REGULAR ARTICLE

Effect of Selenium injection in pregnant camels on selenium status of their new-born and milk

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Abstract

The effect of inoculation of selenium solution to pregnant camels was investigated to assess the impact on selenium status of the new-born and on the selenium concentration in milk. In the trial included 2 groups of 8 camels, the treated one receiving a single injection of selenium solution at the end of pregnancy. In blood, no difference was observed between control and treated group before injection. A significant difference was observed at delivery as well in dam (33.3 vs 44.7 ng/mL respectively) as in calf (28.5 vs 47.6 ng/mL respectively). In milk, the selenium was also significantly in higher concentration in treated group (93 ± 49 ng/mL) than in control one (59 ± 19 ng/mL) at the delivery time. Zinc concentration in milk was positively correlated to selenium content. The improvement of selenium status by a single injection was slight and more efficient supplementation ways could be proposed to the camel farmers.

Key words: Camel, Milk, Colostrum, Selenium, Copper, Zinc

Introduction

The selenium (Se) in milk was regularly investigated in cattle (Ceballos et al., 2009) or in ewe (Davis et al., 2006), but in camel, the references remain scarce. Previous studies were mainly limited to blood status (Faye and Seboussi, 2009) and only 3 references are available on the selenium quantity transferred through milk to the camel calf (Al-Qarawi et al., 2001; Seboussi et al., 2009a; Faye et al., 2011). These publications showed a high variability of the Se content in milk according to the Se status of the mother before calving and to lactation stage after calving. They stated also on the specific Se metabolism in camel regarding the toxicity threshold (Seboussi et al., 2009b) and the supplementation (Faye and

Seboussi, 2009) which cannot be applied directly from cattle requirements. Moreover, those former results obtained in Emirates (Seboussi et al., 2009a) were reported in a context of Se deficiency widely observed in the field with numerous cases of white muscle disease or heart failure due to lack of Se in the mother's diet, and furthermore partly with dams receiving before calving an oral Se supplementation under selenite form. In Saudi Arabia, where selenium status in human population was regarded as low (Al-Saleh, 2000), selenium deficiency was regularly incriminated also in grazing livestock. However, most of the camel farmers did not distribute oral selenium supplementation to their animals, but rather used non-organic selenium solution by injection in pregnant or new-born camels. However, the effect of an unique injection at the end of pregnancy on the selenium status of the new-born and especially on the level of selenium in milk which is the unique source of selenium for the calf, was never studied in camel.

In the present study, the selenium transfer through milk from the dam to the camel calf was analyzed after Se supplementation by injection

before delivery in order to assess the impact on the selenium content in milk and selenium status of the new-born calves. Elsewhere, the interactions with other trace elements as copper and zinc were investigated.

Materials and Methods
Location and animals

This study was carried out in the camel farm of Al -Jouf "Camel & Range Research Center" located in north-west Saudi Arabia, 950 km from Riyadh. Average annual temperature was 20°C, ranging from 12°C to 27°C, and average annual rainfall was 55 mm. The herd was composed by camels of four ecotypes (Malhah, Wadhah, Hamrah and Safrah) but belonging to very close genotype (Abdallah and Faye, 2012; Almathen et al., 2012). The weight of the animals was on average 620 ± 101 kg. Camels were kept in-door throughout the year and housed in pens. Their normal diet was composed of alfalfa (*ad-libitum*), barley (3 kg/day/animal), salt, wheat bran (1kg/day/animal). As the calving season occurred between December and February, all the camels were approximately at the same stage of reproductive cycle. The milk production not including part drunken by camel calves was recorded every day.

