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INFLUENCE OF ORGANIC SELENIUM ON RABIES HUMORAL IMMUNE RESPONSE OF EWES

(*Influência do selênio orgânico na resposta imune humoral antirrábica de ovelhas*)

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ABSTRACT - The objective was to evaluate the effectiveness of mineral supplementation with organic selenium (Se) on rabies humoral immune response in ewes. It was randomly selected 18 Texel ewes without antirabic vaccination in the last 12 months and divided into 2 groups: treated group (TG) supplemented with a mineral mixture additioned of organic selenium and immunized against rabies and a control group (CG) supplemented with mineral mixture without organic selenium addition and immunized. Determination of Se serum was obtained by the enzyme glutathione peroxidase (GSH-Px) activity in two moments, the title and the persistence of anti-rabies neutralizing antibodies in five times by the Rapid Fluorescent Focus Inhibition Test (RFFIT). The mean values found in D210 GSH-Px were higher in TG with 981.8 ± 105.2 U/g Hb and 628.2 ± 188.2 U/g Hb in the CG (p-value < 0.001). The mean values of titers of anti-rabies neutralizing antibodies 30 days after primary vaccination, demonstrated statistically similar titles in the GT and GC, 1,020 IU/ mL in GT and 1,885 IU/ mL CG. Oral mineral supplementation of organic selenium for 210 days did not enhance the humoral immune response and persistence of evidence of rabies neutralizing antibodies, however caused increased serum retention of selenium in ewes.

Key words: Immunity curve; rabies; persistence antibodies; vaccination; mineral supplement.

RESUMO - O objetivo foi avaliar a eficácia da suplementação mineral com selênio (Se) orgânico na resposta imune humoral antirrábica em ovinos. Foram selecionadas, aleatoriamente, 18 ovelhas da raça Texel sem vacinação antirrábica nos últimos 12 meses e divididas em 2 grupos: grupo tratado (GT) suplementado com mistura mineral adicionada de selênio orgânico e vacinados contra raiva e grupo controle (GC)

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suplementado com a mistura mineral sem adição de selênio orgânico e vacinados. A determinação de Se sérico foi obtida através da atividade da enzima glutationaperoxidase (GSH-Px) em dois momentos, e a persistência do título de anticorpos neutralizantes anti-rábicos em cinco momentos pelo Rapid Fluorescent Focus Inhibition Test (RFFIT). Os valores médios encontrados no D210 de GSH-Px foram superiores no grupo GT com 981,8±105,2 U/g Hb e de 628,2±188,2 U/g Hb no GC (valor de p<0,001). Os valores médios encontrados de títulos de anticorpos neutralizantes anti-rábicos 30 dias após a primovacinação, demonstraram títulos similares estatisticamente no GT e GC, 1.020 UI/mL no GT e de 1.885 UI/mL no GC. A suplementação mineral oral de selênio orgânico durante 210 dias não incrementou a resposta imune humoral e a persistência do título de anticorpos neutralizantes antirrábicos, entretanto promoveu aumento da retenção sérica de selênio em ovelhas.

Palavras-chave - Curva de imunidade; raiva; persistência de anticorpos; vacinação; suplemento mineral.

INTRODUCTION

In studies on strategies to increase the organism's defense mechanisms, it has been highlighted the effects of nutrition on the immune response, and among the most studied nutrients are the vitamins and minerals (Santos and Fonseca, 2006).

The selenium (Se) is a micronutrient that is present in the body tissues, essential for the effective functioning of the immune system (Arthur et al., 2003; Paschoal et al., 2006; Goldson et al., 2011), also operates functions on growth, reproduction, prevention of diseases and tissues integrity (Medeiros et al., 2012). Trace minerals are required for immune system integrity, vitamin synthesis, enzyme formation and hormone structure (Rabiee et al., 2010).

Selenium participates in the production of antibodies (Murphy et al., 2006; Carroll and Fosberg, 2007) and as consequence, it raises the immune response (Hintze et al., 2002; Paschoal et al., 2003), protecting leukocytes and macrophages from free radicals formed during phagocytosis of pathogens (Carvalho et al., 2003; Reis, 2008).

