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CHRONIC SELENOSIS IN CAMELS

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ABSTRACT

Three groups of 4 female camels, 2 years-old receiving a basal diet of Rhodes grass and concentrate were supplemented for 90 days with 8, 12 and 16 mg non-organic selenium to each group, respectively. Blood, faeces and urine were collected for selenium, glutathione peroxidase and vitamin E determination. Two camels per group were sacrificed at day 45 and 90. Organs and hair samples were used for histopathological findings and selenium content estimation. The selenium concentration increased significantly in 3 groups with an average value of 321.23 ± 140.5 ng/ml, 443.18 ± 231.06 ng/ml and 298.04 ± 212.13 ng/ml, respectively. Glutathione peroxidase activity varied between 26.85 and 174.16 IU/g Hb. Significant correlations between serum selenium, GSH-Px activity, urine and faecal Se were reported. No significant variation occurred for vitamin E (mean: 0.68 ± 0.36 ng/ml). High selenium level was observed in liver followed by kidney, spleen, lung, heart, pancreas, brain and ovary. Selenium concentration rose significantly in hair. Several symptoms related to selenosis have been reported and histopathological findings showed remarkable lesions in all the organs.

Key words: Camel, glutathione peroxidase, selenium, selenosis, vitamin E

Selenium deficiency is a key factor for nutritional myopathy in various species. In camel, the white muscle disease has been reported in United Arab Emirates with high incidence due to the low selenium daily intake (Elkhouly *et al,* 2001; Seboussi *et al,* 2004). Farmers administered their camels with commercial pellets, mineral blocks or powder mix salts and containing selenium, as prophylactic measure. Due to the lack of data on camel selenium requirements, the supplementation could lead to metabolic disorders in terms of deficiency or toxicity.

Camel sensitivity to trace element imbalances was formerly reported (Faye and Bengoumi, 1994) but no data was available for selenium metabolism in this species. However, in a previous trial comparing cow and camel (Bengoumi *et al,* 1998) where similar selenium supplementation supplied to animals with 2 mg/day for 2 months; a strong higher increase of plasma selenium in camel has been observed (10 times the blood level before supplementation) than in cows (2 times). It has been concluded that plasma selenium level was a very sensitive indicator of oral selenium supply in camel. However, the selenium tolerance in camel is unknown. Present study was aimed to study the range of tolerance to high selenium intake, effect on camel tissues functions and metabolism in order to recommend values for the normal body function.

Materials and Methods

The objective of the trial was to monitor the effect of selenium supplementation in excess on the haematological and biochemical parameters, to investigate the selenium content of the tissues including hair, and finally to observe the symptoms linked to chronic selenosis in camel.

Animals

Twelve young apparently healthy female camels of local breed each 2 years old were shared into 3 groups of 4 each. The average weight was 182.6 kg. The trial was performed at Al-Foha farm belonging to the Food and Agriculture College, UAE University. Camels were weighted on electronic balance every 2 weeks in the morning before feeding, watering and selenium supplementation. Blood samples, rectal temperature, respiratory rate, cardiac frequency, pulse rate and external temperature were recorded every week. Total 24 hour-faeces and urine samples were taken every month. At day 45, one camel of each group was slaughtered and another at the end of the experiment (at day 90).

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Experimental design

Camels were fed with a similar basal diet during the whole experiment, composed from Rhodes grass hay (*Chloris gayana*) with an average quantity of 3 kg DM and 2 kg of pelleted concentrate 10% protein (Soya-Bean Meal, Maize, Barley, Wheat bran, Molasses, Salt, Premix). Camels were provided water *ad libitum*. No vitamin E was added to the basal diet or fed to the animal during the experiment.

Before starting the trial, camels were given rest for 3 weeks and were trained for the adaptation to the experimental procedures. No sampling was done at that time. Selenium supplemented form was sodium selenite (Sodium selenite anhydrous) and given to animals every day, enrobed in date. The quantity of supplied selenium all along the trial was 8 mg (i.e., 17.44 mg sodium selenite), 12 mg (i.e., 26.16 mg sodium selenite) and 16 mg (i.e., 34.88 mg sodium selenite) daily, respectively for each group. Selenium supplementation was stopped immediately at the time of apparition of chronic selenosis and hepatoprotector was given to avoid death. Camels returned to normal good health gradually.

The selenium requirements for camel was assessed similar to cow, i.e., 1mg/day (McDowell, 1992). So, the quantity of selenium given to animals corresponded to high level up to probably chronic toxic level.

Blood sampling

Seven sampling was done at day 0, 15, 30, 45, 60, 75, 90. Jugular blood was collected into 5 ml heparinised vacutainer (H) and 10 ml non heparinised vacutainer (NH) every 2 weeks before food distribution and selenium supplementation. These were transferred immediately for routine haematology and biochemistry analysis at Al-Qattara veterinary laboratory. Serum was harvested after centrifugation of one of the NH tube and stored at -80°C until selenium analysis. Whole blood from H tube was centrifuged; the plasma was harvested then stored at -80°C until vitamin E analysis. For the dosage of GSH-Px in camel erythrocyte, the red blood cells were rinsed 3 times with an isotonic solution of NaCl (0.9%) and centrifuged for 4 min at 4000 rpm. The supernatant was discarded and red blood cells were frozen at -80°C and kept until analysis.

Faecal and urine sampling

Urine and faecal samples were taken every month on each camel. In the experiment farm, camels were placed in an individual compartment and then the total faeces excreted in 24 hours was taken off and weighted. After mixing, a sample of 600 g was taken into plate and placed in stove for 48h at 65°C, grinded and stored in dark and cool place until selenium analysis in labelled pack as follows: type of sample, experiment number, date of sampling, camel number and treatment. Total 24 hours urine of each camel was also taken using a special plastic bag placed on the vulva, weighted and a sample of 20 ml was taken off and stored at – 20°C until selenium analysis.

Feed and water sampling

Selenium content of the camel basal diet and water was also assessed at the beginning, the middle and at the end of the trial. Nutriments were dried, grinded and stored in cool and dark place until analysis. Vitamin E analysis was also performed in the diet components.

Organs sampling

Hairs were taken before slaughtering from the neck and other part of the camel body with sterile scissors. One camel of each group was sacrificed at day 45 and 90 at Swehan slaughter house belonging to the Agriculture and Municipalities Department, Abu Dhabi. The average post mortem body weight was 97.5 kg. Before sampling, organs were weighed and tissue samples were taken using a stainless steel knife. One part of each sample was fixed with formalin 10% and sent to the Al-Qattara veterinary laboratory for histopathological findings. The other part was stored at -80°C until selenium analysis. Sampled organs taken at day 45 were lung, heart, liver, spleen, kidney, pancreas, suprarenal gland, shoulder and femoral muscle, anterior limb bones and posterior limb bones. Same organs were taken at day 90 with addition of brain, intercostal muscles, diaphragm muscle and urinary bladder.

