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Prakash Chandra Sanwal
Department of Veterinary Physiology, College of Veterinary and Animal Science Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan, India

Nalini Kataria
Department of Veterinary Physiology, College of Veterinary and Animal Science Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan, India

Corresponding Author:
Prakash Chandra Sanwal
Department of Veterinary Physiology, College of Veterinary and Animal Science Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan, India

Pattern of extremely higher environmental temperature linked changes in endogenous antioxidant responses of liver in Marwari goat

Prakash Chandra Sanwal and Nalini Kataria

Abstract
Evaluation of the pattern of extremely higher environmental temperature linked variations in endogenous antioxidant responses of liver in Marwari goat was carried out in the present exploration. Liver samples were collected from Marwari breed of goat ageing from 3 to 12 months during extremely higher or hot environmental temperature periods (ETPs) from private slaughter houses. To achieve the objectives of the investigation, analytes were measured in the liver during extremely hot ETPs. The endogenous antioxidant responses of liver included different analytes i. e. vitamin E and vitamin C. A significant ($p \leq 0.05$) decline in the mean values of vitamin E and vitamin C was observed during extremely hot ETP as compared to respective comfortable ETP mean value. The results of the investigation pointed out that male Marwari goat had superior antioxidant potential of liver as compared to female goat. Percent changes in the values reflected the capability of the goats to contrive the reactions during the extremely hot ETP. It can be proposed that goats of arid tracts must be protected from extended exposure to extremely hot environmental temperature.

Keywords: Pattern, extremely higher, environmental temperature, endogenous antioxidant responses

Introduction
Physiology of stress and performance of animal are estimable kits of environment to erect cover and housing methods for animals. It is essential to check the retorts of productivity when stress is present so that strategies related to management can be tailored for the sake of animal welfare (Kataria and Kataria, 2016) [8]. Imbalance of antioxidant and oxidants furnishes oxidative stress with a cellular damage. Free radicals mediate these reactions. Free radicals can bring out serious changes in the cells (Saini *et al*., 2018 and Singhal *et al*., 2018) [18, 22]. The oxidative stress produces liver insult. The mechanism involves changes in contents of DNA, lipids and proteins. Changes in pathways regulating biological tasks may occur. Excessive production of reactive oxygen species (ROS) can jeopardize this homeostasis and can produce oxidative stress (Li *et al*., 2014) [14]. Stress from any source is able to reduce the endogenous antioxidants (Kataria *et al*., 2010a) [12]. Recording of the contents can assist in the explanation of stress level of the animals. Heat stress changes metabolic parameters through free radicals and causes oxidative stress (Kataria *et al*., 2010b, c and Kataria *et al*., 2013a) [11, 13, 9]. It is important to monitor these endogenous antioxidant responses of the liver. Non-enzymatic antioxidants are certain vitamins like C and E. Antioxidants also considered to have a wide scope to confiscate metal ions involved in mechanism of oxidative stress. Oxidative stress is an important issue in the expansion of several disorders (Kataria *et al*., 2012 and Joshi, 2018) [10, 5]. Effect of extreme environmental temperature on various serum physiological parameters has been studied (Kain *et al.*, 2022a, b, Sihag *et al.*,2022 and Singh and Kataria 2022) [6, 7, 20, 21], however, paucity of work is there on liver analytes. Goat bears great economical value in the form of meat industry and milk importance; however, very little scientific awareness has been given over the protective features from the stressors. These animals are transported frequently and grouping and regrouping are common stressors experienced by them. These activities are known to induce emotional stress to the animals. Simultaneously, all the above-mentioned stressors are coupled with the most potent stressor, the higher environmental temperature. Looking towards the worth of Marwari breed of goats with the aim to set the reference values of native breeds, an endeavour associated with antioxidant responses can facilitate researchers and clinicians to put in a nutshell the contemporary implications.

