

Assessment of Erythrocytic Stress Response in Rathi Cattle from Rajasthan during Varying Ambiences

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ABSTRACT

Assessment of erythrocytic stress response in Rathi cattle was done by determining non enzymatic biomarkers of oxidative stress, viz., vitamin A, vitamin C, vitamin E and glutathione in erythrocytes. For this purpose, blood samples were collected from apparently healthy Rathi female calves, heifers and cows from private dairies located in and around Bikaner district, Rajasthan during moderate, extreme hot, extreme cold and rainy ambiances to prepare lysates of erythrocytes. The overall mean values of erythrocytic vitamin A, vitamin C, vitamin E and glutathione were significantly ($p \leq 0.05$) lower during extreme cold, hot and rainy ambiances as compared to moderate ambience. The overall mean values of erythrocytic vitamin A, vitamin C, vitamin E and glutathione in cows were maximum and of calves were minimum ($p \leq 0.05$) in all the ambiances. In calves, calf yearling transition revealed maximum erythrocytic vitamin A, vitamin C, vitamin E and glutathione values, while in heifers category, post-pubertal heifers had significantly ($p \leq 0.05$) higher values of erythrocytic vitamin A, vitamin C, vitamin E and glutathione as compared to pre-pubertal in each ambience. In cows category, among group A animals, non-pregnant milch and in group B animals, multipara had significantly ($p \leq 0.05$) higher values of erythrocytic vitamin A, vitamin C, vitamin E and glutathione in each ambience. In all the physiological states of Rathi cattle, maximum percent variations were observed in rainy ambience. It can be concluded that among all the physiological states, calves showed maximum erythrocytic stress response during rainy ambience.

Key words: Erythrocytic stress response, Non-enzymatic biomarkers, Rajasthan, Rathi cattle.

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INTRODUCTION

Exposure of the animals to varying ambiances may impose stress, which can be observed in the form of changes in blood constituents expressing physiological modulation (Kour and Kataria, 2021). Extreme ambiances situate negative force on the dairy animals moving back the positive processes like growth, production, reproduction and health (Joshi and Kataria, 2018). Arid regions bear the brunt of climate change and Bikaner district of Rajasthan is one of them. A demanding element for researchers is to safeguard Rathi cattle and to employ health programmes, so that deterioration in number of these animals can be prevented with real-time efforts. Low levels of antioxidants may damage intracellular machinery resulting in an alteration in the immune status of animals (Joshi *et al.*, 2017). Erythrocytes are considered as best model to study the development of oxidative stress in the body. There are several endogenous antioxidant systems present in the animal body that are activated during stress *i.e.* biotic or abiotic (Kataria *et al.*, 2010). Oxidative stress can result from diminished antioxidant protection as well as increased free radical production. Erythrocytes are affected by changes in free radicals (Saini *et al.*, 2018).

Most of the studies on calves, heifers and cows have been conducted in well organized dairy farms. However, there is paucity of research on this aspect from unorganized marginal private dairies (Kataria and Kataria, 2008). Generation of

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proper data of physiological strategies in native breeds is considered to be an important mandate of diagnostic laboratories for health management. The object of this investigation was to assess the oxidative stress responses by determining non enzymatic biomarkers in erythrocytes in Rathi female calves, heifers and cows in all the four ambiances.

MATERIALS AND METHODS

For the present study, blood samples were collected from 1200 apparently healthy Rathi female calves ($n= 150$), heifers ($n= 60$) and cows ($n= 90$) ageing two weeks to 12 years old during moderate (300), extreme hot (300), extreme cold (300) and rainy ambiances (300) from private dairies located in and around Bikaner district, Rajasthan. Assessment of erythrocytic stress response was carried out by determining non-enzymatic biomarkers of oxidative stress, *viz.*, vitamin A, vitamin C, vitamin E and glutathione in erythrocytes.

