



## Association of a green tea extract with serum immunoglobulin G status and neonatal vitality in newborn dairy calves

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### ABSTRACT

Poor vigor at birth has been associated with reduced IgG absorption from colostrum and a reduced vitality in neonatal dairy calves. Some natural compounds, such as green tea extract, may improve vitality in compromised calves. The objective of this randomized controlled trial was to evaluate the potential of supplementing a green tea extract (15 mL) to calves to improve vigor and activity behavior for the first 72 h postnatal. Also, this study aimed to investigate the influence of green tea extract supplementation on calf serum IgG concentration and the apparent efficiency of absorption (AEA) of colostrum IgG. Holstein calves ( $n = 24$ ) weighing  $42.49 \pm 1.07$  kg postnatal received a complete random assignment at 3 h of one 15-mL dose of green tea extract (Calf Perk, TechMix) or distilled water orally before tube feeding colostrum replacer (Premolac Plus IgG, Zinpro) at 4 h postnatal. Two observers assessed for calving time and dystocia by live video stream to retrieve all calves within 2 h postnatal. One veterinarian performed a baseline vigor assessment based on heart rate and response to stimuli on all calves at 2.5 h, before colostrum feeding at 3.75 h, as well as at 24, 48, and 72 h postnatal. Calf blood samples were taken to assess total IgG by radial immunodiffusion assay at 2.5, 6, 12, 24, 48, and 72 h. Calf vitality was also observed continuously by video for all calves to determine whether treatment was associated with attempts to stand, lying time, and exploration of their pen environment for the first 24 h. We used an ordinal logistic model to evaluate the odds of green tea extract improving a calf's vigor category from 2.5 h postnatal to 72 h of age. Vigor score was categorized as abnormal ( $\leq 4$ ), average (5), or alert ( $\geq 6$ ), with hour as a fixed

effect. We also ran mixed linear models to evaluate the effect of extract on total IgG and AEA, with time and dystocia as fixed effects. Five dystocia calves were enrolled (2 control, 3 extract), but assistance was minor (e.g., manual assistance and all were assisted within 1 h). Baseline vigor scores and baseline total IgG were not different between groups. Vigor score category was not associated with green tea extract supplementation (odds ratio 1.17; 95% CI: 0.43–3.15) but increased with time compared with controls. We observed no association of treatment with total IgG or AEA in the calves, suggesting green tea extract does not compromise IgG absorption. Calf vitality, lying behavior, and exploratory behavior were not associated with green tea extract treatment. Our findings suggest that green tea extract supplementation does not affect AEA and serum IgG concentration in calves. Future research should evaluate whether green tea extract improves vitality in calves experiencing severe dystocia.

**Key words:** dystocia, supplement, vigor, activity

### INTRODUCTION

Neonatal vigor in mammals is the capacity to live, grow, and develop strength for survival (Apgar et al., 1958), and maximizing a calf's vigor is essential when rearing dairy replacement heifers. Newborn calves can experience hypoxia and blood acidosis due to a poor oxygen environment during dystocia; this has been associated with a lower serum IgG status than calves born under normal conditions (Besser et al., 1990). Research has shown that calves experiencing difficult births take longer to stand, have a delayed suckling reflex, (Barrier et al., 2012), and can have poor colostrum ingestion, contributing to failure of passive transfer in calves (Pearson et al., 2019a). Dystocia, which was reported to affect 5% of dairy cattle (USDA, 2014), has long-lasting effects. For example, calves born to moderate dystocia had a lower likelihood of survival to breeding age compared with calves born without assistance (Barrier et al., 2012). It is possible that calves identified

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with poor vigor may benefit from an intervention to improve their behavioral response and this should be explored.

One approach to assess for postnatal vigor is to evaluate a calf's responsiveness to stimuli, oxygenation, and vital status measures—such as heart rate and respiration rate—using a validated vigor scoring system for dairy calves (Murray et al., 2015). A similar system has also been validated for use with beef calves (Homerovsky et al., 2017). However, some interventions, such as supplementing sodium bicarbonate in the colostrum, have been unsuccessful at helping calves identified with poor vigor (Murray-Kerr et al., 2017). Similarly, calves born to dystocia provided with a nonsteroidal anti-inflammatory drug (NSAID) at birth had similar inflammatory responses and similar metabolic parameters as controls (Pearson et al., 2019b). However, calves with abnormal vigor scores that received an NSAID had improved BW and milk consumption compared with controls (Murray et al., 2016; Gladden et al., 2019). Alternatively, calves born to dystocia provided with an NSAID were observed to have lower circulating IgG at 24 h of age compared with placebo calves (Clark et al., 2020), suggesting a potentially negative effect. This suggests that some research has observed that NSAID usage in newborn calves may compromise IgG absorption. Thus, it is necessary to explore additional methods to improve vigor in calves without compromising IgG absorption.

