Selenium status and supplementation in dromedary camels

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Abstract

The Arabian camel (Camelus dromedarius) is well suited to the desert environment due to its remarkable physiological and metabolic adaptation. Nutrients requirements of the camel are not well defined, particularly that of trace elements. Selenium supplementation is often recommended to treat conditions attributed to Se deficiency such as cardiomyopathy. This paper reports the results of four experiments carried out using pregnant, non-pregnant, young and adult female camels. The commercial source of Se (i.e. sodium selenite) was used to provide supplementary levels between 0 to 16 mg of Se/day. The concentration of Se in serum and the activity of erythrocytes glutathione peroxidase (GSH-Px) were measured.

The Se concentration in serum of camels increased linearly with increased Se supplementation levels from 0 to 4 mg/day, then plateau when levels further increased to a maximum of 16 mg/day. The average Se concentration (expressed as ng/ml) ranged between 100 and 500. Similar trend was observed for the GSH-Px activity. The results suggest a maximum tolerable dose of 8 mg/day and the recommended dose between 2 and 4 mg/day.

Keywords: Camel, Selenium, GSH-Px, metabolism

1. Introduction

Nutritional deficiencies of selenium (Se) and vitamin E, in several species result in muscular dystrophy (MD). Many cases have been reported in camels in their natural habitat (El Khouly et al., 2001; Seboussi et al., 2004). Camel selenium supplementation is often necessary and different methods are used: injection, drenching and trace minerals salt mixture. But, selenium requirements in this species are extrapolated from those of other ruminants. The biological role of selenium in the dromedary is obviously identical to that of the other ruminants, but the metabolism seems differ (Bengoumi et al., 1998). Due to lack of references, the present synthesis aimed to give more details on the serum selenium values based on four experiments focused on selenium intake (Seboussi et al., 2008), excretion (Seboussi et al., 2009a) and tolerance (Seboussi et al., 2009b and 2010) achieved for a better understanding of the selenium metabolism in this species.

2. Material and Methods

2.1. Experiments

Four experiments were performed:

Experiment 1: In the first experiment, 12 non-pregnant and non-lactating female camels shared into three groups received, after a two-week adaptation period, an oral Se supplementation (0, 2 and 4 mg, respectively) under sodium selenite form for three months. Feed intakes were assessed daily, blood samples and body weight were taken weekly, faeces and urine samples were collected every two weeks up to one month after the end of the supplementation period (Seboussi et al., 2008)

Experiment 2: In the second experiment, 12 pregnant females, shared into two groups, received 0 and 2 mg Se respectively under sodium selenite form at the end of their gestation (last three months) and at the beginning of their lactation up to one month. The supplementation was stopped after one month of lactation. As for the previous experiment, faeces and urine samples were collected every two weeks (Seboussi et al., 2009a).

Experiment 3: In the third experiment, eight young female camels shared into four groups of two 2-y old ones received a basal diet enriched with 0, 2, 4 and 8 mg selenium under sodium selenite form for 64 days. At the end of the experiment, one camel in each group was slaughtered at the abattoir and the following organs were sampled: brain (cerebrum and cerebellum), lung, heart (right and left ventricles), liver, spleen, kidney, pancreas, intercostals muscle, psoas muscle, back of the knee muscle, anterior limb bones, posterior limb bones, hair (Seboussi et al., 2009b).

Experiment 4: In the fourth experiment, the quantity of supplied selenium throughout the trial (90 days) was for each group of four camels, 2-yr old, respectively 8 mg (i.e. 17.4 mg sodium selenite), 12 mg (i.e. 26.2 mg sodium selenite) and 16 mg (i.e. 34.9 mg sodium selenite) daily. Selenium supplementation was stopped immediately at the time of apparition of chronic selenosis and liver protective was given to prevent death. Camels returned to normal good health gradually (Seboussi et al., 2010).

All animals in all experiments were subjected to pre-treatment periods to adapt them to their new experimental environments. All, the animals were treated for external and internal parasites using ivermectin (Ivomec N.D.) and were in good health at the beginning of the experiments. During all the length of experiments camels were fed same diet composed of Rhodes grass hay (*Chloris gayana*) (6 kg for adults and 3 kg for young) and of concentrates (2 kg for adults and young) with known selenium content. Camels were supplemented with selenium individually in the morning, enrobed in date. Sodium selenite was the only form of selenium used in these experiments and fed at different level starting from 0, 2, 4, 8, 12, 16 mg/day. No vitamin E was added unless the amount found in the basal ration

Blood was collected in the morning before food and selenium distribution from the jugular vein into 5ml heparinised tubes (H) and 10ml non heparinised tubes (NH). Blood samples from calf-camel were taken immediately after delivery. Serum, plasma and erythrocytes were stored in -80 °C until selenium analysis (serum), vitamin E analysis (plasma) and the erythrocytes glutathione peroxidase activity GSH-PX (erythrocytes). Urine and faeces were also collected regularly from camels, urine samples were stored at -20°C for selenium analysis and faeces were dried and kept in dark and cool place until analysis. Feed and water samples were taken at the beginning, middle and at the end of each trial. Colostrum and milk sampling were also performed and stored at -20 °C until selenium analysis.

