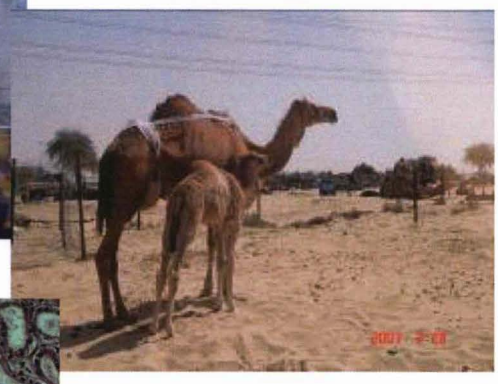
MISSION D'APPUI A LA THESE DE RABIHA SEBOUSSI

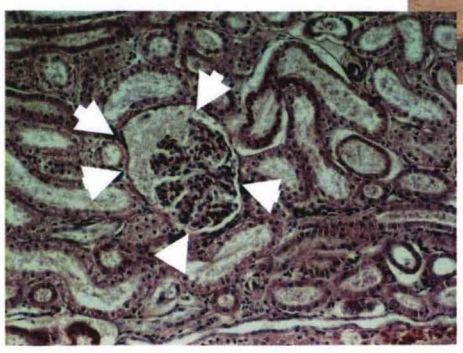
EMIRATS ARABES UNIS

« Métabolisme du Sélénium chez le dromadaire »

4 au 13 mars 2008

Bernard FAYE





Mars 2008

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RESUME

Cette mission était la dernière en appui à la thèse de Rabiha SEBOUSSI sur le métabolisme du sélénium chez le dromadaire. Actuellement en phase de rédaction finale, le travail de thèse est en cours d'achèvement pour une soutenance prévue en juin prochain. La mission a donc été essentiellement consacrée à la rédaction d'articles présentant les principaux résultats. Après une publication acceptée et publiée dans *Biological Trace element Research*, trois autres publications ont été soumises dans *Journal of Veterinary Pharmacology and Therapeuthics* , *Journal of Applied Research in Veterinary Medicine*, and *Journal of Dairy Sciences.*

Par ailleurs, des contacts ont été pris avec l'entreprise française SkuldTech basée à Montpellier, qui souhaite développée une collaboration sur place aux Emirats sur la production laitière caméline (identification des gènes d'intérêt zootechnique).

La poursuite de la collaboration est assurée par une nouvelle thèse et la gestion de l'ISOCARD.

REMERCIEMENTS

J'adresse mes sincères remerciements au collège « Food and agriculture » et à son Doyen, le Dr ALHADRAMI, pour son accueil et sa motivation toujours aussi forte.

Merci aussi à Rabiha SEBOUSSI pour son courage. Je tiens à remercier le Service de Coopération de l'Ambassade de France pour son appui indéfectible.

1 - INTRODUCTION

Dernière mission d'appui pour la thèse en cours avec l'Université des Emirats Arabes Unis, il s'agissait de travailler à la rédaction des articles issus de la thèse, à la correction des différentes parties de la thèse et à la préparation de la soutenance.

2 - RAPPORT D'AVANCEMENT DE LA THESE

Le travail de thèse en est à sa phase finale de rédaction, toutes les analyses ayant été traitées. Pendant la mission, j'ai corrigé la partie introductive, la partie bibliographique ainsi que la partie « matériels et méthodes». Au cours de la mission, la partie discussion a bien avancé et les corrections devraient pouvoir être apportées dans les temps. La partie résultats se faisant sous forme d'articles, nous avons surtout consacré du temps à leur rédaction. Rappelons que plusieurs publications ont été déjà réalisées :

1. FAYE B., SEBOUSSI R., ASKAR M., 2005. *Trace elements and heavy metals in healthy camel blood of United arab Emirates.* J. Camel Res. Pract., 12, 1-6
2. SEBOUSSI R., FAYE B., ALHADRAMI G., 2004. *Facteurs de variation de quelques éléments trace (sélénium, cuivre, zinc) et d'enzymes témoins de la souffrance musculaire (CPK, ALT et AST) dans le sérum du dromadaire (Camelus dromedarius) aux Emirats Arabes Unis.* Rev. Elev. Med. Vét. Pays Trop., 57 (1-2), 87-9
3. SEBOUSSI R., ALHADRAMI G., **FAYE B.,** ASFOUR T., ELKHOULY A., ALBELKHALEK T., ALMASRI J., 2006. *Effect of age, sex, breed and physiological status on selenium (Se), copper (Cu), zinc (Zn) and enzymes indicators of muscular fatigue in dromedrary (Camelus dromedarius).* Proc. 1st Intl Conf. of the International Society of Camelid Research and Development (ISOCARD), Al-Ain, 15-17 April 2006, United Arab Emirates, Poster n°66.
4. SEBOUSSI R.,FAYE B., AL-HADRAMI G., ALKHOULY A., MOHAMED T., ASKAR M., HACEN K., 2007. *Selenium toxicity on camel.* Proc. Intl. Wokshop « Impact of pollution on livestock », B. Faye and Y. Syniavskiy Eds., Springer Publ., NATO series, Almaty (Kazakhstan) (sous presse)
5. SEBOUSSI R., **FAYE B.,** ALHADRAMI G., ASKAR M., IBRAHIM W., HASSAN K., MAHJOUB B., 2008. *Effect of different selenium supplementation levels on selenium status in camel.* Biol. Trace Elem. Res., DOI 10.1007/s12011-008-8107-x.
On line on http://www.springerlink.com/content/12331434458t652n/

Trois autres publications ont été rédigées et soumises. Il s'agit de :

1. **Selenium distribution in camel blood and organs for different levels of supplementation** (publication soumise dans *Journal of Applied Veterinary Medicine*).
   Auteurs: R. SEBOUSSI, B. FAYE, G. ALHADRAMI, M.ASKAR, W. IBRAHIM, K. HASSAN, G. MAHJOUB
2. **Chronic selenosis in camel (**publication soumise dans *Journal of Veterinary Pharmacology and Therapeutics*)
   Auteurs: R. SEBOUSSI, B. FAYE, G. ALHADRAMI, M.ASKAR, M. BENGOUMI, A. EL-KHOULY
3. Effect of **Selenium supplementation on blood status and milk, urine and fecal excretion in pregnant and lactating camel** (publication soumise dans *Journal of Dairy Sciences).*
   Auteurs: R. SEBOUSSI, B. FAYE, M.ASKAR, K. HASSAN, G. ALHADRAMI

Un document de synthèse à visée opérationnelle est également envisagé.

3 - PROPOSITIONS POUR LA POURSUITE DE LA COOPERATION

3-1. Stage Master au Laboratoire vétérinaire

Proposé en 2006, puis en 2007, ce stage pour un étudiant du Master EPSED de l'Université de Montpellier, n'a pu se faire car aucun stagiaire n'a pu être identifié. Cela consistait à mettre en œuvre un travail de conception de fiches « maladies du dromadaire » à partir de cas cliniques et d'études épidémiologiques s'appuyant sur des photos de lésions (y compris sur des données histopathologiques) et un descriptif des cas répertoriés. La fiche de stage est rappelée en annexe. Ces fiches pourront être les premiers éléments d'une « camelpédia » en ligne disponible sur le site web de l'ISOCARD (voir plus loin). Le directeur du laboratoire (Dr Tarik Asfour s'est montré favorable à cette initiative).

3-2. Supervision d'un nouveau travail de thèse

Un projet de thèse est en cours d'élaboration avec une ressortissante soudanaise travaillant au Laboratoire Central Vétérinaire à Al-Ain. La thèse porterait sur les paramètres hématologiques et biochimiques discriminants les principales maladies du dromadaire relevées sur les registres du laboratoire. L'inscription pourrait se faire soit à Montpellier, soit à Khartoum. Les aspects administratifs sont en cours d'instruction au niveau de l'Université des EAU.

3-3. Séjour prolongé à l'Université

Le Doyen du collège « Food and Agriculture» a reformulé une demande de participation à l'organisation d'une session de cours sur une période d'un ou deux mois. Cette période pourrait être mise à profit également pour valoriser le travail de thèse.

3-4. Coopération dans le domaine de la production laitière caméline

La société Skuldtech, une PME basée à Montpellier, est entrée en contact avec moi depuis deux ans pour entreprendre une collaboration avec les Emirats. Cette entreprise a mis au point un outil de diagnostic génomique pour la recherche des gènes candidats à certaines pathologies ou performances animales. Ayant déjà collaboré avec le CIRAD dans le domaine de l'identification de la sensibilité à la trypanosomose, cette société s'est intéressée au marché du dromadaire dans les pays du Golfe. Ayant échoué pour proposer son projet sur l'identification par marqueurs des futurs champions de course, le promoteur de cette entreprise, que j'ai rencontré lors de cette mission, se tourne vers les marqueurs de production laitière ce qui semble intéresser les partenaires émiriens ; A cette occasion, nous avons visité les fermes laitières d'Al-Ain et de Dubaï, très modernes (traite mécanique, ligne de pasteurisation et de transformation laitière, distribution en grandes surfaces). La coopération pourrait se poursuivre avec des expertises possibles pour le secteur privé, la société Skuldtech ayant désormais une implantation à Dubaï.

4 - L'ISOCARD

Issue de la conférence internationale tenue à Al-Ain en avril 2006, l'ISOCARD (*International Society of Camelid Research and Development*) dont j'assume la présidence (notre partenaire le Pr. AL-HADRAMI, Doyen du collège « Food and Agriculture » en assurant le secrétariat) doit organiser sa prochaine réunion du Comité exécutif en avril prochain à Djerba (Tunisie) afin de préparer le prochain congrès qui aura lieu en mars 2009.

5 - CONCLUSION

Avec la fin de la thèse dont la soutenance est prévue en juin, la coopération doit partir sur d'autres bases bien que plusieurs pistes aient été évoquées. Pour la suite, il est donc proposé les actions suivantes qui pourraient être appuyées à partir de 2009.

- ➔ **une mission d'appui pour la valorisation du travail de thèse** afin de rédiger un document de synthèse à visée opérationnelle (voyage + per diem)
- ➔ **un appui à un ou deux étudiants émiriens pour la participation au prochain congrès de l'ISOCARD**
- ➔ **un appui administratif pour un stage d'un étudiant du master PARC** (si un billet d'avion peut être pris en charge, ce serait optimal et sous réserve de trouver un candidat)

ANNEXES

ANNEXE 1

Calendrier de la mission et personnalités rencontrées

Calendrier de la mission et personnalités rencontrées

Mardi 5 mars

- ➢ Départ de Montpellier
- ➢ Arrivée à Al-Ain via Dubaï

Mercredi 6 mars

- ▪ Entretien avec le Pr. AL-HADRAMI, Doyen de la faculté, à l'Université
- ▪ Première séance de travail avec R. SEBOUSSI

Jeudi 7 mars

- ▪ Entretien avec Mrs Didier RITTER et J.P. GIROT (Société SkuldTech)
- ▪ Visite des fermes laitières d'Al-Ain et de Dubaï

Vendredi 8 mars

- ▪ Rédaction des articles et correction de la thèse

Samedi 9 mars

- ▪ Rédaction des articles

Dimanche 10 mars

- ▪ Entretien avec M. Didier GAZAGNADOU, Conseiller de Coopération au SCAC d'Abu-Dhabi
- ➢ Retour sur Al-Ain

Lundi 11 mars

- ▪ Poursuite de la rédaction des articles

Mardi 12 mars

- ▪ Poursuite de la rédaction des articles

Mercredi 13 mars

- ▪ Préparation de la présentation pour la soutenance et soumission des articles aux revues citées dans le texte du rapport

Jeudi 14 mars

- ➢ Départ pour Dubaï et Montpellier via Paris

ANNEXE 2

Extrait du journal de l'Université mentionnant notre coopération

College of Food & Agriculture Newsletter
Issue N° 4 – December 2007

Research Collaboration with CIRAD

Dr. Bernard Faye from Center of Agricultural Research for Developing Countries (CIRAD) visited Prof. Ghaleb Alhadrami, Dean, to discuss their research project about camels on November 6-14, 2007.

An agreement was signed between CIRAD, a French public research institute, and UAEU with the support of the Embassy of France in 2003. Dr. Bernard Faye, with his expertise in camel nutrition, supervises Ms. Rabiha Seboussi who conducts her experiments at the CFA farm for her Ph.D. study on selenium metabolism in racing camels. Prof. Ghaleb Alhadrami and Dr. Bernard Faye deliberated on the importance of continuing collaboration in camel related research. Both researchers played an active role in establishment of ISOCARD (International Society of Camelid Research and Development) and serve as members of the executive committee. ISOCARD will hold its second international conference in Djerba, Tunisia, in 2009. The first conference was organized in Al Ain in 2006.

ANNEXE 3

Article soumis au

« *Journal of Veterinary Pharmacology and Therapeuthics* »

RUNNING TITLE: CAMEL SELENOSIS

Chronic selenosis in camels

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ABSTRACT

As no references on selenium toxicity in camel (*Camelus dromedarius*) was available, three groups of four 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 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 the three 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 varying between 26.85 and 174.16 IU/g Hb. Significant correlations between serum selenium, GSH-Px activity, urine and fecal Se were reported. No significant variation occurred for vitamin E (mean: 0.68 ± 0.36 ng/ml). Highly selenium level was observed in liver followed by kidney, spleen, lung, heart, pancreas, brain and ovary. Selenium concentration rise significantly in hair. Several symptoms related to selenosis have been reported and histopathology findings showed remarkable lesions in all organs.

Key words: camel; glutathione peroxidase; selenium; vitamin E; selenosis.

INTRODUCTION

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). To prevent those cases, farmers supplied their camels with commercial pellets, mineral blocks or powder mix salts and injection pharmaceutical form. Due to the lack of data on camel selenium requirements, the supplementation could be deficient, imbalanced or in excess which in any way could contribute to animal metabolic disorders by deficiency or toxicity.

