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Metaphylactic effect of diphenyl diselenide $(PhSe)_2$ on the health of beef female calves subjected to conventional weaning

Efeito metafilático do disseleneto de difenila $(PhSe)_2$ na saúde de bezerras de corte submetidas ao desmame convencional

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Highlights

$(PhSe)_2$ is an alternative to traditional sources of organic selenium.
$(PhSe)_2$ increased albumin, globulin, and albumin:globulin ratio.
$(PhSe)_2$ enhanced the antioxidant capacity of calves during weaning.

Abstract

The objective of this study was to evaluate the impact of diphenyl diselenide $(PhSe)_2$ on the average daily gain, biochemical parameters, and oxidative status of beef calves subjected to conventional weaning. Thirty female calves, aged six months and weighing 152.42±13.75 kg, were used. The experiment was laid out in a completely randomized design, with the experimental units divided into three groups: control

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group (n=10): 2 mL of NaCl solution; dimethyl sulfoxide group (n=10): 2 mL of dimethyl sulfoxide; and diphenyl diselenide group (n=10): 3 µmol kg^{-1} of $(PhSe)_2$ diluted in 2 mL of dimethyl sulfoxide. Collections and evaluations were conducted at the following time points: -28 (baseline, T1) and -14 (T2) days before weaning; on the initial day of weaning (day 0, T3); and 14 (T4) and 28 (T5) days after weaning. Treatments at T1, T2, and T3 were administered subcutaneously. All animals were subjected to similar handling and feeding conditions throughout the experiment, with free access to water. The assessed parameters included average daily gain, total protein, albumin, globulin, albumin:globulin ratio, reduced glutathione, thiobarbituric acid reactive substances, and total antioxidant capacity determined by the ferric reducing antioxidant power. Among the evaluated parameters, significant differences (P<0.05) were observed in average daily gain according to time points; albumin according to treatments, time points, and treatment × time interaction; globulins according to time points and treatment × time interaction; albumin:globulin ratio according to time points and treatment × time interaction; reduced glutathione according to time points and treatment × time interaction; and ferric reducing antioxidant potential according to time points. The administration of diphenyl diselenide to beef female calves resulted in an increase in albumin, globulin, albumin:globulin ratio, and reduced glutathione during conventional weaning. The beneficial effect of diphenyl diselenide was verified by the treatment × time interactions. These results demonstrate that diphenyl diselenide serves as an alternative to traditional sources of organic selenium, and its use mitigates the challenges faced by beef calves during conventional weaning.
Key words: Beef cattle farming. Oxidative status. Performance. Selenium.

Resumo

O objetivo do trabalho foi avaliar o efeito do disseleneto de difenila $(PhSe)_2$, no ganho médio diário, parâmetros bioquímicos e status oxidativo de bezerras de corte submetidas ao desmame convencional. Foram utilizadas 30 bezerras, com seis meses de vida e 152,42±13,75 kg de peso corporal. Foi utilizado um delineamento inteiramente casualizado sendo as unidades experimentais distribuídas em três grupos: grupo controle (GC, n=10): 2 mL de solução de NaCl; grupo dimetilsulfóxido (GDMSO, n=10): 2 mL de dimetilsulfóxido; e grupo disseleneto de difenila (GDD, n=10): 3 µmol kg^{-1} de $(PhSe)_2$ diluído em 2 mL de dimetilsulfóxido. As coletas e avaliações foram realizadas no M1=basal (-28) e M2 (-14) dia antes do desmame, M3 (0) dia inicial do desmame, M4 (14) e M5 (28) dia após o desmame. Os tratamentos foram administrados no M1, M2 e M3 pela via subcutânea. Todos os animais foram submetidos a condições similares de manejo e alimentação ao decorrer do experimento, tendo livre acesso a água. Os parâmetros avaliados foram: ganho médio diário, proteína total, albumina, globulina, relação albumina:globulina, glutationa reduzida, substâncias reativas ao ácido tiobarbitúrio e capacidade antioxidante total, determinada pelo potencial antioxidante redutor férrico. Dentre os parâmetros avaliados observou-se efeito significativo (P<0,05): no ganho médio diário entre momentos; na albumina entre tratamentos, momentos e interação tratamento x momento; nas globulinas entre momentos e interação tratamento x momento; na relação albumina:globulina entre momentos e interação tratamento x momento; na glutationa reduzida entre momentos e interação tratamento x momento e no potencial antioxidante redutor férrico entre momentos. A administração de disseleneto de difenila em bezerras de corte proporcionou incremento nas variáveis albumina, globulina, relação albumina:globulina e glutationa reduzida durante o desmame convencional, sendo o efeito benéfico do disseleneto de difenila

constatado a partir das interações entre tratamento x momentos. Esses resultados demostram que o disseleneto de difenila é uma alternativa as fontes tradicionais de selênio orgânico, e quando utilizado reduziu os desafios vivenciados pelas bezerras de corte durante o desmame convencional.
Palavras-chave: Bovinocultura de corte. Desempenho. Selênio. Status oxidativo.

