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The effects of colostrum consumption and feed restriction during marketing and transportation of male dairy beef calves: Impact on pre-transport nutritional status and on farm recovery

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ABSTRACT

The aim of this study was to evaluate the effects of colostrum consumption and feed restriction on biomarkers of stress, nutritional and health status, gut functionality, and behavior in male dairy beef calves being marketed and transported. A total of 82 male Holstein calves (42 ± 1.2 kg of body weight and 14 ± 0.9 d of age) were used to study the amount of colostrum given at birth at the dairy farm of origin, the degree of feed restriction suffered at an assembly center simulation (d −4 to d −1), and the effects of a 19 h transportation (d −1). Treatments were as follows: control calves (CTRL; n = 16) were fed 10 L of colostrum at the dairy farm of origin, milk replacer (MR) and concentrate at the assembly center, and were not transported; calves fed high colostrum and milk replacer (HCMR; n = 17) were given 10 L of colostrum at the dairy farm of origin, MR at the assembly center, and transported; calved fed high colostrum and rehydrating solution (HCRS; n = 16) were given 10 L of colostrum at the dairy farm of origin, a rehydrating solution (RS) at the assembly center, and transported; calves fed low colostrum and milk replacer (LCMR; n = 17) were given 2 L of colostrum at the dairy farm of origin, MR at the assembly center, and transported; and calves fed low colostrum and rehydrating solution (LCRS; n = 16) were given 2 L of colostrum at the dairy farm of origin, RS at the assembly center, and transported. Transported calves mimic a 19-h transportation. After transport, all calves were fed 2.5 L of MR twice daily and had ad libitum access to concentrate, straw, and water. Calves' recovery was followed for 7 d. Concentrate intake and health records were collected daily from d −4 until d 7 and body weight (BW) and blood samples were collected on d −4, −1, 0, 1, 2, and 7 of the study. Results showed that the feeding regimen provided at the assembly center reduced BW for the HCRS and LCRS calves compared with the CTRL, HCMR, and LCMR calves. Concentrate intake peaked on d 0 in the transported calves, followed by a reduction of intake on d 1 after transportation. Concentrate intake recovery was lower for the LCRS and LCMR calves. On d −1, nonesterified fatty acids and β-hydroxybutyrate concentrations were greater for the HCRS and LCRS calves compared with the CTRL, HCMR, and HCRS calves. After transportation, serum Cr-EDTA concentration was greater for the HCRS and LCRS calves than the HCMR, LCMR, and CTRL calves. The LCRS calves had the lowest serum concentration of citrulline. Finally, health scores were greater for the LCRS calves from d 0 to 7. In summary, both the greatest degree of feed restriction during the assembly center and the low colostrum consumption at birth negatively affected the recovery of concentrate consumption and BW, gut functionality, health status, and behavior in calves after arrival at the rearing farm.
Key words: dairy beef calf, colostrum, feed restriction, gut functionality

INTRODUCTION

In recent years, the dairy beef industry has developed significantly as an important supply of meat in many countries. However, even though these surplus calves represent an income for dairy producers, efforts put into their postnatal care and nutrition are usually not a priority (Devant and Marti, 2020). Previous studies have revealed differences in management practices with minor attention put on male calves' nutrition, colostrum quality, navel disinfection, and vaccination when compared with heifer calves (Fecteau et al., 2002; Shivley et al., 2016; Renaud et al., 2017; Renaud et al., 2018b). In addition, it has been demonstrated that male calves receive lower volumes of colostrum compared with females (Renaud et al., 2020a) or, in a minority

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of cases, no colostrum at all (Renaud et al., 2017). Because of poor colostrum management practices, the risk of mortality and morbidity increases due to failure on the transfer of passive immunity (Renaud and Pardon, 2022). Colostrum consumption in newborn calves is not only important to gain passive immunity from maternal antibodies (Hulbert and Moisá, 2016), but also because it is composed of nutrients (e.g., carbohydrates, lipids, and proteins) and bioactive substances (e.g., growth factors, cytokines, and enzymes) that have been shown to modulate the development and function of the gastrointestinal tract (**GIT**; Blum and Hammon, 2000). Some of the effects that colostrum exerts on the GIT are related to enhanced cell proliferation and protein synthesis, modulation of microbial population, absorption and motility, and vascular tone, among many others (Blum, 2006). Therefore, colostrum provision may exert long-term effects on calves' lives.

In addition to the poor colostrum management, male calves destinated for the dairy beef production are also exposed to physical and psychological challenges during marketing and transportation from their dairy farm of origin to the rearing facilities. In the marketing process, calves can be transported directly from the dairy farm to the rearing facilities or be mixed and regrouped at auction markets or assembly centers (Pardon et al., 2014; Marcato et al., 2020a; Rot et al., 2022) before being transported to the rearing farms. In Europe, when subjected to assembly centers, calves can stay in these establishments for a period of up to 6 d (European Commission, 2022). During this period, calves are normally fed only rehydrating solutions (**RS**) or in some centers, milk replacer (**MR**), and in addition to the feed and water restriction, they are exposed to commingling, mixing, exposure to new pathogens, loading and unloading from the truck, and physical trauma (Warriss, 1990).

The final transport to the rearing farms involves short- or long-distance transportations. According to European legislation, trucks used for long-distance transportation of unweaned calves must be equipped with water troughs (European Council, 2005). However, these are normally not adapted for young calves, the number of them are not sufficient, and calves are not familiar with them (EFSA AHAW Panel, 2022). Because calves have only 1 h of rest to drink water within 2 consecutives 9-h trips (European Council, 2005), these difficulties aggravate their water restriction and stress during transport.

Altogether, the stress and fasting due to transportation lead to BW losses that are visible on arrival to the rearing facilities. Research has shown that dairy beef calves arrive at veal farms with low BW and compromised health status (Renaud et al., 2018b). The weight loss during marketing and transportation and the decrease in intake during the first days after arrival to the rearing facilities (Hutcheson and Cole, 1986; Loerch and Fluharty, 1999) aggravate their general condition. Hutcheson and Cole (1986) estimated that it can take between 1 and 3 weeks for calves to recover their expected feed intakes after arrival. In accordance, our research group has demonstrated that feed-restricted and fasted unweaned calves take approximately 21 d to recover their concentrate intake after a period of fasting and feed restriction (Pisoni et al., 2022). Back to the importance of colostrum consumption, it has been recently demonstrated that a low colostrum consumption at birth (2 L) can generate losses of approximately 2 kg of BW on arrival to the rearing facilities compared with calves that received higher amounts of colostrum at birth (10 L; Pisoni et al., 2023). Altogether, these data suggest that many factors may be associated and acting together in incrementing BW losses and decreasing feed intake in calves on arrival at the rearing phase.

Finally, another variable associated with drops in concentrate intake and stress is the alteration of gut permeability. Feed restriction can cause modifications in the normal functioning of the intestinal barrier (Zhang et al., 2013; Kvidera et al., 2017b; Pisoni et al., 2022). Under normal conditions, the GIT barrier prevents the absorption of harmful antigens and pathogens and allows the absorption of nutrients (Groschwitz and Hogan, 2009). Consequently, an alteration in this normal functioning allows pathogens' translocation from the intestinal lumen to the bloodstream, leading to immunological activation and increased susceptibility to infection (Berg, 1999). Altogether, it has been shown that the loss of integrity in the gut can directly affect production, metabolic, and inflammatory parameters (Kvidera et al., 2017a).

Poor colostrum provision, marketing, and long-distance transportation are common practices that are detrimental to the recovery of calves on arrival at the rearing facilities. To devise new strategies for improving calves' welfare and performance during this period, it is important to elucidate if these factors exert an individual effect and if they synergistically deteriorate calves' recovery; a concept that, to our knowledge, has not yet been elucidated. Therefore, our initial hypothesis is that male dairy calves with a low colostrum consumption at birth, which suffer feed restriction during marketing and transportation, arrive at the rearing facilities with a compromised nutritional status and gut functionality that will negatively affect their performance, health, and behavior. Therefore, the objectives of this study were to evaluate the effects of colostrum consumption

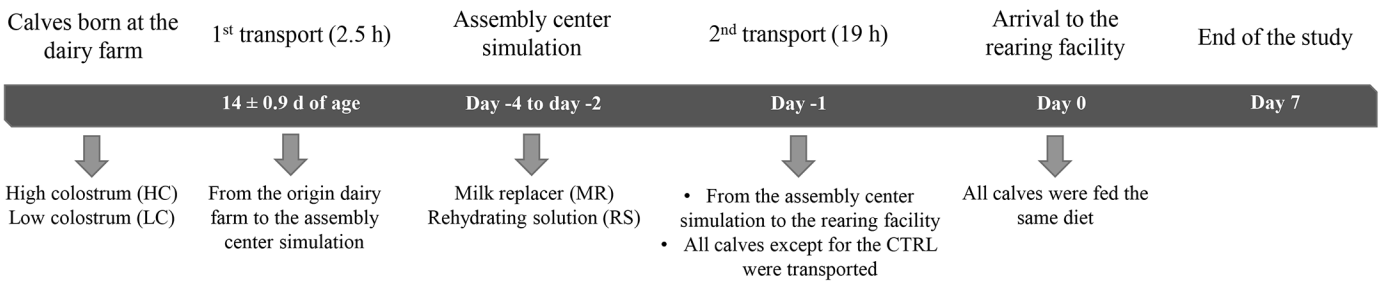

Figure 1. Temporal overview of the study setup of the effects of colostrum consumption and feed restriction during marketing and transportation in the recovery of dairy beef male Holstein calves. CTRL = calves fed 10 L of colostrum at birth, milk replacer at the assembly center simulation, and not transported.

and feed restriction on biomarkers of stress, nutritional and health status, gut functionality, and behavior in male dairy beef calves being marketed and transported.

