

# Plant extracts supplied to pre-weaned dairy calves influence their redox status

## Extratos vegetais fornecidos a bezerros leiteiros pré-desmamados influenciam seu estado redox

Micheli de Paris<sup>1</sup>; Sheila Cristina Bosco Stivanin<sup>1</sup>; Guilherme Heisler<sup>1</sup>; Isabelle Damé Veber Angelo<sup>2</sup>; Arthur Fernandes Bettencourt<sup>2</sup>; Cristiane Matté<sup>3</sup>; Caroline Peres Klein<sup>4</sup>; Maira Balbinotti Zanela<sup>5</sup>; Vivian Fischer<sup>6\*</sup>

### Highlights

The effect of green tea and oregano on redox status was evaluated separately.  
Providing plant extracts improves some antioxidant biomarkers in Jersey calves.  
Oregano extract reduced carbonyl and DCFP concentrations.  
Oregano extract increased the activity of GPx and Catalase.

### Abstract

This study aimed to evaluate the effects of the separate provision of green and oregano tea extracts on the biomarkers of the redox state and health condition in pre-weaned Jersey calves from birth to 60 days of life. Two experiments following the complete randomized design with measures repeated in time were carried out using 38 Jersey calves (17 and 21 calves in experiments 1 and 2, respectively). Calves were distributed according to date of birth into one of three groups: control (CON) - with no addition of extracts; oregano extract (OE) - addition of 70 mg of oregano extract/kg of body weight (BW) and green tea extract (GT) - addition of 35 mg of green tea extract/kg of BW. Eight biomarkers of the redox state were evaluated on days 1, 30, and 60 after birth, and variables measured on day 1 were used as covariates. Body temperature and occurrence of diarrhea were evaluated every two days. Regarding the main results, the supply of oregano extract reduced the concentration of oxidizing biomarkers,

<sup>1</sup> PhD in Animal Science from the Postgraduate Program in Animal Science, Universidade Federal do Rio Grande do Sul, UFRGS, Porto Alegre, Brazil. E-mail: micheli.deparis@gmail.com; sheila\_bosco@hotmail.com; guilhermeheisler@hotmail.com

<sup>2</sup> PhD Students in Animal Science from the Postgraduate Program in Animal Science, UFRGS, Porto Alegre, Brazil. E-mail: isabelle.angelo@hotmail.com; arthurfbettencourt@gmail.com

<sup>3</sup> PhD., Prof<sup>a</sup>, Biochemistry Department, Health Basic Sciences Institute, UFRGS, Porto Alegre, RS, Brazil. E-mail: matte@ufrgs.br

<sup>4</sup> Post-doctoral student in the Biochemistry Department, Health Basic Sciences Institute, UFRGS, Porto Alegre, RS, Brazil. E-mail: lpkl@gmail.com

<sup>5</sup> PhD., Researcher, Brazilian Agricultural Research Corporation, Embrapa Centro de Agricultura Temperada, Capão do Leão, RS, Brazil. E-mail: maira.zanela@embrapa.br

<sup>6</sup> PhD., Prof<sup>a</sup>, Department of Animal Science, UFRGS, Porto Alegre, Brazil. E-mail: vivinha.fischer@hotmail.com

\* Author for correspondence

such as DCFP (oxidation of dichlorofluorescein in plasma) and carbonyl, and increased the activity of antioxidant enzymes, such as GPx and catalase. Green tea extract only reduced DCFP and tended to improve catalase activity. Calves remained healthy (no fever and only a few days with diarrhea), and plant extracts did not improve their health condition. The addition of green tea and oregano extracts into the diet has a positive effect on redox status in pre-weaned Jersey calves.

**Key words:** Green tea. Health. Jersey. Oregano.

## Resumo

Este estudo teve como objetivo avaliar os efeitos do fornecimento separado de extratos de chá verde e orégano sobre os biomarcadores do estado redox e condição de saúde em bezerras Jersey durante o seu aleitamento, desde o nascimento até 60 dias de vida. Foram realizados dois experimentos seguindo o delineamento completamente casualizado com medidas repetidas no tempo, utilizando 38 bezerros Jersey (17 e 21 bezerros nos experimentos 1 e 2, respectivamente). Os bezerros foram distribuídos de acordo com a data de nascimento em um dos três grupos: controle (CON) - sem adição de extratos; extrato de orégano (OE) - adição de 70 mg de extrato de orégano/kg de peso corporal (PV) e extrato de chá verde (GT) - adição de 35 mg de extrato de chá verde/kg de peso corporal. Oito biomarcadores do estado redox foram avaliados nos dias 1, 30 e 60 após o nascimento. A temperatura corporal e a ocorrência de diarreia foram avaliadas a cada dois dias. Quanto aos principais resultados, o fornecimento de extrato de orégano reduziu a concentração dos biomarcadores oxidantes, como DCFP (oxidação da diclorofluoresceína no plasma) e carbonila, e aumentou a atividade das enzimas antioxidantes, como GPx e catalase. O extrato de chá-verde reduziu apenas DCFP e tendeu a melhorar a atividade da catalase. Os bezerros permaneceram saudáveis (sem febre e apresentando diarreia por apenas alguns dias) e os extratos de plantas não melhoraram sua condição de saúde. A adição de extrato de chá verde e de orégano na dieta exerceu efeito positivo no status redox em bezerros Jersey em aleitamento.