Constituted by Se, the glutathione peroxidase (GSH-Px) is an enzyme that acts to protect the cells against the attack of free radicals in level of cytoplasm (Funari Jr, 2008; Reis, 2008). Approximately 75 to 85% of the Se in the ewe's erythrocytes is associated with this enzyme, showing a high correlation between serum selenium levels and the activity of GSH-Px in sheep, so the GSH-Px has been used as the best indicator of metabolic status and the best criterion to define strategies of supplementation with Se (Gierus, 2007; Gromadzinska et al., 2008).

Brazil´s soils are extremely deficient in macro and micro minerals (Carvalho et al., 2003) and grains grown in these soils have low concentration of micro minerals that are essentials for animal health and production (Barbosa, 2009). The concentration of selenium in forages and grains is low, and its ingestion through the natural content of plants and components of the diet is insufficient to reach the nutritional requirements of this element at any stage of animal production, due to that, there is a need for supplementing. Therefore, the supplementation with mineral mixtures containing selenium is essential (Gierus., 2007), once that this mineral supports the antibodies production (Murphy et al., 2006; Carroll and Fosberg, 2007), raising the immune response (Hintze et al., 2002; Paschoal et al., 2003).

Regarding different prophylaxis protocols, researchers have studied the effects of selenium, zinc, copper, and probiotics added in the mineral supplementation of flocks as an alternative to improve the immune response, increasing vaccines performance (Queiroz, et al., 2003; Albas et al., 2005; Arenas et al., 2009; Bun et al., 2011; Gruber et al., 2013; Nagalakshmi et al., 2015; Carneiro et al., 2018).

Queiroz et al. (2003) demonstrated the inefficiency of antirabic vaccines 30 days after administration, and Albas et al. (2005) suggested the application of three doses, with reinforcement at 30 and 180 days after the first dose for better immune response and vaccination coverage, as one dose of vaccine is insufficient to protect the animals against rabies. Arenas et al. (2009) observed a significant increase in the titers of anti-rabies antibodies in cattle supplemented with probiotics, while Carneiro et al. (2018) observed that sheep supplemented with organic zinc obtained better persistence of anti-rabies neutralizing antibodies.

The animal rabies is considered endemic in many regions of the world and stands by the economic losses in livestock farming, owing to their importance in public health, due to 100% of lethality and especially for being a zoonosis (WHO, 2010). Rabies is an infectious disease that affects the central nervous system of man and various species of domestic and wild mammals and due to its continental characteristics and its diversity of fauna, it is difficult to eradicate (Lima et al., 2017). Due to the importance of vaccination for anti-rabies prophylaxis, it has been necessary to find ways to increase the effectiveness of vaccination against rabies (Ferreira et al., 2009).

The objective of this study was to evaluate the effect of mineral supplementation with organic selenium in humoral immune response, the persistence of neutralizing anti-rabies antibodies in healthy sheep and to determine the serum concentration of the activity of selenoprotein GSH-Px.

MATERIAL AND METHODS

The experiment was carried out at property in the region of Londrina, Paraná, Brazil. Eighteen Texel ewes were selected, with an average age of 20 months and 35.9 ± 10.1 kg live weight, never vaccinated against rabies. The animals were randomly divided into 2 groups: 10 animals in the treated group (TG) supplemented with a commercial mineral mixture additioned of organic Se; and 8 animals in the control group (CG) receiving only mineral mixture without addition of organic selenium. The groups remained at 2 pastures of 4 ha, similar topography, containing *Brachiaria brizantha*, managed with continuous grazing, alternating the lots in the pastures every 30 days, offering equal conditions of exposure to both groups.

