

Reduced glutathione, glucose-6-phosphate dehydrogenase and hemoglobin in Indian buffaloes

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ABSTRACT

The reduced glutathione, G-6-PD enzyme activity and hemoglobin level were estimated in 6 different breeds of buffaloes. The overall reduced glutathione level in buffaloes was 19.76 ± 0.12 mg/100 ml blood, with the highest in Murrah (22.79 ± 0.35) followed by Badhawari (21.64 ± 0.48), Nili-Ravi (21.49 ± 0.72), Surti (19.76 ± 0.53), Jaffarabadi (19.35 ± 0.46) and lowest in non-descript breeds as 18.86 ± 0.14 . The effect of breed was highly significant on reduced glutathione level. Higher level of reduced glutathione was in young animals as compared to calves and adults. Three G-6-PD phenotypes determined by screening test on the basis of blood enzyme activity level were normal, heterozygote and deficient types. The G-6-PD activity was highest in the normal phenotype and lowest in deficient phenotype. Whereas the heterozygote phenotype was intermediate. The effect of breeds, age groups, health status and G-6-PD phenotypes were significant on hemoglobin and reduced glutathione while the effect of breeds, age groups and sex was nonsignificant on G-6-PD activity. Healthy animals showed higher levels of G-6-PD activity, reduced glutathione level and haemoglobin concentration. Significant correlations were found between reduced glutathione level with hemoglobin and G-6-PD activity in different breeds, age groups, health status and sex.

Key words: G-6-PD, Buffaloes, Hemoglobin, Murrah, Phenotype, Reduced Glutathione

The glucose-6-phosphate dehydrogenase (G-6-PD) enzyme plays a very important role in the glucose metabolism in the body and is the first line of defense of the body (Ganong 1999). Deficiency of this enzyme leads to acute hemolytic anemia in affected individuals (Yoshida 1973). According to Singari *et al.* (1989) decreased erythrocytic glucose-6-phosphate dehydrogenase (G-6-PD) activity in hemoglobin uric buffaloes may be partially responsible for a decrease in reduced glutathione, thereby causing oxidative stress to erythrocytes, which results in hemolytic syndrome. Although a number of investigations have been carried out in the hematology and blood protein polymorphism systems of buffaloes (Archer and Jeffcott 1977, Bachmann *et al.* 1978, Elamin and Saha 1980), we are unaware of any comprehensive study of blood reduced glutathione (GSH) level in this species of ruminant. In view of the very little information on the above topic in buffaloes, it is deemed important to find out the level of GSH and G-6-PD in Indian

breeds of buffaloes. Accordingly the activity level of GSH, G-6-PD and haemoglobin in 6 different breeds of buffaloes was estimated. We also studied genetic interactions with diseases and production traits in buffaloes.

MATERIALS AND METHODS

Blood samples were collected from 1527 buffaloes of 6 breeds, viz. Murrah, Jaffarabadi, Bhadawari, Surti, Nili-Ravi and non-descript buffaloes. All the blood samples were categorized according to breed, age group (adult, young and calf), health status (healthy and emaciated) and sex (male and female) of animals. Blood samples were collected from slaughterhouse and organized farm. All the blood samples were studied by screening methods originally described by Bernstein (1962) and modified by Singh (1992) for cattle blood samples and with a further modification for buffalo blood samples for present study for different G-6-PD phenotypes by using 2,6-dichlorophenol indophenol. On the basis of the screening test, 3 G-6-PD phenotypes were classified: normal, heterozygote and deficient. The G-6-PD activity levels were estimated by colorimetric methods (Ells and Kirkman 1961) for all blood samples. Glucose-6-phosphate dehydrogenase in red blood cell reacts with glucose-6-phosphate and NADP⁺ forming 6-phospho

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gluconolactone and NADPH. The latter in the presence of phenazine metho sulphate (PMS) reduces a blue dye (2,6-dichlorophenol indophenols) to a colorless state. The rate of dye disappearance is proportional to the G-6-PD enzyme activity. The blood samples which decolorize the dye within 20 min are categorized as normal (homozygote). The samples which cannot decolorize the dye even after 90 min were considered as deficient (homozygote), and the samples which were completely decolorized between 30 to 90 min were considered as heterozygote. So there was a wide range of de-colorization time (30–90 min) in heterozygote.

To find out the reason for taking different timing of de-colorization an experiment was conducted with the mixture of different concentration of normal and deficient whole blood considering normal as having 100% normal cells and deficient having 100% deficient cells. After mixing with different concentration of whole blood of normal and deficient samples the hemolysates were prepared. These hemolysates of different cell concentrations were used and the timing of de-colorization was observed. The statistical analysis of data was done as per Snedecor and Cochran (1989).

