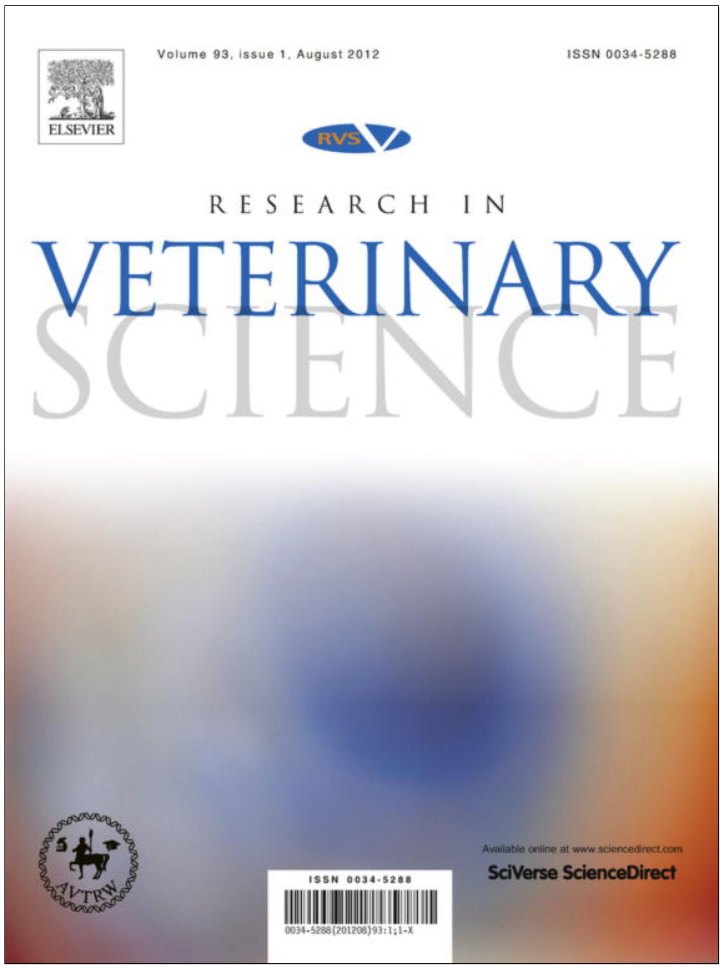

Research in Veterinary Science 93 (2012) 275–278

Contents lists available at SciVerse ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

Effect of selenium supplementation with sodium selenite and selenium nanoparticles on iron homeostasis and transferrin gene expression in sheep: A preliminary study

Gholam Ali Kojouri [a], Samane Jahanabadi [a,b], Mojtaba Shakibaie [c], Ali Mohammad Ahadi [d], Ahmad Reza Shahverdi [e,*]

[a] Department of Clinical Sciences, School of Veterinary Medicine, Shahrekord University, P.O. Box 115, Shahrekord, Iran
[b] Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, P.O. Box 13145 784, Tehran, Iran
[c] Department of Pharmacognosy and Biotechnology, School of Pharmacy, Pharmaceutics Research Center, Kerman University of Medical Sciences, P.O. Box 76175-493, Kerman, Iran
[d] Department of Genetics, Faculty of Basic Sciences, Shahrekord University, P.O. Box 115, Shahrekord, Iran
[e] Department of Pharmaceutical Biotechnology and Biotechnology Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, P.O. Box 14155-6451, Tehran 14174, Iran