Materials and Methods

Liver tissue was collected from the goat after slaughter with the help of sterile B.P. blade for the measurement of parameters of antioxidant responses. After completion of cleaning, each sample was washed with sterile normal saline solution. Then precise weighing of 1g piece of each tissue sample was done. Into a clean dried test tube, 5 ml of normal saline solution was introduced and 1g of liver tissue sample was taken into it. A tissue homogenizer was used for proper homogenization of liver tissue with liquid. The final volume was made to 10 ml by adding normal saline solution with mixing. Due care was taken to maintain the temperature from 4 to 8 °C by the use of chilled distilled water. Then this was shifted to small beaker and vibrated at 1000 rpm for 10 minutes in an electronic digital vibrator (Century). Then again it was shifted to a test tube and centrifuged at 4 °C (10,000rpm) for 20 minutes. Then the tube was put in an incubator at 37 °C for 1hour.This tissue supernatant was used to measure the different liver antioxidant responses as the procedure mentioned for the serum using spectrophotometer (Shimadzu UV-1800) [19]. The levels of antioxidants were determined per mg of protein. Protein in liver supernatant was determined by biuret method (Oser, 1976) [16] with little modification.

Calculation was carried by taking one ml of tissue supernatant signified 0.1g of liver tissue sample. In a particular sample, when the optical densities were found beyond the range, dilutions were made while measuring optical densities and then needed corrections were made while doing the calculations (Anonymous, 2010a) [2].

Following parameter were analyzed to accomplish the present study:

Vitamin E

A spectrophotometric procedure (Nair and Magar, 1955) [15] with modest modification (Anonymous, 2016) [3] was employed for analysis. The procedure is sensitive enough to sense the minute concentration of serum vitamin E. A colour mechanism occurring between phosphomolybdic acid and vitamin E is the basis of method.

Procedure

A clean and dried glass test tube was set in a rack and 1 ml of supernatant and 0.5 ml. of 5% NaOH solution were introduced to it. After genteel shaking for 2 minutes, tubes were positioned for 6 hours at the room temperature. Then dilution of solution was cautiously made with 10 ml double glass distilled water. Introduction of petroleum ether (5 ml) was made. Extracts in petroleum ether were precisely got after 10 minutes of centrifugation at 3000 rpm. Cleansing of extract was vigilantly made by introducing 10 ml distilled water and centrifugation was carried out. Petroleum ether solution was shifted to a clean dried test-tube and the vigilant evaporation of solvent to dryness by employing a boiling water bath and by straight heating was carried out. Introduction of one ml of the phosphomolybdic acid solution was made to residue. Then addition of 3 ml of ethyl alcohol was made after 5 minutes. Solution was vigilantly shaken. Then colour density was measured in a spectrophotometer at 725 mµ using distilled water blank.

Vitamin C

Procedure (Varley, 1988) [24] with modest modification (Anonymous, 2016) [3] was employed for analysis. Procedure incorporated the titration of supernatant vitamin C by 2,6-dichlorophenolindophenol dye. Meticulous mixing of 4 ml of trichloroacetic acid reagent with 4 ml of supernatant was prepared. Then clean dried test tube was centrifuged at 2000 rpm for 5 minutes. In a clean dried small glass beaker, 200µl of the dye solution was added and was titrated by the enough supernatant until the fading of reddish hue.

Calculation

$$\text{Vitamin C } (\mu\text{mol L}^{-1}) = \frac{100}{\text{ml titration}} \text{ X } 2 \text{ X } 0.008 \text{ X} 56.78$$

200 µl of the dye solution = 0.008 mg vitamin C

56.78 = conversion factor for µmol L^{-1} from mg/dl

Then values were converted to µmol mgProtein^{-1}.

Statistical analysis

Present investigation was executed to evaluate the pattern of extremely higher environmental temperature linked variations in endogenous antioxidant responses of liver in Marwari goat from arid tracts. The changes in the means were assessed by Duncan's new multiple range test (Duncan, 1955) [4].

