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Calves fed with milk from cows receiving plant extracts improved redox status

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ABSTRACT

The objective of this study was to evaluate the biomarkers of the redox state of pre-weaned Jersey dairy calves that consumed milk from cows supplemented with green tea (*Camellia sinensis*) or oregano extracts (*Origanum vulgare*). A completely randomized design was used with repeated measures in time. From their birth to 60 days of life, 8, 8 and 7 calves received milk from cows fed on a basal diet without addition of plant extracts (CON), with addition of 10.0 g of oregano extract (OE) per day and with 5.0 g of green tea extract (GT) per day, respectively. On days 1, 30, and 60 after birth redox state biomarkers were evaluated. Body weight was evaluated every two weeks, rectal temperature and fecal score were accessed every two days and concentrate intake was measured every day. Statistical analyses for body weight, body weight gain, concentrate intake, rectal temperature, variables of redox status were performed using the procedure Mixed, evaluating the fixed effects of treatment, day of measurement and their interaction. Statistical analyses for the mean fecal score and the number of days to the first diarrhea occurrence were performed using the procedure Mixed, evaluating the fixed effect of treatment. On day 1, calves fed with milk from GT group had higher plasma glutathione peroxidase activity (GPx) than CON. However, the reverse occurred on day 60. On the day 1, calves in the CON group presented higher concentrations of thiol groups (also known as sulfhydryl groups) than those in GT and OE groups, with reverse occurring on day 30. Calves in the OE group had lower oxidation of dichlorofluorescein in the erythrocytes compared to the others; while calves in the GT group presented higher concentration of GSH and higher activity of the catalase enzyme compared to CON and OE, respectively. Pre-weaned calves fed with milk of cows supplemented with extracts of green tea and oregano did not change physiological and productive variables but they showed improvement in some antioxidant biomarkers.

1. Introduction

The plant extracts have a wide variety of compounds that may present antimicrobial, anti-inflammatory and antioxidant actions (Aristatile et al., 2015; Cyboran et al., 2015), and their inclusion into the diet may enhance animals performance and health (Oh et al., 2017). Previous studies reported that the use of oregano and green tea extracts improved the redox state in lactating goats (Paraskevakis, 2015) and in calves (Elshahawy et al., 2018), respectively. In their study, Paraskevakis (2015) verified significant increase in glutathione peroxidase and glutathione reductase both in blood and milk of lactating goats. According to Elshahawy et al. (2018), green tea increased the enzymatic activities of catalase, reduced glutathione and glutathione peroxidase compared with not supplemented calves. On contrary, Maciej et al. (2016) reported that supplementation with green tea added into the diet of newborn calves did not modify the antioxidant capacity or activity of oxidative damage biomarkers in the early post-natal days using doses of 10 mg of green tea extract /kg of BW.

If the redox status is imbalanced favoring the pro-oxidant state, it may trigger adverse clinical effects (Sies, 2018). The enzymatic system is the primary route of antioxidant defense, being represented mainly by the antioxidant enzymes as glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) that can decrease reactive oxygen species (ROS) and, consequently, the damage to biological

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structures (Belló, 2002).

Pre-weaned dairy calves are subjected to oxidative stress in the first weeks of life (Abuelo et al., 2019). Maternal oxidative and inflammatory status during late pregnancy impact on several health and production variables of neonatal calves (Ling et al., 2018). As the use of plant extracts in dairy cows during the transition period improved some biomarkers of the anti-oxidant status in dairy cows (Paraskevakis, 2015; Oh et al., 2017), the amelioration of the redox status of dams with plant extracts might impact on the redox status of neonatal calves. Moreover the passage of active compounds to milks may also be considered.

Bioactive compounds of plants present distinct resistance to microbial degradation in the rumen. Approximately 60% of carvacrol and thymol were recovered in the rumen (Oh et al., 2017). These essential oils are able to be transferred to mammary gland via gastrointestinal tract (Lejonklev et al., 2013), and thus, oregano extract might ameliorate the redox status of pre-weaned calves. On the other hand, polyphenols in green tea extract undergo extensive biotransformation with processes such as demethylation and conversion to biologically active forms occurring prior to entrance into the small intestine, presenting low detectable amounts of catechins in plasma and raising doubts about their effects on the redox status and health in ruminants (Olagary and Bradford, 2019). Despite alleged anti-oxidants properties of green tea in humans and non ruminants, little is known about the transfer of flavonoids and other metabolites present in green tea or active metabolites after degradation in the rumen to the mammary gland of ruminants.

