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Supplementation with green tea and oregano extracts on productive characteristics, blood metabolites, and antioxidant status of Jersey cows during the transition period

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A B S T R A C T

Plant extracts have been recognized as beneficial to human health and have been evaluated as feed additive for domestic and companion animals. This study evaluated oregano and green tea extracts fed to Jersey cows from approximately 21 d before calving to 21 d after calving on milk production, milk composition, and blood metabolites as well as investigated immunological and antioxidant attributes. Twenty-four Jersey cows with 441 ± 27 kg of BW, 3.5 ± 0.3 of body condition score (**BCS**), and 2.7 ± 1.8 lactations were selected at approximately 28 d before the expected parturition date and were randomly assigned to three treatments with eight cows each: without plant extracts in diet (control – **CON**), addition of 10 g per day of oregano extract (**OR**), and addition of 5 g per day of green tea extract (**GT**). Feed intake, BW, BCS, blood metabolites, hemogram as well as oxidative stress biomarkers were evaluated from approximately 3 weeks *prepartum* to 3 weeks *postpartum* (transition period) while milk production and composition were evaluated during the first 3 weeks of lactation. Plant extracts did not change BW, BCS, and DM intake (**DMI**) throughout the transition period, but OR increased in approximately 20% total digestive nutrients and metabolizable energy intake on days 15 and 16 *postpartum* compared with CON. In the *prepartum*, OR increased in 48% platelets count compared to the CON, while GT augmented in 142% eosinophils compared with CON. Oregano extract reduced the levels of reactive species in the erythrocytes in 40% during *prepartum* and *postpartum* compared with CON, while GT reduced its levels in 24 and 29% during *prepartum* and *postpartum*, respectively, when compared with CON. In the *postpartum* period, OR increased in 60% the carbonylated protein content compared with CON, while GT reduced in 45% the levels of reactive species in plasma compared with CON. During the *postpartum*, both extracts increased in 33% the concentration of reduced glutathione when compared with CON. Moreover, GT tended to decrease feed efficiency in 11% when compared with CON; OE reduced milk pH and somatic cell count when compared with CON. In conclusion, OE and GT did not expressively affect immunological attributes in blood but reduce some oxidative stress biomarkers without compromising productive traits of Jersey cows during the transition period.

Implications

Impaired immunity, reduced feed intake, digestive disturbances, and increased disease propensity are faced by dairy cows in the transition period, which enhance early-lactation risks of culling and mortality. Oregano and green tea extracts have alleged antioxidant capacity and may promote the reduction of oxidative stress, improving immunity and health of dairy cows in the beginning of lactation. In the present study, oregano and green tea extracts added into cow's diet improved some biomarkers of the redox status in dairy cows during the transition period.

Introduction

The transition period is a greatly challenging phase for dairy cows due to the expressive metabolic changes, which adversely affect cow's immune system (Abuelo et al., 2015; Ringseis et al., 2015). This period is characterized by reduced feed intake (McCarthy et al., 2016) and

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the onset of metabolic diseases and inflammatory processes (Bradford et al., 2015). Immunosuppression occurs in consequence of the parturition process, with increases in the expression and release of inflammatory mediators (Abuelo et al., 2015) that may stimulate lipolysis, increasing the release of non-esterified fatty acids (**NEFA**) (Gregor and Hotamisligil, 2011) and promoting the

oxidative stress (Colitti and Stefanon, 2006). Immunosuppression may reduce feed intake and increase energy expenditure (Kasimanickamet et al., 2013).

Natural plant secondary metabolites may act as immunological modulators and might present beneficial effects on animal's oxidative status and health (Winkler et al., 2015; Drong et al., 2016). Oregano extract contains the essential oils carvacrol and thymol and has been used for its antimicrobial (Cho et al., 2015), antimetanogenic (Kolling et al., 2018), and antioxidant properties (Hashemzadeh-Cigari et al., 2014; Paraskevakis, 2015). Green tea extract is rich in catechins, L-theanine and caffeine, and presents antioxidant and anti-inflammatory properties (Chen et al., 2004; Oh et al., 2017).

Previous studies used distinct plant extracts as feed additives in cows' diet during the transition period. Essential oils blend containing rosemary, cinnamon bark, saffron, and clover fed to dairy cows reduced NEFA, insulin, and oxidative stress markers, while increased feed intake and milk production (Hashemzadeh-Cigari et al., 2014). Catechins reduced the levels of triacylglycerides, cholesterol, and NEFA during the first weeks of lactation, while they increased milk production and improved immunity (Winkler et al., 2015) as well as reduced oxidative stress (Colitti and Stefanon, 2006) in dairy cows. On the other hand, other studies reported the absence of effects on feed intake (Winkler et al., 2015) or improvements in the antioxidant capacity (Stefanon et al., 2005; Gessner et al., 2017).

The objective of this study was to evaluate the effects of oregano and green tea extracts as feed additives on milk's production and composition as well as on blood metabolites and antioxidant biomarkers in Jersey cows during the transition period.

