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Original Article
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Supplementation with increasing doses of selenium associated with vitamin E in the treatment of bovines with enzootic hematuria[1]

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ABSTRACT.- Moreira Júnior C.A., Oliveira E.V., Cardoso C.A., Miranda M.P.B., Guedes N.A., Burak D.L., Pfister J.A. & Nunes L.C. 2024. **Supplementation with increasing doses of selenium associated with vitamin E in the treatment of bovines with enzootic hematuria.** *Pesquisa Veterinária Brasileira 44:00, 2024*. Universidade Federal do Espírito Santo, Alto Universitário s/n, Centro, Alegre, ES 29500-000, Brazil. E-mail: louisiane.nunes@ufes.br

Bovine enzootic hematuria (BEH) is a clinical form of poisoning in cattle caused by the consumption of *Pteridium* spp. (bracken fern), which has no treatment. However, selenium (Se) and vitamin E supplementation are feasible. The aim of this study was to evaluate the effects of supplementation with increasing doses of selenium associated with vitamin E in cattle with BEH, compare the levels of Se in whole blood and blood serum, and evaluate the activity of glutathione peroxidase, total antioxidants, and the relative activity of the enzyme monoamine oxidase (MAO). Four groups of cattle with BEH were supplemented parenterally for 13 weeks with increasing doses of Se: Control group, Treatment group 1 (0.05mg/kg), Treatment group 2 (0.1mg/kg), and Treatment group 3 (0.2mg/kg). All groups received 500mg of vitamin E in combination with the Se supplementation. The measured variables included weight, hematuria intensity, hematocrit, total plasma protein, plasma fibrinogen, blood glutathione peroxidase activity, and total antioxidant levels. The blood concentrations of Se and relative MAO activity were evaluated every two weeks. Kruskal-Wallis and Friedman tests ($P<0.05$) were used to assess treatment and time effects, respectively, followed by Dunn's multiple comparison test. For weight, total antioxidant concentration, and relative MAO, there was no significant difference ($P>0.05$) between the treatments, but there was a significant difference over time ($P<0.05$). For hematuria and hematocrit, there were significant treatment differences ($P<0.05$) but no significant time differences ($P>0.05$). For glutathione peroxidase, there was no significant difference ($P>0.05$) among the treatments, but there was a significant difference ($P<0.05$) between treatments M8 and M14 in Treatment group 3. There were no differences in the total plasma protein and fibrinogen levels between treatments or over time ($P>0.05$). Selenium levels were higher in Treatment group 1, reaching the highest concentration (235.3µg/L) in the blood serum at eight weeks. Therefore, supplementation with Se at a dose of 0.05mg/kg associated with vitamin E improved the clinical condition of hematuria but did not interfere with glutathione peroxidase activity or with levels of total antioxidants. MAO activity was reduced by vitamin E supplementation. These results indicate that the serum is the best site for measuring Se levels.

INDEX TERMS: Selenium, supplementation, vitamin E, cattle, bovine enzootic hematuria, clinical aspects.

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RESUMO.- [Suplementação com doses crescentes de selênio associado à vitamina E no tratamento de bovinos com hematúria enzoótica.] A hematúria enzoótica bovina (HEB) é uma forma clínica da intoxicação em bovinos causada pelo consumo de *Pteridium* sp. (samambaia) que não possui tratamento. Contudo, a suplementação utilizando selênio (Se) e vitamina E tem se mostrado viável. Objetivou-se

avaliar os efeitos da suplementação com doses crescentes de selênio associado à vitamina E em bovinos com HEB, bem como, comparar os níveis de selênio em sangue total e soro sanguíneo, avaliar a atividade de glutationa peroxidase, os níveis de antioxidantes totais e a atividade relativa da enzima monoamina oxidase (MAO). Suplementou-se durante 13 semanas, por via parenteral, quatro grupos de bovinos com hematúria enzoótica (HEB), com doses crescentes de Se: Grupo controle, Grupo tratamento 1 (0,05mg/kg de Se), Grupo tratamento 2 (0,1mg/kg de Se) e Grupo tratamento 3 (0,2mg/kg de Se). Todos os grupos receberam em associação 500mg/animal de vitamina E. Quinzenalmente foram avaliadas as variáveis peso, intensidade de hematúria, hematócrito, proteína plasmática total, fibrinogênio plasmático, atividade sanguínea de glutationa peroxidase, níveis de antioxidantes totais, concentração sanguíneas de selênio e atividade relativa de monoamina oxidase. Utilizou-se os testes Kruskal Wallis e Friedman (P<0,05), para avaliar os efeitos dos tratamentos e do tempo, respectivamente, ambos seguidos do teste de comparação múltipla de Dunn. Para as variáveis peso, antioxidantes totais e atividade relativa de monoamina oxidase não houve diferença entre os tratamentos (P>0,05) e houve diferença ao longo do tempo (P<0,05). Para hematúria e hematócrito, houve diferença entre os tratamentos (P<0,05) e não houve diferença ao longo do tempo (P>0,05). Para a variável glutationa peroxidase, não houve diferença significativa entre os tratamentos (P>0,05), porém houve diferença entre os momentos M8 e M14, no Grupo tratamento 3 (P<0,05). Para proteína plasmática total e fibrinogênio não houve diferenças entre os tratamentos e nem ao longo do tempo (P>0,05). Os níveis de Se foram maiores no Grupo tratamento 1 atingindo a maior concentração no momento M8 no soro sanguíneo (235,3µg/L). Portanto, a suplementação com selênio na dose de 0,05mg/kg associada à vitamina E melhorou o quadro clínico de hematúria, mas não interferiu na atividade da glutationa peroxidase e nos níveis de antioxidantes totais. A atividade da monoamina oxidase foi reduzida globalmente pela suplementação de vitamina E. Estes resultados indicam que o soro foi o melhor local para medir o selênio.

TERMOS DE INDEXAÇÃO: Selênio, suplementação, vitamina E, bovinos, hematúria enzoótica bovina, aspectos clínicos.