The knowledge about the effects of green tea and oregano extracts on calves fed milk from cows receiving plant extracts is scarce, being the main contribution of this research. The present study aimed to evaluate the biomarkers of the redox status, in calves from birth to 60 days of age fed milk from dairy cows supplemented with green tea extract (*Camellia sinensis*) or oregano (*Origanum vulgare*) from 21 days pre-partum to 21 days post-partum.

2. Material and methods

2.1. Location Description

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. The experiment was conducted at the Embrapa Clima Temperado Experimental Station, in Rio Grande do Sul State, Brazil, between October 2015 and January 2016. Region's climate is classified as subtropical humid.

During the study, mean values of air temperature, relative air humidity and wind speed were, respectively, 20.1 ± 0.5 °C (mean ± SEM), 85.3 ± 1.6% and 14.2 ± 0.9 km/h. Accumulated rainfall over the entire experimental period was 847.0 mm (Table S1).

2.2. Animals and Management

Approximately 28 days before their respective predicted calving date, 24 Jersey cows were selected based on the proximity of their calving dates from a 60-head experimental herd. Further selection criteria were BCS and parity. Cows were distributed within three groups of eight cows each. All cows were submitted to a seven days adaptation period followed by an experimental period of approximately 42 days (from 21 days before the expected calving date to 21 days after calving). On day 21 before the expected calving date, cows were randomly assigned to one of the following treatments.

The treatments were: control (CON) - without addition of plant extracts into the diet, oregano extract (OE) - addition of 10 g per cow per day of dietary oregano extract, and green tea extract (GT) - addition of 5 g per cow per day of green tea extract into the diet. The extracts and doses in cows' diets used in the present study are the same used in a previous study conducted with dairy cows between 28 and 87 days of lactation (Kolling et al., 2018). These doses are consistent with the levels recommended by feed additive suppliers and the doses reported in the literature (Benchaar and Greathead, 2011; Cobellis et al., 2016; Maciej et al., 2016).

The plant extracts were administered in powder, homogenized in 500 grams of concentrate. Oregano extract (Orego Stim®) has a minimum concentration of 50 g/kg, containing 80-82% Carvacrol, 2.5-3.0% Thymol, 3.5-9.0% p-Cymene and 2.0-5.0% Y-Terpinene. Green tea extract (glycolic extract, marketed by Seiva Bazilis) has a concentration of approximately 56% (± 2.5%) of polyphenols.

The actual number of days cows were fed their dietary treatments during *pre-partum* were 20.1 ± 3.2, 18.5 ± 4.8 and 18.5 ± 4.5 for CON, GT and OR groups, respectively. After parturition cows were fed for 21 days with their respective experimental diets. Diets fed to dairy cows during the pre and post-*partum* are shown in Table S2. More details about the study with the cows supplemented with oregano and green tea extracts are described in Stivanin *et al.* (2019).

At the beginning of the trial, cows in the CON treatment averaged (± SE): 3.0 ± 2.2 lactations, 444.3 ± 9.6 kg of BW and 3.3 ± 0.5 of BCS; in the OR treatment cows averaged 2.7 ± 1.8 lactations, 420.3 ± 41.6 kg of BW and 3.6 ± 0.2 BCS, and the cows in the GT treatment averaged 2.5 ± 1.5 lactations, 458.6 ± 31.3 kg of BW and 3.6 ± 0.2 BCS.

Twenty-three Jersey dairy calves (13 males and 10 females) were separated from their dams immediately after birth and therefore they were not allowed to suck directly at their dams; they were housed in individual shelters for 60 days. Calves received 4 L of colostrum from their dams during the first 12 hours after birth. During the next four days, calves were fed twice daily with 2 L of milk of their dams. From day 6 to 60 of life, calves were fed twice daily with 2L of pooled milk of cows in the same treatment of their dams. Colostrum was not analyzed for IgG nor calves were tested for passive immunity transfer. After the fifth day of age, calves had *ad libitum* access to water, improved range pasture and to a commercial concentrate (composition is shown in Table S3).