**Palavras-chave:** Chá-verde. Jersey. Orégano. Saúde.

## Introduction

Dairy calves experience many stress factors during their first weeks of life, such as separation from the mother, dietary changes, and exposure to various infectious agents, impairing their redox status. Antibiotics and ionophores are used to deal with some of these problems; however, society has concerns about the safety of their use. For this reason, plant extracts are being increasingly used in ruminant feed, including pre-weaned calves (Maciej et al., 2016; Paris

et al., 2020). The redox status of these animals has been associated with health conditions as many free radicals are continuously produced in the cells with the potential to provoke oxidative damage to internal organs. Excessive generation and/or inadequate removal of free radicals result in irreversible damage to exposed cells (Sadiq, 2023). Dairy calves are particularly challenged on the first day of life, during sickness, and following poor colostrum management (Maciej et al., 2016).

Some plant extracts have alleged antioxidant properties already tested in dairy calves, such as green tea extract (Elshahawy, 2018), or tested in goats, such as oregano extract (Paraskevakis, 2015). Antioxidants can be defined as any endogenous or exogenous substance capable of neutralizing a free radical, generally by donating an electron (Martemucci et al., 2022). The mechanisms for inhibiting or reducing damage caused by free radicals can be preventive, preventing their formation, or reconstructive, favoring the repair of damaged structures. Antioxidants can be from enzymatic origin, such as SOD, CAT, and GPx, or non-enzymatic origin, including glutathione (GSH) and vitamins A, C and E (Martemucci et al., 2022).

Catechins are the main flavonoids present in green tea. Although catechins are the dominant phenolic compounds (Sirichaiwetchakoon et al., 2020), several flavonols (up to 4%) and flavones (in trace amounts) are also present in the leaves of this tea. Other related compounds found in green tea are gallic, coumaric, and caffeic acid, as well as the purine alkaloids theobromine and caffeine (Rha et al., 2019). Oregano is composed of approximately 20 different essential oils, with the monoterpenes carvacrol and thymol being those found in greater quantities, 59.7 and 13.7% of the essential oils, respectively (Kosakowska et al., 2021).

Oxidative stress has been involved in intestinal diseases (Kim et al., 2012), and recently, the association between oxidative stress and diarrhea was evidenced by Fu et al. (2023). Previous studies reported that using oregano essential oils or green tea polyphenols decreased the frequency or severity of diarrhea in dairy calves (Maciej et

al., 2016; Katsoulos et al., 2017). Furthermore, Paris et al. (2020) reported that calves receiving milk from cows supplemented with oregano or green tea extracts improved their redox status. On the other hand, Heisler et al. (2020) observed that oregano and green tea extracts anticipated the first rumination of dairy calves, although they did not modify weight gain, diarrhea incidence, or general health conditions. The authors cited provided plant extracts for calves from birth to 10 (Katsoulos et al., 2017) or 60 days of life (Heisler et al., 2020; Paris et al., 2020), as it comprises the most critical phase of life for calves, as well as the pre-weaned period.

In this sense, as far as we know, there are still few studies on the direct feeding of green tea or oregano extracts to pre-weaned calves. Also, the results of these studies are controversial. The hypotheses of the present study are: the supply of green tea or oregano extracts in the diet 1) increases the antioxidant capacity of pre-weaned calves; and 2) improves the health status of dairy calves from birth to 60 days of age. This study aimed to evaluate blood biomarkers of the redox state and health status in pre-weaned dairy calves supplemented with green tea or oregano extract from birth to 60 days of life.

## Material and Methods

### *Ethical declaration*

This study was approved by the Ethics Committee for the Use of Farm Animals of the Universidade Federal do Rio Grande do Sul, protocol number 30756/2016. The experiment was conducted at the Embrapa Clima Temperado Experimental Station in Rio Grande do Sul State, Brazil.

### *Location description, animals, and management*

Two experiments were conducted: the first between March and June 2016 (trial 1) and the second between March and June 2017 (trial 2), at the Embrapa Clima Temperado experimental farm located in Capão do Leão, Rio Grande do Sul, Brazil (farm coordinates: 31°49'02.4" S, 52°27'14.8" W). During trial 1, the values (mean  $\pm$  SD) of air temperature, relative humidity, and wind speed were 16.2  $\pm$  4.94 °C, 88.6  $\pm$  1.86% and 5.9  $\pm$  0.8 km/h, respectively. In trial 2, the values (mean  $\pm$  SD) of air temperature, relative humidity, and wind speed were 18.5  $\pm$  2.9 °C, 84.9  $\pm$  3.8%, and 5.2  $\pm$  2.3 km/h, respectively. The accumulated precipitation was 843.7  $\pm$  147.0 and 633.2  $\pm$  41.9 mm for trials I and II, respectively.

In trials 1 and 2, 17 and 21 Jersey calves were used from birth to 60 days of age, respectively. The treatments, concentrate, and experimental protocol were the same for the two experiments. In each experiment, calves were blocked by birth date and randomly assigned to one of three treatments: control (CON) - with no addition of plant extracts into the diet; oregano extract (OE) - addition of 70 mg/kg BW of oregano extract into the diet; and green tea extract (GT) - addition of 35 mg/kg BW of green tea extract into the diet. Oregano and green tea extract doses were settled based on previous studies (Heisler et al., 2020; Ritt et al., 2023). Adjustment in the quantity of plant extracts was performed every 15 days according to the BW of the animals.

