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ORIGINAL PAPER

INFLUENCE OF SELENITETRIGLYCERIDE SUPPLEMENTATION ON SELENIUM BLOOD STATUS AND SELECTED HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS IN CAMELS (*CAMELUS DROMEDARIUS*)*

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Abstract

This study examined the impact of oral supplementation with a new organic selenium preparation (selenitetriglycerides) on the health of camels (*Camelus dromedarius*). The experiment was conducted on 7 female camels, which were given 240 mg per animal per day of selenitetriglycerides with an oesophageal probe and subsequently monitored for 28 days. Blood samples for tests were collected 10 times (on day 1, 2, 3, 4, 5, 6, 7, 8, 14 and 28) and the following parameters were determined: the level of selenium, vitamin E, the activity of aspartate transaminase (AST), gamma-glutamyl transpeptidase (GGTP), lactate dehydrogenase (LDH), creatine kinase (CK) and the concentration of total protein, glucose, urea and creatinine. The following hematological parameters were determined in whole blood: white blood cell count (WBC), red blood cell count (RBC), hemoglobin level (HGB), platelet count (PLT) and packed cell volume (PCV). Selenium serum level increased significantly on the second day (from 40.18 μg l^{-1} to 198.79 μg l^{-1}), peaked on day 7 (514.76 μg l^{-1}) and remained at a statistically significant level relative to the baseline to the end of the experiment. Clinical observations and analysis of the hematological parameters, liver and kidney function parameters did not show any negative effect of selenitetriglycerides on camel health. The results indicate that selenitetriglycerides can be a safe and effective method of selenium supplementation in camels.

Keywords: selenitriglycerides, camels, selenium, hematological and biochemical parameters.

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INTRODUCTION

Camels (*Camelus dromedaries*) are very important breeding animals in many regions of the world, especially in the Middle East, Africa and Asia, and they are mostly raised on extensive grazing systems. The feeding systems must be adapted to semi-desert and desert conditions, and they depend on the fodder quantity and quality and the climate in the region. Since camels' nutritional demands, especially for micronutrients, are not often met under these grazing systems, the animals should receive micronutrient supplements throughout the year to ensure proper conditions for their growth and development (Alhidary et al. 2016).

Selenium deficit can cause serious health problems in many animal species, which include nutritional muscular dystrophy in ruminants or reproduction disorders, occurring mainly in male animals: bulls, rams and bucks. Deficits of this element significantly decrease the vitality and development parameters in young animals soon after birth, mainly lambs, calves and kids. Because of the important role played by selenium in the stimulation of the immune system, increased susceptibility of an animal to infections is observed with a deficit of this element (Spearset al. 1986).

Camels display a range of physiological adaptations which allow for effective micronutrient management in desert and semi-desert conditions, even with low levels in fodder (Faye 1994). These adaptations include: an increase in the element absorption from fodder in time of deficit, increased accumulation in the body and increased activity of certain enzymes responsible for their transport and metabolism (ceruloplasmin, superoxide dismutase) (Bengoumi et al. 1998, Faye et al. 1999).

According to literature data, average selenium serum levels in adult ruminants range from 70 to 100 μg l^{-1} (Stowe, Heardt 1992) and such levels ensure proper metabolism. Selenium levels in blood serum in camels vary depending on the region, age and sex. The levels measured in dromedaries in Morocco (Hamliri et al. 1990) ranged from 109.1 to 117.8 μg l^{-1}, whereas measurements conducted by Abdel Rahim (2005) in Sudan showed much lower concentrations, from 25 to 53 μg l^{-1}. Symptoms of selenium deficiency in camels were first described in animals kept in zoos and concerned myopathies and cardiomyopathies (Finlayson et. al. 1971, Decker et al. 1977). Similar ailments were described later in young animals kept for economic purposes. Soil and fodder in the UAE (United Arab Emirates) are very poor in selenium, which contributes to frequently observed muscular dystrophies in young camels, both as degeneration of skeletal and cardiac muscles (El-Khouly et al. 2001).

When analyzing the very important physiological role of selenium, it should be noted that the difference between a therapeutic and a toxic dose of the element is very small and depends on genetic predispositions, the way of releasing the element from the body, the type of supplementation (inorganic or organic) and interactions between micronutrients in the fodder.