Camel sensitivity to trace element imbalances was formerly reported (Faye B *et al.,* 1994) but no data was available for selenium metabolism in this species. However, in a previous trial comparing cow and camel (Bengoumi *et al.,* 1998a) where similar selenium supplementation supplied animals (with 2mg/day for 2 months), it has been observed a strong higher increasing of plasma selenium in camel (10 times the blood level before supplementation) than in cow (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. Our concern in the current study was to get the range of tolerance to high selenium intake and effect on camel tissues functions, to study the metabolism and may lead to recommended values for the normal body function.

MATERIAL AND METHODS

The objective of the trial was to monitor the effect of selenium supplementation in excess on the hematological 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 female camels of local breed 2 years old were shared into 3 groups of four. The average weight was 182.6 kg. They were in good health at the beginning of the experiment. The trial was performed at Al-Foha farm belonging to the Food and Agriculture College -UAE University. Camels were weighted on electronic balance every two weeks in the morning before feeding, watering and selenium supplementation. Blood samples, internal temperature, respiratory rate, cardiac frequency, pulse rate and external temperature were reported every week. Total 24h-faeces and 24h-urine samples were taken every month. At day 45 one camel of each group was slaughtered and a second one at the end of the experiment (at day 90).

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 drunken *ad libitum*. No vitamin E was added to the basal diet or fed to the animal during the experiment.

Before starting the trial, camels we bought from the camel market were kept for a 3- wk rest due to the transport stress and were trained for the adaptation to the experimental procedures. No sampling was achieved 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 respectively for each group respectively 8 mg (i.e. 17.44 mg sodium selenite), 12mg (i.e. 26.16 mg sodium selenite) and 16 mg (i.e. 34.88mg sodium selenite) daily. 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 as 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 have been taken at day 0, 15, 30, 45, 60, 75, 90. Blood was collected every 2 weeks before food distribution and selenium supplementation; taken from the jugular vein into 5ml heparinised vacutainer (H) and 10ml non heparinised vacutainer (NH). Two tubes H and NH 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 three times with an isotonic solution of NaCl (0.9%) and centrifuged for 4 min at 4000 g. 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 individual compartment and then the total faecal excreted in 24 hours was taken off and weighted. After mixing, a sample of 600 g was taken into plat 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 up to 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 dark and cool place until analysis. Vitamin E analysis was also performed in the diet components.

Organs sampling

Hair was taken before slaughtering from the neck and other part of the camel body with sterile scissor. One camel of each group was sacrificed at day 45 and at day 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 weighted and tissues samples were taken using a stainless steel knife. One part of each sample was fixed with formalin 10 % and sent to the histopathology laboratory-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, posterior limb bones. Same organs were taken at day 90 with addition of brain, intercostals muscles, diaphragm muscle and urinary bladder.

Laboratory analysis

Before selenium analysis, samples of feed, water, serum, organs, hair, urine and faecal should be 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 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 refrigerator until analysis in screw cap glass. The samples listed up

were digested in the same way with consideration of sample weight and the acid quantities corresponding. Selenium was determined in serum, organs, hair, faecal, urine, diet and water by Inductively Coupled argon Plasma – Atomic Emission Spectrometer (ICP-AES), Varian vista MPX-CCD simultaneous, using 11 points of standard curve of Accu Trace™ Reference Standard solutions from Accustandard® – USA. Quality Control Standard #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's 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 (α-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, 1979).
Following biochemical parameters were determined: glucose (GLU), blood urea nitrogen (BUN), creatinin (CRE), total protein (TP), albumin (ALB), alkaline phosphatase (ALP), creatine kinase (CK), alanine transaminase (ALT), aspartate transaminase (AST), lactate dehydrogenase (LD), gamma glutamyl transferase (GGT). Haematological parameters were estimated also (PVC, 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 analyzer – model: Dimension RXL – Dade Behring – USA, except copper, estimated with atomic absorption spectrophotometer, model SHIMADZU AA 6800.

Organs preparation for anatomopathological findings

Tissues 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), we tested the effect of the supplementation level (3 levels) and of the day of sampling (7 levels for blood and plasma). 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 correlation.

RESULTS

Selenium content in the basal diet and selenium intake

The selenium concentration was 0.49 mg/kg in concentrates, 0.15 in Rhodes grass. There was no Se in water. The daily feed intake was 2 kg of concentrate and 3 kg of grass on 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. So, the dietary Se concentration varied between 1.7 (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

On average 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 at the beginning of the trial was 16.33 ng/ml in fortnight 1 at group 3.

On average young camels excreted 1.09 ± 0.54 kg DM of feces per day and 1.42 ± 0.74 L urine per day. Selenium concentration in urine and feces 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± 930.14µg/day in urine and 1096.02 ± 549.8 µg/day in feces. The urinary Se concentration was higher at month 3 in group 2 receiving 12 mg. 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), 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 average the fecal 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 fecal 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.

Correlations between serum Se, urinary and faecal Se

Se concentration in serum was highly correlated with Se concentration in urine, with total Se excreted in urine and Se concentration in fecal *(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 feces (*P<0.001*) but in a lesser extent with the total fecal quantity excreted (*P<0.05).* Urinary Se excreted was also highly correlated with Se concentration in feces (*P<0.0001).* The quantity of Se excreted in feces 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 ± 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.

Correlations with other blood parameters

Se concentration in serum was not correlated with biochemical and mineral parameters but slightly with TP, WBC, platelets, N, E *(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). Also, GSH-Px was highly positively correlated with creatinin *(P<0.001)* and negatively with AST, LDH *(P<0.05)*. Vitamin E was negatively correlated with creatinin and positively with TP, albumin *(P<0.05)*. No correlations to minerals and hematological 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 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 environment temperature *(P<0.01)*.

Selenium content in organs

The highest total quantity of Se was observed respectively in liver, kidney, spleen, lung, heart, pancreas, brain, and ovary (Fig. 4). The maximum level of Se concentration was observed in camels' 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 day 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 signs occurred within 2 weeks. A hair discoloration was showed followed by alopecia. Alopecia intensity was different from 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 12mg 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 seat alone. In groups 2 and 3, the urinary excretion increased and dark watery diarrhea was also observed. Young camel showed a loss of appetite, thus loss of weight and weakness appeared. Camels from group 3 showed a greatly urine excretion and dark fluid diarrhea as well. Tears with pale mucous were showed as well as an evidence of impairment vision. The alopecia was complete and skin became rough. Dyspneic respiration and pain at auscultation appeared and camel adopted the sternal decubitus position and tended to rest its neck extended (picture 1). Salivation occurred and finally camels showed no desire to eat and drink. The tail was elevated. Fissured pads appeared in all groups but more pronounced in group treated with 12 mg and 16 mg. Consequently, camels found difficulties in walking (picture 2). 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 group3 and treated, and then camels returned progressively within several weeks to the normal under intensive care. But Se was continued

in the 2 other groups for one month. 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, paleness of diaphragm and intercostals muscles, hydrothorax (picture 3), 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 showed a flap heart with necrosis and congestion (picture 4). 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 edema was 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 (picture 5). Vacuolar degenerative changes were seen in convoluted tubules of cortex and collecting tubules of medulla. Severe vascular congestion in medulla zone occurred. Edematous fluid was seen in between the muscular fibers 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 hyalinization (non –inflammatory) and edema were observed in intercostals and diaphragm muscles. Activation in lymphoid follicle was seen in cervical anterior lymph node.

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 muscles fibers were observed and became more eosinophilic and dispersed in edema fluid with picnotic nuclei (non –inflammatory myocardial dystrophy). Hepatic sinusoids congestion with degenerative changes in hepatic cells especially in periportal zone (vacuolar degeneration changes) was observed picture 6). However, non-inflammatory hyalinization in focal area (hyaline degeneration) was reported in shoulder muscle. Focal hemorrhagic 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 hemorrhagic areas in spleen.

Group treated with 16 mg Se daily: albumineous 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 lining epithelial cells of urinary bladder. Heart showed proliferation of Purkinje fibers, capillaries congestion in Purkinje fibers tissues and sub-endocardial tissues, degenerative changes in myofibers. The cardiac tissues showed edematous fluid between more eosinophilic thick myocardial fibers (picture 7). 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 few inflammatory cells infiltration 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 ductile (macroscopic duets). These necrotic areas were infiltrated with few inflammatory cells of fibrosis (picture 8). In addition focal coagulative necrosis areas appeared in pancreatic acinis. Hyalin degeneration of myofibers and edema was also observed in shoulder and intercostal muscle. Severe proliferation of lymphoid follicles and greenish material was engulfed with macrophage (haemosiderin) in cervical anterior lymph node. Focal hemorrhagic 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 camels with 1.43 mg Se per day i.e. 0.28 mg/kg DM that was considered approximatively the requirements for daily cattle (NRC 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. That 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, camels' 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.

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 (LD50) of sodium selenite has been reported to be 1.9 ±1.2 mg of Se/kg LW (Lambourne et al., 1969; Caravaggi *et al.,* 1970; Blodgett & Bevill, 1987). A daily intake of 0.25 mg/kg LW was considered as toxic for sheep and cattle (Muth & 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 & 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 & Raisbeck, 1995). In our study, the first lesions appeared with a selenium intake of approximatively 2.5 mg/kg DM.

Blood parameters values

Selenium level in serum and GSH-Px activity in red blood 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 100ng/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 according to the authors (Hamliri *et al.,* 1990; Liu *et al.,* 1994; Barri & Al-Sultan, 2007). In camel receiving 2mg daily supplementation, the serum selenium value increased up to 200.4 ng/100ml (Bengoumi *et al.,* 1998). In Sultanate of Oman (Faye, unpublished data), high serum value (281ng/mL on average) was observed on camels suspected to be intoxicated with selenium. In a previous study (Seboussi et al., 2008), we have reported 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.

In intoxicated lambs with 4mg/kg LW under sodium selenite form (four 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 (comparatively to our results: after 90 days, 519 ± 97 ng/ml for groups 1 and 2 receiving respectively 2.5 and 3.5 ppm Se in the diet).

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 by Hamliri *et al.*, (1990) (25.8 IU/g Hb) and by Bengoumi *et al.*, (1998) (51.6 IU/g Hb) 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 (Corbera *et al.*, 2003): 298.1 IU/g Hb in female camel. The higher reported value in our study was 174.16 IU/g Hb only. In Australia, the value of GSH-Px in camel was evaluated to 85.8±14.75 IU/g Hb (Agar & Susuki, 1982). In a previous study on supplemented camels with 4mg 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 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 stabilized 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 Se 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 both 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 & 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 organism and cellular events responsible for an immune response. Elevated Se has been shown to promote peroxidative damage in *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 hematocrit was in relation with the absence of anemia in herbivorous in case of selenosis (Jenkins & 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 for camel (Bengoumi *et al.,* 1997). Surprisingly, the CK did not increase in spite of its role as indicator of muscular dystrophy. Similar observation was reported on calves receiving high dietary Se (Jenkins

& Hidiroglou, 1986). The lack of relationship between Se and other trace elements as Cu and Zn was not in accordance with previous results on camel receiving normal Se quantity (Seboussi *et al.,* 2004).

Physical parameters

The normal camel temperature was ranged between 35 to 38.6°C, the pulse rate 45 to 50 and the respiratory rate 5 to 12 pulses 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 high 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 dyspneic and laborious. Such symptoms were reported in Wyoming on 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 *et al.*, 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 disproportionally (Clark *et al* 1996).

In camel, one reference only 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 640µg/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 Ma's study. In the selenium tolerance trial achieved on lamb (Cristaldi et al., 2005) liver had the highest Se concentration (up to 2000 µg/kg) followed by the kidney (around 1000 µg/kg). 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 & 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 & Hidiroglou, 1986). Contrary to Cristaldi *et al.* (2005), a regular increasing of Se concentration with dietary Se level was observed by those authors. According to them, kidney was the major organ involved in 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 & Radostis, 1989), i.e. 12 to 18 times higher than in our results.

Few references on selenium in hair were available in camel. In the wool of Bactrian camel from China, Liu *et al.*, (1994) reported values between 140 and 190µg/kg according to their physiological status. Similar results were published by Ma (1995): 190 to 210µg/kg. Those values were widely below our results: 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 on 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 & 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). These findings were in accordance with lesions observed in heart, lung, liver, kidney and urinary bladder. After the liver, the kidney, particularly the cortex, retained the highest concentration followed by the glandular tissues, especially pancreas and pituitary (IARC, 1975). That 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 histologic 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 & Mason, 1969).

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

Selenium deficiencies in animal, including camel, can result also in damages to the liver, heart, kidney and skeletal muscle (Hammond 1980; El Khouly et al., 2001). So, 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 for 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. approximatively 4-8 mg per day for adult animals or 0.5-1 ppm in the diet.

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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)

	SeExcF				SeConF			
	month							
Se suppl	1	2	3	4	1	2	3	4
8mg	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 faeces (SeConF in ng/ml) according to the treatment (8, 12 or 16 mg Se supplementation) and of the time (in month)

	SeExcU				SeConU			
	month							
Se suppl	1	2	3	4	1	2	3	4
8mg	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**

* $P < 0.05$; ** $P < 0.01$

Table 4. Correlation coefficients between Se serum concentration, GSH-Px and vitamin E in one hand, and blood parameters in a second hand.

