Revista MVZ Córdoba
ISSN: 0122-0268
ISSN: 1909-0544
revistamvz@gmail.com
Universidad de Córdoba
Colombia

Injectable diphenyl diselenide supplementation in dairy sheep

H Biazus, Angeliza; Cazarotto, Chrystian J; Gebert, Roger R; dos Reis, João H.; Zortea, Talyta; Baretta, Dilmar; Machado, Gustavo; Boito, Jhonatan P.; Baldissera, Matheus D.; da Silva, Aleksandro S
Injectable diphenyl diselenide supplementation in dairy sheep
Revista MVZ Córdoba, vol. 23, no. 1, 2018
Universidad de Córdoba, Colombia
Available in: http://www.redalyc.org/articulo.oa?id=69355265005
DOI: https://doi.org/10.21897/rmvz.1239

Artículos Originales

Injectable diphenyl diselenide supplementation in dairy sheep

Suplemento de difenil diselenuro inyectable en ovejas lecheras

DOI: https://doi.org/10.21897/rmvz.1239
Redalyc: http://www.redalyc.org/articulo.oa?id=69355265005

Angeliza H Biazus
Universidade do Estado de Santa Catarina, Brasil
aleksandro_ss@yahoo.com.br

Chrystian J Cazarotto
Universidade do Estado de Santa Catarina, Brasil
aleksandro_ss@yahoo.com.br

Roger R Gebert
Universidade do Estado de Santa Catarina, Brasil
aleksandro_ss@yahoo.com.br

João H. dos Reis
Universidade do Estado de Santa Catarina, Brasil
aleksandro_ss@yahoo.com.br

Talyta Zortea
Universidade do Estado de Santa Catarina, Brasil
aleksandro_ss@yahoo.com.br

Dilmar Baretta
Universidade do Estado de Santa Catarina, Brasil
aleksandro_ss@yahoo.com.br

Gustavo Machado
North Carolina State University, Estados Unidos
aleksandro_ss@yahoo.com.br

Jhonatan P. Boito
North Carolina State University, Estados Unidos
aleksandro_ss@yahoo.com.br

Matheus D. Baldissera
UFSM, Brasil
aleksandro_ss@yahoo.com.br

Aleksandro S da Silva
Universidade do Estado de Santa Catarina, Brasil
aleksandro_ss@yahoo.com.br

Received: 09 January 2017
Accepted: 06 November 2017

Abstract:

Objective. The aim of this study was to evaluate the influence of subcutaneous supplementation with diphenyl diselenide ((PhSe)2) in dairy sheep infected with gastrointestinal nematodes on animal health and possible damage to environment when the feces of these animals will be used for fertilizing. **Materials and methods.** The experiment was performed using 16 primipara

dairy sheep, that were divided into two groups: the group A as control and the group B supplemented with 3 μmol/kg of (PhSe)2 subcutaneously. Blood samples were used to determine the hepatic function, as well as the protein and lipid metabolism in animals. Feces were used to determine the number of helminths eggs per gram of feces (EPG), as well as used for ecotoxicology tests. **Results.** The (PhSe)2 supplementation not affected the helminths reproduction, since the EPG did not differ ($p>0.05$) between groups. Total protein and globulin levels increase ($p<0.05$) in supplemented animals, while the seric alanine aminotransferase (ALT) levels decrease ($p<0.05$) in the end of experimental design. Cholesterol levels increase ($p<0.05$) in the supplemented animals, while triglycerides, albumin and urea not differ between groups ($p>0.05$). The feces of supplemented sheep not interfered the springtails reproduction. **Conclusions.** At the administered dose, the (PhSe)2 is not able to control the parasitism, however, it did increase the globulins and cholesterol levels, that are important to immune response and for sheep reproduction, respectively. Also, the feces of supplemented animals with (PhSe)2 can be used as organic fertilizing, without negative impacts to environment.

Keywords: (PhSe)2, sheep, helminths, springtails.

