J. Dairy Sci. 106:8642–8657
https://doi.org/10.3168/jds.2023-23345

Feeding colostrum and transition milk facilitates digestive tract functionality recovery from feed restriction and fasting of dairy calves

M. Tortadès, S. Marti, M. Devant, M. Vidal, F. Fàbregas, and M. Terré*
Department of Ruminant Production, IRTA (Institut de Recerca i Tecnologia Agroalimentàries), 08140 Caldes de Montbui, Barcelona, Spain

ABSTRACT

The objective of this study was to evaluate the digestive tract recovery and metabolism of feeding either bovine colostrum (BC), transition milk (TM), or milk replacer (MR) after an episode of feed restriction and fasting (FRF) in dairy calves. Thirty-five Holstein male calves (22 ± 4.8 d old) were involved in a 50-d study. After 3 d of feeding 2 L of rehydration solution twice daily and 19 h of fasting (d 1 of study), calves were randomly assigned to one of the 5 feeding treatments (n = 7): calves were offered either pooled BC during 4 (C4) or 10 (C10) days, pooled TM during 4 (TM4) or 10 (TM10) days, or MR for 10 d (CTRL) at the rate of 720 g/d DM content. Then, all calves were fed the same feeding program, gradually decreasing MR from 3 L twice daily to 2 L once daily at 12.5% DM until weaning (d 42), and concentrate feed, water, and straw were offered ad libitum until d 50. Citrulline, Cr-EDTA, β-hydroxybutyrate (BHB), and nonesterified fatty acids (NEFA) in serum and complete blood count (CBC) were determined on d −3, 1, 2, 5, and 11 relative to FRF, except BHB and NEFA at d −3. Volatile fatty acids (VFA), lactoferrin (LTF), IgA, and microbiota (Firmicutes to Bacteroidetes ratio and *Fecalis prausnitzii*) were analyzed in feces on d 5 and 11 before the morning feeding. Health scores were recorded daily from d −3 to d 14 as well as d 23 and 30. Feed concentrate, MR, and straw intake were recorded daily, and body weight on d −3, 1, 2, 5, and 11 and weekly afterward. Calf performance, intake, serum Cr-EDTA, CBC, fecal LTF concentrations and microbiota parameters were similar among treatments throughout the study. Serum NEFA concentrations were greater in TM4, TM10 and C10 calves compared with the CTRL ones from d 2 to 11, and after the FRF, serum concentrations of BHB were lower in CTRL calves than in the other treatments, and on d 11, serum BHB concentrations in the long treatments (C10 and TM10) remained greater than those in the shorter ones (C4 and TM4) and CTRL. Serum citrulline concentrations were similar on d −3 and 1 in all treatments, but they were greater in C4, C10, TM4, and TM10 on d 2 and 5, and on d 11 they were only greater in C10 and TM10 than in CTRL calves. Fecal IgA concentrations tended to be greater in C10 than in CTRL, TM4, and TM10 calves, and in C4 and TM10 than in CTRL animals. Fecal propionate proportion was lesser in C10 than in CTRL, TM4, and TM10 calves, while butyrate was greater in C4 and C10 than in TM4 and CTRL calves. The proportion of non-normal fecal scores of C10 fed calves was greater than TM4 and TM10 calves. Results showed that TM and BC may help to recover intestinal functionality, provide gut immune protection, and increase liver fatty acid oxidation in calves after a FRF episode.

Key words: calf, colostrum, feed restriction, transition milk

INTRODUCTION

In the European Union, 4.3 million head of cattle were traded in 2018 among member countries, being the production cycle the reason behind 70% of the transport in cattle over slaughtering (Rossi, 2020). Particularly in the dairy industry, preweaning calves are transported from multiple origins to assembly centers. After that, calves are sold to rearing and fattening farms until they reach the optimal weight to be slaughtered (Pardon et al., 2014; Wilson et al., 2020). Multiple stressors involved during transportation (new environment, feed restriction, regrouping batches, and environmental factors) have been shown to negatively affect calf health, energetic balance, nutritional and immune status (Knowles et al., 1999; Renaud et al., 2018). This makes these animals more likely to suffer infectious diseases caused by bovine respiratory disease and enteric pathogens (*Escherichia coli*, rotavirus, coronavirus, *Salmonella* spp., and *Cryptosporidium parvum*; García et al., 2000; Step et al., 2008; Earley et al., 2017). Although there exist several prophylactic strategies to cope with these infectious processes (e.g., vaccines and farm management) and the impelling need

Received February 6, 2023.
Accepted June 14, 2023.
*Corresponding author: marta.terre@irta.cat

to reduce the antimicrobial resistances, some nutraceutical products of the dairy industry may be considered a helpful alternative to modulate its nutritional and immunological status.

Bovine colostrum (**BC**) and transition milk (**TM**) contain several bioactive substances that might modulate the immune response including immunoglobulins, proinflammatory cytokines, growth factors as well as antimicrobial molecules similar to lactoferrin (**LTF**; Foley and Otterby, 1978; Pakkanen and Aalto, 1997; Hagiwara et al., 2000). Several authors have described how BC and TM from farm surpluses enhanced preweaning calf health (Blum and Hammon, 2000; Berge et al., 2009; Conneely et al., 2014). Milk replacer (**MR**) supplemented with colostrum decreased the incidence of diseases as well as the associated use of antibiotics (Berge et al., 2009; Chamorro et al., 2017). Similar effects had TM on calf health status when colostrum feeding was followed by 4 subsequent feedings of TM (Conneely et al., 2014). In addition, when transition milk feeding was extended until 21 d of calf life, calves improved growth performance and reduced the occurrence of diarrhea (Kargar et al., 2021). In addition, BC has been used as therapeutic and prophylactic measures in human gastrointestinal and respiratory infectious diseases (Ulfman et al., 2018).

Moreover, the non-nutrient factors of BC and TM, also modulate the gastrointestinal tract (**GIT**) microbial population, development, and immune system functionality (Blum, 2006). Until 24 h after birth the small intestine nonselectively absorbs macromolecules which allows direct access to the circulatory system (Staley et al., 1972; Stott et al., 1979). However, after the GIT closure, prolonged colostrum feedings may promote more local benefits (Blum, 2006). In fact, the high amount of biomolecules contained in BC stimulated small intestine cell proliferation and reduced mucosal epithelial cell turnover (Blättler et al., 2001) as well as increased intestinal development in TM fed calves (Van Soest et al., 2022). Also, intestinal microbiota changed with age and feeding program of preweaning calves mainly due to the variable fermentable subtract (carbohydrate and AA) available for bacterial communities (Song, 2018), which produce different microbial fermentation products, such as short chain fatty acids, that may interfere with host metabolism and gut health (Lührs et al., 2002; Tolhurst et al., 2012; Wang et al., 2012).

The effects of BC and TM feeding on immune and nutritional status have been studied after birth, however BC and TM supplementation later in critical stages during the rearing phase have not been evaluated. Considering the negative effect of feed restriction and fasting (**FRF**) period in young calves (3 wk of age) after transportation (Pisoni et al., 2022), the potential benefits of BC and TM and the need to reduce the systematic use of antibiotics, we hypothesized that BC and TM may help to overcome the negative effects of FRF on preweaning calf intestinal integrity, energy metabolism, and health status. Therefore, the objective of the present study was to evaluate the digestive tract recovery and metabolism of feeding either BC, TM, or MR after an episode of FRF on performance, physiological parameters, gastrointestinal permeability, gastrointestinal immune status, microbiota changes and general health indicators.

MATERIALS AND METHODS

The current study was conducted at IRTA Torre Marimon facilities (Caldes de Montbui, Spain) from October to December 2021 and was approved by the Animal Care Committee of the Government of Catalonia (authorization code 11477).

Animals, Treatments, and Feeding

Thirty-five Holstein male calves were born at a commercial farm located in Lleida, Spain (Selergan S.A.) and fed 4 L of colostrum within the first 6 h and 2 L within the first 12 h after birth via esophageal tube. Thereafter, calves were fed 3 L of whole milk twice daily until transportation for 2 h to IRTA facilities (Caldes de Montbui, Spain). Calves entered to the study in 2 different batches of 15 and 20 calves, respectively, with a delay of 2 weeks. At arrival, calves were weighed (22 ± 4.8 d old and 48 ± 6.0 kg of BW), distributed in individual pens (1.20 m x 1.97 m) and vaccinated against Bovine Respiratory Syncytial Virus and bovine Parainfluenza virus type 3 (PI3; Bovilis Intranasal RSP Live; MSD Animal Health, UK).

