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Benefits of supplementation with diphenyl diselenide in dairy cows in transition period: metabolic, immune and antioxidant effects

[*Benefícios da suplementação com disseleneto de difenila em vacas leiteiras no período de transição: efeitos metabólicos, imunes e antioxidantes*]

C.M. *Rodrigues*[1], M.L. *Moraes*[1], M. *Schneider*[2], S.G. *Collet*[3], A.M. *Prestes*[3], F.W.S. *Cibin*[4], V. *Gomes*[5], M.G. *Blagitz*[6], J.B.T. *Rocha*[1], M.L.R. *Leal*[1]

[1]Universidade Federal de Santa Maria, Santa Maria, RS, Brasil
[2]Unidade Central de Educação, FAEM Faculdades Ltda. (UCEFF Central), Chapecó, SC, Brasil
[3]Universidade do Oeste de Santa Catarina, UNOESC, Xanxerê, SC, Brasil
[4]Universidade Federal do Pampa, Uruguaiana, RS, Brasil
[5]Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, SP, Brasil
[6]Universidade Federal da Fronteira Sul, Realeza, PR, Brasil

ABSTRACT

The aim of this study was to evaluate the effects of diphenyl diselenide (PhSe) supplementation during the transition period on the metabolism, immunity, oxidative status, and milk production of postpartum dairy cows. Twenty-seven Holstein females were divided into groups: (PhSe) (DDG), which received 3 µmol/kg of $(PhSe)_2$ in 4 mL of dimethyl sulfoxide (DMSO) subcutaneously, DMSO (DMSOG), and NaCl (NACLG) which received 4 mL of DMSO and 0.9% NaCl. Evaluation of body condition score (BCS), weighing and administrations were performed at 42, 28, and 14 days prepartum, and on the day of calving (0). On days 0, 7, 14, 21, and 35 postpartum, BCS was evaluated, and blood were collected. Colostrum was obtained from the first postpartum milking. Production was measured, and milk was collected on days 7, 14, 21, and 35 postpartum. DDG showed higher values of total protein (TP) and globulins (GLOB) 7 days postpartum and increase from day 0 to days 21 and 35. DDG immunoglobulin G (IgG) was higher on days 21 and 35, and increased between days 7 and 21. In postpartum TP, GLOB and IgG are physiologically reduced, therefore, the results indicate that supplementation of transition females with (PhSe) stimulated postpartum humoral immunity.

Keywords: animal health, diphenyl diselenide, selenium, transition period

RESUMO

O objetivo deste estudo foi avaliar os efeitos da suplementação com disseleneto de difenila $(PhSe)_2$ durante o período de transição, no metabolismo, na imunidade, no status *oxidativo e na produção pós-parto de vacas leiteiras. Vinte e sete fêmeas Holandesas foram distribuídas nos grupos: $(PhSe)_2$ (GDD), recebeu µmol/kg de $(PhSe)_2$ em 4mL de dimetilsulfóxido (DMSO) subcutâneo; DMSO (GDMSO) e NaCl (GNACL), receberam 4mL de DMSO e NaCl 0,9%. Aos 42, 28 e 14 dias pré-parto e no dia do parto (0), avaliou-se escore de condição corporal (ECC) e realizou-se pesagem e administrações. Nos dias zero, sete, 14, 21 e 35 pós-parto, avaliou-se ECC e coletou-se sangue. Da primeira ordenha pós-parto, obteve-se colostro. A produção foi mensurada, e o leite coletado aos sete, 14, 21 e 35 dias pós-parto. O GDD apresentou valores superiores de proteína total (PT) e globulinas (GLOB) sete dias pós-parto, e incremento do dia zero para os dias 21 e 35. A imunoglobulina G (IgG) do GDD foi superior nos dias 21 e 35, e elevou-se entre os dias sete e 21. No pós-parto PT, GLOB e IgG encontram-se fisiologicamente reduzidas; portanto, os resultados indicam que a suplementação de fêmeas em transição com $(PhSe)_2$ estimulou a imunidade humoral pós-parto.*

Palavras-chave: saúde animal, disseleneto de difenila, selênio, período de transição

Corresponding author: claudiamedeirosrodrigues@gmail.com
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INTRODUCTION

The transition period includes the three weeks preceding and following calving (Grummer, 1995), during which physiological, nutritional, metabolic, and immunological changes occur to prepare the cow for calving, colostrogenesis, lactogenesis, and maintenance of lactation (Sordillo and Raphael, 2013). The late gestation period is also characterized by a reduction in dry matter intake (DMI), leading to a subsequent increase in energy demand, resulting in negative energy balance (NEB) (Grummer *et al.*, 2004).

NEB triggers a redirection of available glucose, which is sent to the mammary gland for lactose synthesis (Grummer, 1995). To meet the energy demand, the body undergoes lipolysis and uses non-esterified fatty acids (NEFA) as an alternative energy source (Sordillo and Raphael, 2013). In the liver, these NEFA can be used as a source of adenosine triphosphate (ATP), converted into ketone bodies, and re-esterified into triglycerides, which are then stored in tissues (Drackley *et al.*, 2001).

High levels of (NEFA) and ketone bodies are associated with compromised immune function, the occurrence of metabolic and infectious diseases, and a reduction in productive and reproductive performance (Sordillo and Raphael, 2013; Leblanc, 2010; Duffield *et al.*, 2009; Walsh *et al.*, 2007). The peripartum period also culminates in increased oxygen consumption and the consequent production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Celi, 2011). In addition to the deficit in the characteristic antioxidant defense capacity of this phase, oxidative stress (OS) may occur, predisposing animals to the occurrence of illnesses (Abuelo *et al.*, 2019).

