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**Does diphenyl diselenide metaphylaxis increase weight gain and immunoglobulin G in holstein calves from the neonatal period to weaning?** A metafilaxia com disseleneto de difenila incrementa o ganho de peso e a imunoglobulina G em bezerras holandesas do período neonatal ao desmame?

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### Abstract

The objective of this work was evaluate metaphylactic effect of diphenyl diselenide (PhSe)<sub>2</sub> of Holstein calves. Twenty female calves from birth to weaning were used, distributed in groups: diphenyl diselenide group (DDG), received 1.5 μmol/kg of (PhSe)<sub>2</sub> in 2 mL of dimethylsulfoxide, subcutaneously; and control group (CG). Administrations and evaluations were performed on days 7, 21, 35, 49, with a final evaluation (70). Higher values of weight and mean daily weight gain happen on day 70 in DDG. Concerning to immunoglobulin G, except for day 7, higher levels were detected in DDG. Metaphylaxis with (PhSe)<sub>2</sub> increases weight gain and immunoglobulin G of calves from the neonatal period to weaning.

**Keywords:** Antioxidants. Mean daily weight gain. (PhSe)<sub>2</sub>. Selenium.

### Resumo

O objetivo deste trabalho foi avaliar o efeito metafilático do disseleneto de difenila (PhSe)<sub>2</sub> em bezerras holandesas. Foram utilizadas 20 bezerras do nascimento ao desmame, distribuídas nos grupos: disseleneto de difenila (GDD), recebeu 1.5 μmol/kg (PhSe)<sub>2</sub> em 2 mL de dimetilsulfóxido, subcutâneo; e controle (GC). Administrações e avaliações ocorreram aos 7, 21, 35, 49 dias, com uma última avaliação (70). Maiores valores de peso e ganho de peso médio diário ocorreram no dia 70 no GDD. Quanto a imunoglobulina G, com exceção do dia 7, maiores teores foram detectados no GDD. A metafilaxia com (PhSe)<sub>2</sub> incrementou ganho de peso e imunoglobulina G das bezerras do período neonatal ao desmame.

**Palavras-chave:** Antioxidantes. Ganho de peso médio diário. (PhSe)<sub>2</sub>. Selênio.

## Introduction

The neonatal period is considered a critical stage in the rearing of dairy calves, since the management they receive during this stage will have implications throughout their productive life, significantly influencing their future performance (Santos et al., 2010).

Ruminants are born without adequate humoral immunity due to the sin epitheliochorial placenta, which protects the fetus against infections by viruses and bacteria, but prevents the passage of immunoglobulins that would migrate from the maternal to fetal circulation (Prestes and Landim-Alvarenga, 2006). Thus, newborns rely on the passive transfer of maternal colostral immunoglobulins (Vogels et al., 2013).

When the transfer of passive immunity is not properly realized, these animals become more susceptible to the occurrence of disease (Godden, 2008), which is responsible not only for mortality, but also for the loss and/or lower weight gain of surviving calves (Beam et al., 2009). It is also worth mentioning that the metabolic stress triggered by weaning can result in oxidative stress (Moraes et al., 2002).

Oxidative stress is a result of excess production of reactive oxygen or nitrogen species, or of the organism's deficient antioxidant capacity (Gaschler and Stockwell, 2017). Regarding protection against reactive species, antioxidants are available, which can act by preventing their formation (Barbosa et al., 2010), intercepting the reactive species and preventing their action (Ellah, 2010).

Among the essential micronutrients, selenium deserves to be highlighted, since it is an integral part of the glutathione peroxidase enzyme (GPx) which is one of the main enzymes of the antioxidant cellular system (Nogueira et al., 2004). Its importance has also been noted for its ability to increase the immune response (Brown and Arthur, 2001).

Among the selenium forms, diphenyl diselenide (PhSe)<sub>2</sub> has been used in research due to its diverse pharmacological properties (Nogueira et al., 2004), including being used in the study of the control of several diseases, by mimicking the activity of GPx (Meotti et al., 2004). Diphenyl diselenide (PhSe)<sub>2</sub> demonstrated antioxidant properties in the tissues of fish submitted to herbicide exposure (Menezes et al., 2012) and improved the quality of meat in quails (Roza et al., 2018).