INTRODUCTION

Numerous diseases pose a risk to the sustainability and permanence of dairy producers, among which plant poisoning stands out. In the state of Espírito Santo, Brazil, the most common cases of poisoning are related to the consumption of plants belonging to the genus *Pteridium*, commonly known as bracken ferns. The clinical forms of toxicosis in cattle resulting from the consumption of *Pteridium* spp. include acute hemorrhagic syndrome, bovine enzootic hematuria (BEH), and neoplasms in the upper alimentary tract (Méndez & Riet-Correa 2001). Silva et al. (2009) demonstrated that dairy farms located in the micro-region of Caparaó, in the southern region of the state of Espírito Santo, Brazil, had a high prevalence of BEH, a clinical form responsible for causing serious economic losses to milk producers in the region. Although the pathogenesis of BEH is well known, there is still no effective treatment for the affected animals (Tokarnia et al. 2012). However, therapies involving selenium (Se) and vitamin E supplementation have shown promising results in mice (Latorre et al. 2011, 2013) and cattle (Latorre et al. 2014, Lanna Neta 2018).

The immunosuppressive effects of ptaquiloside, the active ingredient in *Pteridium* sp., on natural killer (NK) cells have been demonstrated in mice. This substance promotes the reduction of the cytotoxic activity of NK cells by increasing the expression of the metallothionein protein, consequently reducing the intracellular mineral zinc, which is essential for the functioning of the immune system. In the same study, it was found that Se supplementation inhibited the high expression of metallothionein, resulting in an increase in free zinc in NK cells and the recovery of their cytotoxic activity (Latorre et al. 2013). In another study using cattle supplemented with Se and vitamin E, positive immunostimulatory effects on NK cells of the innate immune system of this animal species were observed (Latorre et al. 2014).

Studies on Se supplementation for cattle have also demonstrated an increase in the activity of glutathione peroxidase, an important selenium-dependent antioxidant enzyme responsible for combating free radicals (Deshpande et al. 2018, Gong & Xiao 2018). In addition to promoting an enzymatic increase in glutathione peroxidase, Weiss & Wyatt (2002) reported that owing to the antioxidant properties of Se and vitamin E, their supplementation reduces cellular oxidative stress and thus positively affects the body's health.

In situations involving Se supplementation, monitoring Se levels in animals is important. Blood is considered a relevant means of measuring microelements and can be evaluated using whole blood or serum samples. In a study by Lanna Neta (2018), serum Se levels were successfully measured in fern-poisoned cattle treated with Se and vitamin E supplements. However, Herdt et al. (2000) reported that, although whole blood and serum are valuable means of evaluating the nutritional status of Se, measurement using whole blood is preferable to that of serum because of the greater stability of the microelement, providing more reliable values in cases of long-term ingestion.

Monoamine oxidase (MAO) is an important enzyme responsible for primary amine catalysis in animals. It is found in the central nervous system, blood, kidneys, and the liver. In situations with high MAO activity, excessive production of reactive free radicals may occur, representing a potential risk factor for cellular oxidative stress (Pazini 2013).

Given this, studies on supplementation of the micro-minerals Se and vitamin E, both considered important cellular antioxidants, have been carried out in animals poisoned by ferns. However, there are no reports on the association between the relative activity of MAO in cattle and BEH. Thus, in an attempt to obtain further clarification, the aim of this study was to evaluate the effects of supplementation with increasing doses of Se associated with vitamin E in cattle with enzootic hematuria, to evaluate glutathione peroxidase activity, total antioxidant levels, and relative activity of the enzyme monoamine oxidase, and to compare Se levels in whole blood and blood serum**.**

MATERIALS AND METHODS

Animal Ethics. The experimental protocol was approved by the Animal Use Ethics Committee (CEUA) of the "Universidade Federal do Espírito Santo" (UFES, Alegre) under numbers 03/2017 and 025/2020.

Study location. The study was carried out at the UFES, at the "Centro de Ciências Agrárias e Engenharias" (CCAE) and "Centro de Ciências Exatas Naturais e da Saúde" (CCENS). The analyses were conducted predominantly in the Chemical and Environmental Analysis Laboratory in the Agronomy Research Support Laboratory in the experimental area, the Animal Pathology Laboratory of the Veterinary Hospital – both at CCAE, and the Biochemistry Laboratory – at CCENS.

Fifty-one samples of whole blood and 68 samples of bovine blood serum were analyzed to measure the concentration of the microelement Se and the relative activity of the antioxidant enzyme MAO.

Samples were obtained from 18 female cattle aged between four and 12 years, with BEH confirmed by urine collection through spontaneous urination. The urine was evaluated for the existence of macro-and microhematuria. Clinical-epidemiological data collection took place on the property, and the presence of the plant was verified. The evaluation period was from August 2019 to January 2020. The animals came from different rural properties in the municipalities of Divino São Lourenço and Ibitirama, both belonging to the Caparaó microregion, South of Espírito Santo, Brazil. The presence of *Pteridium* spp. was observed in pastures on all properties. The animals were identified by applying earrings containing identification information to the right ear.

Throughout the experimental period, the animals were kept in their places of origin, respecting the management conditions of the properties' normal routines regarding nutritional, reproductive, and productive aspects. The diet of all animals was based on chopped sugarcane (*Saccharum officinarum*) and *Brachiaria* sp.

The animals were taken to milking parlors and housed in kennels to collect biological material and administer Se and vitamin E. Each animal was physically restrained and supplemented parenterally. After the procedure, the animals were returned to their respective paddocks.

A completely randomized experimental design (DIC) was adopted, and the animals were randomly divided into four groups, with one animal from each farm randomly selected as the control. They received parenteral supplementation, intramuscularly, with different doses of FOSFOSAL® (Control group – physiological solution), Treatment group 1 (0.05mg/kg of Se), Treatment group 2 (0.1mg/kg of Se), and Treatment group 3 (0.2mg/kg of Se), associated with vitamin E® (500mg/animal). In the Control group, the dose of FOSFOSAL® was replaced with a saline solution.

The animals were evaluated at 14 weekly moments, identified by the letter "M" followed by the moment number. M0 corresponded to the initial moment of the experiment, one week before the first supplementation. Supplementation with Se and vitamin E occurred between moments M1 and M13. The experiment ended at M14, corresponding to the last evaluation, and occurred one week after the last application.

Blood samples were collected every 15 days at M0, M2, M4, M6, M8, M10, M12, and M14. Blood samples were collected through a puncture in the tail vein using 5mL tubes with and without anticoagulants (heparin and EDTA). After identification and placement in Styrofoam boxes containing recycled ice, the samples were sent to the Animal Pathology Laboratory at Hovet-UFES for analysis of hematocrit, total serum protein, plasma fibrinogen, glutathione peroxidase, total antioxidants, Se, and monoamine oxidase.

The weight of the animals was assessed each time using a weighing tape suitable for cattle to calculate the dose of the supplement and determine the weight gain. In addition, the intensity of blood in urine was verified by observing spontaneous urination using a subjective semi-quantitative method based on a score ranging from 1 to 4 (1 = absent, 2 = slight, 3 = moderate, and 4 = intense) (Lanna Neta 2018).