Resumen:

Objetivo. El objetivo de este estudio fue evaluar la influencia de la suplementación subcutánea con diselenuro de difenilo (PhSe)2 en ovejas lecheras infectadas con nematodos sobre la salud animal y posible daño al ambiente cuando las heces de estos animales se utilicen para fertilizar. **Materiales y métodos.** El experimento se realizó utilizando 16 ovejas lecheras, que se dividieron en dos grupos: el grupo A se usó como control y el grupo B se suplementó con 3 μmol/kg of (PhSe)2 vía subcutánea. Se utilizaron muestras de sangre para determinar la función hepática, así como el metabolismo de proteínas y lípidos en animales. Las heces se utilizaron para determinar el número de huevos por gramo de heces (EPG), así como para las pruebas de ecotoxicología. **Resultados**. La suplementación (PhSe)2 no afectó la reproducción de helmintos. Los niveles totales de proteína y globulina aumentan ($p<0.05$) en los animales suplementados, mientras que los niveles séricos de alanina aminotransferasa (ALT) disminuyen ($p<0.05$) al final del diseño experimental. Los niveles de colesterol aumentan ($p<0.05$) en los animales suplementados, mientras que los triglicéridos, la albúmina y la urea no difieren entre los grupos ($p>0.05$). Las heces de ovejas suplementadas no interferían en la reproducción de las colas de caballo. **Conclusiones.** A la dosis administrada, el (PhSe)2 no es capaz de controlar el parasitismo; sin embargo, aumenta los niveles de globulinas y colesterol, que son importantes para la respuesta inmune y para la reproducción, respectivamente. Las heces de animales suplementados pueden usarse como fertilizantes orgánicos, sin impactos negativos en el ambiente.

Palabras clave: (PhSe)2, ovejas, helmintos, colas de primavera.

INTRODUCTION

In dairy sheep industry, females in the post-partum period are susceptible to metabolic disorders in consequence to major nutritional requirements, being that this period is particularly important to animal health and consequent female performance due physiologic changes and metabolic stress (1). In attempt to improve the performance and recuperation, the animal supplementation is an interesting approach. The vitamins and mineral, such as selenium, an micromineral with antioxidant properties (2,3), that is able to protects the cell membranes against oxidative degeneration (4), as well as your participation in the composition of glutathione enzymes, a potent antioxidant enzyme (5). Therefore, the selenium is also considered an important stimulant for immunology system, influencing the expression of non-specific, humoral and cellular response (2,3,4,5).

Many studies have demonstrated that sodium selenite supplementation possesses beneficial effects for sheep (6,7,8), however, is important the search for alternative sources of selenium. In this sense, the diphenyl diselenide (PhSe)2, an organic compound derived from selenium with anti-inflammatory, neuroprotective and antioxidant properties (9), may be considered an important source for sheep health improvement. Experimental studies demonstrating the beneficial effects of (PhSe)2 in rats and fish as experimental model, but was not been evaluated in sheep. Based on the effects of (PhSe)2 in the animal metabolism, is possible suggest that the supplementation with (PhSe)2 may exerts beneficial properties, such as decrease of oxidant compounds associated with increase of antioxidant compounds in the blood, as well as the improve of immune system, and consequently control de parasitism, such as observed in infected lambs with Haemonchus contortus supplemented with sodium selenite (10). Similarly, the injectable administration

of sodium selenite and (PhSe)2 in mice experimentally infected with Toxoplasma gondii (11) was able to stimulate the inflammatory response, and consequently increase animal longevity.

The feces of production animals are commonly used as fertilizing in pastures (12). Based in this information, arise the interest whether feces of supplemented animals with (PhSe)2 can be used as fertilizing without consequences for the environment, since several studies demonstrated the negative impact of feces of animals with residues of additives and veterinary drugs for soil microflora (13,14). It is important emphasize that studies demonstrating the use of (PhSe)2 in the livestock are recent, being necessary progress in this lines of research. Therefore, the aim of this study was to evaluate whether subcutaneously (PhSe)2 supplementation can control the sheep parasitic infection during the lactation period, as well as be exerts beneficial properties for animal health. Moreover, a second objective was verifying whether feces of supplemented sheep with (PhSe)2 can be used as organic fertilizing without negative impacts for soil biomass/diversity.