Material and methods

Description of location, animals, and diets

This study was approved by the Animal Ethics Committee of the Federal University of Rio Grande do Sul, n° 29838, on September 29, 2015.

The experiment was conducted at Embrapa Clima Temperado, in Pelotas, Rio Grande do Sul, Brazil, latitude: −31.776, longitude: −52.3594 31° 46′34″ South, 52° 21′34″ W.

Approximately 28 days before their respective predicted calving date, 24 Jersey cows were selected and separated from the experimental herd and distributed within three groups of eight cows each, according to parity and BW. All cows were submitted to a 7-d adaptation period followed by an experimental period of approximately 42 d (from 21 d before the expected calving date to 21 d after calving). On day 21 before the expected calving date, cows were randomly assigned to one of the three treatments: control (**CON**) – without addition of plant extracts in the diet, oregano extract (**OR**) – addition of 10 g per cow per day of oregano extract in the feed, and Green tea extract (**GT**) – addition of 5 g per cow per day of green tea extract in the feed. Doses were chosen upon previous authors' work (Kolling et al., 2018). With the exception of plant extracts, all animals received the same basal diet throughout the experiment (Supplementary Table S1). As some cows did not calve at the expected calving date, the actual number of experimental days *prepartum* was 20.1 ± 3.2, 18.5 ± 4.8, and 18.5 ± 4.5 for CON, GT, and OR groups, respectively.

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 body condition score (BCS); cows in the OR treatment 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.

Plant extracts powder was homogenized in 0.5 kg (as fed) of concentrate and top dressed onto the mixed diet at every feeding. Oregano extract (Orego Stim® Meriden Animal Health Ldta., Northampton, UK, redistributed in Brazil by Advit Animal Nutrition) containing 5% of essential oil of oregano plants (*Origanum vulgare* subsp. *Hirtum*) and 95% of inert substance had a minimum concentration of 50 g/kg, containing 80 to 82% of carvacrol, 2.5 to 3.0% of thymol, 3.5 to 9.0% of p-cymene, and 2.0 to 5.0% of Y-terpinene. Green tea extract (Seiva Brazilis, São Paulo, Brazil) had an approximate concentration of 56% (±2.5%) of polyphenols. Composition of the plant bioactive compounds was based on a general manufacturer's information. Quercetins and caffeine were determined by HPLC, while carvacrol and thymol were determined by gas chromatography coupled to ion trap MS.

Dry matter intake, body weight, and body condition score

As cows grazed during the *postpartum* period, total DM intake (**DMI**) was estimated using chromium oxide (Cr_2O_3). The BW and BCS were evaluated weekly (measurements were taken 7 days apart) in the morning before feeding from day −28 to day 21 relative to expected calving date. The weighing was performed on an electronic scale and the BCS was assigned by trained evaluators on a scale of 1 to 5 (See Supplementary Material S1 for further details).

Blood collection and analysis

The blood was sampled in non-heparin vacutainer collection tubes by venipuncture in the external jugular in the morning (0700 h) before the diet supply on days −7, −4, −2, 0, 2, 4, 7, and 14 relative to calving date. Subsequently, serum samples were analyzed for glucose, calcium, and creatinine levels (see Supplementary Material S1). On days −7, −4, −2, 0, 2, 4, 7, and 14 relative to calving date, blood samples were collected from the coccygeal vein for β-hydroxybutyrate (**BHB**) analysis. On days −7, 7, and 21 relative to calving date, blood samples were collected in EDTA-containing vacutainers by venipuncture in the external jugular for hemogram analysis.

On day −28, 0, 2 and 21 relative to calving date, blood samples from external jugular vein were collected in heparinized vacutainer tubes to determine oxidative stress markers. The following biochemical determinations were performed in the erythrocytes: superoxide dismutase activity, catalase (**CAT**) activity, glutathione peroxidase (**GPx**) activity, oxidation of dichlorofluorescein (**DCF**), and concentration of reduced glutathione (**GSH**). The following biochemical determinations were performed in the plasma: oxidation of DCF, concentration of carbonylated proteins (**CARBO**), and the content of thiol group.

The specific activities of antioxidant enzymes were expressed as U/mg of protein. The results of DCF, GSH, and carbonyls were expressed as nmol/mg protein. The results of thiols were expressed as nmol of TNB/mg of protein. Protein concentration (mg of protein/ml) was measured according to Lowry et al. (1951).

For further details (e.g. methods and references) please refer to the Supplementary Material S1.

Milk collections and analysis

Milk production (kg) was recorded twice a day, at the morning and evening milking using the electronic sensors of the automatic milking machine Delaval® midline. Between the 5 and 21 d of lactation, milk was sampled every day and analyzed for stability in the alcohol test, pH, titratable acidity as well as concentrations of ionic calcium, fat, lactose, protein, total solids extracts, and urea. Milk production was expressed as energy corrected milk **(ECM)**: ECM = [(*0.3246* × *kg of milk*) + (*12.86* × *kg of fat*) + (*7.04* × *kg of true protein*)]; true protein

Table 1.
Effect of providing oregano and green tea extracts on BW and body condition score (BCS) in lactating Jersey cows during prepartum and postpartum.