RESULTS AND DISCUSSION

The blood samples were screened for different G-6-PD phenotypes and the G-6-PD enzyme activity reduced glutathione (GSH), and hemoglobin concentration was estimated for all blood samples. Three G-6-PD phenotypes, normal, heterozygote and deficient were observed on the basis of screening test. The data were analyzed according to G-6-PD phenotypes, breeds, age groups, health status and sex of the animals. The overall GSH level, G-6-PD activity and hemoglobin in buffalo were 19.76 ± 0.12 mg /100 ml blood, 650.94 ± 7.97 m.i.u and 11.03 ± 0.05 g% respectively.

The overall proportions of normal, heterozygote and deficient G-6-PD phenotypes were 26.0, 66.27 and 7.73% respectively in buffaloes. Whereas, the percentage of G-6-PD deficient animals were highest in non-descript buffaloes (10.87%) followed by Jaffarabadi (6.61%), Surti (3.23%), Murrah (0.96%) and Bhadawari (0.81%). G-6-PD deficient phenotype was not observed in Nili-Ravi buffaloes. The data were further analyzed according to age groups, health status and sex in all breeds. In age groups adult animals showed a higher percentage of G-6-PD deficient phenotypes whereas G-6-PD normal phenotypes were more in young group of animals and heterozygote phenotypes were common in calves. Healthy animals showed a higher percentage of G-6-PD normal phenotypes whereas emaciated animals showed higher percentage of G-6-PD deficient phenotypes. The frequency of the G-6-PD deficient gene was highest in the adult age group in all breeds of buffaloes. In males and females, the G-6-PD deficient percentages were almost the same.

The G-6-PD enzyme activity was calculated in all age groups, health status and sexes in 5 breeds and non-descript

Table 1. Level of reduced glutathione (GSH) and glucose-6-phosphate dehydrogenase (G-6-PD) in different age groups, health status and sex in different breeds of buffaloes.

Category	Breeds												
	Murrah (209)		Jaffarabadi (121)		Bhadawari (123)		Surti (93)		Nili-Ravi (24)		Non-Descript (957)		
	GSH	G-6-PD	GSH	G-6-PD	GSH	G-6-PD	GSH	G-6-PD	GSH	G-6-PD	GSH	G-6-PD	
Age group	Adult	22.56±0.55	761.76±25.18	18.81±0.57	519.69±24.82	20.78±0.69	676.55±27.07	19.25±0.83	765.11±23.33	22.30±0.88	794.84±88.23	18.28±0.17	591.44±9.73
	Young	23.91±0.50	851.34±25.12	20.11±0.70	600.78±35.48	22.40±0.67	768.72±28.93	20.15±0.70	797.56±27.20	21.08±0.99	795.31±29.21	19.56±0.22	688.77±10.88
	Calf	19.84±0.96	573.53±29.57	-	-	-	-	-	-	-	-	-	18.90±0.75
Health status	Healthy	23.19±0.36	751.49±17.02	20.86±0.56	600.34±26.92	20.97±0.50	767.04±21.23	21.72±0.54	688.63±35.37	21.94±0.72	717.58±38.34	20.64±0.16	700.39±8.88
	Emaciated	19.62±1.23	696.40±62.18	17.27±0.67	588.49±31.12	17.84±0.19	606.44±43.34	16.01±0.83	629.25±40.15	19.22±2.34	683.00±42.65	16.36±0.18	531.15±9.97
Sex	Male	24.35±0.61	817.27±29.56	19.54±0.87	581.18±43.81	22.22±0.74	741.73±39.52	20.16±0.98	697.21±44.47	22.27±0.79	773.52±81.15	18.97±0.21	652.89±10.77
	Female	22.06±0.43	706.00±19.86	19.25±0.54	541.34±23.31	21.35±0.62	717.02±23.24	19.62±0.63	623.95±19.31	20.90±1.05	808.13±27.59	18.78±0.18	605.51±9.65
Overall (1527)		22.79±0.35	741.44±16.79	19.35±0.46	553.19±20.19	21.64±0.48	725.26±20.27	19.76±0.53	699.38±90.50	21.49±0.72	795.15±34.07	18.86±0.14	625.66±7.22

Figures in parenthesis are the number of observations.