MATERIALS AND METHODS

Handling of Calves at Their Dairy Farm of Origin

All calves used in this study were managed following the principles and guidelines of the Animal Care Committee of Generalitat de Catalunya (Barcelona, Spain; RD 53/2013; project no. 11211). For this study, a total of 82 male Holstein calves (42 ± 1.2 kg of BW and 14 ± 0.9 d of age; mean ± SE) born at a commercial dairy farm (Granja Selergan S.A., Lleida, Spain) were used. At the dairy farm of origin, calves were divided into 2 groups depending on the amount of colostrum consumed at birth: high-colostrum (**HC**; n = 49) calves received 4 L of colostrum within the first 2 h after birth, and 2 L of colostrum in the next 3 feedings within the first 24 h after birth; and low-colostrum (**LC**; n = 33) calves received only 2 L of colostrum (Besser et al., 1991) within the first 2 h after birth (Figure 1). Calves were balanced by birth BW, cow parity (primiparous or multiparous), and birth time (day or night). For all calves, only high-quality colostrum (McGuirk and Collins, 2004) was used and administered via esophageal tube (56.28% CP, 29.48% fat, and 10.07% lactose on a DM basis, and 36,910 IU/L gamma-glutamyl transferase [**GGT**], 1.83 mg/L lactoferrin, 145.44 mg/mL IgG, and 33.11 mg/mL IgG1; analyzed from a single pool of colostrum collected and mixed from the 43 pools fed to the calves). After colostrum consumption, calves were allocated in individual hutches and received 2 L of MR at a concentration of 125 g/L as fed twice daily (21.86% CP, 16.59% fat, 45.50% lactose; Schils BV) and ad libitum access to concentrate, following the standard operating procedures of the commercial dairy farm. Calves remained at the dairy farm of origin until approximately 14 d of age (minimum age required for transportation; European Council, 2005) and then were transported for 2.5 h to an experimental research unit located at IRTA (Institut de Recerca i Tecnologia Agroalimentàries; Torre Marimon, Spain; Figure 1). This short trip was intended to mimic the typical transportation of calves from their dairy farm of origin to the assembly centers or auction markets. To obtain 82 pure Holstein male calves from the same commercial dairy farm, we entered them in 4 groups of 20 with a 2-wk interval between each group.

Handling of Calves During an Assembly Center Simulation (d −4 to −1)

On arrival at the experimental research unit, calves were assigned to an assembly center diet for 3 d (from d −4 to −1 of the study) by BW and age to simulate the feed restriction suffered at assembly centers. During this period, calves were only fed either MR or RS to simulate 2 of the typical diets normally offered in these establishments. A control group that did not suffer any nutritional challenge and was fed high colostrum at birth was also incorporated as follows: control (**CTRL**) calves were fed 2.5 L of MR at a concentration of 125 g/L as fed twice daily and had ad libitum access to a pellet concentrate (17.1% CP, 16.9% NDF, 5.7% ADF, 29.2% starch, 5.5% ether extract, and 5.1% ashes on a DM basis, with the main ingredients being 39% cornflakes, 17% barley, 14% wheat middlings, 13.9% soybean meal, 9% wheat bran, 3% sunflower meal, 1.9% palm oil, 1.3% calcium carbonate, 0.5% premix, and 0.4% salt) and water during their 3 d at the assembly center simulation. The **MR** calves were only fed 2.5 L of MR at a concentration of 125 g/L as fed twice daily with no access to concentrate during their 3 d at the assembly center simulation; and **RS** calves were only fed 2.5 L of RS at a concentration of 60 g/L as fed twice daily (0.39% CP, 0.05% fat, and 84.75% dextrose; Corion) with no access to concentrate during their 3 d at the assembly center simulation. The CTRL and MR

Table 1. Treatment descriptions, effects of colostrum consumption and feed restriction during marketing and transportation in the recovery of dairy beef male Holstein calves[1]

	Colostrum given at birth		Diet offered during the assembly center simulation (d −4 to −1)		19-h transportation (d −1)	
Treatment	2 L (LC)	10 L (HC)	Milk replacer	Rehydrating solution	Yes	No
CTRL		X	X			X
HCMR		X	X		X	
HCRS		X		X	X	
LCMR	X		X		X	
LCRS	X			X	X	

[1]CTRL = control calves; HCMR = calves fed high colostrum and milk replacer; HCRS = calves fed high colostrum and rehydrating solution; LCMR = calves fed low colostrum and milk replacer; LCRS = calves fed low colostrum and rehydrating solution; LC = low colostrum; HC = high colostrum.

calves were fed the same MR offered at their dairy farm of origin (21.86% CP, 16.59% fat, and 45.50% lactose; Schils BV).

Final Treatments

Based on the amount of colostrum fed at birth at the dairy farm of origin (HC or LC) and the type of diet offered during the assembly center simulation (MR, RS, or MR and concentrate), calves were assigned to the final treatments by BW and age as follows: calves fed high colostrum and milk replacer with ad libitum access to concentrate (CTRL; n = 16); calves fed high colostrum and milk replacer **(HCMR**; n = 17); calves fed high colostrum and rehydrating solution (**HCRS;** n = 16); calves fed low colostrum and milk replacer (**LCMR;** n = 17); and calves fed low colostrum and rehydrating solution (**LCRS**; n = 16; Table 1). The technical staff working on this experiment was not blinded to treatments.

Handling of Calves During Long-Distance Transportation (d −1)

In the early afternoon of d −1 of the study (at 1300; after the 3-d assembly center simulation), all calves fitted in the MR and RS treatments were transported for 19 h to mimic a long-distance transportation arriving at the rearing facility in the early morning of d 0 (0800) of the study. The CTRL calves were not transported and stayed at the experimental research unit, where they were fed MR twice daily and given ad libitum access to concentrate and water during the 19-h period (Figure 1). The long-distance transportation was intended to simulate an international purchase of male dairy beef calves. During the 19-h trip, calves had access to water inside the truck during the 1-h rest stop after 9 h of transport, following the regulations from the European Commission for the transport of unweaned calves (European Council, 2005). However, instead of using the water troughs installed in the truck, calves drank from buckets to ensure that all calves had access to water during the rest stop. The vehicle was a commercial semitrailer with 3 decks with side vents with sliding panels. Straw was used for bedding. In total, 4 trips (replicates) with approximately 20 calves each were done following the same route to ensure similar road conditions during the months of October and November 2020 (average ambient temperature of 11°C [range = 4.4–17°C] and 87.5% humidity [range = 82–90%]). Calves were not transported at commercial densities as only the 20 calves of the study for each replicate were transported in 1 deck at a time. The journey was completed with 2 drivers to comply with the legislation.

Handling of Calves on Arrival to the Rearing Facility (d 0 to 7)

After transportation, calves returned to the experimental research unit. From d 0 to 7 of the study, all calves were fed 2.5 L of MR (21.86% CP, 16.59% fat, 45.50% lactose; Schils BV) at a concentration of 125 g/L as fed twice daily and had ad libitum access to the same pelleted concentrate used for the CTRL calves during the assembly center simulation, in addition to straw and water (Figure 1). This period was equivalent to the arrival at a rearing facility where all calves are fed the same diet.

To facilitate understanding of the experimental design, the reader can refer to Table 1 and Figure 1 for a summarized description of the treatments and the timeline of this study. As previously explained, to obtain the total number of calves for this trial we entered them in 4 groups of 20 calves. Therefore, the timeline was repeated 4 times in total, 1 time for each group entering the trial.

Measurements and Sample Collection

Rectal temperature, health, and fecal scores were recorded daily from d −4 to 7 of the study to assess the general health status of the calves and to identify those that might need medical treatment. For the assessment of respiratory disease, the calf health scoring from the University of Wisconsin–Madison (Madison, WI) was used by scoring rectal temperature, nasal discharge, eye discharge, ear disposition, and cough using a scale of 0 to 3, as previously described (McGuirk, 2008). If the sum of each health criteria for each calf and day was ≥5, a calf was considered sick and treated accordingly. In the event of respiratory disease, calves were treated with an intramuscular injection of Florfenicol 300 mg/mL (Florvex, SP Veterinaria S.A.) at a dose of 20 mg/kg of BW twice, with an interval of 48 h. Fecal scores were also recorded daily and scored following Larson et al. (1977). A score of 0 was considered normal (firm but not hard, original form is distorted slightly after dropping to the floor and settling); a score of 1 was for soft feces (does not hold the form, piles but spreads slightly); a score 2 was for runny feces (spreads readily); and a score 3 for watery feces (liquid consistency, splatters). Calves were considered diarrheic when they presented a score ≥2 (Renaud et al., 2020a). Diarrheic calves were treated with a unique intramuscular injection of Marbofloxacin 100 mg/mL (Marbox, CEVA Salud Animal S.A.) at a dose of 8 mg/kg of BW. Body weight was recorded at birth and on d −4, −1, 0, 1, 2, and 7 of the study. From d −4 to 7, concentrate offered and refused was recorded and used to calculate daily DMI. In vivo gut permeability tests were conducted on d −4, −1, 0, 1, 2, and 7 of the study using Cr-EDTA as a marker of total-tract permeability. Chromium-EDTA was orally administered to the calves 2 h after the morning feeding at a concentration of 0.1 g/kg of BW (Sigma-Aldrich Corp.), and a blood sample was taken 2 h after the administration of the marker. The preparation of Cr-EDTA and the concentration given to the calves were based on a previous study (Amado et al., 2019). Blood samples were collected before the morning feeding on d −4, −1, 0, 1, 2, and 7 from the jugular vein using evacuated tubes (BD Vacutainer). Plasma was obtained using 4-mL vacuum tubes with a glycolytic inhibitor (BD Vacutainer Fluoride Tubes), and serum was obtained using 10-mL vacuum tubes with clot activator and silicon coated (BD Vacutainer Serum Tubes). Samples were centrifuged at 1,500 × *g* at 4°C for 15 min, and the obtained serum was aliquoted in individual polypropylene tubes and stored at −20°C until analysis. Serum was collected to analyze energy balance markers (nonesterified fatty acids, **NEFA**; BHB; and glucose), markers of stress (cortisol), inflammation (haptoglobin, **Hp**), muscular damage (creatine kinase, **CK**), dehydration (total protein, **TP**), and gut functionality (citrulline, Cr-EDTA, and D-lactate). On d −4, blood samples were collected for measurements of IgG1 on arrival at the rearing facility. Accelerometer data loggers (Hobo Pendant G Data Logger, Onset Computer Corporation) were used for measurements of standing time, standing duration, and standing bouts records from d −4 to 7 of the study. Standing behavior was recorded at 1-min intervals and accelerometers were placed on the right hind limb of each calf. Each accelerometer was covered with a foamed rubber to protect the calves from abrasions and attached with a self-adhesive bandage wrap. HOBOware software version 3.7.23 (Onset Computer Corporation) was used for data processing.

