Effect of Mineral Supplementation on the Selenium Concentration and Glutathione Peroxidase activity in Cattle and Camels

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ABSTRACT

Five camels and 5 cows were fed with a similar basal diet for six months. They received oral trace element supplementation for 3 months (day 22-112). The supplementation included zinc, copper, selenium, manganese, iodine and cobalt, and corresponded to twice the daily requirement generally recommended for cows. Plasma selenium concentration was significantly lower in the camels (20 ± 2 ng/ml) as compared the cows (37 ± 2 ng/ml). The mineral supplementation induced an important increase in the plasma selenium concentration in camels which reached 220 ± 35 ng/ml. However, for the cows, the increase was very low and did not overreach 65 ± 8 ng/ml. The red blood cell glutathione peroxidase activity was similar in the camel and the cow and varied between 4000 and 6000 IU/100 g hemoglobin. In both species, this activity increased with mineral supplementation and stayed very high even when mineral supplementation was stopped. The results suggested that selenium metabolism in camels is different from that in the cows.

Key words: Selenium, Glutathione Peroxidase, Nutrition, Cow, Camel.

INTRODUCTION

It is well known that camels have some physiological peculiarities for trace-element metabolism due to their adaptation to arid conditions and poor feeding resources (Faye and Bengoumi, 1994). Previous studies (Faye *et al.*, 1992; Bengoumi *et al.*, 1995) concerning the main trace-elements status on camels have shown a lower regulation level of plasma copper and zinc concentration compared to other domestic ruminants. For selenium, there is little evidence to date of clinical deficiencies. Few results on plasma or blood values in field conditions in different areas from Morocco (Hamliri *et al.*, 1990a and b), China (Liu *et al.*, 1994) or in some zoological parks (Finlayson *et al.*, 1971) are available in the literature. Selenium deficiency has been observed in young camels with temperate feeding conditions (Faye *et al.*, 1995). In the Sultanate of Oman, a recent field survey concerning camels and cattle have shown that the plasma selenium concentration was higher in camels affected by clinical symptoms of a non-diagnosed sway disease (unpublished data). It was concluded that camels had a particular selenium regulation. However, no comparative experimental studies between camels and cows are available in the literature. So, the present study was undertaken to compare the effect of mineral supplementation on the plasma selenium concentration and the erythrocyte glutathione peroxidase activity in cattle and camel, under identical feeding and management conditions.

MATERIALS AND METHODS

The study was carried out at the experimental farm station of the I.A.V. Hassan II (Gharb farm), 80 km north of Rabat, Morocco.

Animals

The study included five (5 to 7 years-old) camels originating from South Morocco, and five 4 to 6 years-old multifarious black-pied Friesian cows born at the experimental farm station. All the animals where non-lactating and non-pregnant females. The approximate mean weights were 400 kg for the camels, and 600 kg for the cows. The animals were treated for external and internal

parasites using ivermectine (*Ivomec N.D.*) and were clinically healthy during the whole experiment.

Experimental procedure

During the whole trial, the animals were kept in individual pens. The camels received a basal diet including 3 kg of wheat straw, 1.5 kg of rice meal and 1.5 kg of molasses. The composition of the basal diet for the cows was similar but the quantities were doubled to take into account the weight of the animals and the normal feed intake. Animals were fed individually, there were no refusals at any time. The animals were watered *ad libitum*, the water contained non-detectable selenium concentration. This diet was considered to satisfy the maintenance requirements of the animals for the two species. After an adaptation period of 2 weeks to balance the mineral status of the animals, the experimental period (195 days) consisted of three phases: 1) control period (days 1-21). During this stage, the animals received the basal diet without any mineral supplementation; 2) supplementation period (days 22-112*)* with mineral additives. The mineral mixture including 43.6 mg of sodium selenite was prepared. This additive corresponded to a daily supply for each animal of 2 mg of selenium. These quantities were estimated to be double the requirements usually proposed for cows (McDowell, 1992) and normal for camels (Faye *et al.*, 1992). The mineral supplementation was mixed with the molasses, then with the rice meal and distributed individually each morning. The whole consumption of the mineral supplementation was verified. 3) Post-supplementation period (days 113-195). During this last period of the experiment, animals received the basal diet without supplementation.