Moderate ambience comprised of October-November, extreme cold comprised of December-January, extreme hot ambience of May and June and rainy ambience comprised of July-August-September. Animals were grouped according to age in category I, II and III in each ambience. Category I included Rathi female calves ageing from two weeks to one year. Sub groups included 2 to 3 weeks old (Pre-ruminant phase), 3 to 8 weeks old (Transitional phase), 8 to 16 weeks old (Pre-weaning), 16 to 32 weeks old (Post-weaning) and 32 to 48 weeks old (Calf-yearling transition) female calves. Each group was consisted of 30 animals. Category II having heifers ageing from one year to 3.5 years of age. Grouping of female animals included 1-2.5 years (pre-pubertal) and 2.5 to 3.5 years (post-pubertal) of age group. All post-pubertal animals were non-pregnant. Each category comprised of 30 animals. Category III incorporated Rathi cows ageing 3.5-12 years. They were broadly divided into group A and group B according to physiological state. Animals of group A involved non-pregnant milch (30); pregnant milch (30) and pregnant dry (30) cows. To maintain similarity, all milch animals were sampled between 3 and 4 months of gestation period. Animals of group B were classified according to parity and included primipara (45) and multipara (45) cows. This was irrespective of state like pregnancy and milch. All primipara were between 3.5 and 6 years, whereas all multipara were between 6 and 12 years of age.

Collection of Blood Samples for Erythrocytes

From blood samples collected in di-potassium EDTA, plasma and erythrocytes were separated by centrifugation for 20 min at 1100 g. After aspirating buffy coat carefully, aliquots of erythrocytes were washed twice with isotonic saline solution and then were stored at -20°C until analysis. Erythrocytes were haemolysed with four volumes of ice cold distilled water (Russell *et al.*, 1985). For the determination of analytes the haemolysates were treated with equal volumes of ethanol/chloroform (3:5 v/v) mixture. Then tubes were centrifuged for 20 min at 1100 g. They precipitated the haemoglobin and stroma free haemolysate obtained (Anonymous, 2010) was used to determine the various analytes.

Vitamin C and Vitamin A in erythrocytes were determined by the method described by Varley (1988) with little modifications (Sihag, 2013; Sihag, 2021^{a,b}). Vitamin E in erythrocytes was determined by the spectrophotometric method of Nair and Magar (1955) with little modifications

(Sihag, 2021^a). Glutathione in erythrocytes was determined by the rapid colorimetric micro method of Owens and Belcher (1965) with modifications (Sihag, 2021^b).

RESULTS AND DISCUSSION

Mean \pm SEM values of erythrocytic vitamin E, vitamin C, vitamin A and glutathione of Rathi female cattle, *i.e.*, calves, heifers and cows during moderate, extreme hot, rainy and extreme cold ambiances are presented in Table 1 and 2, respectively. The overall mean values of erythrocytic vitamin E, vitamin C, vitamin A and glutathione obtained from 300 Rathi cattle incorporating calves, heifers and cows during moderate ambience were $0.781\pm 0.005 \mu\text{mol gHb}^{-1}$, $3.60\pm 0.004 \mu\text{mol gHb}^{-1}$, $0.470\pm 0.005 \mu\text{mol gHb}^{-1}$ and $4.63\pm 0.004 \mu\text{mol gHb}^{-1}$, respectively. The range of erythrocytic vitamin E, vitamin C, vitamin A and glutathione were $0.760\text{--}0.802 \mu\text{mol gHb}^{-1}$, $3.39\text{--}3.81 \mu\text{mol gHb}^{-1}$, $0.449\text{--}0.491 \mu\text{mol gHb}^{-1}$ and $4.40\text{--}4.87 \mu\text{mol gHb}^{-1}$, respectively, during moderate ambience.

The overall mean values of erythrocytic vitamin E, C, A and glutathione were significantly ($p\leq 0.05$) lower during extreme cold, hot and rainy ambiances as compared to moderate ambience, however, magnitude of decrease was maximum during rainy ambience and minimum during extreme cold ambience. During rainy ambience, the per cent variation in the values of erythrocytic vitamin E, C, A and glutathione was found to be maximum (-49.67 , 56.66 , -58.72 and -65.87 respectively). Magnitude of decrease in the value of vitamin E, C, A and glutathione during hot ambience was more than that during cold period (Saini, 2017). Sihag (2013) observed lower vitamin E, C, A and glutathione during hot ambience as compared to moderate ambience in buffalo calves.