In sheep, a study performed by our research group to evaluate the distribution of (PhSe)<sub>2</sub> in tissues and plasma, showed that erythrocytes retain (PhSe)<sub>2</sub> and release slowly it into plasma and other tissues. The researchers did not observe any signs of toxicity to the species (Leal et al., 2018). When used in sheep with milk aptitude demonstrated the modulation capacity of oxidative reactions and inflammatory response (Biazus et al., 2019).

The objective of this work was to evaluate if the metaphylaxis with diphenyl diselenide (PhSe)<sub>2</sub> has an effect on weight gain, occurrence of diseases, total protein, immunoglobulin G and oxidative metabolism of Holstein calves from the neonatal period to weaning.

## Material and methods

Ethics committee on animal use

The experimental protocol was approved by the Ethics Committee on Animal Use of the Federal University of Santa Maria (CEUA/UFSM), under number 6250070518.

## Place and period of execution

The experiment was conducted in the municipality of Xanxerê (26° 52' 37" S and 52° 24' 14" O), State of Santa Catarina, Brazil, from February to May 2018.

## Animals

For the study, twenty Holstein calves (n = 20) were used from birth to seventy days of age. The newborn calves were housed in individual stalls of polyethylene with dimensions of approximately 100 x 120 x 210 cm (height, width and depth, respectively), containing wood shavings, bottle support and two buckets for feeding. The maintenance of the stalls was carried out daily, with the waste from that day being removed, in addition to partial removal of the bed if considered necessary. After removing the animals, cleaning and disinfection was performed using acid and alkaline detergents, and chlorine dioxide, in addition to the total replacement of the bed before being used by a new animal.

In the first six hours of life, ingested two liters of colostrum, supplied through a bottle, or through a nasogastric tube when necessary (quality evaluated using a Brix refractometer, considering the limit of 21% as indicative of immunoglobulin concentration > 50 mg/mL, that is, high quality colostrum). After ingestion of colostrum, the calves received, in bottles, a total of six liters of milk at 37°C, fractionated, twice a day. As breastfeeding consisted of supplying discarded milk, consisting of low quality colostrum, transitional milk, or with a high somatic cell count and originating from animals receiving treatment with antimicrobials, it was previously pasteurized at 72°C for 15 seconds, for reducing the risk of transmission of pathogens. Water and concentrate *ad libitum* were available in the stalls. The nutritional and chemical composition of the concentrate is shown in Table 1.

Table 1 - Nutritional and chemical composition of the concentrate.

<b>Ingredients</b>	<b>Quantitie (% MS*)</b>
Corn bran	45,0
Soybean meal	33,0
Soybean hull	16,0
Premix <sup>1</sup>	6,0
<b>Chemical composition</b>	<b>Quantitie</b>
Dry matter (kg)	500,0
Crude protein (% MS)	22,6
Ethereal extract (% MS)	2,9
Neutral detergent fiber (% MS)	188,4
Fiber in acid detergent (% MS)	119,7

\*Dry matter (DM). <sup>1</sup>Composition of premix: calcium (135-165 g/kg), phosphorus (70 g/kg), sulfur (25 g/kg), magnesium (25 g/kg), potassium (30 g/kg), cobalt (3 mg/kg), copper (425 mg/kg), chromium (25 mg/kg), iron (1750 mg/kg), iodine (11 mg/kg), manganese (1700 mg/kg), selenium (13 mg/kg), zinc (1700 mg/kg), biotin (1.5 mg/kg), vitamin A (350000 IU/kg), vitamin D3 (25000 IU/kg), pantothenic acid (126 mg/kg), vitamin B1 (50 mg/kg), vitamin B6 (60 mg/kg), vitamin B12 (1.11 mg/kg), choline (9000 mg/kg), niacin (247.50 mg/kg), riboflavin (50 mg/kg), vitamin C (6000 mg/kg), vitamin K (20 mg/kg), D-limonene (3300 mg/kg), *Saccharomyces cerevisiae* ( $0.75 \times 10^9$  UFC/kg), fluorine (700 mg/kg) and bicarbonate (135 g/kg).