Sampling

Blood samples were collected from the jugular vein into 10-ml heparinized vacutainers. The blood was centrifuged immediately and the plasma was harvested. Red blood cells were rinsed three times with an isotonic solution of NaCl (0.9%) and centrifuged for 10 min. at 3000 g . The supernatant was discarded and red blood cells were frozen. The samples were identified and kept frozen until analysis. The blood sampling was carried out in the morning before feed distribution. During the first stage of the experiment, blood sampling

was performed on days 1, 7 and 17. Twelve samplings were carried out in the course of the second stage, almost once a week: day 24, 31, 38, 48, 55, 62, 69, 80, 87, 94, 101 and 108. In the last stage, blood sampling was performed once a week during the first month (day 115, 122, 129 and 136), then every two weeks up to the end of the trial (day 150, 164, 178 and 195).

In each component of the basal diet, selenium was measured using fluorometry. In the plasma, the method of Bellanger *et al.*, 1992, using spectrofluorometry, was used to measure selenium concentration. The analytical quality of selenium measurements was assured by using seronorm trace elements (Ref. 5337, NYCOMED AS., Pharma diagnostics, Oslo, Norway). Fifteen replicate assays of this reference material were used and the precision was below 5%. Before analysis, red blood cells were thawed to prepare cellular suspension. Lysat suspension was obtained by mixing 0.1 ml of red blood cells with 0.3 of isotonic solution of NaCl. Haemoglobin was measured in the suspension of colorimeter (Boehringer Mannheim kit, ref. 124729). Enzyme activity was measured in the lysat that was taken after mixing 0.1 ml of cellular suspension with 0.9 ml of distilled water. Glutathione peroxidase (EC. 1.11-1.9) activity was measured according to Paglia and Valentine (1967)(Randox kit, ref. RS 505).

Hemoglobin concentration was performed by colorimeter (Boehringer Mannheim, ref. 124 729). Red blood cell enzyme activity was measured according to the specific reaction of each enzyme at 37 °C using the following Boehringer Mannheim kits. The GSH-Px activity was expressed in International Units per 100 grams of haemoglobin (IU/100 gHb) where one International Unit is equivalent to 1 μmole of NADPH oxidized per minute and per 100 grams of hemoglobin.

Analysis of variance was carried out using the SYSTAT software. For each variable to be explained (selenium and GSH-Px), the effects of the species (2 levels: cow or camel), of the mineral supplementation period (3 levels: before, during and after) and of the sampling day (23 levels for blood and plasma) were tested. Previously, normality of distribution was tested by the Skewness and Kurtosis test (test W). Correlations between two variables were studied using the Spearman method. The multiple comparison test of the linear regression model was used to compare camels to cows.

RESULTS

Selenium content in the diet

The selenium concentration was 0.02 ppm in molasses, 0.04 ppm in wheat straw and 0.06 ppm in rice meal. Thus, the selenium intake was 0.24 mg per day for camels and 0.48 mg per day for cows during the period I and III. The mineral mixture bringing 2 mg of selenium per day, the total daily selenium intake was 0.41 mg/kg DM for camels and 0.23 for cows. The total quantity of selenium in the diet during the period II was 2.24 and 2.48 mg/day for camels and cows respectivelly.