Laboratory analysis

Before selenium analysis, samples of feed, water, serum, organs, hair, urine and faeces were digested to release the element from protein bound. In microwave rotator tubes, 2 ml serum of each sample was mixed with 10 ml nitric acid (HNO_3) and 5 ml perchloric acid ($HClO_4$). The acids used were of high purity grade. Digestion programme started using microwave digestion system Milestone MLS-1200 MEGA, Italy. After cooling, digested samples were transferred into flask and diluted to volume by adding deionised water and stored in a refrigerator until analysis in screw cap glass. The samples listed up were digested in the same way with consideration of sample weight and the corresponding acid quantities.

Selenium was determined in serum, organs, hair, faeces, urine, diet and water by Inductively Coupled argon Plasma – Atomic Emission Spectrometer (ICP-AES), Varian vista MPX-CCD, simultaneously, using 11 points of standard curve of Accu Trace™ Reference Standard solutions from Accustandard®, USA. Quality Control Standard no. 1 and Laboratory Performance Check standard have been used. Enzymatic activity of glutathione peroxidase (GSH-Px) in erythrocytes was assessed according to the method of Paglia and Valentine (1967) with commercial kit - Randox (Ransel ND ref RS 505) by Beckman Coulter DU 800 Spectrophotometer. It is expressed in international unit per gram of haemoglobin (IU/g Hb) where one international unit is equivalent to 1 µmole of NADPH oxidised per minute per gram of haemoglobin.

Plasma vitamin E (a-tocopherol) and its content in the basal diet was expressed in µg/ml. Evaluation was monitored in by High Performance Liquid Chromatography system (HPLC-Waters) (Hatam and Kayden, 1979).

Following biochemical parameters were determined: glucose (GLU), blood urea nitrogen (BUN), creatinine (CRE), total protein (TP), albumin (ALB), alkaline phosphatase (ALP), creatine kinase (CK), alanine transaminase (ALT), aspartate transaminase (AST), lactate dehydrogenase (LDH), gamma glutamyl transferase (GGT). Haematological parameters were also estimated [PCV, HB, WBC, and white cells formula (N.L.M.E)]. In addition, some minerals were determined (Cu, Zn, Fe, P, Na, K, Cl and CO_2). The haematology assessment was done with CELL – DYN 3700 system, (Abbot Diagnostics Division). Biochemistry and minerals parameters were analysed with clinical chemistry analyser – model: Dimension RXL – (Dade Behring – USA), except copper, estimated with atomic absorption spectrophotometer, model SHIMADZU AA 6800.

Organs preparation for anatomopathological findings

Tissue specimens were prepared by following 3 steps: (1); Tissues processing using Automatic tissue processor ATP 1 – Triangle Biomedical Sciences, Inc (TBS®), (2); Fixation by wax dispenser – thermo/ Electron Corporation UK and (3) Tissue staining with H & E - Haematoxylin (code H/ 0010 - FSA laboratory Suppliers UK) and Eosin water soluble (code 34027 - BDH chemicals LTD Poole, UK).

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), the effect of the supplementation level (3 levels) and of the day of sampling (7 levels for blood and plasma) were tested. Previously, normality of distribution was tested by the Skewness and Kurtosis test (test W). Interactions between other elements (minerals and biochemical parameters) were tested by Pearson's correlation.

Results

Selenium content in the basal diet and selenium intake

The selenium concentration was 0.49 in concentrates and 0.15 mg/kg in Rhodes grass. There was no Se in water. The daily feed intake was 2 kg of concentrate and 3 kg of grass on an average. Thus, the selenium intake provided by the diet was 1.43 mg per day for camels during all the experiment, the mineral mixture providing 8, 12 and 16 mg of selenium per day. According to the treatment, the total quantity of selenium provided in the diet was 9.4 mg/day for camel in group 1, 13.4 mg in group 2 and 17.4 mg in group 3, respectively. So, the dietary Se concentration varied between 1.7 ppm (group 1) and 3.5 ppm (group 3) DM. Elsewhere, the basal diet provided vitamin E 5.5 µg/g in Rhodes grass and 0.96 µg/g in concentrate i.e., 18.4 µg per day on average.

Mean values of selenium in serum, faeces and urine

The mean value of selenium in serum was 358.3 ± 210.8 ng/ml (n=69) and varied between 16.3 and 899.8 ng/ml. The mean values of selenium in serum was 321.2 ± 140.5 ng/ml in group 1 (8 mg Se), 443.2 ± 231.1 ng/ml in group 2 (12 mg Se) and 298.04 ± 212.13 ng/ml in group 3 receiving 16 mg Se daily. The bi-weekly change showed a significant increase (P>0.001) from fortnight 2 up to the end of the experiment for group 1 and 2 and up to fortnight 3 for group 3 with a value of 767.15 ng/ml. Serum Se concentration decreased significantly in fortnight 4 in group 3 up to the end of the trial to reach a value of 129.86 ng/ml when Se supplementation was stopped at the time of selenosis symptoms appeared (Fig 1). A significant difference occurred between the 3 groups in fortnights 2 to 7. The maximum value (899.87 ng/ml) was observed in group 2 at fortnight 6 and the minimum (16.33 ng/ml) at the beginning of the trial in fortnight 1 at group 3.

On average, young camels excreted 1.09 ± 0.54 kg DM of faeces per day and 1.42 ± 0.74 L urine per

day. Selenium concentration in urine and faeces varied between 33.2 and 2230.5 ng/ml with a mean value 646.6 ± 610.9 ng/ml and between 193.5 and 13487.4 ng/g DM with an average mean 2346.02 ± 2653.9 ng/g DM, respectively. The mean of total Se excreted was 918.45± 930.14 µg/day in urine and 1096.02 ± 549.8 µg/day in faeces. The urinary Se concentration was higher at month 3 in group 2 receiving 12 mg Selenium. Se concentration increased significantly starting from month 2 for 3 groups up to the end of the experiment for groups 1 (8 mg Se) and 2 (12 mg Se), but decreased at month 3 in group 3 (16 mg Se) when Se supplementation was stopped. The maximum value (1496 ng/ml) was observed at month 3 in group 2 and the minimum value (55 ng/ml) was observed at the beginning of the trial at month 1 in group 1. A significant difference was observed in 3 groups starting from month 2. Elsewhere, on an average the faecal or urinary Se total excretion increased significantly at Se supplementation time (Tables 1 and 2). The higher total Se excretion was in group 1 at month 4 with an average 1928 µg/g day. However, the higher value of faecal Se concentration was 7010 ng/g DM at month 2 in group 3. In the same group the lower value (202 ng/g DM) was observed at the beginning of the trial at month 1.

