

**APPRAISAL OF ERYTHROCYTIC ENZYMATIC RESPONSES IN
MAGRA SHEEP EXPOSED TO SUPERIMPOSED STRESSORS****Sunita Pareek*, Mohit Jain, Ruchi Maan and Nalini Kataria**

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Article Received on
11 August 2021,Revised on 31 August 2021,
Accepted on 21 Sept. 2021

DOI: 10.20959/wjpr202112-21871

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ABSTRACT

An exploration was launched to assess the erythrocytic enzymatic responses in *Magra* sheep exposed to superimposed stressors. Superimposed stressors incorporated transportation and regrouping stressors along with extreme ambiances. Blood samples were collected from female *Magra* sheep ageing 3-7 months; 7-11 months; 11-15 months; and 15-19 months during moderate, dry-hot, humid-hot and extreme cold ambiances. Maximum temperature humidity index (THI) values observed were 76.44 ± 0.11 ; 87.23 ± 0.30 ; 89.32 ± 0.07 and 63.22 ± 0.11 , respectively during moderate, dry-hot, humid-hot and extreme cold ambiances. Erythrocytic enzymatic responses were assessed by measuring catalase and glutathione reductase. The values among ambiances altered remarkably ($p \leq 0.05$) and dry-hot and humid-hot ambiances showed maximum changes. *Magra* sheep of all the age groups were found influenced by the impact of superimposed stressors

particularly when transportation and regrouping were associated together during dry-hot and humid-hot ambiances. It was concluded that erythrocytes showed intonations during exposure to superimposed stressors. Recommendations can be made that animals must be provided with proper water and antioxidants especially during stress periods.

KEYWORDS: Erythrocytic enzymatic responses, catalase, glutathione reductase, *Magra* sheep, superimposed stressors.

1. INTRODUCTION

Sheep living under the natural husbandry conditions of arid tracts of Rajasthan commonly encounter to various stressors at a time (superimposed stressors). These stressors are generally in the form of extreme ambiances together with stressors like transportation, regrouping, feed deficit, water deficit etc. The compensatory physiological mechanisms to these stressors are known as stress responses. There is paucity of the studies to find out response of the body organs to superimposed stressors in natural conditions of arid tracts in native breeds. Appraisal of theories based on animal stress responses and emotions are generally based on experiments conducted in the laboratory under artificially developed stressors or highly controlled conditions in the field. Under these conditions the changes were found to be extreme and do not represent the actual condition faced by the animals. However, there is paucity of the studies to find out retort of the blood cells to stressors in natural conditions. Hence, a study was planned to appraise erythrocytic enzymatic responses in *Magra* sheep exposed to superimposed stressors. Simultaneous appraisals are critical in predicting physiological and psychological associates of stress equally.

2. MATERIALS AND METHODS

Ostensibly healthy *Magra* female sheep of 3-7 months; 7-11 months; 11-15 months; and 15-19 months age groups belonging to un-organised sector from in and around Bikaner district, Rajasthan were target group. To achieve the goal of the study, *Magra* female animals were sampled for blood during moderate, dry- hot, humid- hot and extreme cold ambiances. Three hundred blood samples were collected in the morning hours in each ambience. Experiment was carried out with the permission of Institutional Animal Ethics Committee (IAEC), College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan. Superimposed stressors incorporated transportation and regrouping of *Magra* sheep during extreme ambiances *viz.* dry- hot, humid- hot and extreme cold ambiances.

Superimposed stressors were comprised of extreme ambiances and transportation (Set I) and extreme ambiances, transportation and regrouping (Set II). Transportation included short distance travel and for this the sampling was done after the dislodgement (getting off) animals from the vehicle. Regrouping incorporated addition of around 15-20 transported animals to existing 15-20 animals. Control included the screening of the animals during moderate ambience without the presence of any stressor. Extreme cold ambience comprised

of the months of December- January, dry hot ambience comprised of April-May-June and humid-hot comprised of July-August-September month. Erythrocytic catalase was estimated by the spectrophotometric assay method^[1] and Glutathione reductase was estimated by the colorimetric method.^[2] Data obtained in the exploration were expressed as mean \pm SE of mean. Special computer programmes were employed to compute means and standard error^[3] and analyses of variance^[4] to verify the significance of the impacts.^[5]

3. RESULTS AND DISCUSSION

3.1 Erythrocytic catalase

Mean \pm SEM values of erythrocytic catalase of female *Magra* sheep during varying ambiances are presented in Table 1. The range and overall mean values of erythrocytic catalase obtained during moderate ambience in present exploration were similar to the other findings.^[6,7,8] The overall mean value was significantly ($p \leq 0.05$) higher during dry-hot, humid-hot and extreme cold ambience as compared to moderate mean overall value in both the sets of superimposed stressors. During humid-hot ambience, the per cent variations in the overall value was found to be maximum during both the sets of superimposed stressors, however, set II displayed greater per cent variation (+636.17). Assemblage of changes denoted the combined effect of superimposed stressors on the *Magra* sheep irrespective of age groups.