Introduction

The postweaning phase in beef calf production spans from birth to weaning, forming the foundational support for the production system (López-González et al., 2020). Weaning represents a crucial management step and can be executed through various methods (Orihuela & Galina, 2019). Conventional weaning typically occurs between 6-8 months of life (Gottshall, 2009; Enríquez et al., 2010; Barcellos et al., 2019), aiming to provide nutritional support to the calf and restore the body condition of the dam. Weaning weight determines growth potential in the initial life stage, with calves ideally acquiring 40-45% of their adult weight by the end of this phase (Neiva, 2013).

The separation of the calf from its mother inherently induces social stress (Hickey et al., 2003). Weaning is considered a critical period, marked by various stressful factors such as abrupt separation, changes in environment, food and water, handling, crowding, transport, and marketing (Lynch et al., 2019). These conditions alter physiological, immunological, morphological, metabolic, nutritional, and behavioral patterns (National Research Council [NRC], 2016), and exposure to any of these stressors can lead to oxidative stress due to the production of reactive species (Inanami et al., 1999; Eitam et al., 2010). Moreover, it induces immune dysfunctions, heightening the susceptibility to significant diseases at this stage (Lykkesfeldt & Svendsen, 2007).

Selenium, a trace mineral, plays an important role in the health and performance of cattle (Mehdi & Dufrasne, 2016). It functions as an antioxidant, minimizing the effects of oxidative stress, particularly when required at higher levels such as during the weaning and transport of calves (Hall et al., 2013a,b). Similarly, diphenyl diselenide $(PhSe)_2$, an organic compound, possesses pharmacological properties and antioxidant activity (Meotti et al., 2004; Nogueira et al., 2004) and can serve as an alternative to traditional sources of organic selenium in cattle (Prauchner, 2014). In parenteral administration, $(PhSe)_2$ is diluted with dimethyl sulfoxide (DMSO) (Viana, 2019).

In ruminants, $(PhSe)_2$ has been investigated in both sheep (Biazus et al., 2018; Leal et al., 2018) and bovine species (Santos et al., 2019; Rodrigues et al., 2020). Biazus et al. (2018) explored the impact of $(PhSe)_2$ administration, at a dose of 3.0 µmol kg^{-1} diluted in 1.5 mL of DMSO via the subcutaneous route, on the milk composition, oxidative stress, and inflammatory response of recently lambed Lacaune dairy sheep. Leal et al. (2018) assessed the selenium distribution throughout the bodies of five-month-old Texel sheep treated with $(PhSe)_2$ in a single dose of 6 µmol kg^{-1} diluted in 20 mL of DMSO intravenously. Santos et al. (2019) analyzed the effects of $(PhSe)_2$ administration at a dose of 3 µmol kg^{-1} diluted in DMSO subcutaneously, with or without zinc edetate in two administrations at 20-day intervals, on the performance, immune response, protein and lipid metabolism, as well

as oxidative/antioxidant status in the serum and muscle of Holstein calves during weaning. Rodrigues et al. (2020) evaluated whether metaphylaxis with $(PhSe)_2$ at a dose of 1.5 µmol kg^{-1} diluted in 2 mL of DMSO subcutaneously would impact weight gain, the occurrence of diseases, total protein, immunoglobulin G, and the oxidative metabolism of Holstein female calves from birth to weaning at 70 days of age.

The results of administering $(PhSe)_2$ during the weaning of dairy calves revealed that when administered alone, it exhibited significant anti-inflammatory action. When associated with zinc edetate, it demonstrated substantial effects on the animals' development, activating the cellular antioxidant system, reducing the harmful action of free radicals, and stimulating the activity of immune cells (Santos et al., 2019). In weaning dairy female calves, metaphylaxis with $(PhSe)_2$ increased weight gain and immunoglobulin G levels, reinforcing their immunological function (Rodrigues et al., 2020). These findings suggest that the compound warrants investigation in the conventional weaning of beef calves. Therefore, we hypothesize that the use of $(PhSe)_2$ mitigates the negative effects of weight loss, immune dysfunctions, and oxidative stress after weaning. This study thus aims to evaluate the impact of $(PhSe)_2$ on the average daily gain, biochemical parameters, and oxidative status of beef calves subjected to conventional weaning.