To simulate calves staying at an assembly center and further transport, calves were fed restrictively 2.5 L of a rehydration solution (Coriosal, Dilus Laboratories, Santa Eugènia de Berga, Spain) at a concentration of 50 g/L twice daily with ad libitum access to water during 3 d, and a subsequent 19 h of fasting, which represents the maximum journey duration allowed by the Regulation (EC) nº 1/2005 (Council of the European Union, 2005) for unweaned calves. This simulation was based on the severe treatment reported by Pisoni et al. (2022). After that, calves were blocked by BW and age, and randomly assigned to one of the 5 feeding treatments (n = 7; established as d 1 of study): calves were fed either pooled BC (**C4**) or TM (**TM4**) twice daily during 4 d and thereafter MR (22.6% CP and 18.5% fat, Trouw Nutrition España S.A., Madrid, Spain) for 6 d (short duration feeding treatments); or BC (**C10**) or

Table 1. Nutrient and bioactive compounds composition of the feed, milk, colostrum, and milk replacer used in this study

Item	Colostrum	Transition milk	Milk replacer	Concentrate	Straw
Gross energy, kcal/kg DM	5,776	5,567	4,831	—	—
Nutrient, % DM					
DM	21.7	16.2	96.0	87.6	90.6
CP	58.5	56.2	22.6	14.8	4.3
NDF	—	—	—	13.6	80.1
ADF	—	—	—	5.2	51.0
EE[1]	23.5	24.7	18.5	5.2	—
Ash	4.6	6.8	7.5	4.1	6.3
Lactose[2]	12.9	20.9	51.4	—	—
Osmolarity, mOsm/kg	377	404	362	—	—
Biomolecules					
IgG, mg/dL	6,864	3,834	<280	—	—
IgA, mg/dL	657	397	2	—	—

[1]EE = Ether extract.

[2]Lactose in milk replacer was determined by difference (100% − CP% − fat% − ash%) based on Quigley et al. (2007).

TM (**TM10**) twice daily for 10 d (long duration feeding treatments); or MR twice daily for 10 d (**CTRL**). To feed the same BC and TM quality to all the calves and feedings, 265 L of BC and 373 L of TM were collected from several farms and stored at −20°C. Then, they were thawed, pooled in a 200 L milk tank, and individually bottled to achieve 720 g of DM content per bottle (one serving) assuming an average of 25.5% DM content in BC and 18.1% DM content in TM as previously measured in BC and TM samples from our laboratory. Finally, bottles were frozen at −20°C until their use. One hour before feeding, bottles were thawed in a water bath at 55°C. Then, the contained liquid was transferred to feeding bottles and brought up to 3 L at 39°C with warm water to assess the same volume as MR. To be able to apply the feeding treatments, animal's caretakers were aware about the treatments, and it might have led to bias in subjective parameters similar to health scores.

All treatments were initially prepared to be fed at the rate of 720 g/d DM content. Afterward, all calves were fed the same feeding program, restricted amounts of MR at 12.5% DM: 3 L twice daily from d 11 to 15, 2.5 L twice daily from d 16 to 22, 2 L twice daily from d 23 to 29, and finally 2L once daily from d 30 to 43 when calves were weaned. Concentrate feed (14.8% CP, 13.6% NDF; Table 1), water, and straw (3.9% CP, 72.6% NDF, 46.2% ADF) were offered ad libitum until d 50 after fasting.

Measurements and Sample Collection

Feed, milk, and straw intake were daily recorded throughout the experiment. Before the morning feeding on d −3, 1, 2, 5, 11, and weekly afterward until the end of the study calves were weighed. Furthermore, blood samples were collected on d −3, 1, 2, 5, and 11 from the jugular vein, using a nonadditive serum and K2 EDTA tubes (BD Vacutainer, Eysins, Switzerland). Serum tubes were centrifuged at 1,500 × *g* at 4°C for 15 min, and serum was obtained and stored at −20°C until further analysis of NEFA, BHB, and citrulline as an intestinal enterocyte mass marker. Whole blood was used for a subsequent hematological analysis. After that a permeability test was performed by giving orally to calves, 0.1 g of Cr-EDTA/kg of BW (Sigma-Aldrich., Merck KGaA, Darmstadt, Germany) dissolved in 100 mL of warm water (Amado et al., 2019). Two hours later a blood sample was collected using nonadditive serum tubes and following the same procedure described above. All blood samples were analyzed on d −3, 1, 2, 5, and 11, except from BHB and NEFA where d −3 was not determined. Fecal samples were collected by rectal stimulation, on d −3, 2, 5, and 11 in 50 mL sterile containers and frozen at −20°C for further LTF, IgA, VFA, microbiota (Firmicutes to Bacteroidetes (**F/B**) ratio and *Fecalis prausnitzii*) and dry matter analysis. Finally, on d 13, 2 h after the morning feeding, a blood sample was collected using sodium heparin tubes (BD Vacutainer, Eysins, Switzerland) and stored at 4°C to perform an in vitro LPS challenge.

Health scores were recorded daily from d −3 to d 14 as well as d 23 and 30. Calf health scoring criteria were rectal temperature, cough, nasal discharge, eye discharge, ear disposition and fecal consistency using the Calf Health Scoring Chart (University of Madison–Wisconsin School of Veterinary Medicine; https://www.vetmed.wisc.edu/farm/svm-dairy-apps/calf-health-scorer-chs/). Clinical parameters were scored on a scale of 0 through 3, representing 0 for a healthy calf and 3 for a severely affected one. Rectal temperature was also analyzed as a continuous variable.

Chemical Analysis

Concentrate feed, straw, MR, BC and TM samples were collected and analyzed for DM (24 h at 103°C; Regulation [EC] nº 152/2009 [European Commission, 2009]), ash (4 h at 550°C; Regulation [EC] nº 152/2009 [European Commission, 2009]), CP (Kjeldahl method; Regulation [EC] nº 152/2009 [European Commission, 2009]), and fat (gravimetry with acid hydrolysis preparation; Regulation [EC] nº 152/2009 [European Commission, 2009]). Furthermore, the concentrate feed sample was analyzed for ADF and NDF (method 973.18; AOAC International, 1996), and BC and TM samples for lactose content using HPLC—Refractive Index (method 984.22; AOAC International, 1995). Gross energy was determined using an adiabatic calorimeter IKA C 2000 basic (IKA, Staufen, Germany) using the method DIN 51900 for MR and an isoperibol calorimeter (Parr 6400, Moline, IL) for TM and BC. Osmolarity was analyzed using a thermistor cryoscope (European Directorate for the Quality of Medicines & HealthCare, 2012).

Bovine colostrum, TM and MR samples were analyzed for IgG concentrations using a single radial immunodiffusion kit (Radial Immunodiffusion Test, Triple J Farms, Bellingham, WA) according to the manufacturer's recommendations. Bovine colostrum and TM were centrifuged for 15 min at 2,000 × *g* at 4°C to remove the fat from the sample. Whey fraction was collected and diluted with PBS 1:10 (BC) and 1:5 (TM). Five microliters of sample was added per well and incubated at room temperature for 24 h until plate reading.

Serum NEFA concentrations were determined by the enzymatic colorimetric method ACS-ACOD-MEHA (NEFA-HR(2) Assay; Fujifilm Wako Chemicals GmbH, Neuss, Germany). Serum BHB concentrations were determined using a kinetic enzymatic method (D-3-hydroxybutyrate [Ranbut], Randox Laboratories Ltd., Crumlin, UK). Serum NEFA and BHB values were measured using a Beckman Coulter AU400 platform (Beckman Coulter, Brea, CA). Serum citrulline concentrations were measured using a spectrophotometric kit (L-Citrulline Kit, Immundiagnostik AG, Bensheim, Germany). Plates were read at 540 nm using Model 680 Microplate Reader from Bio-Rad (Hercules, CA). Serum Cr-EDTA concentrations were determined by inductively coupled plasma-optical emission spectrometry, using an inductively coupled plasma mass spectrometer (ICP-MS, Agilent 7900, Santa Clara, CA). Blood samples were analyzed for hematology using Element HT5 Veterinary Hematology Analyzer (Heska, Loveland, CO). Hematological variables consisted of hematocrit, hemoglobin, number of white blood cells (**WBC**; including neutrophils, lymphocytes and monocytes), number of red blood cells and platelet counts (**PLT**).

