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Short communication

Dose of selenium in goat kids and its effect on the antigenic response to *Mannheimia haemolytica* and oxidative stress

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ABSTRACT

Selenium (Se) prevents oxidative damage and stimulates the immune system. Currently, there are no data available evaluating the Se-induced antigenic response to *Mannheimia haemolytica* and oxidative stress in goat kids. Twenty-one 6-month-old male Alpine Goat kids (22.9 kg) were immunized against *M. haemolytica* and divided into 3 groups: Basal diet with no additional Se (CG); Se injected subcutaneously at 0.25 mg Se/kg live weight (LW) (SeSG); and Se administered as intraruminal bolus at 0.46 mg Se/kg LW (SeBG). Blood samples were taken from the animals in all groups at 0, 14, 28, 42 and 56 days post-dosing. Erythrocyte Se levels doubled in SeSG during days 14–28 post-dosing (130.6 ng/g Se) as compared to CG. During days 28–42 post-dosing, Se levels decreased ($P < 0.05$) in SeSG (106.9 ng/g Se) *vs.* CG and SeBG (86.2 ng/g Se and 81.7 ng/g Se, respectively). Glutathione (GSH) levels decreased in days 0–28 post-dosing (3.7E–4 nmols/mg of total protein) and then, remained stable up to day 56 (5.6E–4 nmols/mg of total protein). Catalase levels in the SeBG and SeSG (79.9 U) were higher ($P < 0.05$) than in the CG (56.3 U) by day 14 post-dosing. Malondialdehyde (MDA) levels on day 14 post-dosing increased 7-fold in the SeBG (1.28E–3 ng/mg of total protein). IgG levels increased in the groups treated with Se during days 28–42 post-dosing (1.4 nM) *vs.* the CG (0.10 nM) ($P < 0.05$). Treatment with Se improved the immunological response to *M. haemolytica*.

1. Introduction

Infectious diseases and dietary deficiencies are two critical factors causing great losses in goat herds from birth to puberty (Ramírez-Bribiesca et al., 2001). The reported etiologies of pneumonias in goats are *Mycoplasma ovipneumoniae, Pasteurella multocida, Klebseilla pneumoniae, Staphylococcus aureus, Shigella* spp., *E. coli* (Saleh and Allam, 2014), and *M. haemolytica*, which can cause mortality rates in goat kids from 30 to 60% (Mohamed and Abdelsalam, 2008). Specifically, *M. haemolytica* has been commonly isolated from samples of ruminants with pneumonia in Mexico (Jaramillo-Arango et al., 2009). Consequently, it is necessary to induce a good antigenic response to this microorganism. Selenium (Se) supplementation can stimulate humoral immunity (Hall et al., 2013) through the increase in immunoglobulin G (IgG) levels in bovines immunized against pasteurellosis (Rice et al., 2007). Nevertheless, Se deficiency has been observed in a large part of the Mexican territory, especially in the Plateau region, which can cause health problems in deficient animals besides the white muscle disease (Ramírez-Bribiesca et al., 2005).

Infection by several respiratory pathogens increases production of reactive oxygen species (ROS), damaging cell membranes (Khanal and Knight, 2010). An imbalance between oxidants and antioxidants in favor of the former is called “oxidative stress”. There are numerous lines of defense against ROS, including low molecular weight radicals/oxidants such as glutathione (GSH), superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) (Rahmanto and Davies, 2012). Oxidative stress and damage to cell membranes produces malondialdehyde (MDA) as degradation product (Grotto et al., 2009). The joint presence of respiratory disease and Se deficiency in the organism induces oxidative stress (Hefnawy and Tórtora-Pérez, 2010). The application of a subcutaneous dose of Se or administration of intraruminal boluses induces an increase in blood Se levels of neonatal ruminants

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after 20 days post-dosing (Ramírez-Bribiesca et al., 2005; Revilla-Vázquez et al., 2008). Currently, there are no published papers evaluating the antigenic response against *M. haemolytica* in goat kids and their association with Se levels. The aim of this study was to evaluate the antigenic response against *M. haemolytica* through the presence of IgG-specific antibodies and their relationship with oxidative stress markers after administering Se to goat kids as a subcutaneous dose or by oral bolus dosing.

2. Materials and methods

2.1. Standard of ethics and experimental welfare

The animals used for this study were handled under the rules of the Internal Committee for the Care and Use of Experimental Animals approved by the Cuautitlán-UNAM, under registration code CICUAE-FESC C15_03.

2.2. Reception, management, and distribution of the experimental groups

Twenty-one 6-month-old male Alpine Goat kids were selected from Cuautitlán Agricultural Farm. They were housed in 3 corrals (each one with an area of 7 m^2) with a bed of shavings. The animals were fed as a group with sweet alfalfa [93% dry matter (DM) and 17.12% crude protein (CP)] containing 1.11 μgSe/g of Se. Water was given *ad libitum*, and the animals were dewormed with Closantel (10 mg/kg LW). On day 15, all the animals were immunized against *M. haemolytica* by subcutaneously administering 2.5 mL of bacterin-toxoid (Toxo-Bac-INIFAP®, Mexico, 1×10^9 colony forming units/mL, containing the leukotoxin). On the day after the vaccination, all the goat kids were weighed on a digital scale (Torrey®) and sorted into 3 groups based on their body weight, with each group averaging 22.91 ± 4.15 kg. These experimental groups received the following treatments: 1) Control group (CG), did not receive Se supplementation; 2) Subcutaneous Se dose group (SeSG), 0.25 mg Se/kg LW (as $NaSeO_3$. VALNO, lot: 0347C7); and 3) Intrarruminal Se bolus group (SeBG), 0.46 mg Se/kg (as $NaSeO_3$). Both Se products were manufactured in the pharmacy laboratory of FESC-UNAM. Specifically, the Se bolus were prepared with a densifier (reduced iron), a binder (cutin), and a sliding agent (talc) (Revilla-Vázquez et al., 2008) to be degraded in a maximum time of 4 days. Blood samples were taken before the dosing and on day 14 pst-dosing for 4 periods. Blood extraction was performed by venipuncture of the external jugular vein using 20 G-X 38 mm needles and 6 mL Vacutainer® BD vacuum tubes with EDTA anticoagulant. The blood samples were centrifuged at 4000 rpm for 15′ to separate the plasma from the erythrocyte pack and were stored at −80 °C until their analysis.