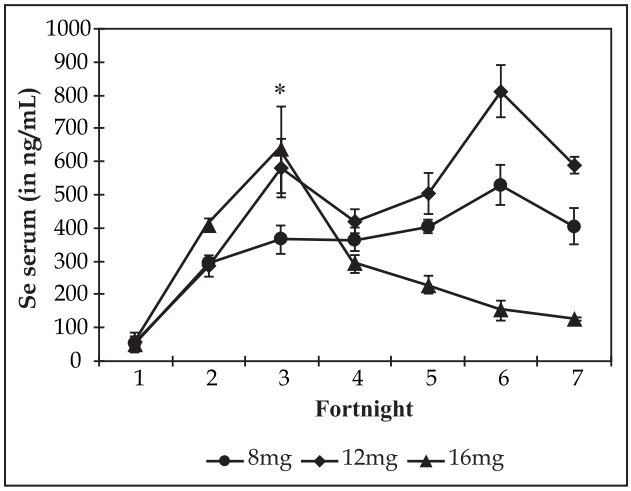

Fig 1. Changes in the Serum Se concentrations according to the selenium supplementation level in camel (Mean and S.E). The * points to the Se supplementation stopping in group 3.

Correlations between serum Se, urinary and faecal Se

Selenium concentration in serum was highly correlated with Se concentration in urine, with total Se excreted in urine and Se concentration in faeces (P<0.001) but not with total urine excreted. Total urinary Se excreted was highly correlated with urinary Se concentration (P<0.0001) and with Se concentration in faeces (P<0.001) but in a lesser extent with the total faecal quantity excreted (P<0.05). Urinary Se excreted was also highly correlated with Se concentration in faeces (P<0.0001). The quantity of Se excreted in faeces was slightly correlated with the total urine excreted (P<0.05). (Table 3).

Mean values of GSH-Px

The GSH-Px activity varied between 26.85 and 174.16 IU/g Hb (n= 69) with a mean value of 79.32

Table 1. Mean and standard-deviation of total Se excretion in faeces (SeExcF in µg) and of Se concentration in faeces (SeConF in ng/g) according to the treatment (8, 12 or 16 mg Se supplementation) and of the time (in month).

Se suppl	SeExcF				SeConF			
	month							
	1	2	3	4	1	2	3	4
8 mg	640±508[a]	1130±573[a]	1467±808[b]	1928±262[b]	230±32[a]	3616±2581[b]	2805±1485[b]	1595±101[b]
12 mg	868±137	1150±317	1282±174	1357±176	225±24[a]	3963±1191[b]	2297±445[b]	2972±479[b]
16 mg	676±289	770±503	1367±301	1453±476	202±5[a]	**7010±3927**[b]	729±22[c]	517±58[c]

[a b c] On line means with different superscripts differ (P < 0.01) Significant differences (P < 0.01) on column are in bold

Table 2. Mean and standard-deviation of total Se excretion in urine (SeExcU in µg) and of Se concentration in urine (SeConU in ng/ml) according to the treatment (8, 12 or 16 mg Se supplementation) and of the time (in month).

Se suppl	SeExcF				SeConF			
	month							
	1	2	3	4	1	2	3	4
8 mg	73± 34[a]	1432±637[b]	**800±67**[b]	1286±332[b]	55±12[a]	782±147[b]	**517±93**[b]	694±115[b]
12 mg	86±24[a]	2231±946[b]	**1239±858**[c]	1576±813[c]	58±18[a]	1047±580[b]	***1496±541***[b]	1472±296[b]
16 mg	63±32[a]	1575±528[b]	101.9±38[a]	91±26[a]	60±12[a]	1215±317[b]	122±9[a]	131±16[a]

[a b c] On line means with different superscripts differ (P < 0.01) Significant differences (P < 0.01) on column are in bold or italic.

Table 3. Correlation coefficient between total Se excretion in faeces (SeExcF), urine (SeExcU), Se concentration in faeces (SeConF), urine (SeConU) and serum Se concentration.

Variables	SeConF	SeConU	SeExcF	SeExcU	Se serum
SeConF	1				
SeConU	0.625**	1			
SeExcF	0.045	0.150	1		
SeExcU	0.498**	0.668**	0.239*	1	
Se serum	0.679*	0.698**	0.193	0.154	1

* P < 0.05; ** P < 0.01

± 30.94 IU/ g Hb. The mean value was significantly higher at the fortnight 5. GSH-Px activity increased starting from fortnight 2. The higher value (121.38 IU/ g Hb) was reported at fortnight 6 in group 1 receiving 8 mg Se daily and the lower value (30.07 IU/ g Hb) was observed at fortnight 1 in at the beginning of the trial in group 3 (Fig 2).

Mean values of vitamin E

The plasma vitamin E (α- tocopherol) was on average 0.68 ± 0.36 µg/ml and varied between 0.20 and 1.56 µg/ml (n=69). There was a significant difference between 3 groups, the maximum value being observed at fortnight 5 in group 1 and the minimum was reported at fortnight 7 in group 2 (Fig 3).

Correlations between serum Se, GSH-Px activity and vitamin E

Se in serum was highly correlated with GSH-Px (r = 0.709; P<0.001) but was not correlated with vitamin E.

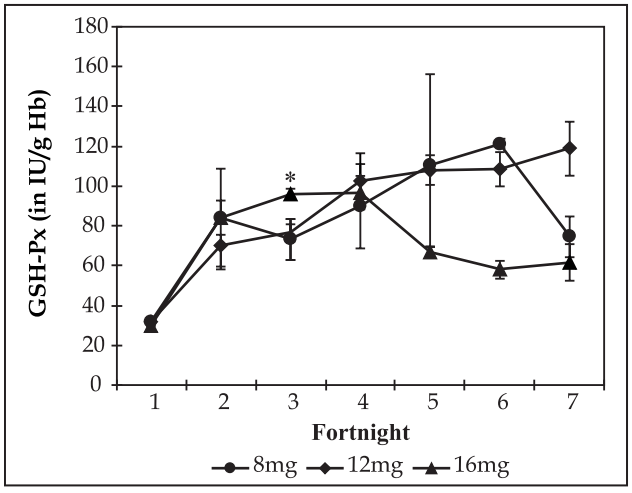

Fig 2. Changes in the glutathione-peroxidase according to the selenium supplementation level in camel (Mean and S.E.). The * points to the Se supplementation stopping in group 3.

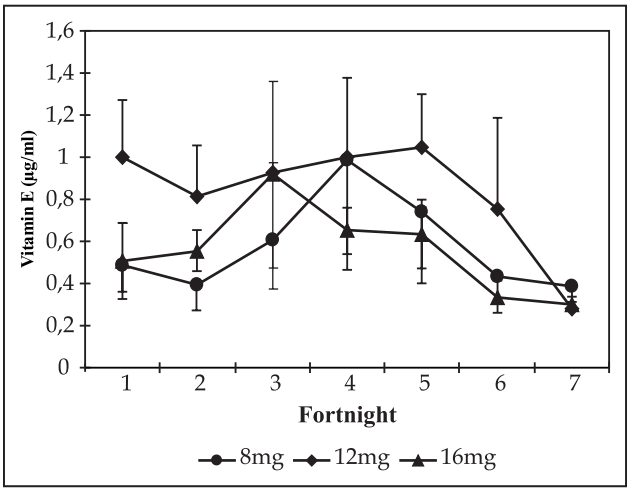

Fig 3. Changes in the vitamin E according to the selenium supplementation level in camel (Mean and S.E.).