MATERIALS AND METHODS

Local and animals. The experiment was performed in a rural property involved in sheep farming, localized in Chapecó (west of Santa Catarina, southern of Brazil – Latitude: 27º 05'47"S; Longitude: 52º 37'06"W). For this study, sixteen primipara newly calved sheep to Lacaune race, with similar age, weight and milk production were used as experimental model. The animals were divided in two groups (A and B), with eight animals each. The group A was used as control (non-supplemented), that received via subcutaneously dimethyl sulfoxide (DMSO) at dose of 1.5 mL (used to dilute the (PhSe)2). The group B was composed by supplemented sheep with (PhSe)2 subcutaneously at 1.5 mL, corresponding at dose of 3 μmol/kg that was applied on days 0, 7, 15, 30 and 45 of experiment.

The dose was determined in a pilot study using 0.5, 1.0, 3.0, and 30.0 μmol/Kg of mineral in four healthy lambs, since (PhSe)2 was never used in sheep. Antioxidant enzyme GPx and liver injury enzimes (alanine aminotransferase and aspartate aminotransferase) were evaluated in serum samples and demonstrated that the two lowest doses did not alter the values of the enzymes. The highest dose, however, intoxicated the lamb (the variables increased and the lamb died, and the histopathology showed lesions of hepatic necrosis). The dose of 3.0 μmol/Kg was considered ideal because it increased GPx activity 8.5 times, without causing hepatic injury (data not published).

The diet was provided to both groups in two periods (7:00 AM and 5:00 PM), and was constituted by corn silage, cynodont hay and concentrated (ground corn, soybean meal, vitamin and mineral core, calcitc limestone and nonensin). Water was provided ad libitum. All animals were contained in the same bay (24 m2), with beaten floor and bed with wood shavings.

Sample collection. At intervals of 15 days, the total blood was collected by jugular tail using vacuolized tubes without anticoagulant (days 0, 15, 30, 45, 60 and 75). The samples were centrifuged at 8000 rpm during 10 min to obtain serum that was stored at -20ºC until biochemical analysis. The feces samples were collected at same experimental period of blood samples, i.e., on days: 0, 15, 30, 45, 60 and 75, to perform the parasitological exam described below. A sample of feces from each animal was collected on day 35, and the feces of each group was homogenized to assessment the ecotoxicological tests.

Biochemical analyses. Seric levels of alanine aminotransferase (ALT), total protein, albumin, triglycerides, cholesterol and urea were performed using a semi-automatized analyzer (BioPlus-2000®) and commercial kits (Analisa®). Globulin values were obtained between total protein and albumin levels.

Coproparasitological analyses. The feces samples were used for gastrointestinal nematodes eggs search using the modified McMaster technique (15) using sucrose solution as flotation fluid for determination the quantity of eggs per gram of feces (EPG). The coproculture was performed to verify the helminths involved in the infection.

Ecotoxicological test. The feces homogenized of each group collected on day 35 were used in the ecotoxicological test to evaluate the springtails reproduction (Folsomia candida). The test was conducted based in the protocol ISO 11267 and as concluded after 28 days (16), with experimental design totally randomized and with 4 replicas. Each replica consisted of plastic container (capacity for 140 mL), filled with 30 grams of soil containing 0, 2, 4, 8 and 16 tons of feces/hectare. In each container were added 10 springtails (F. candida) with age synchronized of 10-12 days (after hatching). On day 14, the springtails were feed with biological ferment (Saccaromyces cerevisiae), and were opened to aeration and water supply weekly. On day 28, the soil of each replica was transferred for another container with major volumetric capacity, that was added water and some drops of black ballpoint ink. After light agitation with glass cane, the numbers of live springtails were counted in the water superficies. Photographs of container were performed to posteriorly count of juveniles of springtails using the software ImageTool (ImageTool 3.0, The University of Texas Health Science Center, San Antonio, TX).

Statistical analyses. The data from the dairy sheep of ALT, triglyceride, total protein, cholesterol, albumin, urea, globulin, OPG, were first analyzed by descriptive statistics for contingency of the information and for further assumptions and what is presented as descriptive is the mean and standard deviation. The data were tested for normality of variance by Kolmogorov-Smirnov test, skewness and homogeneity by Levene's test previously to ANOVA analysis. A one-way ANOVA for repeated measurements to test was used to evaluate the influence of time (an error term was added to accommodate de dependence of subjects {sheep that were resampled), where necessary (statistic difference were found), test was used since it controls the family-wise Type I error rate, by adjusting the observed significance level to the number of multiple comparisons. Secondly, one-way ANOVA was used to analyze all significant parameters that had shown significant difference over time on the repeated measure analysis, mean comparison between groups on each time period were tested (day 0, day 15, day 30, day 45, day 60 and day 75). It was considered significantly different when $p<0.05$. The whole statistical process was carried out with R-language, V.3.3.0 (17).

