



## Effect of green tea and oregano extracts fed to preweaned Jersey calves on behavior and health status



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### ARTICLE INFO

#### Article history:

Received 7 January 2020

Received in revised form

18 March 2020

Accepted 29 March 2020

Available online 20 April 2020

#### Keywords:

calves

*Camellia sinensis*

*Origanum vulgare*

rumination

### ABSTRACT

The aim of this study was to evaluate the effects of green tea (*Camellia sinensis*) and oregano (*Origanum vulgare*) extracts on behavior, weight gain, and health indicators of dairy calves from birth to 60 days of age. Twenty-one Jersey calves were distributed into three treatments: without additives (CON); green tea extract (GT): supply of 30 mg/kg of body weight/day; and oregano extract (OR): supply of 60 mg/kg of body weight/day. Observers registered animal's behavior at 5-minute intervals from 8:00 to 16:00, three times a week, from day 1 to day 60 of life. The behavioral variables considered were time spent in standing, lying down, ruminating, frequency of visits to feeders, frequency of eating pasture and straw, as well as body weight gain, body temperature, heart and respiratory rates, and health conditions. GT and OR anticipated the occurrence of the first rumination and the ingestion of straw in approximately 7 days compared with CON but did not modify the body weight gain, incidence of diarrhea, health conditions, and other behavioral variables. Supply of GT and OR stimulated the onset of dry feed intake and rumination without changing performance and health status.

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### Introduction

Oregano (*Origanum vulgare*) and green tea (*Camellia sinensis*) plants produce secondary metabolites, such as essential oils (e.g., carvacrol) and polyphenols (e.g., catechins), respectively. These plants may act as growth promoters to the microorganisms at the intestine and rumen, enhancing nutrient intake and, in consequence, the performance (Oh and Hristov, 2016). On the other hand, studies *in vivo* reported that intake and weight gain in dairy calves were not improved after supplementation with blends containing oregano (Santos et al., 2015; Kolling et al., 2016) or green tea (Maciej et al., 2016)

Plant extracts may also affect antioxidant status as they decrease reactive oxygen species (Guo et al., 1996; Gladine et al., 2007), and Elshahawy et al. (2018) evidenced weak to moderate positive

effects on antioxidant status in dairy calves supplied with green tea extract (GT). Health condition was improved in studies that reported reduced frequency of diarrhea after the supply of GT (Maciej et al., 2016) and oregano extract (OR) (Katsoulos et al., 2017), but these positive effects were not reported in other studies (de Paris, 2019).

During the first weeks of life, calves are considered physiologically as preruminants, and their transition to functional ruminant is essential to a successful weaning (Chaves et al., 2009). During this transition, calves need to change from a milk-based diet to a dry-feed based diet (Lima et al., 2013) to promote rumen development associated with microbial fermentation in the forestomach and the onset of rumination. The onset of rumination may occur at 1 or 2 weeks after birth (Lopreiato et al., 2018), increasing until the calf is approximately 4 to 6 weeks old (Gilliland et al., 1962; Swanson and Harris, 1958). Rumination is mainly affected by the amount and type of feed; long fibrous particles have been acknowledged as stimulating rumination (Beauchemin, 2018; Lopreiato et al., 2018). Moreover, rumination may be used as an indicator of rumen development or illness detection although variation in rumination time is high in cattle younger than 9 months (Burfeind et al., 2011).

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To the best of our knowledge, the effects of plant extracts on behavior in preweaned calves are not reported. The aim of this research was to evaluate behavior and health indicators in dairy calves supplemented with GT (*Camellia sinensis*) or OR (*Origanum vulgare*) during the preweaning period.

## Material and methods

### Site, animals, and handling description

The experiment was conducted at Embrapa Clima Temperado experimental station, in Capão do Leão, RS, Brazil. Twenty-one Jersey calves were selected at birth and randomly assigned to one of the following treatments: control (CON)—without plant extracts; GT: 30 mg of GT (*Camellia sinensis*)/kg of body weight (BW), OR: 60 mg of OR (*Origanum vulgare*)/kg of BW. The doses of the extracts were adjusted every 15 days according to the BW of the animals. At birth, calves weighed  $28.2 \pm 0.6$  kg,  $29.1 \pm 0.6$  kg, and  $30.4 \pm 0.6$  kg for CON, GT, and OR groups, respectively.