Selenitetriglycerides are a completely new form of organic selenium, in which selenium's oxidation number is four. These compounds contain Se and triglycerides and they are formed by chemical modification of sunflower oil by selenic acid. These compounds are synthesized by esterification of pre-oxidized triglycerides into hydroxyl derivatives with selenic acid (Stańczyk et al. 2010). Selenitetriglycerides are lipophilic and are easily distributed in the body. Studies on rats have shown that oral administration of 2% and 5% solutions of selenitetriglycerides results in the highest selenium levels in kidneys and the liver, with much lower levels found in the brain, spleen, lungs, intestines and heart. Metabolism of this form of selenium in the rat takes place mainly in the liver, and the element is excreted mainly by kidneys – selenium was completely eliminated from the body within 24 hours of the supplementation (Jastrzębski et al. 1997).

The low toxicity of selenium released from selenitetriglycerides is a considerable advantage. According to the literature data, the lethal dose of the element after supplementation in an inorganic form ranges from 1 to 5 mg kg^{-1} BW (Koller, Exon 1986). Studies on rats have shown that the average lethal dose (Se LD_{50}) in supplementation with 2% selenitetriglycerides was 100 mg kg^{-1} BW, and 68 mg kg^{-1} BW with a 10% solution. Such high tolerance to selenitetriglycerides is extremely beneficial and it indicates that they are nearly 30 times less toxic (at the concentration of 2%) in rats than preparations containing sodium selenate.

It is noteworthy that reports on the role and possibilities of selenium supplementation in camels in the literature are scarce. There are no papers on the use of selenitetriglycerides in farm animals. This study aimed to determine the effect of oral administration of selenitetriglycerides (a completely new organic selenium compound) on selenium levels in camel blood serum and on selected hematological and biochemical parameters in these animals.

MATERIALS AND METHODS

All procedures performed in studies were in accordance with ethical standards of the national research committee. All applicable international, national and/or institutional guidelines for the care and use of animals were followed.

The experiment was conducted on a farm in Al Ain, United Arab Emirates. It was conducted on seven adult, 3- to 4-year-old, non-pregnant female camels (*Camelus dromedarius*) with an average body weight of 395±61 kg. During the experiment, the animals were fed individually established feed doses containing ca. 5 kg of hay from rhodes grass (*Chloris gayana)*, 2 kg of feed concentrates, and they had access to water *ad libitum*. Clinical examinations showed no disease symptoms in the animals. The experiment last-

ed 28 days. The camels were given 240 mg of selenitetriglycerides per animal with an esophageal probe for 7 days (starting on day 1). Blood for analyses was collected from the jugular vein from all animals on day 1 (before selenitetriglycerides were administered), on days 2, 3, 4, 5, 6, 7 (after the supplementation) and on days 8, 14 and 28 of the experiment. Blood samples were collected with kits comprising a test tube with EDTA K2 (2 ml) for hematological tests and a test tube with a coagulation activator (9 ml) for assays of selenium, vitamin E, glucose (GLUC), total protein (TP), urea (UREA) and creatinine (CREA) and determination of aspartate aminotransferase (AST), lactate dehydrogenase (LDH), gamma-glutamyl transpeptidase (GGTP) and creatine kinase (CK) activity. The hematological tests in whole blood samples were performed with a Sysmex XT 2000i. The following parameters were determined: white blood cell count (WBC), red blood cell count (RBC), hemoglobin level (HGB), platelet count (PLT) and packed cell volume (PCV). Serum selenium concentration was determined by ICPMS – Perkin Elmer ICP MassSpectrometer Nexion 350X. Vitamin E concentration was determined by high-performance liquid chromatography (HPLC) with a Hewlett Packard HP-1050 chromatograph and ClinRep kits. The blood biochemical tests were performed with a clinical chemistry analyzer Cobas 8000 c702.

Data were entered into Microsoft Excel and processed using SPSS software (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.). The differences between day 1 (before selenium supplementation) and the remaining sampling dates (days 2, 3, 4, 5, 6, 7, 8, 14, 28) were calculated using the Wilcoxon signed-rank test with the Bonferroni adjustment for multiple comparisons. The significance of differences between sampling dates was determined using a P value of ≤ 0.01.