Plant extracts were given to calves as powder diluted in milk. The commercial product (Orego Stim<sup>®</sup>) had a minimum concentration of 50 g/kg of oregano extract,

containing 80-82% Carvacrol, 2.5-3.0% Thymol, 3.5-9.0% p-Cymene and 2-5.0% Y-Terpinene, and the green tea extract (glycolic extract, marketed by Seiva Brasilis) a concentration of approximately 56% ( $\pm$  2.5%) of polyphenols for study 1. In study 2, the commercial product (Oregon OL<sup>®</sup>) had a minimum concentration of 65 g/kg of oregano extract, containing 80-82% Carvacrol, 2.5-3.0% Thymol, 3.5-9.0% p-Cymene and 2-5.0% Y-Terpinene, and the green tea extract was the same used in trial I. In both studies, doses of oregano extract were adjusted to supply the same concentration of carvacrol.

In trial 1, CON and GT groups were composed of three male calves and three female calves each at birth, with (mean  $\pm$  SE) BW of 28.4  $\pm$  0.9 kg and 28.3  $\pm$  1.0 kg, respectively; the OE group consisted of two male and three female calves with BW of 29.2  $\pm$  1.1 kg. In trial 2, the CON group consisted of two male and four female calves with BW of 28.2  $\pm$  1.3 kg; the GT group of two male and five female calves with BW of 29.1  $\pm$  1.1 kg of CP, and the OE group of five male and three female calves with BW of 30.4  $\pm$  1.0 kg.

Immediately after birth, calves were separated from the cows, identified, and housed in individual hutches bedded with straw. Hutches were placed in a flat, rangeland pasture area following an East-West orientation to make shade permanently available. Hutches were displaced three times a week to ensure cleanliness and dryness. During the whole period, calves were fed daily with 4 liters of milk, divided into two meals (08:00 am and 05:00 pm). The concentrate was offered *ad libitum* from the fifth day of life, with no hay offer. The concentrate (Maxxi Milk Terneira Laminada, Rações Supra, Alisul Alimentos S.A., São

Leopoldo, Brazil) presented the following composition: moisture 120g/kg, crude protein 200g/kg, ether extract 30g/kg, acid detergent fiber 120g/kg, mineral matter 100g/kg. The feeder was cleaned daily at 7:30 am, and the concentrate was replaced. Water was supplied *ad libitum* from the third day of life in individual buckets.

The animals were weighed on the first day of life and every 15 days after that, without previous fasting. Shelters provided shade and protection against wind, and straw was provided for bedding.

### *Experimental design*

Data from both experiments were analyzed considering the randomized complete design with repeated measures in time (days). Data were analyzed considering 12 replicates (calves) for control treatment, 13 replications for green tea extract treatment, and 13 replications for oregano extract treatment. The uneven number of replicates was due to calf deaths, which were unrelated to the treatments.

### *Antioxidant profile and redox state*

On days 1, 30, and 60, blood samples were collected from the jugular vein of each animal in five mL tubes containing heparin as an anticoagulant (Vacutainer; Becton-Dickinson, Rutherford, NJ). After blood collection, plasma and erythrocytes were separated by centrifugation at 1000 g for 10 min at 4 °C. Plasma fraction was transferred to microtubes and stored at -80 °C for further analysis. The remaining erythrocytes fraction was diluted 1:10 (v/v) with 0.9% commercial

saline and centrifuged at 1000 g for 10 min at 4 °C three times, and at each step, the supernatant was removed and discarded. At the end of the last centrifugation, the erythrocytes were resuspended in saline at a final dilution of 1:10 and then stored at -80 °C for the following analysis (Kakhniashvili et al., 2004).

Reactive oxygen and nitrogen species were measured in erythrocytes and plasma using 2', 7'-dichlorofluorescein diacetate (DCFH<sub>2</sub>-DA), according to LeBel et al. (1992). DCF fluorescence was measured at excitation and emission wavelengths of 488 nm and 525 nm, respectively, using the SpectraMax Gemini XS Fluorescence Reader (Molecular Devices, Sunnyvale, CA, USA). The standard DCF curve ranging from 0.25 to 10 µM was performed in parallel. Data are expressed as nmol DCF/mg protein.

Total Superoxide dismutase (SOD) enzyme activity was measured in erythrocytes by quantification of the superoxide inhibition-dependent autooxidation at 480 nm (Misra & Fridovich, 1972). Absorbance was measured on a SpectraMax M5 microplate reader (Molecular Devices, Sunnyvale, CA, USA). The activity of SOD is expressed as the amount of enzyme that inhibits the oxidation of epinephrine by 50%, which is equal to one unit. Data are expressed as SOD units/mg protein.

The enzymatic activity of Catalase (CAT) was measured in erythrocytes and tested using microplates (Aebi, 1984). The decrease in absorbance at 240 nm was measured in a medium containing 20 mM hydrogen peroxide and 10 mM potassium phosphate buffer pH 7.0 using the SpectraMax M5 microplate reader (Molecular Devices,

Sunnyvale, CA, USA). The CAT unit is defined as 1  $\mu\text{mol H}_2\text{O}_2$  consumed per minute. The specific activity data are expressed as CAT units/mg protein.