The number of replicates per treatment was 8 (3 males and 5 females), 7 (3 males and 4 females), and 6 (3 males and 3 females) calves for OR, GT, and CON, respectively. Uneven replicates were resulted from removal of calves due to reasons not related to the treatments, for example, one calf was born with morphological problems and the initial data of another two have been lost.

OR (OREGANOL®—Meriden Animal Health Ltd., Northampton, UK) contained 6.5% of essential oil of oregano with 80.9% of carvacrol, 4.7% thymol, 4.6% p-Cymene, and 3.7%  $\gamma$ -Terpinene. GT presented  $56\% \pm 2.5\%$  of polyphenols (Kolling et al., 2018).

From 1 to 60 days of life, calves were lodged in individual shelters placed in a paddock with rangeland pasture improved with *Lolium multiflorum*, with *ad libitum* access to water. During the whole period, calves were fed daily with 4 liters of milk, divided into two meals (08:00 AM and 04:00 PM). The concentrate was offered *ad libitum* from the fifth day of life, and no hay was fed. The animals were weighed on the first day of life, and thereafter every 15 days of life, without previous fasting. Shelters provided shade and protected against wind, and straw was provided for bedding.

### Behavior evaluation

Behavior was registered three times a week, throughout the preweaned period, between the morning meal at 08:00 AM and the afternoon meal at 04:00 PM. The time budgets for rumination,

resting, standing without grazing, and lying were recorded with instantaneous scan sampling of each calf and performed at 5-minute intervals (Silva et al., 2006). The number of visits to the feeder and drinker as well as the number of times calves ingested straw and pasture were observed continuously and recorded whenever they occurred during the 8-h period. Seven previously trained observers registered the behavior, and the observers were switched between treatments on measurement days to avoid bias favoring one of the treatments. Interobserver reliability for the scan observations was not accessed.

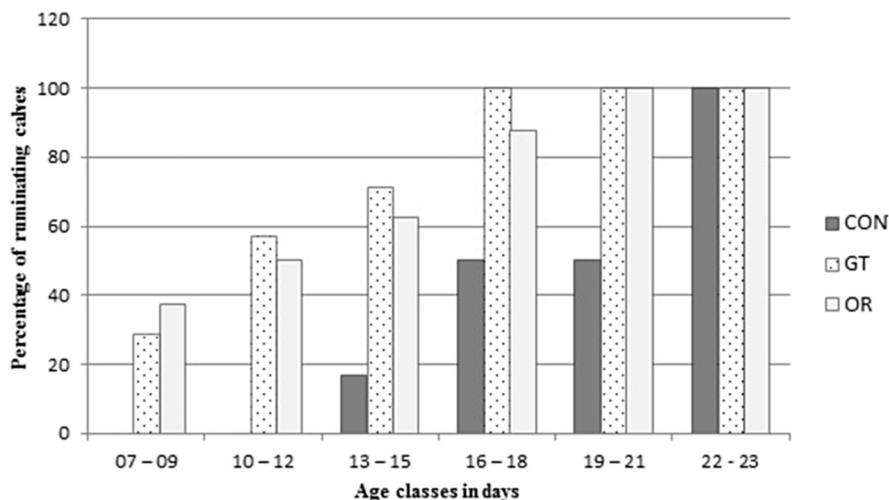
### Health attributes

Respiration rate (RR), heart rate (HR), and body temperature were evaluated daily from 08:30 AM to 09:30 AM during the entire preweaning period. The HR (beats per minute) and RR (breaths per minute) were measured using a stethoscope and stopwatch for one minute. The body temperature was measured by means of a clinical veterinary thermometer inserted near the rectum wall of the animal for three minutes.

Fecal score was attributed to each calf in the morning, daily, as score (0) normal feces, (1) soft feces, (2) muddy feces, and (3) watery feces. The last two scores indicate diarrhea. Frequency of diarrhea was calculated as [total number of days suffered from diarrhea (scores 2 and 3)]/(total number of days inspected  $\times$  100) according to Ishihara et al. (2001).

### Experimental design and statistical analysis

The experimental design was completely randomized, with repeated measures in time (days). Statistical analysis was performed considering the fixed effect of treatments ( $n = 3$ , control, OR and GT) and 6 to 8 replicates (calves). Statistical analysis of the attributes considered treatments, days, and interaction between treatment and days as fixed effects; calves and residue were considered as random effects, using the procedure MIXED of SAS®, version 9.4. The covariance structure selection test was performed using the Bayesian Information Criterion. All variables were previously tested for normal distribution. Means were compared using the LSmeans option, and the interactions were unfolded when significant at 5% of probability. Significant difference was considered when  $P < 0.05$ .



