

Assessment of blood glutathione peroxidase activity in the dromedary camel

Juan Alberto CORBERA^{a*}, Carlos GUTIERREZ^a, Manuel MORALES^a,
Arturo MONTEL^b, José Alberto MONTOYA^a

^aDepartment of Animal Medicine and Surgery, Veterinary Faculty, Universidad de Las Palmas de Gran Canaria, 35416 Arucas, Las Palmas, Canary Islands, Spain

^bServicio de Toxicología, Instituto de Medicina Preventiva
“Capitán Médico Ramón y Cajal”, Madrid, Spain

(Received 25 September 2000; accepted 9 January 2001)

Abstract – Blood glutathione peroxidase (GSH-Px) levels in 709 normal dromedary camels (442 females and 267 males) were assessed in the Canary Islands. All animals were intensively reared, and three different nutritional systems were evaluated, depending on selenium content of the diet. Mean GSH-Px level in the total population was 288.5 ± 157.2 IU·g⁻¹ Hb. Reference ranges were estimated and enzymatic activities below 51 IU·g⁻¹ Hb were considered inadequate. GSH-Px activities obtained in females (298.1 ± 155.7 IU·g⁻¹ Hb) were significantly ($P = 0.037$) higher than in males (272.6 ± 157.2 IU·g⁻¹ Hb). When age groups were compared, only males between 6 and 12 months old exhibited significantly lower mean GSH-Px ($P = 0.006$) than females. A high correlation ($r = 0.88$) between serum selenium concentration and blood GSH-Px activity was estimated, and the regression equation was $y = 2.5101x + 42.423$. Selenium content of the diet above 0.1 mg·kg⁻¹ DM seems to supply adequate selenium requirements for dromedaries under intensive husbandry.

dromedary / camel / nutritional myodegeneration / glutathione peroxidase / selenium

Résumé – Détermination des niveaux sanguins de glutathion peroxydase chez le dromadaire.

Les niveaux de glutathion peroxydase (GSH-Px) dans le sang de 709 dromadaires sains (442 femelles et 267 mâles) ont été mesurés dans les Îles Canaries. Tous les animaux ont reçu un élevage intensif et trois systèmes de nutrition ont été évalués en fonction du contenu en sélénium de l'alimentation. L'activité moyenne de GSH-Px dans l'ensemble de la population a été évaluée à $288,5 \pm 157,2$ IU·g⁻¹ Hb. Les intervalles de référence ont été estimés et les activités enzymatiques en dessous de 51 IU·g⁻¹ Hb ont été considérées insuffisantes. Les activités GSH-Px obtenues chez les femelles ($298,1 \pm 155,7$ IU·g⁻¹ Hb) étaient significativement ($P = 0,037$) plus élevées que chez les mâles ($272,6 \pm 157,2$ IU·g⁻¹ Hb). Quand les groupes d'âge ont été comparés, seuls les mâles âgés de 6 à 12 mois montrèrent une valeur moyenne de GSH-Px significativement ($P = 0,006$) plus basse que les

* Correspondence and reprints

Tel.: (34) 928454305; fax: (34) 928454351; e-mail: corbera@vet.ulpgc.es

femelles. Une forte corrélation ($r = 0,88$) entre la concentration de sélénium dans le sérum et l'activité GSH-Px dans le sang a été mise en évidence et l'équation de régression calculée fut la suivante : $y = 2,5101x + 42,423$. Un contenu en sélénium dans l'alimentation, supérieure à $0,1 \text{ mg}\cdot\text{kg}^{-1} \text{ DM}$, paraît être suffisant pour apporter les besoins en sélénium chez le dromadaire en élevage intensif.

dromadaire / chameau / myodégénération nutritionnelle / glutathion peroxydase / sélénium

1. INTRODUCTION

Nutritional myodegeneration (NMD), also called white muscle disease, is a metabolic disease caused by a nutritional deficiency of selenium and/or vitamin E. The disease occurs world-wide and it has been reported in many species, particularly ruminants, including the camel [12].

NMD has not been reported in camelids breeding in their natural habitat. However, the disease can appear in intensively managed animals, like zoological collections or zoos, where the minimal selenium/vitamin E requirements could not be supplied by the diet [13].

Blood glutathione peroxidase (GSH-Px) activity has been demonstrated as a useful indicator of selenium status in several species. In the dromedary camel, a high correlation between GSH-Px activity and blood selenium has previously been reported [6, 9]. However, the number of animals investigated in these studies (20 and 53, respectively), considered sufficient to determine this correlation, seems to be inadequate for a population study of GSH-Px activity in the dromedaries, particularly due to large standard deviation described in many species [1, 16, 17].