The glutathione peroxidase (GPx) enzyme activity in erythrocytes was tested using microplates (Wendel, 1981). The medium contained 100 mM potassium phosphate buffer, pH 7.7, 1 mM EDTA, 2 mM reduced glutathione (GSH), 0.15 U/mL glutathione reductase, 0.4 mM azide, 0.1 mM NADPH, and 0.5 mM tert-butyl hydroperoxide as the enzymatic substrate. The disappearance of NADPH was monitored at 340 nm using the SpectraMax M5 microplate reader (Molecular Devices, Sunnyvale, CA, USA). The GPx unit is defined as 1  $\mu\text{mol}$  of NADPH consumed per minute, and the specific activity is represented as GPx units/mg of protein.

The concentration of reduced glutathione (GSH) in erythrocytes was measured according to Browne and Armstrong (1998). Initially, proteins in the supernatant were precipitated with metaphosphoric acid (1: 1) and centrifuged at 5,000 g for 10 min at 25 °C. GSH present in the supernatant is reacted with the fluorophore o-phthalaldehyde present in the medium at a concentration of 7.5 mM in addition to 100 mM sodium phosphate buffer pH 8.0 containing 5 mM EDTA. Fluorescence was measured at excitation and emission wavelengths of 350 nm and 420 nm, respectively, using the SpectraMax Gemini XS Fluorescence (Molecular Devices, Sunnyvale, CA, USA) microplate reader. The standard GSH curve from 0.001 to 1 mM was prepared, and a blank sample was run in parallel. Data are expressed as nmol GSH/mg protein.

The thiol content was measured in plasma using microplates (Aksenov & Markesbery, 2001). The assay is based on the reduction of 5,5-dithiobis-2-nitrobenzoic acid (DTNB) by thiols, which become oxidized (disulfide), yielding a yellow derivative (TNB). Absorbance was measured at 412 nm in a medium containing 20 mM sodium phosphate buffer pH 7.4 and 10 mM DTNB prepared in a 0.2 M potassium phosphate solution pH 8.0 using the SpectraMax M5 microplate reader (Molecular Devices, Sunnyvale, CA, USA). Data are expressed as nmol TNB/mg protein.

Carbonylated protein content (CARBO) was measured in plasma according to Reznick and Packer (1994) and Stone et al. (2016) for reading in 96-well microplates. Protein carbonyls react with dinitrophenylhydrazine to form dinitrophenylhydrazone, a yellow compound that was measured at 370 nm using the SpectraMax M5 microplate reader (Molecular Devices, Sunnyvale, CA, USA). Data are expressed as nmol carbonyls/mg protein.

Protein concentration was measured in microplates using bovine albumin as standard (Lowry et al., 1951). The absorbance was measured at 750 nm using the SpectraMax M5 microplate reader (Molecular Devices, Sunnyvale, CA, USA). Data are expressed as mg protein/mL.

Health status was monitored every two days during the experimental period. Calves were observed to detect clinical symptoms of diseases and diarrhea. On the same days, body temperature was measured before feeding using a digital clinical thermometer inserted into the rectal ampulla of the animal at a depth of approximately 3.5 cm for three minutes in the morning and afternoon. Calves did not receive deworming medicines.

The consistency and appearance of the feces were observed daily, and the fecal score was assigned according to their appearance, following classification from 0 to 3 according to Ishihara et al. (2001): (0) normal feces, (1) soft feces, (2) muddy feces, and (3) watery feces. The incidence of diarrhea was calculated according to Ishihara et al. (2001) using the following formula: Frequency of diarrhea (%): (total number of days with diarrhea/total number of days inspected) X 100.

### Statistical analysis

Data were tested for normal distribution (Procedure Univariate, SAS®, using *Shapiro-Wilk* test) and for homogeneity of variances (using Levene's test and Welch option). Differences in oxidative stress biomarkers between CON, OE, and GT groups were analyzed using the MIXED procedure (Enterprise guide version 5.1, 2012, SAS Institute, Cary, NC). We tested the interaction between experiment and treatment (using data on day 1 as covariates). As interactions were not significant, we analyzed the two experiments together. Statistical analysis considered treatments (n = 3), days (30 and 60) and their interactions as fixed effects, and animal and residue as random effects, using method = REML, covariance matrix = AR (1), repeated = day.

The statistical model used was:  $Y_{ijkl} = \mu + \alpha_i + \beta_j + (\alpha^* \beta)_{ij} + b(X_{ij} - X_m) + a_k + e_{ijkl}$ , where  $Y_{ijkl}$  is the observation made for each animal of each experiment on each day of evaluation (n = 2),  $\mu$  is overall mean,  $\alpha_i$  is the effect of the treatment (n = 3),  $\beta_j$  = the day of evaluation,  $(\alpha^* \beta)_{ij}$  is the effect of the interaction between treatment and days,  $b(X_{ij} - X_m)$  is the covariate

measured on day 1,  $a_k$  is the random effect of animal, and  $e_{ijkl}$  is the random error associated with each observation. Means were compared using the Lsmeans option. The significant differences and tendencies were considered when  $P \leq 0.05$  and  $0.05 < P \leq 0.10$ , respectively.