ARTICLE INFO

Article history:
Received 8 January 2011
Accepted 28 July 2011

Keywords:
Iron homeostasis
Selenium
Transferrin
Nanoparticle
Sheep

ABSTRACT

The present research aimed at evaluating the effects of sodium selenite and selenium nanoparticles (Se NPs) on iron homeostasis and the expression of transferrin and its receptor-binding protein genes. Twenty one Lori–Bakhtiary sheep were randomly allocated into 3 groups. Groups 1 and 2 orally received Se NPs and sodium selenite (1 mg kg^{-1}) for 10 consecutive days, respectively. Group 3 served as the control. Blood and sternal bone marrow samples were collected at different supplementation intervals. Various factors such as serum iron concentration, total iron binding capacity (TIBC), and transferrin saturation percent were determined. The expression of transferrin and transferrin binding receptor genes was also studied. Results showed a decreasing trend in serum iron concentration particularly during the early and middle stages of supplementation (0–20 days) with Se NPs or selenium ions. Conversely, the TIBC level increased in sera especially during these periods (0–20 days) in animals that received selenium NPs or selenium ions. Our results also showed that expression of transferrin and its receptor genes was considerably increased during supplementation of the animals by both selenium compounds for 10 or 20 days. After this period, the expression of the mentioned genes significantly decreased, especially in animals that received selenium ions.

1. Introduction

Selenium is an essential micronutrient metalloid with a variety of biological functions. Dietary selenium compounds have an important role with alpha-tocopherol (vitamin E) to prevent cell damage and are necessary for growth, fertility, and immune system health in humans and animals (Schrauzer and Surai, 2009; Hoffmann and Berry, 2008). Selenium deficiency causes scouring, ill thrift, and lowered wool production in weaners and hoggets (Hill et al., 1999). Animals consuming a diet low or deficient in selenium are prone to many problems, including white muscle disease in lambs and calves, calf pneumonia, infertility, and exudative diathesis in chickens and other animals (Van Ryssen and Mavimbela, 1999; Müller et al., 2002). Different oxidation states of selenium (−2, 0, +4, +6) are regularly created in nature except for Se^{+2}. Insoluble Se^{0} can be prepared at nanoscale by reducing higher oxidation states to many allotropic forms (Yang et al., 2008; Raevskaya et al., 2008). Red elemental selenium nanoparticles (Se NPs) not only possess efficiency for up-regulation of selenoenzymes but also, based on several *in vivo* and *in vitro* studies, exhibit lower toxicity compared to other selenium compounds (Wang et al., 2007). Different selenium compounds are used as dietary supplementation in animals, but because of the high biological activity of Se NPs and their lower toxicity, there is more concern about the application of these nanoparticles (Zhang et al., 2001).

However, iron as a vital element is found in all living organisms ranging from the evolutionarily primitive archaea to vertebrates (Walker et al., 2001). Iron is essential to life, because of iron's exclusive ability to serve as electron donor and electron acceptor. Therefore, if iron were free within the cell, the element can catalyze the conversion of hydrogen peroxide into free radicals that cause damage to cellular structures. To prevent that kind of damage, living organisms bind iron atoms to different proteins such as heme, ferritin, and transferrin that allow use of the benefits of iron (Walker

* Corresponding author. Tel.: +98 21 66482706; fax: +98 2166461178.
E-mail addresses: Shahverd@sina.tums.ac.ir, shahverdiar@yahoo.com (A.R. Shahverdi).

0034-5288/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.rvsc.2011.07.029

et al., 2001; Wallander et al., 2006). Some studies have suggested a possible role for dietary selenium ions in moderating iron metabolism (Christensen et al., 2000), but according to our knowledge based on a survey of the literature, the effects of selenium nanoparticles on expression of the transferrin gene and its receptor have not yet been studied. In the present study, we investigated the effects of supplementation with sodium selenite and Se NPs (as a novel selenium compound) on iron homeostasis and the expression of transferrin and transferrin binding receptor genes in ovine individuals.

2. Material and methods

2.1. Preparation of Se NPs

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 (Zhang et al., 2004). 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).

2.2. Animals

Twenty-one healthy, 5- to 12-month-old Lori–Bakhtiary sheep were selected and randomly assigned to three groups. Sheep were orally given 1 mg kg^{-1} of Se NPs (group 1) and 1 mg kg^{-1} of sodium selenite (group 2) for 10 consecutive days. The control group (group 3) was given distilled water. During the study period, sheep were fed alfalfa hay and barley. The experimental procedures carried out in this study complied with the guidelines of Shahrekord University (Shahrekord, Iran) for the care and use of laboratory animals.