Table 2. Correlation between haemoglobin, G-6-PD activity and reduced glutathione in different breeds of buffaloes

Correlation between	Breeds						Overall (1527)
	Murrah (209)	Jaffarabadi (121)	Bhadawari (123)	Surti (93)	Nili-Rabi (24)	Non-Descript (957)	
Hemoglobin and G-6-PD activity	0.54**	0.50**	0.52**	0.068	0.50**	0.71**	0.20**
Hemoglobin and Reduced glutathione (GSH)	0.03**	0.32**	0.36**	0.45**	0.32	0.38**	0.53**
G-6-PD activity and Reduced glutathione (GSH)	0.45**	0.48**	0.49**	0.20*	0.58**	0.46**	0.43**

* Significant at $P<0.05$; ** significant at $P<0.01$; figures in parenthesis are the number of observations.

buffaloes (Table 1). The overall G-6-PD activity was highest in the normal phenotype as 915.55 ± 6.61 m.i.u. and lowest in deficient phenotype as 260.46 ± 7.76 m.i.u. whereas in heterozygote phenotypes it was intermediate as 592.66 ± 9.44 m.i.u. The analysis of variance showed a highly significant ($P<0.01$) effect of G-6-PD types and health status on G-6-PD enzyme activity, which is due to significant difference in G-6-PD enzyme activity level among 3 G-6-PD phenotypes. The effect of breed, age group, and sex was found nonsignificant on G-6-PD activity, but the activity was highest in Nili-Ravi breed and the lowest in non-descript buffaloes. Again the activity was recorded as higher in young animals than in adults and calves. The effect of health status was significant on G-6-PD activity, which may be due to higher activity level in healthy animals than in emaciated animals. Glucose-6-phosphate dehydrogenase also had a correlation with total milk yield (0.08) and average milk yield (0.55).

The reduced glutathione (GSH) levels were estimated calorimetrically in different age groups, health status, and sex in 6 breeds of buffaloes (Table 1). Overall glutathione level in buffalo was 19.76 ± 0.12 mg/100 ml blood. Whereas glutathione levels in Murrah, Jaffarabadi, Bhadawari, Surti, Nili-Ravi and non-descript breeds were 22.79 ± 0.35 , 19.35 ± 0.46 , 21.64 ± 0.48 , 19.76 ± 0.53 , 21.49 ± 0.72 and 18.86 ± 0.14 mg/100 ml blood respectively. The effect of breeds, age groups, health status and G-6-PD phenotypes were highly significant on glutathione level. Where as effect of sex was nonsignificant. This significant effect may be due to significantly higher level of glutathione in Murrah, Bhadawari and Nili-Ravi breeds which did not differ among themselves but differed significantly from Jaffarabadi, Surti and non-descript breeds, in which 3 breeds GSH levels were lower and almost the same. This supports the native geographical distribution of Indian buffalo breeds. The overall glutathione level in adults, young and calves were 19.06 ± 0.16 , 20.54 ± 0.19 and 19.39 ± 0.16 mg/100 ml blood respectively. Levels of glutathione were higher in young animals as compared to adults, which agree with the findings of Annunziata and Iorio (2004). The overall glutathione level was found significantly higher in healthy animals than in emaciated animals in all 6 breeds of buffaloes. These

differences in GSH values in healthy and emaciated buffaloes indicated that GSH content in the blood appeared to be regulated by nutritional status of the animals also. A similar observation was made by Agar and Suzuki (1982) and Singari *et al.* (1989). The glutathione level was 19.96 ± 0.02 in males and 19.63 ± 0.15 mg/100 ml of blood in females, the 2 sexes thus not differ significantly. A good correlation between glutathione level and age at first calving (0.03), total milk yield (0.46), lactation length (-0.23) and average milk yield (0.48) were also found in this study.

Hemoglobin concentration was estimated in all 6 breeds of buffaloes with overall mean of 11.03 ± 0.05 g%, whereas it was 11.07 ± 0.14 , 10.51 ± 0.17 , 11.83 ± 0.17 , 10.76 ± 0.24 , 13.11 ± 0.40 and 10.06 ± 0.07 g% in Murrah, Jaffarabadi, Bhadawari, Surti, Nili-Ravi and non-descript buffaloes respectively. The breeds, age groups, health status, sex and G-6-PD phenotypes has highly significant effect ($P<0.01$) on hemoglobin.

A significant ($P<0.01$) correlation between hemoglobin and G-6-PD activity was found in all breeds of buffaloes except Surti breed. Similarly a significant ($P<0.01$) correlation between hemoglobin and reduced glutathione was found in all breeds except Nili-Ravi. A significant ($P<0.01$) correlation between G-6-PD activity and reduced glutathione was found in all breeds of buffaloes (Table 2).

The between breeds, age groups, health status, sex and G-6-PD phenotypes showed highly significant effect ($P<0.01$) on hemoglobin concentration. All the effects also showed highly significant effect ($P<0.01$) on glutathione also, except the effect of sex, whereas, the G-6-PD activity showed highly significant effect ($P<0.01$) of health status and G-6-PD phenotypes only.

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