Comparative plasma selenium

During the 3 periods of the trial, the mean plasma selenium concentration in camels was 20.8 ± 3.2 ng/ml (P1), 129.6 ± 30.2 (P2) and 82.7 ± 18.7 (P3). For cows, the values were 33.2 ± 3.9, 51.1 ± 5.6 and 36.9 ± 4.9 ng/ml for P1, P2, and P3 respectively. In the camel, the maximum mean value was observed the day before the end of supplementation period: 200.4 ng/ml (fig. 1). In cows, the maximum mean value (64.5 ng/ml) was observed at day 80. So, the oral selenium supplementation has increased plasma selenium concentration 10 fold in camel's vs 2 fold only for cow's, although plasma selenium concentration was significantly lower in camels than cows before supplementation. After the end of supplementation, the decrease of plasma selenium concentration in camels was very rapid. After one month without selenium supply, plasma selenium concentration in camels had again a lower value than in cows.

Erythrocyte GSH-Px activity

The red blood cell (RBC) GSH-Px activity in camels varied from 5163 ± 1039 IU/100 g Hb (P1), 7926 ± 1038 (P2) and 13172 ± 1720 (P3). For cows, the values were 4561 ± 1839, 7128 ± 2271 and 11655 ± 3359 IU/100 g Hb for P1, P2, and P3 respectively. Selenium supplementation induced a significant increase in RBC GSH-Px activity both in camels and cows (fig. 2). There is no significant difference between the two species during the periods I and II.
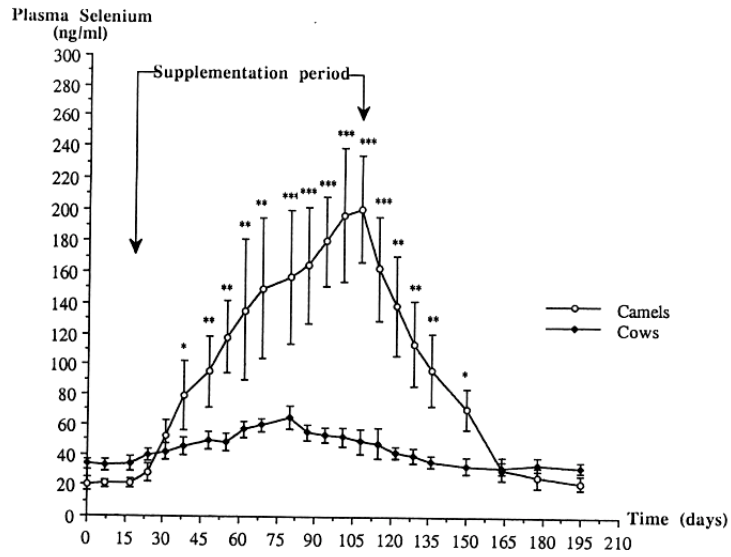
Fig. 1: Change in the plasma selenium concentrations in camels and cows during the 3 periods of the trial. Significant difference between camels and cows. $*p < 0.05$; $**p < 0.01$; $***p < 0.001$. (P1 = d1-21; P2 = d22-112; P3 = d113-195)
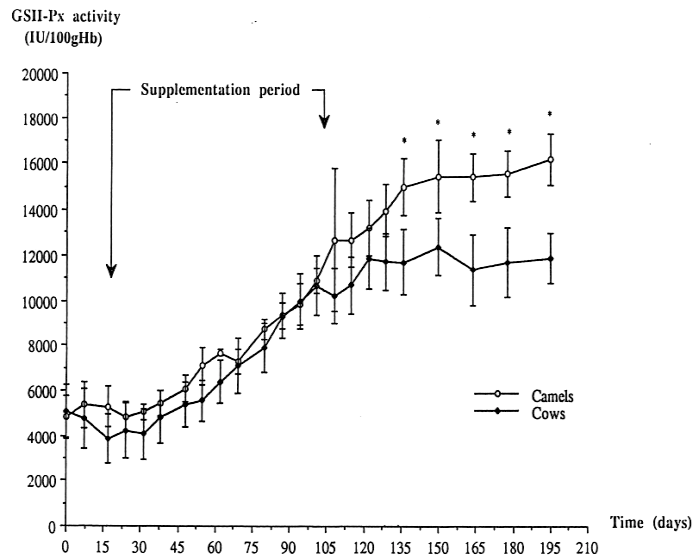

