy metabolism. Hence, the present investigation was planned to find out extreme ambience associated modulations in gluconeogensis in Marwari goat from arid tracts.

MATERIALS AND METHODS

Animals: Six hundred and thirty liver samples of apparently healthy Marwari goat of both sexes ageing 6 months to 4.5 years were collected from private slaughter house during moderate, hot and cold environmental temperature periods. Liver samples were collected immediately after slaughter. In each environmental temperature period 210 liver samples were collected and the animals were grouped into male (105) and female (105). Further each group was divided according to age as below 1 year (70:35 male and 35 female) 1-2 years (70:35 male and 35 female) and 2-4.5 years (70:35 male and 35 female). In case of adult females, sampling was carried out from non pregnant animals only. Mean maximum environmental temperatures during moderate and extreme hot periods were 30.63±0.21 and 45.4±0.03°C and mean minimum environmental temperatures during extreme cold periods was 2.10±0.01°C.

Collection and processing: Liver tissue samples were collected from the animals immediately after slaughter with the help of sterile B.P. blade for the determination of enzyme regulators of carbohydrate metabolism. The liver tissue samples were taken in the ice box and brought to the laboratory. Each sample was cleaned and washed with sterile normal saline solution. Then, 1 g piece of each tissue sample was weighed accurately. Into a tube, 5 mL of Normal Saline Solution (NSS) was taken and 1 g of liver tissue sample was added to it. Proper mixing was carried out in a tissue homogenizer. After mixing the final volume was made to 10 mL by adding normal saline solution. Due care was taken to maintain the temperature from 4-8°C by using chilled water. Then, the fluid was shifted to small beaker and vibrated for 10 min in an electronic digital vibrator (Centuary) at 1000 rpm. The fluid was transferred to a test tube and it was centrifuged at 4°C for 20 min. Then, the tube was kept in an incubator at 37°C for 1 h and the tissue supernatant was collected. This was a modification of method described by Cornelius *et al*. (1959) and Bengoumi *et al*. (1997). This tissue supernatant was used to determine the liver enzymes of gluconeogenesis as the procedure mentioned for the serum.

The modifications were applied after the several pilot trials and it was observed that the values obtained by the simplified technique used in the present study were having a non-significant ($p>0.05$) difference with those obtained by the actual procedures given which were lengthy. Calculation was carried out as for serum. The 1 mL of tissue supernatant represented 0.1 g of tissue sample. Then, by using dilution factor the value was calculated for 1 g of liver tissue as units per g basis. In the case of samples showing optical densities beyond the standard range, dilutions were made while recording optical densities and then necessary corrections were made while doing the calculation.

Analysis: The samples were analysed to find out modulations in gluconeogenesis during extreme environmental temperature periods in Marwari goat. The enzymes invoved in the study were Phosphoenolpyruvate Carboxykinase (PEPCK), Glucose-6-Phosphatase (Glc-6-Pase), Aspartate Amino Transferase (AST) and Alanine Aminotransferase (ALT).

Phosphoenolpyruvate Carboxykinase (PEPCK): It was determined by the methods given by Desai *et al.* (1997) and Smith (2010) with modification. Phosphoenolpyruvate Carboxykinase (PEPC, 4.1.1.32) catalyses the conversion of oxaloacetate into phosphoenolpyruvate and carbon dioxide. Activity was assayed in the presence of NADH and malate dehydrogenase by measuring the rate of incorporation of carbon from bicarbonate into malate. For PEPCK activity 1 g liver tissue was homogenised at 4°C in 4.5 mL of 0.25 M sucrose, 4.5 mL 5 mM tris. HCl and 1 mL 10% ethanol in a tissue homogenizer for 10 min. Then, centrifuged at 5000 rpm for 30 min. The supernatant was used immediately for determination of activity.

Glucose-6-Phosphatase (Glc-6-Pase): It was determined by Spectrophotometric Method (King, 1965). Glucose-6-phosphatase is a phosphomonoesterase which apart from hydrolysing D-glucosamine 6-phosphate (2-amino-2-deoxy-D-glucose 6-phosphate) is specific for D-glucose 6-phosphate. It catalyses the final step in the mobilization of glycogen to glucose and is mainly present in liver and kidney. The assay consists of incubating the sample in the presence of a glucose-6-phosphate substrate buffered to pH 6.5 by means of a citrate buffer and precipitating the proteins to terminate the enzymic reaction and estimating the liberating inorganic phosphate.