Selenium treatment

For the experiment, 16 adult lactating camels 5-18 years old only were available. They were divided randomly into two groups of eight. In spite of the heterogeneous composition of the herd, the groups' composition was comparable (no significant difference) as well for mean age (10.6±6.4 *vs* 8.8±3.3 years for treated and control group respectively) as mean weight (624±78 *vs* 616±121 kg). The camels were in good health all along the experiment. The control group did not receive any selenium supplementation. The treated group was submitted to unique injection of Selepherol© from Vetoquinol Co as preventive dose for selenium deficiency. Selenopherol© contained sodium selenite (23 mg/100ml) and vitamin E as acetate (3.82 g/100ml). The pregnant she-camels received 75 ml (i.e. 17.25 mg Se) by deep IM route at different injection sites to avoid local reaction. The injection was done 3-weeks approximately before the delivery. This level of supplementation corresponded to what was locally practiced by the camel owners to prevent selenium deficiency in camel calf.

Sampling agenda and laboratory analysis

Milk was sampled at the morning milking at the delivery then at day 30 and 60 post-partum in a plastic bottle. Blood samples were collected in the dams just before injection then at the delivery. Blood samples were collected also on camel calves after parturition at the same time of their dam. Samples (blood and milk) were stored in deep freezer at -80°C until laboratory analysis. In blood and milk samples, copper and zinc were determined by Atomic Absorption Spectrophotometer (AA-6650, Shimadzu, Japan) at the IDAC laboratory, Kharj (Saudi Arabia). Selenium was determined in the same laboratory with Hybrid Vapor Generator (HVG-1, Shimadzu, Japan). The data are reported as ng/mL for selenium, and µg/100ml for copper and zinc.

Statistical analysis

The mean and standard deviation was calculated for each parameter and for each group. The variance analysis (ANOVA) for time series was applied to evaluate the difference between control and treated groups all along the experiment. Pearson correlation was determined to assess the relationships between the mineral statuses.

The software XLSTAT (Addinsoft©) was used for the data analysis.

Results and Discussion
Selenium in blood

The effect of selenium injection, yet widely used for preventing selenium deficiency in camel was not studied in this species. Moreover, it is difficult to compare the types of Se supplementation reported in the literature only on the quantitative basis as the form of Se administrated to the animals could differ strongly (injection or oral, organic or non-organic, different doses). So, the effect of the supplementation is essentially assessed by comparing the Se concentration in serum. This concentration is generally regarded as a good short-term indicator of the selenium status in animal. Due to this relatively long apparent terminal half-life, the concentration of Se in serum should be widely independent of small daily variations in Se intake (Haldimann et al., 1996).

In our study, the mean value of selenium concentration in serum was 37.9 ± 0.83 ng/mL in dams and 40.7 ± 1.25 ng/mL in camel calves. There was no difference between control and treated group at the time of injection (36.3 *vs* 37.2 ng/mL respectively), but a significant difference (P<0.01) was observed at delivery as well in dam (33.3 *vs* 44.7 ng/mL respectively) as in calf (28.5 *vs* 47.6 ng/mL respectively) (Table 1). Those values corresponded globally to low level of selenium status. Indeed, on average, normal serum selenium

concentration in camel was regarded as about 100ng/mL (see review of Faye and Seboussi, 2009). For example, in Morocco, Hamliri et al. (1990) observed in whole blood, values varying according to age and sex, between 109.1 and 117.8 ng/mL. Similar figures were recorded by Liu et al. (1994) in China on Bactrian camel with concentrations varying from 97 to 112 ng/mL. However, in Sudan, Abdel Rahim (2005) reported values in whole blood varying between 25 and 53 ng/mL.

In serum from Moroccan dromedaries receiving probably a low Se basal diet, the plasma selenium concentration was quite lower, about 21 ng/mL (Bengoumi et al., 1998a). Recently, in male adult camels in healthy conditions from Iran, the selenium concentration reported in serum was 12.6 ng/mL only (Nazifi et al., 2011). In Saudi Arabia, serum Se values reported in young camels at the slaughterhouse varied between 5.3 and 131 ng/mL with 30% of samples higher than 100 ng/mL (Barri and Al-Sultan, 2007). In the same area than the present study, the serum Se was 50.5 ± 31.5 ng/mL, whatever the physiological stage of the camels (Althamma et al., 2012). In the United Arab Emirates (UAE), the mean value was 200 ± 90 ng/mL in animals with no Se supplementation (Seboussi et al., 2004). In recent experiments with different levels of Se supplementation, selenium content in serum for non-supplemented animals was on average 137.6 ± 18.7 ng/mL in non-pregnant, non-lactating camels (Seboussi et al., 2008), 109.3 ± 33.1 ng/mL in pregnant females, and 103.4 ± 28.7 ng/mL at milking period (Seboussi et al., 2009a). 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 Saudi Arabia, the basal diet could be very low in natural selenium.

