Erythrocyte glutathione concentrations in Nigerian sheep in the Sahel region

I.O. Igbokwe [1] G.S. Bah [1]

Key words

Sheep - Glutathione - Erythrocyte - Nigeria.

Summary

The erythrocyte glutathione (GSH) concentrations of 101 Nigerian Sahel sheep of mixed breeds ranged from 9.2 to 109.4 (45.1 ± 18.8) mg/dl of red blood cells (RBC) or 0.3-3.1 (1.7 ± 0.6) mg/g of hemoglobin (Hb). The majority of sheep (72.3%) had low erythrocyte glutathione concentrations (< 55 mg/dl RBC). The frequency distribution of the values was normal with modal classes at 41-50 mg/dl RBC and 1.6-2.0 mg/g Hb. The erythrocyte GSH values in milligrams per deciliter RBC and milligrams per gram of hemoglobin were strongly correlated, but did not correlate with the packed cell volume and hemoglobin or anemia.

■ INTRODUCTION

An inheritable erythrocyte glutathione (GSH) polymorphism was reported in several sheep breeds according to a review by Agar (1). Sheep having erythrocyte GSH concentrations above 55 mg/dl of red blood cells (RBC) were considered to have high (normal) GSH, while those with values below were considered to have low (deficient) GSH (19). In Finnish Landrace sheep, low GSH was caused by lack of availability of cysteine (a GSH precursor) in the erythrocytes resulting from an impaired permeability of the amino acid into the cells (23, 24). In Tasmanian Merino sheep, low erythrocyte GSH was due to a diminished activity of γ-glutamyl cysteine synthetase (25). A backcross breeding between GSH-deficient Finnish and Merino sheep produced lambs which had inherited both types of GSH deficiencies, and with more severe GSH deficiency (22).

Erythrocytes with low GSH have a shortened life span (18) and tend to be more prone to Heinz body formation than normal ones (21). Sheep with low erythrocyte GSH concentrations have lower ewe and lamb weights at different ages, lower milk production and a disadvantage in the number of lambs born (4, 5), but they produce more wool (11) than those with high GSH.

1. Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Maiduguri, PMB 1069, Maiduguri, Nigeria

Balami, Uda and Yankasa sheep breeds are found in the semi-arid Sahel region of Nigeria, but they are rarely pure breeds, and are used for meat and sometimes wool (9). The purpose of this work was to survey the erythrocyte GSH concentrations in these sheep with reference to packed cell volume and hemoglobin concentrations. This could lay the groundwork for future research to attempt to relate erythrocyte GSH concentrations to productivity traits or erythrocyte abnormalities.

■ MATERIALS AND METHODS

One hundred and one sheep of mixed breeds and both sexes, aged 0.5-5 years and weighing about 8-45 kg, were used. Eighty-one of them were presented at the Maiduguri abattoir for slaughter in October to November 1994, and the other 20 were maintained on the University of Maiduguri farm.

Five milliliters of blood were collected from the jugular vein of each animal with EDTA as anticoagulant. The samples were preserved in ice pack or in the refrigerator at about 4°C, and analyzed within 24 h after collection.

Packed cell volume (PCV) and hemoglobin (Hb) concentrations were determined by the microhematocrit and cyanmethemoglobin methods, respectively. GSH concentration in whole blood was determined spectrophotometrically by the method of Beutler *et al.* (7) using 5,5'-dithiobis- (2-nitrobenzoic acid) (Sigma, UK).

The erythrocyte GSH concentration was calculated using PCV and Hb values, and expressed in milligrams per deciliter of red blood cells and milligrams per gram of hemoglobin.

The data were summarized as means plus or minus the standard deviation, and correlation coefficients (r) between parameters were calculated (10).

■ RESULTS

The mean PCV, Hb, blood and erythrocyte GSH concentrations are presented in table I. The correlation coefficients of blood and erythrocyte GSH concentrations with PCV and Hb values are shown in table II. The frequency distribution of erythrocyte GSH concentrations is illustrated in figures 1 and 2.

Seventy-three (72.3%) of the sheep had low erythrocyte GSH concentrations (< 55 mg/dl RBC) out of a population of 101 sheep, based on the criteria of Tucker and Kilgour (19). The mean erythrocyte GSH concentrations in low and high GSH sheep were 36.4 ± 12.9 mg/dl RBC (n = 73) and 67.7 ± 11.5 mg/dl RBC (n = 28), respectively. The modal classes for erythrocyte GSH concentrations were 41-50 mg/dl RBC and 1.6-2.0 mg/g Hb.