In contrast, serum selenium and GSH-Px activity are not correlated in dromedaries. The use of blood selenium or serum selenium to determine selenium status is unclear in the literature available.

Thus the purpose of our study was to determine the normal reference ranges of the blood GSH-Px activity in dromedaries, according to sex, age, reproductive state and diet of animals, as well as the correlation

between serum selenium and GSH-Px activity.

2. MATERIALS AND METHODS

2.1. Animals

A total of 709 dromedary camels were included in this study, 442 of which were females (59 pregnant females, 64 lactating females, and 319 non-pregnant and non-lactating females) and 267 males (54 intact and 213 castrated males).

A clinical and haematological examination was performed in all animals and those animals that showed signs of disease were excluded. All animals belonged to intensive husbandry farms located in the Canary Islands where camels live (Lanzarote, Fuerteventura, Gran Canaria and Tenerife, Spain).

2.2. Nutrition

With regard to nutritional management observed in the visited farms, diet was classified into three groups, as presented in Table I.

Selenium content of concentrate and forage, respectively, 1.37 and $0.84 \text{ mg}\cdot\text{kg}^{-1} \text{ DM}$ in group A, 0.62 and $0.49 \text{ mg}\cdot\text{kg}^{-1} \text{ DM}$ in group B (plus 40 mg of selenium per kg of mineral block), and 0.17 and $0.09 \text{ mg}\cdot\text{kg}^{-1} \text{ DM}$ in group C.

2.3. Laboratory analysis

After clinical examination, all animals were evaluated haematologically in terms

Table I. Diet used in the studied farms classified by nutritional group.

Nutritional group	Concentrate ^a	Forage ^a	Grazing	Mineral supplementation	Animals (females/males)
A	Corn, barley, oats, dehydrated alfalfa	Silage and wheat straw	+	–	344 (233/111)
B	Corn, barley, oats, dehydrated alfalfa and soya flour or pulp of beet	Corn and wheat straw	–	+	305 (185/120)
C	Corn, barley	Wheat straw	–	–	60 (24/36)

^aIngested daily per animal: 4 kg approximately.

of packed cell volume (PCV), red blood cell (RBC) and white blood cell (WBC) count, a leukogram, by using a cell counter (Sysmex F-800, Boehringer Mannheim, Germany). Haemoglobin concentration was also measured using a spectrophotometer (Hitachi 4020, Boehringer Ingelheim, Germany) with the cyanmethemoglobin method following the manufacturer instructions. Animals that presented an RBC below $7.6 \times 10^9 \text{ mL}^{-1}$, a WBC above or below $2.9\text{--}9.7 \times 10^6 \text{ mL}^{-1}$, neutrophils count above or below 33–77%, lymphocytes count above or below 21–62% and PCV below 24% (normal ranges taken from Higgins and Koch [10]) were excluded ($n = 43$).

Blood samples were collected by jugular venipuncture in lithium heparin tubes. The glutathione peroxidase activity in whole blood was assessed by a spectrophotometer Hitachi 4020 (Boehringer Ingelheim) using the Ransel test (Randox Laboratory, Ardmore, United Kingdom) based on the Plagia and Valentine [15] method.

Serum selenium was determined in a statistically sufficient number of animals ($n = 42$) using a spectrophotometer uv/vis Lambda 1 (Perkin Elmer, USA). Selenium content of concentrate and forage was eval-

uated in a model farm of each nutritional group, using the same spectrophotometer.

2.4. Determination of reference range

In accordance with the conventional procedures for assessing normality provided that the results did not have a Gaussian distribution, we chose the use of percentiles in order to determine the reference ranges, as described by Farver [7]. The 3rd percentile is considered to be a good indicator; like the rest, the 97th percentile is estimated to be the value of the analysis corresponding to the $(n + 1) \times 0.97$ th observation in an ascending array of the analysed values for a sample of n normal animals [7].

2.5. Statistical analysis

Statistical analysis was performed using the SPSS 9.0 statistical software for Windows. The Scheffe test was used to calculate ANOVA, and the Pearson coefficient was used to calculate correlation. Statistical significance was considered when $P < 0.05$. However, P values are always given in the text.