2.3. Measurements

2.3.1. Productivity

Concentrate intake was evaluated every day as the difference between the amount supplied and leftovers. Calves were weighed in the morning on days 1, 15, 30, 45 and 60 without previous fasting but before milk and concentrate feeding. Body weight gain was calculated as the difference between body weight evaluated on days 1, 15, 30, 45 and 60 divided by the number of days between weighings. The control group consisted of 3 male and 5 female calves with mean birth weight of 29.9 ± 2.3 kg of BW; GT group consisted of 5 male and 3 female calves with mean birth weight of 30.1 ± 2.3 kg of BW and OE group by 4 male calves and 3 female calves with mean birth weight of 28.4 ± 2.4 kg of BW.

2.3.2. Antioxidant profile and redox state

On days 1, 30 and 60, blood samples were taken from the jugular vein in 5 ml tubes (Vacutainer; Becton-Dickinson, Rutherford, NJ) containing heparin. Blood sampling was kept consistently throughout the trial: calves were sampled 2 hours after milk morning feeding.

2.3.3. Preparation of samples for biochemical tests

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 during a period of less than four months for further analysis. In order to isolate the erythrocytes, the fractions of platelets and leukocytes, corresponding to the intermediate fraction, were removed and discarded. Remaining fraction containing the isolated erythrocytes was diluted 1:10 (v/v) with 0.9% commercial saline solution and centrifuged at 1000 g for 10 min at 4°C, 3 times; and at each step the supernatant was removed and discarded. At the end of

the last centrifugation, the erythrocytes were resuspended in saline solution at a final dilution of 1:10, and then stored at -80 °C until the biochemical assays described below were performed (Kakhniashvili et al., 2004).

2.4. Determinations of redox state biomarkers

2.4.1. Proteins

Protein concentration was measured according to Lowry et al. (1951), which was adapted for microplates using bovine albumin as standard. 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.

2.4.2. Dichlorofluorescein Oxidation (DCFH)

Reactive oxygen and nitrogen species were measured in erythrocytes (DCFE) and plasma (DCFP) using 2 ', 7′-dichlorofluorescein diacetate (DCFH2-DA) according to LeBel et al. (1992). The DCFH2-DA is cleaved by esterase enzymes producing DCFH2, which is oxidized by reactive species present in the sample, giving rise to DCF fluorescence. 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). Standard DCF curve ranging from 0.25 to 10 μM was performed in parallel. Data are expressed as nmol DCF / mg protein.

2.4.3. Superoxide dismutase activity (SOD)

Total SOD enzyme activity was measured in erythrocytes by quantification of the superoxide inhibition dependent autooxidation at 480 nm (Misra and 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 1 unit. Data are expressed as SOD units / mg protein.

2.4.4. Catalase Activity (CAT)

The enzymatic activity of CAT was measured in erythrocytes and tested according to Aebi (1984), which was adapted for microplates. 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 μmol H_2O_2 consumed per minute. The specific activity data are expressed as CAT units / mg protein.

2.4.5. Activity of glutathione peroxidase (GPx)

The activity of GPx enzyme in erythrocytes was tested according to Wendel (1981) adapted for microplates. 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 μmol of NADPH consumed per minute and the specific activity is represented as GPx units / mg of protein.

2.4.6. Reduced glutathione (GSH)

Concentration of GSH in erythrocytes was measured according to Browne and Armstrong (1998). Initially, proteins in the supernatant were precipitated with meta-phosphoric acid (1: 1) and centrifuged at 5,000 g for 10 min at 25°C. The GSH present in the supernatant is reacted with the fluorophore o-phthaldialdehyde 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 ranging from 0.001 to 1 mM was prepared and a blank sample was run in parallel. Data are expressed as nmol GSH / mg protein.

2.4.7. Thiol levels

The thiol concentration was measured in plasma according to Aksenov and Markesbery (2001) adapted for microplates. The assay is based on the reduction of 50-dithiobis-2-nitrobenzoic acid (DTNB) by thiol groups, which become oxidized (disulphide), 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.

2.4.8. Protein carbonyl (CARBO) content

Carbonylated protein content was measured in plasma according to Reznick and Packer (1994) and adapted by 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.