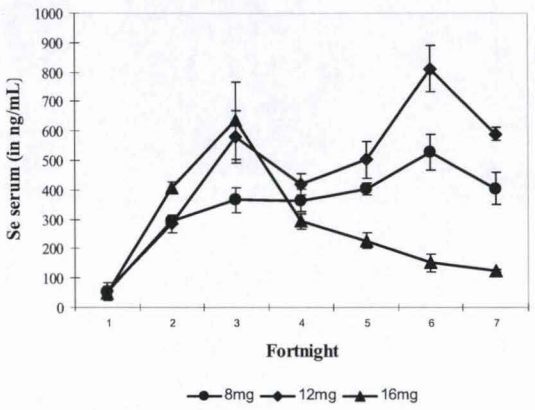
Variables	Se serum	GSH-Px	Vitamin E
PCV	0,022	**-0,284**	0,157
HB	0,034	**0,270**	0,198
RBC	-0.087	**-0.345**	0.108
Platlets	**0.366**	0.107	**0.296**
WBC	**0,287**	0,131	0,213
WBC / N	-0,235	-0,136	0,005
WBC / L	**-0,572**	**-0,662**	-0,042
WBC / M.	**-0,270**	-0,209	-0,122
WBC/ E.	**-0,280**	0,202	**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

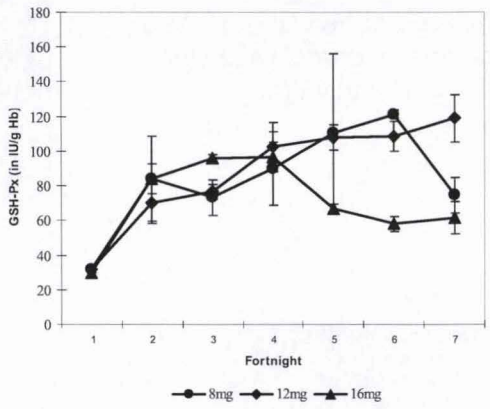
Figure 1. Changes in the Serum Se concentrations according to the selenium supplementation level in camel (Mean and S.E). The * points to the Se supplementation stopping in group 3.

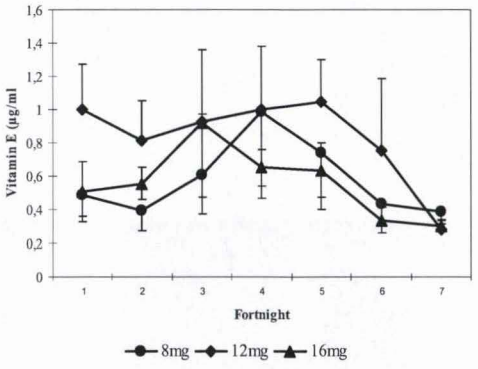
Figure 2. Changes in the glutathione-peroxidase according to the selenium supplementation level in camel (Mean and S.E). The * points to the Se supplementation stopping in group 3.

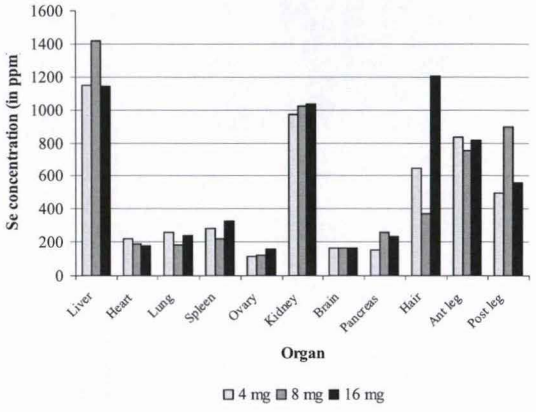
Figure 3. Changes in the vitamin E according to the selenium supplementation level in camel (Mean and S.E).

Figure 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)







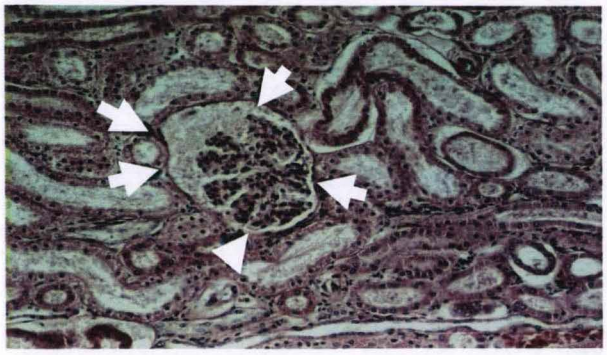


Picture 1.
Camel in sternal decubitus position with neck extended on ground.

Picture 2.
Fissured pads with necrosis on foot of camel receiving 16 mg Se/day

Picture 3.
Hydrothorax in camel receiving 16 mg selenium daily for 45 days

Picture 4.
Heart discoloration and congestion in camel receiving
8 mg selenium per day for 45 days

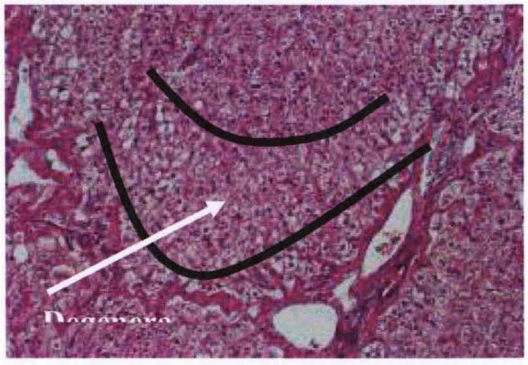


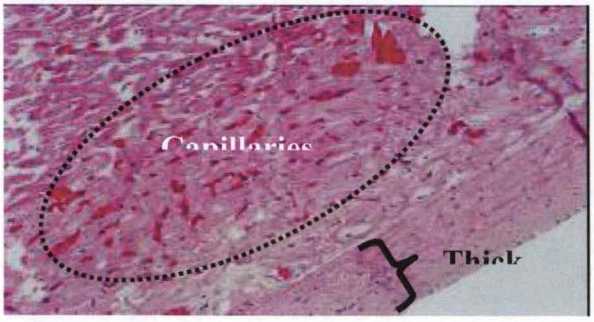
Picture 5.

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)

Picture 6.

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



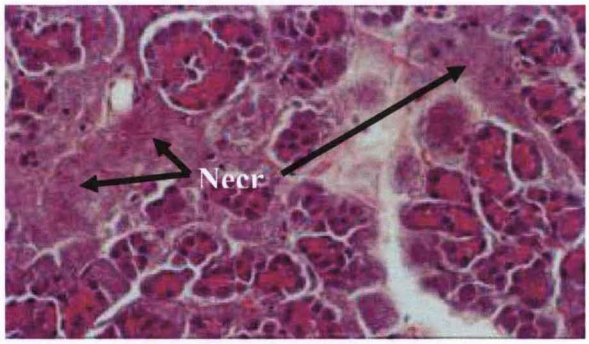


Picture 7.

Proliferation of Purkinje fibers, capillaries congestion in Purkinje fibers tissues and sub-endocardial tissues, degenerative changes in myofibers in cardiac tissues of camel receiving 16 mg Se / day.

Picture 8.

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



ANNEXE 4

Article soumis à

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Selenium distribution in camel blood and organs after different level of dietary selenium supplementation

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ABSTRACT

Eight young female camels shared in four groups of two 2-years received a basal diet enriched respectively with 0, 2, 4 and 8 mg selenium under sodium selenite form for 64 days. Feed intake was assessed daily, blood samples were taken on weekly basis. One camel from each group was sacrificed at the end of the experiment. Se concentration in serum was increased significantly in the supplemented groups with an average of 176.3 ± 18.0 ng/mL in the control group, 382.7 ± 107.6 in group receiving 2 mg Se, 519.8 ± 168.4 in group 4 mg Se and 533.4 ± 158.6 in group 8 mg Se daily. For GSH-Px activity, control group (51.0 IU/g Hb) and group 2 mg (50.5 IU/g Hb) were significantly different than groups 4 and 8 mg (respectively 65.9 and 76.1 IU/g Hb). No significant variation occurred for vitamin E (mean: 0.56 ± 0.23 ng/mL). Significant correlation between serum Se and GSH-Px was reported. Kidney was the richest organ in selenium followed by lung, spleen and liver, but the increase in supplemented groups was more marked in liver and kidney. The hair seemed to be the best indicator of selenium intake in camel.
Key words: camel, glutathione peroxidase, organ, selenium, vitamin E

Introduction

The dromedary camel is well adapted to some trace elements imbalances as reported previously (Faye and Bengoumi, 1994 and 2002). Concerning selenium, few results on plasma or blood values in field conditions in different areas from Morocco, China, Saudi Arabia and Sudan are available in the literature.

In United Arab Emirates (UAE), soils and feedstuffs are generally considered deficient in selenium, and many cases of myocardial dystrophies are reported. To prevent those cases, farmers supplied their camels with commercial pellets, mineral blocks or powder mix salts and injection pharmaceutical form. Due to lack of information's on camel selenium requirements, the supplementation is deficient, imbalanced or in excess which in any way contribute to animal metabolic disorders by deficiency or toxicity.

. In a previous trial comparing cow and camel (Bengoumi *et al.,* 1998a) where similar selenium supplementation supplied animals (with 2mg/day for 2 months), it has been observed a strong higher increasing of plasma selenium in camel (10 times the blood level before supplementation) than in cow (2 times). It has been concluded that plasma selenium level was a very sensitive indicator of oral selenium supply in camel. However, the selenium metabolism is not studied in this species and it is not possible to confirm if

there is a specific sensitivity of camel to selenium deficiency or toxicity. Indeed, the selenium depletion was also faster in the above mentioned trial. After one month without supplementation, the plasma selenium level returned to "normal". It seemed to indicate a better efficiency of selenium absorption and excretion in camel compared to cow.

To understand the effect of selenium supplementation, the present paper aims to study the kinetic of plasma selenium, for different level of selenium supplementation and to assess the selenium storage in the main organs including histopathological changes. The expected references may lead to recommendations in terms of supplementation.

Material and methods

Animal

The study was achieved at Al-Foha farm belonging to the Food and Agriculture College -UAE University, and included eight 2-years female camels of local breed shared into four groups of 2 animals. The mean weight was 196 ± 38 kg. The animals were treated for external and internal parasites using ivermectine (Ivomec N.D.) and were in good health during the whole experiment. They were weighed on electronic balance every two weeks in the morning before feeding and watering and selenium supplementation. Internal temperature, respiratory rate, cardiac frequency and pulse rate was reported every week at the weighing time in order to identify preliminary symptoms of selenium toxicity. At the end of the experiment one camel of each group was slaughtered at Swehan abattoir (Agriculture and municipalities Al-Ain- United Arab Emirates).

Experimental design

During the whole trial, 70 days length, the animals were fed individually with a similar diet composed of 3 kg DM of *Rhodes grass (Chloris gayana)* hay and 2 kg of concentrates (pellets) 10% protein and with known selenium content. The refusals were daily weighed and the quantity of grass adjusted to the mean intake. The animals were drunken *ad libitum.* No additional vitamin E was given during all the experiment.

Before starting selenium supplementation, camels were kept for training period of one week to adapt them to their new experimental environment and no sampling was achieved. The 4 groups of camels received selenium supplementation for 2 months under sodium selenite form enrobed in date given daily early in the morning prior feeding, drinking and sampling as a delicacy. The quantity of supplied selenium all along the trial was:

- 0 mg in group 1 (control group)
- 2 mg in group 2 (i.e., 4,36 mg of sodium selenite)
- 4 mg in group 3 (8,72 mg)
- 8 mg in group 4 (17,44 mg)

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

Blood sampling

Blood was collected in the morning before food and selenium distribution from the jugular vein into 5 tubes, three of 5ml heparinised vacutainer (H) and two of 10ml non heparinised vacutainer (NH). The tubes were centrifuged immediately. The plasma was harvested from H tubes. One was used for haematological analysis; one was used for mineral analysis and in the last one, red blood cells were rinsed three times with an isotonic solution of NaCl (0.9%) and centrifuged for 4 min at 4000 g. The supernatant was discarded and red blood cells were frozen at -70°C and kept until analysis of intra-erythrocyte glutathion-peroxidase. The serum was harvested from NH tubes for selenium analysis.

The blood sampling was carried out weekly in the morning before feed distribution at the days 1, 8, 15, 22, 29, 36, 43, 50, 57, 64.

Feed and water sampling

The elements of the basal diet were sampled at the beginning, the middle and at the end of the trial, dried, grinded and stored in dark and cool place for selenium analysis. A water sampling was also performed at the same time for selenium determination.

Organs sampling

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, gastrocnemia muscle, anterior limb bones, posterior limb bones, hair. After harvesting, the organs were weighed and samples were taken using a stainless steel knife and stored at -80°C up to selenium analysis. Parts were fixed in 10 % formol saline and sent to Al-Qattara veterinary laboratory for histopathological examination. Hair samples were taken from the neck and from different part of the body. Animals were weighed before and after slaughtering.

Laboratory analysis

Selenium was determined in serum, organs and hair by Inductively Coupled argon Plasma – Atomic Emission Spectrometer (ICP-AES), Varian vista MPX-CCD simultaneous, using 11 points of standard curve of Accu Trace™. Reference Standard solutions came 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. In microwave rotator tubes 2 ml serum of each sample was taken and mixed with 10 ml nitric acid (HNO_3) and 5 ml perchloric acid ($HClO_4$). The acids used were high purity grade. After cooling digested samples were transferred into flask and dilute to volume by adding deionised water and finally kept in screw cap glass vial, then stored in refrigerator until analysis. Hair, tissues, *Rhodes grass*, concentrates and water samples were also digested in the same way with respect to the sample weight and the acid quantities corresponding. The enzymatic activity of glutathione peroxidise (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 oxidised per minute per gram of haemoglobin.

Vitamin E (α-tocopherol) quantification was performed in plasma by High Performance Liquid Chromatography (HPLC- Waters) system. It was expressed in µg/ ml (Hatam and Kayden, 1979).