The antioxidant defense system is divided into enzymatic and non-enzymatic components, with minerals in the latter category (Puppel *et al.*, 2015), we emphasize the importance of selenium (Se) in this work. The benefits of this element are well-established in the literature, as researchers have demonstrated since the 1980s that dairy cows receiving diets containing Se show greater capacity to maintain bodily defense mechanisms, including antibody and cytokine production, cell proliferation, prostaglandin metabolism, and cellular function in the innate immune defense system (Smith *et al*., 1984). Adequate intake of this trace mineral is also involved in preventing disorders such as white muscle disease, ovarian cysts, embryonic death, dystocia, postpartum paralysis, retained placenta, and metritis (Mehdi and Dufrasne, 2016) in addition to play a crucial role in mammary gland health, preventing subclinical and clinical mastitis (Machado *et al.*, 2013).

Among the available forms of Se, diphenyl diselenide $(PhSe)_2$, an organic compound, has proven to be an efficient alternative in the treatment and/or prevention of various conditions (Nogueira *et al.*, 2004). The literature describes anti-inflammatory, antioxidant, and immunomodulatory properties (Meotti *et al.*, 2004; Menezes *et al.*, 2012; Stefanello *et al.*, 2015; Doleski *et al.*, 2017; Roza *et al.*, 2018) of this compound in different animal species. Its *in vivo* distribution in ruminants was initially studied in sheep, and through this work, it was established that the primary deposition organ is the liver. From the liver, diphenyl diselenide $(PhSe)_2$ is slowly released into circulation, additionally, there were no apparent signs of toxicity after its administration (Leal *et al.*, 2018).

This study enabled its use in other research involving ruminants, where it demonstrated positive effects on the development of Dutch calves during weaning, anti-inflammatory action, and antioxidant effects when combined with zinc (Santos *et al.*, 2019). In dairy ewes, it demonstrated the ability to modulate oxidative and inflammatory reactions, increase in milk fat and its antioxidant potential, resulting in a reduction in protein oxidation and the production of milk with nutraceutical properties (Biazus *et al.*, 2019). Moreover, when used in Dutch calves from birth to weaning, it increased immunoglobulin G levels and the animals' weight gain (Rodrigues *et al.*, 2020).

Despite studies demonstrating the benefits of selenium supplementation in cows in the transition period, there are no studies using diphenyl diselenide in this animal category. Therefore, we believe that the anti-inflammatory, antioxidant, and immunomodulatory properties of this compound may reduce the deleterious effects triggered by the events in the peripartum period. The aim of this study is to evaluate whether

supplementation with diphenyl diselenide $(PhSe)_2$ during the transition period triggers effects on the metabolism, immunity, oxidative status, and milk production of postpartum dairy cows.

MATERIAL AND METHODS

The project was approved by the Ethics Committee on Animal Use (CEUA) of the Federal University of Santa Maria (UFSM) (protocol number 8663120721). The study was conducted on two commercial dairy farms located in the municipalities of Abelardo Luz (26º 34' 2" S and 52º 20' 2" W) and Faxinal dos Guedes (26º 51' 10" S and 52º 15' 37" W), situated in the western region of the state of Santa Catarina, characterized by a humid subtropical climate. The execution took place between October 2021 and August 2022, with a defined interval between December and March due to the occurrence of thermal stress evident during the summer.

Twenty-seven multiparous Holstein females (*n* = 27) in the transition period were used. Initially, they underwent a physical examination (Dirksen *et al.*, 1993) to assess their health status, ensuring that animals with clinical disorders were not included in the study. The selected females had a body condition score ranging from 3 to 3.75 (where 1 represents thin and 5 obese) and an average daily production in the previous lactation of approximately 35 liters of milk per day.

The cows remained within the routine of the farms and were not subjected to changes in management and diet, keeping the established routine on the farms throughout the study. In the first farm, the cows were kept in a compost barn system during the prepartum period and in a free-stall structure after calving, and in the second farm, they were housed in a compost barn throughout the experimental period. The diet was based on the supply of corn silage, ryegrass hay, concentrate, vitamin and mineral supplement (Table 1), and water *ad libitum*. The animals underwent two daily milkings (05:00 and 17:00).

The twenty-seven animals (*n* = 27) were distributed into three groups, each containing nine animals (*n* = 9/ treatment), based on the body condition score (BCS) presented on day 42 prepartum (Fig. 1). The diphenyl diselenide group (DDG) received 3µmol/kg of $(PhSe)_2$, diluted in 4mL of dimethyl sulfoxide (DMSO), administered subcutaneously (SC). Diphenyl diselenide ($(PhSe)_2$; 98%) was obtained from Sigma-Aldrich® (cat. no. 180629, St Louis, MO, USA), and it was diluted in dimethyl sulfoxide (C_2H_6SO; 99.2%) at the time of administrations. The dimethyl sulfoxide group (DMSOG) and the NaCl group (NACLG) received, respectively, 4 mL of DMSO and 0.9% NaCl, through the same route. Therefore, at 42, 28, and 14 days prepartum, and on the calving day (day 0), the females were weighed, and their body condition score (BCS) was assessed, followed by the administration of treatments.