Results and Discussion

Result and discussion of present study whereas follow

Vitamin E

Sizeable changes in liver vitamin E values were recorded during extremely hot ETP in the form of a significant ($p \leq 0.05$) decrease in liver vitamin E value in Marwari goats as compared to comfortable ETP value. This change was quantified to be highly significant ($p \leq 0.01$) when analysis of variance was executed. Percent change in extremely hot ETP as compared to the value during comfortable ETP was computed to be -36.36.

Table 1: Mean ± SEM values of liver vitamin E (µg g^{-1}) in the Marwari goat during comfortable and extremely hot environmental temperature periods (ETPs) along with percent change during extremely hot ETP

S. No.	Effects	Mean ± SEM values during different environmental temperature Periods (ETPs)		Percent change during extremely hot ETP
		Comfortable ETP	Extremely hot ETP	
1.	Overall ETP (150)	$22.00^{b}\pm0.31$	$14.00^{b}\pm0.30$	- 36.36
2.	Categorization according to gender (I & II categories)			
I.	Males (75), categorization according to age groups as a, b & c			
	Males (75)	$24.00^{bc}\pm0.09$	$16.00^{bd}\pm0.08$	- 33.33
a.	3-6 months (25)	$22.00^{bd}\pm0.006$	$14.00^{bd}\pm0.005$	- 36.36
b.	6-9 months (25)	$24.00^{bd}\pm0.006$	$16.00^{bd}\pm0.005$	- 33.33
c.	9-12 months (25)	$26.00^{bd}\pm0.007$	$18.00^{bd}\pm0.006$	- 30.76
II.	Females (75), categorization according to age groups as a, b & c			
	Females (75)	$20.00^{bc}\pm0.08$	$12.00^{bc}\pm0.09$	- 40.00
a.	3-6 months (25)	$18.00^{bd}\pm0.005$	$10.00^{bd}\pm0.005$	- 44.44
b.	6-9 months (25)	$20.00^{bd}\pm0.004$	$12.00^{bd}\pm0.004$	- 40.00
c.	9-12 months (25)	$22.00^{bd}\pm0.005$	$14.00^{bd}\pm0.005$	- 36.36
3.	Categorization according to age groups irrespective of gender			
a.	3-6 months (50)	$20.00^{be}\pm0.004$	$12.00^{be}\pm0.005$	- 40.00
b.	6-9 months (50)	$22.00^{be}\pm0.004$	$14.00^{be}\pm0.005$	- 36.36
c.	9-12 months (50)	$24.00^{be}\pm0.005$	$16.00^{be}\pm0.005$	- 33.33

Figures in the parenthesis = Number of Marwari goat
ETP = Environmental temperature period
'b' = Significant ($p\leq0.05$) differences between mean values for a row.
'c' = Significant ($p\leq0.05$) differences between overall mean values of males and females for an ETP
'd' = Significant ($p\leq0.05$) differences among mean values of different age groups of a gender for an ETP
'e' = Significant ($p\leq0.05$) differences among mean values of different age groups irrespective of a gender for an ETP
= Percent decrease in the mean value

Vitamin C

Sizeable changes in liver vitamin C values was recorded during extremely hot ETP in the form of a significant ($p\leq0.05$) decrease in liver vitamin C value in Marwari goats as compared to comfortable ETP value. This change was quantified to be highly significant ($p\leq0.01$) when analysis of variance was executed. Percent change in extremely hot ETP as compared to the value during comfortable ETP was computed to be -51.85. Since vitamin C level in the blood is taken as a decent indicator of oxidative stress (Kataria *et al.*, 2010b) [11] along with an immunomodulator (Pareek and Kataria, 2015) [17], the decline in the concentration in liver revealed the presence of oxidative stress in the present investigation during extremely hot ETP.