Following biochemical parameters were determined (glucose (GLU), blood urea nitrogen (BUN), creatinin (CRE), total protein (TP), albumin (ALB), alkaline phosphatase (ALP), creatine kinase (CK), alanine transaminase (ALT), aspartate transaminase (AST), Lactate dehydrogenase (LD), gamma glutamyl transferase (GGT). Haematological parameters were estimated also (PVC, HB, WBC, and D.L.C (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 analyzer – model: Dimension RXL – Dade Behring – USA, except copper, estimated with atomic absorption spectrophotometer, model SHIMADZU AA 6800.

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 (4 levels) and of the day of sampling (10 levels for blood and plasma). 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 correlation.

Results

No specific symptoms were observed on camel in different groups.

Selenium content in the diet and selenium intake

The selenium concentration was 0.49 mg/kg in concentrates, 0.15 in Rhodes grass. There was no Se in water. The daily feed intake was 2 kg of concentrate and 3 kg of grass. Thus, the selenium intake provided by the diet was 1.43 mg per day for camels during all the experiment. The mineral mixture providing 0, 2, 4 and 8 mg of selenium per day, according to the treatment, the total quantity of selenium provided in the diet was 1.4 mg/day for camel in control group, 3.4 mg in group 2, 5.4 mg in group 3 and 9.4 mg/day in group 4. So, the dietary Se concentration varied between 0.25 (control group) and 2 ppm (group 4). 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

On average, the mean values of selenium in the serum was 176.3 ± 18.0 ng/mL in the control group, 382.7 ± 107.6 ng/mL in group receiving 2 mg Se, 519.8 ± 168.4 ng/mL in group receiving 4 mg Se and 533.4 ± 158.6 ng/mL in group receiving 8 mg Se daily.

The weekly change showed a significant increase ($P<0.001$) from week 2 up to the end of experiment in the 3 supplemented groups compared to the control one (Figure 1). There was no difference between the groups receiving 4 and 8 mg Se. A significant difference ($P<0.01$) occurred between group 2 (2mg Se) and the two other supplemented groups in weeks 2, 3, 4 and 6 only. The maximum value (657.3 ng/mL) was observed in the group 3 at week 9 and the minimum at the beginning of the experiment (124.1 ng/mL in group 3 also).

Mean values of GSH-Px

Similar trend occurred for GSH-Px (Figure 2) but a significant difference was observed at the beginning of the experiment. The activity of erythrocyte glutathione peroxidase oscillated between 25.1 and 127.1 IU/ g Hb with an average value of 72.30 ± 23.3 IU/ g Hb. The value in group 4 (76.1 IU/g Hb) and group 3 (65.9 IU/g Hb) was significantly higher than in group 2 (50.5 IU/g Hb) and 1 (51.0 IU/g Hb). No difference was observed in week 2 then a significant difference ($P < 0.001$) was observed from week 3 between the control groups and the supplemented groups. There were no differences between the supplemented groups except in week 9 between groups 3 and 4 in one hand and group 2 in another hand. On average, the GSH-PX values were 42.8 ± 10.6 IU/g Hb in control group for 74.9 ± 15.8, 83.5 ± 17.7 and 87.9 ± 15.0 in the groups 2, 3 and 4 respectively. The maximum value was observed on week 8 in group 3 (103.1 IU/g Hb).

Mean values of vitamin E

The change in vitamin E was not clearly linked to the Se supplementation. On average, the Vitamin E values were 0.68 ± 0.21 µg/mL in control group, 0.57 ± 0.23 in group 2, 0.48 ± 0.21 in group 3 and 0.54 ± 0.18 in the group receiving 8 mg. There were not significantly different between groups. In two cases, at week 4 and week 9, the control group had a higher significant vitamin E value in blood than in groups receiving Se supplementation (Figure 3). The minimum value (0.16 µg/mL) was observed in group 3 on week 9 and the maximum (1.22 µg/mL) in control group at week 8.

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

All over the experiment, the serum selenium plasma was correlated to GSH-Px activity ($r = 0.701$; $P < 0.001$) but no correlation was observed with vitamin E. Within group, the correlation between Se and GSH-Px was significantly negative ($r = - 0.578$; $P<0.05$) in control group and no correlation was observed in the other groups whatever the level of supplementation.

Correlations with other blood parameters

No correlation was observed with blood minerals except a slight relationship between vitamin E and Phosphorus ($r = 0.282$; $P<0.05$). Serum Se and GSH-Px were significantly negatively correlated with creatinine, LDH, total protein and albumin (Table 1), reversely to vitamin E, positively correlated with total protein and albumin as well as ALP. Concerning haematology parameters, serum Se and GSH-Px were negatively linked to PCV and haemoglobin (table 1), while a slight positive relationships was observed between GSH-Px and neutrophile proportion in the WBC.

Selenium content in organs

As four animals only (one per group) were slaughtered, no statistic test was available. The lack of replication was the limit of the present study. Only tendency could be reported. The highest total quantity of selenium was observed by order, in the liver, the kidney, the muscle, the lung and the heart. The total quantity was higher in supplemented groups, but except in kidney and muscle, the quantity was not clearly linked to the Se supplementation level (Table 2). The concentration in the different organ would facilitate the comparison. In control group, kidney (more than 1mg/kg) had the highest concentration, followed by lung (228 µg/kg), spleen (221 µg/kg) and liver (216 µg/kg). Except in hair, where the concentration regularly increased with the Se supplementation level, there was no clear relationships with the Se status of the diet. However, in most of the case the Se concentration was higher in the group receiving 8 mg of Se daily (Table 3). When index 100 was considered as the normal index for control group, the most important increase was observed in hair, liver, lung, ovary and pancreas (Figure 4). Especially in hair, the selenium concentration was 2-fold in group receiving 2 mg Se supplementation compared to control group, 7-fold in group "4mg Se" and 14-fold in group "8mg Se" (Figure 4).

Post mortem and histopathological findings

Camels were weighed after slaughtering with on average of 101.4 kg. Only shoulder muscle discolorations were observed in camel from control group (picture 1). Camel treated with 8 mg showed carcass paleness, pronounced muscle and liver discoloration (picture 2-3). No histopathological lesions were reported in groups 1, 2, and 3.

Camel kidney treated with 8 mg Se showed esinophilic granulated material in diluted Bowman's space, convoluted tubules and degenerative changes in epithelial lining cells (picture 4). Edematous fluid was revealed in between the heart muscular fibers. Vascular degenerative changes were seen in few hepatic cells (picture 5), as well as the congestion in central hepatic vein and hepatic sinusoids.

Discussion

Selenodeficiency is a common fact in United Arab Emirates where cases of degenerative myocarditis are regularly observed on young camel (El-Khouly *et al.*, 2001; Seboussi *et al.*, 2004). Other cases of selenium deficiency on camel were reported in Canary Islands (Corbera *et al.*, 2003), Saudi Arabia (Al-Qarawi, 2001), Morocco (Hamliri *et al.*, 1990), China (Liu *et al.*, 1994) or in zoological parks (Finlay *et al.*, 1971) but no case of selenium intoxication was described. In order to prevent such diseases, commercial concentrates (pellets including soya bean meal, maize, barley, wheat bran, molasses, salt, premix) used for camels were regularly supplemented with selenium salt and vitamin E, generally mixed in a mineral supplement or a pre mixture. The basal diet

of the control group in our experiment supplied camel with approximatively 1.4 mg Se/day i.e. around 0.25 mg/kg DM that was considered approximatively the requirements of dairy cattle according to NRC (NRC, 2000). However, as the mean live weight of the animals was around 200 kg, the selenium supply was 0.75 mg/100kg LW in the control group. The recommendations for beef cattle were higher (1mg/100 kg LW). The groups 2 (1.7 mg/100kg LW), 3 (2.7 mg/100kg LW) and 4 (4.7 mg/100kg LW) received much higher quantity of Se compared to general recommendations for ruminants.

Values of serum selenium

The mean concentration of serum selenium reported in the literature for large animals was 100ng/mL. This value was generally considered as sufficient for the maintenance of suitable metabolic functions (Maas *et al.*, 1990). In cattle receiving different forms of Se supplementation corresponding to 0.07 to 0.30 mg/kg DM, plasma selenium varied from 41.0 to 90.6ng/mL (Cabaraux et al., 2004) and 70 ng/mL was considered as deficiency threshold for cattle.

Few references were available in camel: in Morocco, Hamliri *et al.* (1990) observed on whole blood according to age and sex, values which varied between 109.1 and 117. ng/mL; similar figures were recorded by Liu *et al.*(1994) in China with concentrations varying from 97 to 112ng/mL; in Saudi Arabia, serum Se values reported on young camels at slaughterhouse varied between 5.3 and 131ng/mL with 30% of the samples above 100ng/mL (Barri and Al-Sultan, 2007). Those results were comparable to the values observed at day 1 of our experiment (131.2 ± 10.4ng/mL). In Sudan, Abdel Rahim et al, (2005) reported lower values in whole blood: 25 to 53ng/mL only.

Serum value in our experiment was double on average in group receiving 2mg Se supplementation (382.7ng/100ml). That was higher than the 200.4ng/mL reported by Bengoumi *et al.* (1998) with similar Se supplementation level but with camels having quite lower serum value before supplementation (21ng/mL). In Sultanate of Oman (Faye, unpublished data), high serum value (281ng/mL on average) was observed on camels suspected to be intoxicated with selenium. In a previous study in Emirates (Seboussi *et al.*, 2004), the mean observed value was 200 ± 90ng/mL on adult animals without Se supplementation. In small camelids as lama (Herdt, 2004), the selenium concentration in serum varied on average between 213 and 203ng/mL according to the physiological status.

Serum values in groups supplemented with 4 to 8 mg Se daily passed over 500ng/mL. Such value could be considered probably as a beginning of chronic intoxication. In their study on selenium tolerance in lamb, Cristaldi *et al.* (2005) found maximum serum Se value of 260ng/mL after 32 weeks of supplementation with 2ppm Se. After 12 weeks (duration close to our experiment), the mean serum Se concentration was also 260ng/mL with 4ppm Se in the diet that was higher than the 2.25-3.00ppm maximum in our experiment. Se serum concentration reached 490ng/mL only after one year supplementation with 8ppm. Except in lambs supplemented with 10ppm dietary Se after 20 weeks, serum Se values did not pass over 500ng/mL (Cristaldi *et al.*, 2005).

Values of RBC-GSH-Px activity

GSH-Px, important component in the protection against free radical damage to cells, 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 by Hamliri *et al.*, 1990 (25.8 IU/g Hb) and by Bengoumi *et al.*, 1998a (51.6 IU/g Hb) were not far from our results at the beginning of the experiment (60.9 ± 13.9 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 GHS-Px in camel was evaluated to 85.8±14.75 IU/g Hb (Agar and Susuki, 1982).

Values of plasma vitamin E

Vitamin E, known for its antioxidant properties, is usually found in feedstuffs and supplements. However, even in cases where vitamin E is abundant (fresh hay for example), its level decreases rapidly after storage which lead to deficiencies. There was no significant effect of Se supplementation on vitamin E plasma level. The mean value observed in our experiment (0.56 ± 0.23 ng/mL) was similar than results from literature. In Sudan, Plasma vitamin E on young camels varied between 0.3 and 1.65ng/mL (Barri and Al-Sultan, 2007). Comparable references were reported by Al-Senaidy (1996) and Mousa *et al.* (2006). Those values were lower than those reported in cattle (Nozière *et al.*, 2004).

Relationships between anti-oxidant parameters

The linear relationship between GSH-Px activity and Se in whole blood or serum was a common fact in all species, including camel (Hamliri *et al.*, 1990; Abdel-Rahim, 2005). However, in most of the case, the correlation coefficients were higher than our result. The lack of correlation between Se and vitamin E was also observed in pig (Sivertsen *et al.*, 2007) and calf (Srivanova *et al.*, 2007).

Relationships with other parameters

The serum LDH activity increased usually in case of muscular suffering or myocarditis. So, the negative correlation between Se and enzyme LDH could be interpreted like an opposition between these parameters as indicators of the cellular integrity. Indeed, selenium was an essential element of cellular protection and one could make the assumption that enzyme LDH, indicators of the cellular suffering, were all the more high as the concentration in Se was weak. Surprisingly, no similar negative relationships were observed with AST and ALT, contrary to previous study (Seboussi *et al.*, 2004). Probably, the serum selenium values in our experiment were much higher than the previous one and no cellular suffering linked to low selenium status occurred. At reverse, it was expected an increasing of enzyme activities in the presence of Se toxicity because of tissue necrosis. In fact, since enzyme activities were within normal range, this provided evidence that Se toxicity did not occur.

The negative correlation with albumin and total protein was not clear. In lamb, serum concentration of albumin was not affected by dietary Se supplementation level (Cristaldi *et al.*, 2005). Usually, after intestinal absorption, selenium forwarded by red blood cell to be reduced before passing by in plasma where it was bound to the groupings thiols of proteins, notably albumin (Vitoux *et al.*, 1996). Probably, when selenium concentration increased in serum, the part bound to proteins would be less important than the free selenium. This phenomenon could be more important in camel than other species and could explain the relative high concentration observed in camel after Se supplementation.

Creatinine in urine was normally considered as a convenient indicator of human selenium status (Hojo, 1982) and the relationship between selenium and creatinine clearance was commonly described in the literature (Hagmar *et al.*, 2002). The negative relationship between serum creatinine and selenium in our trial was so unclear.

However all data concerning those enzymes were in the range of normal values reported in camel whatever the Se supplementation level (Bengoumi *et al.*, 1998b).

The positive relationship between phosphorus and vitamin E was rarely reported in spite of a probable synergy between some phosphates and vitamin E (Jentzsch *et al.*, 2001). At reverse, in cattle, the diets rich in concentrates and corn silage were linked to milk richer in phosphorus and poorer in vitamin E (Coulon *et al.*, 2003).