At ten days of age, the calves were relocated to collective stalls containing wood shavings, in groups of 10 animals, where they started to receive Tifton 85 hay (*Cynodon* spp.) (10.5% crude protein) and, during the day, had access to an area of picket with *Cynodon* spp. pasture. Bottle feeding changed to an automatic system, which read the identification earring, and there was a gradual reduction in the quantity supplied, according to the weight and age group presented. The calves remained in these conditions until weaning, performed at seventy days of life, also corresponding to the end of the experimental period.

### Diphenyl diselenide

Diphenyl diselenide (PhSe)<sub>2</sub> presented in a powder formulation with 98% purity and a molecular weight of 312.13 g/mol, was purchased from Sigma-Aldrich (St. Louis, MO, USA).

To determine its dose, toxicity and distribution, our research group conducted a study submitting sheep to the administration of 6 µmol/kg (PhSe)<sub>2</sub> diluted in 20 mL of dimethyl sulfoxide (DMSO) (Leal et al., 2018). Our results indicate a greater affinity with the diffusion of erythrocytes, and its distribution to plasma and other tissues is slow, in addition, the dose administered does not cause any sign of evident toxicity in animals, being considered safe.

Thus, in this experiment, when using diphenyl diselenide as metaphylactic in calves, we chose to fractionate the dose of 6 µmol/kg in four administrations of 1,5 µmol/kg, over the desired period, that is, from the neonatal period to weaning, respecting intervals of 14 days, because it is the period of action of the drug. We also reduced the volume of DMSO used to prepare the solution, setting the volume to 2 mL, which proved to be adequate as a dilution vehicle for (PhSe)<sub>2</sub>, since it has the pharmacological ability to absorb and diffuse other drugs.

### Experimental design

The twenty animals were evenly distributed into two homogeneous groups containing 10 animals, according to body weight at birth. The diphenyl diselenide group (DDG) received 1.5 µmol/kg of diphenyl diselenide, diluted in 2 mL of dimethylsulfoxide 99,2% (DMSO), subcutaneously (SC). The control group (CG) received only DMSO, in the same volume used in the DDG and by the same administration route. The administrations occurred at intervals of 14 days, beginning on day 7, and were later performed on days 21, 35, and 49.

### Clinical evaluation

Clinical evaluations were performed on days 7, 21, 35, 49, and 70. Being the females were weighed using a weight tape for the chest circumference, in addition to being monitored for the health of the flock, by performing a general physical examination according to Dirksen et al.

(1993), which consisted of observing the general condition, attitude, degree of dehydration, coloration of the mucous membranes and consistency of feces. Measurement of capillary filling time, heart rate, respiratory rate, rectal temperature and pulmonary auscultation.

The detection of the occurrence of diseases was carried out using the methodology described by Teixeira et al. (2014), which characterizes diarrhea by observing watery stools for up to three consecutive days, and cases of pneumonia when two or more of the following clinical signs are detected: cough, rectal temperature above 39.5°C, respiratory rate above 40 respiratory movements per minute and/or increased sounds or presence of respiratory gasps.

#### Sample collection and laboratory tests

Blood samples were collected at the same moments of the clinical evaluation (7, 21, 35, 49 and 70 days), by jugular venipuncture, with Vacutainer® coupled system in vacuum tubes containing sodium heparin and without anticoagulant, both with a volume of 10 ml. After collection, the samples in tubes without anticoagulant were centrifuged at 10,000 rpm for 10 minutes to obtain blood serum. The samples stored in tubes with heparin were used in the preparation of the smear slides of the nitroazul tetrazolium reduction test stimulated or not with zymosan particles, as well as for the preparation of erythrocytes for the measurement of glutathione peroxidase, carried out by washing red blood cells in PBS solution followed by centrifugation at 2500 rpm for 10 minutes, three times, obtaining red blood cells. Subsequently, the serum and erythrocytes were distributed in eppendorf tubes (transparent and amber, respectively) properly identified and stored at -80°C until the analyzes were performed.