Correlations with other blood parameters

Se concentration in serum was not correlated with biochemical and mineral parameters but slightly with TP, WBC, platelets, neutrophils and eosinophils (P<0.05). A high negative correlation was observed with lymphocytes percentage (L) (P<0.0001), GSH-Px was also highly negatively correlated with (L) (P<0.0001) and less positively correlated to PCV, RBC (P<0.05). A negative correlation occurred with phosphorus (P). GSH-Px was highly positively correlated with creatinine (P<0.001) and negatively with AST, LDH (P<0.05). Vitamin E was negatively correlated with creatinine and positively with TP, albumin (P<0.05). No correlations to minerals and haematological parameters except platelets (P<0.05) were observed for vitamin E (Table 4).

Correlation with physiological parameters (body temperature, respiratory rate, pulse rate)

Se concentration in serum was highly correlated with the respiratory rate (P<0.001) and less with body temperature and pulse rate (P<0.01). GSH-Px as well was highly correlated with respiratory rate (P<0.001) and less with pulse rate. Vitamin E was not correlated to physiological parameters. On average, the body temperature varied between 36.54°C and 40.04°C with a mean value of 37.77 ± 0.53°C. Respiratory rate was 14.0 ± 2.5 and varied between 9 and 20. Pulse rate varied between 38 and 72 with a mean value of 54.6 ± 5.9. Body temperature was highly correlated to respiratory rate (P<0.001) and to pulse rate (P<0.001). Respiratory rate was highly correlated to pulse rate (P<0.001) and with the environmental temperature (P<0.01).

Table 4. Correlation coefficients between Se serum concentration, GSH-Px and vitamin E with blood parameters.

Variables	Se serum	GSH-Px	Vitamin E
PCV	0.022	-0.284	0.157
HB	0.034	0.270	0.198
RBC	-0.087	-0.345	0.108
Platelets	0.366	0.107	0.296
WBC	0.287	0.131	0.213
WBC / N	-0.235	-0.136	0.005
WBC / L	-0.572	-0.662	-0.042
WBC / M	-0.270	-0.209	-0.122
WBC/ E	-0.280	0.202	0.164
Cu	0.005	-0.156	-0.071
Zn	0.232	0.217	0.108
Fe	0.123	-0.040	0.163
Ca	0.127	-0.049	0.068
Na	-0.107	-0.135	0.080
K	-0.195	-0.133	-0.003
P	-0.068	-0.252	-0.149
Bilirubin	-0.053	0.191	0.037
Glucose	-0.116	-0.180	-0.123
Creatinine	0.207	0.541	-0.247
Total protein	0.254	0.079	0.328
Albumin	0.139	-0.074	0.248
ALP	0.002	0.071	0.028
CK	-0.160	0.203	0.052
ALT	-0.134	-0.215	0.043
AST	-0.206	-0.270	0.014
LDH	-0.156	-0.245	0.012

Selenium content in organs

The highest total quantity of Se was observed in liver, kidney, spleen, lung, heart, pancreas, brain and ovary, respectively (Fig 4). The maximum level of Se concentration was observed in camel's liver from group 2 (1420.3 ng/g). No change in Se concentration was observed between groups in kidney and brain. High Se concentration in ovary and hair was observed in group 3. Between day 45 and 90, the Se concentration in liver was 2-fold in group 1 (from 786 to 1559 ng/g) as well as in group 2 (from 965 to 1874 ng/g). In group 3, the values decreased in the same proportion (from 1622 to 889 ng/g) showing the rapid depletion of Se after stopping supplementation. These changes were similar in kidney but at a lower proportion: from 731 to 1089 (group 1), from 903 to 1140 (group 2) and from 1241 to 813 ng/g (group 3). The changes in other organs were not so clear.

Clinical symptoms

The first clinical sign appeared within 2 weeks. A hair discolouration was shown followed by alopecia of different intensity in groups. It started on the abdomen for group receiving 8 mg, at the base of the neck and on all over the body for camels receiving 12 mg and 16 mg Se daily. At this time body temperature was still in the range (36.67°–37.09°C) for group 1 and 2. But it increased in animals treated with 16 mg (39.13°C). Hypertrophy of the inferior cervical lymph node was seen in 3 groups but was more prominent in the group 3. Camels tended to sit alone. In groups 2 and 3, the urinary excretion increased and dark watery diarrhoea was also observed. Young camel showed a loss of appetite, thus loss of weight and weakness appeared. Camels from group 3 showed an increased urine excretion and dark fluid diarrhoea as well. Tears with pale mucous membranae were shown as well as an evidence of impairment vision. The alopecia was complete and skin became rough. Dyspnoeic respiration and pain at auscultation appeared and camel adopted the sternal decubitus position and tended to rest its neck extended (Fig 5). Salivation occurred and finally camels showed no desire to eat and drink. The tail was elevated. Fissured pads appeared in all groups but were more pronounced in group treated with 12 mg and 16 mg. Consequently, camels found difficulties in walking (Fig 6). The body temperature was elevated to 38°C in group 1 and 2 and reached 40°C in group 3. Se supplementation was stopped at week 3 for all animals of group 3 and treated, and then camels returned progressively within several weeks to the normal under intensive care. But Se was continued in the other 2 groups for one month.

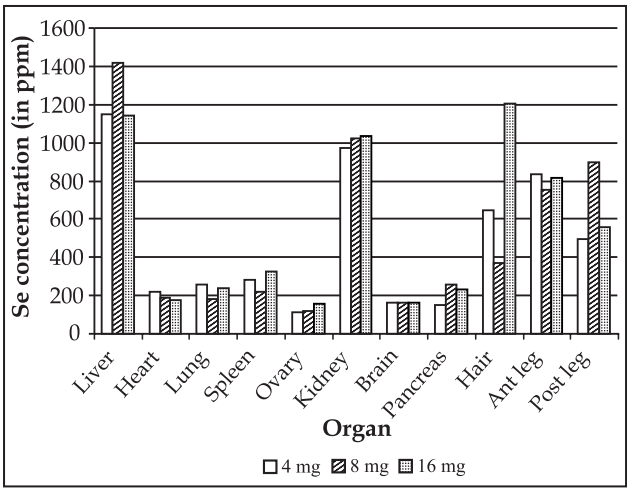

Fig 4. Selenium concentration (in ppm) in the different organs of camel after high Se supplementation (mean of values on slaughtered animals at day 45 and day 90)

At fortnight 5, camels in group 1 and 2 developed a vesicular stomatitis and were treated but selenium supplementation was maintained. Body temperature for group 1 and 2 remained constant.