Hematocrit. Blood samples collected with EDTA anticoagulant were used to determine the hematocrit and to fill capillary tubes using gravity to 3/4 of their capacity. These were then centrifuged in a microcentrifuge at 3,000 rotations per minute for 10 min and read using a specific table.

Total plasma proteins and fibrinogen. As described by Falbo et al. (2005), the total plasma protein, plasma protein, and fibrinogen levels were calculated by subtracting one from the other.

Total plasma protein and fibrinogen were determined simultaneously with hematocrit. Aliquots of blood with heparin were transferred to 1.5mL microtubes (cryotubes type) and stored in a -80°C freezer. Blood serum was obtained by centrifuging blood samples without anticoagulants at 3000 rpm for 10 min in a tube centrifuge. Then, the serum was transferred to 1.5mL microtubes and stored in a freezer -80°C. Both whole blood and serum remained in the freezer -80°C for subsequent measurement of glutathione peroxidase activity, total antioxidants, blood Se levels, and relative MOA.

Glutathione peroxidase. Whole blood samples from all moments were used to carry out the tests for glutathione peroxidase. Samples were thawed in a water bath at 36°C, and the commercial kit (RANDOX, RS 505, SC692 Crumlin, UK) was used according to the manufacturer's instructions.

Total antioxidants. Total antioxidant levels were measured in the blood serum at all time points using a commercial kit (SIGMA-ALDRICK, MAK334, St. Louis/MO, USA) according to the manufacturer's instructions.

Selenium measurement. Selenium was measured in whole blood and blood serum at different time points (initial, intermediate, and final) like that described by Latorre et al. (2014). Serum samples from all time points were used to evaluate the relative MAO activity.

Bovine whole blood and serum samples were sent to the Chemical and Environmental Analysis Laboratory of the Agronomy Research Support Sector, located in the Rive Experimental Area, CCAE, Alegre/ES, to evaluate Se levels. The samples were thawed at room temperature and subjected to prior microwave digestion. Subsequently, they were subjected to the atomic absorption spectrometry technique with hydride generation using the HG3000 device.

Whole blood and serum samples were digested using 2mL of sample (serum or whole blood), 5mL of nitric acid (HNO_3), and 2mL of hydrogen peroxide (H_2O_2). The mixture was gently stirred for 15 seconds. The samples were digested using the MARS 6 and Xpress software.

After digestion, the samples were subjected to atomic absorption spectrometry using hydride generation to measure the Se concentration. The samples were initially acidified using concentrated hydrochloric acid (HCl) to obtain a 50% solution and then gently heated at 70°C for 30 minutes. The mixture was allowed to cool to room temperature before analysis. Acidification was necessary to reduce selenite (Se_2O_3) to selenate (Se_2O_4), which could not be detected using the hydride technique.

Relative monoamine oxidase activity. To evaluate the relative activity of the MAO enzyme in serum, bovine serum samples were sent to the Biochemistry Laboratory (CCENS). The relative activity of the antioxidant enzyme MAO was evaluated by a luminescence assay using the MAO-GloTM kit in a Glomax+ multi-detection system luminometer.

The statistical program GraphPad Prism version 9.0.0 was used to analyze all variables studied. Kruskal-Wallis and Friedman non-

parametric statistical tests were used, with a significance level of 5%. The first test evaluated the treatment effect, and the second evaluated the time effect of each treatment. Both tests were performed using Dunn's multiple comparison test.

Thus, the variables were statistically analyzed for treatment and time effects. For the first analysis, the data obtained at each sampling moment were compared between the different groups. In the second case, the data from different sampling times were compared within each group.

RESULTS AND DISCUSSION

Body weight

No significant differences ($P>0.05$) were observed between the treatments. However, when evaluating the effect of time, a significant difference ($P<0.05$) was noted between M2 and M8 in animals in the Control group, revealing a greater body weight at M2 than at M8 (Table 1). The results obtained in this study differ from those described by Lanna Neta (2018) in a study in which weight gain was verified in dairy cows affected by BEH supplemented for the same period and routes of administration, using inorganic Se in the form of sodium selenite, in doses of 0.05, 0.1, and 0.2mg/kg associated with vitamin E. This difference can be explained by the fact that these properties and animals may have access to nutritional management with different Se levels. Furthermore, Lanna Neta (2018) did not describe the work's development period, which, if carried out during the rainy season, would differ from the current study and would provide greater availability and quality of food for the animals. This would justify the gain in the observed weight. However, the results obtained in the present study align with those observed by Lawler et al. (2004), who did not observe a relationship between weight gain and feed efficiency when steers were supplemented with different sources of selenium.

Some factors may have contributed to these results, such as the stress of collection and parenteral administration of Se. According to Mobiglia et al. (2014), even without external factors, such as the removal of animals from the property and changes in food management, practices such as blood collection result in the immediate release of cortisol into the bloodstream of cattle, which can lead to fatigue and loss of body mass. Thus, it is believed that the method involving moving the animals to the corral, containment, sample collection, and administration of Se corroborates these results.

Regarding the differences in weight observed between M2 and M8 in the Control group, it is believed that some factors, such as the period of development of the study – mostly in the dry period, pregnancy of some animals, and changes in pasture may have influenced the results. According to Santos et al. (2014), certain restrictive climatic factors observed in winter (July, August, and September) compromise the productivity and quality of forage by affecting water availability, solar radiation, and temperature. Therefore, the difference in weight observed can be explained by the fact that collections began during the dry season when the quality and supply of food were reduced.

Hematuria

A significant difference ($P<0.05$) was observed in the reduction of hematuria between the Control and Treatment group 1 at M4, 6, and 14. However, no significant differences were observed ($P>0.05$) in this variable over time in any of the treatments (Table 2). These results indicate a reduction in hematuria after the third week of supplementation with the lowest dose of Se. These results corroborate those obtained by Lanna Neta (2018), who, when supplementing dairy cows affected by BEH using Se and vitamin E, also found a reduction in hematuria in the treatment groups compared to the control.