The LPS challenge was performed within 6 h after sampling. Before the challenge, lyophilized LPS (*Escherichia coli* O111:B4, ref: L3012–5MG, Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) was diluted in PBS to obtain a 1mg/mL LPS solution. In a laminar airflow cabinet, 2 aliquots with 1 mL of whole blood per calf were stimulated either with 50 μL of PBS (nonchallenged control samples) or with 50 μL of 1 mg/mL LPS solution (challenged samples). Aliquots were incubated for 2 h at 39°C with a rotated shaking. Whole blood samples were centrifuged at 2,000 × *g* for 10 min at 4°C. Serum was collected and stored at −80°C until the determination of TNFα. Serum TNFα concentrations were analyzed using a Bovine TNFα ELISA kit (orb437514, Biorbyt LLC, San Francisco). Samples were diluted 1:10 with the kit's dilution buffer to overcome sample matrix interference. Plates were read at 450 nm using Model 680 Microplate Reader from Bio-Rad (Hercules, CA) and the increment of absorbance between the challenged and nonchallenged samples for each calf was calculated.

All fecal samples were analyzed for DM (103°C during 24 h) and results concerning IgA, LTF, and VFA were expressed on DM basis. Fecal extracts for IgA analysis were prepared as Schaut et al. (2019) described with minor modifications. Briefly, 1 mg fecal samples were washed with 10 mL PBS containing 1% Triton-X. Samples were centrifuged at 4°C for 30 min at 3,200 × *g*. The supernatant was transferred to 15-mL tubes centrifuged for a second time at 12,000 × *g* for 20 min at 4°C to further clarify the samples. Supernatants were filter sterilized using 0.2 μm syringe filters and samples were stored at −20°C until analysis. Fecal extracts as well as BC, TM and MR samples were analyzed for IgA concentration using Bovine IgA ELISA kit from Bethyl Laboratories, Inc. (Cat. No. E11–121, Montgomery, TX). Plates were read at 450 nm using EMS Reader MF V.2.9–0. from Labsystems (Vantaa, Finland).

Fecal LTF was extracted adapting Cooke et al. (2020)'s protocol and LTF concentrations were determined using Bovine Lactoferrin ELISA kit from Bethyl Laboratories, Inc. (Cat. No. E11–126, Montgomery, TX). Plates were read at 450 nm using EMS Reader MF V.2.9–0. from Labsystems (Vantaa, Finland).

Semiquantitative analysis of VFA including acetic acid, propionic acid, isobutyric acid, acid butyric, isovaleric acid, and valeric acid in fecal samples was performed using Gas Chromatography with Flame Ionization Detection (GC-FID; HP 6890 Series II GC

Table 2. Primers, annealing temperature (Tm), and optimized DNA primer concentrations of 16S ribosomal RNA for *Firmicutes*, *Bacteroidetes*, universal, and *Fecalis prausnitzii*

Target group	Primer	Sequence (5′→ 3′)	Tm, °C	Concentration, μ*M*
Bacteroidetes	Bac960F	GTTTAATTCGATGATACGCGAG	60	0.50
	Bac1100R	TTAASCCGACACCTCACGG	60	0.50
Firmicutes	Firm934F	GGAGYATGTGGTTTAATTCGAAGCA	60	0.50
	Firm1060R	AGCTGACGACAACCATGCAC	60	0.50
Universal	926F	AAACTCAAAKGAATTGACGG	60	0.50
	1062R	CTCACRRCACGAGCTGAC	60	0.50
Fecalis prausnitzii	PrausF480	CAGCAGCCGCGGTAAA	53	0.50
	PrausR631	CTACCTCTGCACTACTCAAGAAA	53	0.50

System, Agilent, Santa Clara, CA) with an HP-5 capillary column (P/N:19091J-413, 30 m x 0.32 mm × 0.25 μm, Agilent, Santa Clara, CA).

To quantify the F/B ratio and *Fecalis prausnitzii*, the DNA of each fecal sample was extracted using Stool DNA Isolation Kit (Norgen Biotek, Thorold, ON, Canada) according to the manufacturer's instructions with minor modifications. The changes consisted of adding an extra centrifugation step (16,100 × *g* for 2 min at 4°C) before binding the DNA to the column and discarding the pellet as well as eluting the DNA with 50 μL of elution buffer. Real-time PCR was performed using specific primers assembled from Yang et al. (2015) for Firmicutes and Bacteroidetes, Schwiertz et al., (2010) for *Fecalis prausnitzii* and Takai and Horikoshi (2000) for prokaryotic universal rDNA (referred in this article as universal; Table 2). For these quantifications, a total reaction volume of 20 μL was used, containing 10 ng of DNA, 10 μL of SYBR Green (TaKaRa Bio Inc., Kusatsu, Japan), and the optimized primer concentration for each target (Table 2). The PCR reactions consisted of an initial denaturing cycle of 10 min at 95°C, followed by 40 cycles of 10s at 95°C (denaturation), 30s at 60°C for Firmicutes, Bacteroidetes and universal, and 30 s at 53°C for *Fecalis prausnitzii* (annealing; temperature defined at Table 2) and a final extension of 10 min at 72°C. Bacteria quantification was evaluated using the threshold cycle (**Ct**) values, defined as the number of PCR cycles over which product amplification occurs. Therefore, the greater the Ct value, the lower the concentrations of fecal bacteria.

To obtain the F/B ratio, the average Ct value obtained from each primer pair was transformed into a percentage using the following formula (Yang et al., 2015):

$$X = \frac{\left(Eff.Spec\right)^{CtSpec}}{\left(Eff.Univ\right)^{CtUniv}} \times 100,$$

where Eff.Univ is the calculated efficiency of the universal primers (2 = 100% and 1 = 0%) and Eff.Spec refers to the efficiency of the taxon-specific primers. CtUniv and CtSpec are the Ct values registered by the thermocycler. All fecal samples were analyzed with the quantitative PCR assay, and the Ct values were used to calculate the proportion of bacterial taxa in the feces. Then, the ratio between the Firmicutes and Bacteroidetes proportion was calculated.

Statistical Analyses

Performance, Blood, and Fecal Parameters. The study design was a randomized complete balanced design with a covariance adjustment. A power analysis was conducted to determine the experimental units (calf) needed. The type I error rate (α) was 0.05, and the power (1 − β) was set at 80%. The concentration of citrulline was considered our primary outcome based on the differences in citrulline between the severe and control treatment in Pisoni et al. (2022). Data were analyzed using the MIXED procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC) with repeated measures. The statistical model for performance, blood, and fecal parameters and rectal temperature accounted for the random effect of calf as well as the fixed effects of treatment, time and their interaction. Initial BW and batch were included as covariate and batch, respectively, in all the parameters as well as d −3 values in the performance ones. Covariates were removed when they were not significative ($P < 0.05$). Performance parameters and rectal temperature were summarized in 3 periods consisting of from d −3 to −1 (pretreatment period), from d 1 to d 10 (treatment period) and from d 11 to the end of the study (posttreatment period). The first-order autoregressive covariance matrix, the spatial power and the unstructured covariance matrix were tested according to the time points, and the Kenward-Roger degrees of freedom were used based on the lower Bayesian information criterion value. Data were tested for normality using the Shapiro-Wilk test. WBC, PTL, Neutrocytes, BHB, NEFA, Cr-EDTA, IgA, and LTF data were transformed to logarithm to achieve normal distribution. One observation was removed for hemo-

gram analysis because it was an outlier (Mohri et al., 2007) and we suspected of a detection error. Initial BW and age were analyzed using the MIXED procedure of SAS with treatment as a fixed effect and batch was included as a block. Differences were declared significant at $P \leq 0.05$, and trends were discussed at $0.05 < P \leq 0.10$ for all models. Reported means and SEM in the figures were obtained from the output of SAS as well as the P-values except for the transformed data where P-values came from the normalized data. Correlations between serum NEFA and BHB concentration at each sampling day were assessed using Pearson correlation coefficient calculated using JMP software, version 16 (SAS Institute Inc., Cary, NC).