Chemical Analysis

Concentrate and MR samples were analyzed for DM, ash, CP, ADF, and NDF and DM, ash, CP, and sugars, respectively, as previously described (Pisoni et al., 2022). Colostrum samples were analyzed for fat, CP, lactose, and ash, as previously described (Pisoni et al., 2023), and for the concentration of lactoferrin, GGT, IgG, and IgG1. Colostrum lactoferrin concentration was measured using a bovine lactoferrin ELISA kit (Cat. No. E11–126, Bethyl Laboratories, Inc. Montgomery, TX). Concentrations of GGT in colostrum were analyzed using a chemistry analyzer (AU480, Beckman Coulter International S.A.), and IgG and IgG1 were measured by an ELISA kit (Species-Specific Bovine IgG and Bovine IgG1, Bethyl Laboratories Inc.). Serum concentration of NEFA was measured by the enzymatic colorimetric method with NEFA-C reagent (Fujifilm Wako Chemicals Europe GmbH). Serum concentration of glucose was determined following the hexokinase method (glucose hexokinase reagent test, Beckman Coulter Inc.). A kinetic enzymatic method (Randox Laboratories Ltd.) was used to determine serum BHB concentration. Serum cortisol concentrations were determined by an enzyme immunoassay (DRG International Inc.). Serum Hp concentrations were determined by a colorimetric assay (Tridelta Development Ltd.). Serum CK catalytic concentration was determined by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) kinetic method (Creatine Kinase CK-Nac Reagent Test, Beckman Coulter Inc.). The D-lactate was determined by a fluorescence-based assay (Cayman Chemical). Serum IgG1 concentrations were determined by an enzyme-linked immunosorbent assay (Specie-Specific Bovine IgG1, Bethyl Laboratories Inc.). Serum concentration of TP was analyzed by the Biuret method (Total Protein Reagents, Beckman

Coulter Inc.). Levels of NEFA, glucose, BHB, Hp, CK, and TP were determined in a chemistry analyzer (AU480, Beckman Coulter International S.A.). Serum citrulline concentration was measured using a spectrophotometric kit (L-Citrulline Kit, Immundiagnostik AG). Serum Cr-EDTA was determined by inductively coupled plasma-optical emission spectrometry, using an inductively coupled plasma MS (Agilent 7500ce). The intra- and inter-assay coefficients of variation for NEFA, BHB, glucose, cortisol, Hp, CK, D-lactate, IgG1, TP, citrulline, and Cr-EDTA were 1.62% and 4.46%, 0.44% and 2.69%, 0.58% and 1.11%, 2.6% and 6.6%, 4.9% and 5.8%, 1.34% and 3.68%, 3.1% and 3.5%, 3.37 and 9.26%, 0.38% and 0.74%, 9.75% and 5.12%, and <10% and <15%, respectively.

Statistical Analysis

Calf was the experimental unit. A power analysis was conducted to determine the experimental units needed. The type I error rate (α) was 0.05, and the power ($1 - \beta$) was set at 80%. Concentrate intake on d 2 was considered our primary outcome and was based on Pisoni et al. (2022). We expected that concentrate intake of control calves would be around 240 g at d 2 and to find differences of 50% less in concentrate intake in the treatments applied in this study. The study design was a randomized balanced design with a covariate adjustment (BW and age at d −4). The model included the random effect of pen and the fixed effect of treatment, time, and their interaction. Paired-based randomization based on BW at birth, cow parity (primiparous or multiparous), and calving time (a.m. or p.m.) was used to allocate calves in either the HC or the LC treatment at birth. The same criterion was used for the application of the second treatment based on MR or RS feeding. In this case, paired-based randomization was based on age and BW at arrival to the assembly center simulation. Data were analyzed using the MIXED procedure of SAS (version 9.4, SAS Institute Inc.) with repeated measurements for those continuous variables with multiple sampling over time. Non-normal data were log-transformed to achieve normal distributions. The compound symmetry covariance structure and the first-order autoregressive covariance structure were tested according to the time points. Kenward-Roger degrees of freedom were used based on the lower Bayesian information criterion value. Body weight and age at d −4 were analyzed using the MIXED procedure with treatment as a fixed effect. Health scores were binarily categorized considering 0 as indicative of a healthy calf (score 0) and 1 as indicative of a sick calf (scores 1 to 3). A score ≥5 considering the sum of health criteria for each calf and day and was also binary categorized. In this case, if the sum of the health criteria was <5 it was codified as 0 (healthy calf) and if the sum was ≥5 it was considered 1 (sick calf). Health scores were divided into 2 periods: period 1 (from d −4 to d −1) and period 2 (from d 0 to d 7). Fecal score was analyzed as described for health score with 0 as indicative of normal feces (scores 0 and 1) and 1 as indicative of diarrhea (scores 2 and 3). All health parameters were analyzed using the GLIMMIX procedure of SAS with a binomial distribution. The model included calf as a random effect and treatment, period, and their interaction as fixed effects. The model with lower Bayesian information criterion value was selected. Differences were declared significant at $P \leq 0.05$, and trends were discussed at $P \geq 0.05$ and $P \leq 0.10$ for all models.

RESULTS AND DISCUSSION

Verifying Differences Between Treatments Based on the Amount of Colostrum Consumed

To verify that treatments based on the amount of colostrum consumed at birth (HC or LC) were correctly applied, serum concentration of IgG1 was measured on d −4 of the study (right before starting the assembly center simulation). Results showed treatment differences being greater for the CTRL, HCMR, and HCRS calves, compared with the LCMR and LCRS calves ($P < 0.01$; Figure 2). Serum concentration of IgG1 is used as a biomarker for the transfer of passive immunity. Immunoglobulin G1 is the most predominant subclass in colostrum (Korhonen et al., 2000) and, because of this, a better estimate of colostrum consumption. As expected, calves in the HC treatment had greater concentrations of IgG1. A great amount of research exists investigating Ig concentration in young calves, and it has been estimated that maternal Ig has a half-life of approximately 10 d after colostrum consumption (Hassig et al., 2007). However, results from this investigation showed that differences in IgG1 between HC and LC calves can remain beyond 14 d of age (14.2 ± 0.81 d of age; mean ± SE).

BW and Concentrate Intake Recovery

On d −1, after the assembly center simulation period, BW was lesser ($P < 0.01$; Figure 3) for the HCRS and LCRS calves compared with the CTRL, HCMR, and LCMR calves. Correspondingly, a time by treatment interaction ($P < 0.01$; Figure 4) was observed for concentrate intake during the assembly center simulation (from d −4 to −1). This significant interaction indicated that only CTRL calves were able to consume concentrate (average concentrate intake was 78.52 ±

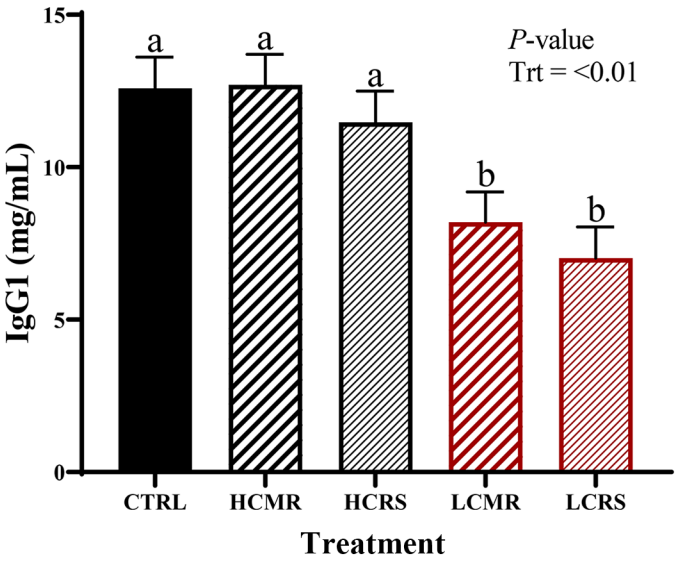

Figure 2. Serum concentration (mean ± SE) of IgG1 on d −4 of the study in male Holstein calves fed 10 L of colostrum at birth, milk replacer (MR) at the assembly center simulation period, and not transported (CTRL); fed 10 L of colostrum at birth, MR at the assembly center simulation period, and transported for 19 h (HCMR); fed 10 L of colostrum at birth, rehydrating solution (RS) at the assembly center simulation period, and transported for 19 h (HCRS); fed 2 L of colostrum at birth, MR at the assembly center simulation period, and transported for 19 h (LCMR); and fed 2 L of colostrum at birth, RS at the assembly center simulation period, and transported for 19 h (LCRS). Different letters within a time point denote differences among treatments ($P < 0.05$); the order of the letters denotes the treatment with the highest value. Trt = treatment.

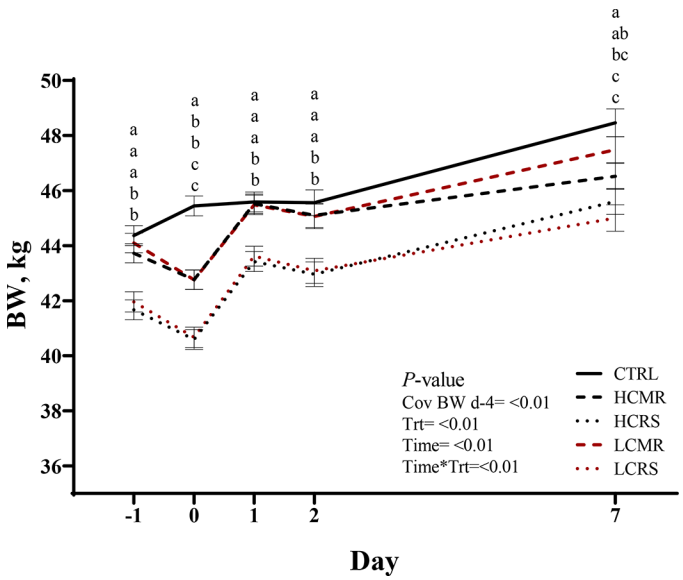

Figure 3. Body weight recovery (mean ± SE) on d −1, 0, 1, 2, and 7 in male Holstein calves fed 10 L of colostrum at birth, milk replacer (MR) at the assembly center simulation period, and not transported (CTRL); fed 10 L of colostrum at birth, MR at the assembly center simulation period, and transported for 19 h (HCMR); fed 10 L of colostrum at birth, rehydrating solution (RS) at the assembly center simulation period, and transported for 19 h (HCRS); fed 2 L of colostrum at birth, MR at the assembly center simulation period, and transported for 19 h (LCMR); and fed 2 L of colostrum at birth, RS at the assembly center simulation period, and transported for 19 h (LCRS). Body weight from d −4 was used as a covariate (cov). Different letters within a time point denote differences among treatments ($P < 0.05$); the order of the letters denotes the treatment with the highest value. Trt = treatment.