Post mortem and histopathological findings

Macro lesions

After slaughtering, camels in all 3 groups showed paleness in all abdominal muscles, diaphragm and intercostal muscles, hydrothorax (Fig 7) and pulmonary emphysema. The texture of liver and lung was not uniform. Heart, liver and kidney were congested and necrosed. In addition to prior lesions, camels receiving 12 mg Se showed a flap heart with necrosis and congestion (Fig 8). However, heart was partially white (fibrosis), congested and necrosed in camel treated with 8 mg. Hepatomegaly was observed in 3 treated groups, while pancreas was atrophied. Brain oedema was also observed in all treatment.

Histopathology lesions

Group treated with 8 mg Se daily: kidney showed eosinophilic granulated material in diluted Bowman's space and convoluted tubules in addition to degenerative changes in epithelial lining cells (Fig 9). Vacuolar degenerative changes were seen in convoluted tubules of cortex and collecting tubules of medulla. Severe vascular congestion in medulla zone occurred. Oedematous fluid was seen in between the muscular fibres and slight congestion of blood capillaries in heart. Vacuolar degenerative changes were observed in few hepatic cells, congestion in central hepatic vein and hepatic sinusoids. In addition, focal areas of muscular hyalinisation (non –inflammatory) and oedema were observed in intercostal and diaphragm muscles**.** Activation in lymphoid follicle was seen in cervical anterior lymph nodes.

Group treated with 12 mg Se daily: kidney lesions showed congestion in blood capillaries of cortex and medulla, degenerative changes in lining epithelial cells of convoluted tubules. Granulated eosinophilic material (albumin) was observed in Bowman's spaces and convoluted tubules' lumens. Heart blood capillaries were congested and hypotrophied cardiac muscle fibres were observed and became more eosinophilic and dispersed in oedema fluid with pycnotic nuclei (non–inflammatory myocardial dystrophy). Hepatic sinusoids congestion with degenerative changes in hepatic cells especially in periportal zone (vacuolar degeneration changes was observed in Fig 10). However, non-inflammatory hyalinisation in focal area (hyaline degeneration) was reported in shoulder muscle. Focal haemorrhagic areas were observed all over the lymphatic tissues of cervical anterior lymph node. Lesions were extended to other tissues with severe vacuolar degeneration in epithelial lining in urinary bladder and sub-capsular focal haemorrhagic areas in spleen.

Group treated with 16 mg Se daily: albuminous material in dilated Bowman's capsule, congestion of blood capillaries of few numbers of glomeruli, an increase in overall glomerular cellularity in few glomeruli. The epithelium lining of convoluted tubules were swollen and more eosinophilic. Congestion of blood capillaries in medulla tissues, and severe degenerative changes were seen in epithelial cells lining of convoluted tubules and collecting tubules with congestion of renal capillaries. Eosinophilic granulated material appeared in diluted Bowman's space and renal tubules. The glomerular tufts disappeared from few Bowman's capsules. Other severe vacuolar degenerative changes appeared in the epithelial cells lining of urinary bladder. Heart showed proliferation of Purkinje fibres, capillaries congestion in Purkinje fibres tissues and sub-endocardial tissues, degenerative changes in myofibres. The cardiac tissues showed edematous fluid between more eosinophilic thick myocardial fibres (Fig 11). There was few leukocytic infiltration and congested blood capillaries were seen, as well as focal necrotic area. Vacuolar degenerative changes were observed all over the hepatic cells of the hepatic lobules. There was infiltration of few inflammatory cells in hepatic sinusoids and slight activation of Kupffer's cells. Non-inflammatory focal necrotic areas and fibrosis were seen all over the pancreatic tissues especially near the pancreatic ductule (macroscopic ducts). These necrotic areas were infiltrated with few inflammatory cells of fibrosis (Fig 12). In addition, focal coagulative necrosis areas appeared in pancreatic acini. Hyaline degeneration of myofibres and oedema was also observed in shoulder and intercostal muscle. Severe proliferation of lymphoid follicles and greenish material was engulfed with macrophage (haemosiderine) in cervical anterior lymph nodes. Focal haemorrhagic areas and blackish green fine granules accumulation were observed in focal areas of spleen. Brain showed perivascular oedema in brain.

Discussion

Poisoning threshold

The basal diet supplied to camels with 1.43 mg Se per day, i.e. 0.28 mg/kg DM was considered approximately the requirements for daily cattle (NRC,

Fig 5. Camel in sternal decubitus position with neck extended on ground.

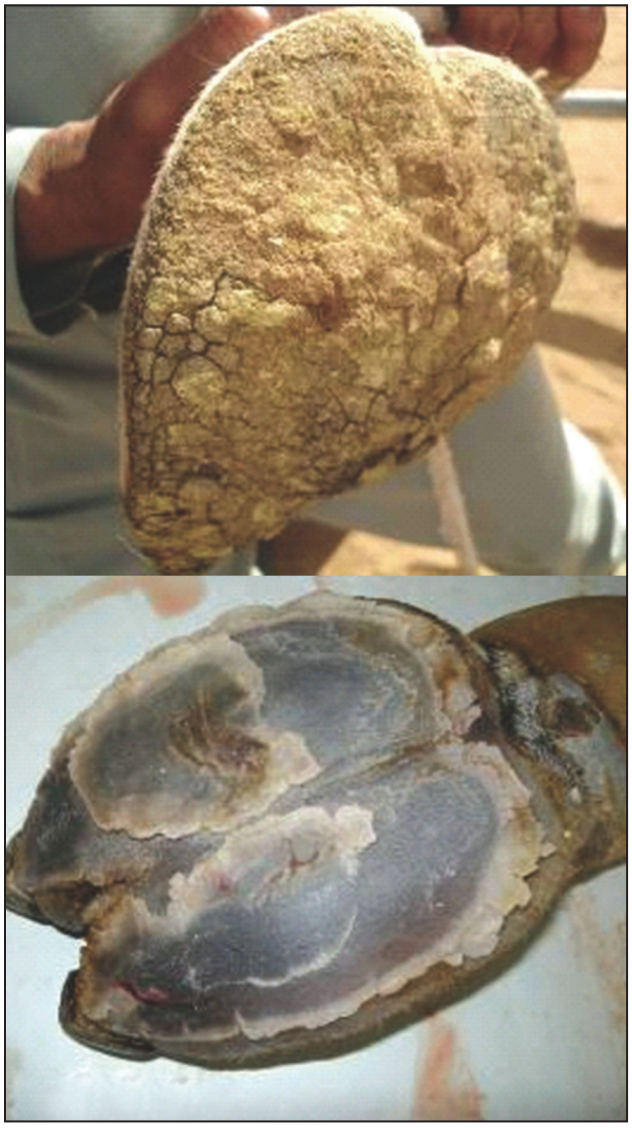

Fig 6. Fissured pads with necrosis on foot of camel receiving 16 mg Se/day.

