

## Maternal transfer of selenium by blood and milk in camels

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### Abstract

Ten pregnant female camels were divided into two groups. The control group received no supplement; the experimental (treated) group received a daily oral supplement of 2 mg Se as selenite for the last three months of pregnancy and the first month of lactation. The Se concentration in serum increased significantly in the supplemented group and was threefold higher ( $305.9 \pm 103.3$  ng/mL) than in the concentration control group ( $109.3 \pm 33.1$  ng/mL). Blood values in camel calves were similar to those of the dams. The selenium concentration increased in similar proportions in milk ( $167.1 \pm 97.3$  ng/mL vs  $86.4 \pm 39.1$  ng/mL in the supplemented and control groups respectively). In the colostrum, Se concentration was higher in both groups, but with a similar difference:  $108.2 \pm 43.9$  ng/mL vs  $302 \pm 94.60$ . Significant correlations were reported between dam serum Se, camel calf serum Se, milk Se and GSH-Px both in dams and calves.

**Keywords:** camel, glutathione-peroxidase, milk, selenium, vitamin E

### Introduction

Selenium is one of the essential trace elements, playing an important role in the metabolism by their inclusion in organic molecules. Yet selenium enters in the composition of an enzyme, the glutathione-peroxidase, which plays a central action in cell protection by anti-oxidative activity. Many studies in domestic animals have shown that selenium supply is linked to a better immune system by the protection of cells involved in the immunity process (white blood cells). Selenium is also involved in reproduction performance and in muscle metabolism. A lack of selenium can lead to infertility, muscle degeneration and heart failure. The metabolism of selenium (Se) is well described in most domestic animals

and the requirements in farm animals have been widely published (Mc Dowell, 1992). There are few references regarding selenium in camel (Faye and Bengoumi, 1994), except for some plasma or blood values under field conditions; however, a review was published recently regarding all aspects of selenium metabolism in camels: ingestion, excretion, deficiency and toxicity (Faye and Seboussi, 2009; Seboussi et al., 2009a).

In Emirates, soils and feedstuffs are generally considered deficient in selenium, and many cases of degenerative myocarditis in young camels have been reported (El Khouly et al., 2001). Thus, all concentrates given to camels are enriched in selenium mainly as selenite, but the impact on selenium status, especially in the pregnant and

lactating female and in the milk-fed camel calf, is unknown.

Therefore, the present paper aims (i) to measure the levels of Se, GSH-Px and vitamin E in pregnant and lactating animals and their calves to provide base data on these variables in normal animals; (ii) to study the effect on these variables of daily supplementation to provide approximately double the daily intake of Se; (iii) to determine the effect of increased levels of selenium in maternal blood on the corresponding levels in the calf; and (iv) to determine the effect on the calf of stopping the supplementation of the dam.

## Material and methods

### Animals

The study included 10 pregnant adult female camels (more than 7 years old) of the local breed randomly divided into two groups of five animals. The mean weight was  $527 \pm 73$  kg with no significant difference in weight between groups. The animals were treated for external and internal parasites using ivermectin (Ivomec N.D.) and were in good health during the whole experiment.

### Experimental design

During the whole trial, the animals were fed individually with approximately 6 kg of Rhodes grass (*Chloris gayana*) hay and 2 kg of concentrates of known selenium content. Water was provided *ad libitum*. The experiment duration was 195 days, starting from the last third of pregnancy to three months after parturition. The trial included three phases: (i) Adaptation period (days

1–15) where the animals received the basal diet without any mineral supplementation. No sampling was undertaken during this period; (ii) Supplementation period with selenium additives (days 15–135): the control group (1) did not receive selenium supplementation and treated group (2) received 4.36 mg anhydrous sodium selenite (i.e., 2 mg of selenium) included in dates given daily as a delicacy. The supplementation period included the three last months of gestation and the first month of lactation; (iii) Post supplementation period (days 135–195) where animals of both groups received the basal diet only. No supplementation was given to camel calves.