10.98 g/d; data not shown). As expected, a clear negative effect of BW losses was seen depending on the type of diet offered during the assembly center simulation period. During this period, in addition to the access to concentrate, the CTRL calves had access to MR, and their average daily DMI was 669 g DM per day compared with HCMR and LCMR calves, which consumed only MR (DMI = 604 g DM per day), and HCRS and LCRS calves, which only consumed RS (DMI = 243 g DM per day; Figure 5). Crude protein and ME intake were also higher for the CTRL calves during this period. On average, the CTRL calves consumed 14.5 g/d of CP and 3.2 Mcal/d of ME, whereas the HCMR, HCRS, LCMR, and LCRS calves consumed an average of 11.4 g/d of CP and 2.6 Mcal/d of ME, 4.5 g/d of CP and 1.0 Mcal/d of ME, 11.5 g/d of CP and 2.6 Mcal/d of ME, and 4.7 g/d of CP and 1.1 Mcal/d of ME, respectively (Figure 6A and B). Differences in DM, CP, and ME intake during the assembly center period are responsible for the BW losses for the RS calves by d −1 of the study. These results agree with a previous experiment conducted by our research group where the effects of fasting and feed restriction were evaluated in unweaned male calves (Pisoni et al., 2022). Results from Pisoni et al. (2022) showed lower BW for calves being fed RS compared with calves fed MR. In addition, these differences remained almost 21 d after arrival at the rearing farm. Marcato et al. (2020b) found similar results in transported calves fed MR versus RS. In their study, calves that were fed a RS lost BW to a greater extent than calves fed MR after 6 h of transportation. The amount of nutrients and energy contained in the MR might have helped to reduce fat mobilization and the consequent use of body reserves as a source of energy (Marcato et al., 2020b), something that is unlikely to occur by feeding RS, which is mainly composed of quickly metabolizable sugars. In the present study, nutritional differences in the diet fed to each treatment caused similar effects. These results confirm that an inadequate feeding regimen (low energy and protein intake) at the assembly centers in young calves causes BW losses before calves are transported.

After 19 h of transport (d 0), all transported calves suffered a shrinkage of approximately 1 kg (Figure 3). However, at d 1 and 2 after transportation, HCMR and LCMR calves had similar BW to CTRL, but LCRS and HCRS continued exhibiting lesser ($P < 0.01$; Fig-

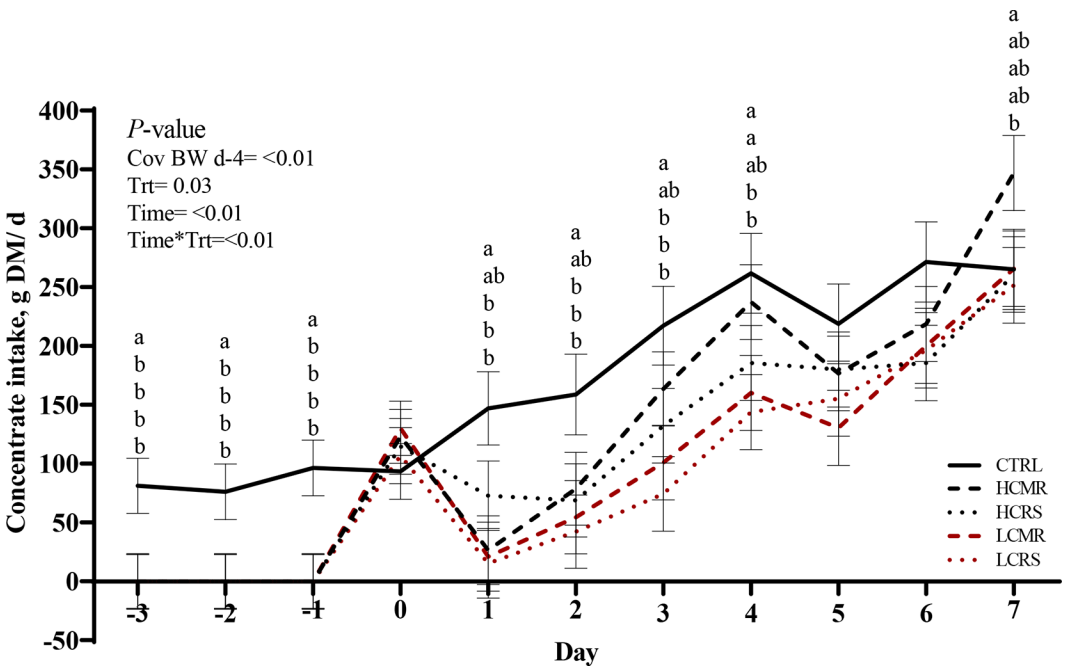

Figure 4. Concentrate intake recovery (mean ± SE) from d −3 (assembly center simulation) to d 7 in male Holstein calves fed 10 L of colostrum at birth, milk replacer (MR) at the assembly center simulation period, and not transported (CTRL); fed 10 L of colostrum at birth, MR at the assembly center simulation period, and transported for 19 h (HCMR); fed 10 L of colostrum at birth, rehydrating solution (RS) at the assembly center simulation period, and transported for 19 h (HCRS); fed 2 L of colostrum at birth, MR at the assembly center simulation period, and transported for 19 h (LCMR); and fed 2 L of colostrum at birth, RS at the assembly center simulation period, and transported for 19 h (LCRS). Body weight from d −4 was used as a covariate (cov). Different letters within a time point denote differences among treatments ($P < 0.05$); the order of the letters denotes the treatment with the highest value. Trt = treatment.

ure 3) BW compared with the rest of the treatments. These results suggest that feeding RS might delay the recovery of BW compared with MR-fed calves. Finally, 7 d after arrival all calves increased their BW, however, the HCMR, HCRS, and LCRS calves continued to show lower BW compared with CTRL calves. After the 19-h transportation period (d 0), concentrate was offered to all calves, and HCMR, LCMR, HCRS, and LCRS calves showed a pronounced increment in concentrate intake ($P < 0.01$; time effect; Figure 4) achieving concentrate intake values close to the CTRL calves; this behavior is most likely explained by the hunger experienced during transportation. However, on d 1 of the study, a significant reduction of concentrate intake was observed for all transported treatments ($P < 0.01$; time effect; Figure 4). Only HCRS calves showed a lesser reduction of concentrate intake close to CTRL calves. The potential reasons for this drop might be in relation to a digestive disorder caused by sudden access to a source of concentrate after a period of feed restriction and transportation. The sudden increment in intake, in addition to an undeveloped or damaged digestive tract, could have potentially caused ruminal acidosis after rapid overconsumption (Owens et al., 1998). Another possible explanation could be that under a condition of stress due to long transport and feed restriction followed by the sudden access to a rich source of rapidly fermented carbohydrates such as concentrate, the gut integrity might have suffered a certain level of disruption allowing the passage of toxins or feed compounds to the bloodstream. Under this circumstance, calves might have reduced their feed intake as part of a defensive physiological mechanism. Also, from d 1 to 7 all transported calves had a numerically lower concentrate intake recovery (Figure 4), compared with the CTRL calves. This could be indicative of a process of gut restoration taking place after the distress caused by the abrupt concentrate intake on d 0. The reduction of concentrate intake 24 h after a period of fasting when concentrate was reoffered to the calves was previously observed (Pisoni et al., 2022). In Pisoni et al. (2022), however, the reduction of concentrate intake was on average 35.9 g DM on d 1, compared with the 84.8 g DM from the present study for the same day (Figure 4). Differences found between both trials might be related to the fact that in Pisoni et al. (2022), calves were not transported but fasted for 9 or 19 h. It is possible that, in addition to the feed and water restriction, the stress due to transportation (e.g., mixing, loading, unloading, environmental conditions)

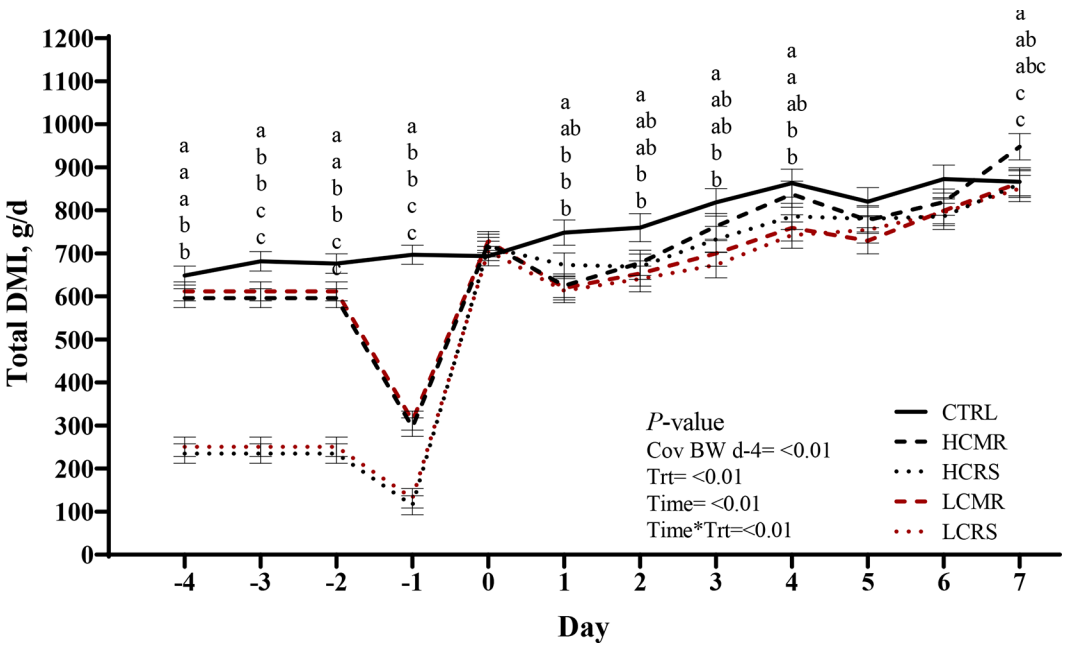

Figure 5. Total DMI (mean ± SE) from d −4 (assembly center simulation) to d 7 in male Holstein calves fed 10 L of colostrum at birth, milk replacer (MR) at the assembly center simulation period, and not transported (CTRL); fed 10 L of colostrum at birth, MR at the assembly center simulation period, and transported for 19 h (HCMR); fed 10 L of colostrum at birth, rehydrating solution (RS) at the assembly center simulation period, and transported for 19 h (HCRS); fed 2 L of colostrum at birth, MR at the assembly center simulation period, and transported for 19 h (LCMR); and fed 2 L of colostrum at birth, RS at the assembly center simulation period, and transported for 19 h (LCRS). Body weight from d −4 was used as a covariate (cov). Different letters within a time point denote differences among treatments ($P < 0.05$); the order of the letters denotes the treatment with the highest value. Trt = treatment.

in the present study could have caused greater BW losses and greater compensatory concentrate intake after transport with a consequent greater drop on d 1. Also, the type of calves and the type of concentrate in Pisoni et al. (2022) were different; calves were Angus-Holstein instead of pure Holstein, and the concentrate was a texturized concentrate with a different ingredient and nutrient composition. From d 2 to 7 of the study, concentrate intake differences began to decrease for the HC calves (HCMR and HCRS) but continued to be visible for the LC calves (LCMR and LCRS) until d 4, showing lower concentrate intake compared with CTRL, HCMR, and HCRS. These results support the idea that a lower amount of colostrum received at birth could negatively affect the digestive capacity to assimilate concentrate, making calves take longer to recover their consumption to levels similar to those for the HC calves. In agreement with our hypothesis, previous studies have demonstrated that colostrum consumption in newborn calves is not only important because of the Ig content but also because it is constituted with bioactive compounds and nutrients that are relevant for the development of the GIT (Blum and Hammon, 2000; Blum, 2006). In the present study, the effects of feed restriction in addition to a lower colostrum consumption at birth might produce changes at a GIT level in these calves that could increase the time needed to recover intake after a feed restriction and transportation period. Both factors (colostrum consumption and feed restriction) probably interact synergistically and seem to be relevant for feed intake recovery in ruminants.