Overall mean values of cows were maximum and of calves were minimum ($p\leq 0.05$) in all the ambiances. Per cent variations in the overall mean values of calves were maximum in all the ambiances as compared to those in cows. In calves, calf yearling transition revealed maximum erythrocytic vitamin E, C, A and glutathione values and pre-ruminants revealed minimum values in each ambience. Per cent variations in pre-ruminant calves were maximum in rainy ambiances. In heifers, post-pubertal animals had significantly ($p\leq 0.05$) higher values of erythrocytic vitamin E, C, A and glutathione in each ambience in comparison to pre-pubertal heifers. Maximum per cent variations were revealed by post-pubertal in rainy ambience. In cows, among group A animals, pregnant dry cows had significantly ($p\leq 0.05$) higher values of erythrocytic vitamin E, C, A and glutathione in each ambience in comparison to others. Maximum per cent variations were exhibited by non-pregnant milch cows in rainy ambience. In group B animals, multipara had significantly ($p\leq 0.05$) higher values of erythrocytic vitamin E, C, A and glutathione in each ambience. Multipara animals showed higher per cent variations in extreme hot and rainy ambiances. In all the types, maximum per cent variations were observed in rainy ambience as compared to moderate ambience. Deeksha (2016) assessed the status of antioxidants in erythrocytes of sheep during extreme environmental temperature periods.

Table 1: Mean \pm SEM values of erythrocytic vitamin E and C ($\mu\text{mol gHb}^{-1}$) in the female Rathi cattle during varying ambiances