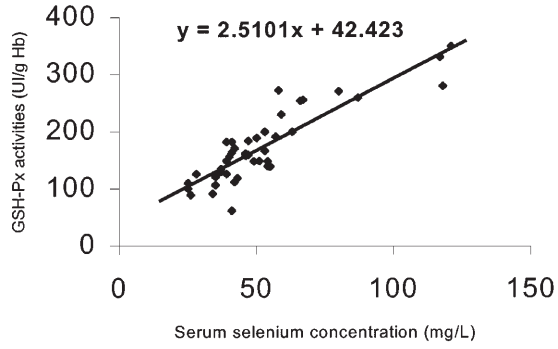


Figure 1. Regression between blood GSH-Px activity and serum selenium concentration.

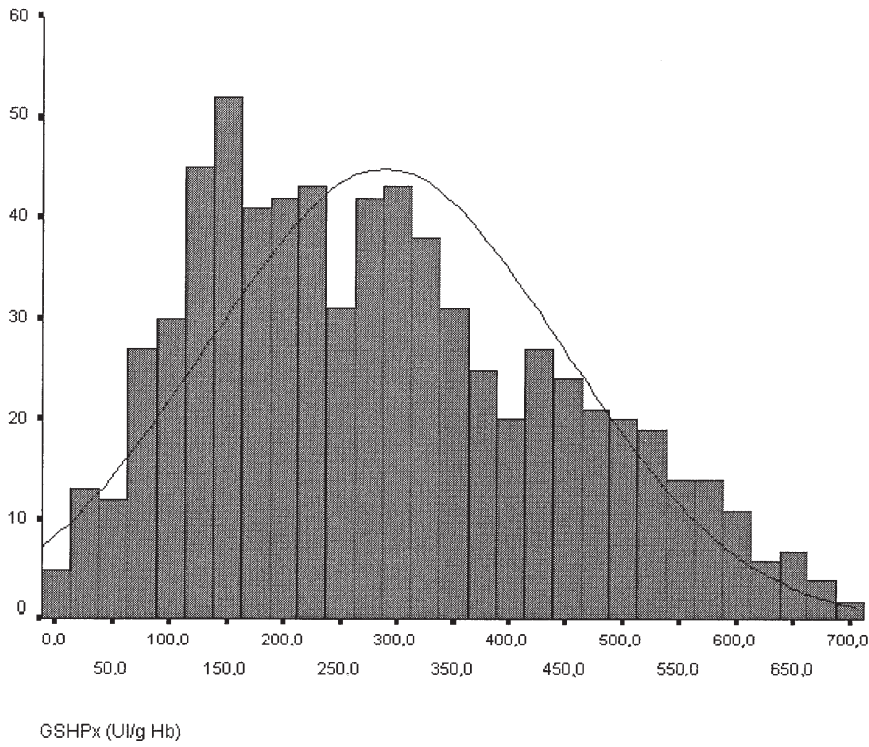


Figure 2. Histogram of GSH-Px activities (UI-g⁻¹ Hb) for the total population.

Table II. GSH-Px activities according to age group and sex.

Age group	Males mean \pm SD (UI·g ⁻¹ Hb)	<i>N</i>	Females mean \pm SD (UI·g ⁻¹ Hb)	<i>N</i>	Total mean \pm SD (UI·g ⁻¹ Hb)
1 week–6 months	202.2 \pm 167.9	12	303.5 \pm 172.3	10	248.3 \pm 173.7
6–12 months	226.6 \pm 115.5 ^a	23	330.9 \pm 130.6 ^a	23	278.8 \pm 132.8
1–2 years	270.6 \pm 154.0	14	221.6 \pm 144.7	18	243.0 \pm 148.5
0–2 years	233.2 \pm 140.3	49	286.9 \pm 149.8	51	260.6 \pm 147.0
2–4 years	273.4 \pm 136.4	21	317.1 \pm 151.4	42	302.6 \pm 146.9
4–10 years	272.3 \pm 163.1	134	288.0 \pm 159.2	221	282.1 \pm 160.6
10–15 years	308.2 \pm 157.2	59	318.8 \pm 156.9	106	315.0 \pm 156.6
>15 years	235.2 \pm 44.3	4	288.4 \pm 183.1	22	280.2 \pm 169.6
Total	272.6 \pm 155.7 ^b	267	298.1 \pm 158.1 ^b	442	288.5 \pm 157.5

^a Statistical differences between sexes ($P = 0.006$).

^b Statistical differences between sexes ($P = 0.037$).

3. RESULTS

3.1. Selenium

The relationship between serum selenium concentration and whole blood GSH-Px activity showed a high correlation ($r = 0.88$, $P < 0.001$) and the regression equation was $y = 2.5101x + 42.423$ (Fig. 1).