According to Tokarnia et al. (2012), hematuria is one of the main clinical signs of BEH resulting from neoplastic or

Table 1. Animals weight distributed in experimental groups at different times subjected to supplementation with increasing doses of selenium (Se) associated with vitamin E in cattle with bovine enzootic hematuria (BEH)

	Animal weight (kg)			
Time	Control	Treatment 1	Treatment 2	Treatment 3
M0	434.0 ^{Aa} (326.3 |-| 506.0)	409.5 ^{Aa} (382.0 |-| 551.0)	405.5 ^{Aa} (332.5 |-| 499.5)	385.5 ^{Aa} (358.0 |-| 422.0)
M2	405.0 ^{Aab} (335.0 |-| 467.8)	388.5 ^{Aa} (361.0 |-| 503.8)	376.0 ^{Aa} (308.5 |-| 442.8)	379.5 ^{Aa} (309.3 |-| 422.0)
M4	385.5 ^{Aa} (330.3 |-| 460.5)	394.5 ^{Aa} (364.0 |-| 484.3)	370.0 ^{Aa} (301.0 |-| 436.0)	364.5 ^{Aa} (306.5 |-| 412.8)
M6	398.0 ^{Aa} (331.0 |-| 472.5)	367.0 ^{Aa} (362.5 |-| 472.0)	382.5 ^{Aa} (318.3 |-| 440.8)	367.0 ^{Aa} (311.5 |-| 453.3)
M8	378.5 ^{Aac} (330.3 |-| 449.0)	391.0 ^{Aa} (340.8 |-| 470.5)	382.0 ^{Aa} (319.8 |-| 430.0)	376.0 ^{Aa} (358.0 |-| 394.0)
M10	395 ^{Aa} (328.8 |-| 454.5)	373 ^{Aa} (358 |-| 443.5)	364 ^{Aa} (309.3 |-| 430)	361.5 ^{Aa} (317 |-| 394)
M12	394.0 ^{Aa} (333.8 |-| 456.0)	394.0 ^{Aa} (351.3 |-| 452.5)	357.0 ^{Aa} (314.8 |-| 428.5)	376.0 ^{Aa} (321.3 |-| 397.0)
M14	391.5 ^{Aa} (325.5 |-| 448.5)	379.0 ^{Aa} (361.0 |-| 489.0)	385.0 ^{Aa} (373.0 |-| 441.0)	367.0 ^{Aa} (348.3 |-| 400.8)

Medians (1st quartile |-| 3rd quartile); ^{A} Medians followed by the same capital letter in the line do not differ from each other using the Kruskal-Wallis test, $^{a-c}$ Medians followed by the same lowercase letter in the column do not differ from each other, according to the Friedman test; Both the tests were performed at a significance level of 5%; Control (physiological solution), Treatment 1 (0.05mg/kg Se), Treatment 2 (0.10mg/kg Se), Treatment 3 (0.20mg/kg Se), associated with the administration of 500mg/animal in all groups.

non-neoplastic lesions in the bladder. In work developed by Silva et al. (2012) in the Caparaó microregion of Espírito Santo, when histopathologically evaluating cattle bladders from slaughterhouses presenting with macroscopic lesions and/or hematuria, they observed neoplastic lesions in 56.52% of the bladders, these being of epithelial origin such as carcinoma and of mesenchymal origin such as hemangioma and hemangiosarcoma. Furthermore, non-neoplastic lesions were observed in 100% of bladders, including vascular changes such as hemorrhage, proliferation, ectasia, dilation, and vascular thickening.

Given the above, some studies conducted in animal models have shown the involvement of oxidative stress in diseases involving blood vessels, such as atherosclerosis. Furthermore, it has been suggested that the consumption of antioxidants has a satisfactory effect on preventing and treating cardiovascular diseases (Rosenblat et al. 2015). Thus, considering the antioxidant and protective effects of Se on cellular lipid membranes against the action of free radicals and lipid peroxides through the enzyme glutathione peroxidase (Marçal et al. 2016), it is suggested that supplementation with Se provides protection and maintains the integrity of the vascular endothelium, in addition to inhibiting inflammatory events. Furthermore, anticancer, antimetastatic, and antiangiogenic properties have been attributed to Se supplementation in human and animal studies (Latorre et al. 2011, 2014, Chen et al. 2013). Therefore, the reduction in hematuria observed between Treatment group 1 and the Control group may be explained by the properties of Se in the regression of neoplastic and non-neoplastic lesions responsible for the clinical condition in animals.

Hematocrit

A significant difference ($P<0.05$) was observed between the Control and Treatment 1 groups at M8 and 14, showing a clear increase in hematocrit and a resulting improvement in anemia. However, when analyzing the time effect, no significant differences ($P>0.05$) were observed among the time points throughout each treatment (Table 3).

Clinical signs of anemia are often observed in clinical situations involving BEH progression in affected animals that do not receive any treatment. In the laboratory, a reduction in hematocrit to values below the limit for the species can be noted, in addition to an increase in erythrocyte fragility and progressive anemia (Radostits et al. 2007, Silva et al. 2009, Ribeiro et al. 2020). However, the data obtained in this study shows an increase in hematocrit in Treatment group 1 compared to the Control, going from values below 24%, the limit for the species, to values within normal limits between 24 and 46% (Jain 1993). These results led us to infer that weekly Se supplementation at the lowest dose after seven weeks improved anemia. Thrall (2015) reported that one of the causes of anemia is blood loss from hemorrhage. Therefore, it is believed that the observed improvement in hematuria reduces anemia, as described by Jean-Blain et al. (1987).

However, increasing the hematocrit level alone may not completely reverse anemia. Therefore, to rule out possible true polycythemia due to dehydration, it is recommended to measure the total plasma proteins, including fibrinogen, which may increase under these conditions (Thrall 2015). These variables were evaluated in this study.

Total plasma proteins and plasma fibrinogen

When evaluating the total plasma proteins and plasma fibrinogen variables in this study, the medians of the groups were within the normal values for cattle in 75% of the samples, confirming that there was indeed an improvement in anemia after supplementation with Se.

Furthermore, total plasma proteins and plasma fibrinogen did not show significant differences ($P>0.05$) in any treatments applied. The same was observed when analyzing the effects of time within each treatment.

