SHORT COMMUNICATION

The levels of glutathione and hemoglobin in sheep erythrocytes as a function of age

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ABSTRACT

The behaviour of the levels of glutathione (GSH) and hemoglobin (Hb) was studied in blood samples from 126 one- to seven-year-old sheep reared in the province of Salerno, Italy. Negative and significant correlation with age both for hemoglobin and glutathione has been reported. A significant difference, on the basis of age, is evidenced only in males (n=24) for Hb (P = 0.001) and only in females (n=102) for GSH (P = 0.001). These results indicate a greater presence of Hb in young sheep and a greater demand for GSH in adult sheep. Moreover, the lowering of GSH and Hb with respect to age may have caused a reduction of the organism defense to environmental stress factors. Therefore, it would be useful, at least in productive animals, to use feed with a high content of polyphenolic compounds that increase the GSH concentration.

The aim of the present work was to study the behaviour of the levels of glutathione and hemoglobin in sheep at different ages.

Key words: Sheep, Glutathione, Hemoglobin

RIASSUNTO

I LIVELLI DI GLUTATIONE E DI EMOGLOBINA NEGLI ERITROCITI DI PECORA IN FUNZIONE DELL’ETÀ

Lo scopo del presente lavoro è quello di studiare il comportamento dei livelli di glutatnine (GSH) e dell’emoglobina (Hb) nelle pecore di differente età. Sono state studiate le variazioni dei livelli di GSH e di Hb in campioni di sangue ottenuti da 126 pecore di età compresa fra 1 e 7 anni allevate nella provincia di Salerno. Correlazioni significative e negative con l’età sono state riportate sia per l’emoglobina che per il glutatnine. Sono state evidenziate differenze significative in relazione all’età solo nei maschi per l’Hb e solo nelle femmine per il GSH. I risultati indicano una maggiore presenza di Hb nelle pecore giovani ed una maggiore richiesta di GSH in quelle adulte. Inoltre, l’abbassamento dei livelli di Hb e di GSH all’aumentare dell’età, potrebbe determinare una riduzione delle difese dell’organismo ai fattori di stress ambientale. Pertanto sarebbe auspicabile, almeno negli animali in produzione, l’uso di alimenti ricchi in composti polifenolici che incrementano la concentrazione del GSH.

Parole chiave: Pecora, Glutatione, Emoglobina
Introduction

Glutathione is a carrier of free radicals and a natural antioxidant that has different properties: it reduces the mutagenicity of aflatoxins; it inhibits the formation of mutagens and is important in the defense against chronic diseases; it is a detoxicating agent; it is an immunomodulator since T-lymphocytes require substantial levels of GSH for their activity and replication; it saves some antioxidative-active vitamins such as vitamins C and E.

Glutathione is the indispensable substrate of the enzyme glutathione peroxidase whose action is the elimination of the peroxides that could compromise the structural integrity of the hemoglobin and molecules of the red blood cells. Moreover, as it is one of the main defense mechanisms against cellular oxidative stress, those components of the diet that increase the GSH concentration in the plasma can be of great importance for human health. Caporaso et al. (2003) showed that the phenolic components of coffee play a fundamental role due to their antioxidant property since it increases the level (16%) of GSH in the plasma.

During aging the glutathione level diminishes within cells and tissues, such as in the heart, in red blood cells and in lymphocytes (Stobs et al., 1984, Marongiu et al., 1993) and in the brain (Ravindranath et al., 1989). Contrasting with Bayón et al. (1994), Tucker and Kilgour (1970) evidenced significant differences between the values of GSH and the concentration of potassium in sheep's blood. Bidik et al. (2002) showed that GSH concentration in the blood was 57% higher in hyperthyroid rabbits than in controls. In buffalo, the GSH level is found, on average, in smaller amounts in TF-II with respect to the other two TF-AA and TF-AI phenotypes that appear to produce higher standards of health and/or welfare (Annunziata et al., 1995). The aim of the present work was to study the behavior of the levels of glutathione and hemoglobin in sheep at different ages.

Material and methods

Blood samples were taken from 126 sheep (1 to 7 years old, 24 males and 102 females) reared in the province of Salerno, Italy. Among 24 males, 6 were one - to three-year-old animals and 18 four- to seven-year-old animals, while among 102 females, 57 were one - to three-year-old animals and 45 were four - to seven-year-old animals. Approximately 24 hours after blood sampling, the levels of hemoglobin (Hb) and glutathione (GSH) were determined.