The selenium requirements for camels were assumed to be similar to those of the cow, i.e., 1 mg/day (Mc Dowell, 1992). The quantity of selenium given to animals corresponded to the level generally supplied by racing camel owners.

### Sampling

In adults and calves, blood was collected every two weeks from the jugular vein in both heparinised (H) and nonheparinised tubes (NH). One NH tube was centrifuged and the serum stored at  $-80^{\circ}\text{C}$  until selenium analysis. From an H tube, 2 ml of whole blood was collected then centrifuged. Plasma was removed and stored at  $-80^{\circ}\text{C}$  until vitamin E analysis. The red blood cells were washed three times with an isotonic solution of NaCl (0.9%) and centrifuged for 4 minutes at 4000 xg. The supernatant was discarded and red blood cells were frozen at -

80°C and kept until glutathione-peroxidase (GSH-Px) analysis.

The blood sampling was carried out twice a month in the morning before feed distribution in adult and young camels. A camel calf's blood sample was taken at the moment of delivery, prior to colostrum feeding, to evaluate the Se status at birth.

Colostrum samples were taken immediately after parturition, and milk was collected when the calf was being suckled. A 20 ml milk sample was collected every two weeks and frozen until analysis.

The elements of the basal diet were sampled at the beginning, the middle and the end of the trial, dried, ground and stored for selenium analysis. Drinking water was also sampled at the same time for selenium determination.

#### *Laboratory analysis*

Selenium was determined in serum by ICP atomic emission spectrometer, Varian Vista MPX-CCD. Quantification of selenium was performed by the standard addition method, using an 11-point standard curve. AccuTrace™ Reference Standard solutions used were Quality Control Standard #1AccuStandard® and Laboratory Performance Check Standard AccuStandard®. GSH-Px activity was measured in erythrocytes according to method of Paglia and Valentine (1967). The GSH-Px activity was expressed in international units per gram of hemoglobin (IU/g Hb), where one international unit is equivalent to 1 mol of NADPH oxidised per minute per gram of hemoglobin. Vitamin E

quantification in plasma was performed by the high-performance liquid chromatography (HPLC) system.

#### *Statistical analysis*

Descriptive analyses (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 selenium supplementation level (two levels: 0 and 2 mg) and of the day of sampling (13 levels for blood and plasma in adult, 7 levels for milk and camel calf blood and plasma). Previously, normality of the distribution was tested by the Skewness and Kurtosis test (test W). Interactions between mother and camel calf parameters were tested by the correlation of Pearson. Significant level at  $P < 0.05$  was retained.

## **Results**

### *Selenium content in the diet and selenium intake*

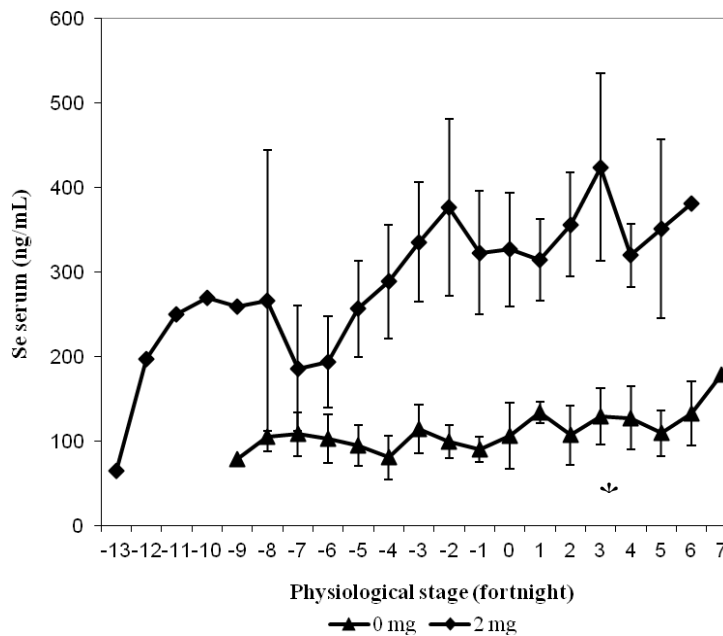
According to selenium concentration in the diet, the selenium intake provided by feeding was 1.8 mg per day. The mineral mixture providing 0 and 2 mg Se per day, the total quantity of selenium provided was 1.8 mg/day for the control camel group and 3.8 mg/day in the treated group, corresponding to 3.6 µg/kg LW in the control group and 6.6 µg/kg LW in the treated group. The diets supplied 0.24 and 0.50 mg/kg respectively. Elsewhere, the basal diet provided vitamin E: 5.5 µg/g in Rhodes Grass and 0.96 µg/g

in concentrate i.e. 32 µg per day on average.