These changes clearly exhibited the presence of oxidative stress in the *Magra* sheep due to stressors. Raised erythrocytic catalase in goats during hot and cold ambient temperatures were explored.^[9] 1.78 times greater erythrocytic catalase was recorded during hot ambience in comparison to moderate ambience in buffalo calves.^[10] Higher levels confirmed the existence of oxidative stress at cellular levels.

Effect of age groups of female *Magra* sheep on erythrocytic catalase

Significant ($p \leq 0.05$) changes among all the overall mean values in all age groups in each ambience in both the sets of superimposed stressors were observed.

Overall mean values in 3-7 months were minimum and of 15-19 months were maximum. This precedent was same for all the ambiances. Both sets of superimposed stressors exhibited same trend. Both the sets of superimposed stressors displayed higher per cent variations during extreme humid-hot. It was concluded that 3-7 months old sheep divulged greater per cent change in the enzymatic biomarkers and also had comparatively lower values of

erythrocytic enzymatic biomarkers. This lucidly indicated the development of oxidative stress to a greater extent in 3-7 months old animals. Further, the effect of oxidative stress was more pronounced in humid-hot ambience. The impact of all the stressors together (Set-II) was greater.

Table no. 1: Mean \pm SEM values of erythrocytic catalase (CAT, kU gHb⁻¹) in the *Magra* sheep during varying ambiances.

S. no.	Effects	Mean \pm SEM values without superimposed stressors		Mean \pm SEM values due to superimposed stressors during varying ambiances		
		Moderate (Control)	Dry-hot	Humid-hot	Extreme Cold	
I	Age groups	Without extreme ambiances & transportation (Control)	Set I: Extreme ambiances & Transportation			
	Overall mean values (300)	2.81 ^{ba} \pm 0.08	11.72 ^{bc} \pm 0.07	16.64 ^{bc} \pm 0.07	6.65 ^{bc} \pm 0.09	
a.	3-7 months (75)	2.00 ^{bef} \pm 0.03	10.02 ^{bd} \pm 0.05	15.01 ^{bd} \pm 0.05	6.02 ^{bd} \pm 0.05	
b.	7-11 months (75)	2.52 ^{beg} \pm 0.04	11.12 ^{bd} \pm 0.02	16.21 ^{bd} \pm 0.02	6.34 ^{bd} \pm 0.03	
c.	11-15 months (75)	3.02 ^{beh} \pm 0.02	12.32 ^{bd} \pm 0.04	17.12 ^{bd} \pm 0.05	6.88 ^{bd} \pm 0.05	
d.	15-19 months (75)	3.68 ^{bei} \pm 0.02	13.45 ^{bd} \pm 0.02	18.21 ^{bd} \pm 0.03	7.34 ^{bd} \pm 0.04	
II	Age groups	Without extreme ambiances, transportation & regrouping (Control)	Set II: Extreme ambiances, Transportation & Regrouping			
	Overall mean values (300)	2.82 ^{ba} \pm 0.08	16.35 ^{bc} \pm 0.09	20.76 ^{bc} \pm 0.12	9.04 ^{bc} \pm 0.10	
a.	3-7 months (75)	2.03 ^{bef} \pm 0.04	14.71 ^{bd} \pm 0.002	19.12 ^{bd} \pm 0.05	8.11 ^{bd} \pm 0.06	
b.	7-11 months (75)	2.58 ^{beg} \pm 0.04	15.82 ^{bd} \pm 0.003	20.04 ^{bd} \pm 0.04	8.88 ^{bd} \pm 0.05	
c.	11-15 months (75)	3.05 ^{beh} \pm 0.04	16.91 ^{bd} \pm 0.002	21.10 ^{bd} \pm 0.03	9.21 ^{bd} \pm 0.05	
d.	15-19 months (75)	3.69 ^{bei} \pm 0.02	17.99 ^{bd} \pm 0.002	22.82 ^{bd} \pm 0.06	9.94 ^{bd} \pm 0.04	

Figures in the parenthesis = Number of *Magra* sheep

'a', 'f', 'g', 'h' & 'i' = Non-significant ($p > 0.05$) differences between mean values

'b' = Significant ($p \leq 0.05$) differences among mean values for a row.