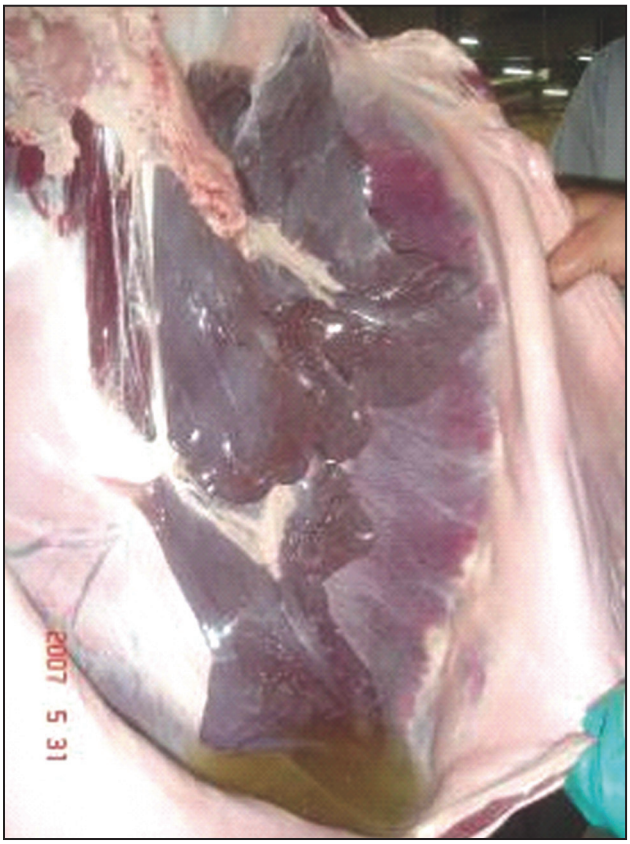

Fig 7. Hydrothorax in camel receiving 16 mg selenium daily for 45 days.

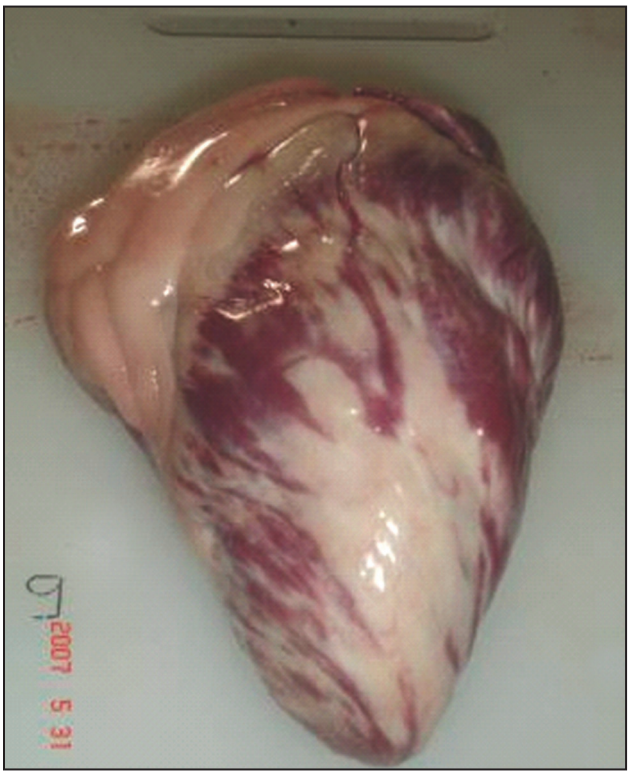

Fig 8. Heart discolouration and congestion in camel receiving 8 mg selenium per day for 45 days.

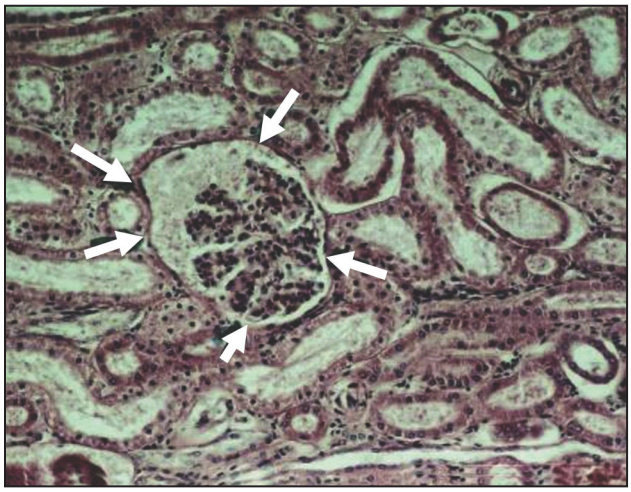

Fig 9. Eosinophilic granulated material in diluted Bowman's space and convoluted tubules in addition to degenerative changes in epithelial lining cells (camel receiving 8 mg Se/day).

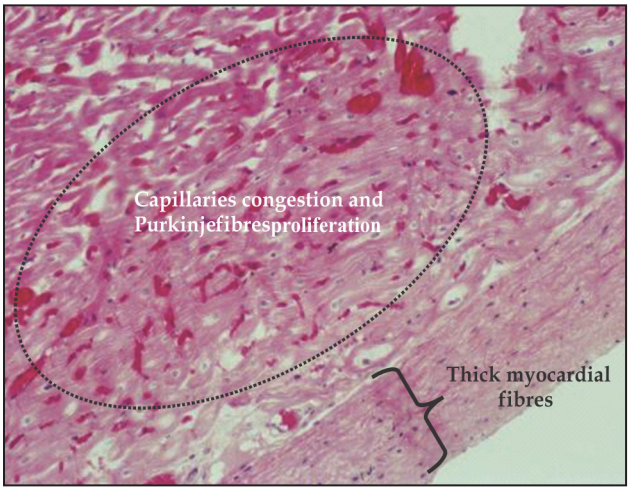

Fig 11. Proliferation of Purkinje fibres, capillaries congestion in Purkinje fibres tissues and sub-endocardial tissues, degenerative changes in myofibres in cardiac tissues of camel receiving 16 mg Se/day.

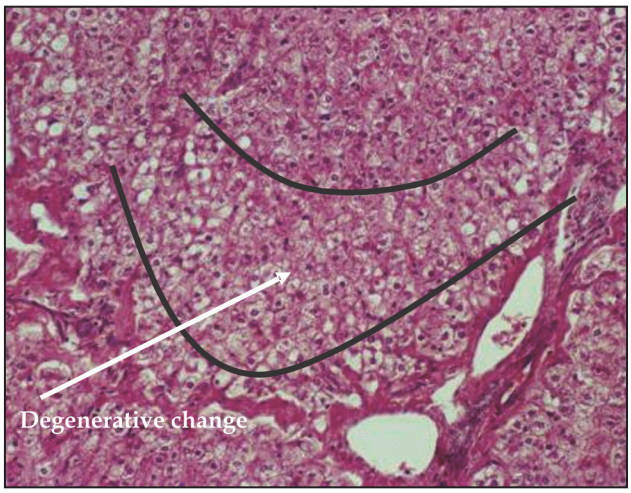

Fig 10. Hepatic sinusoids congestion with degenerative changes in hepatic cells in periportal zone in camel receiving 12 mg Se/day.

