Comparative Clinical Pathology (2020) 29:369–374
https://doi.org/10.1007/s00580-019-03061-3

ORIGINAL ARTICLE

The effects of selenium nanoparticles (SeNPs) on oxidant and antioxidant activities and neonatal lamb weight gain pattern

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Received: 3 June 2019 / Accepted: 16 September 2019 / Published online: 6 November 2019

Abstract

Selenium (Se) is an antioxidant element that prevents the oxidative stress. Se incorporates into proteins such as selenocysteine and prevents oxidative damage to body tissues. In neonatal period, the different types of stress such as hypoglycemia and hypothermia may predispose them to many types of infectious diseases and also reduce growth rate at postnatal period. For these reasons, the present study was conducted on 12 newborn lambs to evaluate the effects of oral selenium nanoparticle (SeNP) supplementation on serum levels of selenium, copper, zinc, thiobarbituric acid reactive substances (TBARS), superoxide dismutase (SOD) and catalase, and also weight gain changes during the first month of their life. Lambs were randomly divided into two groups. The treated group received SeNPs (0.1 mg/kg) and the control group received distilled water orally for seven consecutive days and the blood samples were taken on days 0, 7, 14, and 28. Results showed that in response to the 7-day SeNP administration and compared with the control group, serum selenium concentration was significantly increased, and serum copper and zinc levels were significantly decreased ($P < 0.05$). It was found that on day 14, TBARS activity was higher than that of the control group ($P < 0.05$). On day 28, the SOD level was increased compared with the control group and the TBARS activity decreased ($P < 0.05$). It was also shown that SeNP supplementation can significantly improve the weight gain of lambs on the 14th and 28th day. Overall, the results of the present study indicated the beneficial effects of SeNPs on antioxidant activity and weight gain patterns of newborn lambs which may promote lambs' growth rate in postnatal period.

Keywords Lamb · Selenium · Nanoparticles · Antioxidant · Growth · Postnatal

Introduction

Disturbances in oxidative status of tissues can implicate toxic effects on the cell components (e.g., proteins, lipids, and DNA) through the production of peroxides and free radicals and thus may play a key role in formation of many diseases (Koracevic et al. 2001). In order to overcome oxidative stress, the body is equipped with an advanced antioxidant system (Battin and Brumaghim, 2009). Selenium is a trace element that plays a fundamental role in cells and functions as a redox center of antioxidant enzymes in the body such as glutathione peroxidase (GPx) (Oldfield, 1989). Selenium is also a component of several other proteins such as selenoprotein of muscle, selenoflagellin, Se-transport proteins and the bacterial enzymes, formate dehydrogenase, and glycine reductase. Selenium facilitates significant changes in the metabolism of many drugs and xenobiotics such as arsenic, cadmium, mercury, copper, silver, and lead (Constable et al. 2017). This element utilizes several antioxidant mechanisms to prevent oxidative cellular damage. Selenium prevents membrane damage due to oxidation by increasing glutathione peroxidase activities in scavenging of hydroperoxides and hydrogen peroxide and by alcohol reduction (Battin and Brumaghim, 2009). Constable et al. (2017) explained that the status of selenium and vitamin E in an animal can alter antibody response, phagocytic function, lymphocyte response, and resistance to infectious disease. Kojouri et al. (2012) compared the effect of selenium nanoparticles (SeNPs) and sodium selenite on sheep neutrophil functions and stated that

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SeNPs increased the chemotactic and respiratory burst activities more significantly than sodium selenite. In addition, increasing expression of heat shock protein 90 (HSP90) gene in response to SeNP supplementation was reported by Kojouri et al. (2013) who suggested that the HSP induction protected cells from lethal level of stress. In other words, their finding may explain the beneficial role of short-time oral SeNP supplementation to donkeys in cell stability under stressful conditions such as intense exercise.