To study selenium distribution in organs and hair on slaughtered animals (experiment 3), tissues and hair samples were taken using a stainless steel knife and stored at -80 °C until selenium analysis. Parts were fixed in 10 % formalin saline for histopathology findings.

2.2. Lab analysis
Selenium analysis in serum samples was carried out using inductively coupled argon plasma – atomic emission spectrometer (ICP-AES), Varian vista MPX-CCD simultaneous, using 11 points of standard curve of Accu Trace[TM]. Reference Standard solutions used are

from Accustandard® – USA. Quality Control Standard #1 and Laboratory Performance Check standard have been used. Prior serum selenium analysis, samples digestion was required to freeing the element. This phase was performed by the microwave digestion system milestone MLS-1200 MEGA Italy. The enzymatic activity of glutathione peroxidase (GSH-Px) was measured in erythrocytes according to the method of Paglia and Valentine, 1967 using Randox commercial kit (Ransel ND ref RS 505) by Beckman Coulter DU 800 Spectrophotometer. The GSH-Px activity was expressed in international unit per gram of haemoglobin (IU/G Hb), where 1 international unit is equivalent to 1 µmol of NADPH oxidized per minute per gram of haemoglobin.

2.3. Statistical analysis

Descriptive analysis (mean and standard deviation) were used to give raw results. Variance analysis on repeated measures was carried out using the R software. For each variable to be explained (Se and GSH-Px), we tested the effect of the supplementation level and of the day of sampling by LSD test. Interactions between elements were tested by Pearson correlation.

3. Results

The results of each experiment were already published separately (Seboussi et al., 2008, 2009a, 2009b, 2010) and could be accessible for the readers. In the present communication, only a synthesis of the results are shown by taking in account the whole field of variation in Se supplementation from 0 to 16 mg daily. For the whole experiments, a total of 528 blood samples were achieved. However, for the synthesis, the samples taken after the end of Se supplementation in experiment 1, 2 and 4 were discarded to avoid the depletion period of the experiments. Elsewhere, the camels belonging to treated groups but sampled

at the beginning of the supplementation was considered as received 0 mg of selenium. Finally, 443 samples were analysed including 194 samples for 0 mg Se supplementation in the diet, 117 samples for 2 mg, 66 samples for 4 mg, 37 samples for 8 mg, 21 samples for 12 mg and 8 samples for 16 mg. In the present paper, only results on the concentration in serum (Se) or in erythrocytes (GSH-Px) are presented.

The average serum selenium concentration in non supplemented camels was 100 ng/mL. Values increased significantly ($P<0.05$) in supplemented camels to reach an average of 400 ng/ml with a supplementation of 4 mg Se/day. With prolonged supplementation, concentrations form on average a plateau of 500 ng/ml with a maximum value of 900 ng/mL (fig.1). The first clinical disturbs appeared with a selenium supplementation of 8 mg/day, i.e. on animals having a Se concentration in serum at around 500 ng/ml on average (Seboussi et al, 2009b).
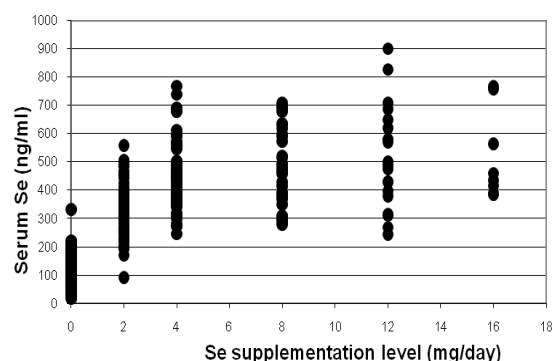

Figure1. Change in serum Se according to the Se supplementation level in adult camels