Aspartate aminotransferase (AST): It was determined by Spectrophotometric Method (King, 1965). Aspartate transaminase is involved in the enzyme system forming urea. The activity of the enzyme is measured by the increase of oxaloacetate with time as the reaction proceeds from right to left. After a fixed time the oxaloacetate formed is determined spectrophotometrically by treating the 2,4-dinitrophenylhydrazine with alkali. Aniline citrate is used to decarboxylate the oxaloacetate formed enzymatically.

Alanine aminotransferase (ALT): It was determined by Spectrophotometric Method (King, 1965). The activity of enzyme is measured by the increase of pyruvate with time. After a fixed time the pyruvate formed from L-alnine and α-oxoglutaric acid according to equation is determined colorimetrically by treating the 2,4-initrophenylhydrazine with alkali.

Statistics: The main parameters were liver enzymes of gluconeogenesis in Marwari goat. The main effects were environmental temperatures, sex and age groups. The subsets were moderate, hot and cold periods male and female and below 1, 1-2 and 2-4.5 years. For each subset data were expressed as mean±SE of mean. For each subset data were expressed as mean±SE of mean. The changes in the means were measured by using multiple mean comparison procedures. For this Duncan's new multiple range test (Duncan, 1955; Steel and Torrie, 1980) was used.

RESULTS AND DISCUSSION

Phosphoenolpyruvate Carboxykinase (PEPCK): Mean±SEM values of liver phosphoenolpyruvate carboxykinase during extreme environmental temperatures, sex and age groups are presented in Table 1. Moderate mean value was 562.00±7.25 U g^{-1} which was obtained from 210 animals during moderate environmental temperature. The mean value of liver PEPCK was significantly ($p \leq 0.05$) higher during hot and cold environmental temperatures in comparison to overall moderate mean value. It is an enzyme in the lyase family used in the metabolic pathway of gluconeogenesis and is a rate-controlling step of gluconeogenesis, the process by which cells synthesize glucose from metabolic precursors. PEPCK is a key enzyme giving pathways to important substrates of fermentation to enter into the gluconeogenetic pathway through oxaloacetate to be converted into phosphoenol pyruvate. In this relation, propionate is important (Beitz, 1996).

Extreme environmental temperatures probably produced stress which was related to higher glucose demand. Earlier researchers have also reported increased PEPCK activity during stress (Hopgood and Ballard, 1973) and related it to higher cortisol activity (Jones *et al.*, 1993). Hamada and Matsumoto (1984) observed that carbohydrate starvation was related with increased cytosolic phosphoenolpyruvate carboxykinase activity. Higher gluconeogenic activity is related to higher glucose

Table 1: Mean±SEM values of phosphoenol pyruvate carboxy kinase (PEPCK, U g^{-1}) in the liver of Marwari goat

	Environmental temperature periods		
Effects	Moderate	Hot	Cold
Overall (210)	562.00±7.25[b]	2411.0±23.13[b]	1125.0±19.60[b]
Sex			
Male (105)	314.00±5.24[d]	2208.0±22.00[d]	923.0±17.60[d]
Female (105)	610.00±9.32[d]	2614.0±24.15[d]	1327.0±20.60[d]
Age (years)			
<1 (70)	326.0±3.910[f]	2101.0±21.13[f]	920.0±16.23[f]
1-2 (70)	548.0±4.360[f]	2414.0±21.44[f]	1126.0±20.31[f]
2-4.5 (70)	612.0±5.150[f]	2718.0±23.94[f]	1329.0±21.41[f]

Figures in the parenthesis indicate number of animals; [b]Marks significant ($p \leq 0.05$) differences among overall mean values of a parameter; [d]Marks significant ($p \leq 0.05$) differences between male and female mean values of a parameter within an environmental temperature; [f]Marks significant ($p \leq 0.05$) differences among mean values of different age groups of a parameter within an environmental temperature. Overall values have been calculated irrespective of environmental temperature

(Murondoti *et al*., 2004) and indicates a better adaptation of metabolism and hepatic glucose production (Van Harten *et al*., 2012). Variation in temperature can affect PEPCK concentration (Hyatt *et al*., 2008) indicating effect of ambience on liver activity.

The sex and age effects were significant ($p \leq 0.05$) in all the environmental temperatures. The mean values were significantly ($p \leq 0.05$) higher in female animals than male animals. Age effect showed a significant ($p \leq 0.05$) increase in the mean values being highest in the animals of 2-4.5 years of age. Higher levels showed the greater significance of gluconeogenesis which is of continual importance in ruminants (Beitz, 1996). Influence of testosterone and oestrogen have been reported on the PEPCK activity (Wimmer, 1989). Age related variations have been recorded by earlier researchers (Sharma and Patnaik, 1984; Haga *et al*., 2008) in animals. Increased PEPCK activity represents enhanced rate of gluconeogenesis resulting in increased glucose production (Murondoti *et al*., 2004).