The lack of relationship between Se and other trace elements as Cu and Zn was not in accordance with previous results (Seboussi *et al.*, 1994). Interaction between selenium, copper or zinc was described for example in rat where zinc induced a decrease in the urinary excretion of selenium (Chmielnicka *et al.*, 1988). But in other cases, no interaction was observed (Hansen and Kristensen, 1980).

The negative relationship between Serum Se or GSH-Px and PCV or Hb could be interpreted, at reverse of the lack of relationships with enzymes, as a chronic selenosis. Anemia was sometimes cited as a Se chronic toxic effect in small carnivores and rodents (Halverson *et al.*, 1966), but not consistently in herbivores (Jenkins and Hidiroglou, 1986; Raisbeck *et al.*, 1996).

Selenium content in organs

The selenium determination in organ was rarely reported because it was without clinical interest. In the wool of Bactrian camel from China, Liu *et al.*, (1994) reported values between 140 and 190µg/kg according to their physiological status. Similar results were published by Ma (1995): 190 to 210µg/kg. Those values corresponded to camels receiving 2mg Se supplementation in our experiment (163.6 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). Part of selenium ingested is involved in hair amino acids synthesis. It was suggested that a level of selenium content should be >120 ppm in cow and calf hair to avoid nutritional myopathy. Season, colour of hair, age and sex affected the selenium content in hair. Selenium concentration was higher in winter than in summer and in the dark colour than the light colour (Lomba et al, 1973). The hair appeared as the most sensitive organ to Se supplementation as it was reported on 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 (Combs and Combs, 1986).

In camel, one reference only 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 640µg/kg) were the organs with the higher Se concentration. Those values, except for liver, appeared much higher than our results. In the selenium tolerance trial achieved on lamb (Cristaldi *et al.*, 2005) liver had the highest Se concentration (up to 2000 µg/kg) followed by the kidney (around 1000 µg/kg). 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). However, in our case only one value per camel group was available. So, the between individual variability could not be taken in account. In calf receiving 3 ppm dietary Se treatment (comparable to our group 4), 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 increasing of Se concentration with dietary Se level was observed by those authors. According to them, kidney was the major organ involved in storage of selenium at low Se supplementation, but at high intakes, the liver became the target organ (Jenkins and Hidiroglou, 1986).

Conclusion

The results seemed to confirm the sensitivity of camel to Se supplementation with an important increase of selenium in serum after oral supplementation. But this increase was not necessary an example for the thrifty physiology, characteristic of the camel behavior. It could be also the mark of a greater sensitivity to toxicity. However, in the present trial, even with 8mg daily Se supplementation, no clinical signs were observed after 10 weeks. Selenium as other trace elements is required in small amount. So, selenium supplementation in excess may be harmful for the camel and can lead to chronic selenosis. The limit margin between selenium deficiency and selenium toxicity has to be considered in further study.

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Table 1. *Correlation coefficients between Se serum concentration, GSH-Px and vitamin E in one hand, and blood parameters in a second hand*

Variables	Se serum	GSH-Px	Vitamin E
PCV	**-0.410**	**-0.265**	0.136
HB	**-0.403**	**-0.252**	0.145
WBC	0.097	-0.025	0.000
WBC / N	0.116	**0.230**	-0.190
WBC / L	-0.101	-0.209	0.139
WBC / M.	0.121	-0.101	-0.067
WBC/ E.	-0.098	-0.144	0.212
Cu	0.055	-0.023	-0.217
Zn	-0.027	0.175	-0.004
Phosphorus	-0.134	-0.197	**0.282**
Sodium	0.108	0.025	0.081
Potassium	0.111	0.126	0.028
Chloride	-0.174	0.050	-0.149
CO^2	0.034	0.032	0.001
Bilirubin	0.133	-0.197	0.011
Glucose	-0.046	-0.051	0.144
Creatinine	**-0.292**	**-0.244**	0.099
Total protein	**-0.259**	**-0.293**	**0.249**
Albumin	**-0.426**	**-0.435**	**0.332**
ALP	-0.098	-0.0716	**0.234**
CK	-0.033	0.029	-0.175
ALT	-0.002	-0.056	-0.124
AST	-0.164	-0.047	-0.067
LDH	**-0.252**	-0.129	0.010
GGT	-0.148	-0.063	-0.037
Fe	-0.181	-0.0763	0.186

Table 2. *Quantity of selenium (in µg) in the different organs according to the Se supplementation level*

	Se supplementation level			
Organ	**0 mg**	**2 mg**	**4 mg**	**8 mg**
Liver	752.2	4616.4	686.8	3070.6
Heart	113.5	165.6	187.7	139.7
Lung	264.6	231.9	1283.9	465.1
Spleen	30.9	33.8	35.6	28.7
Pancreas	20.0	24.7	30.8	37.3
Kidney	704.6	893.3	731.4	1118.1
Ovary	0.56	0.81	0.76	1.07
Brain	61.8	52.4	36.7	84.3
Forelimb	368.7	418.8	665.0	481.2
Hindlimb	286.6	299.8	447.3	458.1

Table 3. *Selenium concentration (in µg/kg) in the different organs according to the Se supplementation level*

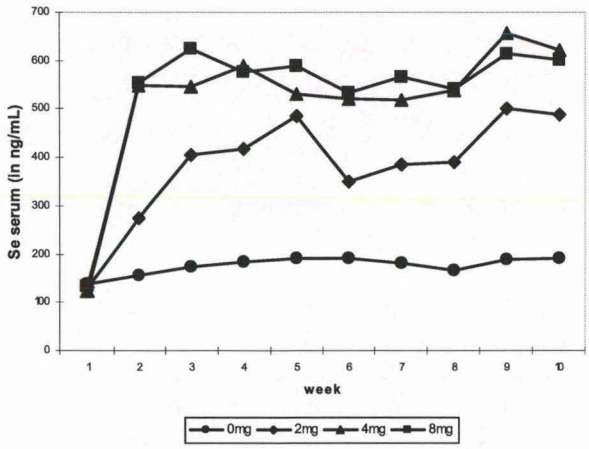
	Se supplementation level			
Organ	**0 mg**	**2 mg**	**4 mg**	**8 mg**
Liver	216.1	1508.4	162.0	1010.1
Heart	138.4	176.2	204.1	179.1
Lung	228.1	178.4	746.5	287.1
Spleen	221.3	211.8	197.7	205.6
Pancreas	105.5	114.8	118.5	207.3
Kidney	1006.6	1488.9	962.5	1552.9
Ovary	141.5	141.6	138.7	358.7
Brain	147.3	163.9	96.7	210.8
Forelimb	131.2	135.1	196.2	154.2
Hindlimb	102.0	96.7	131.2	146.8
Hair	80.7	163.6	563.2	1130.7

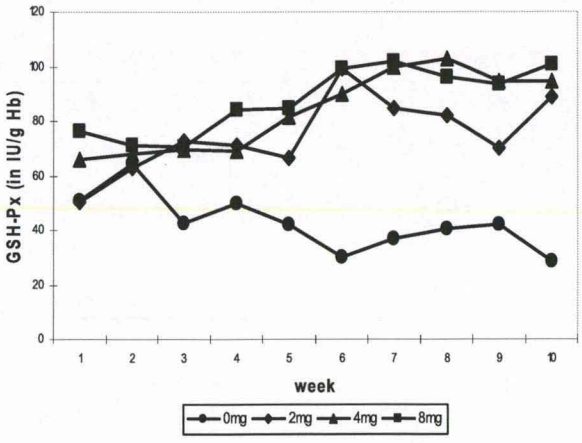
Figure 1. Weekly change in Serum selenium according to the Se supplementation in the basal diet of camels

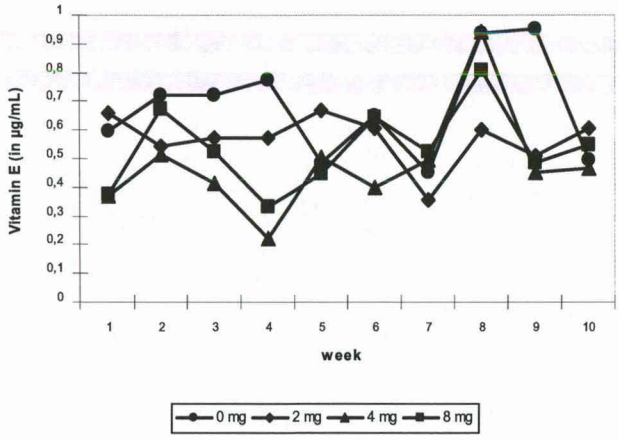
Figure 2. Weekly change in GSH-Px activity according to the Se supplementation in the basal diet of camels

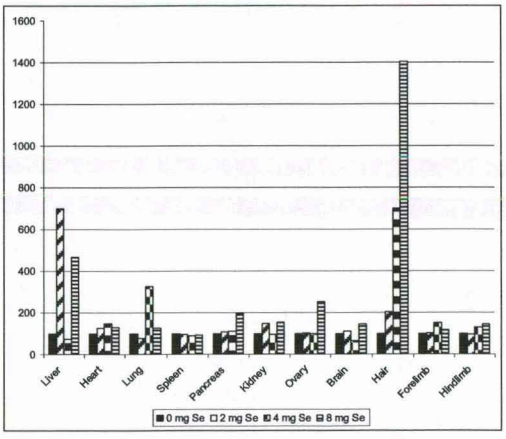
Figure 3. Weekly change in vitamin E according to the Se supplementation in the basal diet of camels

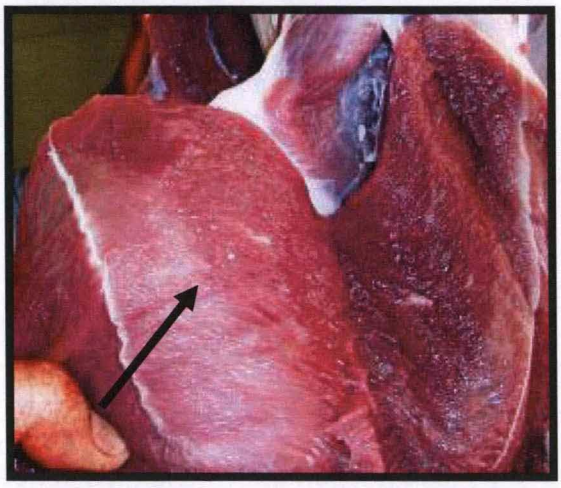
Figure 4. Changes in the Selenium index (=100 for each organ in control group) according to the Se supplementation level





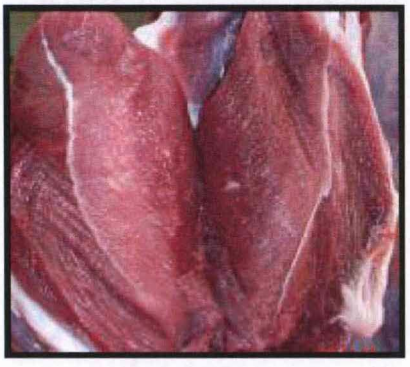






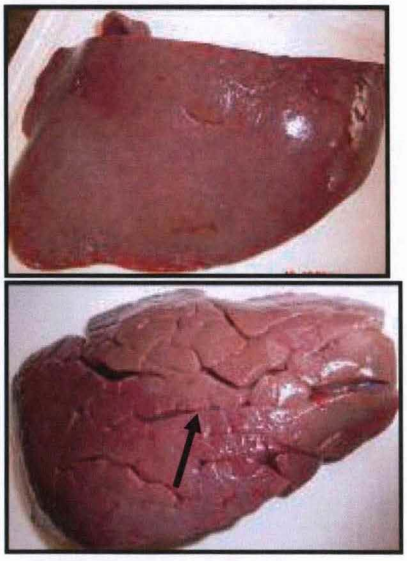
Picture 1

Muscle discoloration (0 mg)
Photo R. Seboussi



Picture 2

Marked muscle discoloration (8 mg)
Photo R. Seboussi

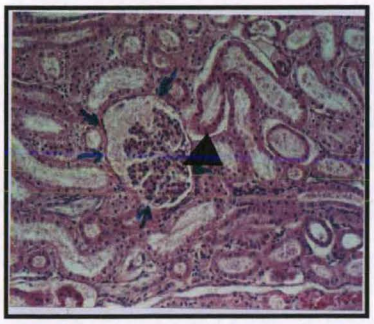


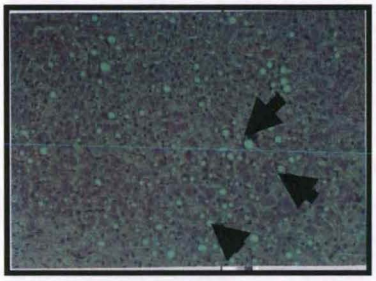
Picture 3

Congested and discoloured liver
and kidney
Photo R. Seboussi

Picture 4

Esinophilic granulations and degenerative changes in epithelial lining cells in kidney of camel receiving 8 mg selenium
Photo A. Elkhouly





Picture 5

Vascular degenerative changes in hepatic cells of camel receiving 8mg selenium
Photo A. Elkhouly

ANNEXE 5

Article soumis à

« *Journal of Dairy Sciences* »

Running Title:

SELENIUM IN PREGNANT AND LACTATING CAMEL

Selenium intake and excretion in pregnant and lactating camel and calf

Seboussi

Selenium deficiency is commonly described in camel provoking white muscle disease in camel calf. To prevent it, oral non-organic selenium supplementation of the dam is achieved on the basis of cattle requirements but no data are available on the selenium metabolism in camel. The present paper aims to assess the impact of selenium supplementation at the end of gestation and at the first month of lactation on blood and milk selenium status in camel dam and their calf as well as on the urinary and fecal excretion.