*Selenium values in serum*

The mean value of serum Se was significantly higher ( $P < 0.001$ ) in the treated group and was threefold higher than in the control group ( $305.9 \pm 103.3$  ng/mL vs  $109.3 \pm 33.1$  ng/mL). On average, Se serum concentration was significantly higher after parturition than before in both groups ( $P < 0.05$ ). Supplementation sharply increased

the levels of serum Se in the pregnant camels between 13 and eight fortnights before parturition. During the next two fortnights the levels fell slightly but were at least twice as high as in the control animals. The difference between groups became greater during the rest of pregnancy and the first four fortnights of lactation, and even persisted for three fortnights after supplementation ceased (Figure 1).

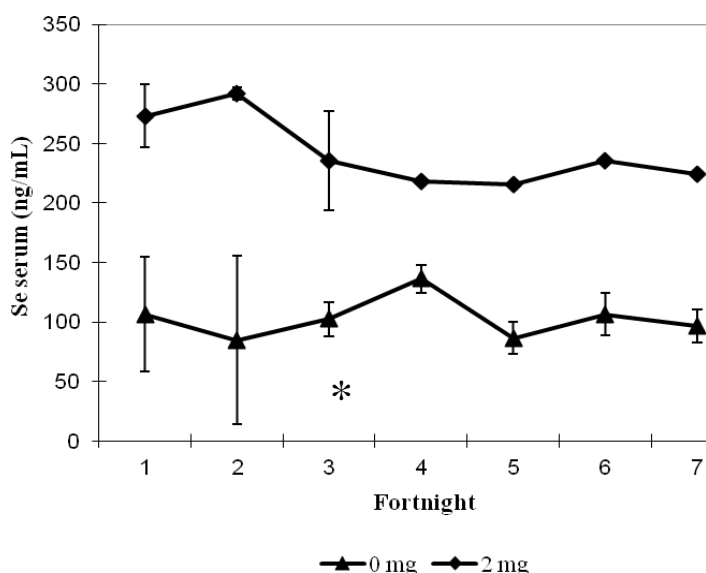


**Figure 1.** Biweekly changes (mean and S.E) in serum selenium concentration (in ng/mL) in camel before and after parturition according to the selenium supplementation level: 0 mg/day (▲) and 2 mg/day (◆)

\* Stopping Se supplementation

The Se serum concentrations in camel calves at parturition were  $273.2 \pm 48.0$  and  $106.3 \pm 26.5$  ng/mL in the treated and control groups respectively. This significant difference ( $P < 0.001$ ) was

maintained for the entire milking period:  $248 \pm 14.1$  vs  $103.4 \pm 28.7$  ng/mL (Figure 2) although the supplementation ceased after one month of lactation.