Variable	Treatments (T)[1]			SD	*P*-value Treatment	*P*-value Day	*P*-value Day × T	*P*-value Contrasts	
	CON ($n = 8$)	OR ($n = 8$)	GT ($n = 8$)					CON × OR	CON × GT
BW (kg)									
Prepartum	446	448	446	3.9	0.93	0.01	0.44	0.74	0.98
Postpartum	406	422	415	6.4	0.22	0.01	0.87	0.09	0.29
BCS (1–5 points)									
Prepartum	3.5	3.6	3.5	0.05	0.60	0.42	0.11	0.37	0.96
Postpartum	3.3	3.3	3.3	0.06	0.97	0.10	0.38	0.94	0.87

[1] CON = control, GT = green tea extract, OR = oregano extract.

was calculated as 95% of the milk CP. For further details (e.g. methods and references), please refer to the Supplementary Material S1.

Experimental design and statistical analysis

A complete randomized design was used and cows were taken as the experimental units. Continuous data were tested for normality (PROC UNIVARIATE; SAS®) using *Shapiro–Wilk* test. Data were analyzed within the *prepartum* and *postpartum* periods. Data collected over time were subjected to a repeated ANOVA measurement considering the fixed effects of treatment, day of assessment, and treatment × day interaction, while animals and residue were considered as random effects (PROC MIXED; SAS®). Parity as well as BW and BCS measured before treatments were started were included in the model as covariates. Analysis of variance was performed to test interaction effect. The contrasts CON × OR and CON × GT with adjusted one-tailed side Dunnett *P*-values were used to compare the means between treatments for all variables. Somatic cells count (**SCC**) values did not follow normal distribution and were logarithmically transformed. Data that did not follow normal distribution even after transformation (e.g. eosinophils, monocytes, and rods during *postpartum* period as well as *postpartum* BW, loss) were analyzed with non-parametric statistics using the Wilcoxon test. The power analysis of the sample size was calculated using the POWER procedure (SAS®). The significant differences between treatments were declared when $P < 0.05$ and a tendency was considered to exist if $0.05 < P < 0.10$. For further details, please refer to the Supplementary Material S1.

Results

The sample sufficiency was confirmed by a power analysis with the result of 0.99 for milk production, milk composition, and antioxidant variables and ranging from 0.76 to 0.99 for the main blood metabolites variables.

Body weight and body condition score

There was no interaction between treatment and day for BW and BCS. The use of plant extracts did not affect BW and BCS (Table 1) during *pre* and *postpartum* periods ($P > 0.05$). Overall BW increased ($P < 0.05$) from 440 ± 3.1 kg at the third week pre-calving to 452 ± 2.8 at calving and further decreased ($P < 0.05$) to 410 ± 4.1 kg at the end of the third week *postpartum* (Fig. 1). Overall BCS was 3.5 ± 0.05 and 3.3 ± 0.05 during *pre* and *postpartum* periods, respectively.

Feed intake

There was no interaction between treatment and day for total DM and feed fractions intakes, except for total digestive nutrient (**TDN**) and metabolizable energy (**ME**) (Table 2). During the *postpartum* period, OR increased the intake of TDN and ME on days 15 and 16 ($P < 0.05$) compared with CON. On the other hand, CON tended ($0.05 < P < 0.10$) to increase TDN intake compared with OR on day 2, while GT tended ($0.05 < P < 0.10$) to increase TDN intake compared with CON on day 8 (Fig. 2). The use of plant extracts did not significantly