3.2. GSH-Px

The results showed a mean GSH-Px activity for the total population of 288.5 ± 157.2 IU·g⁻¹ Hb. The histogram of the GSH-Px activities obtained is showed in Figure 2. Values above the 3rd percentile (51 IU·g⁻¹ Hb) were considered normal and values below 51 IU·g⁻¹ Hb were considered inadequate.

In the total population (Tab. II), statistical differences ($P = 0.037$) were observed in the mean GSH-Px activity between males (272.6 ± 155.7 IU·g⁻¹ Hb) and females (298.1 ± 155.7 IU·g⁻¹ Hb). However, when age groups were compared, no differences

were detected between males and females (Tab. II), except for animals aged 6 to 12 months old.

Furthermore, GSH-Px activity showed statistical differences ($P < 0.001$) between nutritional groups (Tab. III). The highest GSH-Px activities were observed in group A (highest selenium content in the diet), medium values in group B and the lowest values in group C (lowest selenium content in the diet). In relation to nutritional group and sex, statistical differences were observed between males and females only in group C (Tab. III).

In the group ageing between 4 and 10 years old (dromedary reproduction period), blood GSH-Px activity was significantly lower in lactating females (248.6 ± 136.0 IU·g⁻¹ Hb) and in pregnant females (224.1 ± 124.0 IU·g⁻¹ Hb) compared with the non-pregnant and non-lactating females (348.8 ± 171.7 IU·g⁻¹ Hb), ($P = 0.003$ and $P = 0.001$, respectively). Male GSH-Px activity (272.3 ± 163.1) also showed significant difference ($P = 0.005$) compared with the non-pregnant and non-lactating females.

Table III. GSH-Px activities obtained according to nutritional group and sex.

Nutrition group	Males mean \pm SD (UI·g ⁻¹ Hb)	<i>N</i>	Females mean \pm SD (UI·g ⁻¹ Hb)	<i>N</i>	Total mean \pm SD (UI·g ⁻¹ Hb)
A	341.0 \pm 160.0	111	339.2 \pm 155.4	233	339.7 \pm 156.7 ^b
B	244.8 \pm 139.7	120	270.1 \pm 147.8	185	260.2 \pm 145.0 ^b
C	154.1 \pm 74.5 ^a	36	114.3 \pm 41.4 ^a	24	138.2 \pm 65.9 ^b

^a Statistical differences between sexes ($P = 0.021$).

^b Statistical differences between nutrition groups ($P < 0.001$).

4. DISCUSSION

The serum selenium values found in our study and compared with GSH-Px activity resulted in a high correlation ($r = 0.88$). However, the resulting correlation obtained in previous reports using blood selenium concentration was higher ($r = 0.935$) [6, 9]. These findings are in agreement with those reported by Maas et al. [14], whom, by using dairy cattle as the species under study, suggested that blood selenium concentration is a better status indicator than serum selenium.

GSH-Px mean and standard deviation in the dromedary population studied seems to be similar to that reported in other ruminant species, particularly in sheep [1, 17]. However, our mean values were higher than previously reported in the dromedary camels used just for the GSH-Px/blood selenium correlation [6, 9], probably due to the wide standard deviation that is shown in the GSH-Px activity in several species [1, 16, 17].

Considering the 4 to 10 years old age group (dromedary reproduction period) and in relation to sex, higher GSH-Px activities were observed in non-pregnant and non-lactating females than in males. These findings could be attributed to the higher metabolic selenium requirements in the males than in non-pregnant and non-lactating females. However, higher GSH-Px activities were observed in males than in lactat-

ing or pregnant females; these differences could be related to the higher requirements that presented females in these periods [3, 5, 8, 16].

The statistical differences between sexes found only in group C could be due to the lower selenium content of the diet, that does not seem to be sufficient in view of the higher requirements during lactation or pregnancy. These differences have also been described in cattle [2, 4] and sheep [17].

In relation to the age of animals, statistical differences ($P = 0.006$) were only observed between males and females aged 6 to 12 months old, due to the higher growth of males compared to females. This has also been reported in bovine calves [11].

In contrast, GSH-Px activities revealed statistical differences ($P < 0.001$) between nutritional groups. Thus mineral concentrations in pastures grown on semiarid areas contributed to increase the dietary selenium content. Therefore, group A showed the highest GSH-Px activity, group B an intermediate GSH-Px activity, and group C showed the lowest GSH-Px activity found in this study.

In conclusion, the mean GSH-Px activity observed in the dromedary camel population studied was 288.5 ± 157.2 UI·g⁻¹ Hb and values below 51 UI·g⁻¹ Hb were inadequate. When the animals received over 0.1 mg of selenium·kg⁻¹ DM in the diet, the GSH-Px activities in the animals with higher

requirements did not show statistical variations compared to animals with minimal requirements. Finally, by taking GSH-Px activity as the selenium status indicator in the dromedaries, serum selenium values in this study have shown a lower correlation compared with blood selenium values reported previously.