Serum protein values were determined by the use of a refractometer. Immunoglobulin G (IgG) determination was performed by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (Bovine IgG ELISA Kit, Cat. No. E11-118, Bethyl Laboratories, Montgomery, USA). Lipid peroxidation was measured through the production of thiobarbituric acid reactive substances (TBARS) as described by Ohkawa et al. (1979).

While activity of the glutathione peroxidase (GPx) enzyme was determined in a Randox® branded automated biochemical analyzer (RX Daytona® model) using a commercial Randox® kit, according to the technique described by Paglia and Valentine (1967).

The functional activity of neutrophils was evaluated by means of a nitroblue tetrazolium reduction test (NBT) stimulated or not with Zymosan particles (Sigma Aldrich, St. Louis, USA), according to the cytochemical method described by Park and Good (1970), counting one hundred neutrophils in each of the stained blood smears under an optical microscope with an immersion objective (100 x). For stimulated NBT and non-stimulated NBT, neutrophils that reduced nitroblue tetrazolium were considered positive, that is, neutrophils that presented violaceous or blackened cytoplasmic granules (formazan crystals), regardless of the number and size of the granulations. In the stimulated technique, the neutrophils that presented Zymosan particles inside were considered positive for phagocytosis.

#### Statistical analysis

The data were submitted to the Kolmogorov-Smirnov normality test. As normal distribution was not verified, the functional activity of neutrophils was previously submitted to logarithmic transformation ( $\log_{10} X + 1.5$ ). The variables were submitted to two-way analysis of variance (ANOVA) with repeated measures, followed by Tukey's tests, to compare the average between the

moments within the groups, and Bonferroni correction to compare the average of the moments between the groups.

The results are presented in means with their respective standard errors. To evaluate the occurrence of diseases, the unpaired T test was used and the data are arranged in a descriptive way. The level of significance used was 5% ( $p < 0.05$ ). Statistical analyzes were performed using the GraphPad Prism 6® program.

## Results

### Weight and mean daily weight gain

The results of weight and mean daily weight gain (MDWG) are shown in Figures 1a and 1b. Higher values of weight and MDWG were observed on day 70 in the DDG compared to the CG ( $p < 0.05$ ). Within the groups, a gradual increase in weight was observed throughout the experimental period (Figure 1a).

With respect to MDWG, a reduction in values was observed from day 7 to day 21 in the CG, however, on days 35 and 49 there was an increase in MDWG in this group ( $p < 0.05$ ). In the DDG, the same reduction in MDWG was observed at 21 days, followed by an increase in values on days 49 and 70 ( $p < 0.05$ ).

In addition, at 70 days of age, the date when weaning began and the experimental period ended, the DDG animals had a mean weight gain of 7.7 kg over the CG.

### Occurrence of diseases

Regarding the occurrence of diseases, there were no significant differences between groups. On day 7, 70% of the CG animals presented diarrhea, 20% pneumonia, and 10% of the calves did not present any clinical alterations, while 50% of the DDG animals presented diarrhea, 20% pneumonia, and 30% did not show any type of disease.

At 21 days, 40% of CG animals presented diarrhea, 30% pneumonia, and 30% showed no organic changes. However, none of the DDG animals presented disease occurrence. On the 35-day evaluation, the occurrence of pneumonia was observed in 30% of the animals in the CG, combined diarrhea and pneumonia in 10% of the calves, and 60% of the animals did not present clinical alterations. At the same experimental moment, 40% of the DDG animals presented pneumonia and 60% had no disease occurrence.

On day 49, 10% of CG animals had pneumonia, while the rest of the animals (90%) showed no organic changes. In the DDG, 10% of the animals presented diarrhea, 10% pneumonia, and 80% of the animals were healthy. The final evaluation moment (70 days) was marked by the absence of alterations (100%) in the DDG. In the CG, 20% of the animals presented pneumonia and the remainder (80%) presented no clinical changes.

### Total protein

There were no differences in total protein between the experimental groups. In the CG, there was an increase in serum protein (Figure 1c) ( $p < 0.05$ ) between days 21 and 70, and 35 and 70. In the DDG, except for day 49, total protein values were higher on day 70 ( $p > 0.05$ ) in relation to the other moments studied.