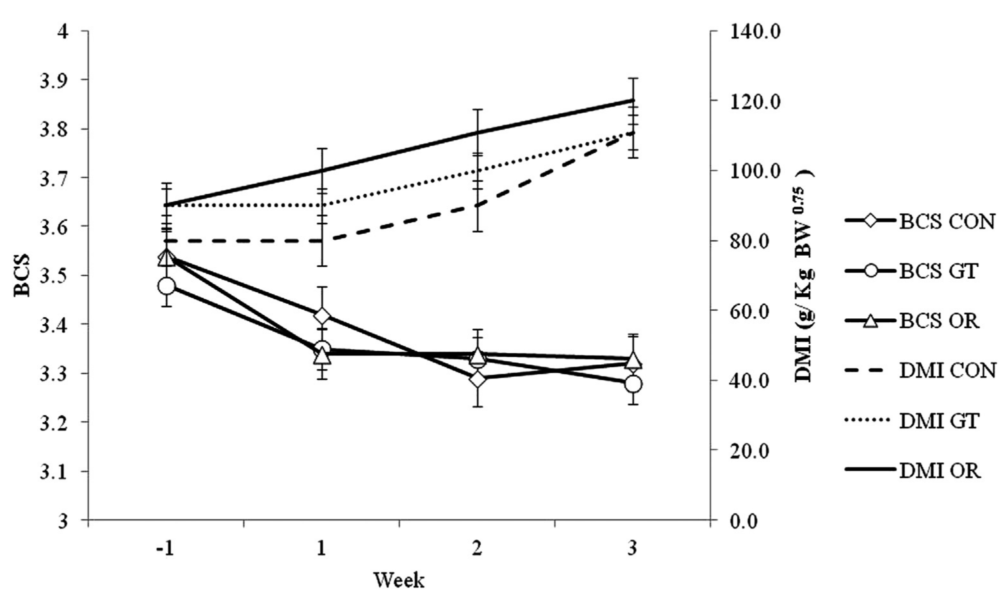

Fig. 1. Ingestion of DM intake (DMI) (g/(kg $BW^{0.75}$ × week)) and body condition score (BCS) according to treatments (CON = control, GT = green tea extract, OR = oregano extract) in Jersey cows between 3 weeks *prepartum* and 3 weeks *postpartum* ($n = 8$ cows per treatment).

Table 2.
Effect of providing oregano and green tea extracts on intake of feed fractions in lactating Jersey cows during the transition period.

Variable	Treatments (T)[1]			SE	*P*-value Treatment	*P*-value Day (D)	*P*-value T × D	*P*-value Contrasts	
	CON ($n = 8$)	OR ($n = 8$)	GT ($n = 8$)					CON × OR	CON × GT
Prepartum									
DM intake (kg/d)	8.0	8.5	8.2	0.4	0.42	<0.01	0.98	0.19	0.59
Metabolizable energy intake (MJ/d)	103.7	110.5	106.0	4.2	0.28	<0.01	0.96	0.24	0.66
Total digestible nutrients intake (kg/d)	5.6	6.0	5.8	0.2	0.29	<0.01	0.95	0.24	0.78
NDF intake (kg/d)	4.10	4.4	4.3	0.2	0.69	0.03	0.98	0.48	0.47
CP intake (kg/d)	1.1	1.2	1.1	0.0	0.11	<0.01	0.54	0.12	0.86
Postpartum									
DM intake (kg/d)	9.0	10.0	9.2	0.5	0.34	<0.01	0.15	0.16	0.84
Metabolizable energy intake (MJ/d)	113.4	123.9	117.7	5.5	0.31	<0.01	0.02	0.15	0.83
Total digestible nutrients intake (kg/d)	6.2	6.7	6.4	0.3	0.33	<0.01	0.01	0.14	0.64
NDF intake (kg/d)	4.2	4.7	4.3	0.3	0.56	<0.01	0.13	0.31	0.91
CP intake (kg/d)	1.2	1.3	1.2	0.6	0.14	<0.01	0.10	0.10	0.96

[1] CON = control, GT = green tea extract, OR = oregano extract.

affect DM, NDF, and CP intakes (Table 2). Dry matter intake was reduced in 0.29 kg DM/day during the pre-calving period ($P < 0.01$), but DMI increased linearly ($P < 0.01$) in the first 16 d of lactation for GT while for OR, DMI began to increase linearly a week before parturition (Fig. 1).

Metabolites in blood and antioxidant enzymatic factors

No significant interactions ($P > 0.05$) were detected between treatment and day of measurement for blood metabolites (Table 3) and hematological profile (for results about hematological profile, please refer to the Supplementary Table S2).

During the *prepartum*, OR increased calcium concentration in *serum* ($P < 0.05$) when compared with CON while during the *postpartum*, OR tended ($0.05 < P < 0.10$) to decrease creatinine compared with CON. Plant extracts did not change ($P < 0.05$) the concentrations of glucose, urea nitrogen, creatinine, and BHB during the *pre* and *postpartum* periods (Table 3).

In the *prepartum*, OR increased ($P < 0.05$) platelet counts compared with CON while GT increased ($P < 0.05$) the percentage of eosinophils compared with CON. Plant extracts did not change erythrocytes, hemoglobin, hematocrit, leucocytes, lymphocytes, rods, and monocytes (for further details, please refer to the Supplementary Table S2).

There were treatment and day interaction ($P < 0.05$) for the antioxidant enzymatic factors (Table 4). During the *prepartum*, OR and GT decreased ($P < 0.05$) the oxidation of DCF in the erythrocytes compared with CON. Oregano extract tended ($0.05 < P < 0.10$) to decrease the concentration of CARBO and the activity of GPx compared with CON (Table 4).

In the *postpartum* period, OR and GT decreased ($P < 0.05$) oxidation of DCF in the erythrocytes compared with CON, while GT decreased ($P < 0.05$) and OR tended ($0.05 < P < 0.10$) to decrease the oxidation of DCF in plasma compared with CON. Both extracts increased ($P < 0.05$) the concentration of GSH compared with CON. Moreover, OR increased the concentration of CARBO compared with CON (Table 4).

Milk production and chemical characteristics

There was a significant ($P < 0.05$) interaction for treatment and day of measurement for ionic calcium in milk (Table 5). On days 7 and 9, OR increased ($P < 0.05$) the ionic calcium concentration in milk when compared with CON. On day 11, GT decreased the ionic calcium content in milk when compared with CON.