Fig. 2: Change in GSH-Px activity in camels and cows during the 3 periods of the trial. Significant difference between camels and cows $*p < 0.05$. (P1 = d1-21; P2 = d22-112; P3 = d113-195)

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However, a different change was observed between cows and camels before the end of the supplementation period. The RBC GSH-Px activity was significantly higher in camels than in cows during the last 75 days of the experiment. In fact, the RBC GSH-Px activity was still increasing in camels even when the selenium supplementation was stopped. In contrast, in cows this activity was kept at the same level during the three months following the selenium supplementation.

Correlation GSH-Px/plasma selenium

The calculation of the correlation between plasma selenium concentration and GSH-Px in erythrocyte was efficient in the first two periods of the trial only, the enzyme activity increasing continuously in spite of the lack of the selenium supplementation during period 3. The correlation coefficient was higher in camels (0.94) than in cows (0.68). However, in all cases the p value was below 0.001.

DISCUSSION

The selenium intake

The selenium content of the basal diet was not sufficient to satisfy the normal requirements of the animals, according to the recommendations of Gueguen *et al.,* (1988), and McDowell (1992), concerning cows. No data were available concerning the exact selenium requirements in camels (Faye and Bengoumi, 1994). The daily intake during the supplementation period corresponded to the normal requirements in non-lactating cows (between 0.1 and 0.2 mg/kg DM).

So, with a daily intake slightly above 2 mg of selenium, the supplemented diet could be considered largely sufficient to satisfy requirements and below the toxicity level (0.5 mg/kg DM in any case in cows).

Plasma selenium concentration

With a similar selenium-deficient basal diet, the normal plasma selenium concentration was lower in camels than in cows. Similar

values were also observed in lambs (Molnar *et al.*, 1996). Concerning camels, referential data are scarce. Preferentially, selenium concentration was evaluated in the whole blood (Diplock, 1987) even in camels (Hamliri *et al.*, 1990a).

Hamliri *et al.* (1990b) have observed similar values in deficient camel and sheep. In China, Liu *et al.* (1994) have reported in whole blood of bactrian camels similar values as the previous authors, but strongly lower values than that reported by (Ma, 1995).

Camels from Sultanate of Oman, affected by a non-diagnosed sway disease, the mean value of plasma selenium concentration was 281 ng/ml (unpublished results) and some very sick camels showed concentrations up to 400 ng/ml. In such cases, a selenium toxicity could be suspected. If the plasma selenium concentration in non-supplemented cows seemed to be normal in our experiment, the camel values could be associated to sub deficient status.

The major difference observed between the two species was quite surprising. In fact, even if camels received, during supplementation period, a double level of selenium reported to dry matter intake, the total daily selenium intake was similar to that in cows. The little difference in the selenium intake per kg of body weight was not sufficient to explain the high increase of plasma selenium concentration observed in camels. As the magnitude of the decrease of the post supplementation plasma selenium concentration was similar to the previous increase, it assumes that plasma selenium concentration in camels is an extremely sensitive indicator of the selenium intake.

It could be concluded that camels have a peculiar selenium metabolism. But, without reliable bibliographical data, we cannot confirm if the dromedary camel is more sensitive to selenium deficiency than cows and other ruminants. Consequently, further experiments in camels with different levels of selenium intake are necessary to propose an interpretation scheme from the plasma results.

GSH-Px activity

The seleno-enzyme glutathione peroxidase was the first selenoprotein among 13 to be described and remains the most comprehensively defined in terms of structure and function (Daniels, 1996). GSH-Px, as one of the primary antioxidant enzymes, is an

important component in the protection against free radical damage to cells and thus is crucial to cell survival. Usually GSH-Px activity was considered as an indicator of selenium status in a variety of species (Ganther *et al.* 1976; Lamand, 1987).