Material and Methods

Animals, location, and experimental period

Thirty beef female calves, aged six months and weighing 152.42 ± 13.75 kg, offspring of multiparous Bos taurus cows, were utilized. The experiment took place on a commercial farm (28°16'54.9" S and 55°40'45.3" W) located in the municipality of Garruchos, Rio Grande do Sul, Brazil, from April to June 2022. This study received approval from the Ethics Committee on the Use of Animals of the Federal University of Santa Maria (CEUA/UFSM) (approval no. 5836200721).

Experimental design

A completely randomized design was employed, with the experimental units divided into three groups: control group (CG, n=10), which received 2 mL of 0.9% NaCl solution; dimethyl sulfoxide group (DMSOG, n=10), treated with 2 mL of 99.2% dimethyl sulfoxide; and diphenyl diselenide group (DDG, n=10), administered with 3 µmol kg^{-1} of 98% $(PhSe)_2$ (Sigma-Aldrich®; St. Louis, MO, USA), diluted in 2 mL of 99.2% dimethyl sulfoxide (Santos et al., 2019). The interval between time points (T) was 14 days, following the premise that the effects of selenium last 14 days (Suttle, 2010). Blood sample collections and body weight assessments were conducted on days -28 (baseline; T1) and -14 (T2) before weaning, on the initial day of weaning (day 0; T3), and 14 (T4) and 28 (T5) days after weaning. Treatments at T1, T2, and T3 were administered subcutaneously.

Management and feeding

All animals remained together in the same 60-ha native pasture paddock, subjected to uniform management and feeding conditions throughout the experiment, with unrestricted access to water. Blood samples were collected and

treatments were administered with the calves brought to a corral containing a squeeze chute suitable for the species.

At T1 and T2, the calves grazed native grasses alongside their mothers in a paddock. Weaning commenced at T3, with the calves spending four days in the corral before being released into paddocks for grazing on native grasses. Throughout T3, the calves were grouped twice daily near feeders offering the concentrate supplement CAMERA BEEF CARCAVIO 15%® supplied at 1% of body weight (composition presented in Table 1). During T4 and T5, the weaned calves remained in a paddock grazing on native grass. The predominant components of the native grass grazing at all times included bahiagrass (*Paspalum notatum*), *Axonopus jesuiticus, Andropogon lateralis,* and *Stipa filiculmis.* The mineral supplement GADO FORTE 40® was available *ad libitum* during T1, T2, T4, and T5 (composition shown in Table 2).

Table 1
Nutritional composition of the concentrate supplement supplied to calves at T3 (day 0, initial day of weaning)

Nutrient	Content
Crude protein (min; g kg^{-1})	150
Ether extract (min; g kg^{-1})	25
Crude fiber (min; g kg^{-1})	200
Acid detergent fiber (max; g kg^{-1})	250
Mineral matter (max; g kg^{-1})	150
Moisture (max; g kg^{-1})	120
Calcium (min; g kg^{-1})	10
Calcium (max; g kg^{-1})	30
Phosphorus (min; mg kg^{-1})	3,000
Sodium (min; mg kg^{-1})	2,800
Sulfur (min; mg kg^{-1})	1,500
Magnesium (min; mg kg^{-1})	2,500
Zinc (min; mg kg^{-1})	50
Iodine (min; mg kg^{-1})	0.80
Fluorine (max; mg kg^{-1})	30
Copper (min; mg kg^{-1})	16.50
Cobalt (min; mg kg^{-1})	0.25
Manganese (min; mg kg^{-1})	33
Selenium (min; mg kg^{-1})	0.16
Vitamin A (min; IU kg^{-1})	1,750
Monensin (mg kg^{-1})	30
Saccharomyces cerevisae (CFU)	3×10^8

Table 2
Mineral supplement supplied to calves at 28 days before weaning (baseline), 14 days before weaning, on the initial day of weaning, and 14 and 28 days after weaning

Nutrient	Content
Calcium (min-max; g kg^{-1})	80-88
Phosphorus (min; g kg^{-1})	40
Sulfur (min; g kg^{-1})	10
Sodium (min; g kg^{-1})	220
Manganese (min; mg kg^{-1})	1,350
Zinc (min; mg kg^{-1})	2,000
Cobalt (min; mg kg^{-1})	16
Copper (min; mg kg^{-1})	450
Iodine (min; mg kg^{-1})	41
Selenium (min; mg kg^{-1})	10
Fluorine (max; mg kg^{-1})	400

At the start of each experimental phase, forage samples from the paddock were collected using a square frame (0.5 × 0.5 m) launched at five different points, measuring the pasture height, followed by cutting and weighing the forage sample. The samples from different points were combined to form a composite sample per phase, frozen at -20 ºC. Before analysis, these samples were placed in an oven at 55 ºC for 72 h until reaching a constant weight. They were then divided into two subsamples, with one ground using a 1-mm-screen sieve for chemical analysis and the other ground using a 2-mm-screen sieve for *in situ* organic matter digestibility analysis (ISOMD). Chemical determinations included dry matter (DM), crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF), mineral matter (MM), and ISOMD, conducted by the Laboratory of Bromatology and Ruminant Nutrition at UFSM (Table 3), following the procedures described by Orlandi et al. (2020).