It is important to recognize that the biological role and toxicity of selenium is related to its composition and chemical formula. Red nano-selenium has a similar biological function as that of sodium selenite, while its toxicity is 7 times less than selenite (Zhang et al. 2001; Huang et al. 2003).

Newborn lambs are susceptible to environmental stress (particularly hypothermia), hence any changes in oxidants or antioxidant activities play a key role against infectious agents (Constable et al. 2017). For these reasons and the importance of the sheep as an economic species, we tried to evaluate the advantages and disadvantages of SeNPs on serum antioxidant capacity and growth rate of newborn lambs.

Materials and methods

Animals

Twelve healthy, 1- to 2-day-old, Kordi lambs were selected and randomly divided into two groups. The treatment group received selenium nanoparticles (0.1 mg/kg) orally for seven consecutive days, and the control group received distilled water.

The experimental procedures carried out in this study complied with the guidelines of Shahrekord University (Shahrekord, Iran) for the care and use of animals.

Nano red selenium preparation

Nano red elemental selenium particles (Nano-Se) were synthesized as described previously by Kojouri et al. (2012). Based on this method, the SeNPs were prepared by dropwise adding of ascorbic acid solution to an aqueous solution of SeO2 (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 a SEM (Philips XL30).

Sampling

Blood samples were collected from the jugular veins at the beginning of the experiment (day 0), and subsequently on days 7, 14, and 28. Also, the lambs were weighted on the mentioned days.

Thiobarbituric acid reactive substances assay

Sample preparation

A serum sample of 0.5 ml was mixed with 0.5 ml sterile distilled water in a labeled 1.5-ml micro-centrifuge tube. A blank sample (for calibration) was also prepared by adding 2 ml of the reagent to 1 ml distilled water.

Reagent preparation

Trichloroacetic acid (10%) (*w*/*v*), 0.375% thiobarbituric acid (TBA) (*w*/*v*), and 0.025 N HCl were mixed and heated gently until TBA was dissolved completely (Rael et al. 2004).

Performing assay

Two milliliters of the reagent were added to each sample, and the microtubes were incubated in a boiling water bath for 10 min. Then, they were placed at room temperature for 15 min and centrifuged at 1000×*g* for 10 min at 4 °C. Finally, the supernatants were transferred to new labeled tubes, and absorbances of the samples were measured at 535 nm against a blank sample. Antioxidative activity was calculated by adding the malondialdehyde index ($1.056 \times 105\ M^{-1}\ cm^{-1}$).

Measurement of superoxide dismutase activity

The NBT (nitroblue tetrazolium) dye reduction test method was used. The method is as follows: 0.1 of sheep serum was added to 2 ml reactive solution containing 0.2 mmol xanthine, 0.12 mM NBT, 0.49 units xanthine oxidase, and 0.1 mol phosphate buffer (PH = 7) and incubated at 37 °C for 20 min. Levels of superoxide dismutase through blue reduction test by using a spectrophotometer at a wavelength of 560 nm were measured, and results were reported as percent inhibition (Sun et al. 1988).

Measurement of catalase activity

Reactant solution was made first. Thus, 50 mM potassium phosphate buffer was added to 10.6 mM hydrogen peroxide and incubated at 37 °C for 60 s. Then, 0.1 ml of serum samples was incubated with 1 ml reactive solution. The reaction was finished by adding 0.5 mmol solution of 32.4 mmol ammonium molybdate thereafter yellow complex of ammonium

molybdate and hydrogen peroxide appeared, and the absorbance was read with a spectrophotometer at 405 nm and compared with the control samples (Goth 1991).