One potential opportunity to improve a calf's vigor is by using green tea extract. Green tea extract contains flavonoids and polyphenols; in particular, it contains epigallocatechin gallate, an antioxidant associated with DNA repair (Wang and Hao, 2015). Furthermore, green tea extract is rich in caffeine, a compound known to have stimulatory effects (Swinbourne et al., 2021), which could increase vigor or affect behavior of the newborn calf. Ishihara et al. (2001) found that supplementing a green tea extract to calves increased the commensal bacterial populations in the lower gut during the first week of life (e.g., bifidobacterial spp. and lactobacillus spp.). Calves fed green tea extract also had a lower risk of receiving antibiotics when treated for diarrhea and bovine respiratory disorder during the preweaning period. Moreover, calves treated for bovine respiratory disorder who received green tea extract as supportive therapy had improved antioxidant-associated enzyme activity (Metwally et al., 2017). Finally, healthy calves receiving green tea extract had improved antioxidant-associated activity (Elshahawy, 2018) and ruminated sooner by ingesting straw sooner than controls (Heisler et al., 2020). However, others observed no effect of green tea extract on calf performance (Maciej et al., 2016). It is possible that neonatal calves may expe-

rience improved vigor from the supplementation of a green tea extract at birth due to its anti-inflammatory properties and stimulatory effects from caffeine, but further investigation is needed.

Calves with poor vitality and born by dystocia have compromised IgG absorption (Besser et al., 1990), and this can compromise calf health and performance (Furman-Fratczak et al., 2011). Thus, it is imperative to first determine if an extract would affect a calf's ability to absorb colostral IgG. It is important to ensure green tea extract does not affect apparent efficiency of absorption of colostral IgG, especially because the gut commences closure within 24 h after birth in calves (Hare et al., 2020).

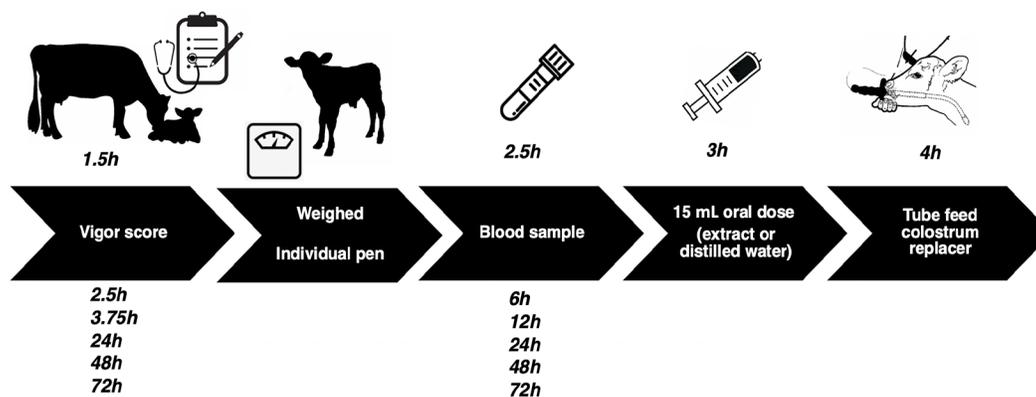
The aim of this randomized control trial was to evaluate if green tea extract was associated with the apparent efficiency of absorption (AEA) of colostral IgG when fed 1 h before colostrum feeding in neonatal calves. Additionally, we evaluated if green tea extract would be associated with an improved vigor category score in neonatal dairy calves born from dams who required minimal to no assistance from birth to 72 h of age, as well as improved activeness for the first 24 h. We predicted that a supplementation of green tea extract to newborn calves would not affect AEA, but would improve calf vigor score and behavior as expressed through more attempts to stand and increased exploratory behaviors during the first 24 h postnatal.

## MATERIALS AND METHODS

### Enrollment Criteria

This randomized control trial was conducted at the University of Kentucky Coldstream Dairy in Lexington, Kentucky, from February to April 2020. The University of Kentucky's Institutional Animal Care and Use Committee approved all procedures for this study under the protocol #2019-3159.

Twenty-four consecutive births of Holstein calves (10 heifers and 14 bulls) weighing  $42.49 \pm 1.07$  kg were enrolled. For simplicity, when referring to time points in this study, hour refers to the calf's age, or h postnatal. The timeline detailing procedural timing is shown in Figure 1. Calves received a complete random assignment (randomized on the website random.org) of either one 15-mL dose of green tea extract (Calf Perk, TechMix) or distilled water orally 1 h before tube feeding one 375-g dose of colostrum replacer (the batch used in this study was verified to contain 150 g of IgG; Premolac Plus IgG, Zinpro) diluted to 3 L with water. One dose of the green tea extract used in this study contained 315 mg of naturally occurring caffeine, 40%



**Figure 1.** The timeline detailing procedural timing of 24 calves offered either a green tea extract or a control (distilled water) at 3 h postnatal. Blood samples were collected to calculate total IgG status, and vigor score assessments were performed to calculate calf vitality at baseline 2.5 h postnatal, and daily at 24, 48, and 72 h of age.

corn syrup as an energy source (glucose), 20% water, and 1% vegetable oil to suspend the solution, as well as the preservatives cassia oil, xanthan gum, and citric acid. The patent information for this product is available online (TechMix, 2022). Treatment assignments were assigned by a researcher not involved with data collection and farm staff was blind to treatment assignments. However, because the treatment appeared visually different from the controls, we were unable to blind the research veterinarian who administered the treatments to the calves.