For the reproduction test with F. candida, the results were submitted to one-way ANOVA followed by Dunnett post hoc test ($p<0.05$), using the software Statistica V 7.0. (YEAR)

RESULTS

The results regarding seric biochemical analysis were showed in Tables 1 and 2. Seric ALT reduced significantly on days 60 and 75 in supplemented dairy sheep with $(PhSe)_2$, while cholesterol levels increased significantly on days 30, 60 e 75, as well as a tendency to increase was observed on day 45. Moreover, an increase of total protein and globulins levels in serum were observed in the supplemented dairy sheep on days 60 and 75 compared to control group. No difference was observed between groups regarding seric levels of triglycerides, albumin and urea.

No difference was observed between groups regarding the number of helminths eggs per gram of feces (EPG) during the experiment (Table 3). The coproculture revealed that eggs counted in the EPG corresponded to the Haemonchus spp. and Trichostrongylus spp.

TABLE 1.

Table 1. Mean and standard deviation of seric alanine aminotransferase (ALT) activity, seric levels of triglycerides and cholesterol in supplemented sheep with diphenyl diselenide (group B) at different days of experiment compared control group (group A).

Variable	Day	Mean ± standard deviation		P value
		Group A	Group B	
ALT	0	17.50 (2.98)	17.12 (3.60)	0.13
(U/L)	15	17.75 (4.59)	18.50 (6.05)	0.78
	30	16.75 (2.96)	15.12 (2.80)	0.27
	45	16.50 (5.13)	14.50 (4.38)	0.41
	60	18.25 (3.85)	13.12 (4.52)	0.05*
	75	17.50 (4.96)	12.50 (3.25)	0.03*
Triglyceride	0	22.57 (6.73)	23.71 (8.56)	0.78
(mg/dL)	15	18.50 (4.72)	17.00 (3.74)	0.49
	30	23.07 (16.05)	24.62 (8.09)	0.81
	45	16.75 (4.68)	18.75 (7.13)	0.51
	60	13.12 (5.57)	17.50 (6.63)	0.17
	75	40.62 (4.41)	40.50 (4.31)	0.95
Cholesterol	0	51.38 (7.80)	49.75 (6.73)	0.66
(mg/dL)	15	62.12 (12.54)	65.38 (10.42)	0.58
	30	59.25 (11.84)	71.38 (8.23)	0.05*
	45	66.38 (11.96)	74.88 (7.73)	0.056
	60	68.88 (11.46)	95.00 (15.93)	0.011*
	75	71.88 (9.66)	83.00 (9.85)	0.027*
* Significant difference between groups				

TABLE 2.

Table 2. Mean and standard deviation of seric levels of total protein, albumin, globulin and urea in supplemented sheep with diphenyl diselenide (group B) at different days of experiment compared control group (group A).

Variable	Day	Mean ± standard deviation		P value
		Group A	Group B	
Total protein	0	6.38 (0.79)	6.86 (0.87)	0.26
(g/dL)	15	5.95 (0.80)	6.36 (0.92)	0.35
	30	6.40 (0.55)	6.33 (1.27)	0.88
	45	5.59 (0.29)	5.69 (0.61)	0.67
	60	6.88 (0.91)	8.65 (0.55)	0.003*
	75	6.90 (1.06)	7.74 (0.95)	0.052
Albumin	0	3.08 (0.42)	3.04 (0.46)	0.86
(g/dL)	15	2.35 (0.75)	2.35 (0.93)	0.10
	30	2.60 (0.55)	2.89 (0.55)	0.31
	45	2.95 (0.24)	2.89 (0.59)	0.78
	60	2.76 (0.45)	3.26 (0.53)	0.06
	75	2.98 (0.53)	2.86 (0.2)	0.59
Globulin	0	3.30 (0.83)	3.83 (0.86)	0.23
(g/dL)	15	3.60 (0.90)	4.01 (1.43)	0.50
	30	3.80 (0.67)	3.44 (1.17)	0.45
	45	2.64 (0.29)	2.80 (0.45)	0.40
	60	4.11 (1.05)	5.39 (0.8)	0.01*
	75	4.01 (0.85)	4.88 (0.94)	0.08
Urea	0	39.00 (8.04)	33.75 (6.71)	0.09
(mg/dL)	15	33.12 (7.94)	33.12 (5.41)	0.10
	30	34.50 (10.14)	42.12 (9.28)	0.13
	45	40.88 (5.00)	43.50 (6.21)	0.36
	60	75.62 (11.17)	67.12 (19.48)	0.30
	75	45.38 (6.23)	46.38 (8.25)	0.78

