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Trace mineral changes in response to organic and inorganic selenium supplementation

Kojouri[1*], Gh., Haghighi[2], N., Aliyari[2], S., Shadkhast[3], M., Eshareghi Samani[2], R., Kojouri[4] A.

[1]Professor, Department of Clinical Sciences, School of Veterinary Medicine, Shahrekord University, Shahrekord, Iran.

[2] DVM, School of Veterinary Medicine, Shahrekord University, Shahrekord, Iran.

[3]Associate professor, Department of Basic Sciences, School of Veterinary Medicine, Shahrekord University, Shahrekord, Iran.

[4]Student, Faculty of Medicine, International Campus of Shahid Sadoghi Medical University, Yazd, Iran.

***Corresponding author**: drgholam_alikojouri@yahoo.com; kojouri@vet.sku.acir*

Abstract

Twenty-one Lori–Bakhtiary sheep were randomly allocated into 3 groups to compare the effects of oral selenium nano particles (Se NPs) and sodium selenite (Na-Se) supplementation on serum selenium, copper, zinc, molybdenum, manganese and cobalt concentration. Groups 1 and 2 orally received Se NPs and Na-Se (1 mg/kg) for 10 consecutive days, respectively and group 3 were considered as control group. Blood samples were taken on the days 0, 10, 20 and 30, and minerals were measured using atomic absorption spectrophotometery. The obtained results showed that selenium, copper and manganese concentration increased and zinc level decreased significantly in both treated groups (P<0.05). In addition, serum molybdenum concentration increased in response to oral Na-Se supplementation on the days 10 and 20 compared to the basal level and also the control group (P<0.05). Findings explain the interaction between selenium derivates and other elements in which the role of Se NPs was considerable in increasing Cu and Mn level and the role of Na-Se noticeable increasing serum molybdenum concentration.

Keywords: Selenium, Nano particles, Mineral, Interaction, Sheep.

Introduction

Selenium (Se) is an essential trace mineral for mammals with a variety of functions, because it plays important roles in the regulation of thyroid hormone metabolism, cell growth and antioxidant defense systems; thus, it protect cells against oxidative stress damage (Zhang *et al.*, 2007).

Generally, selenium deficiency is prone to many problems, including white muscle disease (WMD), pneumonia and infertility. Additionally, it may cause decreased antioxidant defense which is usually defined as oxidative stress (Surai, 2006).

Ruminant diets are usually high in fiber, and considerable digestion of fiber occurs via microbial fermentation in the rumen. However, association of minerals with fiber fractions in feedstuffs and/or binding of minerals to undigested fiber constituents in the gastrointestinal tract may alter bioavailability of some trace minerals in ruminants. The pH in the rumen environment is only slightly acidic (6.0–6.8), and in the rumen, many trace minerals exist largely in an insoluble form. At least some of the metal complexes that are formed in the rumen remain insoluble even under the acidic conditions found in the abomasum (Waghorn *et al*., 1990).

The bioavailabilities of trace elements are also known to be affected by interactions with other elements. These interactions are often antagonistic, but some cases of synergistic interactions have also been observed. For example, whereas the bioavailability of copper is affected negatively by zinc, iron and molybdenum (O'Dell 1989), it has been found that selenium supplementation to sheep grazing on copper deficient pastures actually increases copper absorption (Constable *et al*., 2017).

Kojouri *et al.* (2012) showed that supplementation with Se NPs and Na-Se at early stages decreased the serum iron concentration and raised total iron binding capacity (TIBC) level in sheep. For these reasons, the present study was designed to compare the effects of oral Se NPs and Se-Na supplementation on selenium, copper, zinc, molybdenum, manganese and cobalt status in sheep.

Material and methods

The experimental procedures were carried out with the guidelines of Shahrekord University (Shahrekord, Iran) for the care and use of laboratory animals.