We powered this study to detect a difference in AEA of total IgG in colostrum replacer based on the literature (Hare et al., 2020) to ensure that supplementing the treatment to calves did not compromise total IgG absorption from colostrum. Hare et al. (2020) observed that calves fed one feeding of colostrum within 2 h postnatal followed by a milk replacer feeding had a mean AEA of 36.5% at 25 h postnatal with a reported variation of 2.1%. Therefore, at a power of 90% and an  $\alpha$  level of 0.05, we required 9 calves per treatment to detect a difference. We included 12 calves per treatment in case of loss to followup.

Before calving, closeup cows were in a pen and observed every 2 h by live cameras with playback (Lorex, DP181-42NAE). Cows were observed for signs of labor and dystocia. Delivery time was also monitored to ensure all calves were removed from the dam before suckling and within 1.5 h after calving. Dystocia was scored as follows: 1 – no assistance, 2 – manual assistance, 3 – mechanical assistance with chains, and 4 – surgery (Murray et al., 2015), but only dystocia of 1 or 2 were reported in this study. Dystocia interventions were made by one veterinarian if a calf had abnormal presentation or if signs of hypoxia occurred for more than 30 min during labor (tongue out no progress).

## Data Collection

**Postnatal Management.** After birth, calves were scored for vigor (Table 1) in the maternity pen within 1.5 h by one trained veterinarian before removal from the dam, as adapted from Murray et al. (2015). Briefly, calves with abnormal vigor received lower scores and each calf received a score for responsiveness to stimuli (nasal) and vital scores for heart rate, respiration rate, and rectal temperature. A digital thermometer (GLA M700 Thermometer, GLA Agricultural Electronics) was used to measure calves' rectal temperatures. Calves were then removed from the dam by placing them in a calf cart (Calf-Cart, Reytec LLC). The weights of calves were taken on a scale (Mettler Toledo Ind246) in the calf barn. Calves were placed in an individual pen (3 × 3 m) bedded with shavings within 2 h postnatal. Cameras with night vision (Hikvision GW5091IP) were placed above the individual pens and continuously recorded behaviors onto a digital video recorder (Hikvision, DS-7600).

**Blood Collection.** We took 10-mL blood samples from the calves into nontreated evacuated tubes using jugular venipuncture to determine baseline IgG concentration in grams per liter at 2.5 h and to calculate the AEA rate in the calves after colostrum feeding at 6, 12, 24, 48, and 72 h. The veterinarian performed vigor assessments in the calves at baseline before removal from the dam, after the initial placement in the individual pen, after the green tea extract supplementation at 3.75 h, at 6 and 12 h after colostrum feeding, and at 24, 48, and 72 h of age. Blood samples were allowed to clot at room temperature, and then centrifuged at  $3,000 \times g$  for 15 min at 21°C. The serum was pipetted into 2-mL cryovials in duplicate and frozen at  $-80^{\circ}\text{C}$  for later IgG radial immunodiffusion assay analysis. All

**Table 1.** The neonatal vigor scoring system performed on 24 calves at 1.5, 2.5, 3.75, 24, 48, and 72 h of age; adapted from Murray et al. (2015)<sup>1</sup>

Calf vigor score	0 = Poor vigor, abnormal	1 = Reduced vigor	2 = Normal vigor	3 = Alert
Respiration rate, breaths/min	Fast, >36	Slow, <24	24–36	NA
Heart rate, beats/min	Slow, <80	Fast, >100	80–100	NA
Initial movement	Cannot stand	Stands after stimulation	Stands without stimulation	NA
Straw in nasal cavity	No response	Twitch or flinch	Moves head away	Shakes head
Rectal temperature, °C	<38 or >39.5	38> to ≤39.5	NA	NA

<sup>1</sup>Calves received either 15 mL of green tea extract or distilled water at 3 h postnatal. NA = not applicable.

blood samples were analyzed for IgG concentrations by one laboratory (Zinpro Corporation, North Branch, MN) using radial immunodiffusion assay (Chelack et al., 1993). The laboratory determined the IgG concentration in grams per liter from each sample for calculation of the AEA and apparent persistency of IgG %. The AEA and calf IgG persistency were calculated from Hare et al. (2020). For AEA, serum IgG at discrete time points was subtracted from the initial IgG baseline, relative to the mass of IgG consumed, and corrected by the approximated plasma volume = 0.091 L/kg × birthweight. We calculated the apparent persistency of IgG % in calf serum at 72 h relative to the time of maximal peak IgG concentration for each calf to determine the apparent persistency of IgG. For this study, the apparent persistency of IgG is a calculation of how persistent circulating peak calf IgG concentration is at 72 h relative to a calf's individual peak IgG (Hare et al., 2020).