Sr. No	Effects	Vitamin E ($\mu\text{mol gHb}^{-1}$) during varying ambiances			Vitamin C ($\mu\text{mol gHb}^{-1}$) during varying ambiances				
		Moderate	Extreme Hot	Rainy	Extreme cold	Moderate	Extreme Hot	Rainy	Extreme cold
	Overall values (300)	0.781 ^D \pm 0.005	0.444 ^B \pm 0.005	0.393 ^A \pm 0.005	0.575 ^C \pm 0.005	3.60 ^D \pm 0.004	1.96 ^B \pm 0.003	1.56 ^A \pm 0.003	2.76 ^C \pm 0.003
	Overall values of calves (150)	0.771 ^D \pm 0.001	0.435 ^B \pm 0.001	0.383 ^A \pm 0.001	0.555 ^C \pm 0.001	3.50 ^D \pm 0.001	1.86 ^B \pm 0.001	1.46 ^A \pm 0.001	2.66 ^C \pm 0.001
a	Pre-ruminant (30)	0.761 ^a \pm 0.0001	0.428 ^a \pm 0.0001	0.377 ^a \pm 0.0001	0.526 ^a \pm 0.0001	3.40 ^a \pm 0.0001	1.84 ^a \pm 0.0001	1.44 ^a \pm 0.0001	2.64 ^a \pm 0.0001
b	Transitional (30)	0.766 ^a \pm 0.0001	0.432 ^b \pm 0.0001	0.381 ^b \pm 0.0001	0.542 ^b \pm 0.0001	3.45 ^b \pm 0.0001	1.85 ^b \pm 0.0001	1.45 ^b \pm 0.0001	2.65 ^b \pm 0.0001
c	Pre-weaning (30)	0.771 ^b \pm 0.0001	0.435 ^b \pm 0.0001	0.383 ^b \pm 0.0001	0.555 ^c \pm 0.0001	3.50 ^c \pm 0.0001	1.86 ^c \pm 0.0001	1.46 ^b \pm 0.0001	2.66 ^b \pm 0.0001
d	Post-weaning (30)	0.776 ^b \pm 0.0001	0.438 ^b \pm 0.0001	0.385 ^c \pm 0.0001	0.568 ^d \pm 0.0001	3.55 ^c \pm 0.0001	1.87 ^c \pm 0.0001	1.47 ^c \pm 0.0001	2.67 ^c \pm 0.0001
e	Calf-yearling transition (30)	0.781 ^c \pm 0.0001	0.442 ^c \pm 0.0001	0.389 ^c \pm 0.0001	0.584 ^e \pm 0.0001	3.60 ^d \pm 0.0001	1.88 ^d \pm 0.0001	1.48 ^c \pm 0.0001	2.68 ^c \pm 0.0001
	Overall values of heifers (60)	0.781 ^D \pm 0.001	0.445 ^B \pm 0.001	0.393 ^A \pm 0.001	0.575 ^C \pm 0.001	3.60 ^D \pm 0.001	1.96 ^B \pm 0.001	1.56 ^A \pm 0.001	2.76 ^C \pm 0.001
a	Pre-pubertal (30)	0.777 ^a \pm 0.0001	0.441 ^a \pm 0.0001	0.391 ^a \pm 0.0001	0.573 ^a \pm 0.0001	3.56 ^a \pm 0.0001	1.95 ^a \pm 0.0001	1.55 ^a \pm 0.0001	2.75 ^a \pm 0.0001
b	Post-pubertal (30)	0.785 ^b \pm 0.0001	0.449 ^b \pm 0.0001	0.395 ^b \pm 0.0001	0.577 ^b \pm 0.0001	3.64 ^b \pm 0.0001	1.97 ^b \pm 0.0001	1.57 ^b \pm 0.0001	2.77 ^b \pm 0.0001
	Overall values of cows (90)	0.791 ^D \pm 0.001	0.455 ^B \pm 0.001	0.403 ^A \pm 0.001	0.595 ^C \pm 0.001	3.70 ^D \pm 0.001	2.06 ^B \pm 0.001	1.66 ^A \pm 0.001	2.86 ^C \pm 0.001
	Group A (90), Physiological states: Pregnancy and milch status								
a	Non-pregnant milch (30)	0.781 ^a \pm 0.0001	0.459 ^c \pm 0.0001	0.406 ^c \pm 0.0001	0.576 ^a \pm 0.0001	3.60 ^a \pm 0.0001	2.07 ^c \pm 0.0001	1.67 ^c \pm 0.0001	2.87 ^c \pm 0.0001
b	Pregnant milch (30)	0.791 ^b \pm 0.0001	0.455 ^b \pm 0.0001	0.403 ^b \pm 0.0001	0.595 ^b \pm 0.0001	3.70 ^b \pm 0.0001	2.06 ^b \pm 0.0001	1.66 ^b \pm 0.0001	2.86 ^b \pm 0.0001
c	Pregnant dry (30)	0.801 ^c \pm 0.0001	0.451 ^a \pm 0.0001	0.400 ^a \pm 0.0001	0.614 ^c \pm 0.0001	3.80 ^c \pm 0.0001	2.05 ^a \pm 0.0001	1.65 ^a \pm 0.0001	2.85 ^a \pm 0.0001
	Group B (90), Physiological states: Parity								
a	Primipara (45)	0.781 ^a \pm 0.0001	0.451 ^a \pm 0.0001	0.400 ^a \pm 0.0001	0.584 ^a \pm 0.0001	3.60 ^a \pm 0.0001	2.05 ^a \pm 0.0001	1.65 ^a \pm 0.0001	2.85 ^a \pm 0.0001
b	Multipara (45)	0.801 ^b \pm 0.0001	0.459 ^b \pm 0.0001	0.406 ^b \pm 0.0001	0.606 ^b \pm 0.0001	3.80 ^b \pm 0.0001	2.07 ^b \pm 0.0001	1.67 ^b \pm 0.0001	2.87 ^b \pm 0.0001

Figures in the parentheses indicate number of Rathi animals.