The erythrocytes glutathione peroxidase activity (GSH-Px) followed the same trend than serum selenium, with an average of 20 IU/g Hb in non supplemented camels. In intoxicated camels the GSH-Px activity reachesd100 IU/g Hb with a maximum of 170 IU/g Hb (fig. 2). This activity decreased significantly after delivery and during

lactation period (fig. 3). GSH-Px activity was strongly correlated to serum selenium concentration (r^2 = 0.49, Fig. 4).
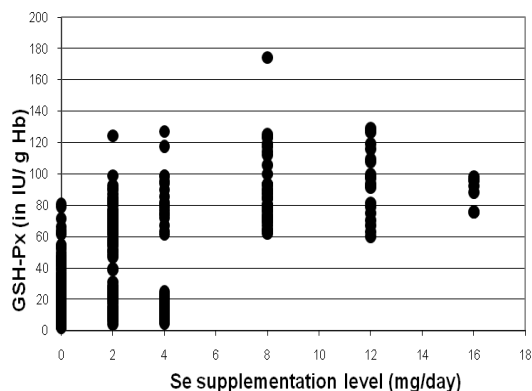

Figure2. Change in Gsh-Px activity according to the Se supplementation level in adult
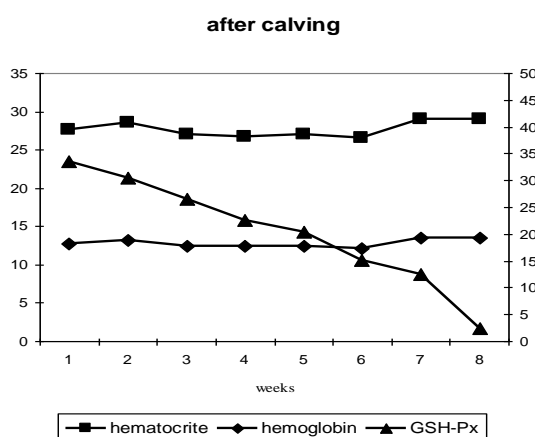

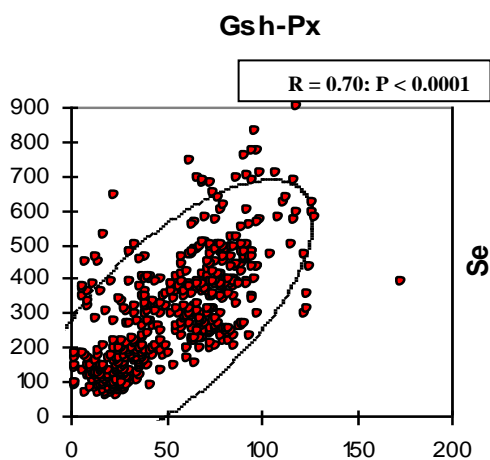Figure 3. Change in GSH-Px activity, hematocrit and hemoglobin in female after camel calving

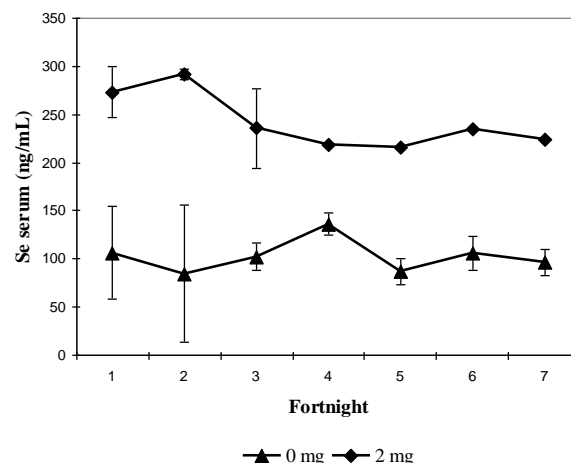Figure 4. Correlation between serum Se and GSH-Px in camel

Figure 5. Change in serum Se in camel calves after calving according to the Se supplementation level of the dam

Selenium status was similar to their mother at birth. The serum selenium concentration values as well as the GSH-Px activity are 3 fold higher in calf camel issued from supplemented camel than those issued from non supplemented camel (fig. 5). The difference was maintained after the end of Se supplementation of the dam.

4. Discussion

The mean serum selenium concentration of 100 ng/ml is considered as sufficient to maintain metabolic functions in large animals (Maas et al., 1990). However, few references were available and were variable in camel serum/plasma. Hamliri et al., 1990 from Morocco reported values varied between 109.1 and 117.8 ng/ml of whole blood in camels of different ages and sex. Similar references were recorded in China by Liu et al., 1994 with concentrations varying from 97 to 112 ng/ml. In Sudan, Abdel Rahim 2005 reported values in whole blood varying between 25 and 53 ng/ml. Without specifying if it was whole blood or serum, Ma, 1995 reported higher values: 274 to 288 ng/ml. The variability was thus high and the range between 12 and 200 ng/ml with an average of 100 ng/ml. However, in most of the reported

values, the selenium status of the diet was unknown even if Se supplementation was not distributed to the animals. In some countries, the basal diet could be very low in natural selenium, or, at reverse, in high quantity under form with a high absorption rate. Also, the analytical procedures, as mentioned above, were not described in all the cases and could change between authors.