Glucose-6-Phosphatase (Glc-6-Pase): Mean±SEM values of liver glucose-6-phosphatase during extreme environmental temperatures, sex and age groups are presented in Table 2. Moderate mean value was 160.00±4.25 mU g^{-1} which was obtained from 210 animals during moderate environmental temperature. The mean value of liver Glc-6-Pase was significantly ($p \leq 0.05$) higher during hot and cold environmental temperature periods in comparison to moderate mean value. It is an important enzyme of gluconeogenesis and glycogenolysis hydrolyzing glucose-6-phosphate resulting in the creation of a phosphate group and free glucose. Glucose is then exported from the cell via glucose transporter membrane proteins. This catalysis completes the final step in gluconeogenesis and glycogenolysis and therefore plays a key role in the homeostatic regulation of blood glucose levels. 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. Significant amount of G-6-Pase are found in liver. This is in accord with the well known function of the liver as the principal source of supply of glucose for the maintenance of blood glucose concentration. Generally G-6-Pase activity is higher than the glucokinase activity which indicates that most of the time liver supplies glucose rather using it (Lehninger *et al*., 1993).

Table 2: Mean±SEM values of glucose-6- phosphatase (Glc-6-Pase, mU g^{-1}) in the liver of Marwari goat

	Environmental temperature periods		
Effects	Moderate	Hot	Cold
Overall (210)	160.00±4.25[b]	560.00±5.13[b]	400.00±4.6[b]
Sex			
Male (105)	101.00±1.24[d]	470.0±6.00[d]	310.0±6.00[d]
Female (105)	219.00±3.32[d]	650.0±7.50[d]	490.0±8.00[d]
Age (years)			
<1 (70)	90.0±3.010[f]	450.0±6.13[f]	290.0±3.23[f]
1-2 (70)	170.0±4.000[f]	550.0±7.44[f]	420.0±4.31[f]
2-4.5 (70)	220.0±2.150[f]	680.0±8.94[f]	490.0±4.01[f]

Figures in the parenthesis indicate number of animals; [b]Marks significant ($p \leq 0.05$) differences among overall mean values of a parameter; [d]Marks significant ($p \leq 0.05$) differences between male and female mean values of a parameter within an environmental temperature; [f]Marks significant ($p \leq 0.05$) differences among mean values of different age groups of a parameter within an environmental temperature. Overall values have been calculated irrespective of environmental temperature

Hot and cold conditions 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). Literature reported the relation between ALT and G-6-Pase activities as glucose can be synthesized from alanine (Bhattacharya and Datta, 1993). Higher ALT activities were recorded in present study during hot ambient temperature period. Gluconeogenesis in ruminants occurs at a high rate even in the fed state (Looney *et al*., 1987).

The sex and age effects were significant ($p \leq 0.05$) in all the environmental temperatures. The mean values were significantly ($p \leq 0.05$) higher in female animals than male animals. Age effect showed a significant ($p \leq 0.05$) increase in the mean values being highest in the animals of 2-4.5 years of age. Higher activity of Glc-6-Pase showed higher rate of gluconeogenesis in females than males and in animals of 2-4.5 years of age as compared to lower age group stock. Purser and Bergen (1969) suggested that changes with age in hepatic gluconeogenesis of ruminants were not dependent on rumen development and volatile fatty acid production.

Aspartate aminotransferase (AST): Mean±SEM values of liver aspartate aminotransferase during extreme environmental temperatures, sex and age groups are presented in Table 3. Moderate mean value was 3.6±0.02 U g^{-1} which was obtained from 210 animals during moderate environmental temperature. The mean value of liver AST was significantly ($p \leq 0.05$) higher during hot and cold environmental temperature in comparison to moderate mean value. AST facilitates the conversion of aspartate and alpha-ketoglutarate to oxaloacetate and glutamate and vice-versa. It provides precursors for gluconeogenesis via transamination.