**Feeding and Health Management.** Calves were offered a milk feeding following colostrum feeding at 0800 or 1700 h (whichever came first relative to time of birth). No calves refused the milk meal after the colostrum feeding. Calves were fed 4.0 L of milk replacer from a nipple bottle divided into 2 daily feedings (Cow's Match; containing a minimum 28% CP, 20% crude fat, 15% crude fiber, 4.87 Mcal of ME/kg) at 0800 and 1700 h until completion of the study at 72 h of age. The veterinarian who delivered the calves also performed health checks daily at 1645 h, which consisted of assessing for the presence of abnormal nasal and eye discharge, head tilt, coughing, fever (McGuirk and Peek, 2014), navel cord swelling (Cantor et al., 2019), and diarrhea (Renaud et al., 2020).

**Calf Behavioral Vitality.** As shown in Table 2, one observer observed the behavioral time budget of 24 calves for the consecutive 24 h after colostrum feeding using an ethogram adapted from Gladden et al. (2019). There was a washout period from placement into the individual pen to 5 min after colostrum tube feeding when behaviors were not assessed due to researcher presence and disturbance to the calves in the individual pens for vigor scoring, treatment administration, and

colostrum tube feeding. Feeding times were recorded when a calf had contact with the bottle nipple and occurred for a maximum of 16 min per d per calf. Farm staff were not permitted to have contact with the calves or enter the pens during this study, and the individual pens had solid siding to avoid stimulation from the external environment. The 2 researchers fed the calves for the duration of the study to ensure consistency of feeding protocol. All individual pens were placed directly under the camera, which had night vision, and no blind spots were observed in the video. Behaviors recorded included the duration and frequency of lying behavior, including transition states from lying to standing; vigilance behavior, such as looking alert in the direction outside of the pen; and exploratory behavior, such as walking, exploring the pen environment, or licking the pen and floor (Table 2). We wanted to assess if green tea extract was associated with these calf vitality behaviors during the first 24 h of life. All calves were placed in the individual pen at the same time (2 h postnatal).

### Statistical Analysis

All statistical analyses were performed with SAS (version 9.4; SAS Institute Inc.). We assessed the data for normality using univariate methods, and the residuals from the models were also assessed for normality from the linear models. Final models presented were obtained using stepwise backward removal. Variables were retained when significance was reported at  $P \leq 0.05$ .

We used an ordinal logistic model to evaluate the odds of green tea extract improving a calf's vigor category at 2.5 to 72 h of age as vigor was non-normally distributed. Vigor was categorized as abnormal ( $\leq 4$ ), average (5), or alert ( $\geq 6$ ), with hour as a fixed effect, and was transformed using cumulative logistic distribution and exponentiated odds were reported. We evaluated the association of treatment with calf serum IgG concentration and AEA using repeated measures linear mixed regression models, but for the AEA model, we only included time to peak total serum IgG concentration at 24 h. For these mixed linear regression models,

**Table 2.** Ethogram of lying, exploratory, and vigilance behavior of 24 calves administered either green tea extract or distilled water at 3 h postnatal; adapted from Gladden et al. (2019)<sup>1</sup>

Category	Behavior	Description
Lying behavior	Lying time	Calf state involves legs positioned under the calf laterally or to the left or right side in contact with the ground. At least 2 knees are in contact with ground. The opposing shoulder is in contact with ground.
Standing behavior	Standing state	Calf is supported by 4 limbs with legs extended for duration of 4 s or more. Knees are not in contact with ground.
Transition	Attempt to stand bouts	Calf is in lying state and initiates partially standing position supported by 1, 2, or 3 limbs extended with the remaining limbs flexed. The head may be up or in contact with the ground. Standing is not achieved within 3 s.
Exploratory bout	Walking	The calf is in a standing state and takes more than 2 steps. Step pauses no longer than 10 s in duration. Can include play behavior such as bucking and kicking.
Exploratory bout	Investigative	The calf is in a standing state. Sniffing, licking, chewing, rubbing, nuzzling, calf physically contacts pen wall, pen gate, shavings, or orally contacts inanimate objects in pen. Bout ended when calf stands idle for at least 10 s.
Vigilance bout	Vigilance	The calf is in a standing or lying state. Calf is motionless with ears forward; head is directed to pen entrance. Bout ended when calf ears laid back or head was turned away from entrance.

<sup>1</sup>Behavioral monitoring began from feeding colostrum at 4 h for 24 h.

we used hour postnatal relative to a calf's birth, and dystocia as fixed effects. The model was repeated by time, calf was the subject, and birthdate was a random effect to account for seasonal effects. The temperature range was considerable during the study, despite its short duration, and ranged from  $-20$  to  $20^{\circ}\text{C}$  at the time of a calf's birth. To account for this variability in temperature, as it could have affected calf AEA, we used birthdate as a seasonal effect. We used the Toeplitz covariance structure with the lowest Akaike's criterion for best model fit, but first order autoregressive, unstructured, and compound symmetry were also explored.