Feed efficiency tended ($0.05 < P < 0.10$) to be lower in cows fed GT when compared with CON. Cows fed OR tended ($0.05 < P < 0.10$) to produce milk with lower pH and produced milk with higher acidity ($P < 0.05$) than CON. Cows fed OR produced milk with lower SCC ($P < 0.05$) than cows in CON (Table 5).

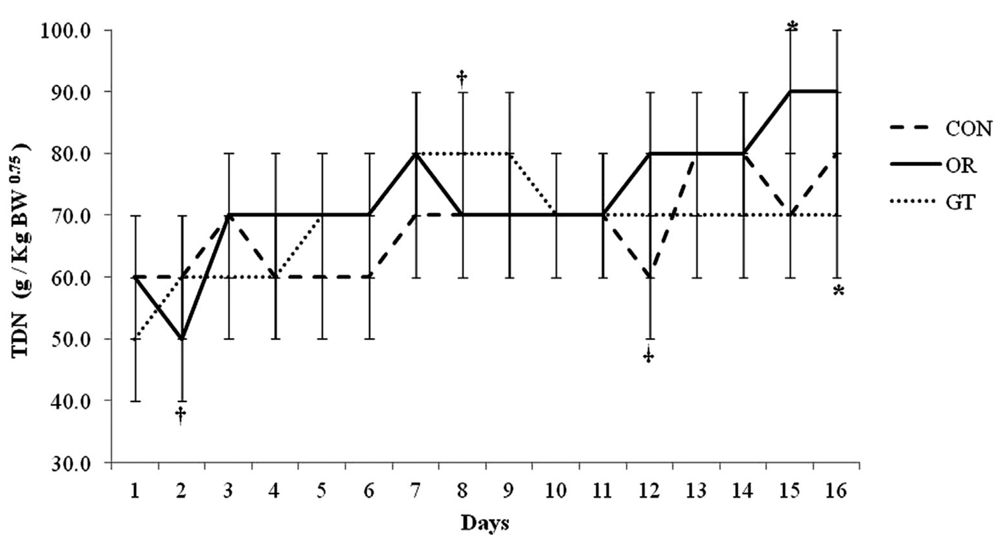

Fig. 2. Total digestible nutrients intake (TDN) (g/(kg BW$^{0.75}$ × week)) according to treatments (CON = control, GT = green tea extract, OR = oregano extract, with eight cows per treatment) in Jersey cows during the *postpartum* period. * $P < 0.05$, † $0.05 < P < 0.10$.

Table 3.
Effect of providing oregano and green tea extracts on blood metabolites in lactating Jersey cows during the transition period.

Variable	Treatments (T)[1]			SD	*P*-value Treat	*P*-value Day (D)	*P*-value T × D	*P*-values Contrasts	
	CON (*n* = 8)	OR (*n* = 8)	GT (*n* = 8)					CON × OR	CON × GT
Prepartum									
Glucose (mg/l)	564.6	557.6	605.3	1.7	0.29	<0.01	0.23	0.72	0.21
Urea nitrogen (mg/l)	228.7	204.9	253.3	2.2	0.43	0.02	0.20	0.49	0.49
Creatinine (mmol/l)	0.87	0.90	1.33	0.1	0.27	0.33	0.26	0.90	0.13
Calcium (mg/l)	92.8	98.0	92.5	0.1	0.08	0.01	0.63	0.04	0.93
BHB[2] (mmol/l)	0.51	0.48	0.54	0.4	0.71	0.24	0.50	0.70	0.62
Postpartum									
Glucose (mg/l)	456.4	502.4	470.1	2.2	0.41	0.07	0.17	0.19	0.68
Urea nitrogen (mg/l)	123.0	137.1	118.2	1.6	0.78	0.22	0.79	0.60	0.85
Creatinine (mmol/l)	1.21	0.66	0.88	0.2	0.18	0.28	0.32	0.07	0.25
Calcium (mg/l)	92.1	94.1	88.1	0.2	0.25	0.64	0.71	0.59	0.24
BHB[2] (mmol/l)	0.87	0.61	0.85	0.2	0.52	0.02	0.97	0.29	0.93

The use of plant extracts did not affect ($P > 0.05$) the following variables: milk production, ECM, stability in the alcohol test, as well as the concentrations of CP, fat, lactose, and total solids in milk (Table 5).

Discussion

This study was designed to evaluate oregano and green tea extracts as feed additives added separately into the diet of lactating Jersey cows from approximately 21 d *prepartum* to 21 d *postpartum*. The present study demonstrated the reduction in the concentration of some oxidative stress biomarkers such as oxidation of DCF in the erythrocytes and in plasma and the increase in the concentration of GSH with the supply of oregano and green tea extracts; those are the main contribution of the present study.

Plant extracts may contribute to increase endogenous antioxidants and to reduce free radicals (Oh et al., 2017) as they may donate electrons to free radicals and induce nuclear factor activation 2 related to erythroid 2 (**Nrf2**), which in turn leads to the activation of several antioxidant enzymes as GPx and CAT (Surh, 2008). Essential oils (Hashemzadeh-Cigari et al., 2014) and catechins (Colitti and Stefanon, 2006) are able to reduce oxidative stress biomarkers in dairy cows, but results reported during the transition period are variable and sometimes controversial (Winkler et al., 2015; Gessner et al., 2017). Recently, studies evidenced that dietary polyphenols can attenuate inflammation and endoplasmic reticulum stress in dairy cows (Bradford et al., 2015; Gessner et al., 2017) and therefore contribute to decrease the occurrence of some metabolic disorders as fatty liver and ketosis (Winkler et al., 2015).