Table 2. Semi-quantitative assessment of the degree of hematuria by clinical assessment of spontaneous urination, classified by scores ranging from 1 to 4, in cattle with bovine enzootic hematuria (BEH) supplemented with increasing doses of selenium (Se) associated with vitamin E at different experimental times

	Hematuria			
Time	Control	Treatment 1	Treatment 2	Treatment 3
M0	4.00 ^{Aa} (1.75|-|4.00)	1.50 ^{Aa} (1.00|-|3.50)	2.00 ^{Aa} (1.25|-|3.50)	3.00 ^{Aa} (2.00|-|4.00)
M4	3.00 ^{AaB} (1.75|-|4.00)	1.00 ^{AaC} (1.00|-|1.75)	1.50 ^{Aa} (1.00|-|3.50)	3.00 ^{Aa} (2.25|-|3.00)
M6	3.50 ^{AaD} (2.00|-|4.00)	1.00 ^{AaE} (1.00|-|1.00)	2.50 ^{Aa} (1.25|-|3.00)	2.00 ^{Aa} (1.25|-|3.50)
M8	3.50 ^{Aa} (2.00|-|4.00)	1.00 ^{Aa} (1.00|-|1.00)	1.00 ^{Aa} (1.00|-|3.25)	3.00 ^{Aa} (2.25|-|3.75)
M10	2.25 ^{Aa} (1.00|-|4.00)	1.00 ^{Aa} (1.00|-|1.00)	1.00 ^{Aa} (1.00|-|3.25)	3.00 ^{Aa} (2.25|-|3.00)
M12	2.25 ^{Aa} (1.75|-|4.00)	1.00 ^{Aa} (1.00|-|1.00)	1.00 ^{Aa} (1.00|-|3.25)	3.00 ^{Aa} (1.50|-|3.75)
M14	3.50 ^{AaF} (1.75|-|4.00)	1.00 ^{AaG} (1.00|-|1.75)	1.00 ^{Aa} (1.00|-|3.25)	4.00 ^{Aa} (2.50|-|4.00)

Medians (1st quartile |-| 3rd quartile); $^{A-G}$ Medians followed by the same capital letter in the line do not differ from each other using the Kruskal-Wallis test, ^{a} Medians followed by the same lowercase letter in the column do not differ from each other, according to the Friedman test; Both the tests were performed at a significance level of 5%; Moment 2 was considered lost because of the small number of samples; Control (physiological solution), Treatment 1 (0.05mg/kg Se), Treatment 2 (0.10mg/kg Se), Treatment 3 (0.20mg/kg Se), associated with the administration of 500mg/animal in all groups; Scores: 1 = absent, 2 = mild, 3 = moderate, 4 = intense.

According to Allison (2015), changes in plasma protein concentrations, such as increase or decrease, are laboratory findings commonly observed in animals, resulting from abnormalities in albumin or globulin content. However, the same author also describes that the increase or decrease in albumin and globulin may not always lead to detectable changes in total plasma protein.

When evaluating plasma fibrinogen, the data were similar to those described by Oliveira (2021), who also did not observe significant differences in this variable in dairy cows affected by BEH and supplemented with doses of 0.05, 0.1, and 0.2mg/kg of body weight of Se associated with vitamin E.

In another study developed by Falbo et al. (2005), where clinical and laboratory changes were observed in bovine females naturally poisoned by ferns in the state of Paraná, Brazil, the occurrence of hyperfibrinogemia in the animals was not verified.

Fibrinogen is a positive acute-phase protein widely used for routine bovine blood counts. In this species, the concentration is very sensitive to the inflammatory processes and may increase despite the white blood cell count indicating no inflammation. This increase may be associated with dehydration (Allison 2015). However, in this study, despite the animals being affected by a chronic disease involving inflammatory processes, the clinical picture of BEH did not trigger marked changes in hypoproteinemia, hyperproteinemia, or hyperfibrinogenemia.

Glutathione peroxidase

When evaluating the effect of time on the activity of the antioxidant enzyme glutathione peroxidase, a significant difference (P<0.05) was found in Treatment group 3 between M8 and M14, with greater activity at M8. No significant differences (P>0.05) were observed among treatments (Table 4). Thus, there was an increase in the enzymatic activity of glutathione peroxidase after the seventh week of supplementation, which was reduced after the last application in the thirteenth week in Treatment group 3.

Glutathione peroxidase governs one of the main enzymatic systems against increased free radicals in the body (Deshpande et al. 2018). It is a Se-dependent enzyme (Bermingham et al. 2014), so its activity is directly dependent on the bioavailability of Se in the body. Therefore, it was expected that, in this study, the groups supplemented with Se would show an increase in glutathione peroxidase activity compared to the control group, which only occurred with the highest supplementation dose.

Studies with supplementation of cows in the prepartum period with Se in the form of enriched yeast and sodium selenite at a dose of 0.3mg Se/kg of dry matter revealed an increase in glutathione peroxidase activity in relation to animals that did not receive Se (Gong & Xiao 2018). Moreover, supplementation in calves in the pre- and post-weaning period in the summer, using 0.1mg/kg of body weight with Se and vitamin E 1mg/kg, among other minerals such as zinc, copper, manganese, and vitamin A, also revealed an increase in the activity of the enzyme glutathione peroxidase after 15, 30, and 45 d of supplementation, in addition to other factors such as better zootechnical performance (Bordignon et al. 2019).

Total antioxidants

When evaluating total antioxidant levels, a significant difference (P<0.05) was noted in the time effect in the Control group between M8 and M12, with lower concentrations at M12. A significant difference (P<0.05) was also observed in the time effect in Treatment group 1 between M0, M14, M12, and M14, with higher levels at M14. When evaluating the treatment effects, a significant difference was observed between the Control and Treatment group 1 at M14, indicating that Treatment group 1 had higher total antioxidant concentrations (Table 5).

It is believed that the reduction in total antioxidant levels observed over time in the Control group was a consequence

Table 3. Evaluation of the hematocrit of cattle with bovine enzootic hematuria (BEH) supplemented with increasing doses of selenium (Se) associated with vitamin E at different experimental times

	Hematocrit (%)			
Time	Control	Treatment 1	Treatment 2	Treatment 3
M0	20.50 ^{Aa} (17.25|-|24.50)	28.00 ^{Aa} (22.00|-|33.25)	24.00 ^{Aa} (21.75|-|27.00)	23.00 ^{Aa} (12.00|-|28.00)
M2	18.00 ^{Aa} (15.25|-|20.75)	26.50 ^{Aa} (13.75 |-|33.25)	26.00 ^{Aa} (18.50|-|27.50)	19.25 ^{Aa} (11.63|-|23.5)
M4	22.25 ^{Aa} (17.25|-|24.00)	26.50 ^{Aa} (25.00|-|32.50)	24.50 ^{Aa} (21.50|-|29.75)	21.50 ^{Aa} (15.25|-|25.50)
M6	21.50 ^{Aa} (18.50|-|23.75)	24.50 ^{Aa} (23.25|-|31.00)	22.50 ^{Aa} (21.25|-|27.50)	23.00 ^{Aa} (17.50|-|26.25)
M8	20.00 ^{AaB} (16.75|-|23.25)	26.00 ^{AaC} (25.25|-|32.00)	23.00 ^{Aa} (22.00|-|27.75)	21.50 ^{Aa} (17.50|-|24.00)
M10	20.50 ^{Aa} (18.75|-|25.25)	26.00 ^{Aa} (24.25|-|30.00)	25.00 ^{Aa} (22.00|-|25.75)	20.00 ^{Aa} (10.50|-|27.25)
M12	20.00 ^{Aa} (17.75|-|21.25)	25.00 ^{Aa} (24.00|-|30.50)	23.50 ^{Aa} (20.00|-|25.50)	20.50 ^{Aa} (13.25|-|26.25)
M14	20.00 ^{AaD} (18.25|-|21.5)	26.00 ^{AaE} (23.50|-|31.50)	22.50 ^{Aa} (17.50|-|23.38)	17.00 ^{Aa} (12.25|-|24.75)