'c' = Significant ($p \leq 0.05$) differences between overall mean values according to stressors for a column

'd' = Significant ($p \leq 0.05$) differences among mean values of different age groups for an ambience

'e', = Significant ($p \leq 0.05$) differences among mean values of different age groups for an ambience within a stressor

3.2 Glutathione reductase

Mean \pm SEM values of erythrocytic glutathione reductase of female *Magra* of different age groups during moderate, dry-hot, humid-hot and extreme cold ambiances are presented in table 2. The observations obtained in the present investigation corroborated the findings of previous researchers.^[6,9,10] The overall mean values of Glutathione reductase were significantly ($p \leq 0.05$) higher during dry-hot, humid-hot and extreme cold ambience as compared to moderate mean overall value in both the sets of superimposed stressors.

A highly significant ($p \leq 0.01$) effect of extreme ambiances i.e. dry- hot, humid-hot and extreme cold ambience were exhibited by analysis of variance. During humid-hot ambience, the per cent variations in the overall value was found to be maximum during both the sets of superimposed stressors, however, set II displayed greater per cent variation (+583.10). Illustration of changes denoted the combined effect of superimposed stressors on the *Magra* sheep irrespective of age groups. Glutathione reductase is considered as an important element of endogenous antioxidant system. Greater value indicated towards the activation of cellular oxidative stress responses to combat oxidative stress.

These changes observed in the study clearly exhibited the presence of oxidative stress in the *Magra* sheep. There is paucity of research on this aspect in *Magra* sheep.

Raised erythrocytic glutathione reductase was observed in goats^[11] and pigs^[12] during hot and cold ambiances.

Effect of age groups of female *Magra* sheep on erythrocytic glutathione reductase

Significant ($p \leq 0.05$) changes among all the overall mean values of all the age groups and in each ambience in both the sets of superimposed stressors were observed.

Overall mean values in 3-7 months were minimum and of 15-19 months were maximum significantly ($p \leq 0.05$). This precedent was same for all the ambiances. Both sets of superimposed stressors exhibited same trend. Both the sets of superimposed stressors displayed higher per cent variations during extreme humid-hot.

It can be concluded that 3-7 months old sheep divulged greater per cent change in the enzymatic biomarkers and also had comparatively lower values of erythrocytic enzymatic biomarkers. This lucidly indicated the development of oxidative stress to a greater extent in 3-7 months old animals. Further, the effect of oxidative stress was more pronounced in humid-hot ambience. The impact of all the stressors together (Set-II) was greater.

Erythrocytic glutathione reductase is a reflection of metabolism.^[13] Glutathione reductase in the erythrocytes of cattle was found related with anaemia in earlier studies.^[14,15] Aging process was tested for bovine erythrocytes and major device used was erythrocytic glutathione reductase in bovine erythrocytes.^[16] Appraisal of the status of antioxidants in erythrocytes of sheep during extreme environmental temperature periods was carried out and erythrocytic glutathione reductase divulged an increase.^[6] Erythrocytic glutathione reductase activity in the buffalo calves was determined and mean value was found greater during hot ambience in comparison to moderate ambience which was ascribed to oxidative stress.^[10] It was deduced that rise in the value during hot ambience was bigger than that of cold period. Higher values denoted the existence of oxidative stress in the *Magra* sheep.

Table no. 2: Mean \pm SEM values of erythrocytic glutathione reductase (GR, kU gHb⁻¹) in the *Magra* sheep during varying ambiances.

S. no.	Effects	Mean \pm SEM values without superimposed stressors		Mean \pm SEM values due to superimposed stressors during varying ambiances	
		Moderate (Control)	Dry-hot	Humid-hot	Extreme Cold
I	Age groups	Without extreme ambiances & transportation (Control)	Set I: Extreme ambiances & Transportation		
	Overall mean values (300)	14.00 ^{ba} \pm 0.194	36.00 ^{bc} \pm 0.03	56.00 ^{bc} \pm 0.06	35.00 ^{bc} \pm 0.02
a.	3-7 months (75)	8.00 ^{bef} \pm 0.01	30.00 ^{bd} \pm 0.01	41.00 ^{bd} \pm 0.01	20.00 ^{bd} \pm 0.01
b.	7-11 months (75)	13.00 ^{beg} \pm 0.01	35.00 ^{bd} \pm 0.01	53.00 ^{bd} \pm 0.01	30.00 ^{bd} \pm 0.01
c.	11-15 months (75)	19.00 ^{beh} \pm 0.01	41.00 ^{bd} \pm 0.01	61.00 ^{bd} \pm 0.01	37.00 ^{bd} \pm 0.01
d.	15-19 months (75)	24.00 ^{bei} \pm 0.01	48.00 ^{bd} \pm 0.01	73.00 ^{bd} \pm 0.01	43.00 ^{bd} \pm 0.01
II	Age groups	Without extreme ambiances, transportation & regrouping (Con	Set II: Extreme ambiances, Transportation & Regrouping		