Calf Health Scores. Individual health criteria scores were categorized binarily considering 0 = healthy calf (score 0) and 1 = unhealthy calf (score 1 to 3) for the statistical analysis. Calf general health score (the overall sum of health criteria score for each calf and day) were categorized binarily as follows: if the resulting sum was < 5 it was codified as 0, and if it was ≥ 5 it was considered as 1. Data were organized in 3 periods to fit the model: from d −3 to −1 (pretreatment period), from d 1 to 10 (treatment period) and from d 11 to the end (posttreatment period). Either the binary general or the individual health criteria scores were analyzed using the PROC GLIMMIX procedure of SAS using a cumulative logit link function and assuming a binary distribution. The statistical model accounted for the fixed effects of treatment, period, and their interaction as well as calf as a random effect. Batch was included as a block.

RESULTS

Intake and Performance Parameters

Initial age and BW values on the pretreatment period (d 3 to −1) before applying the FRF were similar among the different treatment groups (Table 3). During the pretreatment period, animals lost an average of 2.5 kg BW because of the FRF. After, during the treatment and posttreatment periods, calves increased their BW and maintained their ADG similarly among the different feeding treatments. During the application of the feeding treatments (from d 1 to 10), no differences in concentrate feed intake were observed among treatments, but all calves increased concentrate feed intake on d 1 followed by a drastic decline the following days to overcome the FRF. Milk DM intake in TM4, C4, and TM10 calves was lower ($P < 0.001$) than in CTRL calves, and the greater amount of milk refusals was observed in C10 calves compared with the other treatments. All liquid feeds, BC, TM, and MR had similar osmolarity (Table 1). Despite the differences in DM intake; protein and energy intakes were greater ($P < 0.001$, Table 3) in BC and TM treatments than in CTRL calves, being C10 and TM10 diets richer in calories and protein content than the others. Finally, DM intake increased during the posttreatment period (from d 11 to the end) without differences among treatments (Table 3). No differences were observed in feed efficiency (**FE**) in the pretreatment and treatment periods, but FE differed among treatments during the posttreatment period. Long duration treatments (C10 and TM10) had greater ($P < 0.05$) FE than CTRL, TM4 and C4 the week after the end of the feeding treatments (d 11 to 16), and they were similar afterward.

Blood Parameters

Energy Balance. Serum NEFA concentrations were lower ($P < 0.05$, Table 4) in CTRL compared with TM4, TM10, and C10 calves from d 1 to 11. Although no differences were observed in all treatments, on d 1, after the FRF, serum concentration of BHB was greater ($P < 0.001$, Figure 1) than that on d 2. On d 5, serum concentration of BHB was lower ($P < 0.001$) in CTRL calves compared with the other treatments, and on d 11, serum BHB concentration in the long treatments (C10 and TM10) remained greater than that in the shorter ones (C4 and TM4) and CTRL.

Biomarkers of Intestinal Barrier Functionality. Although no differences among treatments were detected on serum Cr-EDTA concentrations, serum Cr-EDTA concentrations increased ($P < 0.001$, Table 4) during the FRF in all treatments, having the greatest serum Cr-EDTA concentrations peak on d 1 followed by a gradual decrease until d 11. Serum citrulline concentrations on d −3 and d 1 were similar in all treatments. Once treatments were applied (d 2), serum citrulline concentration rose ($P < 0.01$; Figure 2) in all treatments except for the CTRL. At the end of the short treatment application (d 5), C4 and C10 calves had greater serum citrulline concentration than the CTRL treatment. Finally, when the application of the long treatments finished (d 11), serum citrulline concentrations tended ($P = 0.06$) to be greater in C10 and TM10 calves than in the CTRL, TM4 and C4 ones; and the short treatments (TM4 and C4) had similar serum citrulline concentrations than CTRL.

Biomarkers of Immune Status. None of the hematological parameters showed differences among treatments. Hemogram values changed ($P < 0.05$, Table 4) during the sampling days except for neutrophils that maintained their count. During the FRF period, all he-

Table 3. Least square means of intake and performance parameters by period of preweaning calves

Item	Treatment[1]					SEM	*P*-value[2]		
	CTRL	TM4	TM10	C4	C10		T	Time	T × time
Pretreatment period (d −3 to 1)									
Initial age, d (d −3)	21.2	22.4	22.2	22.1	21.9	0.81	0.87	—	—
Initial BW, kg (d −3)	48.0	47.3	47.8	48.2	48.1	2.22	1.00	—	—
Rehydrating solution, g of DM/d	250	246	246	248	247	2.9	0.87	0.16	0.62
ADG, kg/d	−0.82	−0.85	−0.73	−0.89	−0.87	0.124	0.90	—	—
Treatment period (d 1 to 10)									
Pretreatment BW, kg (d 1)	45.5	44.8	45.6	45.5	45.5	2.15	1.00	—	—
Milk/colostrum,[1] g of DM/d	720[a]	696[b]	691[b]	693[b]	647[c]	5.9	<0.0001	<0.0001	<0.0001
Concentrate, g of DM/d	225	194	232	212	181	23.4	0.51	<0.0001	0.73
Total DMI, g of DM/d	946[a]	891[ab]	923[a]	906[a]	828[b]	25.7	0.02	<0.0001	0.82
Milk/colostrum[3] GE intake, kcal/d	3478[d]	3560[cd]	3847[a]	3595[c]	3735[b]	32.2	<0.0001	<0.0001	<0.0001
Milk/colostrum[3] CP intake, g/d	163[d]	247[c]	388[a]	250[c]	378[b]	2.9	<0.0001	<0.0001	<0.0001
Milk/colostrum[3] lactose intake, g/d	370[a]	276[b]	145[d]	255[c]	83[e]	1.7	<0.0001	<0.0001	<0.0001
ADG, kg/d	0.73	0.68	0.66	0.72	0.47	0.067	0.07	0.84	0.50
FE,[2] kg/kg DM	0.77	0.76	0.74	0.79	0.57	0.068	0.16	0.17	0.51
Post-treatment period (d 11 to d 50)									
Post-treatment BW, kg (d 11)	52.7	51.7	52.1	52.8	50.2	2.32	0.93	—	—
Final BW, kg (d 50)	90.4	88.7	91.1	86.1	88.9	4.44	0.94	—	—
Milk replacer, g of DM/d	440	438	440	440	440	1.0	0.46	<0.0001	0.62
Concentrate, g of DM/d	1,472	1,417	1,510	1,243	1,431	75.0	0.11	<0.0001	0.89
Straw, g of DM/d	65	72	73	61	72	9.2	0.86	<0.0001	0.33
Total DMI, g of DM/d	1903	1,851	1947	1,669	1,867	80.5	0.14	<0.0001	0.88
ADG, kg/d	0.94	0.93	0.98	0.83	0.97	0.045	0.13	<0.0001	0.53
FE,[4] kg/kg DM	0.52	0.53	0.55	0.52	0.56	0.019	0.43	<0.0001	0.048

[a–e]Within a row, least squares means without a common superscript differ ($P < 0.05$) among treatments.

[1]CTRL = milk replacer (MR) twice daily for 10 d; TM4 = transition milk (TM) twice daily during 4 d and thereafter MR for 6 d; TM10 = TM twice daily for 10 d; C4 = bovine colostrum (BC) twice daily during 4 d and thereafter MR for 6 d; C10 = BC twice daily for 10 d.

[2]T = effect of feeding treatment; time = day of study; T × time = effect of the time by treatment interaction.

[3]According to the established treatment: CTRL, TM4, TM10, C4, C10.

[4]FE = Feed efficiency.

matological parameters, except WBC and neutrophils, increased from d −3 to 1 (data not shown). Then, from d 2 to 11 (treatment period) all hematological parameters gradually decreased. In addition, the production of TNFα did not differ ($P = 0.46$, Table 4) among blood cells of the different treatments after an LPS challenge.

Fecal Parameters

Gastrointestinal Biomarkers of Immune Status. No differences among treatments were detected in fecal LTF concentrations, but they decreased ($P < 0.05$; Table 5) from d 5 to 11 in all treatments. However, fecal IgA differed among feeding treatment and time (Figure 3). Before the application of the feeding treatments (d −3), all calves had similar fecal IgA concentrations. On d 5, all calves increased fecal IgA concentrations except for CTRL calves. Finally, at the end of the feeding treatments application (d 11), TM10 and C10 (long duration treatments) had greater ($P < 0.05$; Figure 3) fecal IgA concentrations than CTRL and TM4 treatments, being C10 feeding treatment above all. In addition, analyses of IgA indicated that BC was 40% richer in IgA content than TM, whereas MR contained less than 0.6% of IgA determined in BC and TM.