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

Small letters = Significant ($p \leq 0.05$) differences among mean values for columns



Table 2: Mean \pm SEM values of erythrocytic vitamin A and glutathione ($\mu\text{mol gHb}^{-1}$) in the female Rathi cattle during varying ambiances

Sr. No	Effects	Vitamin A ($\mu\text{mol gHb}^{-1}$) during varying ambiances				Glutathione ($\mu\text{mol gHb}^{-1}$) during varying ambiances				
		Moderate	Extreme Hot	Rainy	Extreme cold	Moderate	Extreme Hot	Rainy	Extreme cold	
	Overall values (300)	0.470 ^D \pm 0.005	0.244 ^B \pm 0.005	0.194 ^A \pm 0.005	0.374 ^C \pm 0.005	4.63 ^D \pm 0.004	2.07 ^B \pm 0.003	1.58 ^A \pm 0.003	3.78 ^C \pm 0.003	
	Overall values of calves (150)	0.460 ^D \pm 0.001	0.234 ^B \pm 0.001	0.184 ^A \pm 0.001	0.354 ^C \pm 0.001	4.53 ^D \pm 0.001	1.97 ^B \pm 0.001	1.48 ^A \pm 0.001	3.68 ^C \pm 0.001	
	A Pre-ruminant (30)	0.450 ^A \pm 0.001	0.227 ^A \pm 0.0001	0.178 ^A \pm 0.0001	0.325 ^A \pm 0.0001	4.43 ^B \pm 0.0001	1.91 ^A \pm 0.0001	1.43 ^A \pm 0.0001	3.63 ^A \pm 0.0001	
	B Transitional (30)	0.455 ^B \pm 0.0001	0.231 ^B \pm 0.0001	0.182 ^B \pm 0.0001	0.341 ^B \pm 0.0001	4.48 ^B \pm 0.0001	1.95 ^B \pm 0.0001	1.46 ^B \pm 0.0001	3.67 ^B \pm 0.0001	
	C Pre-weaning (30)	0.460 ^C \pm 0.0001	0.234 ^B \pm 0.0001	0.184 ^C \pm 0.0001	0.354 ^C \pm 0.0001	4.53 ^C \pm 0.0001	1.97 ^B \pm 0.0001	1.48 ^B \pm 0.0001	3.68 ^B \pm 0.0001	
	D Post-weaning (30)	0.465 ^C \pm 0.0001	0.237 ^C \pm 0.0001	0.186 ^C \pm 0.0001	0.367 ^D \pm 0.0001	4.58 ^C \pm 0.0001	1.99 ^C \pm 0.0001	1.50 ^C \pm 0.0001	3.70 ^C \pm 0.0001	
	E Calf-yearling transition (30)	0.470 ^D \pm 0.0001	0.241 ^D \pm 0.0001	0.190 ^D \pm 0.0001	0.388 ^E \pm 0.0001	4.63 ^D \pm 0.0001	2.03 ^D \pm 0.0001	1.53 ^D \pm 0.0001	3.72 ^D \pm 0.0001	
	Overall values of heifers (60)	0.470 ^D \pm 0.001	0.244 ^B \pm 0.001	0.194 ^A \pm 0.001	0.374 ^C \pm 0.001	4.63 ^D \pm 0.001	2.07 ^B \pm 0.001	1.58 ^A \pm 0.001	3.78 ^C \pm 0.001	
	A Pre-pubertal (30)	0.466 ^A \pm 0.0001	0.240 ^A \pm 0.0001	0.193 ^A \pm 0.0001	0.373 ^A \pm 0.0001	4.59 ^A \pm 0.0001	2.05 ^A \pm 0.0001	1.57 ^A \pm 0.0001	3.77 ^A \pm 0.0001	
	B Post-pubertal (30)	0.474 ^B \pm 0.0001	0.248 ^B \pm 0.0001	0.195 ^B \pm 0.0001	0.375 ^B \pm 0.0001	4.67 ^B \pm 0.0001	2.09 ^B \pm 0.0001	1.59 ^B \pm 0.0001	3.79 ^B \pm 0.0001	
	Overall values of cows (90)	0.480 ^D \pm 0.001	0.254 ^B \pm 0.001	0.204 ^A \pm 0.001	0.394 ^C \pm 0.001	4.73 ^D \pm 0.001	2.17 ^B \pm 0.001	1.68 ^A \pm 0.001	3.88 ^C \pm 0.001	
	Group A (90), Physiological states: Pregnancy and milch status									
	A Non-pregnant milch (30)	0.470 ^A \pm 0.0001	0.258 ^C \pm 0.0001	0.205 ^C \pm 0.0001	0.375 ^A \pm 0.0001	4.61 ^A \pm 0.0001	2.20 ^C \pm 0.0001	1.71 ^C \pm 0.0001	3.89 ^B \pm 0.0001	
	B Pregnant milch (30)	0.480 ^B \pm 0.0001	0.254 ^B \pm 0.0001	0.204 ^B \pm 0.0001	0.394 ^B \pm 0.0001	4.73 ^B \pm 0.0001	2.17 ^B \pm 0.0001	1.68 ^B \pm 0.0001	3.88 ^B \pm 0.0001	
	C Pregnant dry (30)	0.490 ^C \pm 0.0001	0.250 ^A \pm 0.0001	0.203 ^A \pm 0.0001	0.413 ^C \pm 0.0001	4.85 ^C \pm 0.0001	2.14 ^A \pm 0.0001	1.65 ^A \pm 0.0001	3.87 ^A \pm 0.0001	
	Group B (90), Physiological states: Parity									
	A Primipara (45)	0.470 ^A \pm 0.0001	0.250 ^A \pm 0.0001	0.203 ^A \pm 0.0001	0.383 ^A \pm 0.0001	4.62 ^A \pm 0.0001	2.14 ^A \pm 0.0001	1.65 ^A \pm 0.0001	3.87 ^A \pm 0.0001	
	B Multipara (45)	0.490 ^B \pm 0.0001	0.258 ^B \pm 0.0001	0.205 ^B \pm 0.0001	0.405 ^B \pm 0.0001	4.84 ^B \pm 0.0001	2.20 ^B \pm 0.0001	1.65 ^B \pm 0.0001	3.89 ^B \pm 0.0001	