2.5. Physiological characteristics

Monitoring of health status of calves to rule out clinical diseases comprised rectal temperature and presence/absence of diarrhea. The consistency and appearance of the faeces were observed every two days and the score was assigned according to their appearance, following classification from 0 to 3 according to Ishihara *et al.* (2001): (0) normal faeces, (1) soft faeces, (2) muddy faeces, and (3) watery faeces. Occurrence of diarrhea of each calf was calculated as the number of days to the first time fecal score was equal or above 2 and as a proportion (%) of number of days presenting fecal score equal or above 2 over the total number of days.

2.6. 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). Statistical analysis considered treatments (3), days of evaluation (days 1, 30 and 60) and treatment x day interaction as fixed effects; animal and experimental error were considered as random effects, using the SAS® MIXED procedure, version 9.4. Body weight gain, rectal temperature, concentrate intake, and redox state biomarkers were analyzed following the model: attribute = day + treatment + (day x treatment); method = REML, covariance matrix were Fa(2) and VT and repeated = day. Sex was included in the statistical model but as it did not affect the variables, we removed. As the actual number of days cows received their treatment varied, this information was included into the model as a covariate. A structural selection test was performed using the Bayesian information criterion (BIC). Covariance matrix used were VC for all characteristics except for DCFP, the covariance matrix used was Fa(2). Analysis of variance was performed to test the interaction effect. Comparison of means between treatments were performed using LSmeans (considering treatment, day and the interaction between treatment and day, option = diff) for all variables. The variables CARBO and GSH were not normally distributed and therefore, they were logarithmically transformed. The overall mean fecal score and the number of days to the first diarrhea occurrence were submitted to variance analysis considering the effect of treatment. The power analysis of the sample size was calculated using the POWER procedure (SAS®). Significant differences were declared when $P < 0.05$ and a trend considered to exist if $0.05 < P < 0.10$.

Table 1
Mean values of Thiols and GPx of calves fed milk from cows consuming control diet (CON) or containing green tea extract (GT) or oregano (OE) at the evaluation days.

Variable[2]	Day	Treatment[1]		
		CON	GT	OE
THIOLS (nmol/mg)	1	0.22 a	0.15 d	0.20 cd
	30	0.27 b	0.38 a	0.35 ab
	60	0.20 a	0.24 a	0.25 a
GPx (U/mg)	1	7.52 b	9.16 a	8.28 ab
	30	7.08 a	7.88 a	6.79 a
	60	7.74 a	4.43 b	5.57 b

[1] Treatments: CON = control; GT = 5 g / day green tea extract; OR = 10 g / day oregano extract.
[2] Variables: GPx = glutathione peroxidase.
P-values for interaction treatment x day
Thiols: D1: CON x GT $P = 0.064$
D30: CON x GT $P = 0.009$
GPx: D1: CON x GT $P = 0.049$
D60: CON x GT $P = 0.000$
CON x OE $P = 0.001$

3. Results

The sample sufficiency was confirmed by a power analysis with the result of 0.9 to 0.99 for all antioxidant variables.

The interaction between treatment and day was significant ($P < 0.05$) for GPx activity. On the day 1, calves in GT group presented higher activity of the GPx compared with the CON group ($P = 0.049$), whereas those in the OE group were not statistically different from the others. On day 60, activity of GPx was lower for the calves in GT ($P = 0.0004$) and OE groups ($P = 0.0014$) compared to the CON group, whereas on day 30 GPx activity did not differ between groups (Table 1).

On day 1, calves in GT group tended to have lower thiol concentration ($P = 0.064$) than calves in CON whereas those in the OE group were not statistically different from the others. On day 30 calves in GT presented higher thiol concentration ($P = 0.009$) than CON, while those in OE were not statistically different from the others (Table 1).

Calves in the GT group presented overall higher ($P < 0.05$) concentration for GSH and activity for CAT compared with CON, whereas OE group calves showed overall lower values of DCFP and DCFE concentration than CON. There were no differences between treatments for SOD (Table 2).

There were no differences between treatments ($P > 0.10$) for body weight at birth (29.9, 28.9 and 28.8 kg, SD = 2.5, respectively for CON, GT, OR), final body weight (63.5, 58.7 and 63.6 kg, SD = 7.6, respectively for CON, GT, OR) and average daily body weight gain (0.56, 0.50, 0.58 kg/d, SD = 0.3, respectively for CON, GT, OR). Both OE and GT did not affect ($P > 0.10$) concentrate intake that averaged 0.8 ± 0.2 kg.