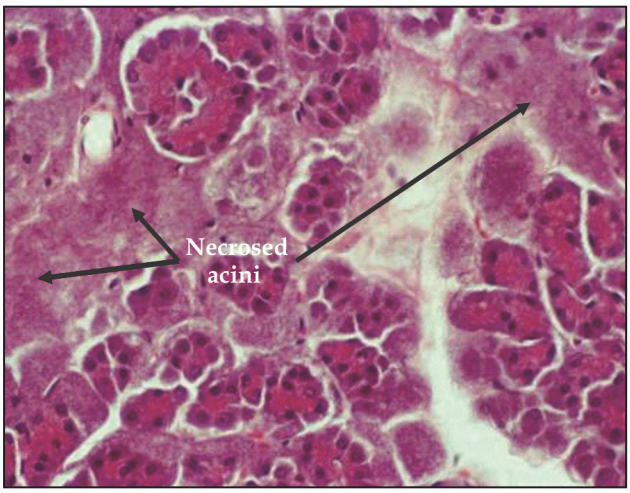

Fig 12. Non-inflammatory focal necrotic areas and fibrosis in the pancreatic tissues of camel receiving 16 mg Se/day.

2000). However, according to the mean weight of the camel in our study (183 kg), the selenium supply with the basal diet was 0.78 mg/100 kg LW. This was lower than recommendations for beef cattle (1 mg/100 kg LW). Selenium is needed in small amounts. The minimum level of selenium in diet that causes chronic selenosis in most animal species is 4-5 mg/kg DM (US NAS/ NRC, 1976) and the minimum level needed to prevent deficiency is 0.02 – 0.05 mg/kg DM (US NAS/ NRC, 1971). As Se deficiencies have been reported in United Arab Emirates, camel's owners supplement their animals to avoid deficiency with a commercial salt mixture and pharmaceutical form by drench or injection. However, no data on camel selenosis has been reported so far.

Chronic Se poisoning is not limited to grazing livestock and can occur from consumption of high Se intake in feed. Oral ingestion of 1 to 2.2 mg of Se/kg LW as sodium selenite has caused appreciable mortality in lambs up to 14 weeks of age (Gabbedy, 1970), but individual susceptibility to selenium toxicosis could be highly variable. Tiwary *et al* (2006) did not observe lamb mortality with an oral sodium selenite up to 4 mg/kg LW. For other authors, the oral median lethal dose (LD_{50}) of sodium selenite has been reported to be 1.9 ±1.2 mg of Se/kg LW (Lambourne and Mason, 1969; Caravaggi *et al,* 1970; Blodgett and Bevill, 1987). A daily intake of 0.25 mg/kg LW was considered as toxic for sheep and cattle (Muth and Binns, 1964). These levels listed previously are higher

than our dietary levels in the present study (0.051 to 0.095 mg/kg LW), which seems to show a high sensitivity of camel species to Se toxicosis. The levels of selenium requirement and toxicity could be very close.

The selenium poisoning was observed with diet containing 44 mg/kg DM for horses and 11 mg/kg DM in pig (Muth and Binns, 1964). Typical lesions of chronic selenium toxicosis were observed on young cattle receiving more than 5 mg/kg DM for 120 days (O'Toole and Raisbeck, 1995). In our study, the first lesions appeared with a selenium intake of approximately 2.5 mg/kg DM.

Blood parameters values

Selenium level in serum and GSH-Px activity in red blood cells reflected usually the dietary Se concentration, and for the group 3, the stopping of Se supplementation was clearly linked to a rapid decreasing of these parameters in blood.

The mean concentration of serum selenium reported in the literature for large animals was 100 ng/ml. This value was generally considered as sufficient for the maintenance of suitable metabolic functions (Maas *et al,* 1990). In camel, the normal values could be considered similar, between 97 and 134 ng/ml (Hamliri *et al,* 1990; Liu *et al,* 1994; Barri and Al-Sultan, 2007). In camel 2 mg daily supplementation, increased the serum selenium value up to 200.4 ng/100 ml (Bengoumi *et al,* 1998). In Sultanate of Oman (Faye, unpublished data), high serum value (281 ng/ml) was observed in camels suspected to be intoxicated with selenium. In a previous study (Seboussi *et al,* 2008) mean values of 301.1 ± 84.1 and 370.8 ± 118.8 ng/ml in camels receiving a daily Se supplementation of 2 and 4 mg, respectively was reported.

In intoxicated lambs with 4 mg/kg LW under sodium selenite form (4 times higher than the group 3 in our experiment); the serum Se increased up to 274 ng/ml only (Tiwary *et al,* 2006). After one month supplementation with 12 ppm Se in the diet, pregnant cattle showed Se values in serum above 420 ng/ml (Yaeger *et al,* 1998). Higher values up to 1500 ng/ml were reported on large animals grazing on seleniferous pastures (Raisbeck *et al,* 1993). In lambs, with diet containing 10 ppm of selenium (Cristaldi *et al,* 2005), no toxicity was observed after one year and the selenium values reached 0.39 ppm in serum (390 ng/ml) after 12 weeks (comparative to our results: after 90 days, 519 ± 97 ng/ml for groups 1 and 2 receiving 2.5 and 3.5 ppm Se in the diet, respectively).

GSH-Px was considered usually as an indicator of selenium status in a variety of species (Ganther *et al,* 1976). Few references were available on camel. The values reported in Morocco were 25.8 IU/g Hb (Hamliri *et al,* 1990) and 51.6 IU/g Hb (Bengoumi *et al*, 1998) and these were not far from our results at the beginning of the experiment (31.3 ± 2.5 IU/g Hb), but quite lower than values reported in Spain 298.1 IU/g Hb in female camel (Corbera *et al,* 2003). The higher reported value in our study was 174.16 IU/g Hb only. In Australia, the value of GSH-Px evaluated in camel was 85.8±14.75 IU/g Hb (Agar and Suzuki, 1982). In a previous study on supplemented camels with 4 mg Se daily (Seboussi *et al,* 2008), GSH-Px value reached 103.1 IU/g Hb only. In case of intoxication, GSH-Px values reached a plateau and did not increase significantly. So the determination of GSH-Px could not be used as an indicator of Se intoxication (Richard *et al,* 1997). Usually, determination of GSH-Px activity is a good indicator of Se status of the animal. But in case of Se overexposure, there was an initial rise in the GSH-Px activity, that later stabilised with no further increase (Deore *et al,* 2005).

There was no significant effect of Se supplementation on vitamin E plasma level. At reverse, the high level of selenium seemed to depress the vitamin E level in plasma (the mean values at the end of the experiment was 0.32 ± 0.05 ng/ml) as it was recently observed in horse affected by selenosis (Crain, 2007). Selenium and vitamin E are antioxidants, both protecting the membranes from oxidative damage. Due to this shared duty, there is a relationship between the compounds, in which one can substitute for the other in a very small way. For instance, more Se is needed when an animal's vitamin E concentrations are low. The sparing effect is an extension of this idea of substitution. In lambs, sodium selenite administration resulted in decreased liver vitamin E concentration (Tiwary *et al,* 2006). The mean value observed in our experiment (0.68 ± 0.36 ng/ml) was similar than results from literature. In Sudan, plasma vitamin E on young camels varied between 0.3 and 1.65 ng/ml (Barri and Al-Sultan, 2005). Comparable references were reported by Al-Senaidy (1996) and Mousa *et al* (2006). Those values were lower than those reported in cattle (Nozières *et al,* 2004).