* Significant difference between groups

TABLE 3.

Table 3. Number of eggs per gram of feces (EPG) of supplemented dairy sheep with diphenyl diselenide (group B) in different days of experiment compared control group (group A).

Variable	Day	Mean ± standard deviation		P value
		Group A	Group B	
EPG	0	1512.50 (1318.48)	1237.50 (1101.87)	0.65
	15	1975.00 (1587.23)	1800.00 (1918.33)	0.84
	30	1425.00 (1525.73)	1612.50 (1776.38)	0.82
	45	1975.00 (2210.20)	2600.00 (2394.64)	0.59
	60	2412.50 (3711.16)	2112.50 (1679.66)	0.83
	75	425.00 (365.47)	500.00 (667.62)	0.78

Note: There was no significant difference between groups.

The number of young springtails did not differ between groups (Figure 1). Therefore, the feces of supplemented dairy cows with $(PhSe)_2$ via injectable not interfere in the springtails reproduction.

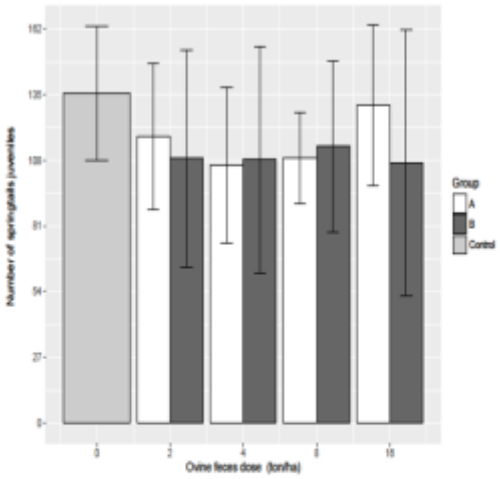

Figure 1. Number of juveniles forms of springtails submitted with organic fertilizing with ovine feces at doses of 2, 4, 8 and 16 ton per hectare (t ha^{-1}). A pool of feces was used of each group. Note: No difference was observed between groups in the tested doses. Group A – Control; and Group B - supplemented dairy sheep with diphenyl diselenide.

FIGURE 1
Figure 1

DISCUSSION

The antioxidant action of selenium is well established, since the mineral protects the cells against deleterious effects and aggressive agents (18,19). This action can be proven by result of seric ALT activity, that is considered an important biomarker of hepatic function. ALT activity reduced in the supplemented sheep with $(PhSe)_2$, that can be considered a protective effect of this selenium form in the liver of sheep during the lactation period. This enzyme is released in the blood after hepatic injury, which can occur during the lactation period of sheep, due the major metabolic requirement and oxidative stress (20). Besides the protective effect of selenium, is important emphasize that supplementation with five doses of $(PhSe)_2$ was not toxic to animals.

Triglycerides, albumin and urea levels did not differ between groups, i.e., the $(PhSe)_2$ no exerts effects in the metabolism or synthesis of these variables. The increase of cholesterol levels can be beneficial for animals, since a positive correlation between increase of cholesterol and progesterone in sheep reflects in a better reproductive performance (21). The augmentation on total protein levels is directly linked to increase of globulins levels in the supplemented animals with $(PhSe)_2$ after 60 days of experiment. This result can be explained by selenium capacity to protects the structure and protein function against oxidation (22), maintaining the protein viability and integrity. Also, the $(PhSe)_2$ can exerts a directly or indirectly effects on cells involved in the immune response, since selenium acts a modulator of immunology system (4,5).