## Results and Discussion

Disturbances in the redox state of the animals can be evaluated using biomarkers (Doğan et al., 2021; He et al., 2023). The present study highlights the beneficial effects of oregano extract, such as the reduction in protein oxidation and general oxidative stress, as well as increased antioxidative enzymes. Oxidative damage to proteins can generate amino acid radicals, producing peroxides of proteins and carbonyls (Pisoschi et al., 2021). DCFP is an indicator of redox shifts and general oxidative stress (Gulcin, 2020).

Mean values of biomarkers of the oxidative status are shown in Table 1. Significant interactions ( $P \leq 0.05$ ) between treatment and days of measurements were detected for DCFP, CAT, and CARBO. CARBO presented a lower concentration in OE compared with CON and GT on day 30, while there were no significant differences between treatments on day 60. DCFP presented a lower concentration for OE and GT than CON on day 30. Conversely, on day 60, DCFP presented a higher concentration in OE and GT than CON. The activity of CAT was higher in CON compared with OE on day 30, presenting intermediary activity in GT. On the other hand, on day 60, the activity of CAT was higher in OE compared with CON and tended to be higher in GT than in CON.

**Table 1**  
Mean (standard deviations) or median (interquartile ranges) values of electrocardiographic variables measured by observers with different levels of experience in dogs

| Variables <sup>2</sup>     | Days <sup>3</sup> | Treatments |          |          | SE   | P-value                       |          |         |
|----------------------------|-------------------|------------|----------|----------|------|-------------------------------|----------|---------|
|                            |                   | Control    | GT       | OE       |      | Treatment <sup>1</sup><br>(T) | Days (D) | T x D   |
| THIOLS (nmol/mg)           | -                 | 0.28       | 0.31     | 0.30     | 0.02 | 0.21                          | 0.96     | 0.23    |
| CARBO (U/mg)               | 30                | 1.16 a     | 1.06 a   | 0.76 b   | 0.12 | 0.20                          | 0.59     | 0.05    |
|                            | 60                | 1.050 a    | 1.05 a   | 1.06 a   | 0.12 |                               |          |         |
| SOD (U/mg)                 | -                 | 45.98 a    | 34.64 b  | 30.53 b  | 5.1  | 0.03                          | 0.30     | 0.38    |
| CAT (U/mg)                 | 30                | 2.22 a     | 1.57 ab* | 1.45 b   | 0.20 | 0.70                          | 0.74     | 0.03    |
|                            | 60                | 1.48 b     | 1.70 ab  | 2.18 a   | 0.20 |                               |          |         |
| GPx (U/mg)                 | -                 | 11.17 b    | 12.30 ab | 16.48 a  | 2.0  | 0.05                          | 0.01     | 0.57    |
| GSH (nmol/mg)              | -                 | 0.14       | 0.13     | 0.13     | 0.01 | 0.93                          | 0.27     | 0.85    |
| DCF <sub>E</sub> (nmol/mg) | -                 | 4016.12    | 4466.3   | 4076.4   | 760  | 0.89                          | <0.0001  | 0.84    |
| DCF <sub>P</sub> (nmol/mg) | 30                | 2444.28 a  | 1717.3 b | 1686.1 b | 162  | 0.76                          | 0.33     | <0.0001 |
|                            | 60                | 1958.5 b   | 2241.2 a | 2439.5 a | 162  |                               |          |         |

<sup>1</sup>Treatments: CON = control; GT = green tea extract; OE = oregano extract; \*GT tended to be different of CON (0.05 < P < 0.10).

<sup>2</sup>Variables: CARBO = carbonyl; SOD = superoxide dismutase; GPx = glutathione peroxidase; DCF<sub>P</sub> = oxidation of dichlorofluorescein in plasma. Variables without interaction: GSH = reduced glutathione; DCF<sub>E</sub> = oxidation of dichlorofluorescein in erythrocytes.

<sup>3</sup>When interaction between treatment and days was significant or tendency, we evaluated the effect of treatment on each day of measurements (30 or 60 days of age)  
SE = Standard error of the mean.

Oregano extract reduced both carbonyl and DCF<sub>P</sub> concentrations on day 30. The enzymatic defense system includes the enzymes SOD, CAT, and GPx, which prevent the oxidation of cell biomolecules and control the levels of free radicals and non-radical species (Doğan et al., 2021; He et al., 2023). The present study evidenced the beneficial effect of oregano enhancing the activity of GPx and CAT, although the last one only occurred on day 60. On the other hand, GT was less effective than OE as it only decreased one biomarker of oxidative stress (DCF<sub>P</sub>). The more expressive effects of oregano extract might be due to essential

oils being poorly or not degraded in the rumen and being transferred via the gastrointestinal tract to the blood of calves (Lejonklev et al., 2013, 2016).

The interactions between treatment and days were significant for CARBO, DCF<sub>P</sub>, and CAT. The lowest values of DCF<sub>P</sub> and CARBO observed on day 30 in OE and GT might be related to the alleged antioxidant properties already reported for both extracts in ruminants (Paraskevakis, 2015; Elshahawy, 2018). Accordingly, oregano extract increased the activity of the antioxidative enzyme GPx on day 30. Reducing pro-

oxidants and increasing the activity of antioxidative enzymes may allow calves to cope better with immune challenges. The immune system development in calves progresses from conception to maturity in approximately six months after birth (Chase et al., 2008), and until 30 days of life, calves rely on passive immunity (Hulbert & Moisés, 2016). Therefore, calves may benefit from less oxidative stress during the pre-weaning period. Moreover, reactive oxidative species increased with the age of calves during pre-weaning and weaning periods (Seibt et al., 2021). In this sense, the higher level of antioxidative enzymes on day 30 (higher activity of GPx in OE) and day 60 (higher activity of CAT in OE and tendency of higher activity in GT) may contribute to the oxidative status of calves. Nevertheless, it is worth to notice the lower values of SOD activity in OE and GT groups compared with not supplemented calves.