RESULTS

The hematological parameters (WBC, RBC, HGB, PLT and PCV) remained at a similar level throughout the experiment with no significant differences between sample collections (Table 1). Similarly, the biochemical parameters (glucose, total protein, urea and creatinine) remained at a similar level to the end of the experiment (Table 2). The serum selenium level was the lowest before supplementation and it then increased significantly ($P \leq 0.01$), starting on day 2 of the experiment, to reach the peak value on day 7 of supplementation and to decrease gradually with consecutive blood sample collections. It was significantly higher ($p \leq 0.01$) than at baseline even on day 28 of the experiment (21 days after selenitetriglycerides supplementation ended) – Table 3. The serum level of vitamin E, as well as the AST, CK, LDH and GGTP activity, remained at a similar level throughout the experiment (Table 3).

Table 1

Hematological parameters in camels orally administered selenitetriglycerides on days 1-7 (mean+SD)

	Days									
Parameter	1	2	3	4	5	6	7	8	14	28
WBC ($10^9 l^{-1}$)	10.71±4.11	10.95±3.93	11.55±3.46	11.88±3.41	11.42±3.86	10.37±3.83	11.29±4.02	10.88±3.39	10.75±4.19	11.85±4.79
RBC ($10^{12} l^{-1}$)	8.14±0.69	8.42±1.13	8.69±0.78	8.85±0.37	9.21±0.81	8.42±0.78	8.57±0.79	8.76±0.69	7.85±0.73	8.12±1.11
HGB (g dl^{-1})	11.15±0.81	11.42±1.13	11.14±1.06	11.23±0.78	11.71±0.95	11.14±1.21	11.59±0.81	11.19±0.89	10.14±1.06	10.42±1.39
PLT ($10^9 l^{-1}$)	309.42±35.05	316.12±53.87	261.27±60.67	269.14±99.96	225.71±94.41	236.14±72.98	279.42±54.14	255.12±62.34	231.28±67.67	262.28±82.85
PCV (%)	23.85±1.67	24.85±2.03	24.42±1.81	25.42±1.71	27.13±2.16	25.15±2.44	25.33±1.63	25.49±1.61	23.28±1.64	24.28±2.36

No statistical difference was noted between pre-treatment values and any of the days after treatment.

Table 2

Serum concentrations of glucose, total protein, urea and creatinine in camels orally administered selenitetriglycerides on days 1-7 (mean+SD)

	Days									
Parameter	1	2	3	4	5	6	7	8	14	28
Glucose (mmol l^{-1})	4.81±0.15	4.31±0.25	4.68±0.27	5.02±0.32	4.31±0.45	4.92±0.63	4.51±0.61	4.17±0.31	4.29±0.54	4.22±0.43
Total protein (g dl^{-1})	5.54±0.51	5.74±0.44	5.57±0.49	5.71±0.48	5.75±0.52	5.67±0.51	5.88±0.45	5.62±0.57	5.52±0.66	5.65±0.56
Urea (mmol l^{-1})	1.67±0.53	1.65±0.46	1.42±0.36	1.29±0.33	1.53±0.36	1.38±0.42	1.92±0.89	1.95±0.71	1.88±0.31	1.85±0.82
Creatinine (µmol l^{-1})	139.42±17.69	116.71±15.86	130.22±17.11	126.42±14.75	136.85±13.83	130.87±14.89	132.35±18.38	120.15±11.48	116.12±21.13	133.05±22.81

No statistical difference was noted between pre-treatment values and any of the days after treatment.

Table 3
Serum concentrations of selenium, vitamin E and activity of AST, CK, LDH and GGTP in camels orally administered selenitetriglycerides on days 1-7 (mean+SD)

	Days									
Parame-ter	1	2	3	4	5	6	7	8	14	28
Se ($\mu g\ l^{-1}$)	40.18±11.29	198.79*±29.51	298.09*±27.85	366.24*±39.06	409.61*±64.59	458.78*±75.67	514.76*±83.17	502.94*±90.11	243.78*±31.26	197.83*±32.34
Vit. E ($\mu g\ ml^{-1}$)	0.89±0.33	0.99±0.42	0.76±0.21	0.84±0.29	0.91±0.47	0.86±0.23	0.95±0.34	0.79±0.24	0.81±0.37	0.93±0.39
AST ($U\ l^{-1}$)	69.88±21.74	70.21±22.69	71.48±22.71	72.42±22.28	70.65±18.71	66.91±20.76	66.61±21.62	69.31±.22.81	76.15±24.13	73.13±23.44
CK ($U\ l^{-1}$)	111.71±33.73	143.15±53.24	118.28±50.11	101.85±18.33	130.14±28.43	144.13±22.33	124.5727.45	110.61±21.62	138.50±27.76	113.44±31.33
LDH ($U\ l^{-1}$)	464.28±62.44	420.71±64.47	421.28±67.73	527.71±55.08	502.85±82.67	500.57±65.37	447.57±72.54	445.85±68.28	480.28±84.76	443.18±71.03
GGTP ($U\ l^{-1}$)	7.57±1.13	8.12±1.15	7.42±0.97	8.38±1.15	6.69±0.81	7.27±1.09	7.85±1.34	7.14±1.21	7.73±1.34	7.43±1.03