Gut Microbiota and Microbiota Metabolites. To study the gastrointestinal microbiome and its interaction with the feeding treatment, fecal VFA, the F/B ratio and the quantification of *Fecalis prausnitzii* were determined. There were no differences in total fecal VFA concentrations among treatments. However, the molar percentage of fecal propionate was lower ($P < 0.05$, Table 5) in C10 than in CTRL, TM4 and TM10 calves, and similar to C4 calves. The proportion of fecal butyrate was greater ($P < 0.05$, Table 5) in C4 and C10 calves than in CTRL and TM4 ones, as well as it decreased ($P < 0.01$) from d 5 to 11 in all treatments. The proportion of fecal acetate tended to be greater ($P = 0.07$, Table 5) in TM4 and CTRL compared with TM10 calves, but it increased ($P < 0.05$) from d 5 to 11 in all treatments.

The F/B ratio, the *Fecalis prausnitzii*, and the bacterial taxons did not differ among treatments, but *Fecalis prausnitzii* specific Ct values increased from d 5 to d 11 ($P < 0.01$, Table 5). The overall mean relative abundance of Firmicutes and Bacteroidetes were 34.15% and 65.5%, respectively.

Table 4. Least squares means of energy balance, intestinal permeability, hemogram, and immune parameters in blood on d −3 to 10 relative to feed restriction and fasting (FRF) of preweaning calves

	Treatment[1]						*P*-value[2]		
Item	CTRL	TM4	TM10	C4	C10	SEM	T	Time	T × time
Energy balance parameters									
BHB,[3,4] mmol/L	0.12[b]	0.18[a]	0.17[a]	0.17[a]	0.18[a]	0.012	<0.0001	<0.0001	<0.0001
NEFA,[3,4,5] mmol/L	0.31[c]	0.37[a]	0.33[ab]	0.31[bc]	0.31[ab]	0.023	0.02	<0.0001	0.41
Intestinal barrier functionality parameters									
Citrulline, μmol/L	37.7[c]	42.2[bc]	51.7[a]	50.6[ab]	48.7[ab]	3.26	0.01	<0.0001	<0.01
Cr-EDTA,[7] mg/L	1.00	0.98	1.21	0.99	1.08	0.116	0.81	<0.0001	0.74
Hemogram parameters									
Hematocrit, %	33.37	30.65	29.38	33.76	31.38	2.032	0.51	<0.0001	0.71
Hemoglobin, g/dL	10.82	10.05	9.46	10.97	10.23	0.728	0.59	<0.0001	0.54
RBC,[6] $\times 10^{12}$/L	9.32	8.85	8.71	9.27	9.12	0.475	0.87	<0.0001	0.47
WBC,[7] $\times 10^{9}$/L	9.58	10.88	10.36	9.77	10.13	0.878	0.83	<0.0001	0.54
Neutrophiles,[3] $\times 10^{9}$/L	4.49	5.14	4.78	4.80	5.08	0.602	0.87	0.008	0.79
Lymphocytes, $\times 10^{9}$/L	4.78	5.40	5.26	4.66	4.75	0.415	0.63	<0.0001	0.27
Monocytes, $\times 10^{9}$/L	0.25	0.28	0.26	0.26	0.23	0.023	0.71	<0.0001	0.04
Platelets,[3] $\times 10^{7}$/L	8.82	8.68	10.23	9.52	9.07	0.911	0.88	<0.0001	0.78
Immune parameters									
TNFα, increase of absorbance	0.011	0.020	0.020	0.006	0.009	0.0067	0.46	—	—

[a–c]Within a row, least squares means without a common superscript differ ($P < 0.05$) among treatments.

[1]CTRL = Milk replacer (MR) twice daily for 10 d; TM4 = Transition milk (TM) twice daily during 4 d and thereafter MR for 6 d; TM10 = TM twice daily for 10 d; C4 = Bovine colostrum (BC) twice daily during 4 d and thereafter MR for 6 d; C10 = BC twice daily for 10 d.

[2]T = effect of feeding treatment; time = day of study; T × time = effect of the time by treatment interaction.

[3]*P*-values were obtained from the logarithm transformed data.

[4]Least squares means were obtained from d 1 to d 10 relative to FRF.

[5]NEFA = nonesterified fatty acids.

[6]RBC = Red blood cell.

[7]WBC = White blood cell.

Calf Health Scores

The general proportion of animals considered ill using the binary scale (being 0 = healthy calf and 1 = unhealthy calf) did not differ among treatments. The binary general score indicated that during the treatment period (d 1 to 10) calves were less healthy ($P < 0.001$, Table 5) than during the pretreatment (d −3 to 1) and posttreatment period (d 11 to the end). Moreover, no differences in individual health criteria scores were found among treatments (data not shown) except for the fecal scores, which varied throughout the experiment (Figure 4). During the pretreatment period, the fecal proportion of non-normal feces was similar among treatments, but once the feeding treatments were applied (treatment period, from d 1 to 10) fecal scores increased in all the treatments except from TM4. The proportion of non-normal fecal scores of C10 fed calves was greater ($P < 0.05$; Table 5) than the ones fed with TM (TM4 and TM10). During period 3 (from d 11 to 50), C10 calves had a greater fecal proportion of non-normal feces than TM4 ones, while C4, TM10, and CTRL did not differ between C10 and TM4. No differences in mean rectal temperature were found among treatments.

DISCUSSION

In the present study different performance traits, blood parameters, fecal biomarkers, and health scores were used to evaluate recovery of feeding calves either BC, TM, or MR after an episode of FRF. Until now, several authors described how FRF associated with transport negatively affected calf energy balance (Knowles et al., 1999), immunity (Marcato et al., 2020), health status (Renaud et al., 2018), and their intestinal barrier integrity (Zhang et al., 2013).

One of the main findings in this study was that FRF and feeding treatments affected serum BHB and NEFA concentrations. During the prolonged FRF period, calves underwent to a negative energy balance status and NEFAs were released from adipose tissue to the blood due to the insufficient intake of glucose-precursors. Consequently, calves were unable to metabolize the increasing NEFA concentration to triacylglycerol through the glucose-depending Krebs's cycle, and the cycle precursor acetyl-CoA was accumulated (Adewuyi et al., 2005). Then, acetyl-CoA was completely oxidated into ketonic bodies such as BHB. In our study, serum BHB and NEFA concentrations increased twice after the FRF period (d 1) compared with expected values for