Bioactive compounds of plants present distinct resistance to microbial degradation in the rumen. Oh et al. (2017) reviewed the subject and reported that approximately 60% of carvacrol and thymol were recovered in the rumen. On the other hand, polyphenols in green tea

Table 4
Effect of providing oregano and green tea extracts on enzymatic antioxidant factors and reactive species in the blood of lactating Jersey cows during the transition period.

Variable	Treatments (T)[1]			SD	*P*-value Treatment	*P*-value Day (D)	*P*-value T × D	*P*-value Contrasts	
	CON (*n* = 8)	OR (*n* = 8)	GT (*n* = 8)					CON × OR	CON × GT
Prepartum									
SOD (U/mg of protein)[2]	15.65	16.04	15.37	0.77	0.85	0.04	0.23	0.73	0.80
THIOL (nmol TNB/mg of protein)[3]	0.19	0.16	0.17	0.02	0.69	0.26	0.40	0.40	0.56
CARBO (U/mg of protein)[4]	1.82	1.48	1.61	0.12	0.19	0.32	0.40	0.07	0.30
DCFP (nmol/mg of protein)[5]	40.595	37.847	56.406	8.381	0.61	0.93	0.87	0.91	0.35
DCFE (nmol/mg of protein)[6]	18.202	10.727	13.922	4.053	0.01	<0.01	0.38	0.02	0.03
GSH (nmol/mg of protein)[7]	0.12	0.13	0.12	0.01	0.63	0.7	0.39	0.62	0.60
CAT (U/mg of protein)[8]	1.35	1.9	1.77	1.67	0.12	0.1	0.38	0.54	0.12
GPx (U/mg of protein)[9]	0.7	0.3	0.5	0.15	0.17	0.39	0.43	0.06	0.1
Postpartum									
SOD (U/mg of protein)[2]	15.65	16.04	15.37	0.77	0.85	0.04	0.23	0.73	0.80
THIOL (nmol TNB/mg of protein)[3]	0.16	0.18	0.15	0.02	0.50	0.29	0.52	0.51	0.71
CARBO (U/mg of protein)[4]	1.69	2.74	2.13	0.41	0.04	0.06	0.55	0.04	0.18
DCFP (nmol/mg of protein)[5]	44.134	32.298	24.033	4.605	0.01	0.73	0.81	0.07	0.04
DCFE (nmol/mg of protein)[6]	15.361	9.642	10.859	8.912	0.04	<0.01	0.05	0.03	0.01
GSH (nmol/mg of protein)[7]	0.09	0.12	0.12	0.01	0.04	0.02	0.74	0.02	0.04
CAT (U/mg of protein)[8]	1.06	1.15	1.03	0.13	0.79	<0.01	0.11	0.55	0.98
GPx (U/mg of protein)[9]	0.84	1.28	0.98	1.03	0.30	0.43	0.71	0.12	0.36

[1] CON = control, GT = green tea extract, OR = oregano extract.
[2] SOD = superoxide dismutase.
[3] THIOL = concentration of thiols.
[4] CARBO = carbonyls.
[5] DCFP = oxidized dichlorofluorescein diacetate in plasma.
[6] DCFE = oxidized dichlorofluorescein diacetate in the erythrocytes.
[7] GSH = reduced glutathione.
[8] CAT = catalase.
[9] GPx = glutathione peroxidase.

Table 5.
Effect of providing oregano and green tea extracts on production and physical–chemical composition of milk of lactating Jersey cows during the beginning of lactation.

Variable	Treatments (T)[1]			SD	*P*-value Treat	*P*-value Day (D)	*P*-value T × D	*P*-value Contrasts	
	CON ($n = 8$)	OR ($n = 8$)	GT ($n = 8$)					CON × OR	CON × GT
Milk production (kg/d)	16.1	18.3	14.0	0.9	0.02	<0.01	0.94	0.13	0.11
ECM (kg/d)[2]	17.2	19.7	17.2	1.3	0.37	0.41	0.53	0.21	0.97
Feed Efficiency (ECM[2]/kg DMI)	1.9	1.9	1.7	0.1	0.10	0.93	0.24	0.95	0.06
Alcohol stability (°GL)	74	70	74	1.7	0.17	<0.01	0.14	0.10	0.88
pH	6.6	6.5	6.6	0.3	0.01	<0.01	0.07	0.08	0.88
Acidity (°D)	16.9	21.0	17.3	1.2	0.05	0.04	0.64	0.02	0.84
Ionic calcium (mg/l)	150	157	149	8.6	0.78	<0.01	0.02	0.55	0.93
Fat (g/100 g)	3.5	3.5	3.9	0.2	0.52	0.61	0.34	0.99	0.31
Total protein (g/100 g)	3.6	3.6	3.7	0.6	0.66	<0.01	0.74	0.74	0.55
Lactose (g/100 g)	4.3	4.5	4.3	0.8	0.27	0.07	0.80	0.21	0.86
Total solids (g/100 g)	12.6	12.7	13.1	0.28	0.56	0.04	0.09	0.86	0.30
Somatic cell count ($\times 10^3$cel/ml)	1 496	563	1 123	195	0.12	0.03	0.14	0.04	0.39