To evaluate the effect of treatment on the duration of lying behavior and the duration of exploratory behavior for the first 24 h of life, we used a general linear mixed model in which behavior was summarized by day and sex was used as a fixed effect. The remaining behaviors were non-normally distributed, and thus we evaluated the association of treatment with attempts to stand, exploratory bouts, vigilance duration, and vigilance bouts using Kruskal-Wallis tests.

## RESULTS

Most calves (19/24) were born without assistance during calving (10 control and 9 extract), but 5 out of 24 (2 control and 3 extract) were manually pulled by the veterinarian due to hypoxia (e.g., tongue out, no progress). We observed 5 heifers and 7 bulls per treatment. Vigor scores conducted 15 min before colostrum replacer feeding at 3.75 h were  $4.08 \pm 0.34$  for control calves and  $4.33 \pm 0.41$  for calves receiving the green tea extract. Baseline total IgG at 2.5 h was  $0.42 \pm 0.05$  g/L for control calves and  $0.65 \pm 0.14$  g/L for calves

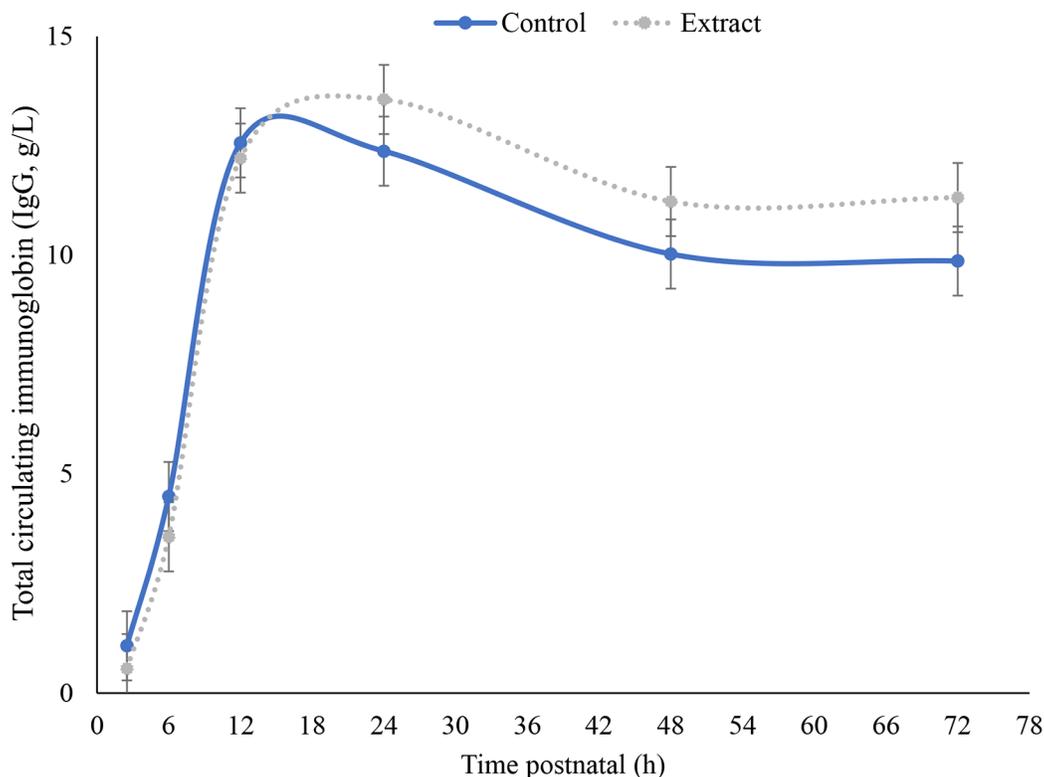
receiving the green tea extract. The mean birthweight of these calves was  $42.49 \pm 1.07$  kg postnatal. All calves completed the study at 72 h postnatal and remained healthy.

### Vigor Assessment

Vigor score was not associated with green tea extract supplementation (odds ratio 1.17; 95% CI: 0.43–3.15;  $P > 0.10$ ) compared with controls. However, time was associated with the probability of a different vigor category ( $P < 0.001$ ). Specifically, compared with 2.5 h postnatal, calves at 72 h were less likely (odds ratio 0.21; 95% CI: 0.08–0.56;  $P = 0.001$ ) to be in a lower vigor category. At 2.5 h, 54% (13/24), 33% (8/24), and 13% (3/24) of calves had abnormal, normal, and alert vigor status, respectively. By 72 h, 21% (5/24), 42% (10/24), and 38% (9/24) of calves had abnormal, normal, and alert vigor status, respectively.