Gut Functionality

Serum concentrations of Cr-EDTA, citrulline, and D-lactate were used to evaluate the effects of feed restriction and transportation on the gut functionality of calves. Results from serum citrulline concentration showed differences between treatments being lower ($P = 0.05$; Figure 7A) for LCRS, compared with CTRL and LCMR treatments, although calves in the HCMR and HCRS treatments showed intermediate values. Citrulline is a nonessential AA key intermediate in the urea cycle formed exclusively in enterocytes (Kaore and Kaore, 2014). Thus, a decrease in plasma citrulline concentration is associated with a decrease in the functional mass of enterocytes (Oliverius et al., 2010). Results from serum citrulline concentration for the LCRS treatment were expected due to the potential negative effects of low-colostrum consumption and the greater severity of the feed restriction on the normal physiology and functioning of the GIT. However, HCRS calves did

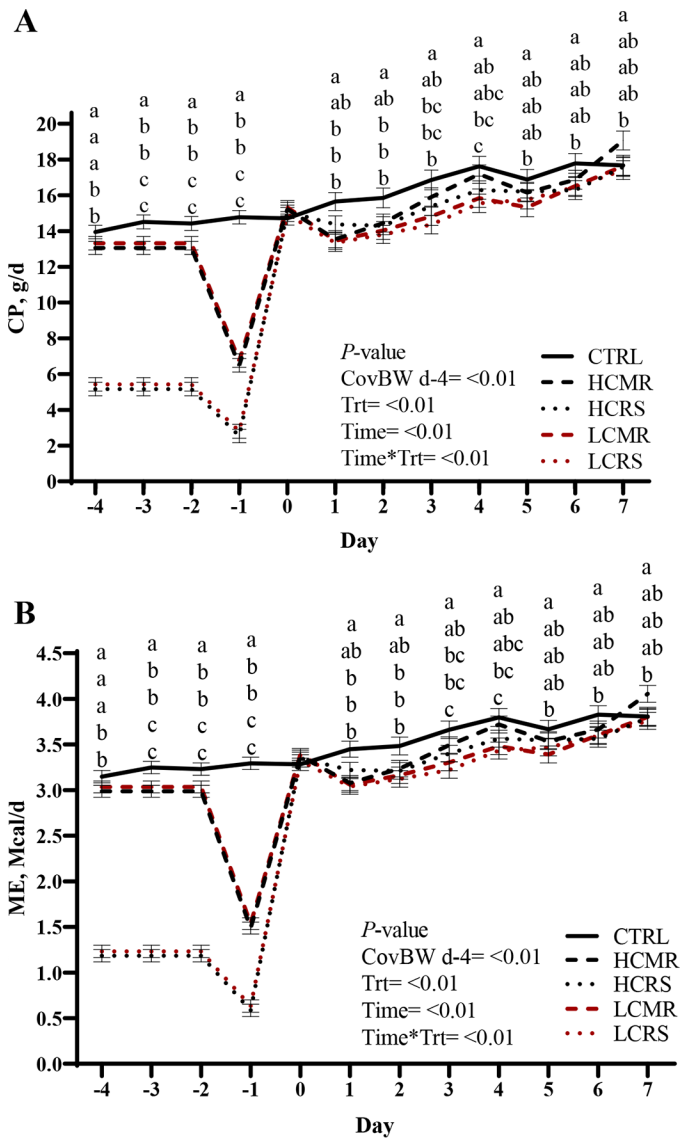

Figure 6. Crude protein (A) and metabolizable energy (B) intake (mean ± SE) from d −4 (assembly center simulation) to d 7 in male Holstein calves fed 10 L of colostrum at birth, milk replacer (MR) at the assembly center simulation period, and not transported (CTRL); fed 10 L of colostrum at birth, MR at the assembly center simulation period, and transported for 19 h (HCMR); fed 10 L of colostrum at birth, rehydrating solution (RS) at the assembly center simulation period, and transported for 19 h (HCRS); fed 2 L of colostrum at birth, MR at the assembly center simulation period, and transported for 19 h (LCMR); and fed 2 L of colostrum at birth, RS at the assembly center simulation period, and transported for 19 h (LCRS). Body weight from d −4 was used as a covariate (cov). Different letters within a time point denote differences among treatments ($P < 0.05$); the order of the letters denotes the treatment with the highest value. Trt = treatment.

not show a decrease in serum citrulline. In agreement with our hypothesis an additive effect might explain the small amount of colostrum consumed and the severity of the feed restriction on gut functionality. Similar results were observed in a previous study conducted by our research group where serum citrulline concentration was lower in calves fed RS and fasted during 9 or 19 h, compared with a control group fed MR and not fasted (Pisoni et al., 2022).

Results from serum Cr-EDTA concentration showed a time by treatment interaction ($P < 0.01$; Figure 7B). Serum Cr-EDTA is a biomarker of intestinal permeability to large molecules through the paracellular space of adjacent enterocytes (Bjarnason et al., 1995) that has been extensively used in calves (Hunt et al., 2002; Wood et al., 2015; Wilms et al., 2019). Because it is an indigestible probe, it can be used for assessments of total-tract permeability and it can be measured in plasma, serum, and urine (Bischoff et al., 2014). In the present study, no differences between treatments were observed by d −1 after the assembly center simulation. However, on d 0 after 19 h of fasting due to transportation, HCRS calves showed greater ($P < 0.01$) serum Cr-EDTA concentrations compared with CTRL, HCMR, and LCMR calves, whereas CTRL calves showed the lowest concentrations of serum Cr-EDTA. The lack of differences on d −1 suggests that neither feed restriction nor colostrum consumption at birth or their combination might have been challenging enough to exert differences in gut permeability between treatments. However, when calves were exposed to the stress and fasting of a 19 h transportation, those RS-fed calves (HCRS and LCRS) showed greater increments of serum Cr-EDTA. Previous studies have demonstrated that short-term or progressive fasting and feed restriction suffered during marketing and transportation can generate changes in the intestinal function of ruminants (Zhang et al., 2013; Kvidera et al., 2017b; Pisoni et al., 2022). Reduction in the number of intestinal cells and villus height, increments in cell apoptosis (Ferraris and Carey, 2000), and a decrease in the absorptive capacity of the gut (Moeser et al., 2012) due to fasting and malnutrition have been demonstrated. Other effects on gastrointestinal functionality can also be explained based on colostrum consumption at birth. As previously stated, colostrum is composed of bioactive substances that play an important role in GIT development and modulation (Blum et al., 2002; Blum, 2006). These bioactive substances are components of colostrum that are more predominant in high-quality colostrum, which coincides with the first milkings (Blum and Hammon, 2000). Some of the described effects are related to small intestine epithelial growth (Bühler et al., 1998), enhanced survival of epithelial cells, crypt cell proliferation, and enzymatic activity (Blättler et al., 2001). Based on our hypothesis, it was expected that a higher colostrum consumption with a greater amount of bioactive substances would have incremented gut growth and development and made it more resilient to the stress

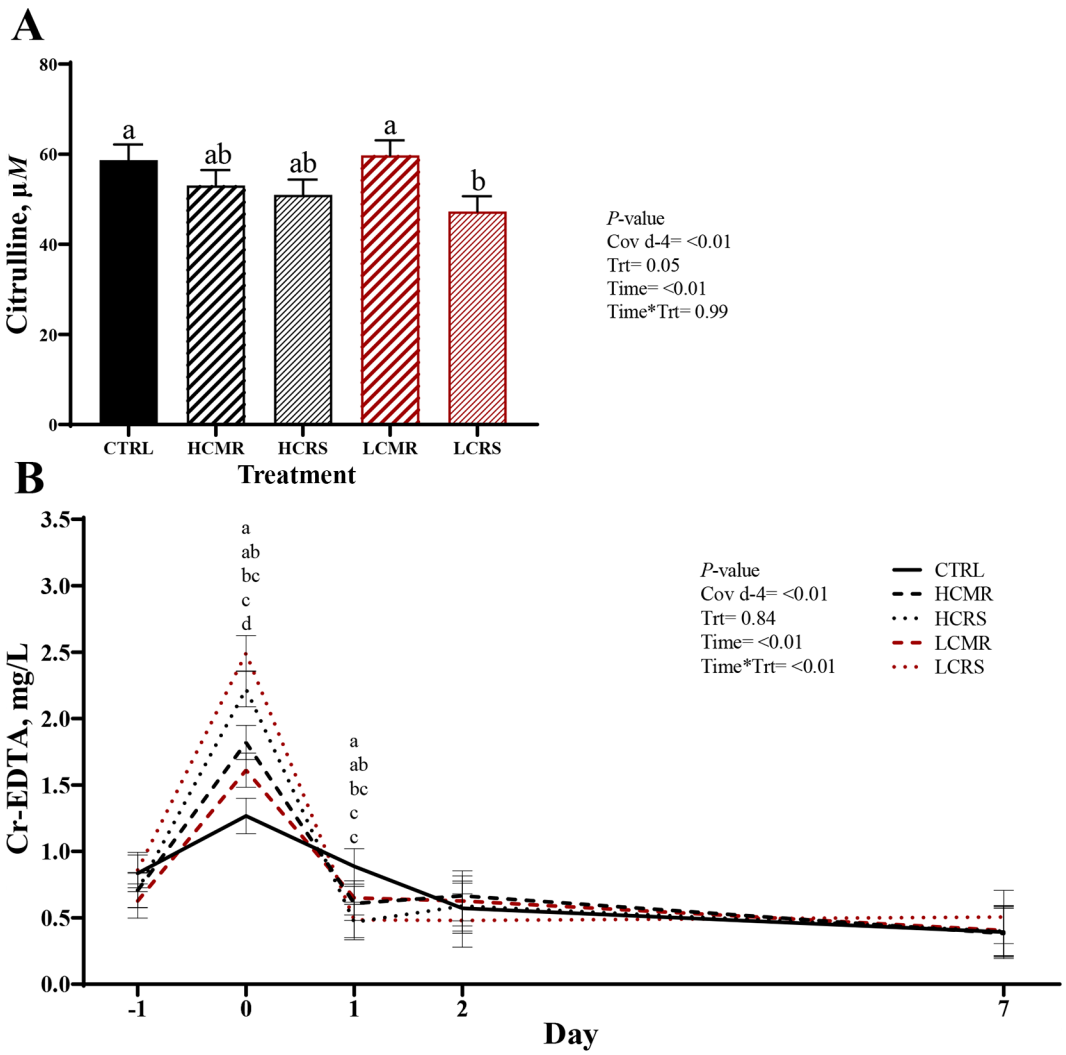

Figure 7. Serum concentration (mean ± SE) on d −1, 0, 1, 2, and 7 of citrulline (A) and Cr-EDTA (B) in male Holstein calves fed 10 L of colostrum at birth, milk replacer (MR) at the assembly center simulation period, and not transported (CTRL); fed 10 L of colostrum at birth, MR at the assembly center simulation period, and transported for 19 h (HCMR); fed 10 L of colostrum at birth, rehydrating solution (RS) at the assembly center simulation period, and transported for 19 h (HCRS); fed 2 L of colostrum at birth, MR at the assembly center simulation period, and transported for 19 h (LCMR); and fed 2 L of colostrum at birth, RS at the assembly center simulation period, and transported for 19 h (LCRS). Serum concentrations of citrulline and Cr-EDTA from d −4 were used as a covariate (cov). Different letters within a time point denote differences among treatments ($P < 0.05$); the order of the letters denotes the treatment with the highest value. Trt = treatment.