Table 2: Mean ± SEM values of liver vitamin C (µg g^{-1}) in the Marwari goat during comfortable and extremely hot environmental temperature periods (ETPs) along with percent change during extremely hot ETP

S. No.	Effects	Mean ± SEM values during different environmental temperature Periods (ETPs)		Percent change during extremely hot ETP
		Comfortable ETP	Extremely hot ETP	
1.	**Overall ETP (150)**	$\mathbf{0.27^{b}\pm0.01}$	$\mathbf{0.13^{b}\pm0.01}$	**- 51.85**
2.	**Categorization according to gender (I & II categories)**			
I.	**Males (75), categorization according to age groups as a, b & c**			
	Males (75)	$0.29^{bc}\pm0.008$	$0.15^{bd}\pm0.007$	- 48.27
a.	3-6 months (25)	$0.27^{bd}\pm0.002$	$0.13^{bd}\pm0.001$	- 51.85
b.	6-9 months (25)	$0.29^{bd}\pm0.001$	$0.15^{bd}\pm0.001$	- 48.27
C	9-12 months (25)	$0.31^{bd}\pm0.001$	$0.17^{bd}\pm0.001$	- 45.16
II.	**Females (75), categorization according to age groups as a, b & c**			
	Females (75)	$0.25^{bc}\pm0.007$	$0.11^{bc}\pm0.009$	- 56.00
a.	3-6 months (25)	$0.23^{bd}\pm0.001$	$0.09^{bd}\pm0.001$	- 60.86
b.	6-9 months (25)	$0.25^{bd}\pm0.001$	$0.11^{bd}\pm0.001$	- 56.00
c.	9-12 months (25)	$0.27^{bd}\pm0.002$	$0.13^{bd}\pm0.001$	- 51.85
3.	**Categorization according to age groups irrespective of gender**			
a.	3-6 months (50)	$0.25^{be}\pm0.007$	$0.11^{be}\pm0.007$	- 56.00
b.	6-9 months (50)	$0.27^{be}\pm0.008$	$0.13^{be}\pm0.008$	- 51.85
c.	9-12 months (50)	$0.29^{be}\pm0.007$	$0.15^{be}\pm0.007$	- 48.27

Figures in the parenthesis = Number of Marwari goat
ETP = Environmental temperature period
'b' = Significant ($p\leq0.05$) differences between mean values for a row.
'c' = Significant ($p\leq0.05$) differences between overall mean values of males and females for an ETP
'd' = Significant ($p\leq0.05$) differences among mean values of different age groups of a gender for an ETP
'e' = Significant ($p\leq0.05$) differences among mean values of different age groups irrespective of a gender for an ETP
= Percent decrease in the mean value

Research community is giving huge attention to this parameter as one of the tools to assess endogenous antioxidant responses (Abhimanu *et al.*, 2015; Pareek and Kataria, 2015 and Joshi, 2018) [1, 17, 5]. The dearth of reserach on this viewpoint in Marwari goats from the arid tracts has led the launch of this investigation with the aim that data collected will assist in interpretation of clinical conditions. Declined liver vitamin C value in goats during extremely hot ETP attempted to clarify the presence of oxidative stress in apparently healthy Marwari goats.

Evaluation of the pattern of extremely higher environmental temperature linked variations in endogenous antioxidant responses of liver in Marwari goat.

Evaluation of the pattern of extremely higher environmental temperature linked variations in endogenous antioxidant responses of liver in Marwari goat divulged the presence of oxidative stress in the animals during extremely higher environmental temperature period. This was evaluated by measuring the levels of analytes of the endogenous antioxidant responses of liver i. e. vitamin E and vitamin C.