2.3. Sampling and biochemical parameters

Blood and sternal bone marrow samples were collected at different supplementation intervals (0, 10, 20, and 30 days). The blood serum was separated by centrifugation at 2000*g* for 20 min. Serum iron was determined by the Ferene end-point colorimetric method (Ziest Chemie, Iran), which is a superior iron chelating agent forming a complex with ferrous iron with a maximum absorption at 593 nm (Hennessy et al., 1984). Furthermore, the total binding iron capacity (TIBC) was determined. Excess iron was added to serum to saturate both transferrin binding sites; non-bound iron was precipitated with magnesium carbonate, and bound iron was determined with a TIBC kit (Darman Kave, Iran) (Ramsay, 1997). The transferrin saturation percent (TS%), an indicator of body iron stores, was determined by dividing the serum iron level by the TIBC (Mainous et al., 2004). To evaluate the expression of transferrin and transferrin binding receptor genes, bone marrow specimens were collected from the sternum and stored at −70 °C.

2.4. Analysis of genes expression

Total RNA was isolated from frozen samples using the RNX plus solution (Cinnagen, Iran) according to the supplier's instructions. Briefly, RNX solution (1 mL) was added to the tube containing the sample and incubated at room temperature for 5 min. Chloroform was added to the solution and centrifuged for 12,000 rpm at 4 °C for 15 min. The upper phase was then transferred to another tube, and an equal volume of isopropanol was added. The mixture was centrifuged for 15 min at 12,000 rpm, and the resulting pellet was then washed with ethanol. The extracted RNA was dissolved in DEPC-treated water and stored at −70 °C.

cDNA was synthesized from each RNA sample with the reverse transcription method. The reaction was started with the incubation of a mixture containing total RNA (10 μL), primers (20 pmol L^{-1}), and sterile DEPC solution (1 μL) at 65 °C for 5 min. The mixture was then briefly quenched on ice and added to a mixture containing 4 μL RT reaction buffer, 2 μL dNTP, and 1 μL of M-MLV reverse transcriptase. The RT reaction was carried out at 42 °C for 1 h and terminated by incubation at 70 °C for 10 min. The cDNA product (20 μL was stored at −70 °C for further experiments). The PCR was performed using *Taq* Polymerase, and forward and reverse primers for transferrin (TGAATATTTCAGCGCAGGC and TGAAAGCCCCTGTATAGCC) and transferrin receptor (TCTCAGTCATCAGGATTGCCC and CCTTCAGCACATTGTTCACGC). Polymerase chain reaction amplifications were performed using 5 μL of cDNA with 0.15 μL of *Taq* DNA polymerases (5 U μL^{-1}), 0.75 μL of $MgCl_2$, 2.5 μL of PCR buffer (10×), 0.5 μL of dNTP, 0.5 μL of each primer (20 pmol L^{-1}), and 15.1 μL of sterile double distilled water. The PCR reaction was conducted under the following conditions: an initial denaturation at 94 °C for 4 min, 30 cycles of denaturation at 94 °C for 30 s, annealing at 52 °C for 40 s and elongation at 72 °C for 30 s, and a final extension at 72 °C for 4 min. The housekeeping gene glyceraldehydes-3-phosphate dehydrogenase (GAPDH) was used as a control. The PCR products were separated on an acryl amide gel (8%) and visualized by silver nitrate staining. Scanned gel images were imported into Uvitec software (Uvitec, Cambridge, UK), and the volume density of the amplified products was calculated and normalized against GAPDH control values. Each gene analysis was repeated three times, and the average density value was used in the final analysis.

2.5. Statistical analysis

Software SPSS 11.5 for windows (SPSS Inc., Chicago) was used for statistical analysis. Data are presented as the means ± SE. The differences between groups were determined by using one way analysis of variance (ANOVA) test and to evaluate the time effect two way analysis of variance (ANOVA) test was used. *P*-values of less than 0.05 were considered significant.

3. Results

The SEM image of Se NPs shows the sphere-like nanoparticles ranging in size <220 nm with some aggregations (Fig. 1). Elemental composition analysis of prepared NPs confirm the presence of selenium atoms with absorption peaks at 1.37, 11.22, and 12.49 keV for signals of SeLα, SeKα, and SeKβ, respectively (Fig. 2).