A linear relationship between erythrocyte GSH-Px and whole blood Se concentration was described in different species (Rea *et al.*, 1979), notably in the camel (Hamliri *et al.*, 1990a). In our study, this relationship seemed to be higher in camels than in cows when selenium supply occurred. Erythrocyte GSH-Px activity on camels has been measured only by Hamliri *et al.*, (1990a) who found values between 1500 to 3600 IU/100 g Hb.

In our trial, GSH-PX activity was similar in cows and in camels during the first two periods in spite of a high difference in the plasma selenium concentration. According to Avissar *et al.* (1989), 12% of the selenium in plasma is accounted for by GSH-Px protein. This value was probably lower in camels during supplementation period seeing that selenium concentration was strongly higher than in cows. So, it seems that the camel was able to store selenium in the plasma in higher quantity than other ruminants.

After the end of supplementation period, the camel was able to increase GSH-Px activity probably because of the high quantity of selenium stored in the plasma. The selenium induces the biosynthesis of the glutathione peroxidase, seleno-dependent enzyme. When the selenium supply was stopped, the plasma selenium level decreased but the GSH-Px activity was maintained in cattle, either by the biosynthesis induction due to stored selenium or by the long plasmatic half-life of this enzyme.

In camels, the plasmatic GSH-Px activity continued to increase, even if selenium supply was stopped and even if the plasma selenium concentration decreased. This increase could be explained by maintenance of the biosynthesis induction in the camel erythrocytes from the selenium probably stored in the erythrocytes, and a longer plasmatic half-life of GSH-Px compare to cattle. In fact, the erythrocyte GSH-Px activity being closely related to the half-life to the red blood cells, the enzymatic activity is higher in camels than in cows when selenium was depleted because of the longer survival of camel erythrocyte (Yagil, 1974).

It could be suggested that this high capacity of plasma storage in camel's compared to cow's is a facet of the camel's adaptability to hard desert feeding conditions. Similar results have been found

concerning copper and zinc: the trace-element post supplementation depletion was always slower in camels than in cows (Bengoumi *et al.*, 1977a; Faye and Bengoumi, 1997). This ability allows the camel to assume the maintenance of enzymatic activity (GSH-Px for Se, ceruloplasmin for Cu, superoxide dismutase for Zn) when the trace elements in the diet become deficient. We did not measure the quantity of selenium in the urine which is the main method of secretion, as for other anions (Thompson and Robinson, 1986).

It is well known that the camel kidney has a high ability to recycle water, urea and electrolytes (Yagil, 1985; Bengoumi *et al.*, 1993). Elsewhere, the camel kidney has a higher enzymatic activity than the liver, contrary to most other mammals (Bengoumi *et al.*, 1997b). Contrary to other ruminants, the camel kidney has also a higher selenium concentration than the liver (Ma, 1995). It can be suggested that the camel regulates the excretion of selenium more efficiently than the cow and promotes a high plasma storage, useful for deficient period. It is a good example of the thrifty physiological, characteristic of camel behavior.

CONCLUSION

The present study has managed to determine the plasma selenium concentration and the erythrocyte GSH-Px activity in camels and their variations according to the selenium intake. However, if this experiment demonstrated that selenium metabolism is different in camels than in cows, more questions are raised. If we can assert that the camel has a better ability to store selenium in plasma than the cow, the limit of tolerance to selenium intake in the former is not known, neither the real distribution of selenium and GSH-Px activity in the different tissues, nor the importance of selenium excretion through urine and feces.

The higher increase of the plasma selenium concentration in camels during selenium supplementation shows that the camel is more adapted to selenium deficiency but probably more sensitive to selenium intoxication. Obviously, further investigations using different levels of selenium intake for a long period might be undertaken.

ACKNOWLEDGMENTS

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