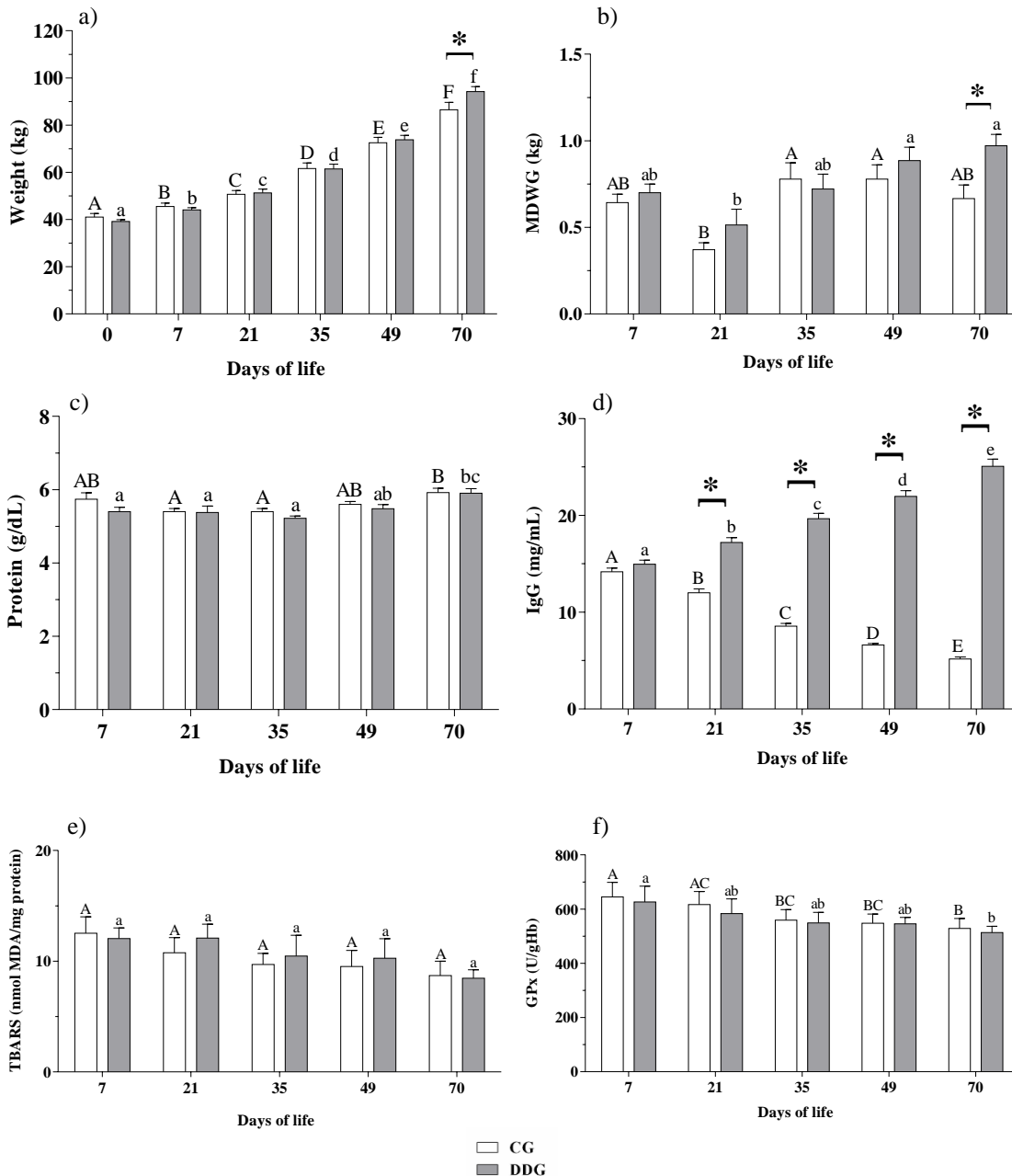


Figure 1 - Average values and standard errors of **a)** weight (day 0 indicates birth weight, while day 70 weight at weaning), **b)** mean daily weight gain (MDWG), **c)** serum protein, **d)** immunoglobulin G (IgG), **e)** thiobarbituric acid reactive substances (TBARS), **f)** glutathione peroxidase (GPx), of calves from the control group (CG) and treated with diphenyl diselenide (DDG). Upper case letters refer to differences ( $p < 0.05$ ) between days within the control group, while lower case letters refer to differences ( $p < 0.05$ ) between days within the group treated with diphenyl diselenide. \*represents difference between groups ( $p < 0.05$ ).