Table 3
Results of chemical analyses of samples collected from grazing paddocks during the different evaluation time points

Time point*	DM (g kg^{-1} as-fed)	g kg^{-1} DM					
		CP	EE	NDF	ADF	MM	ISOMD
T1	421.1	82.1	14.9	760.1	428.0	85.0	437.5
T2	423.7	89.9	17.3	733.0	401.7	75.4	433.2
T3	410.4	103.3	18.1	752.7	409.8	86.7	423.4
T4	341.8	136.8	17.5	715.9	358.8	90.0	523.2
T5	350.4	124.6	15.5	708.9	372.1	92.5	492.3

*Time points: T1 = 28 days before weaning (baseline); T2 = 14 days before weaning; T3 = initial day of weaning (0); T4 = 14 days after weaning; and T5 = 28 days after weaning. Abbreviations: DM - dry matter; CP - crude protein; EE - ether extract; NDF - neutral detergent fiber; ADF - acid detergent fiber; MM - mineral matter; and ISOMD - in situ organic matter digestibility.

Collection and preparation of blood samples

Blood collection occurred at time points 1, 2, 3, 4, and 5 through jugular venipuncture, utilizing a needle and vacuum tube system. Serum was obtained by centrifuging tubes without anticoagulant at 3000 x g for 15 min. Following centrifugation, the samples were cooled before processing, transferred to labeled microtubes, and stored at -20 ºC.

Evaluated parameters

All parameters were assessed at time points 1, 2, 3, 4, and 5. Body weight was measured on a mechanical scale, and average daily gain (ADG) was calculated between assessments. Total protein (TP) and albumin (AL) were determined using a Mindray BS-120® automatic biochemical analyzer with commercial kits (Bioclin®). Globulin (GL) was derived by calculating the difference between TP and AL (GL = TP - AL), and the albumin:globulin ratio (AL:GL) was obtained by dividing AL by GL (AL:GL = AL/GL) (González & Silva, 2017). Reduced glutathione (GSH-NPSH) was estimated through spectrophotometry (Ellman, 1959). Lipid peroxidation was assessed by measuring thiobarbituric acid reactive substances (TBARS) (Ohkawa, 1979), wherein malondialdehyde (MDA) reacts with thiobarbituric acid (TBA) to form a colored complex. The total antioxidant capacity was determined using the ferric reducing antioxidant potential (FRAP) method, which assesses the reducing power of iron ions and employs ascorbic acid as a classic antioxidant (Benzie & Strain, 1996).

Statistical procedures

Assumptions of normality, homoscedasticity, and independence of residuals were tested beforehand. The response variables were analyzed using a linear mixed model with repeated measurements over time. The model included the effects of animals as random and treatments as fixed. The statistical model is represented as follows:

$$Y_{ijkl} = \mu + TREAT_i + TIME_j + (TREAT*TIME)_{ij} + ANIMAL_k + e_{(i)k} + \varepsilon_{ijkl}$$

where Y_{ijkl} = response variables; μ = overall mean of all observations; $TREAT_i$ = treatments (CG, DDG, and DMSOG); $TIME_j$ = evaluation time points (1, 2, 3, 4, or 5); $(TREAT*TIME)_{ij}$ = interaction effect between treatments and evaluation time points; $ANIMAL_k$ = random effect of animal; $e_{(i)k}$ = random error associated with each experimental unit, assuming NID (0, σ_e^2), ε_{ijkl} = random error associated with each observation, assuming ~ NID (0, σ_ε^2). Subsequently, Tukey's mean comparison test was applied ($P<0.05$). and the MIXED procedure of the SAS statistical software (Cody, 2015) was used.

Results and Discussion

The stress associated with weaning affects nutritional requirements (NRC, 2016) and prompts the utilization of the body's own reserves for maintenance purposes (Tzou et al., 1991). In our experiment, we observed weight loss in all three groups at T4 (Tables 4 and 5). This decline is likely linked to the diverse stressors encountered by the calves during weaning. Notably, at T5, there was an increase in body weight across all groups (Table 5). The induced catabolic state during weaning also triggers the production of reactive species, challenging antioxidant defenses (Eitam et al., 2010). This diversion of energy toward generating antioxidant defenses instead of tissue development is well-documented (Russell et al., 2016). The amalgamation of challenges during weaning, along with associated complications, elevates the susceptibility to diseases (Wang et al., 2019).