Effect of Selenium Supplementation on Blood Status and Milk, Urine and Fecal Excretion in Pregnant and Lactating Camel

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ABSTRACT

Ten pregnant female camels divided into two groups received after a 2 weeks adaptation period, an oral selenium (Se) supplementation (0 and 2 mg respectively) under sodium selenite form for 6 months from the three last months of gestation up to the three first months of lactation. Feed intake was assessed daily. Blood samples and body weight were taken on a bi-weekly basis, both in dams and their camel calves after parturition. Feces and urine samples were collected monthly, and milk on a bi-weekly basis. The Se concentration in serum increased significantly in the supplemented group and was threefold higher than the concentration compared to the control group, respectively 305.9 ± 103.3 ng/mL and 109.3 ± 33.1 ng/mL. The selenium concentration increased in similar proportion in milk (86.4 ± 39.1 ng/mL in the control group *vs* 167.1 ± 97.3 ng/mL in treated group), in urine and feces. The gluthathione-peroxidase (GSH-Px) activity varied between 18.1 ± 8.7 in control group and 47.5 ± 25.6 IU/g Hb in treated group but decreased after parturition in both groups. Vitamin E did not change significantly and was on average1.17 ± 0.72 ng/mL and 1.14 ± 0.89 ng/mL in the control and treated group respectively. Significant correlations were reported between serum Se, milk Se, GSH-Px, fecal and urinary excretion or concentration. Blood values in camel calves were similar to those of the dams. The results seemed to confirm the sensitivity of camel to Se supplementation with an important increase of selenium in serum and milk.

Keywords : selenium, glutathione-peroxidase, camel milk, excretion

INTRODUCTION

The metabolism of selenium (Se) is well described in most of the domestic animals and the requirements in farm animals widely published (Mc Dowell, 1992). However, there are few references concerning selenium in camel (Faye and Bengoumi, 1994). Only some plasma or blood values in field conditions in different areas from Morocco (Hamliri et al., 1990), China (Liu et al. 1994) and Saudi Arabia (Al-Qarawi, 2001) are available in the literature. Recent data were published in United Arab Emirates (Seboussi et al., 2004 and 2008) and Saudi Arabia (Abdel Rahim, 2005). In Emirates, soils and feedstuffs are generally considered deficient in selenium, and many cases of degenerative myocarditis on young camel are reported (El-Khouly et al., 2001). Thus, all concentrates given to camels are enriched in selenium mainly under selenite forms, but the impact on selenium status especially on pregnant and lactating female is unknown as well as the selenium status of the milking camel calf.

Therefore, the present paper aims to study in pregnant and lactating camel the kinetic of plasma selenium and other biochemical and mineral parameters at different level of selenium supplementation under sodium selenite form and to assess the selenium excretion in milk, urine and feces in order to understand the metabolism of this mineral in this species.

MATERIAL AND METHODS

The present paper is a part of a study aiming to assess the selenium metabolism (intake, storage, excretion, blood references) and variability of selenium status in pregnant and lactating camels and their calves by inducing a variation in the selenium supply.

Animals

The study was achieved at Al-Foha farm which belongs to the Food and Agriculture College of the UAE University and included 12 healthy adult female camels (more than 7 years old) of local breed randomly divided into two groups of 6 animals. The mean weight was 527 ± 73 kg. No significant difference in the weight occurred at the beginning of the experiment, but there was a significant weight difference between groups the last month of gestation and the weeks 2, 4 and 5 after parturition. The animals were treated for external and internal parasites using ivermectin (Ivomec N.D.) and were in good health during the whole experiment. They were weighed on electronic balance every two weeks in the morning before feeding and watering. All the females were pregnant at the beginning of the trial. They were chosen among 15 females mated in the same time of the year. The trial started approximately 3 months before the parturition and stopped three months after the parturition. Probably because early abortion, two camels did not finish their pregnancy and were discarded from the experiment. Finally, five camels were monitored in each group.

Experimental Design

During the whole trial, the animals were fed individually with approximatively 6 kg of Rhodes grass (*Chloris gayana*) hay and 2 kg of concentrates with known selenium content. The refusals were daily weighed and the quantity of grass adjusted to the mean intake. There was no concentrate refusal. The animals were provided water *ad libitum.* The experiment duration was approximatively 6 months (195 days) starting from the last third of pregnancy to 3 months after parturition. The trial included 3 phases:

1- An adaptation period (days 1-15) where the animals received the basal diet without any mineral supplementation. No sampling was achieved during this period.

2- Supplementation period with selenium supplementation additives (days 15-135). Control group (1) did not receive selenium supplementation. Treated group (2) received 2 mg selenium supplementation under sodium selenite form (i.e., 4.36 mg of sodium selenite) included in dates given daily as a delicacy. The supplementation period included the three last months of gestation and the first month of lactation

3- A post supplementation period (days 135-195). After one month lactation, selenium supplementation was stopped. Animals of both groups received the basal diet only. No supplementation was given to camel calves

The selenium requirements for camel was assessed as similar to cow, i.e., 1mg/day (Mac Dowell, 1992). The quantity of selenium given to animals corresponded to level generally supplied by racing camel owners.

Blood Sampling

In both females and camel calves, blood was collected from the jugular vein in both heparinized (H) and nonheparinized tubes (NH). Two tubes of H and NH were transferred to Alqatara veterinary laboratory (agriculture and municipalities department – AbuDhabi) for hematology and biochemistry analysis. One NH tube was centrifuged

and the serum was stored at -80°C until selenium analysis. From an H tube, 2 ml of whole blood was collected then centrifuged. Plasma was removed and stored at -80°C until vitamin E analysis. The red blood cells were watched three times with an isotonic solution of NaCl (0.9%) and centrifuged for 4 min at 4000xg. 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 colostrum feeding to evaluate the Se status at birth.

Colostrum and Milk Sampling

Colostrum samples were taken immediately after parturition and milk was collected at the milking time when the calf was suckling. The sampled milk was harvested every two weeks in a 20 ml tube then frozen up to analysis.

Fecal and Urine Sampling

The feces were sampled for 24 hours on each animal every month all along the experiment. For this, animals were placed in individual box and all the fecal excretion on the cleaned ground was harvested. The whole excretion was weighed. A sample of 600g was taken out, dried (at 65°C in a stove for 48 hours), ground then placed in a plastic bag labeled (date, animal number, type of sample) and placed in a dark, dry and cool place until selenium analysis. Urine was collected for 24 h with a plastic bag tied close to the vulva. The whole samples were weighed and acidified with HCl 0.1 M, and a portion was poured into a 4-ml cryogenic flask then frozen at -20°C until the analysis. Urine sampling was performed at the same interval as feces sampling.

Feed and Water Sampling

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

Laboratory Analysis

Selenium was determined in serum, urine and feces. GSH-Px activity was measured in erythrocytes. Quantification of selenium was performed by the standard addition method, using 11 point standard curve. AccuTrace™ Reference Standard solutions used were Quality Control Standard #1AccuStandard® and Laboratory Performance Check Standard AccuStandard®. The analysis of selenium required the digestion of the samples to destroy proteins and amino acids to release the molecules of Se related to proteins. The serum (2ml) was mixed in the tubes of rotator's digester with 10 ml nitric acid (HNO_3) then 5 ml perchloric acid ($HClO_4$). The acids used in the sample preparation were high-purity grade. The tubes were placed in the rotator then introduced into the microwave digestion system using Milestone MLS-1200 MEGA, Sorisole, Italy. After cooling, the digested sample was transferred to a volumetric flask and stored in refrigerator until analysis. The same digestion protocol was followed for urine and fecal sample. For selenium determination in different samples inductively coupled argon plasma – ICP atomic emission spectrometer, Varian Vista MPX–CCD Simultaneous was used. Selenium in grass, concentrates, and water was also determined with ICP.

Hemoglobin was measured in the suspension by colorimetry (Boehringer Mannheim kit, ref. 124729). GSH-Px activity was measured with Beckman Coulter DU 800 Spectrophotometer by using the RANDOX kit, ref. RS 505 according to the method of Paglia and Valentine (11). 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 oxidized per minute per gram of hemoglobin.

Vitamin E quantification in plasma was performed by the high-performance liquid chromatography (HPLC) system. For vitamin E determination, 1-ml samples of plasma were extracted using, for one time, the sample volume of ethanol and, for two times, the sample volume of hexane. Vitamin E was measured in the extracts as ά-tocopherol by HPLC using a 3.9x150-mm silica column and UV detection at 292 nm. The mobile phase was hexane/chloroform (85/15) with isocratic elution (12).

In plasma, the other mineral parameters were trace elements (copper, zinc and iron). The following biochemical parameters were determined in the serum: glucose, creatinine, total proteins, albumin, bilirubin, CPK, Alkaline phosphatase, ALT, AST, LDH, GGT and vitamin E). The hematological parameters analyzed in routine included hematocrit, WBC, hemoglobin, and blood formula (WBC/N, WBC/L, WBC/M, and WBC/E for neutrophile, lymphocyte, monocyte and eosinophile percentages).

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 (thirteen levels for blood and plasma in adult, six levels for urine and feces, seven 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 other elements (minerals and biochemical parameters) and 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

The selenium concentration was 0.49 mg/kg in concentrates, 0.15 in Rhodes grass. There was no Se in water. The feed intake was on average 5.5 kg D.M of grass and 2 kg of concentrate and did not vary significantly all along the experiment and between groups. Thus, the selenium intake provided by the diet alone was 1.8 mg per day. The mineral mixture providing 0 and 2 mg of selenium per day, according to the treatment, the total quantity of selenium provided in the diet was around 1.8 mg/day for camel in group 1 and 3.8 mg/day in group 2. According to the mean weight in each group, the selenium intake corresponded to 3.6 µg/kg LW in control group and 6.6 µg/kg LW in treated group. The diets supplied 0.24 and 0.50 ppm 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 selenium content in serum was significantly higher ($P < 0.001$) in supplemented group (2 mg) and was threefold higher than the concentration compared to the control group, respectively 305.9 ± 103.3 ng/mL and 109.3 ± 33.1 ng/mL. The maximum level was observed 2 weeks before calving in the group receiving 2 mg (638.7 ng/mL). The selenium level at parturition was still significantly higher ($P < 0.001$) in treated group in spite of slight decrease around calving period (Figure 1). On average, Se serum concentration was significantly higher after parturition than before in both groups ($P < 0.05$) in spite of supplementation stopping in treated group (Table 1).

The Se serum concentrations in camel calf at parturition were 106.3 ± 26.5 and 273.2 ± 48.0 ng/mL in the control and treated groups respectively. This significant difference ($P < 0.001$) was maintained for the entire milking period: 103.4 ± 28.7 and 248 ± 14.1 ng/mL in the control and treated groups respectively (Figure 2).

Erythrocyte GSH-PX Activity

The GSH-Px activity varied from 2.2 to 98.9 IU/g Hb according to the sampling date and was on average significantly higher in supplemented she-camels (P <0.001): 18.1 ± 8.7 in control group *vs* 47.5 ± 25.6 IU/g Hb in treated group. 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 one (P < 0.001) (Table 1). 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 QSH-Px values threefold higher than the control calves: 73.8 ± 2.9 *vs* 25.0 ± 3.2 IU/g Hb (P < 0.001). A high significant difference was observed for the entire trial even after supplementation stopping with on average of 73.0 ± 14.1 IU/g Hb in the treated group *vs* 22.8 ± 4.5 IU/g Hb in the control one (Figure 4).

Plasma Vitamin E

No significant changes was observed for plasma vitamin E for the entire experiment in both groups and no difference occurred between treated and control animals (Figure 5) where the mean values were 1.17 ± 0.72 ng/mL and 1.14 ± 0.89 ng/mL respectively.

In camel calf, vitamin E varied from 0.002 to 4.67 IU/G Hb with a mean of 0.82 ± 1.06 IU/g Hb. The plasma vitamin E was significantly higher (P < 0.05) in the treated group than in the control one at the second month of lactation only (Figure 6). The mean value in the control group was 0.65 ± 0.49 *vs* 0.82 ± 1.08 ng/mL in the treated one.

Selenium Excretion in Milk

The Se concentration in milk varied from 39.5 to 482.6 ng/mL with an average of 86.4 ± 39.1 ng/mL in the control group and 167.1 ± 97.3 ng/mL in treated group. At birth, Se concentration in colostrum was threefold higher in treated group: mean value 302 ± 94.60 *vs* 108.2 ± 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 7).

Selenium Excretion in Feces and Urine

The mean fecal and urine excretion of she-camel for the entire experiment was 2.17 ± 0.88 kg/24h DM and 2.45 ± 1.31 L/24h respectively. There was no significant difference both between groups and physiological stages (pregnant and lactating periods). Two parameters were retained: the total Se excretion (SeExcF in feces and SeExcU in urine) and the Se concentration (SeConF in feces and SeConU in urine). The extreme values were 29.4 – 1676.7 µg for SeExcU, 26.1 – 642.3 ng/mL for SeConU, 148.2 – 4539.5 µg for SeExcF and 107.5 – 1214.4 ng/g for SeConF.

On average, all parameters were significantly higher in the treated group than in the control one at P < 0.001 (Table 2). No significant changes were observed in the control group before and after parturition. At reverse, in the treated group, all the excretion parameters increased significantly after parturition (P < 0.05). Except at the beginning of the trial, three months before parturition, Se urinary concentration was significantly different in the two groups for the entire experiment (Figure 8). Selenium fecal excretion was significantly higher in the treated group from the beginning of the trial (Figure 9).

Relationships between Antioxidant Parameters in Dam and Camel Calf

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 (Table 3).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 mother (r = -0.757; $P < 0.001$).