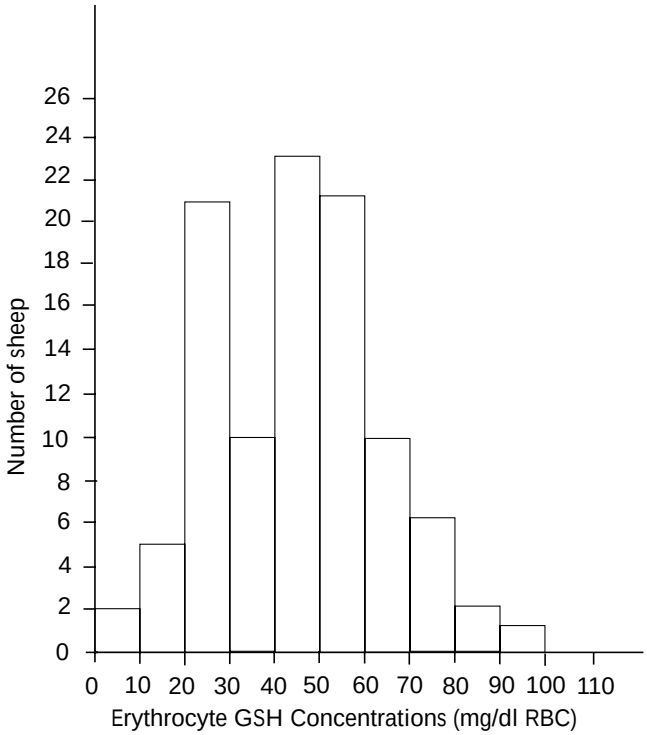

Figure 1: *Frequency distribution of erythrocyte GSH concentrations (mg/dl RBC) of Nigerian Sahel sheep.*

Table I

Packed cell volume, hemoglobin and erythrocyte glutathione concentrations in Nigerian sheep

Parameters	Mean ± SD	Range
PCV (%)	29.2 ± 6.4	10-44
Hb (g/dl)	7.7 ± 1.6	2.9-12.0
Blood GSH (mg/dl)	12.9 ± 5.8	3.1-28.1
Erythrocyte GSH		
mg/dl RBC	45.1 ± 18.8	9.2-109.4
mg/g Hb	1.7 ± 0.6	0.3-3.1

Table II

Correlation coefficients (r) in the relationship of blood and erythrocyte glutathione concentrations with packed cell volume and hemoglobin concentrations in Nigerian sheep

Parameters	r
Blood GSH *vs.* PCV	0.42[a]
Blood GSH *vs.* Hb	0.42[a]
Erythrocyte GSH	
mg/dl RBC *vs.* PCV	-0.18[b]
mg/g Hb *vs.* Hb	0.10[b]
mg/dl RBC *vs.* blood GSH	0.76[a]
mg/g Hb *vs.* blood GSH	0.83[a]
mg/dl RBC *vs.* mg/g Hb	0.81[a]
PCV *vs.* Hb	0.78[a]

[a] Significant, P < 0.01
[b] Not significant, P > 0.05

Nine sheep with PCVs below 21% had a mean erythrocyte GSH of 62.1 ± 28.0 mg/dl RBC, which was significantly (P < 0.05) higher than a mean value of 42.1 ± 19.2 mg/dl RBC in 23 sheep with PCVs above 33%. Thus, sheep with higher PCVs tended to have lower erythrocyte GSH concentrations than those with lower PCVs. This relationship was slightly reflected in an insignificant negative correlation (r = -0.18; P > 0.05) between erythrocyte GSH and PCV values. It is noteworthy that the lowest erythrocyte GSH concentration of 9.2 mg/dl RBC was found in sheep with PCV of 34%.

There was no correlation (r = 0.01) between erythrocyte GSH in milligrams per gram of hemoglobin and hemoglobin values. Fourteen sheep with Hb values below 6.0 g/dl had mean erythrocyte GSH concentrations of 1.8 ± 0.5 mg/g Hb, which were not significantly (P > 0.05) different from the mean of 1.6 ± 0.7 mg/g of hemoglobin from 22 sheep with Hb values above 9.0 g/dl.

PCV and Hb values showed significant positive correlations with blood GSH concentrations. PCV was positively correlated with Hb values. There was a positive correlation between blood and erythrocyte GSH concentrations on one hand, and the values of erythrocyte GSH in milligrams per deciliter RBC and milligrams per gram of hemoglobin, on the other (table II).