Table 3: Mean±SEM values of aspartate aminotransferase (AST, U g^{-1}) in the liver of Marwari goat

	Environmental temperature periods		
Effects	Moderate	Hot	Cold
Overall (210)	3.6±0.02[b]	8.6±0.01[b]	5.2±0.06[b]
Sex			
Male (105)	2.5±0.03[d]	7.7±0.04[d]	4.3±0.03[d]
Female (105)	4.7±0.02[d]	9.5±0.01[d]	6.1±0.02[d]
Age (years)			
<1 (70)	2.4±0.03[f]	7.2±0.02[f]	4.2±0.02[f]
1-2 (70)	3.5±0.02[f]	8.7±0.01[f]	5.1±0.04[f]
2-4.5 (70)	4.9±0.02[f]	9.9±0.03[f]	6.3±0.02[f]

Table 4: Mean±SEM values of alanine aminotransferase (ALT, U g^{-1}) in the liver of Marwari goat

	Environmental temperature periods		
Effects	Moderate	Hot	Cold
Overall (210)	2.6±0.01[b]	7.6±0.01[b]	4.2±0.02[b]
Sex			
Male (105)	1.6±0.01[d]	6.8±0.02[d]	3.2±0.03[d]
Female (105)	3.6±0.02[d]	8.4±0.01[d]	5.2±0.02[d]
Age (years)			
>1 (70)	1.5±0.02[f]	6.1±0.02[f]	3.3±0.02[f]
1-2 (70)	2.4±0.01[f]	7.9±0.03[f]	4.0±0.01[f]
2-4.5 (70)	3.9±0.02[f]	8.8±0.03[f]	5.3±0.02[f]

Figures in the parenthesis indicate number of animals; [b]Marks significant ($p \leq 0.05$) differences among overall mean values of a parameter; [d]Marks significant ($p \leq 0.05$) differences between male and female mean values of a parameter within an environmental temperature; [f]Marks significant ($p \leq 0.05$) differences among mean values of different age groups of a parameter within an environmental temperature. Overall values have been calculated irrespective of environmental temperature

The results were in accordance to the observations of earlier researchers (Tibbo *et al*., 2008). Climate associated variations in the activity of AST may indicate stress to liver. Gahlot *et al*. (1987) emphasised the possible role of aspartic acid in dietary fixation of ammonia and amination of keto acids thereby helping the animals to combat the stress of heat. Kataria and Bhatia (1991) suggested better heat adaptation due to higher AST activities during hot conditions.

The sex and age effects were significant ($p \leq 0.05$) in all the environmental temperatures. The mean values were significantly ($p \leq 0.05$) higher in female animals than male animals. Age effect showed a significant ($p \leq 0.05$) increase in the mean values being highest in the animals of 2-4.5 years of age. Sex and age effects were also shown by earlier researchers in animals (Tibbo *et al*., 2008).

Alanine aminotransferase (ALT): Mean±SEM values and analysis of variance of liver alanine aminotransferase during extreme environmental temperatures, sex and age groups are presented in Table 4. Moderate mean value was 2.6±0.01 U g^{-1} which was obtained from 210 animals during moderate environmental temperature. The mean value of liver ALT was significantly ($p \leq 0.05$) higher during hot and cold environmental temperature in comparison to moderate mean value. Alanine aminotransferase is an enzyme of alanine cycle. When muscles produce lactate during the condition of decreased oxygen, they also produce alanine. This alanine is shuttled to the liver where it is used to make glucose. ALT catalyses the transfer of an amino group from alanine to a-ketoglutarate, the products of this reversible transamination reaction being pyruvate and glutamate. In gluconeogenesis glucose is formed from non carbohydrate precursors and amino acids being one of them. Transamination of amino acids facilitates the entry of carbon skeleton directly into the cycle as pyruvate or oxaloacetate or indirectly via TCA cycle.

Nazifi *et al*. (2003) reported higher ALT activity in heat stressed sheep. Higher cortisol in heat stressed animals probably stimulated ALT synthesis. The intracellular cytoplasmic level of ALT is several times that of extracellular fluid. The sex and age effects were significant ($p \leq 0.05$) in all the environmental temperatures. The mean values were significantly ($p \leq 0.05$) higher in female animals than male animals. Age effect showed a significant ($p \leq 0.05$) increase in the mean values being highest in the animals of 2-4.5 years of age. Sex and age effects were also observed by earlier researchers (Tibbo *et al*., 2008; Saeed and Hussain, 2006).

CONCLUSION

Variation in the activities of all the enzymes studied clearly indicated the greater effect of hot environmental temperature on the modulation of gluconeogenesis. Modulation of gluconeogenesis is an important aspect to understand beneficial turning of metabolic processes during stress period. It was observed that effect of hot period was of greater magnitude in comparison to cold period. It was consistent for each subset. Hot period related change was maximum in the activity of PEPCK (4.29 fold) followed by Glc-6-Pase (3.5 fold), ALT (2.92 fold) and AST (2.38 fold). PEPCK and Glc-6-Pase are considered important enzymes of gluconeogenesis and their enhanced activities showed adaptive response to combat the stress of environmental temperature with increased glucose production.

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