Measurement of Se, Cu, and Zn

The elements (Se, Cu, and Zn) were measured by atomic absorption (PERKIN ELMER 4100). First, 2 ml of serum was added to 1 ml of 5% solution of ammonium pyrimidine carbamate and mixed for 20 min in the shaker, until the elements formed organometallic complexes in the solution. Then, 1 ml of methyl isobutyl ketone was added and mixed for 30 min; after 10 min, the solution was centrifuged at 2500 (rpm) until the element was converted to the organic phase. After setting the oven and device, calibration curves of the elements were drawn using standard and palladium matrix modifier software win Lab 32, and the amount of selenium, copper, and zinc was measured in solution.

Statistical analysis

Data were statistically analyzed by SigmaStat (the 3.1 version) program and one way analysis of variance tests. Also, Tukey test and Dunnett's method were used for further evaluations at $P < 0.05$.

Results

Results showed that normal serum selenium concentration was significantly increased during the first 28 days of the lamb's life (Table 1). But the increasing trend in response to SeNP supplementation was significantly higher than that of the control ones at days 7, 14, and 28 ($P < 0.05$).

As seen in Table 1, serum selenium level of the control group was significantly increased at day 7 compared with the basal level (day 0) and also a significant increase of Se in days 14 and 28 is observed compared with days 0 and 7 ($P < 0.05$). In the treated group, serum selenium concentration increased in all days and showed significant improvement compared with the previous days and the control group, too ($P < 0.05$).

In the control group, serum copper and zinc concentration were increased significantly on days 14 and 28 compared with days 0 and 7 ($P < 0.05$). But in the treated group at day 7, the amount of copper was significantly lower than day 0 and also lower than the control group ($P < 0.05$). The pattern of serum Cu concentration of the treated group was changed on day 14 and increased significantly compared with day 7 ($P < 0.05$). Serum zinc level in the treated group was significantly decreased at day 7 compared with day 0 and the control group, but on days 14 and 28 and compared with days 0 and 7, was significantly increased ($P < 0.05$).

As shown in Table 2, TBARS values of the control group were not significantly changed during the days of sampling. While in the treated group and on day 14, the mean of TBARS value was increased significantly compared with days 0, 7, and 28 ($P < 0.05$). Results also showed that TBARS level at days 14 and 28 was significantly higher than the control group ($P < 0.05$).

The decreasing patterns of SOD and catalase activities of the control group were summarized in Table 2. These changes were only significant for SOD at days 7, 14, and 28 compared with day 0 (basal level). In the treated group, the similar decreasing pattern occurred on day 14 which was significant compared with the basal level (day 0). After that, SOD activity was increased significantly on day 28 in comparison with day 14 and the control group, too ($P < 0.05$).

The weight gain pattern of newborn lambs was shown in Table 3. In all periods of sampling, the increase of weight gain in the control group was slight, but in the treated group, the trend of increase was quite noticeable. As seen in the Table 3 in days 14 (7.07 kg) up to 28 (9.49 kg), the weight gain was 2.42 kg, and compared with the control group whose weight gain was 1.01 kg, it is quite significant.

Results of the Pearson correlation test indicated the presence of strong negative and/or positive correlation between parameters. In the control group and at day 0, strong negative

Table 1 Changes in (Mean ± SEM) serum mineral concentration (ng/ml) during the different days of sampling

Minerals	Group	Days			
		0	7	14	28
Selenium (ng/ml)	Control	3.66 ± 1.71^{a}	20.84 ± 4.12^{b}	36.51 ± 7.92^{c}	36.41 ± 7.39^{c}
	Treatment	4.36 ± 0.98^{a}	53.28 ± 8.4^{c}	80.61 ± 6.14^{d}	122.92 ± 17.57^{e}
Copper (ng/ml)	Control	39.26 ± 4.5^{a}	46.13 ± 5.54^{a}	52.33 ± 5.35^{c}	59.85 ± 6.04^{c}
	Treatment	36.01 ± 3.96^{a}	16.46 ± 5.05^{b}	42.4 ± 3.41^{ac}	58.33 ± 2.47^{ac}
Zinc (ng/ml)	Control	71.4 ± 7.32^{a}	98.73 ± 5.91^{b}	114.76 ± 7.1^{b}	119.08 ± 10.79^{b}
	Treatment	74.21 ± 3.97^{a}	68.03 ± 1.94^{a}	109.4 ± 6.33^{b}	114.33 ± 3.28^{b}