Significant ( $P < 0.05$ ) effects for treatment were detected for SOD and GPx. The activity of SOD was higher in CON than in OE and GT. Conversely, GPx activity was higher in OE compared with CON. Plant extracts did not modify the concentration of thiols and DCFE ( $P > 0.10$ ).

Significant effects ( $P \leq 0.05$ ) for days of measurements were detected for DCFE and GPx. The concentration of DCFE decreased from day 30 to day 60 (7041 x 1331.5 nmol/mg), while the activity of GPx increased from day 30 to day 60 (10.78 x 15.86 U/mg).

Positive effects on oxidative status in calves supplemented with green tea extract were previously detected by Paris et al. (2020) and Elshahawy (2018), evidencing higher concentration of GSH and higher activity

of the catalase enzyme compared to not supplemented calves, while in the present study, we detected a tendency for higher catalase activity in GT. Polyphenols in green tea extract undergo extensive conversion to biologically active forms before entering the small intestine (Olagaray & Bradford, 2019). Even though phenols are transferred to milk (Jordán et al., 2010), they may affect redox biomarkers in calves.

There are controversial results concerning the effects on redox status in ruminants supplemented with green tea and oregano extracts. Paraskevakis (2015) added 30 g of dried oregano leaves (equivalent to 1 mL of essential oil) to the diet of dairy goats and observed an improvement in enzymatic and non-enzymatic antioxidant defenses in blood and milk. Maciej et al. (2016) reported that calves supplemented with 10 mg/day of quercetin (a secondary compound present in green tea) or 10 mg/kg of BW of green tea extract did not alter the metabolism and antioxidant status. Conversely, Zhong et al. (2011) found that lower dosages (2 g/kg DM) of catechin supplementation promoted a better antioxidant action when compared to higher dosages (3 or 4 g/kg DM).

Clinical symptoms of diseases were not detected during the trial except for diarrhea. Overall mean diarrhea frequency was 8% without differences between treatments ( $P > 0.10$ ). The number of days with fecal scores  $\geq 2$  was 4.7, 5.3, and 5.8 (se = 0.8) for the CON, GT, and OE groups, respectively. The age at which the fecal score was equal to or higher than 2 for the first time was similar between groups: mean of 18.3, 17.2, and 24.9 days (se = 3.5) for CON, GT, and OE groups, respectively.

The absence of effects of plant extracts on the health status of calves was probably because all calves were not challenged by diseases as they did not present clinical symptoms other than diarrhea, which, in turn, presented low frequency. In previous studies, using oregano essential oils or green tea polyphenols decreased the frequency or severity of diarrhea in dairy calves (Maciej et al., 2016; Katsoulos et al., 2017).

Pre-weaned dairy calves usually face challenges during the short period between birth and weaning. Therefore, supplementation with natural products could improve redox and health status without raising concerns about residual products and microorganism resistance. The present study hypothesized that the supply of green tea or oregano extracts in the diet 1) increases the antioxidant capacity of pre-weaned calves; and 2) improves the health status of dairy calves from birth to 60 days of age. The first hypothesis was accepted as oregano extract increased the activity of antioxidative enzymes (e.g., GPX and CAT) and reduced the concentration of biomarkers of cellular reactive oxidant species (DCFP) compared with not-supplemented calves. Similarly, green tea extract also reduced the concentration of cellular reactive oxidant species (DCFP) biomarkers and tended to enhance catalase activity compared with not-supplemented calves. We could not accept the second hypothesis, as extracts of oregano and green tea did not improve health conditions, probably because calves were healthy.

## Conclusion

The addition of green tea and oregano extracts into the diet positively affects redox status in pre-weaned Jersey calves. Well-managed and healthy calves may not benefit from supplementation with oregano and green tea extracts to improve health condition.

## Conflict of Interest Statement

The authors declare no conflict of interest.

## References

- Aebi, H. (1984). Catalase in vitro. *Methods Enzymology*, 105(1), 121-126. doi: 10.1016/s0076-6876(84)05016-3
- Aksenov, M. Y., & Markesbery, W. R. (2001). Changes in thiol content and expression of glutathione redox system genes in the hippocampus and cerebellum in Alzheimer's disease. *Neuroscience Letters*, 302(2-3), 141-145. doi: 10.1016/S0304-3940(01)01636-6
- Browne, R. W., & Armstrong, D. (1998). Reduced glutathione and glutathione disulfide. *Methods in Molecular Biology*, 108(1), 347-352. doi: 10.1385/0-89603-472-0:347
- Chase, C. C. L., Hurley, D. J., & Reber, A. J. (2008). Neonatal immune development in the calf and its impact on vaccine response. *Veterinary Clinics of North America: Food Animal Practice*, 24(1), 87-104. doi: 10.1016/j.cvfa.2007.11.001