Animals

Twenty-one healthy, 5 to 12 months old, Lori–Bakhtiary sheep were selected and randomly divided into three groups. Groups 1 and 2 were orally given Se NPs (1 mg/kg BW) and Na-Se (1 mg/kg BW) for 10 consecutive days, respectively. The control group was given distilled water (group 3). Animals were housed in box stalls and fed hay silage, straw, and barley met the recommended nutrient requirements (NRC, 1982).

Selenium nano particles (Se NPs) preparation

Se NPs were synthesized with a method described previously with some minor modification (Zhang *et al.*, 2004). Based on this method, the Se NPs were prepared by drop-wise adding of ascorbic acid solution to an aqueous solution of SeO_2 (1 mM) that was vigorously stirred until the concentration of ascorbic acid in the mixture reached 4 mM. During this process, a visible red precipitate was formed, and this color was observed as a provisional marker showing the conversion of Se^{4+} ions to Se^0 NPs. To observe the NP surface features and to determine the elemental composition of NPs, a SEM (scanning electron microscope) equipped with an EDX (energy dispersive X-ray) microanalysis attachment was employed. For SEM observation, NPs were mounted on specimen stubs with double-sided adhesive tape and coated with gold in a sputter coater device (model SCD 005; Bal-Tec). Samples were analyzed by using an SEM (Philips XL30).

Sampling

Blood samples were taken from jugular vein before the beginning of the experiment (Day 0) and subsequently on the days 10, 20 and 30. Serum concentration of selenium, copper, zinc, molybdenum, manganese and cobalt was determined by atomic absorption spectrophotometry (Kojouri, 2006; Kojouri and Shirazi, 2007; Kojouri *et al.*, 2009a; Kojouri *et al.*, 2009b).

Analytical method

Data (Mean ± SE) were analyzed by Sigmastat 3.1 version (Sigma Stat, Jandel Sci, San Rafaael, CA) program, using One Way Analysis of Variance (ANOVA) and Pearson correlation at the level of $p<0.05$.

Results

Three different ration samples were taken and selenium, copper, zinc, molybdenum, manganese and cobalt concentration were measured by atomic absorption spectroscopy (UNICAM 939; Unicam, Kassel, Germany). The mean values on the basis of dry matter were as follows; 0.49±0.17, 19.06±7.1, 15.22±3.01, 0.1±0.014, 118.14±19.21, 0.87±0.77 mg/kg, respectively. Results showed that there were no significant differences in the basal level (Mean ± SEM) of selenium, copper, zinc, molybdenum, cobalt and manganese of 3 groups ($p>0.05$). As shown in table 1, serum concentration of selenium was significantly increased in both treated groups ($P<0.05$). In response to oral selenium derivates supplementation, serum Cu and Mn level increased and Zn concentration decreased. A positive correlation was also found between Se/Cu in Se NPs supplemented group ($r = +0.886$, P value = 0.0452). In Na-Se supplemented group serum molybdenum concentration increased on the days 10 and 20 compared to day 0 and the control group (P values= 0.035 and 0.033, respectively). The cobalt concentrations were not affected by oral Se supplementation.

Discussion

Serum selenium concentration in both treated groups increased significantly compared to the basal level and the control ones. These results were similar to Pan *et al.* (2007) findings who reported that selenium supplementation increased muscle, liver, kidney and spleen selenium level compared to the control group. Wang *et al.* (2007) stated that antioxidant and pro-oxidant effects, or bioavailability and toxicity of selenium depends on its chemical form. Elemental selenium powder in the redox state of zero (0) is not soluble but is generally considered to be biologically inert. Nanotechnology holds promise for medication and nutrition because materials at the nanometer dimension exhibit novel properties different from those of both isolated atom and bulk material (Albrech *et al*., 2006; Zhang *et al*., 2007). Serum selenium level significantly increased in both treated groups, but in group 2 the increase of selenium level occurred 10 days sooner than Se NPs ($P<0.05$). However, no remarkable differences were observed between the two treated groups. This finding may explain the various bioavailabilities Se NPs and Na-Se in sheep.

In Se NPs group, the increasing trend of the serum selenium concentration started more slowly than that in the Na-Se group, wherein it increased sharply. This may relate to new characters of nano size selenium which is absorbed by active transportation (amino acid transport systems) from duodenum (Zhang *et al.*, 2001; Qin *et al.*, 2007; Wang *et al.*, 2009).