■ DISCUSSION

The erythrocyte GSH concentrations in the Nigerian sheep ranged from 9.2 to 109.4 mg/dl RBC, in agreement with previous reports in several sheep breeds in other parts of the world, where erythrocyte GSH polymorphism has been reported (2, 8, 11, 17, 19, 20). The majority (72.3%) of the Nigerian Sahel sheep had low erythrocyte GSH (< 55 mg/dl RBC), a situation that has been similarly reported in Indian sheep breeds, where low GSH sheep represented 68.78% of the population (1). This is in contrast with the

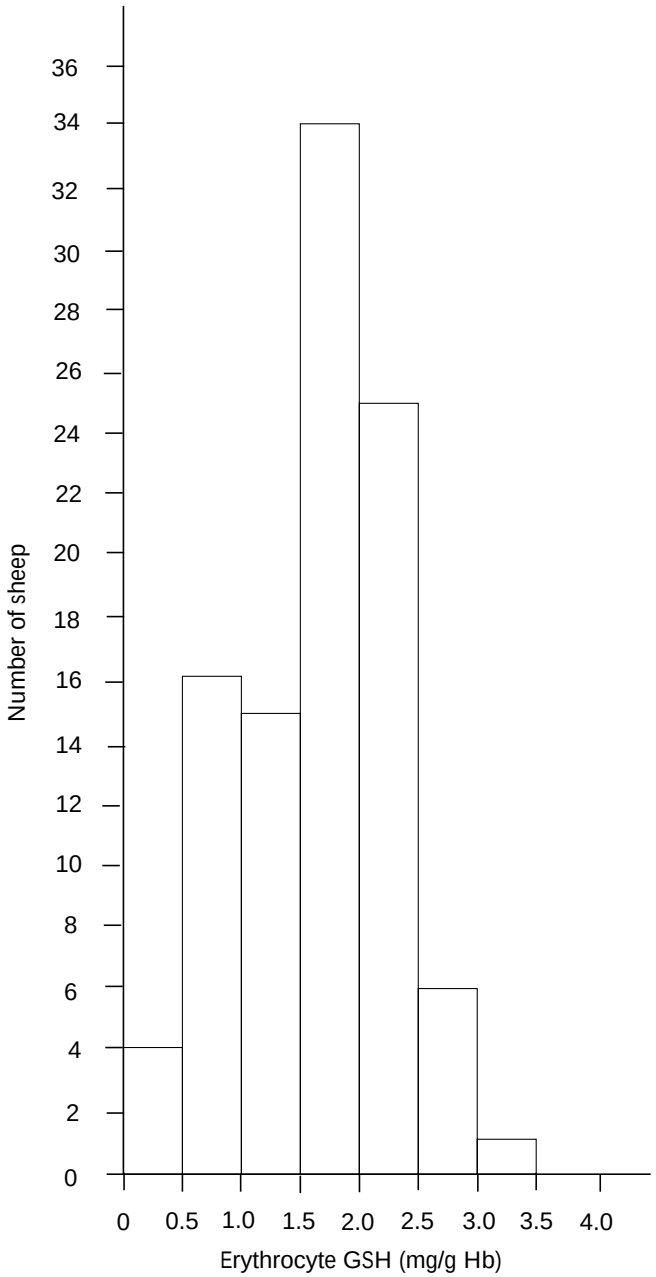

Figure 2: *Frequency distribution of erythrocyte GSH concentrations (mg/g Hb) of Nigerian Sahel sheep.*

situation among sheep breeds in the UK, USA, and Australia, where low GSH sheep only represent 0-43% of the population (1). Nigerian Sahel goats also have a high incidence of low erythrocyte GSH concentrations, even with values lower than 9 mg/dl RBC (13).

The lowest erythrocyte GSH concentration in sheep ever reported was 4.5 mg/dl RBC (17), and the lowest erythrocyte GSH concentration in Nigerian Sahel sheep was 9.2 mg/dl RBC. The low values of erythrocyte GSH concentrations in the Nigerian sheep were similar to those reported in Tasmanian Merino and Finnish Landrace sheep breeds, where biochemical abnormalities in the erythrocytes capable of affecting GSH synthesis were reported (23-25), with increased erythrocyte susceptibility to Heinz body formation and reduced erythrocyte life span (18, 21). However, low erythrocyte GSH concentrations in the sheep of the present study were not associated with anemia, which agrees with the report of Smith and Osburn (17). It was rather often associated with high PCV. This may be an indication of a physiological adaptation to the low erythrocyte GSH. The insignificant negative correlation between erythrocyte GSH and PCV contrasts with the positive correlation between the parameters in cattle from previous studies (12, 14). In the Nigerian Sahel goats, the erythrocyte GSH did not correlate with PCV or anemia (13). In humans, erythrocyte GSH was inversely related to PCV (6), and low erythrocyte GSH concentrations as found in the sheep resulted in anemia (15). In the sheep, the low erythrocyte GSH may have a biochemical basis and pathophysiological implications, which need to be studied.