On days 0 (calving day), 7, 14, 21, and 35 postpartum, blood samples were also collected by coccygeal venipuncture with a 21 G needle, using a vacuum collection system (BD Vacutainer®, Franklin Lakes, NJ, USA), in tubes containing ethylenediaminetetraacetic acid (EDTA) (4 mL) and without anticoagulant (9mL).

From the first postpartum milking (day 0), colostrum was obtained and stored in a sterile universal collection bottle (50mL) at -20ºC. The milk production was measured using an automatic weighing system, and milk samples were collected in sterile bottles (50 mL) containing bronopol and azidiol preservative tablets on days 7, 14, 21, and 35 postpartum. The bottles remained refrigerated (1ºC to 7ºC) for a maximum period of 72 hours until arrival at the laboratory, where the analyses were conducted. Shipment was carried out in thermal boxes containing ice for temperature maintenance.

Plasma fibrinogen was determined according to the methodology described by Kaneko *et al.* (2008). Beta-hydroxybutyrate (BHB) was measured using a portable digital device, Free Style Optium Neo® (Abbott®, Chicago, IL, USA), using blood β-ketone test strips (Abbott®, Chicago, IL, USA). Biochemical parameters: calcium (Ca), fructosamine (FRUC), total protein (TP), and albumin (ALB) were determined using commercial kits (Bioclin®, Belo Horizonte, MG, BR) on an automatic biochemical analyzer (BS 230 Mindray®, Mahwah, NJ, USA). To obtain globulin values (GLOB) and the albumin-globulin ratio (A:G),

the difference between TP and ALB values (TP – ALB = GLOB) and the ratio between ALB and GLOB fractions (ALB/GLOB = A:G) were calculated, respectively.

Table 1. Composition of the diet fed to Holstein cows (prepartum and postpartum), submitted to supplementation or not with diphenyl diselenide at 42, 28, and 14 days prior to calving, and on parturition date (0), and distributed in groups: diphenyl diselenide (DDG), dimethyl sulfoxide (DMSOG) and Na Cl (NACLG).

Ingredients		Values	
		Prepartum	Postpartum
Corn silage	(% DM[3])	64.07	45.01
Ryegrass hay	(% DM[3])	12.00	20.49
Corn grain	(% DM[3])		10.21
Soybean meal, extruded	(% DM[3])	11.23	13.44
Soybean meal, expellers	(% DM[3])		1.73
Soybean hulls	(% DM[3])	10.63	6.85
Urea	(% DM[3])		0.19
Premix	(% DM[3])	2.07[1]	2.08[2]
Chemical composition			
DM[3]	(%)	49.80	57.60
CP[4]	(% DM[3])	11.30	13.40
ADF[5]	(% DM[3])	26.50	24.40
NDF[6]	(% DM[3])	42.60	39.80
Starch	(% DM[3])	23.30	23.90
Fatty acids	(% DM[3])	3.42	3.90
Ca[7]	(% DM[3])	0.51	0.60
P[8]	(% DM[3])	0.31	0.32
ME[9]	(Mcal/kg)	2.57	2.60
NEL[10]	(Mcal/kg)	1.70	1.71
Se[11]	(mg/kg)	0.41	0.61

[1]Minimal vitamin and mineral levels per kg of product (prepartum): calcium (106 g); phosphorus (30 g); sulfur (90 g); magnesium (20 g); sodium (31 g); chlorine (130 g); cobalt (12 mg); cooper (600 mg); chromium (30 mg); iron (600 mg); iodine (60 mg); manganese (1.600 mg); selenium (16 mg); zinc (2.400); biotin (80 mg); vitamin A (480.000 IU); vitamin D3 (200.000 IU); vitamin E (12.000 IU); *Saccharomyces cerevisiae* (1.5 x 10^9 CFU); monensin (500 mg); fluorine (300 mg).[2]Minimal vitamin and mineral levels per kg of product (postpartum): calcium (160 g); phosphorus (20 g); magnesium (40 g); sodium (85 g); sulfur (18 g); chlorine (88 g); cobalt (55 mg); cooper (680 mg); iodine (60 mg); manganese (1.100 mg); selenium (25 mg); zinc (3000 mg); chromium (15 mg); fluorine (200 mg); vitamin A (300.000 IU); vitamin D (100.000 IU); vitamin E (2.000 IU); biotin (60 mg); monensin sodium (600 mg); protected choline (150 mg); *Saccharomyces cerevisiae* (1.5 x 10^{10} CFU); *Bacillus cereus* (5.2 x 10^8 CFU); *Enterococcus faecium* (5.2 x 10^8 CFU); *Lactobacillus acidophilus* (5.2 x 10^8 CFU); *Ruminobacter amylophilum* (4.5 x 10^8 CFU); *Ruminobacter succinogenes* (4.5 x 10^8 CFU); *Succinovibrio dextrinisilvens* (4.5 x 10^8 CFU); adsorbent (60 g); yeast cell extract (12.50 g); antioxidant (150 mg).[3]Dry matter (DM); [4]Crude protein (CP); [5]Acid detergent fiber (ADF); [6]Neutral detergent fiber (NDF); [7]Calcium (Ca);[8]Phosphorus (P); [9]Metabolizable energy (ME); [10]Net energy for lactation (NEL); [11]Selenium (Se).