The single Se injection improved slightly the Se status of the camel, appreciated by the increase in serum concentration. However, with daily oral supplementation, a most important effect was reported. In two groups of pregnant females receiving 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 (Seboussi et al., 2009a), the mean value of selenium content in serum was significantly higher in supplemented group (2 mg) and was three-fold higher than the concentration compared to the control group (305.9 ± 103.3 ng/mL and 109.3 ± 33.1 ng/mL respectively). The selenium level at parturition was still significantly higher in the treated group in spite of a slight decrease around the calving period. In the trial of Al-Qarawi et al. (2001) involving selenodeficient camels with muscular dystrophy, treatment involving selenium – vitamin E (Bo-SE, Schering – Plough Animal health, 2.19 mg sodium selenite + 50 mg vitamin E) by IM injection at a dose rate of 0.5 mg/kg body weight for two consecutive days allowed getting an increase of selenium concentration on average 2.3 ng/mL up to 23.7 ng/mL, i.e. with a similar trend to that observed by Bengoumi et al. (1998a) who reported a multiplication by 10 of the serum Se after supplementation.

Table 1. Selenium (Se) concentrations in camel serum and milk (mean and S.D.) in Control (C) and Treated (T) groups in mother (at injection and delivery time) and calf for serum, and at delivery and every month for milk

	Serum (ng/mL)			Milk (ng/mL)		
	Injection	Delivery	Calf	Delivery	D30	D90
Se-C	36.3 ± 6.0^a	33.3 ± 2.3^a	28.5 ± 7.8^a	59.1 ± 19.2^a	50.0 ± 29.3^a	58.9 ± 13.2^a
Se-T	37.2 ± 10.7^a	44.7 ± 8.2^b	47.6 ± 8.7^b	93.2 ± 49.0^b	69.1 ± 30.0^a	72.2 ± 17.2^b

[a,b] Means in column with a different letter in superscript differ (P < 0.05)

Table 2. Copper (Cu) and zinc (Zn) concentrations in camel serum and milk (mean and S.D.) in Control (C) and Treated (T) groups in mother (at injection and delivery time) and calf for serum, and at delivery and every month for milk.

	Serum (µg/100mL)			Milk (ppm)		
	Injection	Delivery	Calf	Delivery	D30	D90
Cu-C	70.1 ± 18.9	59.0 ± 24.1	78.2 ± 13.0	0.08 ± 0.01	0.08 ± 0.02	0.06 ± 0.02
Cu-T	81.8 ± 25.2	80.3 ± 29.9	63.0 ± 25.4	0.14 ± 0.07	0.07 ± 0.02	0.07 ± 0.02
Zn-C	77.7 ± 13.9	71.8 ± 12.2	77.6 ± 23.9	15.96 ± 3.2	3.59 ± 1.6	3.35 ± 0.8
Zn-T	71.5 ± 15.0	68.3 ± 18.9	47.6 ± 20.4	8.60 ± 14.8	2.54 ± 0.8	3.09 ± 0.7

In new-born animals, the serum selenium values reflected generally the Se status of the dam, with a positive correlation between the serum concentration in dam and in new-born (r = 0.622; P > 0.01). In the same area than our study, Athamma et al. (2012) found 37.2 and 46.1 ng/mL in dam and new-born respectively. In Emirates, with camel receiving 2mg/day oral Se supplementation, the Se serum concentrations in camel calf at parturition were 273.2 ± 48.0 and 106.3 ± 26.5 ng/mL in the treated and control groups respectively (Seboussi et al., 2009a) i.e. a similar proportion than in dams.