The effects of prepared Se NPs as well as selenium ions on the iron homeostasis of sheep were investigated during this study. Results showed that the mean levels of iron (203.1 ± 13.6 μg dL^{-1}) and TIBC (325.86 ± 22.4 μg dL^{-1}) in day 0 were near the normal values reported in the literature (179–207 and 298–370 μg dL^{-1}) (Table 1) (Wallander et al., 2006). Compared to the control group, the blood iron concentration in sheep was statistically decreased after 20 and 30 days of supplementation for group 1 (which received Se NPs), and after 30 days for group 2 (which received selenium ions), respectively ($P < 0.05$). The TIBC level was increased in sheep sera especially at early stages of supplementation (day 10) in both mentioned groups. However, in contrast to the control group,

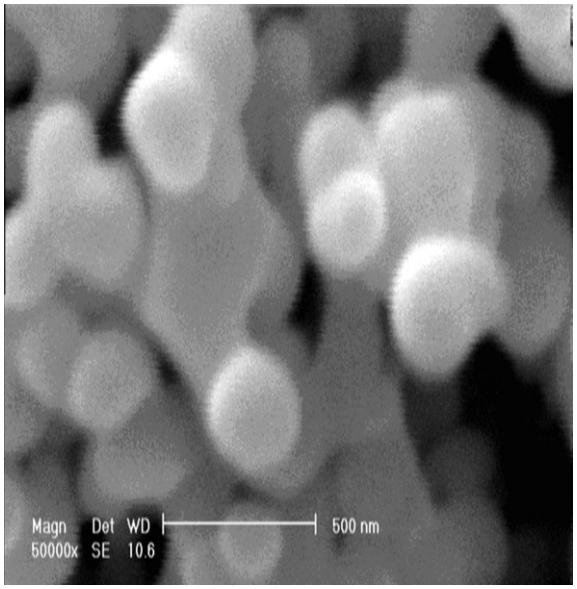

Fig. 1. SEM micrograph of Se NPs prepared by reducing the SeO_2 solution with ascorbic acid.

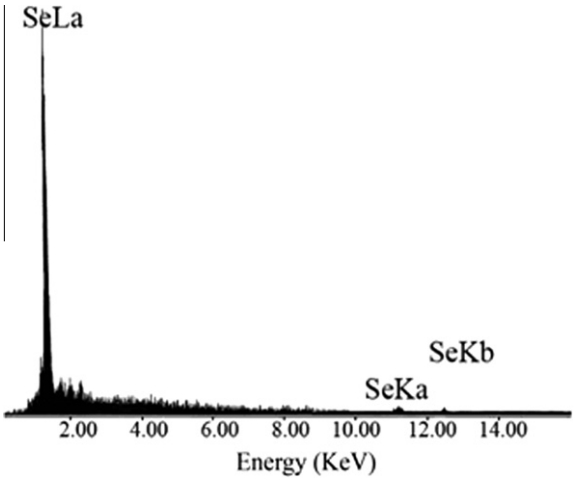

Fig. 2. Energy dispersive X-ray spectrum of Se NPs.

which received placebo, the level of TIBC in sheep sera in group 1 and 2 showed some fluctuation during supplementation. For example, although the TIBC level increased at day 20 of Se NPs supplementation (group 1), a significant decrease in the TIBC level was observed after 30 days of supplementation with these nanoparticles ($P > 0.05$) (Table 1). Moreover, the effect of Se NPs on the transferrin saturation percent was generally more than the selenium ions in the tested animals. The lowest amount of TS% was observed after 20 days in the sheep that received selenium nanoparticles. However, the TS% in the control group did not change significantly during this period (30 days) and approximately remained constant (Table 1).

Also in this study, the expression of transferrin and transferrin binding receptor genes was evaluated using RT–PCR (Table 2). Results indicated that expression of transferrin and its receptor genes increased significantly during supplementation of animals by both selenium compounds for 10 or 20 days. After this period, the expression of the mentioned genes decreased significantly ($P < 0.05$), especially in the animals that received selenium ions (Table 2).