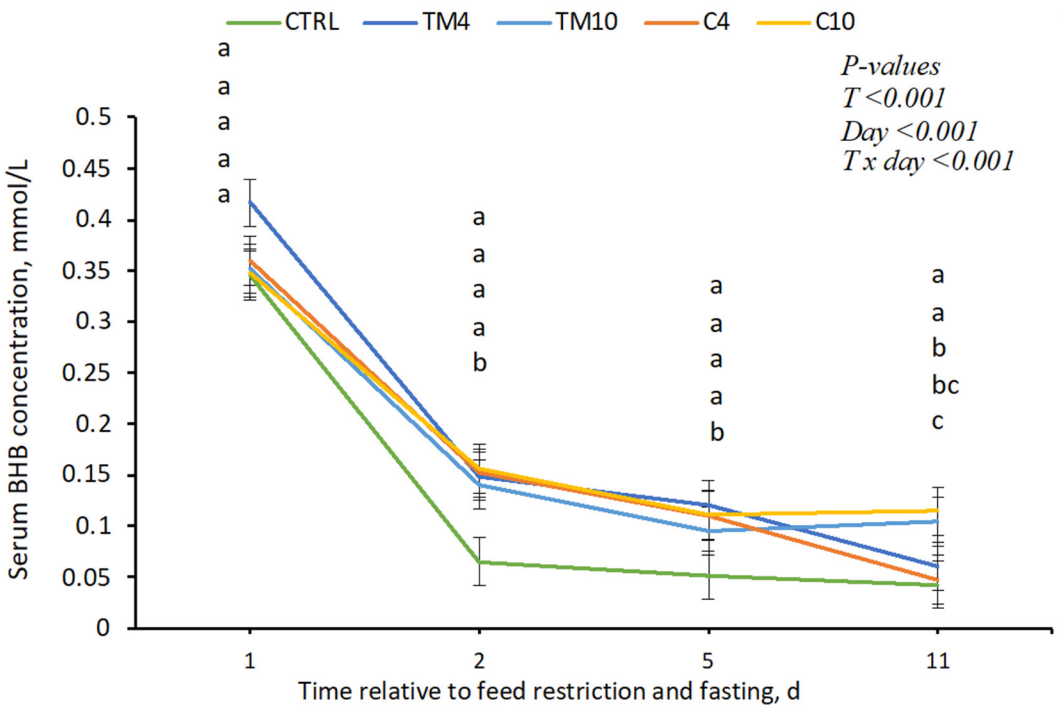

Figure 1. Serum BHB evolution by experimental day after a feed restriction and fasting period in preweaning calves fed either milk replacer (MR) twice daily for 10 d (CTRL), transition milk (TM) twice daily for 4 d and thereafter MR for 6 d (TM4), TM twice daily for 10 d (TM10), bovine colostrum (BC) twice daily during 4 d and thereafter MR for 6 d (C4) or BC twice daily for 10 d (C10) from d 1 to 10 of study. Letters (a–c) within the same measurement point indicate that least squares means without a common superscript differ ($P < 0.05$) among treatments. Letters represent the treatments in order they appear in each sampling point.

this age reported in Knowles et al., (2000), which was also described in Pisoni et al. (2022) for the severe FRF treatment. In fact, during this period our calves lost an average of 2.5 kg of BW, which may indicate the need of calves to mobilize body fat reserves into NEFA (Table 3). After that, all calves recovered their age-expected normal serum NEFA concentrations 24 h after the FRF period, suggesting a similar decrease of the need of fat mobilization in all treatments when they had access to feed. However, BC and TM fed calves had still greater serum BHB concentrations than CTRL calves on d 2 and 5, indicating a possible effect of the energy source of feeds on serum BHB levels recovery. Calves in CTRL groups received MR, which was richer in lactose and poorer in fat content in contrast to BC and TM (Table 1), and it is consistent in the literature on the ketogenic effect of rich-fat diets (Wulff Helge, 2002; Vidali et al., 2015). This hypothesis was reinforced by the values of BHB serum concentration on d 11, because when short treatments (C4 and TM4) fed calves changed their diet to MR, serum BHB concentration decreased. Although we expected a similar evolution of NEFA and BHB, because feeding calves high-fat MR also increased NEFA concentrations (Kuehn et al., 1994; Echeverry-Munera et a., 2023), and it may explain the lower NEFA concentration in CTRL calves, serum NEFA concentration did not follow the same pattern after FRF as BHB. Although in the present study, BHB and NEFA were correlated (0.62, 0.40, 0.67, 0.35, in d 1, 2, 5, and 11, respectively, $P < 0.05$) at the different sampling points, the correlations were weaker at d 2 and 11 compared with d 1 and 5, suggesting differences in the hepatic metabolism. These differences might be attributed to individual variation to adapt to the different diet changes during the treatment period, but it is difficult to elucidate with the present data, and further studies would be needed to confirm and understand these differences.

In the literature, Welboren et al. (2021) showed that calves fed at the same DM level with a high-fat MR (39.9% lactose and 24.6% crude fat) had greater ADG than those fed a high-lactose MR (46.1% lactose and 18% crude fat) due to a greater energy intake in high-fat fed calves. However, calves fed ad libitum a high-lactose/low-fat MR (44% lactose and 17% fat) had greater ADG than those fed a high-fat MR (37% lactose and 23% fat) during the preweaning period because they were able to regulate their intake according to the caloric density of the diet (Echeverry-Munera et al., 2021). In our study, differences in milk

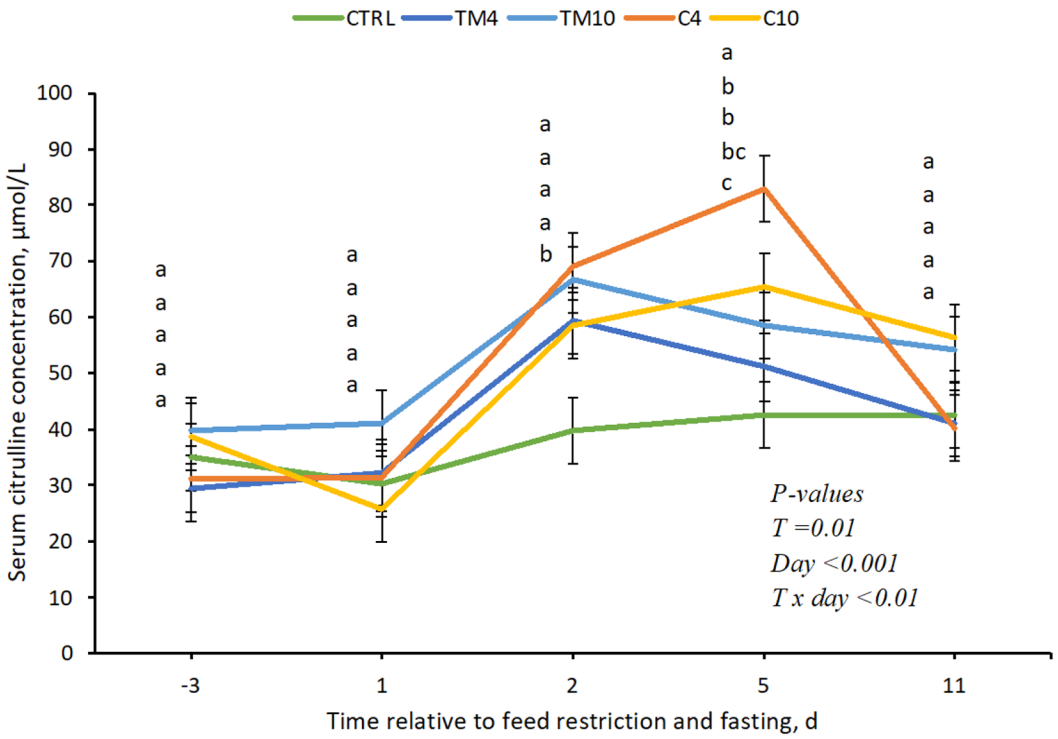

Figure 2. Serum citrulline evolution by experimental day before and after a feed restriction and fasting period (from d −3 to −1) in preweaning calves fed either milk replacer (MR) twice daily for 10 d (CTRL), transition milk (TM) twice daily during 4 d and thereafter MR for 6 d (TM4), TM twice daily for 10 d (TM10), bovine colostrum (BC) twice daily during 4 d and thereafter MR for 6 d (C4) or BC twice daily for 10 d (C10) from d 1 to 10 of study. Letters (a–c) within the same measurement point indicate that least squares means without a common superscript differ ($P < 0.05$) among treatments. Letters represent the treatments in order they appear in each sampling point.

DM intake were due to the lower DM content of TM and BC obtained from the pooled samples (**Table 1**) compared with the assumed DM content when bottled the individual feedings (25.5% DM content in BC and 18.1% DM content in TM). In addition, BC and TM fed calves still consumed more energy and protein than the MR fed ones, without differences in performance among treatments, this may be explained partially, due to the short duration of the feeding treatments, or the low number of animals per treatment used. The FRF period also affected concentrate feed intake. On d 1, after a 3-d feed restriction when calves had access to a concentrate feed, they increased their concentrate feed intake. However, the day after, calves decreased concentrate feed intake. In a similar study, Pisoni et al. (2022) also observed this concentrate consumption pattern after a FRF period. This may be due to a transient subclinical ruminal acidosis for the sudden concentrate feed consumption (Pederzolli et al., 2018) and or to gut transient functionality loss (Pisoni et al., 2022).