- Doğan, E., Merhan, O., Erdağ, D., Karamancı, E., Bozukluhan, K., & Doğan, A. N. C. (2021). The effect of vitamin C on oxidant and antioxidant parameters in anthrax vaccine administered cattle. *Van Veterinary Journal*, 32(3), 109-113. doi: 10.36483/vanvetj.958358
- Elshahawy, I. I. (2018). Oxidant/antioxidant status in calves supplemented with green tea extract. *International Journal of Animal and Veterinary Sciences*, 12(4), 108-111. doi: 10.5281/zenodo.1316299
- Fu, Z. L., Yang, Y., Ma, L., Malmuthuge, N., Guan, L. L., & Bu, D. P. (2023). Dynamics of oxidative stress and immune responses in neonatal calves during diarrhea. *Journal of Dairy Science*, 107(2), 1286-1298. doi: 10.3168/jds.2023-23630
- Gulcin, İ. (2020). Antioxidants and antioxidant methods: an updated overview. *Archives of Toxicology*, 94(3), 651-715. doi: 10.1007/s00204-020-02689-3
- He, F., Liu, R., Tian, G., Qi, Y., & Wang, T. (2023). Ecotoxicological evaluation of oxidative stress-mediated neurotoxic effects, genetic toxicity, behavioral disorders, and the corresponding mechanisms induced by fluorene-contaminated soil targeted to earthworm (*Eisenia fetida*) brain. *Science of the Total Environment*, 871(1), 162014. doi: 10.1016/j.scitotenv.2023.162014
- Heisler, G., Fischer, V., Paris, M., Angelo, I. D. V., Panazzolo, D. M., & Zanela, M. B. (2020). Effect of green tea and oregano extracts fed to preweaned Jersey calves on behavior and health status. *Journal of Veterinary Behavior*, 37(1), 36-40. doi: 10.1016/j.jveb.2020.03.002
- Hulbert, L. E., & Moisé, S. J. (2016). Stress, immunity, and the management of calves 1. *Journal of Dairy Science*, 99(4), 3199-3216. doi: 10.3168/jds.2015-10198
- Ishihara, N., Chu, D. C., Akachi, S., & Juneja, L. R. (2001). Improvement of intestinal microflora balance and prevention of digestive and respiratory organ diseases in calves by green tea extracts. *Livestock Production Science*, 68(2-3), 217-229. doi: 10.1016/S0301-6226(00)00233-5
- Jordán, M. J., Moñino, M. I., Martínez, C., Lafuente, A., & Sotomayor, J. A. (2010). Introduction of distillate rosemary leaves into the diet of the Murciano-Granadina goat: transfer of polyphenolic compounds to goats milk and the plasma of suckling goat kids. *Journal of Agricultural and Food Chemistry*, 58(14), 8265-8270. doi: 10.1021/jf100921z
- Kakhniashvili, D. G., Bulla, L. A., & Goodman, S. R. (2004). The human erythrocyte proteome: analysis by ion trap mass spectrometry. *Molecular and Cellular Proteomics*, 3(5), 501-509. doi: 10.1074/mcp.M300132-MCP200
- Katsoulos, P. D., Karatzia, M. A., Dovas, C. I., Filioussis, G., Papadopoulos, E., Kiossis, E., Arsenopoulos, K., Papadopoulos, T., Boscós, C., & Karatzias, H. (2017). Evaluation of the in-field efficacy of oregano essential oil administration on the control of neonatal diarrhea syndrome in calves. *Research in Veterinary Science*, 115(1), 478-483. doi: 10.1016/j.rvsc.2017.07.029
- Kim, Y. J., Kim, E. H., & Hahm, K. B. (2012). Oxidative stress in inflammation-based gastrointestinal tract diseases: challenges and opportunities. *Journal*