4. Discussion

In the current study, the animals' diet was further supplemented by selenium. Iron is essential for many biological processes and is supplied to cells by the internalization of iron-loaded transferrin bound to its receptor. Free iron has the ability to generate oxidative radicals that damage essential biologic components such as lipids, proteins, and DNA (Roy and Enns, 2000). Chronic iron overload in mice was associated with a decrease in heart tissue concentrations of selenium and GPX activity, and researchers have shown that supplementation with sodium selenite can decrease heart tissue concentrations of iron and oxidative stress in the mouse (Bartfay and Bartfay, 2002; Bartfay, 2003). Furthermore, Oppenheimer (2001) proved that bacterial growth in serum was inhibited by decreasing the free iron concentration. Our findings showed that supplementation with selenium (Se NPs and sodium selenite) at early stages decreased the serum iron concentration and raised the TIBC level in sheep (Table 1). The selenium supplementation can be gradually decreased the serum iron concentrations in sheep after 30 days of supplementation (Table 1). However, in many eukaryotic systems, expression of transferrin and ferritin genes is tightly regulated through the coordinated action of the iron responsive element/iron regulatory protein (IRE/IRP) machinery (Danzeise et al., 2006). The expression of receptors for the primary iron transport protein, transferrin, is the major determinants of cellular iron uptake. The expression of this glycoprotein is tightly regulated in response to intracellular iron concentration, a mechanism that Fahmy and Young (1993) showed to be under translational control. In the current study, selenium supplementation initially stimulated the expression of transferrin and its receptor genes and finally suppressed their expression in sheep (Table 2). This effect might be related to hypoferremia and cell internalization of iron. Transferrin receptors are expressed ubiquitously on proliferating cells. Several findings suggest that the interaction between transferrin and its receptor play a crucial role in cell growth (Besancon et al.,

Table 1
Serum concentration of iron (μg dL^{-1}), TIBC (μg dL^{-1}) and TS (%) during selenium nanoparticles and sodium selenite supplementation (1 mg kg^{-1}) in sheep. (All data are presented as mean ± SD and in each group $n = 7$).

Time (day)	Group 2 (sodium selenite)			Group 3 (control)			Group 1 (Se NPs)		
	Iron (μg dL^{-1})	TIBC (μg dL^{-1})	TS (%)	Iron (μg dL^{-1})	TIBC (μg dL^{-1})	TS (%)	Iron (μg dL^{-1})	TIBC (μg dL^{-1})	TS (%)
0	201.6 ± 17.2	301.4 ± 27.5	66.9 ± 8.2	204.0 ± 6.9	352.6 ± 23.0	57.8 ± 5.2	203.8 ± 16.7	323.6 ± 16.7	62.9 ± 6.3
10	179.0 ± 7.3[C]	354.2 ± 38.7[a]	50.5 ± 5.1	202.4 ± 4.8	308.0 ± 24.9	65.7 ± 6.1	184.8 ± 14.0	369.8 ± 19.9[a]	50.0 ± 3.0[a,e]
20	186.8 ± 19.3	291.2 ± 31.7	64.1 ± 9.3	211.4 ± 26.8	306.0 ± 16.9	69.0 ± 10.4	168.4 ± 13.3[a,e]	414.4 ± 24.3[a,f]	40.6 ± 3.1[a,d,e]
30	128.2 ± 10.0 [a,b,c,e]	269.0 ± 7.8	47.6 ± 2.8[a,e]	190.8 ± 18.6	291.4 ± 25.0	65.5 ± 5.9	151.2 ± 17.2[a,e]	304.0 ± 7.8[b,c]	49.7 ± 5.0[a]

[a] Was significant compared to day 0, $P < 0.05$.
[b] Was significant compared to day 10, $P < 0.05$.
[c] Was significant compared to day 20, $P < 0.05$.
[d] Was significant compared to group 2 and 3, $P < 0.05$.
[e] Was significant compared to control group, $P < 0.05$.
[f] Was significant compared to group 2 and 3, $P < 0.05$.

Table 2
Semi-quantitative RT–PCR results of transferrin and transferrin receptor genes expression in sheep during selenium nanoparticles and sodium selenite supplementation (1 mg kg^{-1} day^{-1}). (All data are presented as mean ± SD and in each group $n = 7$).