The animals of both groups went through an initial period of mineral supplementation during 90 days, before starting the anti-rabies vaccination protocol, receiving mineralization of commercial salt in identical formulation containing Se in the inorganic form (Sodium Selenite) already contained in the formulation of commercial salt, in the dose of 25 mg/kg of the product, whereas only the TG received the additional source of organic Se (Sel-Plex®- Alltech in Brazil) at the dose of 0.66 mg/head/day orally, followed by an experimental period of 120 days simultaneously with the vaccination protocol, totalizing two hundred and ten days of Se supplementation. The mineral supplementation was provided daily, in collective feedlots for each group into the sheep pen, and added a palatalizing agent, citrus pulp, stimulating the animals' consumption. At the beginning of this experiment all animals were identified, weighed, dewormed with nitroxinil, and monitored fortnightly by the Famacha® method for control of nematode parasites until the end of the experiment.

At the first day of supplementation (D.0), all animals were subjected to the jugular venipuncture for blood collection in heparinized tubes for indirect determination of Se concentration through the enzyme glutathione peroxidase (GSH-Px), being performed new collection at the end of the experimental period (D.210). The heparinized blood was centrifuged for 10 minutes at 450 g, in refrigerated centrifuge for the plasma obtention. The red blood cells were washed with PBS for three cycles for obtaining the concentrate of red cells, which were stored in Eppendorfs amber color, and kept frozen for a maximum period of 30 days at -80º C for later determination of activity of GSH-Px, performed through the method of colorimetric determination, according to the commercial kit from Randox® brand (Ransel, Code RS 505), being used the concentrate of red cells of erythrocytes in the dilution of 200 µl (Paglia and Valentine, 1967; Prohaska et al., 1977)

After 90 days (D.90) of selenium supplementation, the animals were subjected to vaccination against rabies, inactivated commercial vaccine, at a dose of 2 ml subcutaneously as indicated by the manufacturer, and received a booster dose 30 days after the primary vaccination (D.120), in compliance to the recommendations of the Ministry of Agriculture, Livestock, and Supply (BRASIL, 2009).

Blood samples were collected with intervals of 30 days (D.90, D.120, D.150, D.180, D.210) for obtaining blood serum and determination of the curve of persistence of neutralizing antibodies anti-rabies titers, through the technique of serum-neutralization in BHK cells21 (RFFIT), according to Smith et al. (1996), being the methodology validated in the Instituto Butantan (MOURA et al., 2008). These samples were collected into dry tubes and the aliquots of blood serum, obtained after centrifugation at 160 g for 10 minutes, packed in containers Eppendorf type and kept frozen at -20º C until later sent to the Instituto Butantan.

At the beginning of the experiment period (D.0) and at the end of the experiment (D.210), samples were collected from the pastures, using the simulated grazing method - hand plucked (DE VRIES, 1995) (approximately 40 cm of height), and forwarded to the bromatological analysis for the determination of the quantity of Se total received by the groups, with a detection limit of 0.050 mg/L. Fluxogram of the experiment is shown in Figure 1.