* Significant differences at $P \leq 0.01$ between sampling dates (day 1 vs. day 2, day 1 vs. day 3, day 1 vs. day 4, day 1 vs. day 5, day 1 vs. day 6, day 1 vs. day 7, day 1 vs. day 8, day 1 vs. day 14, day 1 vs. day 28).

DISCUSSION

The results of the hematological tests indicate that selenium supplementation did not affect the WBC or RBC count, HGB, platelet count or PCV. A comparison of the results with literature data (Chaudhary, Iqbal 2000, Hassan et al. 2018) shows that they lay within the reference ranges for camels. Some authors (Seboussi et al. 2009) have noted the negative correlation of the WBC count in camels with supplementation with inorganic selenium forms (sodium selenate), attributing it to higher exposure to oxidative changes in lymphocyte cellular membranes. It was emphasized in a study conducted by Hassan et al. (2018) that a selenium deficit in camels was accompanied by a decrease in HGB level and the PCV and RBC counts, which suggested the occurrence of anemia in the animals. The findings of studies on cows (Kaur et al. 2005) indicate that selenium supplementation can increase the RBC and WBC counts and HGB level. No such relationships were observed in this experiment.

An analysis of the serum glucose concentrations shows that the selenitetriglyceride supplementation did not affect this parameter. The results were similar throughout the experiment and remained within the reference standards for dromedaries given in the literature (Elitok, Cirak 2018). Literature data (Juniper et al. 2006) concerning selenium supplementation in dairy cattle indicate a similar lack of effect of the concentration of this microelement on glucose levels. However, some authors (Fontenelle et al. 2018) suggest the potential impact of selenium on glucose transformations through stimulation of glucose intake and regulation of metabolic processes such as glycolysis, gluconeogenesis, fatty acid synthesis or the pentosephosphate pathway.

The total protein concentration was similar throughout the experiment, which indicates that this parameter is not affected by the supplementation. These results resembled the values reported by other authors (Elitok, Cirak 2018, Hassan 2018). Hassan et al. (2018) found that a selenium deficiency can cause hypoproteinemia and hypoalbuminemia in camels. The literature data obtained on goats (Reczyńska et al. 2019) also indicate that selenium can stimulate protein biosynthesis and its supplementation increases protein concentration in ruminant serum.

The urea and creatinine analyses indicate that there is no significant effect of selenitetriglyceride supplementation, which proves the absence of any adverse effect of selenium supplementation on the kidney function. The urea concentration was quite low in all tests and was slightly different than that reported in the literature. The creatinine level in serum remained within the reference range for camels (Elitok, Cirak 2018). The results of this experiment were confirmed by a cattle study (Shinde et al. 2009, Bagnicka et al. 2017), in which it was proven that therapeutic doses of selenium did not have an adverse effect on kidney function. An increase in the

urea and creatinine concentration is a rather sensitive indicator of the organ damage caused by hyperselenosis and such symptoms have been observed in calves after overdosing this microelement (Kumar et al. 2008).

The selenium serum level increased significantly on all sampling days. It was the highest on day 7 of the supplementation and it remained at a significantly increased level compared to the baseline value until day 21, following the end of supplementation with selenitetriglycerides. The dose of the selenium preparation was determined based on a study which demonstrated a low selenite triglyceride toxicity level in rats (Jastrzębski et al. 1997) and an experiment conducted on sheep (Zagrodzki et al. 2000) and it was 240 mg Se per animal per day, which gives approximately 0.6 mg Se kg^{-1} BW.

It should be highlighted that this is the first investigation of the effectiveness of selenitetriglyceride application in camels. The concentrations obtained in this experiment (maximum selenium concentration of 514.76 μg l^{-1} on day 7) are quite high and exceed the reference levels for this element for camels (Seboussi et al. 2010). The selenium concentration of 40.18 μg l^{-1} at the beginning of the experiment was low, which could indicate a deficiency in tested animals. Other authors (Seboussi et al. 2008) applied long-term (90 days) oral supplementation with sodium selenate in camels at 2 mg daily and observed much lower Se concentrations. Similar results were observed in cows (Juniper et al. 2006), which demonstrated that the preparation used in the current study was effective.