Time (day)	Relative expression of transferrin (% of control)		Relative expression of transferrin receptor (% of control)	
	Group 1 (Se NPs)	Group 2 (sodium selenite)	Group 1 (Se NPs)	Group 2 (sodium selenite)
0	100.0 ± 3.5	100.0 ± 3.5	100.0 ± 5.8	100.0 ± 5.8
10	115.1 ± 9.0[a]	129.8 ± 6.7[a]	117.1 ± 5.2[a]	117.9 ± 2.9[a]
20	123.7 ± 8.1[a,b]	89.3 ± 5.7[a,c]	122.0 ± 1.4[a,b]	106.0 ± 1.9
30	64.8 ± 4.8[a,c]	69.1 ± 6.4[a,c]	81.9 ± 4.0[a,c]	86.9 ± 5.6[a,c]

[a] Was significant compared to day 0, $P < 0.05$.
[b] Was significant compared to group 2, $P < 0.05$.
[c] Was significant compared to day 10 and day 20, $P < 0.05$.

1987). The number of transferrin receptors notably increases when stationary cells are stimulated to proliferate. In contrast, the numbers of transferrin receptor diminish when the cells stop dividing (Besancon et al., 1987). Earlier studies demonstrated that nanoscale elemental selenium (Nano-Se) has efficacy comparable to selenite in up-regulating selenoenzymes and tissue selenium levels (Zhang et al., 2001; 2005). The present study revealed that Se NPs and sodium selenite approximately have similar patterns on the expression of transferrin and its receptor gene (Table 2). The relative expression of transferrin and its receptor gene in group 1 (day 20) was significantly higher than group 2 ($P < 0.05$) (Table 2). This may due to the mild release of selenium from nanoparticles. Furthermore after 20 days by decreasing the serum concentration of iron in group 1 and 2 (Table 1) in a related pattern expression of transferrin and its receptor gene were significantly decreased ($P < 0.05$).

In other animals, Se NPs exhibited much lower toxicity as indicated by the median lethal dose (LD_{50}), liver injury, and short-term toxicity (Zhang et al., 2007). However, in this study, a fixed dose of Se NPs and selenium ions (1 mg kg^{-1}) was used for evaluating different parameters, and further work should be carried out using different amounts of Se NPs on iron homeostasis in sheep. Se NPs exhibited much lower toxicity (Zhang et al., 2007) and in future after toxicological studies, Se NPs may serve as a new agent for selenium supplementation in sheep.

Acknowledgments

This work was financially supported by Shahrekord University (Shahrekord, Iran), and the authors would like to thank the Biotechnology Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences (Tehran, Iran) and the Iranian Nanotechnology Initiative Council for their admirable participation in this study.