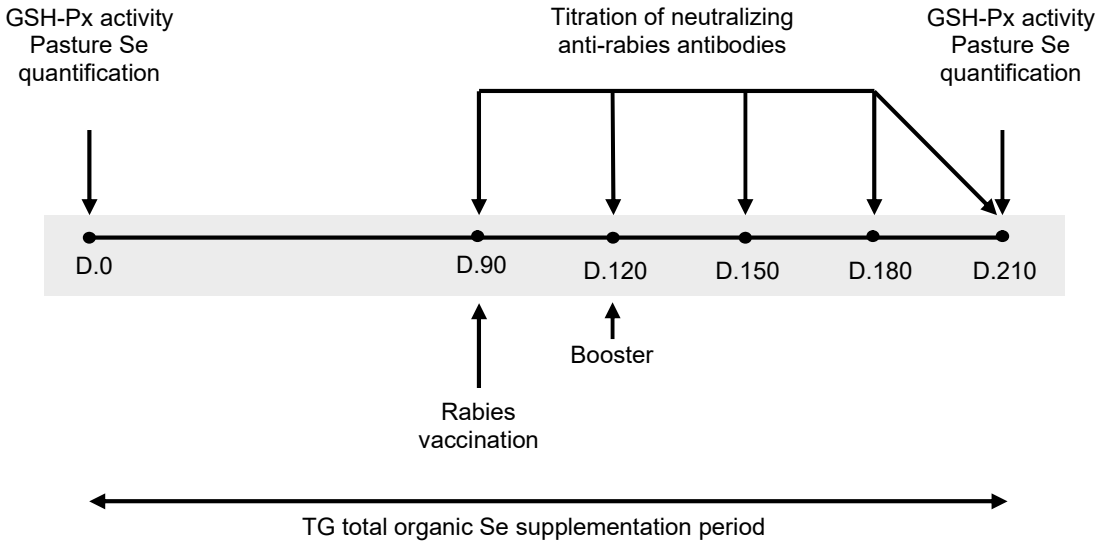

Figure 1 – Fluxogram of the experiment on the procedures applied to the GC and GT.

The experiment was carried out respecting the rules of the Ethics in the use of animals in research (approved by the Committee of Ethics for animal use: CEA/UNOPAR

n° 017/12). For statistical analysis of data statistical package Minitab 13.0 was used and the quantitative data by means of analysis of variance with minimum level of significance of 5%, the averages were compared by the Tukey test, the comparison of different moments were evaluated by paired t test.

RESULTS

The samples of *Brachiaria brizantha* pasture subjected to bromatological analysis for the determination of total quantity of Se received by groups indicated the absence of the element, therefore the amount of Se consumed was 0.00 mg/kg DM in forage. The consumption of mineral mixture was 22 g/head/day in both groups (TG /CG), totalizing the consumption of inorganic Se (60% of absorption) of 0,33 mg/head/day. In the CG the basal intake (pasture + mineral mixture) of inorganic Se (Sodium Selenite) totalized 0,33 mg/head/day. In the TG the consumption of an additional organic Se, in the dose of 0,66 mg, totalized a consumption of 0,66 mg/head/ day of organic Se and 0,33 mg/head/day of inorganic Se from the basal diet.

The Indirect determination of serum selenium concentration was performed through the selenoprotein glutathione peroxidase (GSH-Px). The results of the mean values of serum GSH-Px found at D.0 and at the D.210 of the experiment are shown in Tab. 1. The mean GSH-Px values found in D.210 were higher in TG with 981.8 ± 105.2 U/g Hb comparing to CG with 628.2 ± 188.2 U/g Hb, (p-value<0.001).

Table 1 - Average values of serum glutathione peroxidase (GSH-Px) in sheep vaccinated against rabies with two doses and supplemented with selenium.

GROUPS	**GSH-Px (U / g Hb)**	
	First adaptation day	D.210
CG	616,3 ± 15,7^{aA}	628,2 ± 188,2^{aA}
TG	655,9 ± 172,4^{aA}	981,8 ± 105,2^{bB}
p-value	0,243	0,000

Lower-case letters at the same column indicate no significant difference (p<0.05).
Capital letters at the same row indicate no significant difference (p<0.05).

The results of the titers of neutralizing antibodies anti-rabies are shown in Tab. 2. The means of titers of neutralizing antibodies anti-rabies obtained in D.90 were 0,048 IU/mL in GT and 0,039 IU/mL in CG (Tab. 2), showing that the animals had no anti-rabies antibodies before vaccination, demonstrating that there was no antigenic stimulation prior to the experiment. The World Health Organization considers as an indicator for evaluating the efficacy of anti-rabies vaccine and immune response, titer equal to or above 0.5 IU/mL (WHO, 1992).

Table 2 – Average titers of anti-rabies neutralizing antibodies (IU/mL) determined by serum-neutralization in BHK cells21 (RFFIT) in the D.90, D.120, D.150, D.180, D.210.

GROUPS	**Neutralizing antibodies titers (IU mL^{-1}) 1**				
	D.90	D.120	D.150	D.180	D.210
CG	0,039^{aA}	1.885^{aB}	7,763^{aC}	1,350^{aB}	0,775^{aB}
TG	0.048^{aA}	1.020^{aB}	5,070^{aC}	1,440^{aB}	0,720^{aB}
p-value	0,203	0,269	0,289	0,839	0,846

Lower-case letters at the same column indicate no significant difference (p<0.05).
Capital letters at the same row indicate no significant difference (p < 0.05).
Days of experiment: D.90 = before vaccination; D.120 = 30 days after the first vaccine dose; D.150 = 30 day after the booster. D.180 = 60 day after the booster. D.210 = 90 day after the booster.