It is common knowledge that selenium can be toxic even at a small overdose. Clinical observations of the camels in the experiment did not show any disease symptoms associated with the administration of selenitetriglycerides. There are no reports in the literature on selenium poisoning in camels, but there are descriptions of such cases in other ruminants. A cattle study (Kaur et al. 2005) revealed symptoms of selenium poisoning after administration of a dose of 2.5 mg Se kg^{-1} BW for 21 days. The first signs of subacute systemic toxicity, i.e. anorexia, redness of the eye, diarrhea, swelling of the joints and the base of the ear, and wound formation in the pastern region, were observed by the sixth day of the experiment and two of the animals died within 18-21 days. Stiffness of the neck, respiratory distress and subnormal body temperature were observed in the terminal stages. In another study (Tiwary et al. 2006), lambs were administered a single ruminal bolus containing sodium selenate (0, 1, 2, 3 or 4 mg Se kg^{-1} BW) or selenomethionine (0, 1, 2, 3, 4, 6 or 8 mg Se kg^{-1} BW). The animals were observed for seven days. Sodium selenate doses higher than 2 mg Se kg^{-1} BW and selenomethionine doses higher than 4 mg Se kg^{-1} BW resulted in tachypnea and/or respiratory distress after minimal exercise. Higher doses induced multifocal myocardial necrosis and pulmonary alveolar vasculitis with pulmonary edema and hemorrhage.

The current study showed a rapid increase in the serum selenium level following the supplementation. This element level increased nearly five-fold

within 24 h, which shows that this form of selenium preparation is highly available to camels.

In studies performed on the same animal species using oral supplementation of an inorganic form of selenium (Seboussi et al. 2008), a significant increase in the serum Se concentration was not achieved until four weeks of the supplementation and it peaked in week 11 of the experiment. The limited effectiveness of oral administration of short-acting, inorganic selenium species was confirmed by other authors (Ortman, Pehrson 1999), who did not observe a satisfactory increase in the element level in ruminants following such supplementation. The selenium level in the current experiment peaked on day 7 of selenitetriglyceride administration, when it was more than 12 times higher than at baseline.

The serum concentration of vitamin E remained similar throughout the experiment, which indicates that there is no correlation between selenium supplementation and this parameter. The serum vitamin E levels were quite low (below 1 $\mu g\ ml^{-1}$), which suggests that the nutrient supply was rather poor. Such low concentrations of vitamin E can be attributed to its low levels in fodder, since other authors (Seboussi et al. 2010) determined the level of this parameter in rhodes grass (which was the main component of the camel fodder used in this study) to be 5.5 $\mu g\ g^{-1}$.

The activity of the liver enzymes (AST, LDH and GGTP) determined in the current study indicates that they are not affected by the supplementation with the selenium preparation. The dose of selenitetriglycerides (240 mg per animal per day) did not have an adverse impact on the liver function, which shows that this form of selenium is well tolerated. The findings of another study (Seboussi et al. 2008), in which different doses of an inorganic selenium preparation were used, were also similar. The results of studies on sheep (Zaki et al. 2018) demonstrated that an increase in the liver enzymes (especially AST) activity is a sensitive parameter of selenium poisoning. Notably, the GGTP activity was lower in the current study than in another study (Seboussi et al. 2010), which indicated that the full integrity of liver cells was preserved.

As in the case of liver enzymes, no changes in the activity was observed for creatine kinase, which is the most specific indicator of muscular degeneration caused by selenium deficiency in ruminants (Abutarbush, Radostits 2003). The activity of this enzyme determined in the current study did not differ from the data published for camels by other authors (Seboussi et al. 2008) and it remained within the reference range for the species.

The results of this study indicate that oral administration of selenitetriglycerides is a safe and effective method of selenium supplementation in camels. The increase in the serum level of the element was much higher than in studies where inorganic forms of selenium were used. The research described in this paper was a pilot study. It seems necessary to conduct further research on a larger number of animals to demonstrate the useful-

ness of this form of selenium supplementation in camels, which is of scientific and practical importance in the breeding of these animals in the Middle East.

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