References

Bartfay, W.J., 2003. Selenium status and the pathogenesis of iron-overload cardiomyopathies: Cause or Consequence. Queen's Health Science Journal 6, 40–46.
Bartfay, W.J., Bartfay, E., 2002. Decreasing effects of iron toxicosis on selenium and glutathione peroxidase activity. Western Journal of Nursing Research 24, 119–131.
Besancon, F., Silbermann, F., Dron, M., Tovey, M.G., Thang, M.N., Bourgeade, M.F., 1987. Relationship between inhibition of cell growth and of transferrin receptor expression by interferon (IFN) α: studies in IFN-sensitive and IFN-resistant Daudi cells. Journal of General Virology 68, 2647–2654.
Christensen, M.J., Olsen, C.A., Hasen, D.V., Ballif, B.C., 2000. Selenium regulates expression in rat liver of genes for proteins involved in iron metabolism. Biological Trace Element Research 74, 55–70.
Danzeise, R., Achsel, T., Bederke, U., 2006. Superoxide dismutase 1 modulates expression of transferrin receptor. Journal of Biological Inorganic Chemistry 11, 489–498.
Fahmy, M., Young, S.P., 1993. Modulation of iron metabolism in monocyte cell line U937 by inflammatory cytokines: changes in transferrin uptake, iron handling and ferritin mRNA. Biochemical Journal 296, 175–181.
Hennessy, D.J., Reid, G.R., Smith, F.E., Thompson, S.L., 1984. Ferene: a new spectrophotometric reagent for iron. Canadian Journal of Chemistry 62, 721–724.
Hill, G.M., Link, J.E., Meyer, L., Fritsche, K.L., 1999. Effect of vitamin E and selenium on iron utilization in neonatal pigs. Journal of Animal Science 77, 1762–1768.
Hoffmann, P.R., Berry, M.J., 2008. The influence of selenium on immune responses. Molecular Nutrition & Food Research 52, 1273–1280.
Mainous, A.G., Wells, B., Carek, P.J., Gill, J.M., Geesey, M.E., 2004. The mortality risk of elevated serum transferrin saturation and consumption of dietary iron. Annals of Family Medicine 2, 139–144.
Müller, A.S., Pallauf, J., Most, E., 2002. Parameters of dietary selenium and vitamin E deficiency in growing rabbits. Journal of Trace Elements in Medicine and Biology 16, 47–55.
Oppenheimer, S.J., 2001. Iron and its relation to immunity and infectious disease. Journal of Nutrition 131, 616S–635S.
Raevskaya, A.E., Stroyuk, A.L., Kuchmiy, S.Y., Dzhagan, V.M., Zahn, D.R.T., Schulze, S., 2008. Annealing-induced structural transformation of gelatin-capped Se nanoparticles. Solid State Communications 145, 288–292.
Ramsay, W.N.M., 1997. The determination of the total iron-binding capacity of serum. Clinica Chimica Acta 259, 25–30.
Roy, C.N., Enns, C.A., 2000. Iron homeostasis: new tales from the crypt. Blood 96, 4020–4027.
Schrauzer, G.N., Surai, P.F., 2009. Selenium in human, animal nutrition: resolved, unresolved issues. A partly historical treatise in commemoration of the fiftieth anniversary of the discovery of the biological essentiality of selenium, dedicated to the memory of Klaus Schwarz (1914–1978) on the occasion of the thirtieth anniversary of his death. Critical Reviews in Biotechnology 29, 2–9.
Van Ryssen, J.B.J., Mavimbela, D.T., 1999. Broiler litter as a source of selenium for sheep. Animal Feed Science and Technology 78, 263–272.
Walker, B.L., Tiong, J.W.C., Jefferies, W.A., 2001. Iron metabolism in mammalian cells. International Review of Cytology 211, 241–278.
Wallander, M.L., Leibold, E.A., Eisenstein, R.S., 2006. Molecular control of vertebrate iron homeostasis by iron regulatory proteins. Biochimica et Biophysica Acta (BBA)- Molecular Cell Research 1763, 668–689.
Wang, H., Zhang, J., Yu, H., 2007. Elemental selenium at nano size possesses lower toxicity without compromising the fundamental effect on selenoenzymes: Comparison with selenomethionine in mice. Free Radical Biology and Medicine 42, 1524–1533.
Yang, L.B., Shen, Y.H., Xie, A.J., Liang, J.J., Zhang, B.C., 2008. Synthesis of Se nanoparticles by using TSA ion and its photocatalytic application for decolorization of cango red under UV irradiation. Material Research Bulletin 43, 572–582.
Zhang, J., Wang, H., Yan, X., Zhang, L., 2005. Comparison of short-term toxicity between nano-Se and selenite in mice. Life Sciences 76, 1099–1109.
Zhang, J., Wang, X., Xu, T., 2007. Elemental selenium at nano size (Nano-Se) as a potential chemopreventive agent with reduced risk of selenium toxicity: comparison with se-methylselenocysteine in mice. Toxicological Science 101, 22–31.
Zhang, J.S., Gao, X.Y., Zhang, L.D., Bao, Y.P., 2001. Biological effect of a nano red elemental selenium. Biofactors 15, 27–38.
Zhang, S.Y., Zhang, J., Wang, H.Y., Chen, H.Y., 2004. Synthesis of selenium nanoparticle in the presence of polysaccharides. Materials Letters 58, 2590–2594.