Within columns, values with different superscripts are significantly different ($P < 0.05$)

Within rows, values with different superscripts are significantly different ($P < 0.05$)

Table 2 Changes in (Mean ± SEM) serum TBARS, SOD, and catalase level during the different days of sampling

Parameters	Group	Days			
		0	7	14	28
TBARS (μM/L)	Control	0.52 ± 0.069	0.53 ± 0.019	0.42 ± 0.068^{a}	0.41 ± 0.06^{a}
	Treatment	0.67 ± 0.17^{a}	0.45 ± 0.23^{ac}	1.5 ± 0.16^{b}	0.96 ± 0.15^{bc}
SOD (%inhibition)	Control	11.36 ± 0.8^{a}	7.68 ± 0.79^{b}	6.94 ± 0.74^{b}	6.81 ± 0.54^{b}
	Treatment	11.77 ± 0.59^{a}	11.55 ± 3.27^{ab}	7.86 ± 1.02^{b}	13.45 ± 1.93^{ac}
Catalase (KU/L)	Control	12.91 ± 2.9	10.4 ± 1.31	9.87 ± 1.04	10.46 ± 1.09
	Treatment	13.82 ± 2.206	11.79 ± 1.44	10.12 ± 1.12^{b}	8.93 ± 0.68

Within columns, values with different superscripts are significantly different ($P < 0.05$)
Within rows, values with different superscripts are significantly different ($P < 0.05$).

correlations were observed between TBARS/Cu ($r = -0.875$, $P = 0.0225$) and catalase/Zn ($r = -0.972$, $P = 0.0011$) but this result was not seen in the treated group. In day 0, in the treated group, there is a positive correlation between Zn/Cu ($r = +0.837$, $P = 0.0377$) and also for the control group ($r = +0.804$, $P = 0.053$) on day 7.

In the treated group, in day 7, a positive correlation was observed between TBARS/Cu ($r = +0.822$, $P = 0.0447$), and in day 14, a negative correlation was seen between TBARS/Zn ($r = -0.883$, $P = 0.0198$). It is important to know that on day 28, a positive correlation was observed between SOD/catalase ($r = +0.856$, $P = 0.0298$) and Zn/Cu ($r = +0.956$, $P = 0.0029$).

Discussion

The role of trace minerals in the animal body include vitamin synthesis, hormone production, enzyme activity, collagen formation, tissue synthesis, oxygen transport, and other physiological processes related to growth, reproduction, and health (Paterson et al.1999). From the obtained results, serum selenium level in the treated group was significantly increased on days 7, 14, and 28 compared with the control group ($P < 0.05$). Of course, this is somewhat obvious because selenium supplementation increased its serum levels.

Based on Suttle's (1975) work, copper absorption is high (70–85%) in milk-fed lambs but decreases to < 10% after weaning, while our results showed that serum concentration of copper and zinc in the treated group decreased after consumption of SeNPs and then again resumed its upward trend. This could be due to the distribution in proper absorption of copper and zinc by SeNP consumption that leads to decreased absorption of these elements in gastrointestinal system (Heyland et al. 2005). The present findings are inconsistent with the results of Kojouri and Shirazi (2007). They found that after injecting vitamin E and selenium compound to the pregnant ewes, the concentration of iron, copper, and zinc in sera of newborn lambs was increased during the first week of life (Kojouri and Shirazi, 2007). Larson (2005) stated that balance among the trace minerals is also an important consideration and often poses a considerable challenge to the trace mineral status of the animal due to antagonist interactions that can occur between minerals.