Plant extracts did not affect ($P > 0.10$) the occurrence of diarrhea. Overall frequency (%) of days without diarrhea (fecal scores ≤ 2) were 79.4 ± 2.7, 85.2 ± 2.7 and 80.5 ± 3.4 for CON, GT, and OE, respectively. The first occurrence of fecal score ≥ 2 did not vary between treatments and it was on day 9.9 ± 1.6, 8.5 ± 1.6 and 5.4 ± 2.0 for CON, GT, and OE, respectively

There were no differences ($P > 0.10$) between treatments for rectal temperature. Overall means for rectal temperature (°C) were 38.9 ± 0.1, 38.7 ± 0.1 and 38.8 ± 0.2 for CON, GT, and OE, respectively.

4. Discussion

Previous reports showed improvement of antioxidant state in calves with green tea (Elshahawy et al., 2018) but to the best of our knowledge the effects of oregano on the redox status in dairy calves was not accessed. The main contribution of the present study was to highlight some positive effects of maternal supplementation with green tea and oregano extracts during the late gestation and early lactation periods (transition period) on the redox status of pre-weaned calves.

In general, all groups of calves showed a variation in the redox state during the pre-weaning period. Previous studies reported higher oxidative stress at birth, with increasing antioxidant capacity in the next days after calving (Jaffari-Dehkordi et al., 2015). The higher GPx activity noticed on day 1 in calves born to GT-fed cows are probably due to the higher antioxidant activity in the colostrum of these cows. As we did not measured the concentration of IgG and the oxidative/anti-oxidative profile in the colostrum of each cow, we cannot ruled out the differences in the natural antioxidant activity in the colostrum nor its variability between cows (Abuelo et al., 2014) and thus we may not attribute this effect solely to the green tea extract supplementation. Other beneficial effects on redox biomarkers in calves born to GT-cows were noticed later such as the higher value for thiol concentration on day 30 and the overall higher concentration of GSH and higher activity of CAT. Previous studies with cows supplemented with GT also reported increased GSH concentration in the blood of cows (Kolling, 2016; Vizzotto et al., 2020). Although catechins undergo extensive degradation in the rumen, phenols are transferred to milk (Jordán et al., 2010) and thus affect redox biomarkers in calves. Tea polyphenols present antioxidant activity, resulting mainly from hydrogen atom transfer (HAT) or single electron transfer (SET) reactions, or both involving hydroxyl groups. Tea catechins are thought to interrupt lipid

Table 2
Mean values of redox biomarkers of calves fed milk from cows consuming control diet (CON) or containing green tea extract (GT) or oregano (OE).

Variables[2]	Treatments[1]			SE	*P* – values		
	CON	GT	OE		CON x GT	CON x OE	GT x OE
Redox status profile							
THIOLS (nmol/mg)*	0.23	0.25	0.27	0.03	0.42	0.19	0.53
CARBO (nmol/mg)	2.44	3.31	2.56	0.36	0.01	0.73	0.04
DCFP (nmol/mg)	48124	47838	30390	3600	0.65	<0.00	<0.00
DCFE (nmol/mg)	4884	4790	3264	282.7	0.7	<0.00	<0.00
GSH (U/mg)	0.09	0.14	0.06	0.03	0.04	0.37	0.01
SOD (U/mg)	56.87	57.56	52.07	3.22	0.82	0.14	0.10
CAT (U/mg)	2.20	2.61	2.05	0.16	0.02	0.20	0.01
GPx (U/mg)*	7.45	7.16	6.88	0.40	0.56	0.12	0.53

[1] Treatments: CON = control; GT = 5 g / day green tea extract; OR = 10 g / day oregano extract.
[2] Variables: CARBO = carbonyl; DCFP = oxidation of dichlorofluorescein in plasma; DCFE = oxidation of dichlorofluorescein in erythrocytes; GSH = reduced glutathione; SOD = superoxide dismutase; CAT = catalase; GPx = glutathione peroxidase.
SE = standard error.
* For the interaction between treatment x days, please refer to Table 2.

peroxidation by HAT reaction and quench free radicals such as oxygen, nitrogen, and chlorine radical species by SET reactions (Lambert and Elias, 2010). Both mechanisms may have a sparing effect on antioxidant enzymes oxidative and signaling compounds, explaining the observed higher activity of GPx and higher levels of thiol groups in calves fed milk from GT-cows.