REFERENCES

- [1] Andrés S., Mañé M.C., Sánchez J., Barrera R., Zaragoza C., Jiménez A., Response to barium selenate supplementation in sheep kept at pasture in the Mediterranean Area, *Vet. Res.* 28 (1997) 539-545.
- [2] Campbell D.T., Maas J., Weber D.W., Hedstrom O.R., Norman B.B., Safety and efficacy of two sustained-release intrarecticular selenium supplements and the associated placental and calostriferous transfer of selenium in beef cattle, *Am. J. Vet. Res.* 51 (1990) 813-817.
- [3] Cappa V., Importancia y papel del selenio en la alimentación de los bovinos, *Vet. Prax.* 11 (1996) 15-20.
- [4] Chaiyabutr N., Loypetjra P., Pichaicarnarong A., Survey of selenium status of buffaloes and cattle in northeastern Thailand, *Thai. J. Vet. Med.* 13 (1983) 92-103.
- [5] Edmondson A.J., Norman B.B., Suther D., Survey of state veterinarians and state veterinary diagnostic laboratories for selenium deficiency and toxicosis in animals, *J. Am. Vet. Med. Assoc.* 202 (1993) 865-872.
- [6] El-Magawry S.M., Ibrahim I.A., Dowidar M.F., Yousif M.A., Glutathione peroxidase activity in camel erythrocytes (*Camelus dromedarius*) in relation to blood selenium concentration, *Assiut Vet. Med. J.* 20 (1988) 21-24.
- [7] Farver T.B., Concepts of normality in clinical biochemistry, in: Kaneko J.J. (Ed.), *Clinical biochemistry of domestic animals*, 4th ed., Academic Press, San Diego, 1989, pp. 1-20.
- [8] García-Belenguer S., Gascón M., Purroy A., Aceña C., Distrofia muscular nutricional por deficiencia de selenio y/o vitamina E en rumiantes, *Medicina Veterinaria* 9 (1992) 84-93.
- [9] Hamliri A., Khallaayoune K., Johnson D.W., Kessabi M., The relationship between the concentration of selenium in the blood and the activity of glutathione peroxidase in the erythrocytes of the dromedary camel (*Camelus dromedarius*), *Vet. Res. Commun.* 14 (1990) 27-30.
- [10] Higgins A., Kock R.A., A guide to the clinical examination, Chemical restraint and medication of the camel, in: Higgins A. (Ed.), *The camel in health and disease*, Bailliere Tindall, London, 1986, pp. 149-163.
- [11] Kleczkowski M., Klucinski W., Sitarska E., Winnicka A., Sikora J., Ladysz R., Wojewoda L., Dziekan L., Skowronski M., Glutathione peroxidase activity in calves and their dams originating from the Lomz Landscape Park of the Narew river valley, *Acta Academiae Agriculturae ac Technicae Olstenensis* 32 (1996) 31-36.
- [12] Kock R.A., Chapman M.J., Management, feedings and health of camels kept under intensive husbandry, in: Higgins A. (Ed.), *The camel in health and disease*, Bailliere Tindall, London, 1986, pp. 149-163.
- [13] Kovác G., Michna A., Bartko P., Reichel P., Mudroc P., Seidel H., An outbreak of nutritional muscular dystrophy in camels (*Camelus bactrianus*) in Košice Zoo, *Folia Vet.* 40 (1996) 33-37.
- [14] Maas J., Galey F.D., Peauroi J.R., Case J.T., Reply: blood selenium or serum selenium?, *J. Vet. Diagn. Invest.* 5 (1993) 146-147.
- [15] Paglia D.E., Valentine W.N., Studies on the quantitative and qualitative characterization of erythrocytes glutathione peroxidase, *J. Lab. Clin. Med.* 70 (1967) 158-169.
- [16] Sáez T., Ramos J.J., Marca M.C., Sanz M.C., Fernández A., Verde M.T., Haematological and biochemical changes in the blood of ewes and lambs after selenium and vitamin E injection, *J. Appl. Anim. Res.* 9 (1996) 51-60.
- [17] Zachara B.A., Trafikowska U., Kaptur M., Kimber C., Lejman H., The effect of barium selenate injection on selenium concentration and glutathione peroxidase activity in blood of pregnant ewes fed selenium deficient diet, *Biol. Trace Elem. Res.* 32 (1992) 415-419.