Relationships with other blood components

The negative correlation with lymphocytes could be explained by the interferences between high selenium level in an organism and the cellular events

responsible for an immune response. Elevated Se has been shown to promote peroxidative damage *in vitro* and *in vivo* systems. Lymphocyte cell membranes are especially, susceptible to free radical damage (Bjornstedt *et al,* 1996). The lack of relationships with RBC and haematocrit was in relation with the absence of anaemia in herbivores in case of selenosis (Jenkins and Hidiroglou, 1986).

The LDH and AST activity increased usually in case of muscular suffering or myocarditis. So, the positive correlation between GSH-Px and enzymes LDH and AST could be linked to the heart damage. Elsewhere, the mean values of all the enzymes (LDH, AST and ALT) were all above the normal range in camel (Bengoumi *et al,* 1997). Surprisingly, the CK did not increase, in spite of its role as an indicator of muscular dystrophy. Similar observation was reported on calves receiving high dietary Se (Jenkins and Hidiroglou, 1986). The lack of relationship between Se and other trace elements as Cu and Zn was not in accordance with previous results in camel receiving normal Se quantity (Seboussi *et al,* 2004).

Physiological parameters

The normal camel temperature was ranged between 35 to 38.6°C, the pulse rate 45 to 50 pulse per minute and the respiratory rate 5 to 12 per minute. According to these references, camels in our study showed high body temperature, especially in group 3 treated with 16 mg, high pulse rate and respiratory rate. The high correlation between serum Se and respiratory rate was explained by the respiratory tract failure linked to the selenosis. The respiration became dyspnoeic and labourious. Such symptoms were reported in Wyoming in cattle suffering from selenosis (Beath, 1982).

Selenium in hair and organs

After absorption, Se was distributed in different organs. Heart, erythrocytes, pancreas, liver, kidney, stomach and gastrointestinal tract mucosa tend to accumulate normally Se compounds (Hanson and Jacobson, 1966).

In all the cases, the liver contained higher level of Se than kidney, which was consistent with observations in sheep (Cristaldi *et al,* 2005; Ewan *et al,* 1968). Upon our results, the liver seemed the target organ in highly dietary Se intake as reported previously by Smyth *et al* (1990). It was reported in non Se supplemented sheep that higher Se level was observed in kidney rather than in liver, but in case of Se supplementation, the liver Se concentration increased disproportionaly (Clark *et al,* 1996).

In camel, only one reference was available for selenium concentration in organs (Ma, 1995). In this study, kidney (3100 to 3900 µg/kg), liver and heart (1100 to 1500 µg/kg), muscle and brain (620 to 640 µg/kg) were the organs with the higher Se concentration. Those values, except for liver and muscle, appeared much higher than our results, in spite of the normal Se status of the camels. In the selenium tolerance trial achieved on lamb liver had the highest Se concentration (up to 2000 µg/kg) followed by the kidney (around 1000 µg/kg) (Cristaldi *et al,* 2005). In another study on sheep, selenium was found in the highest concentration in the kidney, followed by the liver, pancreas, heart and skeletal muscle (Combs and Combs, 1986). No linear trend of the liver Se concentrations according to the Se supplementation level was observed in lamb (Cristaldi *et al,* 2005). In calf receiving 3 ppm dietary Se treatment, the Se concentrations were 4740 µg/kg in liver, 3420 in kidney, 1380 in heart and 340 in muscle (Jenkins and Hidiroglou, 1986). Contrary to Cristaldi *et al* (2005), a regular increase of Se concentration with dietary Se level was observed by these authors. According to them, kidney was the major organ involved in the storage of selenium at low Se supplementation, but at high intakes, the liver became the target organ. In case of chronic selenosis, the sheep hepatic levels of selenium were about 20 to 30 mg/kg (Blood and Radostits, 1989), i.e. 12 to 18 times higher than in our results.

Few references on selenium concentration in hair were available in camel. In the wool of bactrian camel from China, Liu *et al* (1994) reported values between 140 and 190 µg/kg according to their physiological status. Similar results were published by Ma (1995) and values were 190 to 210 µg/kg. Those values were widely below our results, i.e. 740.9 µg/kg on average with a range of 301.4 to 1971.8 µg/kg. In lamb, the wool Se concentration varied between 500 and 2500 µg/kg according to the dietary Se level (Cristaldi *et al,* 2005). The hair appeared as the very sensitive organ to Se supplementation as it was reported in lamb (Cristaldi *et al,* 2005) and cattle (Perry *et al,* 1976). However, as for other minerals, the selenium concentration in hair is of limited interest, especially, because the variability is very high (Combs and Combs, 1986).

Clinical and necropsy findings

The clinical symptoms showed in this study were in accordance with previous signs observed in chronic poisoning in other species (Casteel *et*

al, 1985; Harrison *et al,* 1983; Beath, 1982; Tiwary *et al,* 2006). When selenosis injury occurred, the selenium accumulated mainly in the circulatory and respiratory system, as well as, in the organs of elimination (Beath, 1982). which is evident by lesions observed in heart, lung, liver, kidney and urinary bladder. After the liver, kidney (particularly the cortex) retained the highest concentration followed by the glandular tissues, especially, pancreas and pituitary (IARC, 1975). This explained the high Se level in kidney, the lesions occurring in cortex and medulla, the degenerative changes and necrosis found in the current study. The gross and histological lesions reported in camel were comparable to those observed in lambs (Tiwary *et al,* 2006) and suggest that the heart, as target organ of selenium intoxication, failed, leading to pulmonary oedema and hydrothorax (Lambourne and Mason, 1969).

The foot lesions with the necrosis of keratocytes were comparable to those observed in alkali disease (chronic selenosis) in cattle (O'Toole and Raisbeck, 1995) and horse (Raisbeck *et al,* 1993) in spite of the lack of hooves in camel.

Selenium deficiencies in animal, including camel, can also result in damages to the liver, heart, kidney and skeletal muscle (Hammond and Beliles, 1980; El Khouly *et al,* 2001). Comparable necropsy lesions were reported on Se deficiency and toxicity. The lack or the excess of selenium seems leading to similar cell damage.

Conclusion

The main conclusion of this description of selenium intoxication in camel for the first time was the high susceptibility of this species to selenosis. Young camels started showing toxicity symptoms at dose of 8 mg Se daily within 3 weeks under sodium selenite form. According to dietary Se supply and to mean weight of the animal from the group 1, 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. Those values were 5 times less than in sheep and cattle. According to such results, it could be important 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.

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