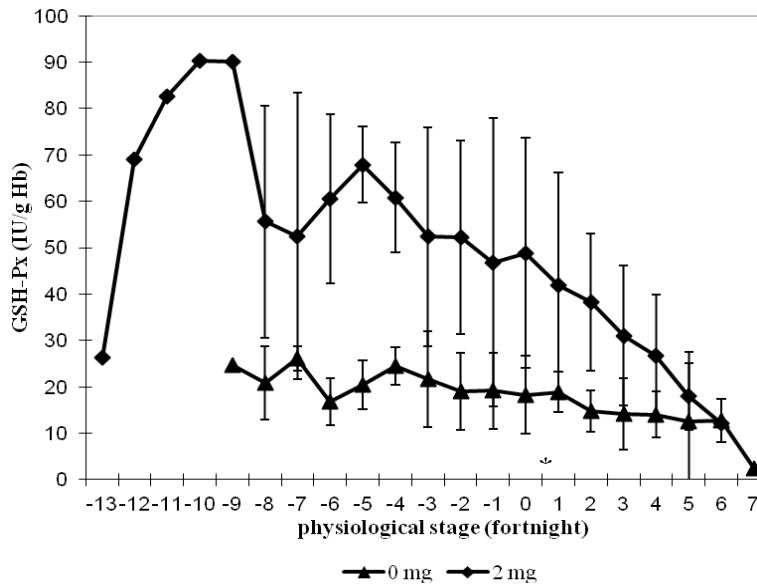
**Figure 2.** Biweekly changes (mean and S.E) in serum selenium concentration (in ng/mL) in camel calf according to the selenium supplementation level: 0 mg/day (▲) and 2 mg/day (◆).

\* Stopping Se supplementation

#### *Erythrocyte GSH-Px activity*

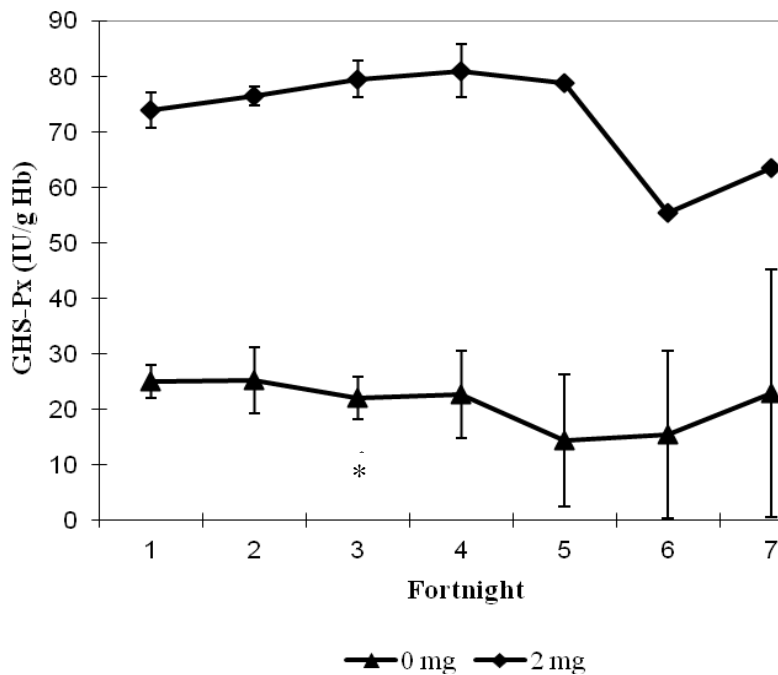
The Se supplementation increased the GSH-Px activity according to a similar pattern of selenium in serum between the 13<sup>th</sup> and 6<sup>th</sup> fortnight before parturition. As a whole, the GSH-Px activity varied from 2.2 to 98.9 IU/g Hb and was on average significantly higher in supplemented dams ( $P < 0.001$ ):  $47.5 \pm 25.6$  vs.  $1 \pm 8.7$  IU/g Hb. There was a difference in GSH-Px activity before and after parturition in both groups but reverse to Se selenium with a significant decrease ( $P < 0.05$ ) in control group

and in treated group ( $P < 0.001$ ). Indeed, the GSH-Px activity decreased highly and regularly from the last month of gestation (Figure 3). At parturition the camel calves born from supplemented dams had GSH-Px values threefold higher than the control calves:  $73.8 \pm 2.9$  vs  $25.0 \pm 3.2$  IU/g Hb ( $P < 0.001$ ). A significant difference was observed for the entire trial even after supplementation stopped, with an average of  $73.0 \pm 14.1$  IU/g Hb in the treated group vs  $22.8 \pm 4.5$  IU/g Hb in the control group (Figure 4).