The helminths oviposition was not affects by $(PhSe)_2$ supplementation. We expected an indirectly action of selenium, i.e., the $(PhSe)_2$ supplementation would stimulate a response against gastrointestinal helminths, and consequently would reduce the parasitic infection and oviposition. This could occurred because selenium acts in the expression of L-selectin and interleukin-8R genes, and in the TLR-4 neutrophils receptor in sheep, that are involved in the recognition and response to bacterial and parasitic pathogens (23).

Ecotoxicological test is very important to verify the homeostasis between production and environment, since this test evaluate whether residues of chemicals would cause impact on soil microfauna. In this study, the residues of $(PhSe)_2$, or our metabolites, did not affected the springtails reproduction. The quantification of springtails is the initial point to understanding the ecological processes of nutrient cycling, because they are considered indicators of anthropogenic interventions and soil quality (24). Recent, research to evaluate the effects of pig manure, from diets incorporating veterinary pharmaceuticals, on survival and reproduction of F. candida observed that application of these residues should be regulated not only using a volume-based criterion, but should incorporate data on soil properties (25). Thus, we believe that test for new supplements can be accomplished with an ecotoxicological assessment, seeking environmental safety.

The protocol with $(PhSe)_2$ at administered dose was not able to control the parasitic infection or reduce the helminths oviposition, although has been observed an increase in globulin levels, a fraction of protein composed by immunoglobulins and inflammatory protein of acute phase. The increase of cholesterol levels in the supplemented dairy sheep with $(PhSe)_2$ may have been a beneficial effect, since cholesterol is a cofactor for progesterone synthesis, hormone directly linked with better reproductive performance. Moreover, the feces of supplemented animals with $(PhSe)_2$ can be used for organic fertilizing without significant negative impacts to soil springtails fauna, an important marker of soil quality and environmental contamination.

Ethics Committee.

This Project was approved by Ethics Committee for Animal Experimentation of Universidade do Estado de Santa Catarina (CETEA/UDESC), under protocol number 5446050216.

REFERENCES

1. Caroprese M, Albenzio M, Annicchiarico G, Sevi A. Changes occurring in immune responsiveness of single and twin-bearing Comisana Ewes durin the transition period. J Dairy Sci. 2006; 89(2):562-568.
2. Underwood EJ, Suttle NF. The Mineral Nutrition of Livestock. Wallingford: CABI. 1999, 3:614.
3. Battin, EE, Perron NR, Brumaghim JL. The central role of metal coordination in selenium antioxidant activity. Inorg Chem. 2006; 45(2):499–501.
4. McDowell LR, Williams SN, Hidiroglou N, Njeru CA, Hill GM, Ochoa L, Wilkinson NS. Vitamin E and selenium suplementation for the ruminant. Anim Feed Sci Technol. 1996; 60(3-4):273-296.
5. Riaz M, Mehmood KT. Selenium in human health and disease: A Review. J Postgrad Med Inst. 2012; 26(2): 120-133.
6. Sadeghian S, Kojouri GA, Mohebb A. Nanoparticles of Selenium as Species with Stronger Physiological Effects in Sheep in Comparison with Sodium Selenite. Biol Trace Elem Res. 2012; 146(3):302-308.
7. Kamdev S, Dass RS, Garg AK, Sahu S, Gogoi S. Effect of different selenium sources (Selenium yeast and Sodium selenite) on haematology, blood chemistry and thyroid hormones in male goats (Capra hircus). Indian J Anim Res. 2015; 49(6):788-792.
8. Faixová Z, Piešová E, Maková Z, Cobanová K, Faix S. Effect of dietary supplementation with selenium-enriched yeast or sodium selenite on ruminal enzyme activities and blood chemistry in sheep. Acta Vet Brno. 2016; 85(2):185-194.
9. Freitas AS, Prestes AS, Wagner C, Sudati JH, Alves D, Porciúncula LO, Kade IJ, Rocha JBT. Reduction of diphenyl diselenide and analogs by mammalian thioredoxin reductase is independent of their gluthathione peroxidase-like activity: a possible novel pathway for their antioxidant activity. Molec. 2010; 15(11):7699–7714.
10. Nicolodi PRSJ, Camargo EV, Zeni D, Rocha RX, Cyrillo FC, Souza FN, Della Libera AMM, Bondan C, Leal MLR. Perfil proteico e metabolismo oxidativo de cordeiros experimentalmente infectados pelo Haemonchus contortus e suplementados com selênio e vitamina E. Cienc Rural. 2010; 40(3):561-567.