### Immunoglobulin G (IgG)

Higher levels of IgG were observed in the DDG compared to the CG (Figure 1d) on all days, with a significant difference being observed between groups on days 21, 35, 49 and 70. Within DDG, it was observed that the calves showed a gradual increase in IgG over all experimental periods ( $p < 0.05$ ). However, within the CG there was a reduction in the values of this variable in experimental animals from 7 to 70 days ( $p < 0.05$ ).

### Thiobarbituric acid reactive substances (TBARS)

Serum TBARS values are shown in Figure 1e. There were no differences between the experimental groups or between the moments within each group ( $p > 0.05$ ).

### Glutathione peroxidase (GPx)

The mean values of GPx can be visualized in Figure 1f. There were no differences in the activity of this enzyme between the experimental groups ( $p > 0.05$ ). In the CG, higher GPx values were detected on day 7 in relation to days 35, 49, and 70 ( $p < 0.05$ ), as occurred between days 21 and 70 ( $p < 0.05$ ). In the DDG greater activity of this enzyme was observed on day 7 compared to day 70 ( $p < 0.05$ ).

### Oxidative metabolism and neutrophil phagocytosis (NBT)

There were no differences in oxidative metabolism and phagocytosis of the neutrophils in the stimulated technique either between the groups or within the experimental groups (Table 2). In the non-stimulated technique, no significant values were observed between the groups, and in the DDG. However, in the control group, higher values of negative neutrophils (non-stimulated technique) were detected on day 7 compared to day 70 ( $p < 0.05$ ). In the non-stimulated technique, the neutrophils positive for NBT, that is, those that presented the formazan crystal inside, presented higher values ( $p < 0.05$ ) on day 70 compared to those obtained on day 7 in the CG.



Table 2 - Effect of treatments in the control (CG) and diphenyl diselene (DDG) groups on oxidative metabolism and phagocytosis of blood neutrophils.