Erythrocyte GSH concentrations in sheep are usually expressed in milligrams per deciliter RBC (1). There was a strong positive correlation between the values of erythrocyte GSH in milligrams per deciliter RBC and milligrams per gram Hb, perhaps because of the concurrent positive correlation between PCV and Hb. Similar situation has been reported in cattle (12) and goats (13).

Greater productivity in terms of better growth rate and greater number of lambs born were favored by selection of high erythrocyte GSH levels in sheep (4), and sheep with low erythrocyte GSH produced more wool (11). The Nigerian sheep are mainly used for meat and should, preferably, be selected for fast growth and better body weight at maturity. In the future, the relationship of productivity traits to erythrocyte GSH levels in Nigerian sheep should be examined.

In conclusion, the majority of Nigerian Sahel sheep had low erythrocyte GSH concentrations, which did not significantly correlate with PCV or Hb.

REFERENCES

1. AGAR N.S., 1975. Glutathione polymorphism in the sheep red blood cells. *Int. J. Biochem.*, **6**: 843-852.

2. AGAR N.S., ROBERTS J., EVANS J.V., 1972. Erythrocyte glutathione polymorphism in sheep. *Aust. J. Biol. Sci.*, **25**: 619-626.

3. AGAR N.S., ROBERTS J., MULLEY A., BOARD P.G., HARLEY J.D., 1975. The effect of experimental anemia on the levels of glutathione and enzyme activities of the erythrocytes of normal and glutathione-deficient sheep. *Aust. J. Biol. Sci.*, **28**: 233-238.

4. ATROSHI F., 1979. Phenotypic and genetic association between production/reproduction traits and blood biochemical polymorphic characters in Finn sheep (Thesis). Helsinki, Finland, Government Printing Centre, p. 37-42.

5. ATROSHI F., SANDHOLM M., 1982. Red blood cell glutathione as a marker of milk production in Finn sheep. *Res. vet. Sci.*, **33**: 256-259.

6. BAUER J.D., 1980. Laboratory investigation of red cell pathology. In: Sonnenwirth A., Jarett L. Eds., Vol. 1, 8th ed. London, UK, CV Mosby Company, p. 915.

7. BEUTLER E., DURON D., KELLY B.M., 1963. Improved method for the determination of blood glutathione. *J. lab. clin. Med.*, **61**: 882-888.

8. BOARD P.G., ROBERTS J., EVANS J.V., 1974. The genetic control of erythrocyte reduced glutathione in Australian Merino Sheep. *J. agric. Sci.*, **82**: 395-398.

9. BOURN D., WINT W., BLENCH R., WOOLEY E., 1994. Nigerian livestock resources survey. *World Anim. Rev.*, **78**: 49-58.

10. CHATFIELD C., 1983. Statistics for technology. A course in applied statistics, 3rd. ed. London, UK, Chapman and Hall.

11. KALLA S.D., GHOSH P.K., 1975. Blood biochemical polymorphic traits in relation to wool production efficiency in Indian sheep. *J. agric. Sci.*, **84**: 149-152.

12. IGBOKWE I.O., BAH G.S., OBAGAIYE O.K., SAROR D.I., ESIEVO K.A.N., 1996. Erythrocyte glutathione concentrations and the correlations with packed cell volume, haemoglobin and plasma ascorbic acid concentrations in Nigerian Wadara cattle. *Revue Elev. Méd. vét. Pays trop.*, **49**: 263-265.

13. IGBOKWE I.O., RIBADU A.Y., BUKAR M.M., 1998. Erythrocyte glutathione concentrations in Nigerian Sahel goat. *Small Rumin. Res.*, **30**: 1-6.

14. IGBOKWE I.O., UMAR I.A., OBAGAIYE O.K., SAROR D.I., ESIEVO K.A.N., 1995. Erythrocyte glutathione concentrations in Nigerian Zebu and Ndama cattle. *Revue Elev. Méd. vét. Pays trop.*, **48**: 177-179.