11. Barbosa CF, Tonin AA, Da Silva AS, Azevedo MI, Monteiro DU, Waczuk EP, Duarte T, Hermes C, Camillo G, Vogel FF, Faccio L, Tonin PT, Wolkmer P, Leal MR, Duarte MMF, Moresco RN, Lopes STA, De La Rue ML. Diphenyl diselenide and sodium selenite associated with chemotherapy in experimental toxoplasmosis: influence on oxidant/antioxidant biomarkers and cytokine modulation. Parasitol. 2014; 141(13):1761-1768.

12. Azizi P. Characterization of Humic Fertilizers from Horse, Sheep and Cattle Dung by their Density. Asian J Chem. 2007; 19(5):3677-3680.

13. Gil-Díaz MDM, Perez-Sanz A, Martín M, Lobo MC. Potential diffusion of doramectin into a soil amended with female pig manure. A Field Experiment. J Agric Food Chem. 2011; 59(19):10635–10640.

14. Zhao Z, Zhang Y, Xuan Y, Song W, Si W, Zhao Z, Rao Q. Ion-exchange solid-phase extraction combined with liquidchromatography-tandem mass spectrometry for the determination of veterinary drugs in organic fertilizers. J Chromat. 2016; 1022(1):281-289.

15. Gordon HM, Whitlock HV. A new technique for counting nematode eggs in sheep faeces. J Counc Sci Ind Res. 1939; 12(1):50-52.

16. ISO 11267. Soil Quality – Inhibition of Reproduction of Collembola (Folsomia candida by Soil Pollutants. International Organization for Standardization, Geneva, Switzerland. 1999.

17. R-Core-Team R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2013.

18. Cemek M, Büyükben A, Büyükokuroglu ME, Aymelek F, Tür L. Protective roles of vitamin E (-tocopherol), selenium and vitamin E plus selenium in organophosphate toxicity in vivo: a comparative study. Pest Biochem Physiol. 2010; 96(3):113–118.

19. El-Demerdash FM. Antioxidant effect of vitamin E and selenium on lipid peroxidation, enzyme activities and biochemical parameters in rats exposed to aluminium. J Trace Elem Med Biol. 2004; 18(1):113–122.

20. Piccione G, Casella S, Assenza A, Fazio F, Caola G. Evaluation of serum homocysteine and oxidative stress during lactation in ewes. Czech J Anim Sci. 2008; 53:462-465.

21. Bianchi AE, Macedo VP, França RT, Lopes STA, Lopes LS, Stefani LM, Volpato A, Lima HL, Paiano D, Machado G, Da Silva AS. Effect of adding palm oil to the diet of dairy sheep on milk production and composition, function of liver and kidney, and the concentration of cholesterol, triglycerides and progesterone in blood serum. Small Rum Res. 2014; 117: 78–83.

22. Reddy KP, Sailaja G, Krishnaiah C. Protective effects of selenium on fluoride induced alterations in certain enzymes in brain of mice. J Environ Biol. 2009; 30(5):859–864.

23. Hugejiletu H, Bobe G, Vorachek WR, Gorman ME, Mosher WD, Pirelli GJ, Hall JA. Selenium supplementation alters gene expression profiles associated with innate immunity in whole-blood neutrophils of sheep. Biol Trace Elem Res. 2013; 154(1):28–44.

24. Cutz-Pool LQ, Palacios-Vargas JG, Castañomeneses G, García-Calderón, NE. Edaphic Collembola from two agroecosystems with contrasting irrigation type in Hidalgo State, México. Appl Soil Ecol. 2007; 36(1):46-52.

25. Maccari AP, Baretta D, Paiano D, Leston S, Freitas A, Ramos F, Sousa JP, Klauberg-Filho O. Ecotoxicological effects of pig manure on Folsomia candida in subtropical Brazilian soils. J Hazardous Materials. 2016; 314:113-120.