- of *Gastroenterology and Hepatology*, 27(6), 1004-1010. doi: 10.1111/j.1440-1746.2012.07108.x
- Kosakowska, O., Weglarz, Z., Jabrucka, E. P., Przybyl, J. L., Krasniewska, K., Gniewosz, M., & Baczek, K. (2021). Antioxidant and antibacterial activity of essential oils and hydroethanolic extracts of greek oregano (*O. vulgare* L. subsp. *hirtum* (Link) letsvaart) and common oregano (*O. vulgare* L. subsp. *vulgare*). *Molecules*, 26(4), 988. doi: 10.3390/molecules26040988
- LeBel, C. P., Ischiropoulos, H., & Bondy, S. C. (1992). Evaluation of the probe 2',7'-dichlorofluorescein as an indicator of reactive oxygen species formation and oxidative stress. *Chemical Reseach in Toxicology*, 5(2), 227-231. doi: 10.1021/tx00026a012
- Lejonklev, J., Kidmose, U., Jensen, S., Petersen, M. A., Helwing, A. L. F., Mortensen, G., Weisbjerg, M. R., & Larsen, M. K. (2016). Short communication: Effect of oregano and caraway essential oils on the production and flavor of cow milk. *Journal of Dairy Science*, 99(10), 7898-7903. doi: 10.3168/jds.2016-10910
- Lejonklev, J., Løkke, M. M., Larsen, M. K., Mortensen, G., Petersen, M. A., & Weisbjerg, M. R. (2013). Transfer of terpenes from essential oils into cow milk. *Journal of Dairy Science*, 96(7), 4235-4241. doi: 10.3168/jds.2012-6502
- Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, 193(1), 265-275. doi: 10.1016/S0021-9258(19)52451-6
- Maciej, J., Schäff, C. T., Kanitz, E., Tuchscherer, A., Bruckmaier, R. M., Wolfram, S., & Hammon, H. M. (2016). Short communication: Effects of oral flavonoid supplementation on the metabolic and antioxidative status of newborn dairy calves. *Journal of Dairy Science*, 99(1), 805-811. doi: 10.3168/jds.2015-9906
- Martemucci, G., Costagliola, C., Mariano, M., D'andrea, L., Napolitano, P., & D'Alessandro, A. G. (2022). Free radical properties, source and targets, antioxidant consumption and health. *Oxygen*, 2(2), 48-78. doi: 10.3390/oxygen2020006
- Misra, H. P., & Fridovich, I. (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *Journal of Biological Chemistry*, 247(10), 3170-3175. doi: 10.1016/S0021-9258(19)45228-9
- Olagaray, K. E., & Bradford, B. J. (2019). Plant flavonoids to improve productivity of ruminants – A review. *Animal Feed Science and Technology*, 251(1), 21-36. doi: 10.1016/j.anifeedsci.2019.02.004
- Paraskevakis, N. (2015). Effects of dietary dried Greek Oregano (*Origanum vulgare* ssp. *hirtum*) supplementation on blood and milk enzymatic antioxidant indices, on milk total antioxidant capacity and on productivity in goats. *Animal Feed Science and Technology*, 209(1), 90-97. doi: 10.1016/j.anifeedsci.2015.09.001
- Paris, M., Stivanin, S. C. B., Klein, C. P., Vizzotto, E. F., Passos, L. T., Angelo, I. D. V., Zanela, M. B., Stone, V., Matté, C., Heisler, G., & Fischer, V. (2020). Calves fed with milk from cows receiving plant extracts improved redox status. *Livestock Science*, 242(1), 104272. doi: 10.1016/j.livsci.2020.104272

- Pisoschi, A. M., Pop, A., Iordache, F., Stanca, L., Predoi, G., & Serban, A. I. (2021). Oxidative stress mitigation by antioxidants - An overview on their chemistry and influences on health status. *European Journal of Medicinal Chemistry*, 209(1), 112891. doi: 10.1016/j.ejmech.2020.112891
- Reznick, A. Z., & Packer, L. (1994). Oxidative damage to proteins: spectrophotometric method for carbonyl assay. *Methods in Enzymology*, 233(1), 357-363. doi: 10.1016/s0076-6879(94)33041-7
- Rha, C.-S., Jeong, H. W., Park, S., Lee, S., Jung, Y. S., & Kim, D.-O. (2019). Antioxidative, anti-inflammatory, and anticancer effects of purified flavonol glycosides and aglycones in green tea. *Antioxidants*, 8(8), 278. doi: 10.3390/antiox8080278
- Ritt, L. A., Orso, C., Silveira, A. K., Frazzon, J., Vargas, D. P., Wagner, R., Oliveira, F. C., Nörnberg, J. L., & Fischer, V. (2023). Oregano extract fed to pre-weaned dairy calves. Part 1: Effects on intake, digestibility, body weight, and rumen and intestinal bacteria microbiota. *Livestock Science*, 269(1), 105165. doi: 10.1016/j.livsci.2023.105165
- Sadiq, I. Z. (2023). Free radicals and oxidative stress: signaling mechanisms, redox basis for human diseases, and cell cycle regulation. *Current Molecular Medicine*, 23(1), 13-35. doi: 10.2174/1566524022666211222161637
- Seibt, K. D., Ghaffari, M. H., Scheu, T., Koch, C., & Sauerwein, H. (2021). Characteristics of the oxidative status in dairy calves fed at different milk replacer levels and weaned at 14 weeks of age. *Antioxidants*, 10(2), 260. doi: 10.3390/antiox10020260
- Sirichaiwetchakoon, K., Lowe, G. M., & Eumkeb, G. (2020). The free radical scavenging and anti-isolated human ldl oxidation activities of *Pluchea indica* (L.) Less. Tea compared to green tea (*Camellia sinensis*). *BioMed Research International*, 2020(4183643), 1-12. doi: 10.1155/2020/4183643
- Stone, V., August, P. M., Stocher, D. P., Klein, C. P., Couto, P. R. G., Silva, Y. D., Sagini, J. P., Salomon, T. B., Benfato, M. S., & Matté, C. (2016). Food restriction during pregnancy alters brains antioxidant network in dams and their offspring. *Free Radical Research*, 50(5), 530-541. doi: 10.3109/10715762.2016.1152361
- Wendel, A. (1981). Glutathione peroxidase. *Methods in Enzymology*, 77(1), 325-333. doi: 10.1016/s0076-6879(81)77046-0
- Zhong, R., Xiao, W., Ren, G., Zhou, D., Tan, C., Tan, Z., Han, X., Tang, S., Zhou, C., & Wang, M. (2011). Dietary tea catechin inclusion changes plasma biochemical parameters, hormone concentrations and glutathione redox status in goats. *Asian-Australasian Journal of Animal Sciences*, 24(12), 1681-1689. doi: 10.5713/ajas.2011.11007