Medians (1st quartile |-| 3rd quartile); $^{A-E}$ Medians followed by the same capital letter in the line do not differ from each other using the Kruskal-Wallis test, ^{a} Medians followed by the same lowercase letter in the column do not differ from each other, according to the Friedman test; Both the tests were performed at a significance level of 5%; Control (physiological solution), Treatment 1 (0.05mg/kg Se), Treatment 2 (0.10mg/kg Se), Treatment 3 (0.20mg/kg Se), associated with the administration of 500mg/animal in all groups.

of the same factors described for weight, such as the low nutritional quality of the forage in the dry period, the evolution of pregnancy in some animals, and changes in the pasture. According to Samóra (2014), dairy cows face several metabolic challenges in their reproductive and productive cycles, which can lead to negative energy balances and a consequent deficit in vitamins and minerals, including those with antioxidant properties, such as Se and vitamin E.

In the present study, it was observed that supplementation with Se associated with vitamin E at a dose of 0.05mg/kg (Treatment group 1) caused an increase in total antioxidant levels throughout the treatment, pointing to higher levels of antioxidants after 13 weeks of supplementation, demonstrating the effectiveness of the antioxidant properties of Se and vitamin E. Furthermore, when analyzing the hematuria and hematocrit variables, Treatment group 1 also presented the best clinical results for reducing hematuria and increasing hematocrit. According to Weiss & Wyatt (2002), supplementing animals with Se and vitamin E reduces oxidative stress by removing peroxides and reducing the peroxidation of fatty acids in the cells, thereby positively affecting the health status of the organism. It has been suggested that the antioxidant effect of Se associated with vitamin E is responsible for the observed clinical improvement.

Selenium

The blood Se concentrations in all cattle in the present study remained within normal levels during the supplementation

Table 4. Activity of the glutathione peroxidase enzyme in whole blood of cattle with bovine enzootic hematuria (BEH) supplemented with increasing doses of selenium (Se) associated with vitamin E at different experimental times

	Glutathione peroxidase			
Time	Control	Treatment 1	Treatment 2	Treatment 3
M0	142.5 ^{Aa} (142.8|-|172.5)	169.5 ^{Aa} (160.8|-|236.0)	168.5 ^{Aa} (141.3|-|183.0)	135.5 ^{Aa} (128.0|-|182.0)
M4	129.0 ^{Aa} (120.3|-|153.0)	140.5 ^{Aa} (117.0|-|205.3)	127.5 ^{Aa} (117.8|-|132.8)	157.5 ^{Aa} (118.0|-|172.3)
M6	122.5 ^{Aa} (100.5|-|154.0)	163.5 ^{Aa} (129.0|-|237.8)	144.5 ^{Aa} (127.8|-|169.5)	187.0 ^{Aa} (155.0|-|227.3)
M8	183.5 ^{Aa} (168.3|-|257.5)	227.5 ^{Aa} (179.3|-|266.8)	217.5 ^{Aa} (154.8|-|254.8)	246.5 ^{Aab} (190.5|-|265.0)
M12	120.0 ^{Aa} (75.0|-|205.8)	134.5 ^{Aa} (75.0|-|283.3)	159.5 ^{Aa} (59.5|-|269.3)	77.5 ^{Aa} (62.5|-|182.5)
M14	184.5 ^{Aa} (159.0|-|208.5)	199.0 ^{Aa} (83.5|-|261.3)	182.0 ^{Aa} (89.25|-|247.8)	71.00 ^{Aac} (38.5|-|182.3)

Medians (1st quartile |-| 3rd quartile); ^{A} Medians followed by the same capital letter in the line do not differ from each other using the Kruskal-Wallis test; $^{a-c}$ Medians followed by the same lowercase letter in the column do not differ from each other, according to the Friedman test; Both the tests were performed at a significance level of 5%; Moments 2 and 10 were considered lost portions owing to the low number of samples; Control (physiological solution), Treatment 1 (0.05mg/kg Se), Treatment 2 (0.10mg/kg Se), Treatment 3 (0.20mg/kg Se), associated with the administration of 500mg/animal in all groups.

Table 5. Total antioxidant levels of cattle with bovine enzootic hematuria (BEH) supplemented with increasing doses of selenium (Se) associated with vitamin E at different experimental times

	Total antioxidant levels			
Time	Control	Treatment 1	Treatment 2	Treatment 3
M0	0.065 ^{Aa} (0.058|-|0.078)	0.082 ^{Aae} (0.067|-|0.092)	0.086 ^{Aa} (0.078|-|0.094)	0.072 ^{Aa} (0.065|-|0.090)
M2	0.090 ^{Aa} (0.078|-|0.102)	0.100 ^{Aa} (0.090 |-|0.113)	0.090 ^{Aa} (0.082|-|0.102)	0.087 ^{Aa} (0.083|-|0.131)
M4	0.084 ^{Aa} (0.072|-|0.095)	0.091 ^{Aa} (0.074|-|0.099)	0.076 ^{Aa} (0.068|-|0.089)	0.083 ^{Aa} (0.062|-|0.104)
M6	0.081 ^{Aa} (0.068|-|0.091)	0.088 ^{Aa} (0.071|-|0.093)	0.082 ^{Aa} (0.068|-|0.092)	0.094 ^{Aa} (0.076|-|0.094)
M8	0.097 ^{Aab} (0.081|-|0.102)	0.096 ^{Aa} (0.093|-|0.096)	0.090 ^{Aa} (0.084|-|0.094)	0.087 ^{Aa} (0.081|-|0.091)
M10	0.078 ^{Aa} (0.074|-|0.104)	0.100 ^{Aa} (0.090|-|0.110)	0.086 ^{Aa} (0.078|-|0.103)	0.085 ^{Aa} (0.074|-|0.097)
M12	0.063 ^{Aac} (0.058|-|0.073)	0.072 ^{Aaf} (0.068|-|0.080)	0.078 ^{Aa} (0.059|-|0.087)	0.073 ^{Aa} (0.057|-|0.092)
M14	0.078 ^{AaD} (0.076|-|0.085)	0.101 ^{AagH} (0.100|-|0.116)	0.088 ^{Aa} (0.078|-|0.111)	0.084 ^{Aa} (0.070|-|0.094)