### Immunity

Green tea extract supplementation was not associated with IgG concentration in the calves [green tea extract ( $13.56 \pm 0.80$  g/L) and control ( $12.38 \pm 0.80$  g/L) LSM  $\pm$  SEM  $P > 0.10$ ; Figure 2]. Even though the IgG treatment  $\times$  hour interaction was not significant for IgG concentration ( $P > 0.10$ ), it is displayed in Figure 2 to demonstrate peak IgG concentrations and total IgG concentrations (LSM  $\pm$  SEM) across time by treatment. Green tea extract supplementation was not associated with AEA ( $P > 0.10$ ), and the hour  $\times$  treatment interaction was also not significant ( $P > 0.10$ ). However, AEA was significantly associated with hour ( $P < 0.001$ ). Interestingly, AEA at 24 h (25.73



**Figure 2.** Total IgG values (LSM  $\pm$  SEM) from a mixed linear regression model for calves at birth, at 2.5 h postnatal, and after colostrum feeding at 6, 12, 24, 48, and 72 h postnatal for 24 Holstein calves. Calves were tube-fed 3 L of colostrum replacer (150 g of IgG) at 4 h postnatal.

$\pm 1.17\%$ ) was comparable to the AEA of IgG at 12 h ( $24.40 \pm 1.17\%$   $P > 0.10$ ). The AEA for 12 and 24 h were different from the AEA of IgG at 6 h ( $<0.001$ ). Figure 3 shows a box plot for AEA by treatment at maximum peak total IgG at 24 h for the raw data. Finally, the apparent persistency of IgG was also not associated with treatment (control  $36.7 \pm 1.7\%$ , green tea extract  $34.0 \pm 1.6\%$   $P > 0.10$ ).

### Calf Vitality Behavior

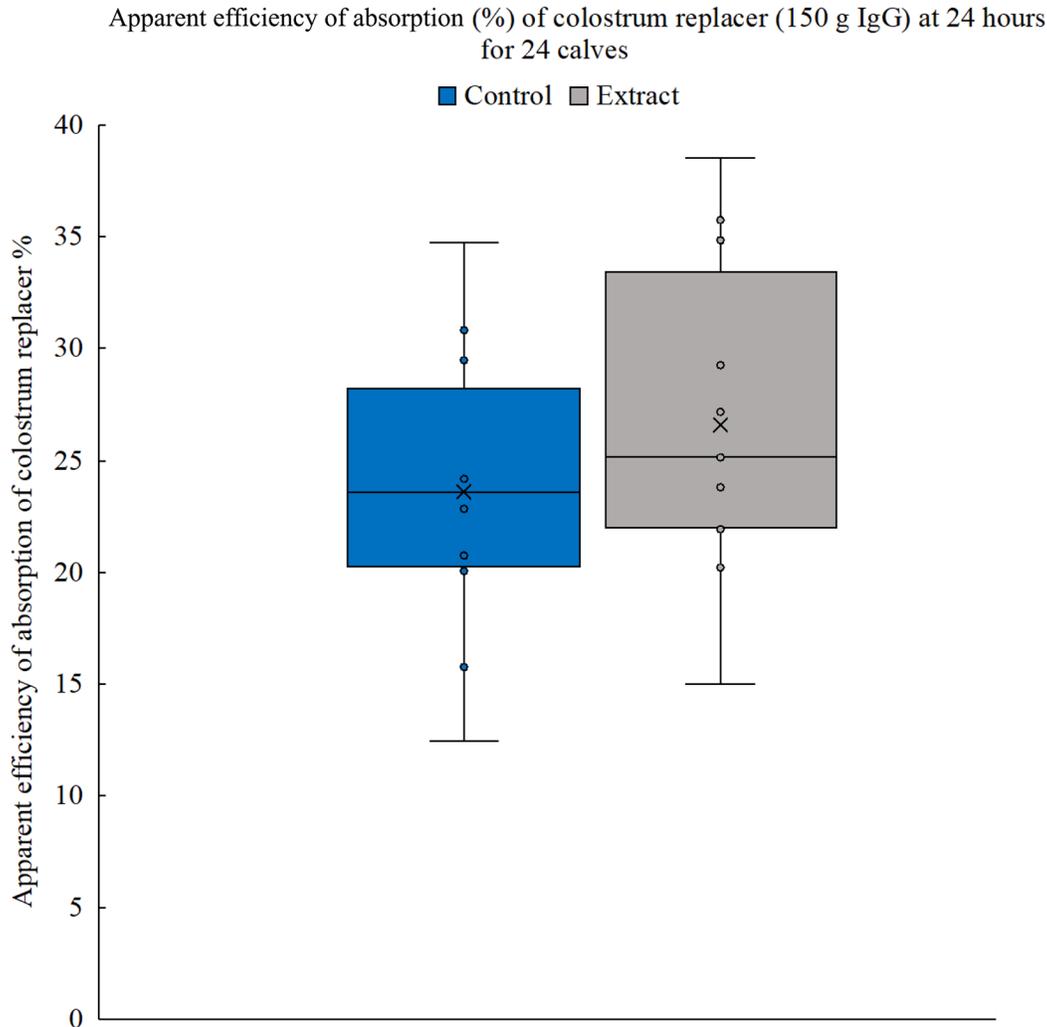
Lying time (LSM  $\pm$  SEM) was similar in the calves (green tea extract  $14.7 \pm 2.3$  h and control  $18.2 \pm 2.2$  h,  $F_{1,2} = 1.54$ ;  $P = 0.23$ ). Furthermore, the duration of exploratory behavior for the calves investigating the pen and walking around in the pen was also similar between treatments (green tea extract  $31.0 \pm 4.7$  min and control  $37.2 \pm 4.7$  min,  $F_{1,2} = 0.87$ ;  $P = 0.36$ ). Treatment was not associated with exploratory bouts, attempts to stand, or vigilance behavior in the first 24 h of life ( $P > 0.10$ ; Table 3). Briefly, median vigilance behavior, attempts to stand, and exploratory bouts were similar by treatment. Thus, calves spent the majority of these 24 h lying down, with 30 min spent on vigilant and exploratory behavior cumulatively. The