**Figure 1.** Frequency distribution, in percentage, of calves engaged in rumination in control (CON), green tea extract (GT), and oregano extract (OR) groups.

**Table 1**  
Mean age at the first event and body weight of calves supplemented with oregano extract or green tea extract

Variable	Treatments			SEM	P-value
	CON	GT	OR		
Age (days) at 1st event of					
Rumination	19.33 <sup>a</sup>	12 <sup>b</sup>	12.37 <sup>b</sup>	1.15	0.0109
Ingestion of straw	14.16 <sup>a</sup>	8.71 <sup>b</sup>	6.25 <sup>b</sup>	1.23	0.0232
Ingestion of pasture	6.50	9.71	9.12	0.57	0.0601
Ingestion of concentrate	9.16	7.71	10.50	0.98	0.5111
Body weight (kg)					
Birth	28.17	29.14	30.37	0.63	0.3821
Weaning	61.67	58.14	61.62	0.93	0.2180
Weight gain (kg/day)	0.558	0.483	0.521	0.018	0.2881

CON, control; GT, green tea extract; OR, oregano extract; SEM, standard error of the mean.

## Results

There was no interaction between treatments and days for any attribute. Calves in the GT and OR groups anticipated first rumination in 7 days compared with the CON ( $P < 0.05$ ). In the GT and OR groups, 100% and 88% of calves ruminated for the first time until 19 days of age ( $P < 0.05$ ), whereas only 50% of the calves in CON initiated rumination until 19 days of age (Figure 1). Calves in the GT and OR groups anticipated ( $P < 0.05$ ) the first ingestion of straw in 5.6 and 8 days, respectively, compared with CON. Calves in the GT and OR groups delayed ( $P < 0.05$ ) the first ingestion of pasture in approximately 3 days compared with CON. The age at first ingestion of concentrate was similar between the groups (Table 1).

The BW gain and physiological measures were similar between groups. Average daily time spent eating, ruminating, standing, and lying did not change between treatments. The frequencies of visits to the feeder and drinker were similar between the groups (Table 2). As the preweaning period progressed, calves spent more time ( $P < 0.05$ ) in rumination and standing as well as they increased the frequency of visits to the feeder, while they decreased time spent in resting and lying (Figure 2).

The overall frequency of diarrhea (fecal score  $\geq 2$ ) was similar between groups, averaging  $11.7 \pm 2.5\%$ ,  $13.3 \pm 2.3\%$ , and  $10.2 \pm 2.2\%$  in CON, GT, and OR groups, respectively. The first observation of diarrhea was similar between treatments, averaging  $20.7 \pm 4.4$ ,  $27.0 \pm 3.8$ , and  $19.7 \pm 4.1$  days of age in CON, GT, and OR groups, respectively. No other clinical symptoms of other diseases were observed in the calves.

## Discussion

The present study highlighted that preweaned calves supplemented with GT and OR anticipated the onset of rumination and ingestion of straw in 7 days. Some studies reported that essential oils affect rumen microbiota, favoring the production of butyrate that in turn may enhance rumen papillae development (Lane et al., 2002). Bioactive compounds present in green tea products, particularly flavonoids, present effects on inflammatory mediators and pathways, barrier integrity, and/or gut microbiota composition (Gil-Cardoso et al., 2016). Rumination had commenced for all calves by 12 days after birth in the GT and OR groups, whereas calves not supplemented with plant extracts (CON) ruminated by 19 days after birth. The average age at first rumination of calves in CON was similar to the results previously reported by Yavuz et al., 2015. Early initiation of rumination may lead to earlier weaning, without possibly impacting the growth rate of the animals after weaning, when the rumen must be developed and capable of absorbing and metabolizing the final fermentation products (Bittar et al., 2009).