In the present study, several parameters of endogenous antioxidant responses of liver i.e. vitamin E and vitamin C exhibited significant ($p \leq 0.05$) changes in the mean values during extremely hot ETP as compared to respective comfortable ETP mean values. Liver vitamin E, vitamin C revealed significant ($p \leq 0.05$) decline in the mean values during extremely hot ETP as compared to respective comfortable ETP mean values. A bigger crash of oxidative stress can be handled by enhancing the levels of antioxidants principally enzymes as the part and parcel of the natural ploy of the body (Upreti *et al.*, 2002) [23]. It can be surmised that body tends to employ endogenous antioxidants like vitamin E and vitamin C to offset the oxidative stress and to bump the flooded free radicals. Extremely hot ETP in the present investigation affected the contents of parameters of endogenous liver antioxidant responses with a higher propensity. Antioxidant responses were found to be influenced during extremely hot ETP. It was surmised that depletion of antioxidants like vitamin C and vitamin E presented a biological crusade between free radicals and antioxidants. In order to fight free radicals, vitamin C and vitamin E were utilized and the concentrations reflected a decrease. Simultaneously, body attempted to increase the responses of antioxidant potential by raising the manufacture of enzymatic antioxidants like superoxide dismutase; catalase and glutathione reductase (Kataria *et al.*, 2010b) [11]. Suppositions can be derived from the interpretation of the observations collected in the present study regarding the values of non-enzymatic antioxidants in the liver. Percent changes were observed to be utmost for each parameter during extremely hot ETP in the female goats and in the animals of 3-6 months of age.

Vitamin C indicated -51.85% decline. That observations also demonstrated the potency of antioxidant potential in Marwari goats during harsh environmental conditions. The higher extent of alterations in the mean values of above discussed analytes revealed the veracity that oxidative stress happened during extremely hot environmental temperature period but Marwari goats had the adequate physiological strategies in the form of responses to protect the animals. The alterations observed in the parameters investigated, it can be reiterated that female goats and goats of 3-6 months of age were invaded by the greater bang of extremely hot environmental temperature period.

In the present investigation, three parameters where explored, the most sensitive parameter was found to be vitamine C which showed the maximum percent change. Excessive production of free radicals to a level that can strike internal defending antioxidant system of the body can culminate in oxidative stress. All the time, endogenous antioxidant arrangement may not be adequate to safeguard the body from gratuitous free radical formation and consequently to oxidative insult. For that reason, amplification of antioxidant hoard may be required to remodel antioxidant profile.

The findings of the present study have attempted to offer a new insight to vitamin C and vitamin E levels and changes according to the extremely hot environmental temperature. Endogenous antioxidant responses of liver during extremely hot environmental temperature will help in making the plans for the health improvement of native breeds of arid tracts. Exploration of the previous research clarified the scarcity of research on this aspect, hence the upshots of the present investigation will assist in developing antioxidant supplementation plans during harsh climatic conditions. The research has provided an imminent approach to the liver functions in goats by finding the possible modulations in the reactions influencing the antioxidant responses, particularly during the presence of abiotic stressors. Based upon these observations, strong commendations can be made regarding enriching the ration of the animals with suitable antioxidants to protect and maintain the health of native animals.

Conclusion

The conclusion of the exploration will confer aid in supervising the stratagem for placate of the native breeds of goat. Eloquent stipulations of the present study have attempted to divulge that liver cells are fraught with a blend of antioxidants. Resolution of research have tried to focus on the relative worth of the changes in the levels of antioxidants associated with the extreme ly hot environmental temperature. This may be the first investigation where serum analytes of iron indices and parameters of antioxidant responses of liver have been appraised at one stage in Marwari goat. Research contribution of the present study can be employed in strengthening the clinical aspect of Physiology in Veterinary sciences in the field of antioxidant status and in systematizing the scientific supervision of the animals during adverse ambiences. The vibrancy of alterations regarding antioxidant responses of liver revealed the existence of oxidative stress. Findings acquired in the investigation will assist in increasing the endorsement of contrivances to have gist about the damaging effects of harsh ambiences in the goat. Results will be temptingly valuable in crafting scientific tactics for Marwari goat to assist the marginal farmers and goat raisers from arid tracts and for researchers associated in the scientific execution of practices in goat sector. It can be concluded that present study evaluated efficiently the pattern of extremely higher environmental temperature linked variations in endogenous antioxidant responses of liver in Marwari goat from arid tracts.

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