In our study, the breed composition of each group was composite and not similar. However, the breed effect on the selenium status of animal was not clearly stated. In non-pregnant sheep, Ramirez-Perez et al. (2000) did not report a significant difference between Rambouillet and Suffolk breed. At our knowledge, a genetic variability of selenium status was never reported on camel. Moreover, the camel ecotypes participating to the present experiment were regarded as very close genotypes (Al-Swailem et al., 2007). The age variability of camels in our trail was high. It was not stated from the literature an age effect on selenium status. In human, for example, no significant association was found between selenium and age (Akbaraly et al., 2010). Similar observations could be done for other trace-elements as copper and zinc in camel for which the age effect was not clearly stated in the literature (see review of Faye and Bengoumi, 1994).

Selenium in milk

The selenium content in milk was significantly higher in treated group (93 ± 49 ng/mL) than in control one (59 ± 19 ng/mL) at the delivery time (P >0.05). The difference was not significant one month later, but again slightly higher in treated group (P > 0.05) at the second month of lactation (figure 1). Those values appeared low compared to the results of Faye et al., (2011) in Emirates where the Se concentration in milk varied from 39.5 to 482.6 ng/mL with an average of 167.1 ± 97.3 ng/mL in treated group receiving 2 mg daily in oral supplementation before the delivery, and 86.4 ± 39.1 ng/mL in the control group. In this last study, both in control and treated groups, Se milk concentration decreased and difference was observed after one month as in our study. In their study on camel milk, but without specification on the lactation stage, Al-Awadi and Srikumar (2001) reported quite lower values (13.9 ± 2.4 ng/mL). In dairy cow, 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). According to the meta-analysis of Caballos et al. (2009), the selenium concentration in cow milk varied between 9.2 and 16.3 ng/mL with a maximum of 29.2 ng/mL observed in cattle supplemented with Se yeast. In ewe milk, the values varied from 32 to 81 ng/mL in non-supplemented animals (Davis et al., 2006).
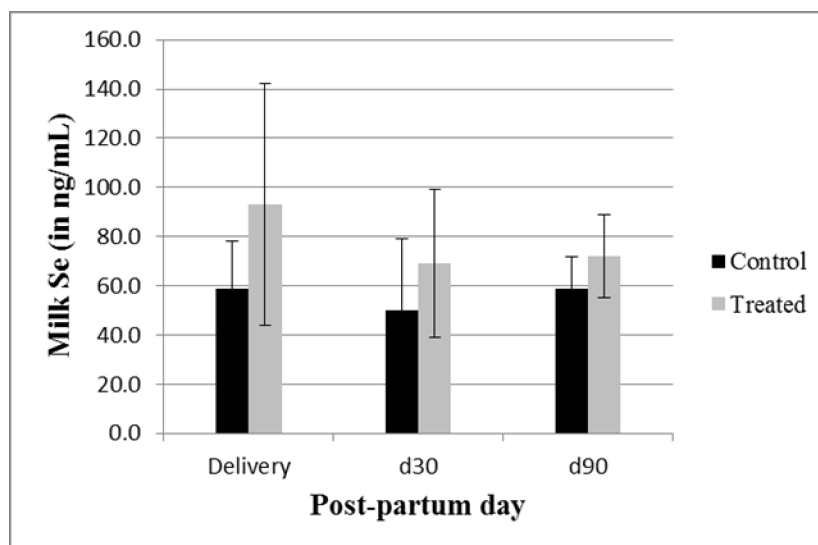

Figure 1. Changes in selenium concentration in camel milk in control and treated groups receiving Se injection.

Contrary to the results reported previously (Seboussi et al., 2009a), the colostrum Se concentration was not a clear reflect of the serum Se of the dam. In our results, the correlation was not significant (P=0.06), even if a tendency was observed. However, by comparing to the literature data in other dairy species (Ceballos et al., 2009; Davis et al., 2006), and in spite of the relative low Se status in serum, camel milk seems richer in selenium (Faye et al., 2011).