Immunoglobulin G (IgG) was measured by sandwich ELISA according to the methodology described by Reber *et al*. (2008). Lipid peroxidation was measured through the formation of thiobarbituric acid reactive substances (TBARS) (Ohkawa, 1979). Meanwhile, glutathione (GSH) was determined by spectrophotometry using the Ellman method (1959).

The colostrum was thawed at room temperature and divided into two portions, one of them was used for evaluation in the Brix refractometer (model BTX-1, Vee Gee Scientific®, Vernon Hills, IL, USA). While the other part of the sample was centrifuged at 10,000 xg for 30 minutes to obtain the liquid/protein fraction, in which the concentrations of IgG were determined by sandwich ELISA (Reber *et al.*, 2008).

The samples containing bronopol were used for somatic cell count (SCC), and those containing azidiol for total bacterial count (TBC), both

determined by flow cytometry, following ISO 13366-2 (IDF 148-2) and ISO 4833-1, ISO 16297 (IDF 161), ISO 21187 (IDF 196), respectively. The analyses were carried out at the Centralized Milk Analysis Laboratory of the Milk Herd Analysis Program (PARLEITE), accredited in the Brazilian Milk Quality Network (RBQL).

The assumptions of normality, homoscedasticity, and independence of residuals were previously tested using the Shapiro-Wilk test. The response variables were analyzed using a linear model with repeated measures over time, satisfying the following statistical model: $Y_{ijkl} = \mu + TREAT_i + MOMENT_j + (TREAT \times MOMENT)_{ij} + \varepsilon_{ijkl}$; in wich: Y_{ijkl} = response variables; μ = overall mean of all observations; $TRAT_i$ = treatments (GNACL, GDMSO and GDD); $MOMENT_j$ = assessment moments (0, 7, 14, 21 and 35 days); $(TRAT \times MOMENTO)_{ij}$ = interaction effect between treatments and assessment timepoints; ε_{ijkl} = random error associated with each observation, assumed to be ~ NID (0, σ^2_ε). If there was a statistical difference, the Tukey's mean comparison test was performed. For this analysis, the R Studio statistical software (R CORE TEAM, 2013, Vienna, AUT) was used with a significance level of 5% ($p < 0.05$). The results are presented as mean and standard error.

RESULTS AND DISCUSSION

In order to monitor the energy metabolism of females throughout the postpartum period, BCS, BHB, and FRUC (Fig. 1) were determined, as one of the processing methods of NEFAs in the liver is their conversion into ketone bodies, giving rise to the intermediate metabolite acetone, acetoacetate, and BHB (Drackley *et al.*, 2001). In this study, there was no significant difference ($p = 0.280$) in BHB levels between the groups, however, in the comparison between time points, the DMSOG showed a significant increase between days 0 and 14 ($p = 0.011$). Gong and Xiao (2021) also did not detect differences in BHB concentrations in dairy cows supplemented with Se-yeast. As BHB is commonly used in determining the intensity and adaptation to NEB (Leblanc, 2010), we can conclude that the animals were able to metabolize mobilized NEFAs, thereby releasing low concentrations of BHB into circulation.

The results of FRUC (Fig. 1) confirm this hypothesis, as they also remained relatively stable, only DMSOG showed significantly higher values compared to DDG on day 0 and to the other groups NACLG and DDG on day 7 ($p = 0.001$). However, no differences were observed in the comparison between moments within the groups ($p = 0.589$). When observing FRUC levels, values above the reference range for the species (213 to 265 µmol/L) (Jensen *et al*., 1993) were noted in all groups during the experimental period. We believe this result occurred due to the NEB, characteristic of transitioning cows, since the determination of fructosamine is as an alternative to measuring glucose when assessing energy metabolism, as both have a positive relationship, and fructosamine concentrations vary according to glycation that occurred during the previous two weeks, signaling early deficiencies in energy supply (Thrall *et al.*, 2007).

Among the factors that may have contributed to these findings are the average BCS values (Fig. 1), which varied between groups on day 7 postpartum, with DDG showing a higher score than NACLG ($p < 0.001$). Despite the difference between treatments, the values remained below 3.75. It is known that cows with a higher BCS, i.e., > 3.75, consume less food, consequently, more intensely reduce body condition, mobilize more lipids for use as an alternative source of energy, and exhibit higher blood concentrations of BHB, which is closely related to the occurrence of metabolic and infectious disorders (Bernabucci *et al.*, 2005). Moreover, the mean values presented for BHB remained < 1.4 mmol/L, thus demonstrating the absence of subclinical ketosis (Leblanc, 2010), with a single exception on day 14 for DMSOG where values were higher. In the DMSO and DD groups, this significant reduction in BCS ($p < 0.001$), possibly triggered by the mobilization of fat reserves for milk production, occurred between days 28 and 14 prepartum and 35 postpartum (DMSOG), and from day 14 prepartum to days 21 and 35 postpartum (GDD).