Table 4
Probability values for treatment, time points, and treatment × time interaction for average daily gain, biochemical parameters and oxidative status of beef calves subjected to conventional weaning

Variable	Treatment	P-value		
		Treatment	Time point	Treatment × Time
ADG (kg day^{-1})	CG DDG DMSOG	0.1935	<0.0001	0.5300
TP (g dL^{-1})	CG DDG DMSOG	0.4184	0.1383	0.2075
AL (g dL^{-1})	CG DDG DMSOG	0.0016	<0.0001	<0.0001
GL (g dL^{-1})	CG DDG DMSOG	0.8746	<0.0001	0.0001
AL:GL	CG DDG DMSOG	0.2685	<0.0001	<0.0001
GSH (NPSH) (nmol mL^{-1})	CG DDG DMSOG	0.4758	<0.0001	0.0199
TBARS (nmol MDA mL^{-1})	CG DDG DMSOG	0.2703	0.1099	0.4121
FRAP (µg Eq. AA)	CG DDG DMSOG	0.9569	<0.0001	0.1111

*Variables: ADG - average daily gain, TP - total protein, AL - albumin, GL - globulin, AL:GL - albumin:globulin ratio, GSH - reduced glutathione, TBARS - thiobarbituric acid reactive substances; and FRAP - ferric reducing antioxidant potential of beef calves subjected to conventional weaning. Distributed into experimental groups: CG - control group, DDG - diphenyl diselenide group, DMSOG - dimethyl sulfoxide group, at time points -28 (baseline; T1) and -14 (T2) days before weaning, on the initial day of weaning (0; T3), and 14 (T4) and 28 (T5) days after weaning.

Table 5
Mean values and standard error for treatment, time points, and treatment × time points interaction for average daily gain, biochemical parameters, and oxidative status of beef calves subjected to conventional weaning

Variable	Treatment	P-value					Mean
		T1	T2	T3	T4	T5	
ADG (kg day^{-1})	CG	0.0±0.0	0.209±0.040	0.628±0.180	-0.659±0.139	0.413±0.120	0.171±0.09
	DDG	0.0±0.0	0.325±0.040	0.579±0.160	-0.121±0.117	0.500±0.104	0.260±0.06
	DMSOG	0.0±0.0	0.311±0.040	0.591±0.107	-0.221±0.152	0.422±0.110	0.229±0.06
	Mean	0.0±0.0^{B}	0.369±0.090^{A}	0.599±0.080^{A}	-0.319±0.090C	0.445±0.060^{A}	0.221±0.04
TP (g dL^{-1})	CG	7.05±0.15	6.77±0.16	7.03±0.15	7.01±0.31	6.96±0.11	6.96±0.10
	DDG	6.77±0.16	6.75±0.15	6.89±0.19	6.84±0.09	6.82±0.15	6.81±0.10
	DMSOG	6.97±0.10	6.59±0.10	6.50±0.17	6.69±0.13	7.06±0.16	6.76±0.01
	Mean	6.93±0.08	6.70±0.08	6.80±0.10	6.84±0.11	6.95±0.08	6.84±0.04
AL (g dL^{-1})	CG	3.47±0.03^{Ba}	4.14±0.05^{Aa}	4.15±0.07^{Aa}	3.30±0.06Ba	3.35±0.05^{Ba}	3.69±0.06
	DDG	3.39±0.05^{Ba}	4.16±0.06^{Aa}	3.32±0.06^{Bc}	3.26±0.07Ba	3.34±0.05^{Ba}	3.49±0.05
	DMSOG	3.34±0.04^{Ca}	3.99±0.04^{Aa}	3.75±0.06^{Bb}	3.15±0.06Ca	3.35±0.05^{Ca}	3.51±0.05
	Mean	3.40±0.02	4.09±0.03	3.74±0.07	3.23±0.04	3.35±0.03	3.56±0.03
GL (g dL^{-1})	CG	3.58±0.15^{Aa}	2.63±0.14^{Ba}	2.88±0.16^{Ba}	3.71±0.26Aa	3.61±0.09^{Aa}	3.27±0.10
	DDG	3.38±0.13^{Aa}	2.59±0.11^{Ba}	3.57±0.19^{Aa}	3.58±0.09Aa	3.48±0.14^{Aa}	3.32±0.08
	DMSOG	3.64±0.09^{Aa}	2.60±0.08^{Ba}	2.75±0.17^{Bb}	3.54±0.11Aa	3.71±0.15^{Aa}	3.25±0.08
	Mean	3.54±0.07	2.61±0.06	3.06±0.12	3.60±0.09	3.60±0.08	3.28±0.05
AL:GL	CG	0.99±0.04^{Ba}	1.62±0.09^{Aa}	1.49±0.11^{Aa}	0.94±0.04Ba	0.93±0.03^{Ba}	1.20±0.05
	DDG	1.02±0.04^{Ba}	1.64±0.06^{Aa}	0.94±0.05^{Bb}	0.91±0.03Ba	0.98±0.04^{Ba}	1.10±0.04
	DMSOG	0.93±0.03^{Ba}	1.56±0.05^{Aa}	1.42±0.07^{Aa}	0.89±0.03Ba	0.91±0.04^{Ba}	1.14±0.04
	Mean	0.98±0.02	1.61±0.04	1.29±0.06	0.91±0.03	0.94±0.02	1.15±0.03
GSH (NPSH) (nmol mL^{-1}	CG	505.45±17.38^{Aa}	474.36±19.99^{ABa}	456.88±39.09^{ABa}	342.23±19.98Ba	400.86±42.02^{ABa}	437.87±15.25
	DDG	433.81±16.54^{ABa}	518.05±40.57^{Aa}	551.00±38.14^{Aa}	451.43±24.47Aba	343.55±14.70^{Ba}	459.57±16.12
	DMSOG	513.04±31.29^{Aa}	495.72±33.20^{Aa}	472.40±34.23^{ABa}	388.01±25.28Aba	350.49±14.34^{Ba}	443.93±15.04
	Mean	485.03±14.61	496.03±18.40	492.75±21.97	395.42±15.55	364.50±15.41	447.08±8.90