Oxidative stress is defined as reduced antioxidant defense and increase in the formation of reactive oxygen in body tissues. Cells and biological fluids typically have natural protective antioxidant mechanisms. Heyland et al. (2005) have described oral selenium consumption as a factor in support of antioxidant activity and reduces mortality in acute disorder. Our study showed that the level of TBARS in the treated group was significantly more than the control group at days 14 and 28. Sadeghian et al. (2012), after 10 days' oral administration of sodium selenite and selenium nanoparticle to ewes, reported that TBARS increase in both groups at days 20 and 30, so that the increase was significant compared with the control group ($P < 0.05$). Therefore, they conclude that oral administration of SeNPs may lead to an unexpected increase in oxidant activity

Table 3 Changes in weight gain (Mean ± SEM) of lambs during the different days of sampling

	Group	Days			
		0	7	14	28
Weight gain (kg)	Control	3.33 ± 0.25^{a}	4.93 ± 0.3^{b}	5.7 ± 0.45^{bc}	6.71 ± 0.48^{c}
	Treatment	3.31 ± 0.29^{a}	5.66 ± 0.44^{b}	7.07 ± 0.63^{c}	9.49 ± 0.91^{d}

Within columns, values with different superscripts are significantly different ($P < 0.05$)
Within rows, values with different superscripts are significantly different ($P < 0.05$)

in a shorter time in comparison with sodium selenite. Because changes in serum TBARS is a good marker of lipid peroxidation in the body, its return to baseline depends on the body's antioxidant system performance (Zhang et al. 2001). Haung et al. (2003) mentioned the effects of the antioxidant activities of SeNPs and stated that a small particle is very effective in trapping free radicals and preventing the damage to DNA. This is clearly seen in the current study, so that on day 14, the activity of serum SOD in the treated group significantly decreased. In other words, the SOD is utilized for neutralization of oxidants. Also, the Pearson correlation test showed the presence of a negative and significant relationship between serum Zn and TBARS value. But on day 28, serum SOD activity increased and TBARS decreased. Previously, studies showed copper and zinc's role as required components of superoxide dismutase (Spears 2003).

In the present study, however, no significant changes occurred in serum levels of catalase, but in the treated group at day 28, a significant and positive correlation was presented between SOD and catalase activities, which indicates the beneficial role of SeNPs on antioxidant capacity. Kumar and colleagues suggested that selenium has no effect on serum total protein, albumin/globulin ratio, and SGOT and SGPT activities but increases the capacity of immune system to protect cells against free radicals (Kumar et al. 2008). Wang et al. (2007) compared the effect of selenium nanoparticles and selenoprotein on GST activity and preferred the SeNP function.

The results of oral SeNP supplementation on weight gain changes were considerable. The difference between the average of lamb's weight at days 7, 14, and 28 was 0.73, 1.37, and 2.78 kg, respectively. Lawler et al. (2004) stated that in selenium-rich areas, the muscle growth and increase in weight gain are higher than selenium-poor areas. Zhang et al. (2007) reported that in goats fed 0.3–1 mg/kg DMI nano-selenium for 95 days, the growth rate and antioxidant activity (superoxide dismutase and glutathione peroxidase levels) are more than the control group ($P < 0.05$). They declared that this is probably due to the positive effects of nano-selenium on growth hormone and insulin levels (Zhang et al. 2007). Zhou and Wang (2011) reported that growth rate in broilers fed with 0.3 mg/kg nano-selenium was better than the control group.

Conclusion

The results of this study show positive effects of selenium nanoparticles on weight gain and protection of cells against oxidant activities in newborn lambs.

Acknowledgments The authors would like to thank the directorate of postgraduate studies.

Funding information This study was financially sponsored by the Shahrekord University (Shahrekord, Iran).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval The experimental procedures carried out in this study complied with the guidelines of Shahrekord University (Shahrekord, Iran) for the care and use of animals. This article does not contain any studies with human participants or animals performed by any of the authors.

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