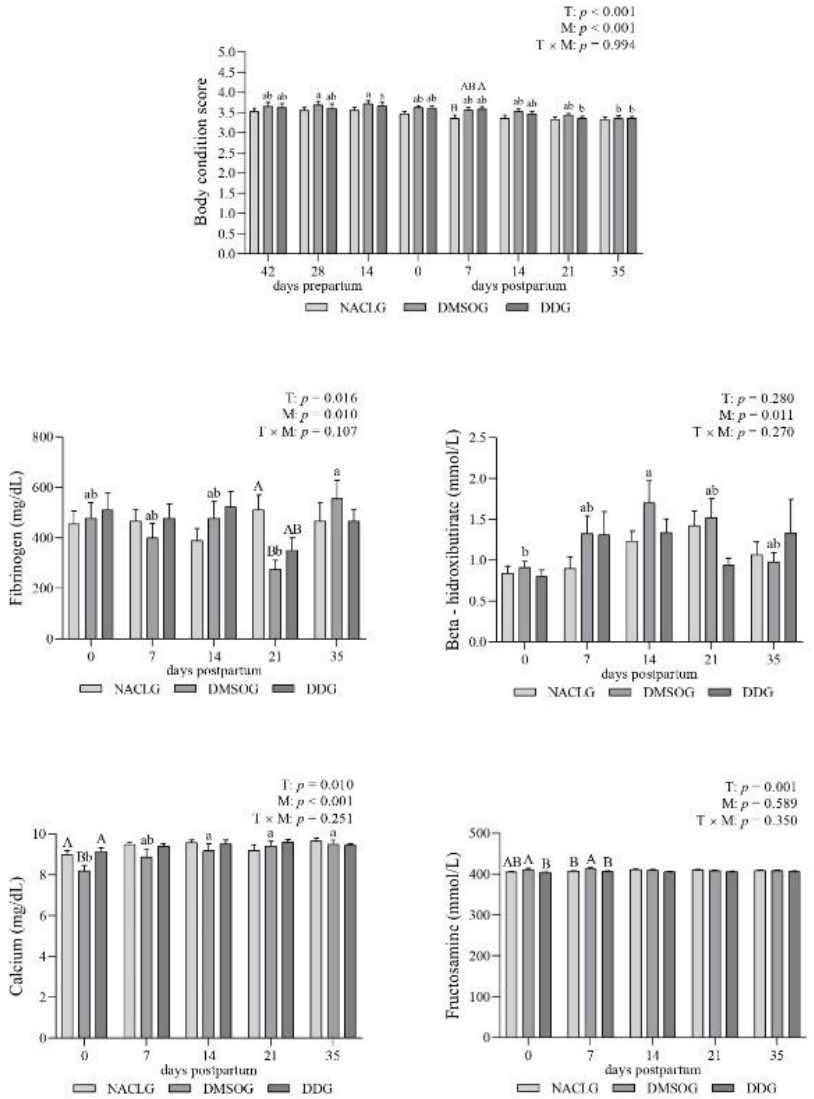

Figure 1. Average ± standard error and *p* value of body condition score, fibrinogen (mg/dL), beta-hidroxibutirate (mmol/L), calcium (mg/dL) and fructosamine (mmol/L), of Holstein cows from parturition (day 0) to 7, 14, 21 and 35 days postpartum, who were submitted to supplementation or not with diphenyl diselenide at 42, 28, and 14 days prior to calving, and on the date of parturition (day 0). The cows were distributed into groups: diphenyl diselenide (DDG), dimethyl sulfoxide (DMSOG) and Na Cl (NACLG) (average ± standard error, *n* = 9/treatment). Distinct capital letters refer to differences ($p < 0.05$) between groups on the respective days, while lowercase letters refer to differences ($p < 0.05$) between days within groups. T = treatment effect, M= moment effect, and T × M = interaction treatment moment.

During the transition period, the establishment of a pro-inflammatory state is also commonly described (Sordillo and Raphael, 2013), however, in this study, only the fibrinogen levels (Fig. 1) in the NACLG were found to be higher ($p = 0.016$) than those in the DMSOG on day 21, and throughout the experimental period, values for both groups remained within the reference range for the species (300 to 700 mg/dL) (Kaneko *et al.*, 2008). In the comparison between days within the groups, an increase was observed only between days 21 and 35 in the DMSOG ($p = 0.010$). Since plasma fibrinogen is a positive acute-phase protein, meaning it rises in response to an inflammatory process (Thrall *et al*., 2007),

we can consider the possibility that this situation did not occur during the study.

The serum calcium concentration (Fig. 1) of the animals was also measured, as it is a parameter that allows the identification and monitoring of hypocalcemia, a condition that is involved in the prevalence and incidence of other diseases (Leblanc, 2010). The cows in the DMSOG group had lower serum concentrations than the other groups on the day of calving (day 0) ($p = 0.010$), indicative of subclinical hypocalcemia. Because of the low levels detected on the calving day, the DMSOG showed a significant increase in serum calcium concentration on days 14, 21, and 35 ($p < 0.001$). The remaining values in all groups remained stable within the physiological range (8.5 to 10 mg/dL) (Goff *et al*., 2014), indicating that the animals remained healthy throughout the rest of the postpartum period.

According to the proximity of the calving date, there is a physiological decline in total protein (TP) concentrations due to the transfer of maternal immunoglobulins for colostrum synthesis in the mammary gland (Saut and Birgel Junior, 2008). In this study, we can observe that the animals that received supplementation with $(PhSe)_2$ showed an increase in total protein (TP) values (Fig. 2) on day 7 postpartum compared to the DMSOG ($p < 0.001$). Furthermore, the behavior of GLOB (Fig. 2) followed that of TP, with the DDG also showing higher values ($p < 0.001$) on day 7 compared to DMSOG. Within the groups, TP and GLOB exhibited mimetic activity, with DMSOG showing a significant increase in values ($p < 0.001$) from days 0 and 7 to days 21 and 35, and DDG from day 0 to days 21 and 35.