Group	Days	Stimulated				Non- Stimulated	
		Negative (%)	NBT + (%)	ZYM + (%)	NBT + ZYM + (%)	Negative (%)	NBT+ (%)
CG	7	50,10 ( $\pm$ 12,03) <sup>A</sup>	14,90 ( $\pm$ 6,57) <sup>A</sup>	29,30 ( $\pm$ 10,56) <sup>A</sup>	5,70 ( $\pm$ 2,22) <sup>A</sup>	89,80 ( $\pm$ 2,24) <sup>A</sup>	10,20 ( $\pm$ 2,24) <sup>BCD</sup>
	21	49,10 ( $\pm$ 7,91) <sup>A</sup>	20,20 ( $\pm$ 4,53) <sup>A</sup>	23,20 ( $\pm$ 5,30) <sup>A</sup>	7,50 ( $\pm$ 3,02) <sup>A</sup>	77,50 ( $\pm$ 4,78) <sup>AB</sup>	22,50 ( $\pm$ 4,78) <sup>ABCD</sup>
	35	31,30 ( $\pm$ 8,12) <sup>A</sup>	29,70 ( $\pm$ 5,64) <sup>A</sup>	22,70 ( $\pm$ 4,70) <sup>A</sup>	16,30 ( $\pm$ 3,77) <sup>A</sup>	76,00 ( $\pm$ 7,66) <sup>AB</sup>	24,00 ( $\pm$ 7,66) <sup>ABC</sup>
	49	47,20 ( $\pm$ 9,26) <sup>A</sup>	19,20 ( $\pm$ 6,75) <sup>A</sup>	25,20 ( $\pm$ 5,83) <sup>A</sup>	8,40 ( $\pm$ 2,33) <sup>A</sup>	74,30 ( $\pm$ 6,64) <sup>AB</sup>	25,70 ( $\pm$ 6,64) <sup>AB</sup>
	70	44,50 ( $\pm$ 10,61) <sup>A</sup>	22,50 ( $\pm$ 7,42) <sup>A</sup>	25,80 ( $\pm$ 8,89) <sup>A</sup>	7,40 ( $\pm$ 2,45) <sup>A</sup>	61,20 ( $\pm$ 8,31) <sup>B</sup>	38,80 ( $\pm$ 8,31) <sup>A</sup>
DDG	7	40,10 ( $\pm$ 8,22) <sup>a</sup>	11,90 ( $\pm$ 3,93) <sup>a</sup>	42,00 ( $\pm$ 8,89) <sup>a</sup>	6,00 ( $\pm$ 2,37) <sup>a</sup>	83,70 ( $\pm$ 4,63) <sup>a</sup>	16,30 ( $\pm$ 4,63) <sup>a</sup>
	21	51,10 ( $\pm$ 7,94) <sup>a</sup>	12,80 ( $\pm$ 1,97) <sup>a</sup>	26,10 ( $\pm$ 6,81) <sup>a</sup>	10,00 ( $\pm$ 4,38) <sup>a</sup>	78,90 ( $\pm$ 5,34) <sup>a</sup>	21,30 ( $\pm$ 5,36) <sup>a</sup>
	35	50,10 ( $\pm$ 9,98) <sup>a</sup>	12,00 ( $\pm$ 3,13) <sup>a</sup>	31,50 ( $\pm$ 8,43) <sup>a</sup>	6,40 ( $\pm$ 1,89) <sup>a</sup>	84,50 ( $\pm$ 2,13) <sup>a</sup>	14,90 ( $\pm$ 2,04) <sup>a</sup>
	49	50,20 ( $\pm$ 8,68) <sup>a</sup>	18,70 ( $\pm$ 2,98) <sup>a</sup>	19,70 ( $\pm$ 5,02) <sup>a</sup>	11,40 ( $\pm$ 3,84) <sup>a</sup>	67,70 ( $\pm$ 6,12) <sup>a</sup>	31,80 ( $\pm$ 6,09) <sup>a</sup>
	70	39,10 ( $\pm$ 9,48) <sup>a</sup>	29,00 ( $\pm$ 7,90) <sup>a</sup>	21,80 ( $\pm$ 5,45) <sup>a</sup>	10,10 ( $\pm$ 3,55) <sup>a</sup>	65,40 ( $\pm$ 3,55) <sup>a</sup>	34,60 ( $\pm$ 6,08) <sup>a</sup>

Results are expressed as average  $\pm$  standard error. Upper case letters refer to differences ( $p < 0.05$ ) between days within the control group, while lower case letters refer to differences ( $p < 0.05$ ) between days within the group treated with diphenyl diselenide. \*  $p < 0.05$  when compared to the difference between the control and diphenyl diselenide groups on the respective days.

## Discussion

Selenium (Se), administered in the form of diphenyl diselenide (PhSe)<sub>2</sub>, is an trace element that plays a fundamental role in animal health. Among its most well-known functions, it can be cited as an essential component of the antioxidant enzyme GPx, in addition to also composing the 5-iodothyronine deiodinase enzyme, essential in the activation of tetraiodothyronine (T4) in triiodothyronine (T3) (NRC, 2001), known for being widely involved in the mechanisms of growth and weight gain (Thompson et al., 1995).

The use of (PhSe)<sub>2</sub> in the present study significantly increased the calves' weight and MDWG at the end of the experimental period, that is, at the time of weaning (70 days), a phase that is usually marked by a reduction in weight gain and greater occurrence of diseases. Guyot et al. (2007), reported the importance of Se in Belgian Blue calves. These animals are characterized by the “double muscle”, thus, they have a greater demand for Se. In this work, the researchers supplemented cows with 0,5 ppm of yeast enriched with Se and observed a significant increase in the growth rate of the calves, thus demonstrating the importance of Se administration in animal performance. When administering a single dose of (PhSe)<sub>2</sub> combined with zinc edetate, to male Dutch calves, in the pre and post-weaning period, Santos et al. (2019) also observed beneficial effects on the development of animals, justified by the action of zinc associated with selenium in stimulating the immune system of animals. In this sense, it is important to note that Se deficiency, whether borderline or even short-term, directly affects the development of animals, in addition to triggering white muscle disease and nutritional muscular dystrophy (Suttle, 2010).