caused by feed restriction and transportation. However, under the conditions of the present study, no clear effect of colostrum consumption itself explained differences in serum Cr-EDTA or serum citrulline concentration. However, as mentioned previously, those calves with LC consumption showed numerically lower concentrate intake recovery compared with HC-fed calves. Taking this into consideration, a potential relationship seems to exists between the affection of the gut barrier integrity due to stress and feed restriction and its capacity for restoration afterward depending on the previous access to bioactive substances contained in colostrum. Based on these results, it can be reasoned that calves with the lowest colostrum consumption and the highest degree of feed restriction had greater intestinal dysfunctionality and lower intake recovery rates.

Finally, no differences were observed among treatments for serum concentration of D-lactate ($P = 0.61$; data not shown). D-Lactate is a normal product from bacterial fermentation (Terpstra et al., 2016). In cases of intestinal ischemia, the gut loses integrity, increasing its permeability and the subsequent translocation of bacteria and D-lactate to the bloodstream (Sun et al., 2001; Nielsen et al., 2011). In this study, and considering that feed restriction has been shown to alter the normal functioning of the intestinal barrier (Zhang

et al., 2013; Kvidera et al., 2017b), a rise in serum D-lactate was expected. However, results from the present study showed no differences among treatments suggesting that D-lactate might not be an indicator of gut permeability as sensitive as serum citrulline and Cr-EDTA.

Energy Balance

A time by treatment interaction ($P < 0.01$) was seen for serum concentrations of NEFA and BHB (Figure 8A and B, respectively); on d −1 after the assembly center simulation period, these concentrations were greater for the HCRS and LCRS calves, compared with calves in the rest of the treatments. In addition, a time by treatment interaction ($P < 0.01$; Figure 8C) was found for serum concentration of glucose being lower for HCRS and LCRS calves in the same period. Measurements of NEFA, BHB, and glucose are normally used as indicators of energy balance. Rises in serum NEFA and BHB concentrations right after the assembly center simulation reflect a greater body fat mobilization to use free fatty acids as a source of energy (Pénicaud et al., 2000; Grigor et al., 2001). This energy deficiency was caused by the negative energy balance triggered by the administration of RS instead of MR during the assembly center simulation in HCRS and LCRS calves, which could not satisfy their energy demands based on the energy obtained by the absorption of nutrients in the diet. In addition to the lack of energy from the diet, the stress derived from hunger raises plasma catecholamine concentrations, which also mobilize fat reserves (Fröhli and Blum, 1988). Comparable results to these parameters were observed in previous studies where serum NEFA concentrations increased after a period of transportation and feed restriction (Bernardini et al., 2012; Pisoni et al., 2022). Concerning serum BHB, its concentration was influenced to the same extent by the negative energy balance suffered. These results on serum NEFA and BHB were in accordance with the losses on BW found for HCRS and LCRS calves after the assembly center simulation period previously described. Moreover, on d −1, differences in NEFA and BHB between HCRS and LCRS calves were found to be be greater for the HCRS calves. It could be hypothesized that because HCRS calves received greater amounts of colostrum at birth than LCRS calves, these animals might have had greater body fat reserves and were able to mobilize more lipids in that negative energy balance condition. Another hypothesis could be a possible positive effect triggered by the bioactive compounds contained in colostrum that could affect gut physiology and, consequently, its metabolite absorption capacity. In any case, the influence of the feed restriction was, as expected, more pronounced than the influence of colostrum consumption. As expected, on d 0 after transportation, serum concentrations of NEFA and BHB were greater ($P < 0.01$) for all transported calves, compared with the CTRL. Calves in the CTRL treatment showed the lowest concentrations of these metabolites during the assembly center simulation and transportation periods as those calves were not transported and were fed MR and concentrate during this time. At d 1, the LCRS calves showed lower concentrations of serum NEFA, compared with the rest of the treatments, and the HCRS calves had higher concentrations of serum BHB, compared with the CTRL calves. From d 2 to 7, no differences among treatments were observed for serum NEFA or BHB concentrations. Following a similar approach regarding energy balance, calves that were fed only RS were expected to show a lower concentration of glucose, compared with calves being fed MR. On d 0, after the 19 h transportation, serum glucose concentrations were greater for the CTRL calves, compared with the rest of the treatments, due to the access to MR and concentrate during this time. At d 1 and stimulated by the increment in concentrate intake after transportation, serum glucose concentration increased for all transported calves, showing greater concentrations for the HCRS and LCRS calves, compared with CTRL calves. Differences in serum glucose concentration between the HCRS and LCRS and the CTRL calves cannot be explained based on the concentrate intake because no differences were seen between these treatments. Previous research has shown a negative correlation between serum NEFA and BHB concentration and reduced insulin responsiveness (Oikawa and Oetzel, 2006). Based on Oikawa and Oetzel (2006), we hypothesized that the higher increment in NEFA and BHB concentrations for the HCRS and LCRS calves might have led to an insulin resistance condition or lower insulin secretion that incremented glucose concentration, compared with the CTRL calves on d 1. From d 2 to 7, no differences among treatments in serum glucose concentrations were observed. Rapid increments in serum glucose concentration after transportation, when calves start eating concentrate, have been previously described (Mormede et al., 1982; Knowles, 1999; Marcato et al., 2020b). However, because low serum concentrations of glucose have been associated with increments in mortality in newly arrived calves (Mormede et al., 1982), avoiding large drops in its concentration should be taken into consideration. This is especially important when these drops last a couple days, such as in the case of LCRS and HCRS calves, which had serum low glucose concentrations during d −1 to 0.

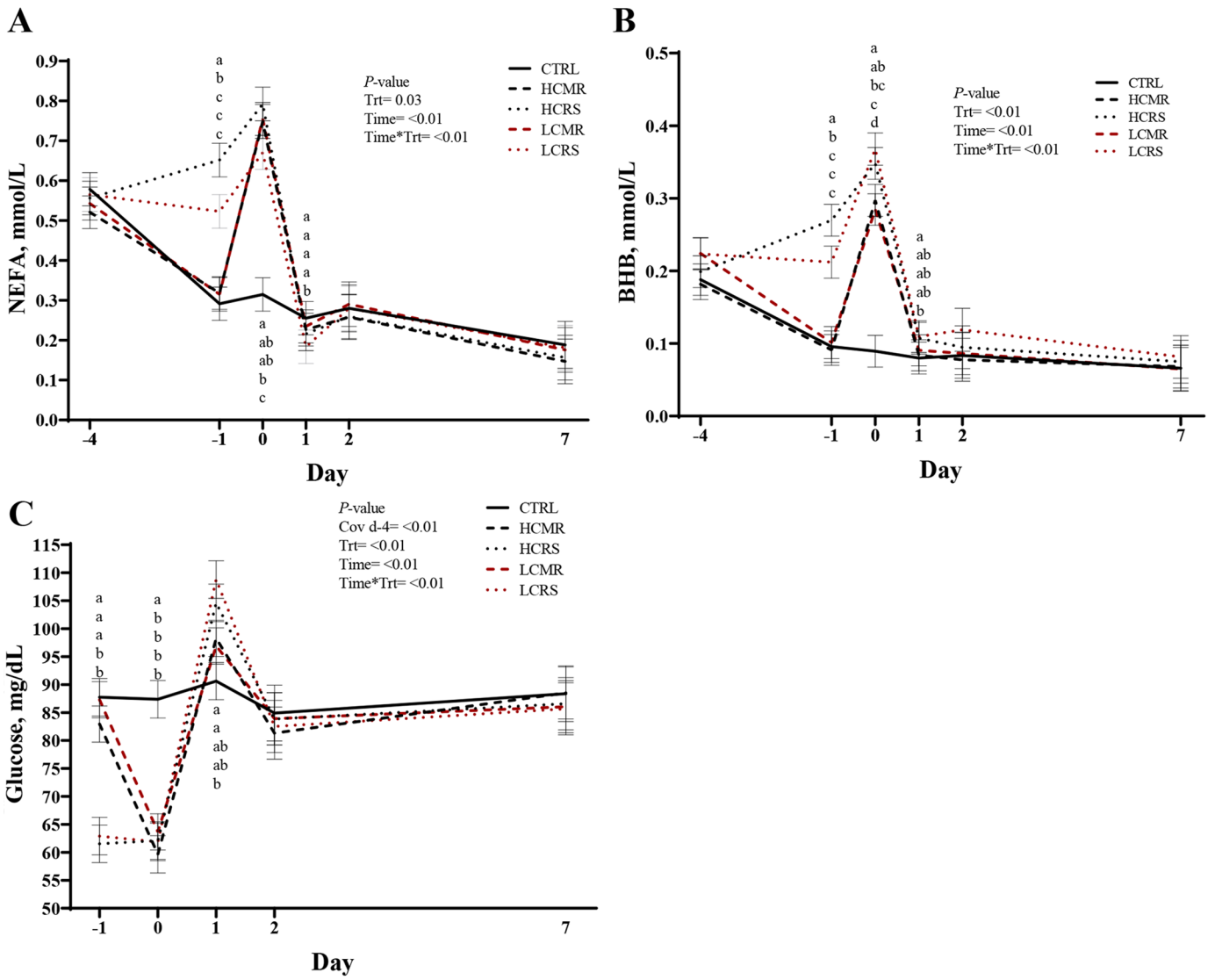

Figure 8. Serum concentration (mean ± SE) of nonesterified fatty acids (NEFA, A), BHB (B), and glucose (C) on d −4, −1, 0, 1, 2, and 7 for NEFA and BHB and d −1, 0, 1, 2, and 7 for glucose in male Holstein calves fed 10 L of colostrum at birth, milk replacer (MR) at the assembly center simulation period, and not transported (CTRL); fed 10 L of colostrum at birth, MR at the assembly center simulation period, and transported for 19 h (HCMR); fed 10 L of colostrum at birth, rehydrating solution (RS) at the assembly center simulation period, and transported for 19 h (HCRS); fed 2 L of colostrum at birth, MR at the assembly center simulation period, and transported for 19 h (LCMR); and fed 2 L of colostrum at birth, RS at the assembly center simulation period, and transported for 19 h (LCRS). Serum concentration of glucose from d −4 was used as a covariate (cov). Different letters within a time point denote differences among treatments ($P < 0.05$); the order of the letters denotes the treatment with the highest value. Trt = treatment.