Wang *et al.* (2009) stated that inorganic selenium compounds, such as selenite, are passively absorbed (only 29 percent) throughout the small intestine and 50 to 75% of total ingested Se is excreted in the urine. Mehdi *et al.* (2013) explained that low absorption rate of selenite and selenate in ruminants is believed to be the result of selenide formation in the rumen (Zhang *et al.*, 2001; Qin *et al.*, 2007; Wang *et al.*, 2009).

Hu *et al.* (2012) showed that the transfer of Se NPs from the intestinal lumen to the body was higher than that of selenite, while the intestinal retention of Se NPs was lower compared toselenite.

Table 1**:** *Serum concentration changes of selenium, copper, zinc, Zn/Cu ratio, molybdenum, cobalt and manganese during seleniumnanoparticles and sodium selenite supplementation in sheep. [All data are presented in Mean and in each group (n=7); Significant changes are bolded].*

	Selenium nano particles supplementation (Se NPs) (Group 1)				**Control (Group 3)**				**Sodium Selenite (Group 2)**			
	Day				**Day**				**Day**			
	0	**10**	**20**	**30**	**0**	**10**	**20**	**30**	**0**	**10**	**20**	**30**
Selenium (μg/ml)	0.29	0.43	**0.55**$^{\alpha,\delta}$	**0.55**$^{\alpha,\delta}$	0.32	0.34	0.37	0.31	0.3	**0.5**$^{\alpha,\delta}$	**0.53**$^{\alpha,\delta}$	**0.51**$^{\alpha,\delta}$
Cu (μg/dl)	107.4	**123.4**$^{\delta}$	**103.2**$^{\beta}$	**98.2**$^{\beta}$	97.2	100	95.4	85.6	98.2	116.4	**92.6**$^{\beta}$	**86.4**$^{\beta}$
Zn (μg/dl)	147.64	**89.84**$^{\alpha,\delta}$	**102.58**$^{\alpha}$	**106.08**$^{\alpha}$	145.76	139.18	130.06	127.62	137.46	**75.72**$^{\alpha,\delta}$	**90.66**$^{\delta}$	**89.94**$^{\delta}$
Zn/Cu	1.38	**0.756**$^{\delta}$	**0.99**$^{\beta,\delta}$	**1.09**$^{\beta,\delta}$	1.51	1.41	1.4	1.49	1.47	**0.65**$^{\alpha,\delta}$	**0.97**$^{\beta,\delta}$	**1.05**$^{\beta,\delta}$
Mo (ppm)	61.2	50.6	98	90.4	59.4	71.8	68.4	53	63.6	**127.4**$^{\alpha,A}$	**131.4**$^{\alpha,\delta}$	136.2
Co (ppm)	38.4	46	44	39.2	35.8	31.6	35	37.5	38.8	39.8	37.4	43
Mn (ppm)	41	50.6	**72**$^{\alpha,\delta}$	**61.4**$^{\alpha}$	44.9	35	32	39	47.4	26.8	**59.4**$^{\beta,\delta}$	**31.6**$^{\gamma,A}$

α: Was significant to the basal level (Day 0), P<0.05.
β: Was significant to day 10, P<0.05.
γ: Was significant to day 20, P<0.05
δ: Was significant to the control group, P<0.05.
A: Was significant to the group 2 (Na selenite), P<0.05.

Evidence for interactions between Se NPs and copper obtained as copper serum levels on the day 10 significantly increased compared to the control group (P<0.05). At this point, there was a significant positive correlation between Se/Cu. This finding is in agreement with the result of a New Zealand study, in which administration of selenium to sheep grazing on copper deficient pastures increased the copper absorption and improved the growth rate of lambs (Constable *et al.*, 2017).