2.3. Laboratory analysis

The hematological samples were analyzed with the following tests. *Se levels in the erythrocytes:* The samples were analyzed according the modified Capelo method (Capelo et al., 2006). *Reduced glutathione (GSH) levels in the erythrocytes:* GSH was determined according the modified Eyer method, sulfosalicylic (Eyer and Podhradský, 1986). *Catalase (CAT) activity in the erythrocytes:* A rapid test as described by Iwase and colleagues (Iwase et al., 2013) was used. *Measurement of malondialdehyde (MDA) levels:* It was conducted according to the modified methods previously described (Lykkesfeldt, 2001). *Concentration of immunoglobulin G (IgG) – ELISA test:* The IgG concentration was estimated according to the method described by Morales (Morales-Alvarez et al., 1993).

2.4. Statistical analysis

The treatments were assigned to each group using a 3-level completely randomized factorial design (CG, SeSG, and SeBG). The levels of Se, GSH, CAT, MDA and IgG against *Mannheimia haemolytica* were selected as the response variables. A comparison of means was performed with the Tukey test considering a significant statistical difference ($p < 0.05$).

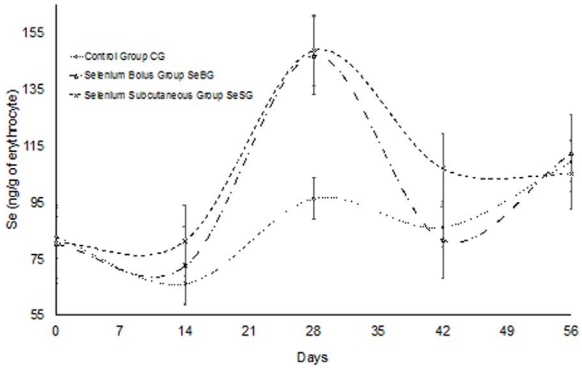

Fig. 1. Levels of erythrocyte selenium in goat kids with or without selenium supplementation.

3. Results

The data are presented as figures showing the means and their standard deviations in the 3 experimental groups. Fig. 1 shows the erythrocyte Se levels. On days 0–14 post-dosing, the average Se levels remained low in the 3 groups (81.12 ± 1.23 ng/g Se and 73.36 ± 7.65 ng/g Se, respectively; $P > 0.05$). During the days 14–28 post-dosing, the Se levels doubled ($P < 0.05$) in SeSG and SeBG (130.66 ± 29.77 ng/g Se), while the CG maintained an average Se level below 95 ng/g. During the days 28–42 period post-dosing, the levels of Se decreased ($P < 0.05$) between SeSG (106.93 ± 15.19 ng/g Se) *vs.* CG and SeBG (86.22 ± 12.37 ng/g Se and 81.69 ± 10.19 ng/g Se, respectively). On day 42 post-dosing, an interaction effect ($P < 0.05$) was observed between the CG and the SeBG. On day 56 post-dosing, an average of 108.87 ± 3.76 ng Se/g ($P > 0.05$) was obtained among the groups. Fig. 2 shows the GSH content in the erythrocytes. On day 0, the GSH levels in the 3 groups averaged 9.6E–4 ± 1.118E–5 nmols/mg of total protein ($P > 0.05$). GSH levels decreased during days 0–28 (3.7E−4 ± 4.714E−6 nmols/mg of total protein) in SeBG and SeSG, and then, they remained stable up to day 56 (5.6E−4 ± 8.49E–5 nmols/mg of total protein). An interaction effect was observed ($P < 0.05$) between CG and the SeSG and SeBG groups from day 14 up to day 45 post-dosing. Fig. 3 shows the CAT content in the erythrocytes. The mean CAT level at the beginning of the experiment was 73.10 ± 1.85 U. On day 14, the CAT levels in SeBG and SeSG were higher ($P > 0.05$; 79.90 ± 3.93 U) than in the CG (56.33 ± 1.69 U). Subsequently, from day 14 to day 28 post-

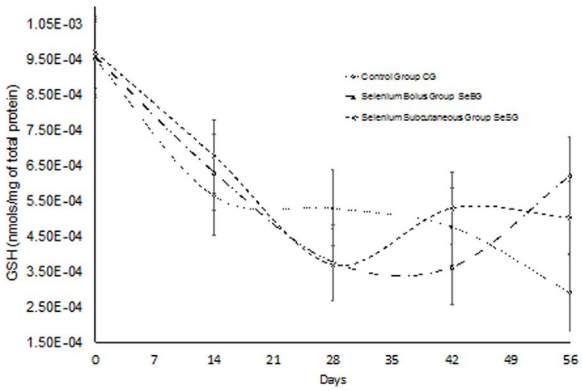

Fig. 2. Levels of erythrocyte GSH in goat kids with or without selenium supplementation.