Figures in the parentheses indicate number of Rathi animals.
 Capital letters = Significant ($p \leq 0.05$) differences among mean values for a row.
 Small letters = Significant ($p \leq 0.05$) differences among mean values for columns

Sihag (2013) observed lower vitamin E, C, A and glutathione during hot ambience as compared to moderate ambience in buffalo calves.

Minka and Ayo (2011) explored goats during the hot dry season to observe the impact of 12 h road transportation on some basic blood cells. Ascorbic acid addition before transportation improved the unfavourable bang of transportation stress on neutrophil and lymphocyte ratio and eosinopenia of the goats. Chaturvedi and Kataria (2013) recorded control value of vitamin C as $2.42 \pm 0.05 \mu\text{mol gHb}^{-1}$ in goats during moderate environmental temperature, which was reduced significantly during hot and cold ambiances. The magnitude of reduction was higher during hot than cold environmental temperature. Kankofer *et al.* (2010) explored ante- and postpartum plasma antioxidative and oxidative profiles of cows having retained placental membranes and normal cows. The maximum antioxidant and oxidant activity was observed at 2 and 1 week ante-partum advocating the existence of oxidative stress. Erythrocytic glutathione in dry and lactating cows was also measured by Esievo and Moore (1979), while Chaturvedi and Kataria (2013) made an appraisal of oxidative stress on the basis of erythrocytic glutathione level in goats during hot and cold ambiances.

CONCLUSION

Outline of variations in the values of erythrocytic vitamin A, vitamin C, vitamin E and glutathione put forward the view that erythrocytic stress response was maximum during rainy ambience. This response was in the form of depletion of vitamin A, C, E and glutathione values in erythrocytes probably in an attempt to battle the developed oxidative stress due to stressful ambience. Among calves, heifers and cows, maximum impact of rainy ambience was observed on calves as maximum depletion in the overall mean values was observed in this category. It was concluded that extreme ambiances produced oxidative stress in the Rathi cattle to a greater extent which resulted in depletion of the level of each of the antioxidant vitamin A, C, E and glutathione in erythrocytes.

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