Medians (1st quartile |-| 3rd quartile); $^{A-H}$ Medians followed by the same capital letter in the line do not differ from each other using the Kruskal-Wallis test, $^{a-g}$ Medians followed by the same lowercase letter in the column do not differ from each other, according to the Friedman test; Both the tests were performed at a significance level of 5%; Control (physiological solution), Treatment 1 (0.05mg/kg Se), Treatment 2 (0.10mg/kg Se), Treatment 3 (0.20mg/kg Se), associated with the administration of 500mg/animal in all groups.

period. Furthermore, it was observed that serum Se concentrations were between 81 and 315μg/L, showing good levels of the element in the animals at the beginning of the study. According to Villar et al. (2002), Se levels equal to 9.7+7.2μg/L in plasma and less than 35μg/L in whole blood are considered severe deficiency in dairy cattle. Lucci et al. (1984) stated that concentrations of 60μg/L represent average levels, and concentrations above 60μg/L represent good levels of Se in dairy cattle serum. This information corroborates the data described by Arshad et al. (2021), who reported adequate Se levels above 180μg/L in whole blood and 80μg/L in plasma for cattle. Thus, when analyzing Se concentrations in whole blood, there was no significant difference (P>0.05) among the treatments. The same was observed when the concentrations of the elements were analyzed throughout each treatment (Table 6). However, when analyzing Se concentrations in the blood serum, a significant difference was found between Treatment groups 1 and 2 at M8, with a higher Se concentration in Treatment group 1. However, when evaluating Se concentrations throughout each treatment, there were no significant differences (P>0.05) between any of the treatments (Table 6).

The results of this study indicate a Se peak at M8 in Treatment group 1, which was supplemented with a dose of 0.05mg/kg of Se. In the same group, as previously described, there was an improvement in the clinical picture of hematuria after three weeks of supplementation, in addition to an improvement in anemia with an increase in hematocrit at M8. Therefore, it is believed that the gradual progression of Se concentration in the serum until its peak at M8, using a dose of 0.05mg/kg, could improve hematuria and reverse anemia in animals.

Latorre et al. (2013) found that ptaquiloside is responsible for the immunosuppression of animal NK cells, which facilitates the emergence of neoplasms. The same authors verified that the immunosuppressive effects of ptaquiloside on NK cells were indirectly linked to the increased expression of metallothionein through the Mt1 and Mt2 genes, which are proteins responsible for regulating intracellular zinc. Zinc ions are essential for the correct functioning of the immune system, and a decrease in their intracellular levels is associated with the impaired activity of immune system cells, including NK cells. When mice were supplemented with Se, they found that Se inhibited the increased expression of metallothionein, in addition to increasing free zinc in NK cells. Selenium compounds act as oxidants and react quickly with metallothionein clusters, releasing Zn ions into the cellular environment. These mechanisms explain what was observed in the present study: significant clinical improvements were observed in the clinical changes caused by *Pteridium* sp. poisoning as serum Se levels increased in cattle.

Notably, a previous study sought to compare the highest level of Se with increased activity of the enzyme glutathione peroxidase in the dairy cattle serum supplemented with different doses of Se and vitamin E but found no significant difference (Oliveira 2021). However, Cardozo et al. (2013) demonstrated that dairy cows supplemented with 5mg/100 kg sodium selenite showed an increase in glutathione peroxidase activity from 30 to 90 days after Se administration. A noteworthy finding of this study was that the element concentrations in the serum were higher than those in the whole blood. Therefore, the serum is the best place to measure Se levels in dairy cattle affected by BEH supplemented with increasing doses of Se and vitamin E. However, Herdt et al. (2000) highlighted that when working with serum, hemolysis must be avoided, as its presence can directly influence the results.

According to Anjos et al. (2008), the concentration of Se in whole blood is the sum of the microelements present in erythrocytes and serum, with 60% of the Se found in erythrocytes. It should be noted that the present study was carried out on animals with chronic fern poisoning, which presented with hematuria and, consequently, anemia. This indicates that anemia in these animals was due to the loss of erythrocytes. A decrease in erythrocytes may explain the lower concentration of Se in the whole blood of these animals. Therefore, our study suggests that serum is the best place to measure Se.

Relative monoamine oxidase activity

There was no significant difference (P>0.05) among the different treatments regarding the relative activity of MAO. However, a significant difference (P<0.05) was noted in the time effect for all treatments. In the Control group, the moments M2 and M10, M2 and M12, and M2 and M14 differed significantly, with greater MAO expression occurring at M2 than in the other groups. In Treatment group 1, there was a significant difference between M2 and M12 and M2 and M14, with greater enzyme activity at M2. In Treatment group 2, a difference was observed between M2 and M14, with greater enzyme activity

Table 6. Selenium concentrations in blood and total serum of cattle with bovine enzootic hematuria (BEH) supplemented with increasing doses of selenium (Se) associated with vitamin E at different experimental times

Time	Selenium blood concentrations (μg/L)							
	Control		Treatment 1		Treatment 2		Treatment 3	
	Whole blood	Serum blood	Whole blood	Serum blood	Whole blood	Serum blood	Whole blood	Serum blood
M4	181.6 ^{Aa}	250.7 ^{Aa}	186.7 ^{Aa}	260.9 ^{Aa}	136.0 ^{Aa}	229.5 ^{Aa}	258.9 ^{Aa}	188.4 ^{Aa}
	(156.4|-|225.9)	(182.1|-|297.9)	(170.8|-|199.2)	(151.1|-|344.7)	(127.0|-|207.4)	(165.3|-|408.7)	(128.2|-|259.7)	(101.8|-|357.9)
M8	168.6 ^{Aa}	219.2 ^{Aa}	175.9 ^{Aa}	337.5 ^{AaB}	203.4 ^{Aa}	192.0 ^{AaC}	164.3 ^{Aa}	145.9 ^{Aa}
	(155.4|-|199.9)	(186.4|-|237.8)	(147.1|-|216.7)	(273.1|-|586.9)	(174.8|-|273.5)	(107.6|-|213.5)	(148.2|-|353.9)	(108.5|-|316.9)
M14	284.1 ^{Aa}	158.9 ^{Aa}	235.3 ^{Aa}	176.1 ^{Aa}	200.1 ^{Aa}	182.3 ^{Aa}	234.0 ^{Aa}	140.5 ^{Aa}
	(248.8|-|324.4)	(87.51|-|225.1)	(194.0|-|307.0)	(99.99|-|250.5)	(193.5|-|201.7)	(130.8|-|219.6)	(213.4|-|256.5)	(98.12|-|230.5)