[1] CON = control, GT = green tea extract, OR = oregano extract.

extract undergo extensive degradation and modification in the rumen and intestine, presenting low detectable amounts of catechins in plasma and raising doubts about their effects on the redox status and health in ruminants (Wein et al., 2016; Olagaray and Bradford, 2019).

Therefore, our results confirmed some beneficial effects of oregano extract and in lesser extend of green tea extract on the antioxidant defense system such as the reduction in the oxidation of DCF in the erythrocytes and in plasma and the increase in the concentration of GSH, which are considered as biomarkers of antioxidant defense system (Marrocco et al., 2017).

On the other hand, we noticed an increment in CARBO in cows in OR compared to CON during the *postpartum* period. CARBO is usually considered as a marker of protein oxidation (Firuzi et al., 2006), so this increase can be considered as a negative effect. It is worth to point out that this rise in CARBO was not followed by increments in creatinine and plasma urea nitrogen that are positively related to protein catabolism (Nozad et al., 2012).

Controversial results have been reported about the effect of plant extracts on oxidative stress biomarkers, partially related to distinct doses and products. Colitti and Stefanon (2006) and Hashemzadeh-Cigari et al. (2014) detected reduction in reactive species and an increase in the activity of antioxidant enzymes in dairy cows receiving chestnut tannins and a blend of essential oils, respectively. On the contrary, Stefanon et al. (2005) and Winkler et al. (2015) did not report any effect of plant extracts on the reduction of oxidative stress during the transition period.

Oregano and green tea extracts did not change total DM, CP, and fiber intakes, but we noticed an increase in TDN and ME intakes at the end of the third lactation week (days 15 and 16) in cows fed oregano extract. Kolling et al. (2018) fed the same extracts and doses to Holstein and Girolando (Holstein × Gyr) cows between 28 and 87 d of lactation and reported that both extracts decreased methane emissions while increased the digestible fraction of the ingested DM, which could explain the higher ME and TDN intakes observed in the present study.

The absence of effects of oregano and green tea extracts on BW and BCS during *prepartum* and *postpartum* was probably due to the similar feed intake. The small increase in TDN intake observed in the end of the trial was not enough to increase BW and BCS. These results are in agreement to those reported by Kolling et al. (2018).

The similar feed intake between treatments explains the absence of differences in BHB, usually used as indicator of lipid catabolism and mobilization of body reserves (Akbar et al., 2015). β-hydroxybutyrate mean values were below the values usually reported in the literature for subclinical ketosis, 1.0 to 1.4 mmol/l, during the *postpartum* period (Duffield et al., 2009). One of the reasons might have been the moderate metabolic challenge provoked by the moderate ECM production (18.0 ± 1.3 kg), which is one of the limitations of the present study. Consequently, BCS losses during the first 3 weeks *postpartum* (±0.19 points) were below the 0.5 point score that is usually considered as capable of causing metabolic diseases (Garnsworthy, 2006).

The absence of effects of OR and GT on milk production compared with CON is in agreement with Hristov et al. (2013) and Kolling et al. (2018), and is related to the similar nutrient ingestion and moderate milk production levels. Moreover, differences in milk production were more consistently reported after the first 3 or 4 weeks of lactation (Olagaray and Bradford, 2019), so the experimental protocol used in the present study might have precluded the detection of significant differences. Wincker et al. (2015) supplemented cows with a blend composed by green tea and curcuma and reported increased milk production during the first 9 weeks of lactation.

As cows in OR produced milk with lower SCC, it could be expected a positive effect on milk production as high SCC reduces milk production (Hadrich et al., 2018). It is noticeable that cows in OR produced numerically more milk ($P = 0.13$) than cows in CON. The lowest SCC in the milk of cows in OR may be related to the alleged antibacterial properties reported for oregano (Cho et al., 2015), as its main components, carvacrol and thymol are able to affect cytoplasmic membrane properties and may inhibit bacterial adhesion to mammary epithelial cells (Souza et al., 2013).

The lower SCC explains the lowest pH values in milk from OR as infected mammary gland increased capillary permeability, altering mineral flow between blood and mammary gland, and thus increasing pH of milk. Previous studies also reported lower pH in milk from cows fed oregano (Cervinkova et al., 2013). Higher ionic calcium concentration in milk from OR in the first days of lactation (Fig. 3) might be explained by its highest acidity and lowest pH. Such increase in ionic calcium is probably responsible for the tendency of reduction in milk ethanol stability (Horne, 2015) from OR when compared with CON. Unfortunately, we were not able to detect why GT presented lower ionic calcium in milk than CON in day 11.