In our study, hemogram parameters, health scores and cytokine TNFα were used to assess the general health status of animals. Hematology mean values obtained were within the corresponding age range (Mohri et al., 2007), when references were found, or the adult reference range for cattle (Radostits et al., 1994) when no references values were found for these young age, except for a mild neutrophilia and thrombocytosis found in all treatments. During FRF calf hemogram values suggested worse calf health, because of the increase in hematocrit, lymphocytes, and platelets, probably indicating a mild dehydration (Roland et al., 2014), or/and reactive thrombocytosis to stress (Roland et al., 2014). In addition, the increase in lymphocytes could be also related to an innate immune response to vaccination applied at calf arrival to our facilities (Ellis, 2017). Furthermore, the proportion of animals with a bad general health score increased from the FRF period to the treatment period, whereas it decreased afterward. The general health status and hemogram recovery within the first 10 d of study may be related to the lack of differences in the LPS challenge among treatments. Apart from a decrease in fecal consistency of BC fed calves during the treatment period (which will be discussed later), neither respiratory nor rectal temperature was affected by the feeding treatments, which agrees with Berge et al. (2009) and Chamorro et al. (2017) health studies related to feeding MR supplemented with colos-

Table 5. Least squares means of fecal immune and microbiota biomarkers of preweaning calves on d 5 and 11, and health scores from d −3 to d 14, 23, and 30 of study

	Treatment[1]						*P*-value[2]		
Item	CTRL	TM4	TM10	C4	C10	SEM	T	Time	T × time
Immune biomarkers									
IgA,[5] μg/mg of DM feces	7.77	9.66	9.67	16.50	21.96	4.814	0.05	<0.001	0.01
Lactoferrin,[5] ng/mg of DM feces	226.5	207.4	227.8	213.9	263.3	26.75	0.51	0.02	0.22
Microbiota biomarkers									
Total VFA, μmol/g of DM feces	426	377	467	350	412	49.5	0.50	0.31	0.33
Molar % of VFAs									
Acetate	56.7	57.2	49.0	53.4	52.7	2.15	0.07	<0.01	0.88
Propionate	25.1^{a}	24.1^{a}	24.3^{a}	22.9^{ab}	20.1^{b}	1.11	0.03	0.19	0.27
Butyrate	11.5^{bc}	11.3^{bc}	13.1^{ab}	13.5^{a}	13.8^{a}	0.69	<0.05	<0.01	0.77
F/B Ratio, Ct	0.56	0.59	0.62	0.47	0.57	0.060	0.49	0.59	0.47
Fecalis prausnitzii,[5] Ct	24.7	24.8	25.3	25.6	26.5	1.20	0.83	<0.01	0.96
Bacterial taxa relative abundance, %									
Firmicutes	36.8	35.7	34.6	30.1	34.7	2.11	0.24	0.41	0.48
Bacteroidetes	70.8	62.7	61.7	65.2	65.2	3.37	0.38	0.60	0.20
Health scores									
General score[3]	0.13	0.17	0.07	0.13	0.19	0.058	0.58	<0.001	0.78
Fecal score[4]	0.19^{b}	0.15^{b}	0.19^{b}	0.20^{ab}	0.42^{a}	0.065	0.08	<0.001	0.02
Rectal temperature, °C	38.7	38.5	38.5	38.6	38.5	0.08	0.33	<0.001	0.99

[a–c]Within a row, least squares means without a common superscript differ ($P < 0.05$) among treatments.

[1]CTRL = Milk replacer (MR) twice daily for 10 d; TM4 = Transition milk (TM) twice daily for 4 d and thereafter MR for 6 d; TM10 = TM twice daily for 10 d; C4 = Bovine colostrum (BC) twice daily during 4 d and thereafter MR for 6 d; C10 = BC twice daily for 10 d.

[2]T = effect of feeding treatment; time = day of study for all the parameters except for the health score where time units were period; T × time = effect of the time by treatment interaction.

[3]General health score (the overall sum of health criteria for each calf and day) was categorized binarily: if the resulting sum was <5 it was codified as 0, and if it was ≥5 it was considered as 1. Finally, the proportion contemplates 0 = healthy calf (score 0) and 1 = unhealthy calf.

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

[5]*P*-values were obtained from the logarithm transformed data.

trum replacer to dairy calves after an initial colostrum feeding.

Focusing on calf gastrointestinal tract functionality, we used different biomarkers of intestinal barrier functionality (Cr-EDTA and citrulline), immune status (LTF and IgA), and microbiota (VFA, F/B ratio, and *Fecalis prausnitzii*) together with fecal scores to assess the effects of our feeding treatments. Foley and Otterby (1978) reported a laxative effect of colostrum when it was undiluted compared with diluted BC with water or whole milk. They concluded that when total solids were equalized to whole milk levels, an increase in the incidence of scours should not be expected. In addition, some authors described improvements in gut health whether they prolonged colostrum or TM administration after the first day of life (Berge et al., 2005; Kargar et al., 2021; Van Soest et al., 2022). In fact, when MR was supplemented with colostrum replacer, newborn calves reduced the incidence of diarrhea as well as the need for antibiotic therapy (Berge et al., 2009; Chamorro et al., 2017). Kargar et al. (2021) described how feeding TM for 3 weeks period improved the newborn growth performance and reduced the occurrence of diarrhea. Also, feeding TM for 4 d after one colostrum feeding stimulated intestinal development as well as improved health scores compared with MR fed calves (Van Soest et al., 2022). Contrary to the previous authors' reports and despite diluting the BC and TM with warm water, getting similar osmolarity values in BC, TM, and MR, we observed a decrease in fecal consistency in C10 treatment between d 1 and 10. Although our investigation was not designed to study the incidence of diarrhea, the poor fecal scores in C10 treatment cannot be attributed to differences in the feed osmolarity. To assess the intestinal barrier functionality, it was tested with Cr-EDTA as a marker of gut permeability and citrulline, a nonprotein AA, as an enterocyte mass biomarker (Amado et al., 2019; Gultekin et al., 2019). Elevated Cr-EDTA serum concentrations indicate an increase in paracellular permeability, which makes blood more accessible to large molecules (Zhang et al., 2013). In our study, the elevated serum Cr-EDTA concentrations during the FRF period could be explained as a dysfunctionality of the gut barrier by the feed restriction and stress for the long hours of fasting in all treatments, possibly producing an increase in gastrointestinal permeability. Moreover, a high citrulline concentration would be indicative of greater enterocyte mass and a healthier intestinal barrier (Gultekin et al., 2019). Concretely in mammals, citrulline is a product

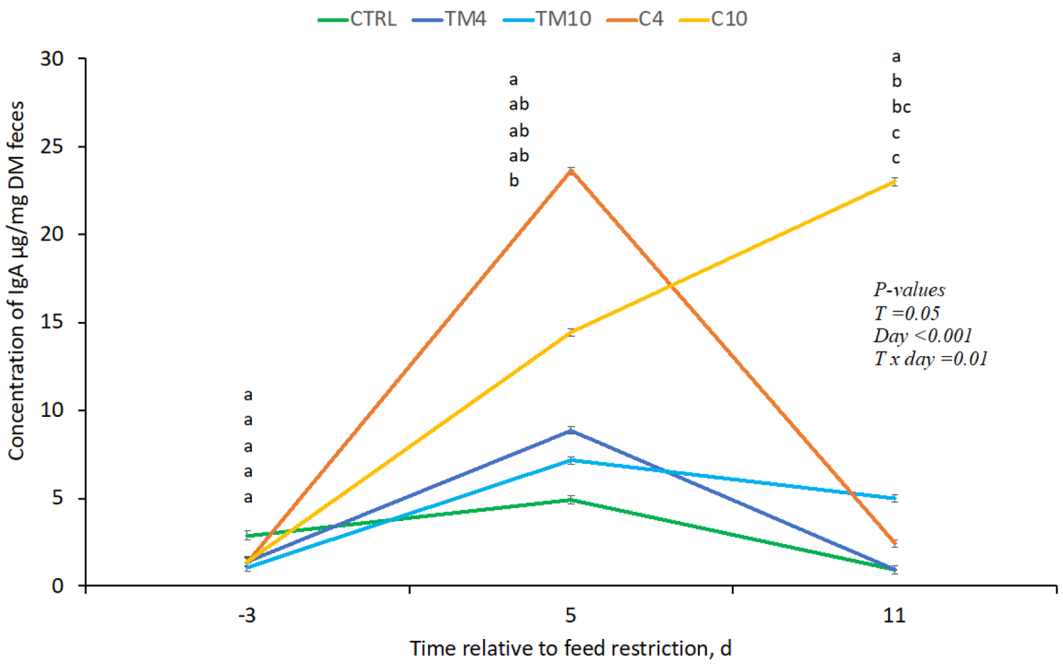

Figure 3. Fecal IgA evolution by experimental day before and after a feed restriction and fasting period (from d −3 to −1) in preweaning calves fed either milk replacer (MR) twice daily for 10 d (CTRL), transition milk (TM) twice daily during 4 d and thereafter MR for 6 d (TM4), TM twice daily for 10 d (TM10), bovine colostrum (BC) twice daily during 4 d and thereafter MR for 6 d (C4) or BC twice daily for 10 d (C10) from d 1 to 10 of study. Data were back transformed from fecal IgA logarithm least squares means while error bars represented fecal IgA logarithm standard errors. Letters (a–c) within the same measurement point indicate that least squares means without a common superscript differ ($P < 0.05$) among treatments. Letters represent the treatments in order they appear in each sampling point.