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TBARS (nmol MDA mL^{-1})	CG	15.83±1.19	15.91±0.90	13.85±0.59	23.29±11.62	10.54±0.55	15.73±2.14
	DDG	14.73±0.95	15.58±0.88	14.40±0.81	12.14±0.42	10.15±0.45	13.40±0.42
	DMSOG	14.20±0.92	14.80±1.03	15.33±1.00	11.98±1.03	10.45±0.40	13.35±0.46
	Mean	14.89±0.58	15.41±0.53	14.56±0.48	15.42±3.50	10.38±0.26	14.12±0.72
FRAP (µg Eq, AA)	CG	147.17±5.22	148.33±3.41	162.17±2.49	145.70±5.99	144.17±5.92	149.59±2.25
	DDG	144.00±10.16	137.00±5.90	162.83±7.93	176.33±15.86	133.67±7.01	150.77±4.88
	DMSOG	141.55±6.07	144.58±4.39	183.36±17.75	149.42±5.80	136.24±6.36	151.03±4.65
	Mean	144.15±4.14^{B}	143.34±2.74^{B}	169.90±6.87^{A}	157.28±6.29AB	137.97±3.69^{B}	150.48±2.39

*Different uppercase letters in the row and lowercase letters in the column differ statistically by Tukey's test at 5% significance (P<0.05). Variables: ADG - average daily gain, TP - total protein, AL - albumin, GL - globulin, AL:GL - albumin:globulin ratio, GSH - reduced glutathione, TBARS - thiobarbituric acid reactive substances; and FRAP - ferric reducing antioxidant potential of beef calves subjected to conventional weaning. Distributed into experimental groups: CG - control group, DDG - diphenyl diselenide group, DMSOG - dimethyl sulfoxide group, at time points -28 (baseline; T1) and -14 (T2) days before weaning, on the initial day of weaning (0; T3), and 14 (T4) and 28 (T5) days after weaning.

Regarding ADG, a significant difference was observed only for time points (P<0.0001) (Table 4). Diphenyl diselenide partially mimics the "physiological chemistry" of selenium in mammalian bodies (Nogueira & Rocha, 2010). As an organic compound, when absorbed, it is directly incorporated into muscle proteins selenomethionine and selenocysteine (Combs, 2015; NRC, 2016). Organic sources exhibit a greater capacity to express the gene in muscle selenoproteins (Zhang et al., 2019). Selenium is a crucial component of 20 to 25 selenoproteins (Lobanov et al., 2009), including iodothyronine deiodinase, regulating the production of the active hormone T3 (triiodothyronine) in peripheral tissues and the thyroid, aiding in metabolism and animal growth (Rooke et al., 2004). Therefore, selenium plays a pivotal role in the development of young animals, particularly during the initial growth stages (Ganie et al., 2010). In sheep, a single intravenous administration of $(PhSe)_2$ maintained high plasma selenium concentrations for 15 days without signs of toxicity throughout the experimental period (Leal et al., 2018). Santos et al. (2019) reported that dairy calves treated with $(PhSe)_2$ at weaning gained more weight compared to other groups at 270 days of life. In the study by Rodrigues et al. (2020), an increase in weight and ADG was observed in the group of dairy female calves treated with $(PhSe)_2$ throughout the entire experimental period, with the highest weight and ADG values detected at the time of weaning at 70 days of life, where treated female calves gained 7.7 kg. It is essential to note that when weaning beef calves, suckling is stopped abruptly, whereas weaning dairy calves occurs gradually, with less expected weight loss.