The concentrations of ALB and the A:G ratio (Fig. 2) did not indicate differences between groups ($p = 0.086$; $p = 0.725$). The same situation occurred within the groups for ALB values ($p = 0.751$), however, in the DMSOG, there was a decrease in the ratio between calving day (0) and days 21 and 35 ($p = 0.001$). Throughout the entire experimental period, the females in the DDG showed numerically higher values of TP, GLOB, and serum IgG compared to the other treatments, resulting in higher IgG results (Fig. 2) for DDG on days 21 and 35 postpartum, when compared to the other groups ($p = 0.002$), additionally, there was a significant increase between days 7 and 21 within the DDG ($p = 0.023$).

We believe that the administration of $(PhSe)_2$ contributed to the enhancement of humoral immunity in these animals, as concentrations of TP, GLOB, and IgG are physiologically reduced in the immediate postpartum period. Additionally, positive correlations between selenium levels and increased concentrations of IgG in serum and colostrum have been reported in dairy cows, consequently leading to elevated serum levels in their calves (Swecker *et al.*, 1995; Awadeh *et al.*, 1998; Hefnawy and Tortora-Pérez, 2010).

In a study comparing the provision of inorganic (sodium selenite) and organic Se sources (selenomethionine or selenium-enriched yeast), researchers found similar results to ours, for the animals receiving organically sourced Se experienced an increase in plasma immunoglobulin concentrations. The authors concluded that organic sources have advantages in improving animal immunity and are therefore a more suitable choice for bovine supplementation (Huang *et al*., 2023).

Although the mechanism by which $(PhSe)_2$ enhances immunoglobulin production is not fully elucidated, several plausible hypotheses exist. These include stimulation for selenoprotein synthesis (De Bem *et al.*, 2013), and modulation of anti-inflammatory and antioxidant pathways (Dias *et al.*, 2014). Furthermore, the immunostimulating effects of different chemical forms of Se have been frequently described in recent years (Golin *et al.*, 2023).

The Brix index, obtained through a Brix refractometer, provides a correlation to the total solids content of a solution, in the case of colostrum, which has an IgG concentration accounting for approximately 85 to 90% of the total solids, it is possible to infer colostral IgG concentrations through this evaluation (Godden *et al*., 2019). It is important to note that, in addition to the benefits attributed to immunoglobulins, colostrum also contains high levels of nutrients and bioactive compounds that stimulate postnatal growth and development (Hammon *et al.*, 2013).