median lying bouts, vigilance bouts, and exploratory behavior bouts were similar and around 25 to 30 frequencies per behavior.

### DISCUSSION

The supplementation of green tea extract to newborn calves did not affect the AEA of IgG from the colostrum replacer, which was consistent with our hypothesis. Green tea extract supplementation also did not affect the exploratory behavior, vigilance behavior, or calf activeness when compared with controls. The process of birth is a challenge for a calf, as the calf must breathe on its own, stand, suckle, and regulate its body temperature moments after being born (Murray-Kerr et al., 2017). Thus, it is possible that the calves did not have vigor affected by treatment due to the ceiling effect of parturition. Alternatively, the supplementation of green tea extract does not affect calves born to nonsevere dystocia.

In the current study we observed that dystocia occurred in one-fifth of the calves born, but dystocia involved manual extraction only. Larger studies in dairy cattle have observed that severe dystocia is rare (USDA 2014). Similarly, minimally invasive dystocia was re-



**Figure 3.** The 24-h postnatal apparent efficiency of absorption rates of colostrum IgG in 24 Holstein calves supplemented with green tea extract or not at 3 h postnatal. Calves were tube-fed colostrum replacer (150 g of IgG) at 4 h postnatal. The box plot represents the minimum (bottom tail), maximum (top tail), first quartile (lower box line), median (middle box line), third quartile (upper box line), and mean (x) of the control and green tea extract calves.

ported in one-fifth of dairy calves born (as reviewed by Mee, 2013). It is important to manage calves who experience dystocia, as calves born by moderate dystocia were less likely to survive to breeding age than calves born without assistance (Barrier et al., 2012). Furthermore, calves born with reduced vigor have a reduced suckle reflex, which has been associated with increased morbidity (Homerosky et al., 2017). We hypothesized that calves receiving a green tea extract dose before colostrum replacer feeding would have stimulatory properties by increasing the odds of improved vigor within 72 h or would have improved early life vitality in the first 24 h because the process of parturition is an inflammatory process for the calf (Mee, 2008). Studies which evaluated green tea extract supplementation did

not administer it to calves immediately postnatal, making comparisons difficult. Indeed, supplementation of green tea extract has improved the antioxidant activity in preweaning calves compared with controls (Elshahawy, 2018). This may be because green tea extract was observed to have anti-inflammatory properties and high antioxidant activity without affecting cell function *in vitro* (Cyboran et al., 2015; Aristatile et al., 2015). However, supplementing calves with 10 mg/kg of BW of green tea extract had no effect on antioxidant capacity, or changes in oxidative biomarker status in calves in early life (Maciej et al., 2016). Therefore, we suggest that one dose of green tea extract does not negatively affect calf behavior when fed postnatal, but our findings suggest that it does not appear to affect the behavioral

**Table 3.** The behavioral repertoire for the 24 h after colostrum feeding of individually housed Holstein dairy calves (n = 24) offered either green tea extract or distilled water before feeding colostrum replacer (3 L of 150 g IgG) at 4 h postnatal<sup>1</sup>

Behavior	Green tea extract	Control	$\chi^2$	<i>P</i> -value <sup>2</sup>
Attempt to stand bouts <sup>3</sup>	27.5 ± 1.9 (95% CI: 23.5–32.0)	26.0 ± 1.6 (95% CI: 23.9–30.8)	0.44	0.50
Exploratory bouts <sup>4</sup>	32 ± 3.2 (95% CI: 24.3–38.4)	25.5 ± 3.5 (95% CI: 21.6–36.9)	0.96	0.33
Vigilance min	30.5 ± 5.1 (95% CI: 20.1–42.4)	26.5 ± 5.9 (95% CI: 16.2–43.3)	0.03	0.86
Vigilance bouts	26.0 ± 2.1 (95% CI: 21.4–31.7)	24.5 ± 2.3 (95% CI: 21.1–30.5)	0.14	0.74

<sup>1</sup>The association of treatment with behavior was generated using Kruskal-Wallis testing (median ± SE; 95% CI).

<sup>2</sup>Significance:  $P \leq 0.05$ .

<sup>3</sup>Attempt to stand bouts were a transition state from lying to standing using an ethogram adapted from Gladden et al. (2019).