Relationships with Excretion in Feces and Urine

The serum Se concentration was significantly correlated with the total Se excreted and Se concentration both in feces and urine. The Se excretion in urine was correlated with Se excretion in feces also (Table 4).

Correlations with other Blood Parameters

Selenium concentration in serum was positively correlated to WBC, eosinophile (E), creatinin, and negatively correlated with RBC, lymphocyte (L), trace elements (copper, zinc and iron) and enzymes (ALT, AST). GSH-Px was mostly correlated to hematological parameters and negatively to most of the enzymes (ALT, AST, LDH and GGT). Except with WBC, there was no link with vitamin E (Table 5).

In camel calf, very few blood parameters were correlated with Se serum, except potassium and CK. GSH-Px was negatively linked to zincemia and Se in milk was correlated to PCV, RBC and Hb, to creatinine and CK but negatively to copper and ALT (Table 6).

DISCUSSION

Because of selenodeficient soils and consequently fodders, common cases of degenerative myocarditis on camel are regularly observed in the United Arab Emirates (El-Khouly et al., 2001). Therefore, the commercial concentrates used for camels were supplemented with selenium in various forms (sodium selenite, selenium combination and vitamin E), generally mixed in a mineral supplement or a pre mixture. The basal diet without supplementation supplied 0.36 mg/100kg LW that was lower than recommendations for beef cattle (1mg/100 kg LW). Even with 2 mg Se supplementation daily, i.e. 0.66 mg/100 kg LW, the recommendations were not reached. The selenium supplementation could be under an organic nutritional form e.g. selenium yeast, but this form was not used in Emirates for the supplementation of camels.

Usual Values of Serum Selenium, GSH-Px andVvitamin E in Camel

The mean concentration of serum Se reported in the literature for large animals was approximately 100 ng/mL, a value considered as sufficient for the maintenance of suitable metabolic functions (Maas et al., 1990). However, few references were available in camel serum. In the dromedary from Morocco, Hamliri et al. (1990a) observed on whole blood, according to age and sex, values that varied between 109.1 and 117.8 ng/mL which was similar to those reported on sheep in the same area (Hamliri et al., 1990b). Similar figures were recorded by Liu et al. (1994) in China 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.

In Morocco, in dromedaries receiving a low Se basal diet, the serum selenium concentration was quite lower, about 21ng/mL (Bengoumi et al., 1998a), but the concentration increased up to 200.4 ng/mL after 2 mg Se supplementation. In Saudi Arabia, serum Se values reported on young camels at slaughterhouse varied between 5.3 and 131 ng/mL with 30% of the samples above 100 ng/ml (Barri and Al-Sultan, 2007). In a previous study in Emirates (Seboussi et al., 2004), the mean value was 200 $\pm$90 ng/mL on camels without Se supplementation. Therefore, our current results seemed in accordance with the literature for the non-supplemented camels but quite higher for supplemented animals. No data were 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 2 groups. The maternal transfer of selenium was efficient and the oral supplementation of pregnant camel is an effective method of increasing selenium

concentration in serum of their camel calves. Those results were in accordance of those reported in cow (Weiss et al., 1984). Weiss et al. (1984) suggested that selenium concentration in blood serum of the dam near parturition can be used as an index of serum selenium status of the neonatal calf.

GSH-Px as one of the primary antioxidant enzymes is an important component in the protection against free radical damage to cells and, thus, is crucial to cell survival. Usually GSH-Px activity was considered as an indicator of selenium status in a variety of species. In a study achieved in Sudan, GSH-Px activity determined on whole blood was 6.32 EU/mL in male camels and 18.64 EU/mL on female camel (Abdel Rahim, 2005). In Morocco, values of 25.8 IU/g Hb were reported by Hamliri et al. (1990a and 51.6 IU/g Hb by Bengoumi et al. (1998a) on non supplemented camels. In comparison, the values revealed by Corbera et al. (2003) appeared very high (298.1 IU/g Hb in female camel). In 2mg-Se supplemented camels reported in Morocco, GSH-Px activity increased up to 131.7 IU/g Hb (Bengoumi et al., 1998a), values higher than our results. As for serum Se, no data were available for GSH-Px in new born camel calves. The observed values in the calves reflected those revealed in the dam at parturition as expected.

As the date of delivery for one camel from supplemented group was not correctly estimated, Se supplementation started 6 months before parturition at the place of 3 months for all other camels. That explained the mean difference and very high S.E in supplemented group at the beginning of the trial.

The vitamin E concentration in plasma in our results was quite similar to those described in the literature: for example 0.3 to 1.65 ng/ml in young camels from Sudan (Barri and Al-Sultan, 2007). Similar results were reported by Al-Senaidy (1996) and Mousa et al. (2006). Those values were similar to those reported in sheep but quite lower than in cattle (Mitsioulis and Judson, 2000).

Physiological Changes in Antioxidant Parameters

In camel, the increase of selenium in serum seemed very sensitive to the Se supplementation as it was reported in previous studies (Bengoumi et al., 1998a. Seboussi et al., 2008). On average, the Se concentration in serum was threefold higher in 2 mg-Se treated group than in the control, before and after supplementation. Such response was clearly higher that it was reported in cattle receiving similar quantity of Se supplementation (Juniper et al., 2006).

It has been reported that the GSH-Px activity increased by about 23% until the end of pregnancy in human (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 (Carine et al., 1993). In our study, such increase was not observed in control group. At reverse, a significant decrease of GSH-Px after parturition was already described in sheep (White et al., 1989) in spite, 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 et al., 2007) linked to lactation stress, but others reported a slight increase as it has been observed in the control group (Gürdogan et al., 2006). Contrary to our previous study achieved on non-pregnant and non-lactating camel (Seboussi et al., 2008), the GSH-Px activity was not maintained after the end of supplementation.

The plasma α-tocopherol level (vitamin E) did not change on average for the entire experiment, but a significant decrease was observed in 2mg-Se treated group at parturition (Figure 5) as it was reported in cow (Chawla and Kaur, 2004). This decrease in plasma Vitamin E concentration at parturition in cows has been attributed to colostrum production and secretion.

A linear relationship between erythrocyte GSH-Px and whole blood Se concentration was already described in camel (Hamliri et al., 1990a; Abdel Rahim, 2005). In our study, the relationship was highly significant but the correlation coefficient was lower than those reported by Corbera et al. (2003), Abdel Rahim (2005) and Bengoumi et al. (1998a): respectively 0.88, 0.88 and 0.94. The low correlation coefficient between these two

parameters in our study was linked to the changes reported above around the parturition, the GSH-Px decreasing after parturition while serum Se was increased. No data on correlation between Se and Vitamin E were available in camel, but a similar lack of correlation was observed in other species (Sivertsen et al., 2007).

Selenium Excretion

One reference only was available on selenium content of camel milk (Al-Awadi and Srikumar, 2001) and the reported value (13.9 ± 2.4 ng/mL) was quite lower than in our study, but the authors did not mention the lactation stage. 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). The colostrum Se concentration was a clear reflect of the serum Se of the dam. After parturition, in 2mg-Se group, selenium concentration in milk dropped especially when Se supplementation was stopped in mother, but the serum Se concentration in camel calf was maintained at high level compared to those of the literature. If our results are confirmed, it should underline the richness of camel milk in selenium. On average, on the three first months of lactation, the selenium intake by camel calves was 8 µg/kg LW in the control group.

The main excretion routes for Se are feces and urine (Leng et al., 2000). As for dairy cattle, fecal and urinary Se excretion increased when animals were supplemented. In our previous study in non pregnant and non lactating camel (Seboussi et al., 2008) similar trend was reported, but contrary to Juniper et al. (2006), no linear effect was observed between Se excretion and dietary selenium. In dairy cow, the Se fecal concentration was between 370 to 780 ng/g with Se dietary supplementation between 0.15 and 0.40 ppm (Juniper et al., 2006), that was close to our results (on average 225 and 817 ng/g in the control and the treated group respectively). However, the urinary excretion was on average slightly higher in our study (on average 62 and 194 ng/mL in the two groups) than in dairy cattle: 20 to 140 ng/ml (Juniper et al., 2006). The urinary selenium concentration is considered to be a sensitive indicator of sodium selenite consumption in excess of nutritional requirements (Leng et al., 2000). Elsewhere, the camel is well-known for its water metabolism and its ability to excrete a more concentrate urine although the watering was *ad libitum* in our experiment. The high Se urinary concentration particularly in camel receiving Se in their diet, compared to cattle, seems to demonstrate a peculiar sensitivity to Se supplementation.

Correlation with other Blood Parameters

The negative correlation with lymphocytes was observed on adult camels only and could be explained by the interferences between selenium level in organism and cellular events responsible for an immune response. Elevated Se has been shown to promote peroxidative damage in *in vitro* and *in vivo* systems. Lymphocyte cell membranes are especially susceptible to free radical damage (Bjornstedt et al., 1996).

The negative relation between Se and other trace elements, as Fe, Cu and Zn, was unclear and not in accordance with previous results (Seboussi et al., 2004). There was probably interaction between selenium (Se^{-}) and iron, copper or zinc (Fe^{++}, Cu^{++} or Zn^{++}), and interaction at molecular level was reported in human.

The negative correlation between Se and enzyme ALT, ALT and even with LDH and GGT for GSH-Px could be interpreted like an opposition between these parameters as indicators of the cellular integrity. Indeed, selenium was an essential element of cellular protection and one could make the assumption that those enzymes, indicators of the cellular suffering, were all the more high as the concentration in Se was weak. However all data concerning those enzymes were in the range of normal values in camel (Bengoumi et al., 1998b).

CONCLUSIONS

On average, the camel is able to maintain its metabolic functions according to its live weight and its production performance by eating 20% less than the cattle. The ability of this animal to recycle nitrogen, water and minerals is a common described feature. Therefore, the Se supplementation has to take care with the particular physiology of the camel. Indeed, the results seemed to confirm the sensitivity of camel to Se supplementation with an important increase of selenium in serum and milk. But this increase could be also the mark of a greater sensitivity to toxicity, although the tolerance level to selenium toxicity in camel was not known.

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Table1. Mean ± standard error of serum selenium (Se), gluthatione peroxidase (GSH-Px) and vitamin E (VitE) in camel before and after parturition according to the Se supplementation

[a-c] Means with different superscript differ ($P < 0.05$)

	Serum Se (ng/mL)		GSH-Px (IU/g Hb)		VitE (ng/mL)	
Se suppl.	**0 mg**	**2 mg**	**0 mg**	**2 mg**	**0 mg**	**2 mg**
Before parturition	97.4 ± 25.7[a]	272.7 ± 105[b]	21.2 ± 7.5[a]	58.1 ± 23.9[b]	1.06 ± 0.48[a]	1.16 ± 1.01[a]
After parturition	121.6 ± 25.5[a]	349.7 ± 85.5[c]	14.8 ± 8.8[a]	33.4 ± 20.7[c]	1.28 ± 0.90[a]	1.12 ± 0.71[a]

Table 2. Mean ± standard error of total selenium excreted in urine (SeExcU) and feces (SeExcF), selenium concentration in urine (SeConU) and feces (SeConF) female camel before and after parturition according to the Se supplementation.

[a-c] Means with different superscript letters differ ($P < 0.05$)

	Control group	Treated group
	Before parturition	
SeExcU (µg)	147.3 ± 115.1[a]	394.1 ± 217.3[b]
SeConU (ng/mL)	72.9 ± 56.1[a]	230.3 ± 150.0[b]
SeExcF (µg)	408.1 ± 190.2[a]	1533.8 ± 533.9[b]
SeConF (ng/g)	211.7 ±56.6[a]	724.2 ± 205.9[b]
	After parturition	
SeExcU (µg)	111.0 ± 62.7[a]	629.3 ± 330.5[c]
SeConU (ng/mL)	51.8 ±15.2[a]	252.5 ± 114.0[c]
SeExcF (µg)	487.9 ± 261.7[a]	2290.0 ± 942.7[c]
SeConF (ng/g)	238.9 ± 63.3[a]	940.8 ± 126.5[c]

Table 3. Correlation coefficients between antioxidant parameters in camel dam and camel calf: Se serum in the mother (Semoth), camel calf (Secalf) and milk (SeMilk), GSH-Px in mother (GPXmoth) and calf (GPXcalf), vitamin E in mother (VitEmoth) and calf (VitEmoth

Parameters	Se serum	GSH-Px	Vitamin E
PCV	-0,069	**0,263**	0,064
HB	-0,065	**0,255**	0,084
WBC	**0,203**	0,020	**0,176**
RBC	**-0,194**	**0,236**	0,067
platlets	0,144	**-0,182**	0,135
WBC / N	0,145	**0,223**	-0,155
WBC / L	**-0,319**	**-0,176**	0,091
WBC / M.	-0,004	-0,039	-0,101
WBC/ E.	**0,233**	-0,086	0,134
Cu	**-0,244**	-0,164	0,088
Zn	**-0,393**	-0,118	-0,005
Ca	**-0,228**	-0,160	0,001
P	-0,060	-0,119	0,140
Na	0,014	**-0,246**	-0,005
K	-0,003	0,055	-0,031
Fe	**-0,209**	-0,017	0,033
Bilirubin	-0,051	-0,090	-0,048
Glucose	**-0,197**	0,052	-0,022
Creatinine	**0,201**	**0,374**	0,063
Total protein	0,080	**0,185**	0,002
Albumin	-0,083	0,113	0,050
ALP	**-0,179**	-0,009	0,007
CK	-0,020	-0,017	0,039
ALT	**-0,240**	**-0,430**	-0,097
AST	**-0,195**	**-0,322**	0,025
LDH	-0,088	**-0,260**	0,003
GGT	0,124	**-0,464**	**0,198**

Table 4. Correlation coefficients between urinary or fecal excretion in camel and serum Se concentration

* $P < 0.05$; ** $P < 0.01$

	SeExcU	SeConU	SExcF	SeConF
SeExcU				
SeConU	**0.663****			
SeExcF	**0.516****	**0.553****		
SeConF	**0.671****	**0.722****	**0.771****	
Se serum	**0.591****	**0.618****	**0.583****	**0.830****

	SeMoth	GPXMoth	vitEMoth	SeCalf	GPXCalf	VitECalf
SeMoth						
GPXMoth	0.289					
vitEMoth	0.049	-0.003				
SeCalf	**0.682***	**0.624***	0.163			
GPXCalf	**0.839****	**0.509***	0.056	**0.830****		
VitECalf	-0.043	0.052	-0.187	-0.018	0.091	
SeMilk	**0.364***	**0.527***	-0.024	**0.596***	**0.495***	-0.200

* $P < 0.05$; ** $P < 0.01$

Table 5. Correlation coefficients between she-camel Se serum concentration, GSH-Px and vitamin E in one hand, and blood parameters in a second hand.