The main bioactive components of oregano are phenolic components and essential oils of which thymol and carvacrol are the major components (Oh et al., 2017), capable of slowing down lipid peroxidation, reducing free radicals formation (Hać-Szymańczuk et al., 2018). The overall reduction in the concentration of reactive substances in the erythrocytes (DCFE) and plasma (DCFP) showed by calves born to OE-cows compared with not supplemented cows is related to the reduced free radicals formation, what was already observed in cows fed oregano extract (Vizzotto et al., 2020, *in press*) and to the active compounds transferred to milk. Essential oils are poorly or not degraded in the ruminal environment and are transferred via gastrointestinal tract to mammary gland and to milk of cows (Lejonklev et al., 2013; 2016), explaining at least partially the positive results observed in group during the pre-weaning period.

Our results about the improvement in some biomarkers of the redox status in calves fed milk from green tea or oregano extracts-supplemented cows are in line with their mechanism and actions already described in other papers, which basically are reducing of formation of radical species by e.g. diminishing or interruption of lipid peroxidation and scavenging of free radical species (Lambert and Elias, 2010; Hać-Szymańczuk et al., 2018).

The absence of effects of plant extracts on body weight gain and concentrate intake is related to the similar amounts of milk and concentrate consumed by calves in all treatments. Oregano and green tea were mixed into the milk which was entirely consumed by the calves. Essential oils in oregano and polyphenols in green tea may alter smell and taste in feeds (Oh et al., 2017) but apparently it was not enough to change calves' intake. These results are in agreement to results of previous studies when oregano and green tea extracts were fed directly to heifers and dairy cows and did not significantly affect productive variables such body weight gain and diet intake (Aemiro et al., 2016; Kolling et al., 2016; 2018; Stivanin et al., 2019).

The results open new perspectives in cow's supplementation with green tea and oregano extracts during the transition period and initial stage of lactation of dairy cows as besides the beneficial effects on cow's health and digestion, we may expect positive effects on pre-weaned calves' redox status. Researches focusing on the potential use of plant extracts in feeding dairy calves are still limited and the published results are variable, but many of them indicate potential beneficial effects.

The authors acknowledge some of the limitations of the present study such as calves' passive immunity transfer was not measured immediately after birth (e.g. concentration of IgG) and the secondary compounds were not evaluated in the milk of the cows in order to verify their passage to the milk. Thus, the effects on calves' biomarkers of redox status observed during the pre-weaning period might be not only attributable to the transport of active compounds of oregano or green tea into milk as calves born to oxidatively-stressed cows during late gestation also show redox imbalance (Abuelo et al., 2019).

5. Conclusions

Supplementation of cows with oregano and green tea extracts during the transition period may allow transfer of active compounds into the milk and thus improve some biomarkers of redox status of calves during the pre-weaning period. Calves born to green tea extract-fed cows exhibited higher glutathione peroxidase and catalase activities; while those from oregano extract-fed cows, lower concentration of reactive substances in plasma and erythrocytes. Improving redox status might benefit calves' health. Further research is needed to establish the direct effect of plant extracts on calf's redox status as well the effective dosage and ways of supplementing the green tea and oregano extracts.

Author Statement

M. de Paris was the main investigator conducting the trial at the field, project conceptualization and writing; **S.C.B. Stivanin** was responsible for the statistical analysis; **E.F. Vizzotto, L.T. Passos** and **I.D.V. Angelo** were the secondary investigators conducting the trial at the field; **M.B. Zanela** was responsible for the field activities administration; **V. Stone, C.P. Klein** and **C. Matté** run the biomarkers redox essays and helped in the interpretation of the results; **G. Heisler** was responsible for the review, editing and **V. Fischer** was the main investigator responsible for funding acquisition, project administration and editing of the manuscript.

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Ethics statement

This study was approved by the Ethics Committee for the Use of Farm Animals from the Federal University of Rio Grande do Sul, protocol number 30756.

Declaration of Competing Interest

None

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.livsci.2020.104272.

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