**Figure 3.** Biweekly changes (mean and S.E) in GSH-Px activity (in IU/g Hb) in she-camels before and after parturition according to the selenium supplementation level: 0 mg/day (▲) and 2 mg/day (◆).

\* Stopping Se supplementation



**Figure 4.** Biweekly changes (mean and S.E) in GSH-Px activity (in IU/g Hb) in camel calves according to selenium supplementation level: 0 mg/day (▲) and 2 mg/day (◆).

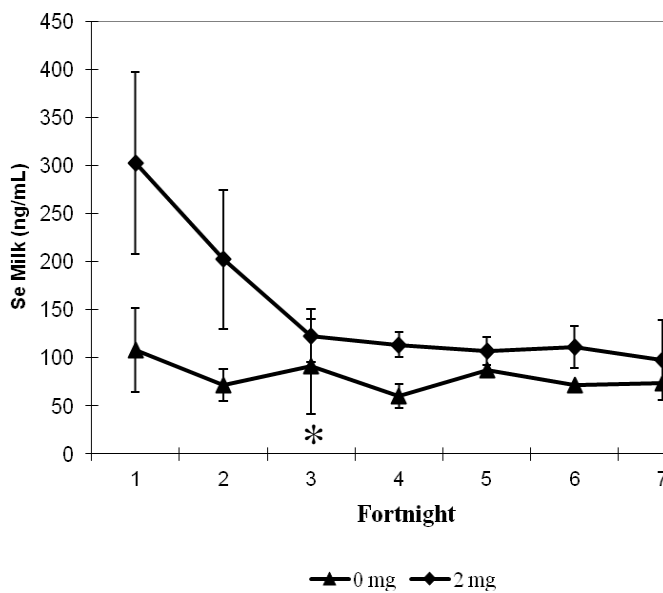
\* Stopping Se supplementation

### Plasma vitamin E

No significant changes were observed for plasma vitamin E for the entire experiment in both groups and no difference occurred between treated and control animals where the mean values were  $1.17 \pm 0.72$  ng/mL and  $1.14 \pm 0.89$  ng/mL respectively. In camel calves, vitamin E varied from 0.002 to 4.67 IU/G Hb with a mean of  $0.82 \pm 1.06$  IU/g Hb. The plasma vitamin E was significantly higher ( $P < 0.05$ ) in the treated group than in the control group at the second month of lactation only.

### Selenium excretion in milk and correlations

The Se concentration in milk varied from 39.5 to 482.6 ng/mL with an average of  $167.1 \pm 97.3$  ng/mL in the treated group and  $86.4 \pm 39.1$  ng/mL in the control group. At birth, Se concentration in colostrum was threefold higher in the treated group: mean value  $302 \pm 94.60$  vs  $108.2 \pm 43.9$  ng/mL ( $P < 0.001$ ). In both groups Se milk concentration decreased and, after the second milk sampling, no significant difference was observed (Figure 5).



**Figure 5.** Biweekly changes (mean and S.E) in milk selenium concentration (in ng/mL) in she-camels for the three first months of lactation according to the selenium supplementation level: 0 mg/day (▲) and 2 mg/day (◆).

\* Stopping Se supplementation

In spite of the reverse trend after parturition, serum Se concentration was correlated with GSH-Px both in dam ( $r = 0.338$ ;  $P < 0.001$ ) and in camel calf ( $r = 0.819$ ;  $P < 0.001$ ). A negative correlation was observed between GSH-Px and vitamin E in dam ( $r = -0.167$ ;  $P < 0.05$ ) but not in camel calf. Except for vitamin E, the antioxidant status of the mother was significantly correlated with that of the camel calf, both for selenium in serum or in milk and for GSH-Px. By considering the Se concentration in colostrum and the status of the mothers and of their camel calves at parturition, positive correlations were observed with serum Se in mother ( $r = 0.659$ ;  $P < 0.05$ ) and in calf ( $r = 0.689$ ;  $P < 0.05$ ), with GSH-Px in mother ( $r = 0.739$ ;  $P < 0.05$ ) and in calf ( $r = 0.811$ ;  $P < 0.001$ ). At reverse, a negative correlation was reported with vitamin E concentration in the mother ( $r = -0.757$ ;  $P < 0.001$ ).