Few papers relate the impact of selenium complementation on the mineral status of camel and, generally, the doses applied for selenium deficiency control were those recommended for cattle. To our knowledge, the first trial achieved to assess the effect of selenium supplementation level on the plasma selenium status was reported by Bengoumi et al. (1998). In this experiment, the selenium status of camels was compared with that of cattle with similar weight and receiving daily 2 mg Se *per os* under sodium selenite form for two months. The results showed a sharper increase of plasma selenium occurring in camels (10 times the plasma level before supplementation) compared to cows (twice the starting level). As the magnitude of the decrease of plasma selenium concentration after stopping supplementation was similar to the previous increase, it was supposed that plasma (or serum) selenium concentration in camel was an extremely sensitive indicator of selenium intake. The fast selenium depletion at the end of the supplementation period seemed also to indicate a better efficiency of selenium absorption and excretion in camel compared to cow.

For its transport in blood, selenium is linked to specific protein (selenoprotein) including glutathione peroxidase (GSH-Px). The values of GSH-Px reported in Morocco by Hamliri et al., 1990 (25.8 IU/g Hb) were not far from our results at the beginning of the experiment (19.1 ± 16.8 IU/g Hb), but quite lower than values reported in Spain (Corbera *et al.*, 2003):

298.1 IU/g Hb in female camel. Indeed, even in highly supplemented group, GSH-Px value reached 103.1 IU/g Hb only. In Australia, the value of GSH-Px in camel was evaluated to 85.8±14.75 IU/g Hb (Agar and Susuki, 1982). In the comparative study of Bengoumi et al., (1998), the increase of GSH-Px activity was similar in camels and cows for the supplementation period with a higher correlation in camels (r = 0.94) than in cows (r = 0.68). As for other species, GSH-Px is a good indicator of the Se status of camel. However, after the end of the supplementation, GSH-Px activity continued to increase in camels' blood while it was stable in cows'.

This increase could be explained by the 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 compared to those of cattle. In fact, the erythrocyte GSH-Px activity being closely related to the half-life to the red blood cells, the enzymatic activity was higher in camels than in cows when selenium was depleted because of the longer survival of camel erythrocytes (Yagil et al., 1994).

The linear relationship between erythrocyte GSH-Px and whole blood Se concentration was described in camel by several authors (Hamliri et al., 1990; Abdel-Rahim, 2005; Barri and Al-Sultan, 2007) but with variable correlation coefficients. At our knowledge, only experimental selenosis has been reported (Faye and Seboussi, 2008, Seboussi et al., 2009 b). The first clinical disturbs appeared with a selenium supplementation of 8 mg/day. The first physiological symptoms were an increase of the respiratory rate, pulse rate, and internal temperature up to 40 °C. So, the toxic level could be considered at approximately 8 mg Se/day.

Elsewhere, this toxic level was confirmed by the quantification of selenium excretion in faeces and urine

(Seboussi et al., 2009a, b and 2010) according to the variable levels of Se supplementation in the diet. Se faecal excretion increased slowly up to 4 mg Se in the diet, then highly from 8 mg daily supplementation up to 16 mg. The total faecal excretion varied from 637.9 ng/day in non-supplemented camels up to 4084.4 ng/day in camels receiving 16 mg Se/day in the diet. The total faecal excretion was comparable to urinary excretion when administering up to 4 mg supplementation, but the main part of Se excretion after 8 mg of supplementation was of faecal origin. The total urinary excretion varied from 518.5 ng/day (control groups) up to 1795.9 ng/day (16 mg Se supplemented group). Forty-five percent of the excreted Se was from urine in non-supplemented animals *vs* 26-30% only in highly supplemented camels. Moreover, Se concentration in serum was highly correlated with Se concentration in urine and faeces.

5. Conclusion

The metabolism of selenium in camel is quite comparable to that of the other herbivorous. However, a high concentration in blood with high Se supplementation could be observed. According to dietary Se supply and mean weight of the animal, selenosis appeared with 0.05 mg/kg LW Se supply only. Severe intoxication occurred with 16 mg Se supplementation, i.e. 0.10 mg/kg LW. These values were 5 times lower than those for sheep and cattle. Based on these results, it seems essential to limit Se supplementation in camel at 0.01-0.02 mg/kg LW, i.e. approximately 4-8 mg per day for adult animals or 0.5-1 ppm in the diet.

6. Acknowledgement

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