The mean values found in titers of neutralizing antibodies anti-rabies 30 days after the first vaccination (D.120), demonstrated statistically similar titles in GT and CG, 1.020 IU/ml in GT and 1.885 IU/ml in GC, where the two groups remain initially protected against rabies in the first dose of vaccine, with titers higher than those recommended by WHO (1992). However, the boost dose applied 30 days after the first vaccination potentiated the humoral immune response, significantly raising the levels of antibodies 60 days (D.150) after the first vaccination, in both groups.

DISCUSSION

It needs to be clear that the rabies vaccine was used only as a model to ascertain the effect of organic Se supplementation in the interface of nutrition and effectiveness of rabies vaccine. This model is advantageous in healthy sheep that have never been in contact with the virus, since contact with virus in animals that have not been previously vaccinated will necessarily cause clinical manifestation (Daher, 2005).

Research demonstrate that Brazilian soils are extremely deficient in macro and micro minerals (Carvalho et al., 2003) and forage plants and grains grown in these soils have low concentration in essential micro minerals for animal production (Barbosa, 2009). In Brazil, selenium is not easily available to animals reared in grazing regime, since most of our soils are highly leached and with low fat content of chelated material, Se is lost easily (Carvalho et al., 2003). Thus, the absence of the element Se found in the bromatological analysis is consistent with the observations of the national literature.

The results concerning the amount of Se in the diet, considering the estimated DM intake of 3% of live weight (LW) and an absorption of only 60% (NRC, 2007) in pasture of *Brachiaria brizantha*, resulted in a final amount of Se consumed in the forage plants below the recommendation (NRC, 2007) for sheep, which is 0.3 mg/kg DM per day. These data show the necessity of mineral supplementation of the component Se in this study. Gierus (2007) in an extensive review of the literature pointed out that the concentration of selenium in forages and concentrates is low, and its ingestion through the natural content of plants and components of the diet is insufficient to reach the nutritional requirements of this element at any stage and is categorical affirming that a supplementation with mineral mixtures containing selenium is indispensable.

As described by Pugh (2005) the consumption of mineral mixture for sheep is estimated between 10 to 28 g/animal/day, therefore, the final consumption in both groups remained within the expected range. Considering the supplementation provided in mineral mixture, the average final intake of Se (pasture+ mineral mixture) in the control group and in the treated group were within the recommendation of the NRC (2007), not exceeding the maximum limit of 0.7 mg/animal/day.

Although the need of Se in the diet of sheep has been known for decades, the chemical source and dosage for an excellent productive health remain obscure (Hall et al., 2011). The bioavailability of Se is not linear, because there are large variations in the Se content in foods, determined by a combination of geographical conditions and environmental factors, and chemical forms that can be absorbed and metabolized (Fairweather-Tait et al. 2010). Usually, Se is present in organic form, as selenomethionine (SeMet) or selenocistein (SeCys), and in the inorganic form as sodium selenate (Uden et al., 2004).

The GSH-Px has been widely used for studying the response to supplementation of Se to the animals (Zachara, 1992; Arthur and Becket, 1994), being the best indicator of metabolic status and the best criterion to define strategies of supplementation with this mineral (Gierus, 2007; Gromadzinska et al., 2008). Therefore, in the present study it was used the determination of the activity of selenoprotein GSH-Px as the criterion of serum Se evaluation.

The results of the concentration of GSH-Px, both in GT and the GC, are within the reference values (Jaramillo et al., 2005), considered desirable above 130 U/g Hb. Thus, the TG presented a significant increase, greater than 50%, the concentration of GSH-Px in relation to the CG (Tab. 1), indicating the increase of absorption of Se, supplied through organic supplementation to TG in the two hundred and ten days of the experiment.