## Discussion

### *Usual values of serum selenium, GSH-Px and vitamin E in camels*

The mean concentration of serum Se, reported in the literature for large animals, was approximately 100 ng/mL. However, few references were available in camel serum. The reported values varied from 20 to 120 ng/mL, according to the physiological or feeding status of the camels (Hamliri et al., 1990; Liu et al., 1994; Abdel Rahim, 2005; Barri et al., 2007; Seboussi et al., 2008). In Morocco, in dromedaries receiving a low Se basal diet, the serum selenium concentration was quite lower, about 21ng/mL, but the concentration increased to 200.4 ng/mL after 2 mg Se supplementation (Bengoumi et al, 1998).

No data was available on serum selenium in new born camel calves. The observed serum Se values in our study were similar to those of the dams with the same ratio between the two groups. The maternal transfer of selenium was efficient, and the oral supplementation of pregnant camels

was an effective method of increasing selenium concentration in serum of their calves. The results were in accord with those reported in cows (Weiss et al., 1984).

Similar values in camels were reported in different countries (Abdel-Rahim, 2005; Hamliri et al., 1990; Bengoumi et al., 1998), except in Spain where the values appeared very high (Corbera et al., 2003: 298.1 IU/g Hb). As for serum Se, no data were available for GSH-Px in newborn camel calves.

The observed values in the calves reflected those revealed in the dam at parturition as expected. The vitamin E concentration in plasma in our results was quite similar to those described in the literature (Abdel-Rahim, 2005; Al-Senaïdy, 1996; Mousa et al., 2006).

### *Correlation between Se and GSH-Px*

GSH-Px activity is usually regarded as a good indicator of the Se status in all species, including camel (Faye and Seboussi, 2009). However, the different patterns between Se and GSH-PX in the *peri-partum* observed in our experiment conducted to a relative lower correlation coefficient in camel, compared to cow (Bengoumi et al., 1998). It has been reported that the GSH-Px activity increased by about 23% until the end of pregnancy in humans (Uotila et al., 1991). Indeed, it has been indicated that cells used enzymes such as GSH-Px against the oxidative damage, which occurs in pregnancy (Chawla and Kaur, 2004). In our study, such an increase was not observed in the control group. Conversely, a significant decrease of GSH-Px after parturition has been described in sheep (White et al., 1989), despite, in our study, of the serum Se increase in the same period. Some authors reported a decrease of serum Se at the beginning of lactation in sheep (Travnicek, 2007) linked to lactation stress, but others reported a slight increase, as observed in the control group (Gürdoğan et al., 2006). Contrary to our previous studies achieved on non-pregnant



and non-lactating camels (Bengoumi et al., 1998; Seboussi et al., 2008), the GSH-Px activity was not maintained after the end of supplementation.

#### *Selenium excretion in milk*

One reference only was available on selenium content of camel milk (Al-Awadi and Srikumar, 2001) and the reported value ( $13.9 \pm 2.4$  ng/mL) was quite lower than in our study, but the authors did not mention the lactation stage. In dairy cows, the milk Se concentration varied from 19.4 to 53.7 ng/mL with Se dietary selenium between 0.15 and 0.40 ppm (Juniper et al., 2006). The colostrum Se concentration was a clear reflection of the serum Se of the dam. After parturition, in the 2mg-Se group, selenium concentration in milk dropped, especially when Se supplementation was stopped in the mothers, but the serum Se concentration in the calves was maintained at a high level compared to those of the literature. If our results are confirmed, it should underline the richness of camel milk in selenium.

In conclusion, Se supplementation has to be administered according to the particular physiology of the camel. Indeed, the results seemed to confirm the sensitivity of camels to Se supplementation with an important increase of selenium in serum and milk. However, this increase could be also the mark of a greater sensitivity to toxicity, as a recent study on the tolerance level to selenium toxicity in camel has shown (Seboussi et al., 2009b).

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