Biomarkers of Stress and Health Status

No differences were observed in serum cortisol concentration during the assembly center simulation (d −4 −1). However, a time by treatment tendency ($P = 0.10$; Table 2) was observed at d 0 of the study for serum cortisol concentrations being greater for the LCRS compared with the CTRL, HCMR, and LCMR calves, whereas LCRS and HCRS calves had similar concentrations. This increment in cortisol was almost certainly associated with the stress due to transportation having a greater effect in the RS-fed calves. Cortisol is a glucocorticoid hormone involved in the stress response and regulated by the hypothalamic-pituitary-adrenocortical axis. It is the primary hormone used as a biomarker in studies of stress response. Rises in plasma cortisol levels in unweaned transported calves have been previously described (Fazio et al., 2005; Bernardini et al., 2012). In addition, high levels of circulating cortisol are responsible for changes in cytokine levels and, there-

Table 2. Serum concentration (mean ± SE) of cortisol, haptoglobin (Hp), and total protein (TP) in male Holstein calves measured on d −4 and −1 (pre-transport) and d 0, 1, 2, and 7 (post-transport) and on d 0 and 1 for creatine kinase (CK)[1]

	Treatment						*P*-value		
Item[2]	CTRL	HCMR	HCRS	LCMR	LCRS	SEM	Trt	Time	Time × Trt
D −4 and −1									
Cortisol, ng/mL	11.50	12.55	15.21	13.18	14.98	0.129	0.32	—	—
Hp, mg/mL	0.17	0.12	0.14	0.12	0.10	0.025	0.14	—	—
TP, g/dL	5.70[a]	5.56[a]	5.57[a]	4.98[b]	4.96[b]	0.103	<0.001	—	—
D 0, 1, 2, and 7									
Cortisol, ng/mL	9.62	10.72	10.83	10.38	15.05	1.736	0.31	<0.001	0.10
Hp, mg/mL	0.15	0.13	0.15	0.12	0.13	0.018	0.93	<0.001	0.16
TP, g/dL	5.26[a]	5.23[a]	5.04[ab]	4.78[bc]	4.74[c]	0.094	<0.001	<0.001	0.84
CK, IU/L	115.69	118.75	115.60	116.79	120.55	2.736	0.26	<0.001	0.31

[a–c]Values with different superscripts within a row differ with a *P*-value ≤0.05.

[1]Treatments: CTRL = calves fed 10 L of colostrum at birth, MR at the assembly center simulation period, and not transported; HCMR = calves fed 10 L of colostrum at birth, MR at the assembly center simulation period, and transported for 19 h; HCRS = calves fed 10 L of colostrum at birth, rehydrating solution (RS) at the assembly center simulation period, and transported for 19 h; LCMR = calves fed 2 L of colostrum at birth, MR at the assembly center simulation period, and transported for 19 h; and LCRS = calves fed 2 L of colostrum at birth, RS at the assembly center simulation period, and transported during 19 h. Trt = effect of colostrum consumption and feed restriction; Time × Trt = effect of the time by treatment interaction.

[2]Day −4 was used as a covariate in the pre-transport analysis.

fore, in alterations of the immune function (Vegas et al., 2011). For this reason, cortisol has been proposed as a potential biomarker of disease in young calves (Marcato et al., 2018). In addition, and of interest in this investigation, cortisol produced under stressful situations was shown to disrupt intestinal permeability affecting normal digestive functions and decreasing feed intake (Lambert, 2009). However, based on the present results, it could be inferred that chronic exposure to stressful conditions with the consequent rises in cortisol might be necessary to cause visible changes in the immune system or to a gut permeability level. Bernardini et al. (2012) found cortisol levels just slightly higher than normal values (18.4 n*M*; Marcato et al., 2018) in transported calves (37 ± 6 d of age), which returned to normal values after 2 d. In the present study, the average cortisol concentration for all treatments was 14.1 ng/mL (equivalent to 38.8 n*M*), doubling the reference value for calves. In addition, just for the LCRS calves, the average concentration of serum cortisol was 15.05 ng/mL (equivalent to 41.4 n*M*), meaning that when feed restriction was severe and colostrum provision was low, serum cortisol concentrations increased well above reference values.

Surprisingly, serum concentrations of Hp showed no differences between treatments during the assembly center simulation or for post-transport days ($P = 0.77$; Table 2). Levels of Hp in serum are incremented by cytokines in response to several stressors (Tóthová et al., 2008). On the other side, Hp is considered one of the most reliable markers in detecting diseases in calves because it is mainly triggered by pathological damage and has high sensitivity (Marcato et al., 2018; Saco and Bassols, 2023). In the present study, the average concentration of Hp for all treatments was 0.13 mg/mL, which has been proposed as a threshold to differentiate healthy from sick calves of approximately 2 (Yu et al., 2019) and 5 weeks of age (Gånheim et al., 2003). However, in the present study, Hp was not able to differentiate feed-restricted and transported calves from the control. The increase from 0.10 to 0.13 mg/mL observed in LCRS from the assembly center simulation period to the post-transport period may be in relation to the increased number of calves with a score >5 for the same treatment shown in Figure 9 (period by treatment tendency; $P = 0.07$). The sum of health criteria (score >5) for each calf day was also calculated and showed a period by treatment tendency ($P = 0.06$; Table 3; Figure 9). During period 1, no differences were seen between treatments for the sum of health criteria. During period 2, after the assembly center simulation and transportation (d 0 to 7), LCRS calves increased their health scores from period 1 to period 2. From these results, it can be hypothesized that after the feed restriction suffered during the assembly center simulation and transportation, the additive effect of low colostrum consumption and feed restriction based on feeding RS aggravated the calves` condition making them more prone to get sick. Results from the fecal scores showed period by treatment differences ($P < 0.01$; Figure 10), with an increment in the diarrhea score for the LCRS calves on period 2 compared with CTRL, HCMR, and HCRS calves. In addition, the HCMR calves had higher fecal scores during period 1 that decreased by period 2. This reduction in the fecal score in the HCMR calves versus the increase in the LCRS calves could be as-

Table 3. Proportion of male Holstein calves with calf health score parameters greater than 0, measured from d −4 (beginning of the assembly center simulation) to d 7 (end of the study)[1]

	Treatment						*P*-value		
Health score[2]	CTRL	HCMR	HCRS	LCMR	LCRS	SEM	Trt	Period	Period × Trt
Nose	0.09	0.07	0.12	0.06	0.17	0.04	0.46	0.64	0.15
Eye	0.14	0.12	0.16	0.14	0.10	0.04	0.91	<0.01	0.56
Cough	0.04	0.04	<0.01	0.03	0.07	0.02	0.78	0.94	0.98
Fecal[3]	0.17	0.19	0.24	0.22	0.30	0.29	0.43	<0.01	<0.01
Rectal temperature	0.41	0.39	0.34	0.37	0.25	0.07	0.57	0.29	0.11
Score >5[4]	0.24	0.12	0.19	0.24	0.22	0.05	0.35	0.40	0.07

[1]Treatments: CTRL = calves fed 10 L of colostrum at birth, MR at the assembly center simulation period, and not transported; HCMR = calves fed 10 L of colostrum at birth, MR at the assembly center simulation period, and transported for 19 h; HCRS = calves fed 10 L of colostrum at birth, rehydrating solution (RS) at the assembly center simulation period, and transported for 19 h; LCMR = calves fed 2 L of colostrum at birth, MR at the assembly center simulation period, and transported for 19 h; and LCRS = calves fed 2 L of colostrum at birth, RS at the assembly center simulation period, and transported for 19 h. Trt = effect of colostrum consumption and feed restriction. Period = period 1 from the assembly center simulation to transportation (d −4 to d −1) and period 2 from arrival to the end of the study (d 0 to d 7). Period × Trt = effect of the period by treatment interaction.

[2]Health scores greater than 0; the health score "ear" was excluded because of its low incidence.

[3]Fecal score was binary transformed considering 0 = normal feces (scores 0 and 1) and 1 = diarrheic feces (scores 2 and 3).

[4]Score >5 (the sum of health criteria for each calf and day) was categorized binary: if the resulting sum was <5 it was coded as 0, and if it was ≥5 it was considered as 1. The proportion considered 0 = healthy calf (score 0) and 1 = sick calf. Because a score >5 evaluates respiratory disease, the "fecal" score was not considered in the calculation.

sociated with the greater effect of feed restriction and lower colostrum consumption on the health status of the calves.

Measurements of serum CK have been described as a potential biomarker of physical challenges associated with tissue damage, fatigue, and muscle degradation (Marcato et al., 2018). Previous studies have found increments in serum CK activity in young cattle after a period of transportation (Warriss et al., 1995; Knowles, 1999; Averós et al., 2008). These increments in CK are associated with muscle damage and physical exhaustion and, because of this, CK has been considered an indicator of welfare in calves during transportation (Averós et al., 2008). No differences between treatments were observed for serum catalytic concentration of CK in the present study ($P = 0.25$; Table 2), indicating that no substantial muscular damage occurred during transport. In addition, CK serum catalytic concentrations from the present study were within the reference range for calves in the first weeks of age (Knowles et al., 2000). Some differences between previous research and the present study are seen in relation to the origin of the calves and the transport conditions. In the present study, all calves belonged to the same dairy farm and did not go through a process of commingling and mixing with calves from different origins. The stocking density in the truck was lower during the 19 h transportation than in commercial situations, as only 20 calves were transported in a commercial trailer without space limitation in one deck; therefore, plenty of space was available for all calves to lie down during the journey. Finally, the straw bedding provided during transporta-