For the total protein variable, no significant effects of treatments (P = 0.4184), time points (P = 0.1383), or treatment × time point interaction (P = 0.2075) were observed (Table 4). Reference ranges for total protein in cattle are between 6.74-7.46 g dL^{-1} (Kaneko et al., 2008). Total protein comprises albumin, globulin (α, β, γ), and fibrinogen, with its synthesis occurring in the liver. It serves as an indicator of the overall health and nutrition status of cattle (González & Silva, 2017). In the bloodstream, selenium is associated with α- and β-globulins, and albumin (Schrauzer, 2000). Santos et al. (2019) noted an increase in total protein levels in Holstein calves treated with $(PhSe)_2$ at a dose of 3 μmol kg^{-1}, combined with zinc edetate; the levels in this group were highest on day 70 after administration. Rodrigues et al. (2020) observed no difference in the total protein of Holstein female calves between the groups; however, they detected higher values on day 70 (weaning) in the $(PhSe)_2$ group, after four administrations at a dose of 1.5 μmol kg^{-1}.

The albumin variable was significantly affected by treatments (P = 0.0016), time points (P<0.0001), and treatment × time interaction (P<0.0001) (Table 4). There was a statistical difference between treatments for albumin at T3, with the CG exhibiting the highest means (Table 5). Concerning the differences within the DDG over time points, there was an increase in values from T1 to T2, followed by a significant decrease from T2 to the subsequent time points (T3, T4, and T5) (Table 5). Reference ranges for albumin in cattle are between 3.03-3.55 g dL^{-1} (Kaneko et al., 2008). Albumin, the principal and most abundant plasma protein, constitutes around 50% of total proteins and is the most stable fraction (González & Silva, 2017). Exclusively synthesized by the liver, albumin serves as a crucial source of plasma sulfhydryl, acting as a significant extracellular antioxidant (Halliwell & Gutteridge, 1989). It is also considered a negative acute phase protein, with its synthesis and concentration decreasing in inflammatory processes (Ceciliani et al., 2012). The observed oscillation in DDG may be attributed to selenium's predominant association with albumin in plasma, where albumin contributes 80% of plasma osmolarity, functioning as a selenium transporter (Schrauzer, 2000; Mehdi & Dufrasne, 2016). This suggests that weaning poses a challenging management scenario for calves body homeostasis.

Globulin showed a significant effect of time points (P<0.0001) and treatment × time interaction (P = 0.0001) (Table 4). There was a statistical difference between DMSOG and the other treatments (CG and DDG) for the globulin variable at T3, with DMSOG displaying the lowest means (Table 5). Regarding the differences within the DDG over time points, there was a reduction in values from T1 to T2, followed by a significant increase from T2 to the subsequent time points (T3, T4, and T5) (Table 5). Reference ranges for globulin in cattle are between 3.00-3.48 g dL^{-1} (Kaneko et al., 2008). Globulin, comprising α, β, and γ fractions, may reflect changes in humoral immunity, inflammatory or infectious processes, and can be informative about adaptation to stress (González & Silva, 2017). The γ globulin fraction includes immunologically active substances, and antibody formation relies exclusively on the percentage of this protein fraction, which consists of immunoglobulins

A, M, E, G, and some enzymes; IgG typically represents 85% of this fraction (Kaneko et al., 2008). The administration of $(PhSe)_2$ may have led to a significant increase from T2 to the subsequent time points (T3, T4, and T5), as selenium in the blood is linked to the α and β-globulin fractions, playing an important role in selenium transport (Schrauzer, 2000). During the weaning of Holstein calves, Santos et al. (2019) also observed an increase in globulin in the $(PhSe)_2$ group associated with zinc edetate, with the highest levels on day 70. In the study by Rodrigues et al. (2020), with Holstein calves from the neonatal period until weaning at 70 days of age, metaphylaxis with $(PhSe)_2$ increased immunoglobulin G, enhancing humoral immunity.

For the albumin:globulin ratio, significant effects of time points ($P<0.0001$) and treatment × time interaction ($P<0.0001$) were detected (Table 4). There was a statistical difference between the DDG and the other groups for this variable at T3, with the DDG showing the lowest means (Table 5). As regards the differences within the DDG across time points, there was an increase in values from T1 to T2, followed by a significant decrease from T2 to the subsequent time points (T3, T4, and T5) (Table 5). Reference ranges for the albumin:globulin ratio in cattle are between 0.84-0.94 (Kaneko et al., 2008). To maintain blood osmotic balance, serum concentrations of globulin and albumin are inversely proportional, and alterations in this ratio often manifest as early signs of changes in the protein profile (Kaneko et al., 2008). Therefore, an increase in globulin appears to correspond with a simultaneous reduction in albumin, possibly involving an exchange between albumin and α-globulin (Herz & Hod, 1969; Jain, 1986). This ratio also provides information about the nutritional status of cattle (Amorim et al., 2007), being particularly relevant in cases of infections (Silva et al., 2008).