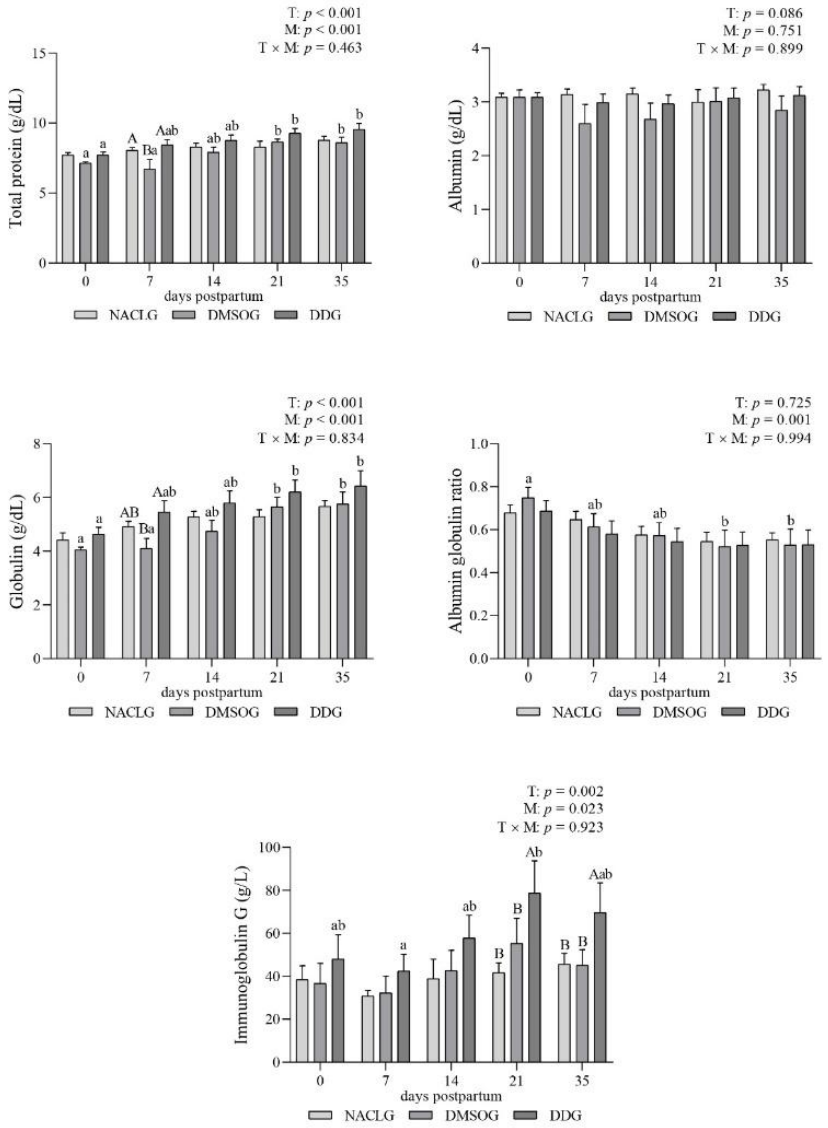

Figure 2. Average ± standard error and *p* value of total protein (g/dL), albumin (g/dL), globulin (g/dL), albumin globulin ratio and immunoglobulin G (g/L), of Holstein cows from parturition (day 0) to 7, 14, 21 and 35 days postpartum, who were submitted to supplementation or not with diphenyl diselenide at 42, 28, and 14 days prior to calving, and on the date of parturition (day 0). The cows were distributed into groups: diphenyl diselenide (DDG), dimethyl sulfoxide (DMSOG) and Na Cl (NACLG) (average ± standard error, n = 9/treatment). Distinct capital letters refer to differences ($p < 0.05$) between groups on the respective days, while lowercase letters refer to differences ($p < 0.05$) between days within groups. T = treatment effect, M= moment effect, and T × M = interaction treatment moment.

The colostral variables Brix and IgG (Fig. 3) did not differ between the groups (p = 0.874; p = 0.913), when observing their values, we realized that the colostrum obtained from the animals participating in the study is of excellent quality, as they exhibit a Brix index ≥ 22% and IgG concentrations ≥ 50g/L (Godden *et al.*, 2019). Despite the absence of statistical differences, colostrum samples from the studied females demonstrated values indicating the quality of the produced colostrum, that will have the primary function of provide passive immunity to newborn

calves until their own immune system is developed.

Regarding milk production, there were no significant diferences between treatments (p = 0.659) and moments (p = 0.466) (Fig. 3). These results are similar to those found by Biazus *et al*. (2019), who, when supplementing dairy ewes with diphenyl diselenide, also did not observe any effects on the volume of milk produced.

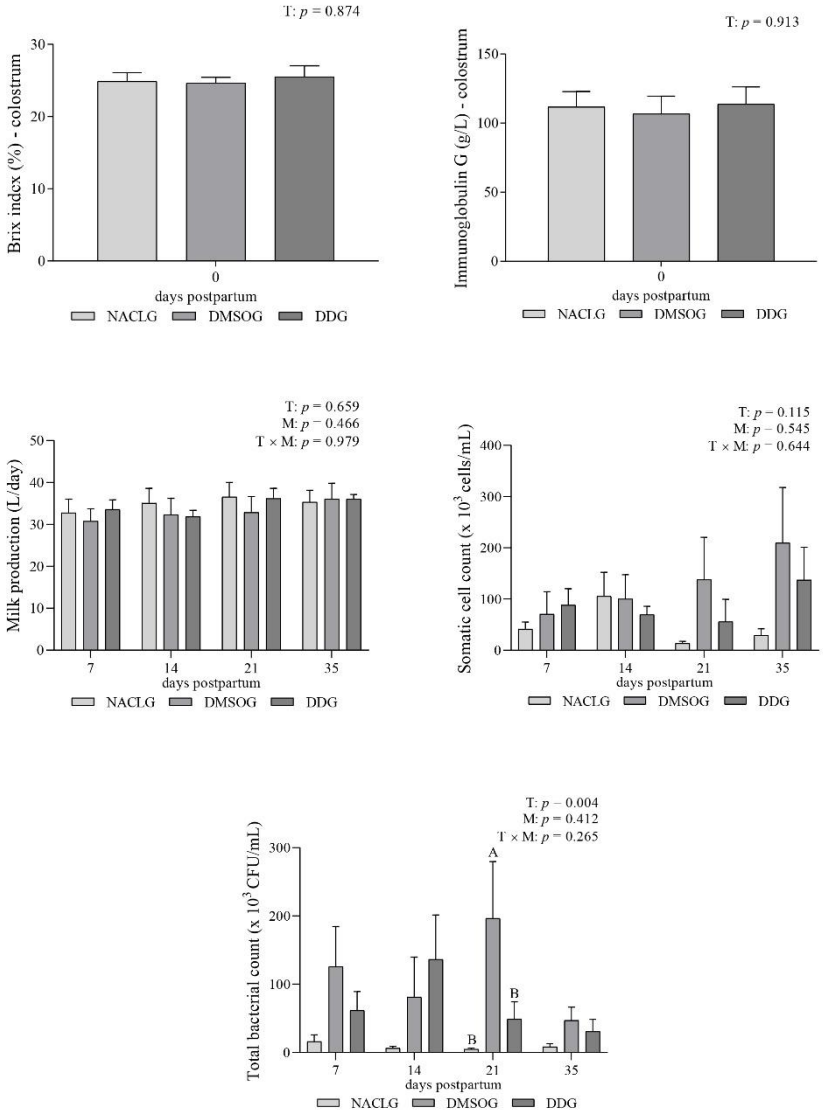

Figure 3. Average ± standard error and p value of colostrum brix index (%) and immunoglobulin G (g/L), milk production (L/day), somatic cell count (x10^3 cells/mL) and total bacterial count (x10^3 CFU/mL), of Holstein cows from parturition (day 0) to 7, 14, 21 and 35 days postpartum, who were submitted to supplementation or not with diphenyl diselenide at 42, 28, and 14 days prior to calving, and on the date of parturition (day 0). The cows were distributed into groups: diphenyl diselenide (DDG), dimethyl sulfoxide (DMSOG) and Na Cl (NACLG) (average ± standard error, n = 9/treatment). Distinct capital letters refer to differences ($p < 0.05$) between groups on the respective days, while lowercase letters refer to differences ($p < 0.05$) between days within groups. T = treatment effect, M= moment effect, and T × M = interaction treatment moment.