Kojouri and Shirazi (2007) explained that injection of selenium and vitamin E compound to pregnant ewes increased copper concentration of their offspring which in turn could prevent lambs from disorders related to copper deficiency. Copper is an essential component of a number of mammalian enzymes. Some of the medically important copper-containing enzymes are (1) the cytosol form of superoxide dismutase (copper and zinc), (2) cytochrome oxidase (c and aa_3), (3) lysyl oxidase, (4) ascorbic acid oxidase, and (5) ceruloplasmin. In addition, copper appears essential for absorption and transportation of iron to the liver and reticuloendothelial system and is therefore necessary for hemoglobin formation. Furthermore, copper is essential for preventing cellular oxidative damage and its role in iron and sulfur metabolism is also important (Smith, 2015).

Conversely, the serum zinc concentration and the Zn/Cu ratios decreased in both treated groups. House and Welch (1989) stated that selenium had an antagonistic effect on zinc absorption in zinc depleted rats, and also had an antagonistic effect on selenium absorption in zinc adequate rats.

Selenium has been increasingly recognized as an essential element in biology and medicine. Its biochemistry resembles that of sulfur yet differs from it by virtue of both redox potentials and stabilities of its oxidation states (Underwood and Suttle, 1999). Selenium can substitute for the more ubiquitous sulfur of cysteine and therefor plays an important role in more than a dozen selenoproteins. Selenium compounds release zinc from zinc/thiolate coordination environments, thereby affecting the cellular thiol redox state and the distribution of zinc and likely of other metal ions (Jacob *et al.,* 1999).

Kojouri and Shirazi (2007) also showed that selenium supplementation in pregnant ewes increased their offspring serum concentrations of Cu and Fe during the first 4 weeks of life and disturbed their Zn:Cu and Zn:Fe ratios at the end of 4th week which in turn may lead to the zinc deficiency. Underwood and Suttle (1999) explained the inverse role of Zn and Cu in ruminants and emphasized that the presence of each of these elements resulted in a decrease in absorption of another. Zinc is a component of the enzyme carbonic anhydrase, which is located in the red blood cells and parietal cells of the stomach and is related to the transport of respiratory carbon dioxide and the secretion of hydrochloric acid by the gastric mucosa. Zinc is also associated with RNA function and related to insulin, glucagon, and other hormones. It also has a role in keratinization, wound healing, calcification, and somatic and sexual development. Because it has a critical role in nucleic acid and protein metabolism, a deficiency may adversely affect the cell mediated immune system (Constable *et al.*, 2017).

Our results also showed increasing trend in serum concentration of manganese in response to selenium derivates supplementation. But cobalt concentration was not affected by Se supplementation.

Trace minerals including zinc, copper, and manganese are necessary for normal keratinization of horn cells or excessive selenium may contribute to the development of sand cracks (Anderson and Rings, 2009). Phosphorus, cobalt, and molybdenum were considered as nutritional elements that may affect parasite infestation. Supplementation with phosphorus has been shown to prevent worm establishment.

Cobalt deficiency has also been associated with reduced immunity to gastrointestinal nematodes and the addition of molybdenum at 6 to 10 mg/day to the diet decreased worm burdens in lambs. This effect was not attributable to the expected copper deficiency. Molybdenum may have a role in increasing jejunal mast cells and blood eosinophil numbers that may prevent parasite establishment (Smith, 2015).

Manganese plays an active role in bone matrix formation, and in the synthesis of chondroitin sulfate, responsible for maintaining the rigidity of connective tissue. In manganese deficiency, these are affected deleteriously and therefor skeletal abnormalities result.

Sadeghian *et al.* (2012) stated that the thiobarbituric acid reactive substances (TBARS) value in Se NPs group were reduced after the 20th day, but in group 2 (Na-Se), the TBARS value was in a high level till the 30th day. These findings showed that increasing trend of anti-oxidant activities in Se NPs group reduced the TBARS value sooner.

In the present study, the interaction between selenium derivates and other elements such as Cu and Mn was established in which the role of Se NPs is more considerable than Na-Se. As previously described these elements (Se, Cu and Mn) have useful roles in anti-oxidant activities, which protect cells from lipoperoxidation damage in conjunction with reduction in TBARS value.

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