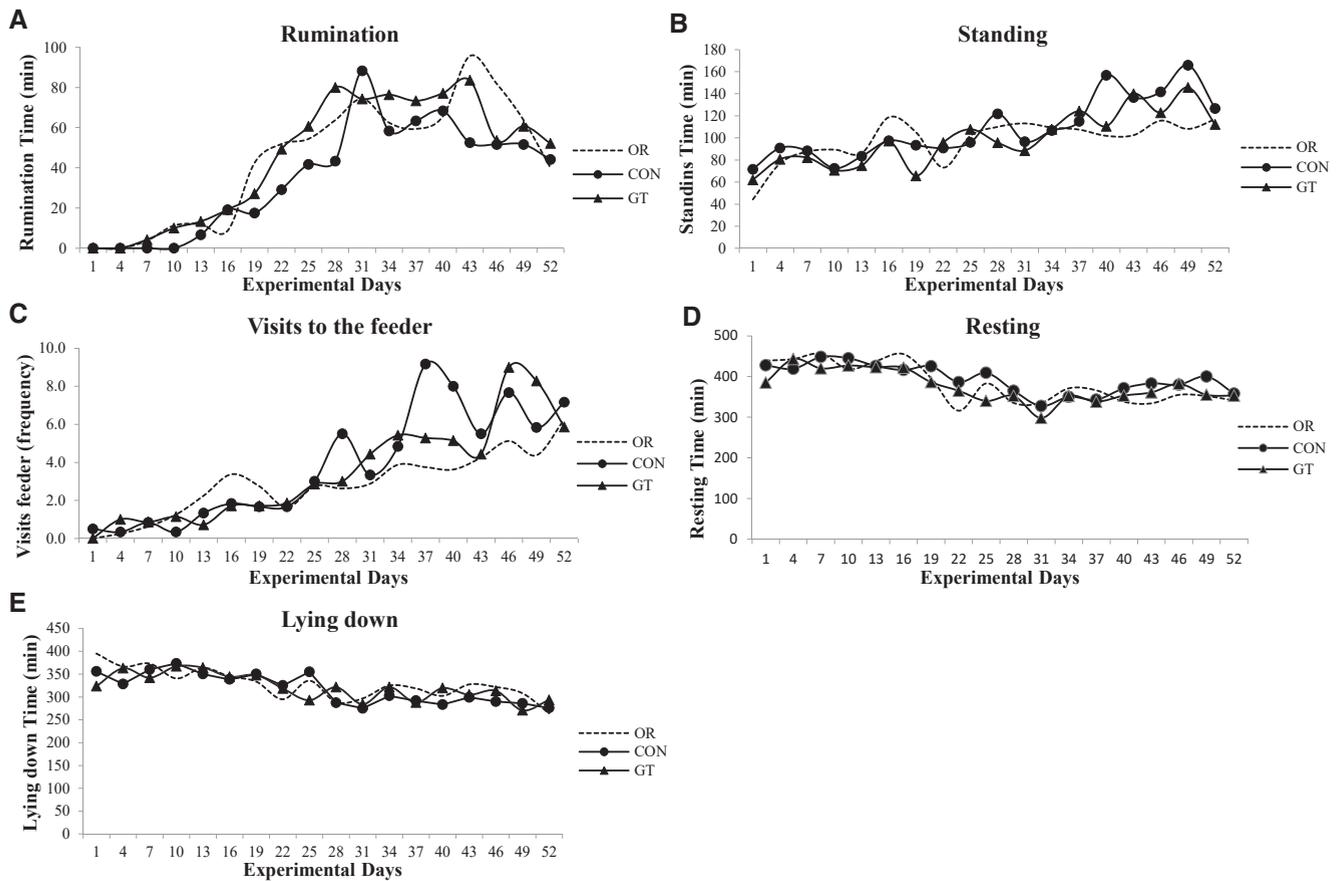
We did not measure the intake of concentrate, pasture, and straw, which is a limitation of the present study. Nevertheless, we observed the frequency of visits to the feeder (concentrate) and registered the onset of ingestion of concentrate, straw, and pasture as well the frequency of ingestion of these feeds during the preweaning period. As calves in the GT and OR groups started to eat straw earlier than calves in CON and the frequency of visits to the concentrate feeder and the onset of concentrate ingestion was statistically similar between groups, we may relate the earlier straw intake in the GT and OR groups to the onset of rumination. Intake of

**Table 2**  
Averages for behavioral activities and health attributes of calves fed control diet and diets with oregano extract or green tea extract during the preweaning period