Interactions with other trace elements

The interactions between trace elements were reported in many publications. For example, for long time, studies have revealed an inverse relationship between zinc and selenium in human milk, and maternal selenium status was found to influence the protein binding pattern of zinc in human milk (Brätter et al. 1997). Zinc and copper have been found to be bound partially to the same proteins, e.g. lactalbumin, in colostrum and transitional milk (Kantola and Vartiainen, 2001), and a direct correlation has been found between copper and selenium in human milk (Perrone et al., 1994).

The interaction between selenium metal ions and other trace elements can alter their respective availability and cause deficiencies, with unforeseen consequences for the activity of enzymes requiring these trace elements as cofactors. Most studies have reported that, in different situations, the level of one element is (or is not) affected by the presence of the other one. The presence of selenium could reduce the availability of metal ions blocking them in insoluble compounds. On the other hand, selenium deficiency has been reported to cause an overload of iron and unbalanced in vivo distributions of other elements, such as magnesium, calcium, copper and zinc (Chareonpong-Kawamoto and Yasumoto, 1995).

In our study, copper concentrations in serum were in the normal range for camel (Table 2), with values between 31 and 121 µg/100mL. Similar values were reported by Athamma et al. (2012) in the same area: 70.3±19.8 and 58.6±13.9 µg/100 ml for copper in female camels and their new-born respectively.

The range for zinc concentrations (38 to 112 µg/100 mL) was in the upper range than reported in the review of Faye and Bengoumi (1994). There was no difference between control and treated groups both for copper and zinc.

Regarding milk, few data were available. Our results for copper (Table 2), i.e. 85 ± 42µg/L on average was comparable to the findings of Bengoumi et al. (1998b) in Morocco (113±49 µg/L), but lower

than the values reported by Dell'Orto et al. (2000) in camel from the Horn of Africa (370 to 400 µg/L on average according to the mineral supplementation) and those published in Saudi Arabia by Mehia et al. (1995). There was no difference between the groups in the copper concentration in milk whatever the date of sampling. In our study the average of zinc concentration in the milk was 7.5 ±9.6 mg/L which was quite higher than the values reported by Dell'Orto et al. (2000) and Bengoumi et al. (1998b) respectively 2.52 to 3.16 mg/L, and 2.87 ± 0.8 mg/L. Contrary to copper, a slight significant difference (P<0.05) was observed at the delivery with a higher value in control group (15.9 ± 3.2 mg/L) than in treated one (8.6 ± 14.8 mg/L).

Contrary to the minerals in serum which had no correlations, the minerals' (Cu, Zn and Se) concentrations in milk were positively correlated: copper concentration was correlated to zinc (r =0.537; P<0.01) and zinc was correlated to selenium (r =0.415; P<0.05). In a previous study (Faye et al., 2009), a negative correlation was observed between Zn and Se in camel serum, but the analysis included animals with selenosis which provoked inflammation process leading to a drastic decrease of zinc and iron in serum. Probably, the negative interaction between selenium and zinc in milk reported by some authors (Brätter et al., 1997) could be observable within a certain range of concentration when one element saturated the binding sites as it was observed between zinc and copper in camel serum (Bengoumi et al., 1998c).

Conclusion

The selenium supplementation by a single injection in pregnant camel at the end of the gestation was commonly used by the camel farmers in Saudi Arabia, in a context of a wide deficiency of the soil and forages in selenium. It appeared that this practice could improve slightly the selenium status of the new-born calves by increasing Se in milk at least in the colostrum. But the improvement seemed to have short effect. Other ways for selenium supplementation, as organic selenium distributed in the diet which was never tested in camel, could be applied and proposed to the camel farmers. A new experiment is currently testing the effect of organic selenium on the status of camel in this essential element.

Acknowledgements

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