Parameters	Se serum	GSH-Px	Vitamin E
PCV	-0,069	**0,263**	0,064
HB	-0,065	**0,255**	0,084
WBC	**0,203**	0,020	**0,176**
RBC	**-0,194**	**0,236**	0,067
platlets	0,144	**-0,182**	0,135
WBC / N	0,145	**0,223**	-0,155
WBC / L	**-0,319**	**-0,176**	0,091
WBC / M.	-0,004	-0,039	-0,101
WBC/ E.	**0,233**	-0,086	0,134
Cu	**-0,244**	-0,164	0,088
Zn	**-0,393**	-0,118	-0,005
Ca	**-0,228**	-0,160	0,001
P	-0,060	-0,119	0,140
Na	0,014	**-0,246**	-0,005
K	-0,003	0,055	-0,031
Fe	**-0,209**	-0,017	0,033
Bilirubin	-0,051	-0,090	-0,048
Glucose	**-0,197**	0,052	-0,022
Creatinine	**0,201**	**0,374**	0,063
Total protein	0,080	**0,185**	0,002
Albumin	-0,083	0,113	0,050
ALP	**-0,179**	-0,009	0,007
CK	-0,020	-0,017	0,039
ALT	**-0,240**	**-0,430**	-0,097
AST	**-0,195**	**-0,322**	0,025
LDH	-0,088	**-0,260**	0,003
GGT	0,124	**-0,464**	**0,198**

Table 6. Correlation coefficients between camel calf Se serum concentration, GSH-Px , vitamin E and Se concentration in milk in one hand, and blood parameters in a second hand.

Parameters	Se serum	GSH-Px	Vitamin E	Se milk
PCV	0,177	0,168	-0,231	**0,533**
HB	0,162	0,152	-0,231	**0,521**
WBC	-0,223	-0,185	-0,125	0,095
RBC	0,151	0,157	-0,233	**0,488**
platlets	-0,043	0,078	0,147	-0,245
WBC / N	-0,013	-0,117	-0,224	0,307
WBC / L	0,034	0,125	0,212	-0,279
WBC / M.	0,059	0,036	-0,005	0,028
WBC/ E.	-0,061	0,039	0,166	-0,263
Cu	-0,123	-0,116	0,061	**-0,309**
Zn	-0,196	**-0,342**	-0,202	-0,231
Ca	0,083	0,184	0,038	0,097
P	0,232	0,180	0,012	-0,031
Na	0,017	-0,021	-0,100	0,027
K	**0,361**	0,176	-0,087	0,264
Cl	0,059	-0,105	0,147	-0,052
Mg	-0,229	-0,023	-0,028	-0,147
Fe	0,065	0,168	0,035	-0,008
Glucose	0,136	0,233	-0,015	-0,144
Bilirubin	-0,233	-0,212	-0,149	0,184
Creatinine	-0,274	-0,282	-0,169	**0,323**
Total protein	-0,021	0,012	0,130	-0,286
Albumin	-0,108	0,005	0,249	-0,254
ALP	-0,016	-0,191	0,044	0,065
CK	**0,402**	0,208	-0,098	**0,425**
ALT	-0,021	-0,109	0,054	**-0,337**
AST	0,227	0,130	-0,030	0,026
LDH	0,274	0,220	-0,055	0,114
GGT	-0,037	-0,172	-0,284	-0,016

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 (♈).

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 (♈).

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

Figure 4. Biweekly changes (mean and S.E) in GSH-Px activity (in IU/g Hb) in camel calf according to the selenium supplementation level: 0 mg/day (♀) and 2 mg/day (♈).

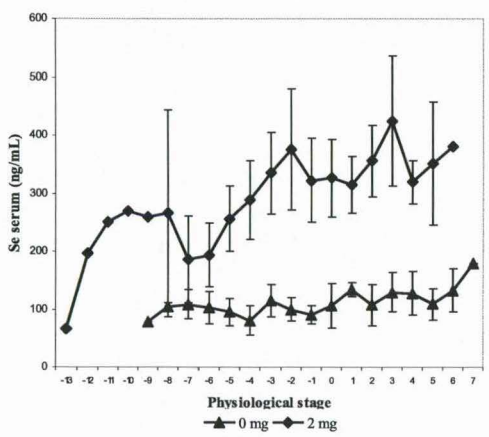
Figure 5. Biweekly changes (mean and S.E) in plasma vitamin E concentration (in µg/mL) in camel before and after parturition according to the selenium supplementation level: 0 mg/day (♀) and 2 mg/day (♈).

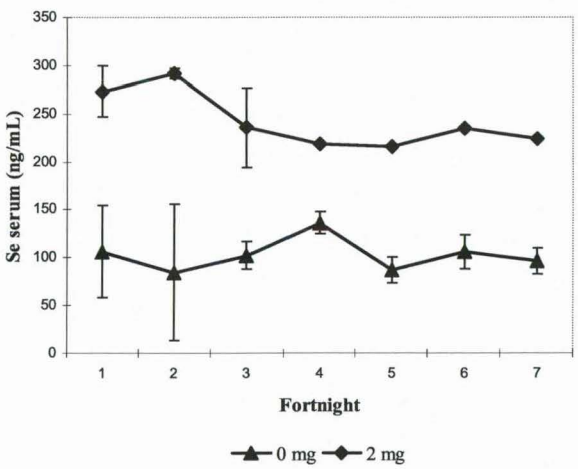
Figure 6. Biweekly changes (mean and S.E) in plasma vitamin E (in ng/mL) in camel calf according to the selenium supplementation level: 0 mg/day (♀) and 2 mg/day (♈).

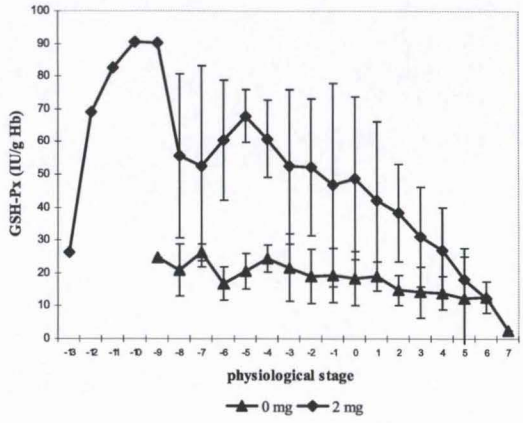
Figure 7. Biweekly changes (mean and S.E) in milk selenium concentration (in ng/mL) in she-camel for the three first months of lactation according to the selenium supplementation level: 0 mg/day (♀) and 2 mg/day (♈).

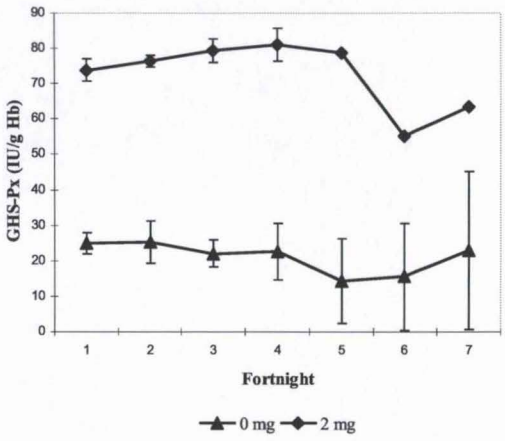
Figure 8. Monthly changes (mean and S.E) in Se urinary concentration (in ng/mL) in she-camel at the end of gestation and beginning of lactation according to the selenium supplementation level: 0 mg/day (♀) and 2 mg/day (♈).

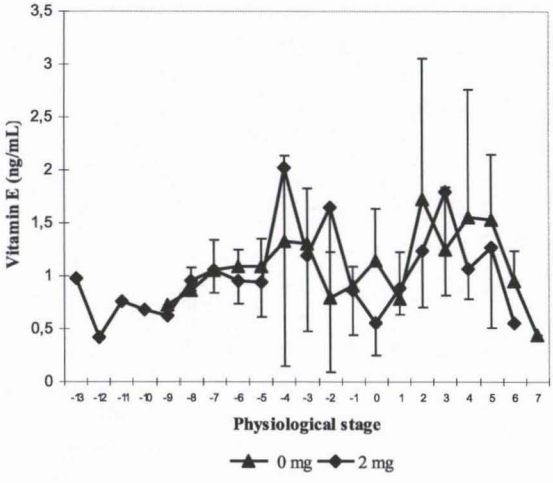
Figure 9. Monthly changes (mean and S.E) in Se fecal concentration (in ng/g) in she-camel at the end of gestation and beginning of lactation according to the selenium supplementation level: 0 mg/day (♀) and 2 mg/day (♈).

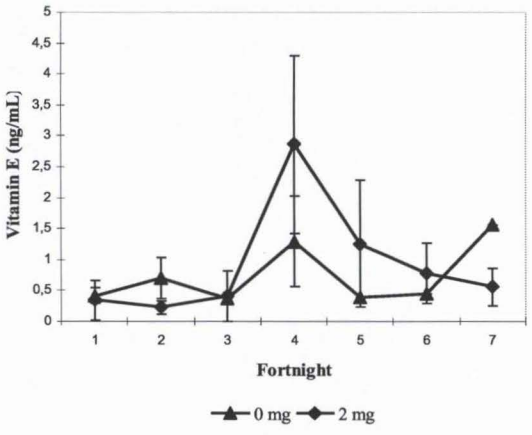


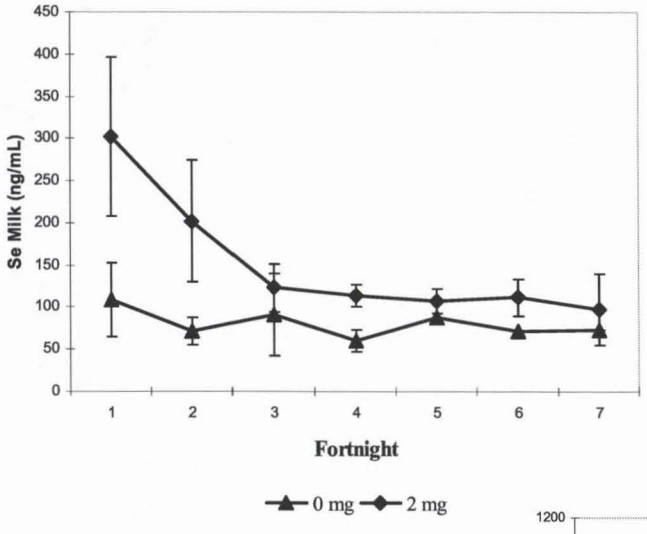



GSH-Px (IU/g Hb)
physiological stage
0 mg
2 mg


GHS-Px (IU/g Hb)
Fortnight
0 mg
2 mg


Vitamin E (ng/mL)
Physiological stage
0 mg
2 mg


Vitamin E (ng/mL)
Fortnight
0 mg
2 mg


Se Milk (ng/mL)
Fortnight
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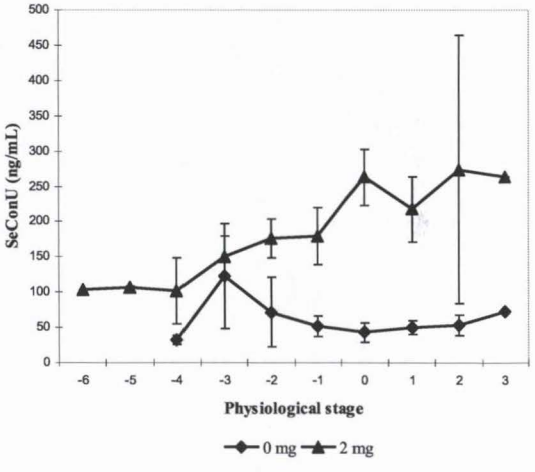
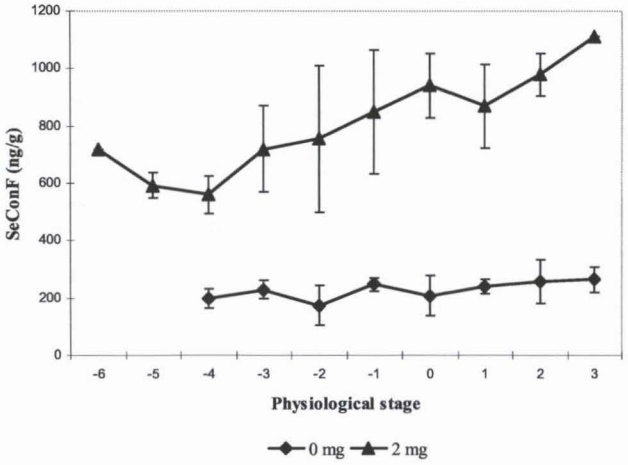
2 mg


SeConU (ng/mL)
Physiological stage
0 mg
2 mg


SeConF (ng/g)
Physiological stage
0 mg
2 mg