Medians (1st quartile |-| 3rd quartile); $^{A-C}$ Medians followed by the same capital letter in the line do not differ from each other using the Kruskal-Wallis test, ^{a} Medians followed by the same lowercase letter in the column do not differ from each other, according to the Friedman test; Both tests with a significance level of 5%; Control (physiological solution), Treatment 1 (0.05mg/kg Se), Treatment 2 (0.10mg/kg Se); Treatment 3 (0.20mg/kg Se), associated with the administration of 500mg/animal in all groups.

Table 7. Relative activity of the serum monoamine oxidase (MAO) enzyme in cattle with bovine enzootic hematuria (BEH) supplemented with increasing doses of selenium (Se) associated with vitamin E at different experimental times

	Relative activity of MAO			
Time	Control	Treatment 1	Treatment 2	Treatment 3
M0	0.475 [Aa] (0.267|-|0.857)	0.535 [Aa] (0.330|-|0.762)	0.655 [Aa] (0.292|-|0.890)	0.770 [Aal] (0.630|-|1.000)
M2	0.475 [Aab] (0.267|-|0.857)	0.535 [Aaf] (0.330 |-|0.762)	0.655 [Aai] (0.292|-|0.890)	0.770 [Aam] (0.630|-|1.000)
M4	0.620 [Aa] (0.565|-|0.665)	0.635 [Aa] (0.600|-|0.655)	0.650 [Aa] (0.575|-|1.018)	0.540 [Aa] (0.450|-|0.71)
M6	0.435 [Aa] (0.395|-|0.460)	0.465 [Aa] (0.435|-|0.480)	0.405 [Aa] (0.392|-|0.462)	0.380 [Aa] (0.380|-|0.520)
M8	0.440 [Aa] (0.390|-|0.460)	0.455 [Aa] (0.422|-|0.532)	0.450 [Aa] (0.440|-|0.542)	0.440 [Aa] (0.410|-|0.460)
M10	0.370 [Aac] (0.325|-|0.422)	0.420 [Aa] (0.395|-|0.437)	0.375 [Aa] (0.352|-|0.397)	0.350 [Aa] (0.290|-|0.410)
M12	0.335 [Aad] (0.312|-|0.347)	0.335 [Aag] (0.270|-|0.340)	0.330 [Aa] (0.297|-|0.340)	0.340 [Aan] (0.300|-|0.350)
M14	0.325 [Aae] (0.295|-|0.362)	0.300 [Aah] (0.295|-|0.315)	0.300 [Aaj] (0.290|-|0.317)	0.340 [Aa] (0.310|-|0.420)

Medians (1st quartile |-| 3rd quartile); [A] Medians followed by the same capital letter in the line do not differ from each other using the Kruskal-Wallis test, [a-n] Medians followed by the same lowercase letter in the column do not differ from each other, according to the Friedman test; Both the tests were performed at a significance level of 5%; Control (physiological solution), Treatment 1 (0.05mg/kg Se), Treatment 2 (0.10mg/kg Se), Treatment 3 (0.20mg/kg Se), associated with the administration of 500mg/animal in all groups.

in M2. In Treatment group 3, the difference was between M0 and M12 and M2 and M12, with the highest enzyme activity occurring at M0 and M2. It was also observed that enzymatic activity was similar throughout each treatment, including the Control group. These strains initially showed high MAO activity, and a significant reduction in enzymatic activity was observed at the end of the supplementation period (Table 7). In this study, all groups received the same dose of vitamin E (500mg/kg) and different Se doses. Therefore, the fact that the enzymatic behavior of the Control group was similar to that of the other groups suggests that vitamin E associated (or not) with different doses of Se was able to reduce enzymatic activity after the ninth week of supplementation.

The reduction in MAO activity after administration of vitamin E was also verified by Xu et al. (2003), who evaluated the effect of vitamin E supplementation on memory and brain monoaminergic neurotransmitter levels in rats with chronic episodic hypoxia. These authors observed an increase in the monoamines norepinephrine, dopamine, and 5-hydroxytryptamine levels in different brain regions, indicating an inhibitory action of vitamin E on MAO activity. Similarly, Tang et al. (2008) supplemented rats with inorganic (sodium selenite) and organic (selenium-enriched yeast) forms orally at a dose of 2mg/kg of diet and observed a significant reduction in the activity of the MAO-B isoenzyme in the brains of rats and concluded that supplementation with Se could reduce the activity of the brain MAO-B enzyme owing to the antioxidant capacity of Se. However, there have been no studies on the relative activity of MAO and its association with the action of vitamin E and Se in animals poisoned by ferns. Considering that ptaquiloside has a cytotoxic action on different tissues, it is believed that poisoning in cattle by this toxic principle would lead to an increase in the relative activity of MAO, which would lead to the excess production of free radicals. Thus, it is understood that if supplementation with Se and vitamin E has an immunomodulatory effect, it could reduce the cytotoxic activity of ptaquiloside, in addition to reducing the relative activity of MAO. Furthermore, it is assumed that animals chronically poisoned by ferns and treated with Se and vitamin E have low relative MAO activity. A noteworthy finding of this study was that MAO may be an important indicator of the severe cytotoxicity caused by ptaquiloside. Based on this finding, supplementation with Se and vitamin E could be indicated.

CONCLUSIONS

Parenteral selenium supplementation at a dose of 0.05mg/kg, associated with vitamin E at a dose of 500mg/kg in cattle with bovine enzootic hematuria (BEH), improves the clinical picture of hematuria and anemia after three and seven weeks of supplementation, respectively, but did not interfere with glutathione peroxidase activity and total antioxidant levels.

Monoamine oxidase activity is reduced by vitamin E supplementation.

Furthermore, serum was the best option for measuring selenium levels, increasing the concentration in the eighth week of supplementation.

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