Table 1. Average, standard deviation and correlation (r) of Hb and of GSH in sheep.

| Age (years) | n. | Hb g/100 ml | GSH mg/100 ml |
|------------|----|-------------|---------------|
|            |    | x ± σ       | x ± σ         |
| 1          | 6  | 10.75 ± 3.66| 12.45 ± 4.59  |
| 2          | 10 | 8.53 ± 1.05 | 14.37 ± 3.09  |
| 3          | 47 | 9.24 ± 1.24 | 11.97 ± 3.84  |
| 4          | 29 | 9.36 ± 1.38 | 10.09 ± 3.83  |
| 5          | 20 | 8.58 ± 0.74 | 9.75 ± 4.09   |
| 6          | 10 | 8.21 ± 0.82 | 10.63 ± 4.12  |
| 7          | 4  | 8.49 ± 1.10 | 9.11 ± 5.11   |
| Total      | 126| 9.07 ± 1.44 | 11.20 ± 4.06  |

Hb-age: \( r = -0.24^* \); GSH-age: \( r = -0.27^{**} \).

* \( P \leq 0.05 \)
** \( P \leq 0.001 \)
were determined. Total Hb were determined by quantitative colorimetric determination in whole blood at 530 – 550 nm (Sigma Diagnostic Kit) while the GSH level were determined on whole blood according to the technique of Beutler et al. (1963). For statistical analysis two groups based on age classes and sex were formed: one group (years 1 – 3) from 63 animals (57 female and 6 male) and the second group (years 4 – 7) from 63 animals (45 female and 18 male). The data obtained were processed with the SPSS statistical package (Norusis, 1989). Statistical significance of differences for hemoglobin and glutathione among mean observed group of animals were calculated using the Student t – test.

Results and discussion

In addition to the decrease in value of means and standard deviation among 1 – 7 years, Table 1 also reports a negative and significant correlation with age for both hemoglobin and glutathione (r = - 0.24; P ≤ 0.05 and r = - 0.27; P ≤ 0.001). Table 2 shows a reduction in the means between age classes 1 (years 1-3) and 2 (years 4-7) for the whole population (n=126) which is significant only for GSH (P ≤ 0.001). These significant differences are evidenced only in males (n=24) for Hb (P ≤ 0.001) and only in females (n=102) for GSH (P ≤ 0.001).

These results indicate a greater presence of Hb in young male and female sheep while a greater demand for GSH only in adult female sheep.

Conclusions

Given the importance of GSH in cells and tissues and its strict relation with age, it would be useful, at least in adult animals, to use feeds particularly rich in polyphenolic compounds that would increase the GSH concentration. The lowering of Hb and GSH levels as a function of age could result in a diminished defense of sheep against environmental stress even if the therapeutic value of the free radicals (reactive oxygen species, ROS) should be reevaluated in light of the new research carried out by Segal's group (Ahiuwalla et al. 2004). Therefore, further biochemical and molecular studies are required on antioxidant enzymatic systems (Cu, Zn SOD; Glutathione peroxidase, Glutathione reductase, etc.) to understand the role that GSH plays in regulating aging and development.

Table 2. Average, standard deviation and “t” of Hb and GSH for years and for sex in sheep.

| Sex (n.) | group 1 (n.) | group 2 (n.) | Hb g/100 ml | GSH mg/100ml |
|---------|-------------|-------------|-------------|--------------|
|         | f | m | f+m | 1 - 3 | 4 - 7 |         |         |
|         | 57 | 45 | 102 | 9.22 | 1.71 | 12.65 | 3.77 |
|         | 8.87 | 1.32 | 9.75 | 4.16 |
|         | 9.07 | 1.56 | 11.37 | 4.19 |
|         | 9.81 | 0.23 | 10.04 | 4.00 |
|         | 8.88 | 0.76 | 10.66 | 3.41 |
|         | 9.11 | 0.78 | 10.51 | 3.49 |
|         | 9.27 | 1.64 | 12.39 | 3.84 |
|         | 8.88 | 1.18 | 10.01 | 3.95 |
|         | 9.07 | 1.44 | 11.20 | 4.06 |
|         | 6  | 18  | 24  | 63 | 63 |         |         |
| Hb: t (group 1-group 2)= 4.58**; DF = 22
GSH: t (group 1 - group 2)= 3.44**; DF = 124; t (group 1 - group 2)= 3.64**; DF = 100
**P ≤ 0.001
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The paper should be attributed equally to the authors.

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