The individual assessment of SCC and TBC (Fig. 3) was used as a tool for the management and monitoring of mammary gland health and milk quality, and despite the well-known beneficial effects of selenium supplementation on mammary gland health (Mehdi and Dufrasne, 2016), there were no differences between treatments in SCC values (p = 0.115). As for TBC, higher values were detected on day 21 in the DMSOG compared to NACLG and DDG (p = 0.004). However, throughout the experimental periods, no differences were detected within the groups to SCC (p = 0.545) and TBC (p = 0.412). Nevertheless, the results in all groups during the experimental period remained within the acceptable maximum values for these variables, which are respectively between 500,000 cells/mL and 300,000 CFU/mL (Brazil, 2018), demonstrating the absence of subclinical mastitis cases.

The concentrations of thiobarbituric acid reactive substances (TBARS) are used to determine lipid peroxidation indices (Jentzsch *et al*., 1996). Regarding this parameter, there was no difference between the groups during the experimental period (Fig. 4; p = 0.526). Within the moments of each group, DMSOG showed a significant reduction between days 7 and 21 (p = 0.003). Another indication of the absence of OS is the fact that glutathione (GSH) levels (Fig. 4) also did not differ statistically between treatments (p = 0.520) and moments (p > 0.811). This finding is relevant because GSH is known to be essential for the enzyme glutathione peroxidase (GPx), which has selenium in its active site, to break down H_2O_2 into H_2O (Lu, 2013).

Although the antioxidant potential of Se is widely recognized, there is some discrepancy in the literature, because in certain studies, selenium supplementation has been shown to improve the ability to combat OS during the peripartum period (Sun *et al*., 2017, 2021; Gong and Xiao, 2018). However, non-significant relationships with the antioxidant status of lactating cows have been reported in other research (Hall *et al*., 2014; Hachemi *et al*., 2023). We believe that the differences in the literature regarding supplementation may be associated with the Se status of the animals (Respati *et al*., 2023). Since the selenium status of the females undergoing supplementation was unknown in this study, and considering our results and the referenced works, we believe that more studies are necessary to clarify the potential antioxidant effects of $(PhSe)_2$ in transition cows.

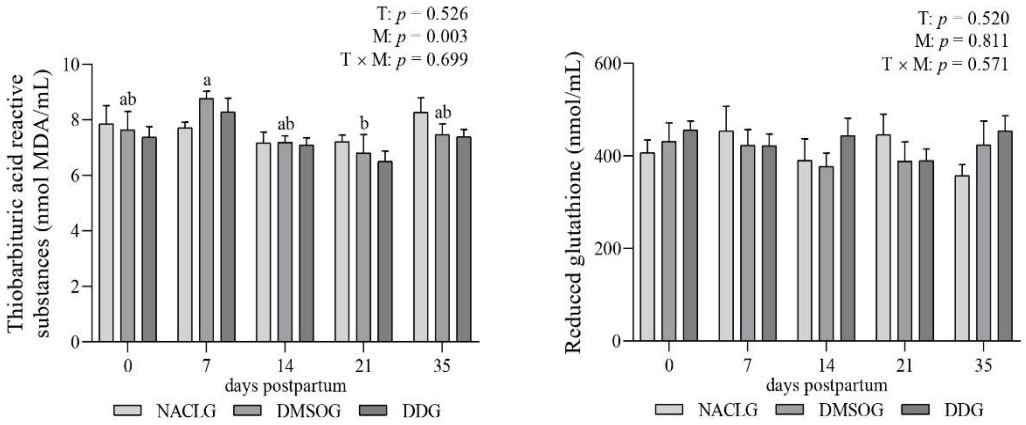

Figure 4. Average ± standard error and p value of thiobarbituric acid reactive substances (nmol MDA/mL) and reduced glutathione (nmol/mL), of Holstein cows from parturition (day 0) to 7, 14, 21 and 35 days postpartum, who were submitted to supplementation or not with diphenyl diselenide at 42, 28, and 14 days prior to calving, and on the date of parturition (day 0). The cows were distributed into groups: diphenyl diselenide (DDG), dimethyl sulfoxide (DMSOG) and Na Cl (NACLG) (average ± standard error, n = 9/treatment). Distinct capital letters refer to differences (p < 0.05) between groups on the respective days, while lowercase letters refer to differences (p < 0.05) between days within groups. T = treatment effect, M= moment effect, and T × M = interaction treatment moment.

CONCLUSION

The results allow us to conclude that supplementation with the organoselenium compound diphenyl diselenide $(PhSe)_2$, carried out during the transition period, was able to stimulate the humoral immunity of females in the postpartum period. Therefore, this study highlights a property of diphenyl diselenide that has been little reported so far – its immunomodulatory capacity. We believe that supplementation with this compound is a viable alternative for use in strategic moments, such as the transition period, during which cows are subjected to high metabolic demands and have reduced immune and antioxidant defenses, wich can result in incidence of diseases detected at this stage of the production cycle. We also emphasize that $(PhSe)_2$ can be administered parenterally, ensuring that the recommended dose is adequately applied, furthermore there were no signs of toxicity in the animals subjected to supplementation, demonstrating the safety of using this element.

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