Reduced glutathione differed significantly in response to time points ($P<0.0001$) and the treatment × time interaction ($P = 0.0199$) (Table 4). However, no statistical difference between treatments was found for this variable (Table 5). Regarding the differences within the DDG across time points, a significant reduction in activity was noted from T2 and T3 to T5 (Table 5). The primary antioxidant capacity is derived from non-enzymatic antioxidants, such as reduced glutathione (Halliwell & Gutteridge, 1989), characterized by its low molecular weight. Reduced glutathione, a tripeptide formed by glycine, glutamic acid, and cysteine, constitutes the most abundant reducing thiol in the intracellular environment (Ribeiro et al., 2005). Its reducing activity is attributed to its sulfhydryl group (Vasconcelos et al., 2007). While it is primarily found in plasma, reduced glutathione is also present in other extracellular and intracellular fluids. The activity of reduced glutathione is directly dependent on selenium levels in the body, making it a valuable indicator of long-term selenium status in the blood (González & Silva, 2017). The antioxidant activity of $(PhSe)_2$ is, at least in part, attributed to the induction of reduced glutathione. Although it is relatively easy to increase reduced glutathione levels by administering precursor agents like cysteine, replenishing depleted levels in the body is considerably more challenging (Nogueira & Rocha, 2010). Low concentrations of reduced glutathione may be linked to compromised

animal health, as it plays a fundamental role in regulating the immune response, particularly when combined with cysteine (Dröge et al., 1994). Selenium is also an integral part of the enzyme glutathione peroxidase, which can decompose hydrogen peroxide and organic hydroperoxides (Nogueira et al., 2004). In studies by Santos et al. (2019) and Rodrigues et al. (2020), evaluating the weaning of dairy calves and female calves, respectively, erythrocyte glutathione peroxidase activity was assessed with $(PhSe)_2$. Santos et al. (2019) observed an increase in glutathione peroxidase activity with the administration of $(PhSe)_2$ alone or in combination with zinc edetate. Rodrigues et al. (2020) reported no difference in glutathione peroxidase activity between the groups. Meanwhile, Biazus et al. (2018), working with recently lambed dairy sheep, detected higher serum activity of glutathione-S-transferase and glutathione peroxidase in the $(PhSe)_2$ group.

Thiobarbituric acid reactive substances were not significantly affected by treatments (P = 0.2703), time points (P = 0.1099), or their interaction (P = 0.4121) (Table 4). Thiobarbituric acid reactive substances, indicative of lipid peroxidation byproducts (Ohkawa, 1979), are particularly associated with the oxidation susceptibility of polyunsaturated lipids (Miller et al., 1993) and are recognized as reliable biomarkers for oxidative stress (Georgieva, 2005). In a study by Santos et al. (2019), the $(PhSe)_2$ group, in conjunction with zinc edetate, exhibited reduced blood and muscle TBARS concentrations compared to isolated $(PhSe)_2$ and zinc edetate groups, suggesting diminished lipid peroxidation. Contrarily, Rodrigues et al. (2020) found no variance in TBARS levels between groups or time points within each group, indicating a consistent absence of oxidative stress throughout the experimental period. Biazus et al. (2018) reported lower serum TBARS concentrations in the $(PhSe)_2$ group only on days 45, 60, and 65, implying lipid peroxidation during these specific experimental intervals.

Ferric reducing antioxidant potential exhibited significant differences only between time points (P<0.0001) (Table 4). Total antioxidant capacity can be analyzed using several methods, one of which is the ferric reducing capacity of the sample (Benzie & Strain, 1996). In the absence of reference values, comparing the impact between treated and untreated groups provides insights into nutritional and health status (Celi, 2011). Weaning systems involving transport and entry into the feedlot often lead to reduced antioxidant capacity, which is fully restored within 28 to 60 days (Chirase et al., 2004; Pregel et al., 2005). Total antioxidant capacity reflects the cumulative action of all antioxidants in the sample, offering an integrative parameter that describes the dynamic equilibrium between oxidants and antioxidants (Ghiselli et al., 2000). Diminished antioxidant concentrations may compromise the body's ability to detoxify oxidants generated by cells during aerobic metabolism.

Conclusions

The administration of diphenyl diselenide to beef female calves led to increased levels of albumin, globulin, and albumin:globulin ratio, along with increased

reduced glutathione during conventional weaning. The positive impact of diphenyl diselenide was further validated by the observed interactions between treatment and time points. These findings demonstrate the potential of diphenyl diselenide as an alternative to conventional sources of organic selenium, and its use minimizes the challenges faced by beef calves during the process of conventional weaning.

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