15. KONRAD P.N., RICHARDS F., VALENTINE W.N., PAGLIA D.E., 1972. γ-glutamyl cysteine synthetase deficiency. A cause of hereditary haemolytic anaemia. *New Eng. J. Med.*, **286**: 557-561.

16. SMITH J.E., LEE M.S., MIA A.S., 1973. Decreased γ-glutamyl-cysteine synthetase: the probable cause of glutathione deficiency in sheep erythrocytes. *J. lab. clin. Med.*, **82**: 713-718.

17. SMITH J.E., OSBURN B.I., 1967. Glutathione deficiency in sheep erythrocytes. *Science*, **158**: 374-375.

18. TUCKER E.M., 1974. A shortened life span of sheep red cells with a glutathione deficiency. *Res. vet. Sci.*, **16**: 19-22.

19. TUCKER E.M., KILGOUR L., 1970. An inherited glutathione deficiency and a concomitant reduction in potassium concentration in sheep red cells. *Experientia*, **26**: 203-204.

20. TUCKER E.M., KILGOUR L., 1972. A glutathione deficiency in the red cells of certain Merino Sheep. *J. agric. Sci.*, **79**: 515-516.

21. TUCKER E.M., KILGOUR L., 1973. The effect of anaemia on sheep with inherited differences in red cell reduced glutathione concentrations. *Res. vet. Sci.*, **14**: 306-311.

22. TUCKER E.M., KILGOUR L., YOUNG J.D., 1976. The genetic control of red cell glutathione deficiencies in Finnish Landrace and Tasmanian Merino sheep and in crosses between these breeds. *J. agric. Sci.*, **87**: 315-323.

23. YOUNG J.D., ELLORY J.C., TUCKER E.M., 1975. Amino acid transport defect in glutathione-deficient sheep erythrocytes. *Nature*, **254**: 156-157.

24. YOUNG J.D., ELLORY J.C., TUCKER E.M., 1976. Amino acid transport in normal and glutathione-deficient sheep erythrocytes. *Biochem. J.*, **154**: 43-48.

25. YOUNG J.D., NIMMO I.A., 1975. Glutathione synthesis in glutathione-deficient erythrocytes from Finnish Landrace and Tasmanian Merino sheep. *Biochem. Biophys. Acta*, **404**: 132-141.

Reçu le 17.8.98, accepté le 27.1.99

Résumé

Igbokwe I.O., Bah G.S. Concentrations du glutathion érythrocytaire chez des moutons nigérians de la région du Sahel

Les concentrations du glutathion érythrocytaire de 101 moutons de plusieurs races du Sahel nigérian ont varié de 9,2 à 109,4 (45,1 ± 18,8) mg/dl de globules rouges ou de 0,3 à 3,1 (1,7 ± 0,6) mg/g d'hémoglobine. La majorité des moutons (72,3 p. 100) avaient de faibles concentrations érythrocytaires (< 55 mg/dl de globules rouges). La distribution des fréquences des valeurs a été normale avec des classes modales allant de 41 à 50 mg/dl de globules rouges et de 1,6 à 2,0 mg/g d'hémoglobine. Les valeurs du glutathion érythrocytaire étaient fortement corrélées en termes de milligrammes par décilitre de globules rouges et de milligrammes par gramme d'hémoglobine, mais ne l'étaient pas avec l'hématocrite, l'hémoglobine et l'anémie.

Mots-clés : Ovin - Glutathion - Erythrocyte - Nigeria.

Resumen

Igbokwe I.O., Bah G.S. Concentraciones de glutation eritrocitario en ovinos Sahelinos de Nigeria

Las concentraciones de glutation eritrocitario (GSH) de 101 ovejas Sahelinas de Nigeria, de razas mixtas, varió de 9,2 a 109,4 (45,1 ± 18,8) mg/dl RBC o 0,3-3,1 (1,7 ± 0,6) mg/g Hb. La mayoría de las ovejas (72,3%) presentaron concentraciones eritrocitarias bajas (< 55 mg/dl RBC). La distribución de las frecuencias de los valores fueron normales, con clases modales de 41-50 mg/dl RBC y 1,6-2.0 mg/g Hb. Los valores de GSH eritrocitario en mg/dl RBC y mg/g Hg se correlacionaron fuertemente, pero no se correlacionaron con PCV y Hb o anemia.

Palabras clave: Ovino - Glutation - Eritrocito - Nigeria.