<sup>4</sup>Exploratory bouts were a combination of walking and investigative behavior using an ethogram adapted from Gladden et al. (2019).

status of calves in early life. Future research should determine if a larger dose of green tea extract is associated with improved behavior in calves born to normal births, as we did not observe any findings.

Despite research on the biological activity of extracts and polyphenolic compounds contained therein, the mechanism of molecular interaction of green tea extract in the body is lacking (Cyboran et al., 2015). This may be one of the possible explanations as to why we found no effects of the green tea extract supplementation on calf vigor or early life behavior. It is possible that, in this study, a lack of a response was observed because all calves were healthy, and three-quarters of calves were born to normal births. However, it was important to conduct this study without calves born to extreme dystocia, as difficult calvings are associated with decreased odds of achieving successful passive transfer of immunity (Pearson et al., 2019a). Thus, this study had to first confirm that green tea extract supplementation would not compromise AEA in the calves. Some researchers have observed that the flavonoids derived from green tea extract are most beneficial for sick or stressed calves (Maciej et al., 2016). However, others observed that offering a green tea extract to calves during the preweaning phase decreased the likelihood of digestive and respiratory diseases in calves (Ishihara et al., 2001), decreased the latency to first ruminate and consume straw (Heisler et al., 2020), and improved overall health and performance in calves (Metwally et al., 2017). Thus, there is also the possibility that supplementing green tea extract to preweaning calves has cumulative effects, but this is speculation. However, we can suggest from this study that vigor category was not improved in healthy calves when fed a 15-mL dose of green tea extract. Future research should investigate if

green tea extract fed for multiple days in early life has the capacity to improve calf vigor status in compromised calves born by dystocia.

In this study, we did not observe an effect of green tea extract supplementation on AEA of colostrum IgG. However, the AEA rates and calf IgG concentration in this study were comparable to others who observed that calves had low baseline IgG concentrations before colostrum feeding, and peak circulating IgG occurs at 24 h after colostrum feeding in the calves (Wilm et al., 2018). Furthermore, the AEA of the colostrum IgG for calves in this study was comparable to others (Halleran et al., 2017). Thus, we can suggest that supplementing green tea extract to calves before feeding colostrum replacer did not negatively affect IgG absorption.

In this study, we did not observe any association of green tea extract supplementation with lying time, attempts to stand, or exploratory behavior such as interacting with the pen or walking, or vigilance attentive behavior directed toward the pen entrance. This disagreed with our expectations, as we expected that green tea extract supplementation would stimulate the calf due to the anti-inflammatory and antioxidant effects observed (Maciej et al., 2016). Furthermore, green tea extract contains caffeine, which is a hydrophobic stimulant that is readily absorbed in the lower gut and blocks adenosine receptors, thus providing a normal cellular environment in abnormal conditions as reviewed by (Swinbourne et al., 2021). Caffeine has been suggested as a potential stimulant to improve vigor response in neonates, but there was a lack of evidence when fed to piglets to improve performance (Dearlove et al., 2018). Caffeine also did not reduce metabolic acidosis status or hypercapnia in foals (Giguère et al., 2007, 2008), or asphyxia in calves (Balıkcı and Yildiz,

2009). Indeed, most benefits were observed when caffeine was supplemented to the dam during parturition rather than the offspring, which increased the survival of lambs (Robertson et al., 2018) and piglets (Superchi et al., 2016). However, more research is needed before we can conclude these benefits to cattle. As an example, negative effects on the human fetus were observed in women taking high doses of caffeine as reviewed by Swinbourne et al. (2021). We conclude that in this study, at a dose of 15 mL of green tea extract which contained 315 mg of naturally occurring caffeine, the neonatal calves' behavior was not affected.

It is interesting to note that although median bouts of behavior and mean durations were similar in this study, behavioral bouts had a lot of individual variation independent of treatment. Exploratory and vigilance behavior are associated with temperament and individual traits in calves (Neave et al., 2020), and we believe that this may be why we observed individual variation in behavioral bouts, though median bout behaviors were similar by treatment. To our knowledge, few studies have observed neonatal calf behavior in the first day of life. Gladden et al. (2019) observed that neonatal calves had long lying times and low exploratory activity. However, we were surprised that the lying times of the neonatal calves in this study were comparable to the lying times observed in older calves (Cantor et al., 2019). We did not expect exploratory behavior to occur for over a half-hour in the first 24 h of life as feral cattle hide their calves during the first day postpartum (von Keyserlingk and Weary, 2007). Thus, we suggest that this study also provides insight into the behavior of a young neonate raised in a dairy operation during the first hours of life.

## CONCLUSIONS

Green tea extract supplementation had no significant association with serum IgG concentration, AEA of colostral IgG, vigor score, or exploratory and activity behavior for the neonatal dairy calves in this study. We suggest that behavior and vigor score were not associated with 15 mL of green tea extract supplementation in healthy neonate calves. Future research should evaluate whether green tea extract can improve vigor in calves at a higher dosage, or in calves born by stressed conditions such as severe dystocia.

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