Variable	Treatments			SEM	P-value		
	CON	GT	OR		Treatments	Days	Treatments $\times$ days
Behavior (min/observation period)							
Rumination	8.04	10.75	10.61	1.41	0.34	<0.0001	0.9872
Resting	92.05	89.18	89.44	1.42	0.32	<0.0001	0.9720
Standing	25.36	23.55	22.86	1.30	0.39	<0.0001	0.4806
Lying down	74.72	76.38	77.19	1.28	0.39	<0.0001	0.5713
Frequency (N <sup>o</sup> observations/period)							
Visits to drinkers	2.46	1.66	2.24	0.28	0.12	<0.0001	0.4660
Visits to the feeder	4.49	4.03	3.38	0.36	0.10	<0.0001	0.4977
Health and physiological attributes							
Respiratory rate (bpm/min)	47.08	47.66	49.38	2.42	0.76	<0.0001	0.6170
Heart rate (bpm/min)	116.18	119.71	115.42	2.43	0.39	<0.0001	0.2073
Rectal temperature ( $^{\circ}$ C)	38.35	38.30	38.33	0.05	0.68	<0.0001	0.1218
Fecal score <sup>a</sup> mean	1.10	1.21	1.04	0.14	0.39	0.0004	0.7757

CON, control; GT, green tea extract; OR, oregano extract; SEM, standard error of the mean.

<sup>a</sup> Fecal score (0) normal feces, (1) soft feces, (2) muddy feces, and (3) watery feces. The last two scores indicate diarrhea (scores 2 and 3).



**Figure 2.** Average time spent rumination (A), standing (B), frequency of visits to the feeder (C), resting (D), and lying down (E) by Jersey calves during the preweaning period.

solid feeds is recognized as necessary to stimulate rumination (Beauchemin, 2018), and calves ate straw used for bedding that stimulated rumination (Lopreato et al., 2018).

The absence of significant differences in BW gain between treatments is probably explained by the similar health status and the feeding protocol adopted as calves were offered similar amounts of milk and concentrate that they ingested without leftovers. Values of BW gain are compatible with those reported by Shivley et al. (2018). Average daily weight gain is similar to the values reported by Uys et al. (2011) and compatible with values reported in calves fed moderate amount of milk (Omid-Mirzaei et al., 2015).

The overall fecal score is within values reported by the treated calves (Ishihara et al., 2001; Selvi and Tapki, 2019). Calves did not present any other clinical symptoms, and their physiological measurements such rectal temperature and HR and RR were in the normal range, 38.0°C–39.3°C, 60–120 bpm and 15–40 rpm, respectively (Reece, 2015; El-Sheikh et al., 2012) and most of dairy farms are challenged with diarrhea in dairy calves, especially during the preweaning period (Hötzel et al., 2014). Diarrhea is the first cause of mortality (Leal et al., 2008) and morbidity (Meganck et al., 2014) of preweaned calves. The absence of effects of plant extracts on fecal score, diarrhea occurrence, and age at first manifestation of diarrhea may be due to the dose of the extracts used, the number of animals used, and to the fact that diarrhea has multifactorial causes (Meganck et al., 2014). Moreover, Katsoulos et al. (2017) and Selvi and Tapki (2019) reported reduction in neonatal diarrheal syndrome in calves supplemented with 12.5 and 23 mg/kg BW of oregano essential oils, respectively. Ishihara et al. (2001) verified decrease in neonatal diarrheal syndrome in calves supplemented

with 38 mg/kg BW of GT. The doses used by those authors are greater than those used in the present study.

## Conclusions

The main effect of providing GT or OR to Jersey calves during the preweaning period is the anticipation of the first straw ingestion and rumination that may enhance success in early weaning.

## Ethical considerations

This study was run in compliance with ethical standards and was approved by the Research Ethics Committee on the Use of Farm Animals of Universidade Federal do Rio Grande do Sul under Protocol 30756.

## Conflict of interests

The authors declare no conflict of interests.

## Acknowledgments

The authors would like to acknowledge Empresa Brasileira de Pesquisa Agropecuária—EMBRAPA Clima Temperado for providing the animals and infra-structure; Mr. Ivan dos Santos and ADVET Nutrição Animal for the supply of oregano extract; National Council for Scientific and Technological Development and Coordination for the Improvement of Higher Level -or Education- Personnel for research scholarships and financial resources (CNPq 473562/2012-0).

**Authors' contributions:** The idea for the paper was conceived by Guilherme Heisler and Vivian Fischer. The experiments were designed by Vivian Fischer, Micheli de Paris, and Maira Balbinotti Zanella. The experiments were performed by Guilherme Heisler, Micheli de Paris, Isabelle Damé Veber Angelo, and Dejana Maíra Panazzolo. The data were analyzed by Guilherme Heisler and Vivian Fischer. The paper was written and reviewed by Guilherme Heisler and Vivian Fischer.

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