Steen et al. (2008) working with supplementation of organic and inorganic selenium in ewes and newborn lambs, found serum concentrations of selenium, significantly elevated in animals that received organic selenium. In a study by Calamari et al. (2009) was observed a positive correlation between the concentration of plasmatic Se and the activity of GSH-Px in horses supplemented with Se-yeast. In general, organic forms are absorbed and retained by ruminants more readily than the inorganic forms (Qin et al., 2007).

Knowles and collaborators (1999) also found an increase in serum concentrations of GSH-Px in dairy cows supplemented with Se when compared to animals without suplementation, however, didn´t found effect of the source of the glutathiones. Small difference in the activity of GSH-Px was observed in sheep, when sources of organic and inorganic Se were compared (Van Ryssen et al., 1989). Paiva (2006) affirms that the responses of concentration in serum and GSH-Px in the liver of lambs were similar in the supplementation with organic and inorganic Se, unlike the results of this research where the Se-yeast was effective in increasing the activity of GSH-Px.

Most of the studies show an increase in the activity of glutathione peroxidase in animals supplemented with sources of selenium, mainly with sources of organic selenium (Qin et al., 2007; Steen et al., 2008; Calamari et al., 2009; Hall et al., 2011). However, it is necessary to consider the time of Se supplementation, because for a significantly increased activity there is a need supplementation from 5 to 7 weeks (Knight and Tyznik, 1990). The refitting period of the enzyme GSH-Px in most cells is around 105 days. Part of Se, present in the unactivated enzyme, can be recycled within the organism (Figueira, 2009).

In this study, the supplementation of Se organic was held for a period of 210 days, beginning 90 days before vaccination, precisely to ensure enough Se in the blood. This fact probably explains the high concentrations of GSH-Px in the group GT with 981.8 ± 105.2 U/g Hb and 628.2 ± 188.2 U/g Hb in CG ($p<0.001$) demonstrating the effective incorporation of the Se mineral. Jaramillo and collaborators (2005) working with heifers obtained average concentrations of GSH-Px of 389 ± 184 U/g Hb, and in some animal values of 936 U/g Hb. Paiva (2006) found an average of 350 U/g Hb in the liver of lambs supplemented with Se.

Particularly, trace elements are related to cells mediators of the humoral immunity and non-specific immune response, as in the function of T-B cells, activity of NK cells and the release of cytokines (Marcos et al., 2003). In this way, during the immune response, the level of minerals such as selenium (Se), zinc (Zn) and copper (Cu) in the blood

decreases dramatically and, on the other hand, the absorption is increased and, consequently, in these situations, the dietary requirement of these minerals can be larger (Ribeiro et al., 2008). It was expected that the greatest increase of the activity of GSH-Px, and the largest contribution of Se serum is evidenced in the results of this study, promoting an anti-rabies humoral immune response vaccine more effective.

The selenium plays an important role for the optimal functioning of the immune system, although the exact mechanism through which this mineral interacts with the immune system remains unknown (Brummer, 2012). In studies with mice, the status of Se affected the RNAm expression of certain cytokines modulators of the immune system (Li; Beck, 2007) and interleukin 2 receptors (Roy et al., 1994). The change in the proliferation of lymphocytes has also been associated with the status of Se (Roy et al., 1994). Some authors have demonstrated that both the cellular immune response as the humoral, are incremented in animals which receive supplements of Selenium (Nemec et al., 1990; Bires et al., 1993; Morgante et al., 1996). Other studies show that Se participates in the production of antibodies (Murphy et al., 2006; Carroll; Fosberg, 2007) and therefore raises the immune response (Hintze et al., 2002; Paschoal et al., 2003), in addition to protecting leukocytes and macrophages from free radicals formed during phagocytosis of pathogenic agents (Carvalho et al. 2003; Reis, 2008).

None of the animals used in this study showed titers of antibodies in serum before the beginning of vaccinations, demonstrating that there was no antigenic stimulus preceding the experiment. When analyzing the results of the titers of neutralizing antibodies anti-rabies in Tab. 2, we observed that there was no statistically significant difference between the TG and CG, showing that the supplementation with different sources of selenium did not influence the humoral immune response to anti-rabies vaccination.