	Overall mean values (300)	trol)			
		14.20 ^{ba} ± 0.03	65.00 ^{bc} ± 0.03	97.00 ^{bc} ± 0.04	56.00 ^{bc} ± 0.02
a.	3-7 months (75)	8.01 ^{bcf} ± 0.01	53.00 ^{bd} ± 0.01	81.00 ^{bd} ± 0.01	48.00 ^{bd} ± 0.01
b.	7-11 months (75)	13.10 ^{beg} ± 0.01	60.00 ^{bd} ± 0.01	92.00 ^{bd} ± 0.01	54.00 ^{bd} ± 0.01
c.	11-15 months (75)	19.20 ^{beh} ± 0.01	68.00 ^{bd} ± 0.01	103.00 ^{bd} ± 0.01	61.00 ^{bd} ± 0.01
b	15-19 months (75)	24.10 ^{beci} ± 0.01	74.00 ^{bd} ± 0.01	110.00 ^{bd} ± 0.01	69.00 ^{bd} ± 0.01

Figures in the parenthesis = Number of *Magra* sheep

‘a’, ‘f’, ‘g’, ‘h’ & ‘i’ = Non-significant ($p \geq 0.05$) differences between mean values

‘b’ = Significant ($p \leq 0.05$) differences among mean values for a row

‘c’ = Significant ($p \leq 0.05$) differences between overall mean values according to stressor for a column

‘d’ = Significant ($p \leq 0.05$) differences among mean values of different age groups for an ambience

‘e’ = Significant ($p \leq 0.05$) differences among mean values of different age groups for an ambience within a stressor

Interactions of ambiances with superimposed stressors

The interactions were computed for catalase and glutathione reductase as ambiances & transportation; ambiances, transportation & regrouping; age groups; and ambiances & transportation X ambiances, transportation & regrouping X age groups. They were found to be highly significant ($p \leq 0.01$) which revealed the impact of superimposed stressors on the female *Magra* sheep of all age groups.

4. CONCLUSION

The cellular enzymatic biomarkers exhibited similar swatch and revealed significant increase during dry-hot, humid-hot and extreme cold ambiances as compared to respective moderate ambience mean value in each age group. The magnitude of increase was maximum in humid-hot. Set I and set II of superimposed stressors exhibited similar design, however, the effect of superimposed stressors was more pronounced in set II i.e. extreme ambiances, transportation & regrouping. It was assessed by the greater extent of increase of enzymatic biomarkers. Regarding age effect, 3-7 months old age group divulged minimum values in each ambience of both the sets of superimposed stressors. Concomitantly, 15-19 months old age group revealed maximum value in each case. Humid-hot ambience divulged maximum per cent variation in each age group. It was observed that 3-7 months old sheep divulged greater per cent change in the enzymatic biomarkers and also had comparatively lower values of serum

enzymatic biomarkers. This clearly indicated the development of oxidative stress to a greater extent in 3-7 months old animals. Further, the effect of oxidative stress was more pronounced in humid-hot ambience. The impact of all the stressors together (Set-II) was greater. Erythrocytic catalase exhibited maximum overall per cent change (+636.17) during humid-hot with set II of superimposed stressors. It can be taken as most sensitive parameter among serum non-enzymatic biomarkers. Both the cellular enzymatic biomarkers exhibited similar archetype. They showed significant ($p \leq 0.01$) increase during dry-hot, humid-hot and extreme cold ambiances as compared to respective moderate ambience mean value. This was evident in each age group also. The magnitude of increase was maximum in humid-hot as in each case. Set I and set II of superimposed stressors exhibited similar archetype. The effect of superimposed stressors was more pronounced in set II i.e. extreme ambiances, transportation & regrouping stress.

5. ACKNOWLEDGMENT

The authors would like to thank Dean, college of veterinary and animal science, Rajasthan University of Veterinary and Animal Sciences, Bikaner for providing the facilities, encouragement, support and permission to publish this paper.

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