According to Mcguirk (2008), the factors that can significantly change the morbidity and mortality rates are related to the age of the animals, passive immunity transfer, management used in the farms, types of accommodation, season, country, region and data source. Being the cases of enteritis and pneumonia considered the most commonly linked to the death of dairy calves. Teixeira et al. (2014), reported the effect of treatment with minerals in reducing cases of diarrhea, and Guyot et al. (2007), observed a reduced incidence of diseases during the first two weeks of life in calves whose mothers were supplemented with yeast enriched with Se. We did not observe any influence of (PhSe)<sub>2</sub> administration on the occurrence of diseases, although we also detected diarrhea and pneumonia as the main diseases that affected the calves during the experimental period.

In addition to the functions mentioned above, Se acts in the regulation of pinocytosis, promoting the absorption of immunoglobulins (Kamada et al., 2007). Thus, the lack of this mineral can also affect the levels of IgG and the function of T cells, determining a higher prevalence and severity of diseases (Arthur et al., 2003). Its effects, both in organic and inorganic forms, on the immune response are already established in the literature (Chauhan et al., 2014), since its administration assists in the phagocytic activity of macrophages (Salles et al., 2014). It can also be attributed to its antioxidant properties, improvements in the functions of the humoral and cellular immune system (Teixeira et al., 2014). In the present study, we detected a significant and exponential increase in the levels of immunoglobulin G in calves since the second administration of (PhSe)<sub>2</sub>, while in the animals in the control group, serum IgG concentrations significantly decreased over the experimental period.

As for the total protein values, there was no difference between groups. Menezes et al. (2016), also did not detect influence of (PhSe)<sub>2</sub> in the total protein content, in carps fed with a diet containing the compound and which presented oxidative stress due to exposure to fipronil.

TBARS is one of the by-products of lipid peroxidation (Ohkawa et al., 1979), thus, by observing the linearity of its values over the experimental period, indirectly, we concluded that there was no oxidative stress. Fact that justifies the stable values in the activity of GPx, since there was no increase in the production of reactive species. Menezes et al. (2016), detected a reduction in the levels of TBARS in the liver, gills, brain and carp muscle exposed to fipronil, however, the animals were previously submitted to the formation of ER, not endogenous, and presented oxidative stress, which required greater activity in the antioxidant system. Roza et al. (2018), also observed a reduction in lipid peroxidation in the serum, liver and muscle of quails when employed (PhSe)<sub>2</sub>; in this same study, the authors reported a significant increase in GPx activity.

Through GPx, Se is involved in the metabolism of arachidonic acid. It is probably through this relationship that its supplementation benefits the phagocytic capacity of neutrophils (NRC, 2001). The process of phagocytosis performed by neutrophils is an important defense mechanism of the host against invading microorganisms, and the production of oxidative substances within it, which occurs through increased respiratory activity, which is fundamental for the adequate efficiency of this process (Tizard, 2014). Neutrophils from newborn calves have a low capacity to respond to the NBT test (Costa et al., 2004), however, with age, changes occur, with an increase in oxidative capacity during the first four months of life (Costa et al., 2008). We did not observe the influence of (PhSe)<sub>2</sub> on oxidative metabolism and neutrophil phagocytosis between the groups in stimulated and non-stimulated techniques.

However, Camargo et al. (2010), observed, in adult sheep, experimentally infected with *Haemonchus contortus*, that supplementation with Se was able to promote a decrease in the ability to reduce NBT in the non-stimulated technique, thus demonstrating the role of Se in modulating the oxidative metabolism of neutrophils.

## Conclusion

The results obtained in this study allow us to affirm that diphenyl diselenide (PhSe)<sub>2</sub> metaphylaxis increases the weight gain and immunoglobulin G of Holstein calves from the neonatal period to weaning.

## Conflict of interest

The authors declare no conflicts of interest.

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