Several plant components containing essential oils and catechins activate potential receptor channels in neurons, intestines, pancreas, immune cells, and other tissues (Holzer, 2011). This means that bioactive compounds in plant extracts could bind to and enable transient receptor potential channels to act as secondary transducers of cell activation or ion transporters (Oh et al., 2017) and may have the ability to modify immune cells, including macrophages, neutrophils, T lymphocytes, and B lymphocytes, stimulating or inhibiting the secretion of cytokines and antibodies (Drong et al., 2016). The effects of plant secondary compounds as immune-regulators may depend on the challenge on the immune status of experimental animals (Oh et al., 2017). In the present study, the absence of effects of plant extracts on most of the blood attributes related to immune cells might be related to the low degree of inflammation presented by the cows, although we did not measure

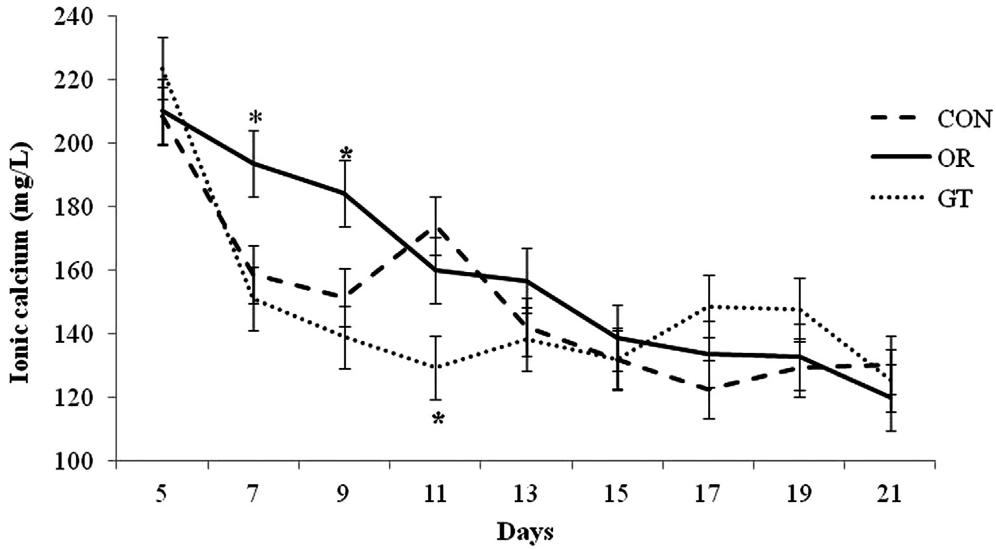

Fig. 3. Ionic calcium concentration in the milk of Jersey cows receiving no additive (control = CON) oregano extract (OR) or green tea (GT) extracts in the beginning of lactation ($n = 8$ cows per treatment). $^{*}P < 0.05$.

inflammatory biomarkers, and also it might be related to the low challenge cows were exposed due to moderate milk production. The highest values of platelets and eosinophils observed in OR and GT (Supplementary Table S2), respectively, are within the reported ranges for healthy cattle (Islam et al., 2014). It was not possible to relate the reduction in the concentration of reactive oxygen species and the increase in the activity of antioxidant enzymes with improvements in immunity, in disagreement with Bradford et al. (2015) and Trevisi and Minuti (2017). Oregano and green tea extracts were able to reduce several attributes of oxidative stress while enhancing the activity of some antioxidant enzymes during the transition period but not enough to improve blood attributes related to immune cells. Oregano and green tea extracts did not cause adverse effects on productive variables.

Conclusion

Oregano extract and green tea extracts improve several biomarkers of antioxidant defense system reducing free radicals in Jersey cows during the transition period. Since feed intake was similar and milk production level was moderate for all cows, such positive effects were not enough to improve milk production, milk composition, and blood metabolites pattern.

Feeding oregano extract at 10 g per cow per day reduces some oxidative stress markers in Jersey cows during the transition period without adverse effect on animal's performance such as feed intake, milk production, and feed efficiency.

Feeding green tea extract at 5 g per cow per day reduces some oxidative stress markers in Jersey during the transition period but it impaired feed efficiency.

Supplementary materials

Supplementary data to this article can be found online at https://doi.org/10.1016/j.animal.2020.100032

Ethics approval

This study was approved by the Animal Ethics Committee of the Federal University of Rio Grande do Sul, n° 29838, on September 29, 2015, project entitled as “Behavior, feed intake evolution, oxidative stress, milk production and quality of dairy cows under distinct feeding strategies during the transition period”.

Authors statement statement

E.F. Vizzotto was the main investigator conducting the trial at the field, project conceptualization and writing, **S.C.B. Stivanin** was the second investigator conducting the trial at the field, project conceptualization and responsible for the statistical analysis **M. de Paris 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; and **V. Fischer** was the main investigator responsible for funding acquisition, project administration and editing of the manuscript.

Data and model availability statement

None of the data were deposited in an official repository.

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Declaration of interest

None.

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