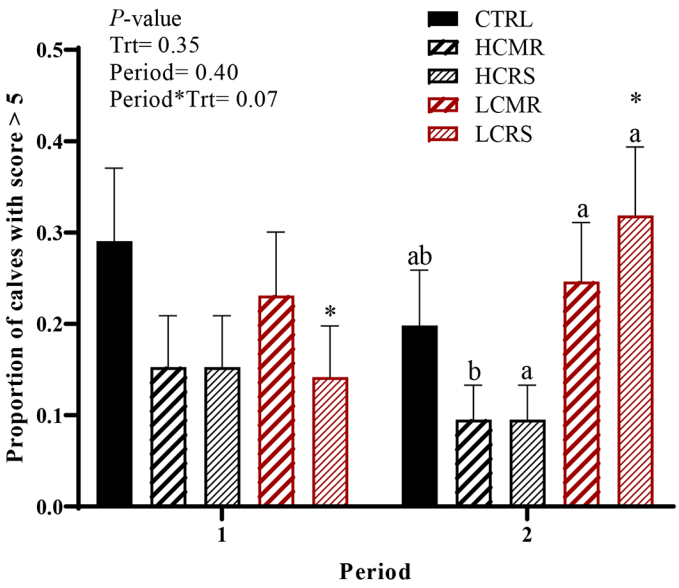

Figure 9. Proportion of calves with a calf health score >5 (± SE) from d −4 to −1 (period 1) and from d 0 to 7 (period 2) of the study in male Holstein calves fed 10 L of colostrum at birth, milk replacer (MR) at the assembly center simulation period, and not transported (CTRL); fed 10 L of colostrum at birth, MR at the assembly center simulation period, and transported for 19 h (HCMR); fed 10 L of colostrum at birth, rehydrating solution (RS) at the assembly center simulation period, and transported for 19 h (HCRS); fed 2 L of colostrum at birth, MR at the assembly center simulation period, and transported for 19 h (LCMR); and fed 2 L of colostrum at birth, RS at the assembly center simulation period, and transported for 19 h (LCRS). Asterisks denote differences among periods. Different letters within a time point denote differences among treatments ($P < 0.05$); the order of the letters denotes the treatment with the highest value. Trt = treatment.

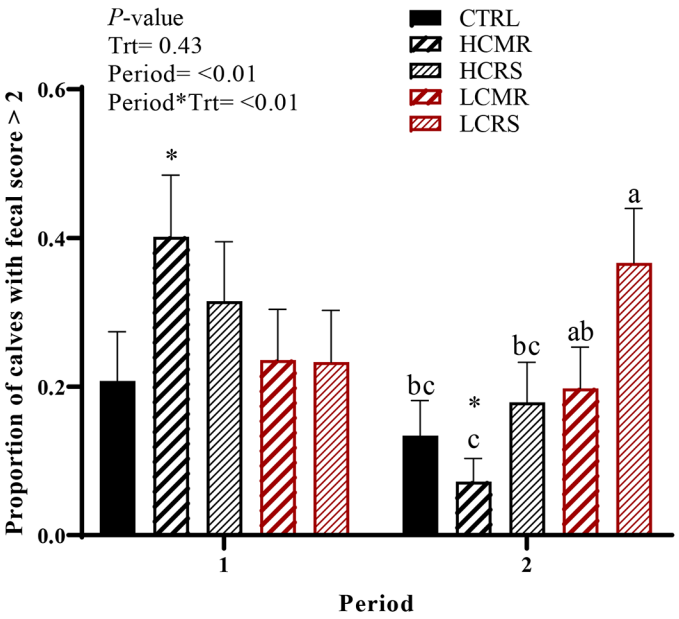

Figure 10. Proportion of calves with a fecal score >2 (± SE) from d −4 to −1 (period 1) and from d 0 to 7 (period 2) of the study in male Holstein calves fed 10 L of colostrum at birth, milk replacer (MR) at the assembly center simulation period, and not transported (CTRL); fed 10 L of colostrum at birth, MR at the assembly center simulation period, and transported for 19 h (HCMR); fed 10 L of colostrum at birth, rehydrating solution (RS) at the assembly center simulation period, and transported for 19 h (HCRS); fed 2 L of colostrum at birth, MR at the assembly center simulation period, and transported for 19 h (LCMR); and fed 2 L of colostrum at birth, RS at the assembly center simulation period, and transported for 19 h (LCRS). Asterisks denote differences among periods. Different letters within a time point denote differences among treatments ($P < 0.05$); the order of the letters denotes the treatment with the highest value. Trt = treatment.

tion was sufficient to cushion calves and avoid bruises. These differences in management might have ameliorated the negative effects of transportation and therefore diluted differences in CK catalytic concentration between treatments.

Greater concentrations in serum TP pre- and post-transport reflect increments in colostral Ig (Hogan et al., 2015), and this is because, in addition to albumins, Ig are the greatest constituents of TP in newborn calves' blood (Elsohaby et al., 2015), allowing them to be used as an estimator of colostrum consumption in calves. Serum TP levels are also indicative of the hydration status of calves (Heller and Chigerwe, 2018), and their concentration increases during a dehydration condition. In the present study, treatment differences were observed for serum TP concentration being greater for CTRL, HCMR, and HCRS calves, compared with LCMR and LCRS calves ($P < 0.01$; Table 2). Because calves did not exhibit signs of dehydration at the time of sampling, the differences observed in TP concentration may be indicative of the amount and quality of colostrum consumed at birth and not an indication of the hydration status of the calves.

Behavior

Finally, behavior results showed time by treatment differences for standing time ($P = 0.05$; Figure 11). At d −3, LCRS and HCRS calves showed longer and shorter standing times, respectively. These differences disappeared by d −2. During transportation on d −1, LCRS continued showing longer standing times, compared with the other treatments. These increments in standing times could reflect the stress originated from hunger in RS-fed calves, compared with the MR-fed ones. However, this was only true for LCRS but not for HCRS calves. On d 0, standing times continued to increase, coinciding with the previously mentioned raise in concentrate intake on arrival at the rearing facility for all transported calves. In addition, by d 1, standing times decreased for all treatments, also coinciding with the reduction of concentrate intake observed for the same day. The RS-fed calves (LCRS and HCRS) showed lower standing times, compared with the other treatments for d 1 (Figure 11). From d 1 to 7, CTRL calves showed longer standing times, compared with the transported calves. However, a decrease in the standing time for the transported calves (HCMR, HCRS, LCMR, and LCRS), compared with CTRL, was observed as a consequence of exhaustion during the periods under feed restriction and transportation. No differences between treatments were observed for the standing duration and standing bouts. The stress derived from hunger during the assembly center simulation and the 19-h transportation not only affected serum concentrations of NEFA, BHB, glucose, and the corresponding losses on BW but also increased standing times, something that was particularly evident for the calves in the LCRS treatments. Again, an additive effect of these 2 factors (colostrum consumption and feed restriction) was observed. Results from the present study agree with results observed in a previous study conducted by our research group, where increments in standing times during a period of fasting and feed restriction in Angus-Holstein male calves that were being fed RS and fasted for 19 h were observed, compared with calves fed MR and fasted for 9 h (Pisoni et al., 2022). Finally, the present study results encourage further investigation into other effects of normal commercial situations (commingling, stocking densities, mixing, physical trauma during loading or unloading, among others) and the role these might have as aggravators of the general conditions of calves on arrival to the rearing facilities.

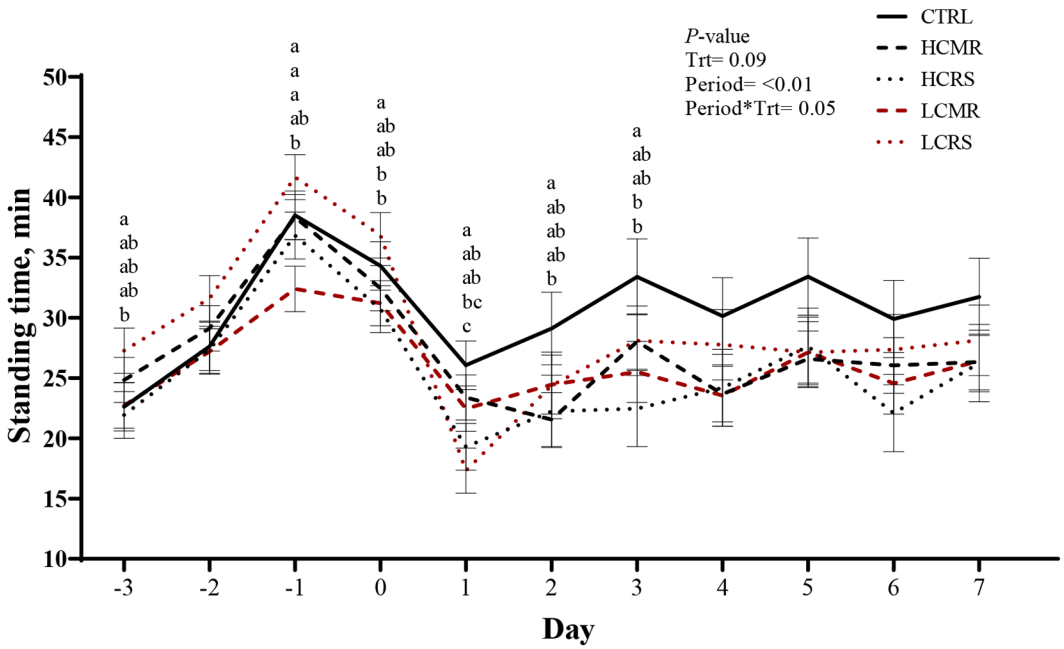

Figure 11. Pendant data loggers' records (mean ± SE) on standing time (min) from d −3 to 7 in male Holstein calves fed 10 L of colostrum at birth, milk replacer (MR) at the assembly center simulation period, and not transported (CTRL); fed 10 L of colostrum at birth, MR at the assembly center simulation period, and transported for 19 h (HCMR); fed 10 L of colostrum at birth, rehydrating solution (RS) at the assembly center simulation period, and transported for 19 h (HCRS); fed 2 L of colostrum at birth, MR at the assembly center simulation period, and transported for 19 h (LCMR); and fed 2 L of colostrum at birth, RS at the assembly center simulation period, and transported for 19 h (LCRS). Different letters within a time point denote differences among treatments ($P < 0.05$); the order of the letters denotes the treatment with the highest value. Trt = treatment.

CONCLUSIONS

The degree of feed restriction negatively affected parameters related to BW and intake recovery, gut permeability, and behavior. Moreover, a synergistic effect between the amount of colostrum consumed at birth and the degree of feed restriction suffered during marketing and transportation was also observed in concentrate intake recovery and biomarkers of energy balance (serum NEFA and BHB) and enterocyte mass (serum citrulline). However, the effect of colostrum consumption and feed restriction on health status and stress and gut permeability biomarkers such as Hp, CK, cortisol, and D-lactate was not so evident. Results from this study showed that it is clear that feeding RS has a severe negative effect on BW losses, but feeding 2 L of MR twice daily also does not cover the requirements of those animals. This practice of feeding low amounts of MR in surplus calves that is common at dairy farms of origin, at assembly centers, or at rearing farms needs further evaluation as improper nutrition has a direct effect on animal health, behavior, and welfare.

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