In contrast, Carneiro (2018) working with immunity against rabies in sheep supplemented or not with organic zinc reported that, in the first dose of the vaccine only 87.5% of the animals in the control group were with titles considered protective, compared with the animals of the group supplemented with organic zinc where 100% of animals already had protective titles only with the first dose of the vaccine, thus demonstrating an increase in the humoral immune response in sheep supplemented. The humoral immune response requires proliferation of lymphocytes during several days prior to contribute significantly to the protection of the host (Fernandes et al., 2013), thus, the micro mineral Se seems not to have the same effectiveness in the proliferation of lymphocytes that the Zn.

The results in Tab. 2 shows that in D.150, D.180 and D.210, the curve of persistence of anti-rabies neutralizing antibodies, presents a sharp decline from thirty days after the booster dose (D.150), and observing the individual values of each animal in the experiment in D.210, 40% of the treated group showed evidence of less than 0.5 IU/mL and 50% in the control group, leaving the animals exposed to infections. The lack of the immune response persistence set in Table 2 weakens the control measures, as the recommendation of MAPA (Brazil, 2009) is revaccinating animals annually in endemic areas and in those areas of epidemic proportions the mandatory vaccination should be carried out in 6 to 6 months. Therefore, when continuing the physiological decline of persistence observed in Tab. 2, from 150 days, probably most sheep vaccinated would be unprotected with titers lower than 0.5 IU/mL, indicating the need for revaccination.

The humoral system is also affected by the deficiency of Se (Arthur et al., 2003), both the adaptive and innate immunity are compromised in individuals with disabilities of this mineral, and changes in the ingestion of Se affects the immune response to different etiologic agent, including antiviral immunity and response to vaccines (Verma et al., 2011). Studies in production animals demonstrate the effects of deficiency of the immune response (Spalholz et al., 1990). In most cases, these studies found an increase of both immune responses mediated by humoral and cell, where the levels of Se intake increased. However, the results are highly informative about the benefit of supplementation above the appropriate levels to confer additional immune protection (Verma et al., 2011). The heterogenic titers of anti-rabies antibodies observed in this study may have occurred by the level of supplementation area near the appropriate maximum limit in the two groups.

Funari Jr. (2008) working with broilers concluded that selenium supplementation showed no effect on the humoral immunity in both organic and inorganic sources. The immune response is influenced by several factors such as stress, the supplementation of cholecalciferol and other nutrients. Supplementation with organic and inorganic Se proposed in this study may not have been sufficient to increase a humoral response and stressful factors as the successive attacks by predators (dogs) during the experimental period, may have caused the release of cortisol and justify the absence of a humoral immune response more effective and persistent in GT.

Under stress conditions, as happens during sheepherd management situations, there may have an increase in the plasma concentration of glucocorticoids, especially cortisol (Hopster et al., 1999). The increase in serum cortisol concentration as a result of stress is associated with reduced lymphocyte response to mitogen stimulation (Blecha and Minocha, 1983). Cortisol is an inhibitor of antibody production, phagocytosis,

lymphocyte-activating factors, and T-cell maturation (Khansari et al., 1990), interfering with the immune response.

Several authors have studied the immune response in cattle and observed that the response was heterogeneous, with non- responsive animals, interspersed with animals that presented low, medium, and high values (Ciuchini et al., 1981; Albas et al., 1998; Tizard, 2002). It seems that, in relation to the Se and the sheep, the same phenomenon may have been presented, where animals after the booster dose responded with titers of anti-rabies neutralizing antibodies remarkably high, on the order of 16 IU/mL, others with median response with values from 5 to 6 IU/mL and some with minimal responses with values of 1 IU/mL.

Another highlighting factor is that the anti-rabies immunoprophylaxis proved to be an efficient immune experimental model, due to its characteristic of high mortality of rabies, decreases the possibility of new antigenic stimuli, thus facilitating the research that uses a longer experimental period, such as minerals traces, which require several months of supplementation for the desired effect.

CONCLUSIONS

Oral mineral supplementation of organic selenium did not enhance the humoral immune response and persistence of anti-rabies neutralizing antibody, however caused increased serum retention of selenium in ewes.

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