of the glutamine conversion in enterocytes and it is metabolized in the kidney to provide Arg to the urea cycle (Curis et al., 2005). In our investigation, all calves maintained the same citrulline levels at arrival and 24 h after the FRF period. However, CTRL calves sustained their lower citrulline levels throughout the study, while calves fed either BC or TM increased their serum citrulline, suggesting a benefit of BC and TM on intestinal cells. Based on literature (Krácmar et al., 2007, Terré et al., 2021), BC is richer in Glu than conventional MR, which it may explain the greater serum citrulline concentration observed in BC or TM fed calves. Biomarkers used to assess immune status were IgA, that plays an important role in the defense against the entry of enterotoxins and pathogenic organisms, and LTF as a marker of neutrophil activity in the gastrointestinal tract (Celi et al., 2019). IgA is not only secreted by the intestinal mucosa, it could be also provided with the diet. Although human colostral IgA has antitrypsin resistance, it is sensible to pepsin in acid pH (2.5), and the digestion of IgA depends on the development of the proteolytic activity of digestive enzymes in human neonates, which is very low up to 3 mo of life (Parkin et al., 1973). However, no literature about colostral IgA digestion in calves was found, but the intestinal secretion of IgA was described in preruminant calves (Porter et al., 1972). In our study, fecal IgA was highly detected on BC fed calves' feces compared with the CTRL ones on d 5, and continued in greater concentrations until d 11 in C10 calves, which could be due to the differences in IgA concentrations present in BC, TM, and MR. The detection of fecal IgA could explain a possible role of IgA in the preservation of the intestinal epithelial barrier and the development of immune tolerance to commensal gut microbiota depending on the colostrum treatment provided (Celi et al., 2019). Unfortunately, we cannot distinguish if these differences could come from either feeding treatments or intestinal secretion. On the other side, LTF is excreted from neutrophils and is found in most exocrine secretions, including milk and intestinal mucus (Celi et al., 2019), but it is also present in BC (1.84 g/L) and TM (0.86 g/L; Blum and Hammon, 2000). In our study, fecal LTF decreased during the feeding treatments, but it was similar among them, which might suggest a reduction of gastrointestinal inflammation (Celi et al., 2019) after the stress period of feed restriction. Similar to IgA, we could not ensure if fecal LTF detected came from the feeding treatments or intestinal mucosal secretion.

Finally, some authors described the role of the gut microbiome in metabolic health in preweaning calves, because they contribute in the development of the in-

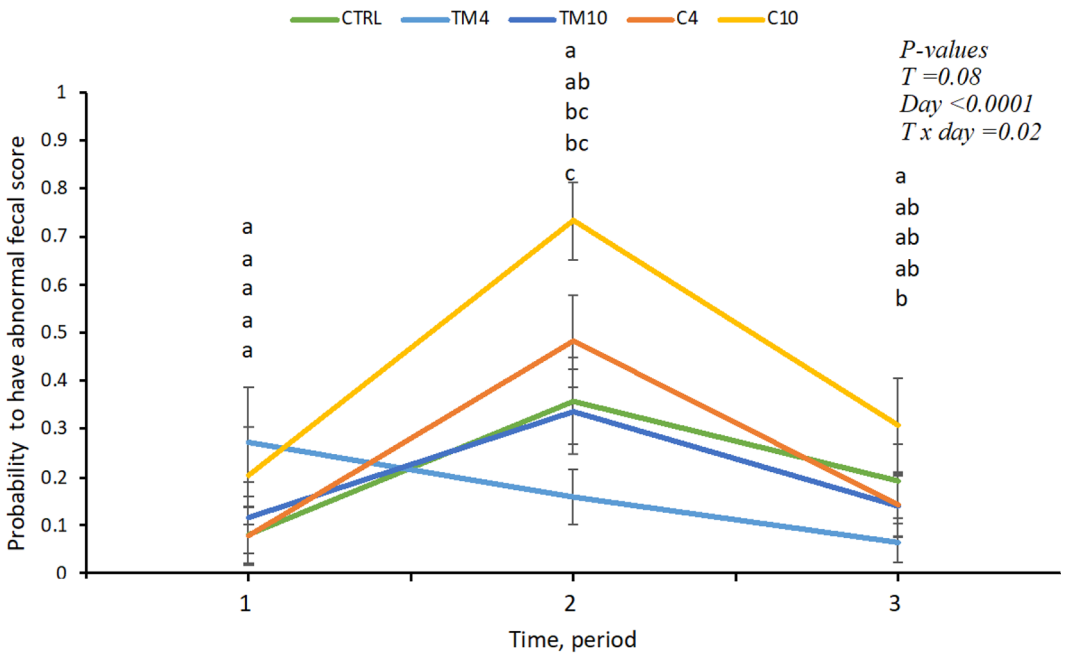

Figure 4. Probability to have abnormal feces (1) versus normal ones (0) by period of time (pretreatment period (with feed restriction and fasting) = from d −3 to −1, treatment period = from d 1 to 10 and posttreatment period = from d 11 to the end of the study) in preweaning calves fed either milk replacer (MR) twice daily for 10 d (CTRL), transition milk (TM) twice daily during 4 d and thereafter MR for 6 d (TM4), TM twice daily for 10 d (TM10), bovine colostrum (BC) twice daily during 4 d and thereafter MR for 6 d (C4) or BC twice daily for 10 d (C10) from d 1 to 10 of study. Letters (a–c) within the same measurement point indicate that least squares means without a common superscript differ ($P < 0.05$) among treatments. Letters represent the treatments in order they appear in each sampling point.

testinal mucosal epithelium, mucosal immune system, and hindgut digestion and fermentation (Guan, 2022). Particularly, Dietert and Silbergeld (2015) suggest multiple interactions between and within the microbiome, host immune system and the exposure to exogenous actions, which may modulate the composition and metabolism of gut microbiota by altering fatty acid production and regulating epigenetic modifications. Undigested carbohydrates and proteins are the main substrates for bacterial fermentation, which result in a wide range of metabolites such as VFA (Macfarlane and Macfarlane, 2012). In the present study we used fecal VFA to assess intestinal microbial fermentation (Macfarlane and Macfarlane, 2012) and the F/B ratio as a marker of gut dysbiosis (Yang et al., 2020). This ratio represents the proportion of "potential beneficial microbes" (including major species from the genera *Bifidobacterium*, *Lactobacillus*, *Faecalibacterium*, *Eubacterium*, and *Ruminococcus*) to "potential detrimental microbes" (that include some species from the genera *Clostridium*, *Enterobacter*, *Enterococcus*, *Bacteroidetes*, and *Ruminococcus*; Yang et al., 2020). Some human and animal studies report that an increase in the F/B ratio is associated with several pathologies such as obesity and inflammatory bowel disease (Magne et al., 2020; Stojanov et al., 2020; Yang et al., 2020). In our study, despite the decrease of fecal consistency during the feeding treatments (treatment period), especially in C10 calves, we did not detect differences the in the F/B ratio among treatments. However, we observed a different VFA profile, especially in BC calves, in which they increased butyrate proportion in their feces in comparison to CTRL calves in detriment of propionate proportion. Bovine colostrum is richer in oligosaccharides compared with mature milk (Fischer-Tlustos et al., 2020), and they can serve as a metabolic substrate for colonic bacteria (Playford and Weiser, 2021). Milk enriched with oligosaccharides increased the abundance of *Bifidobacterium*, but also a small increase of the butyrate-producing bacterium *Fecalis prausnitzii* (Fu et al., 2019). However, in our study, we observed a decrease in *Fecalis prausnitzii* concentrations from d 5 to 11, indicating that the increase in butyrate could not be attributed to this specie. We considered the increase of butyrate in BC fed calves as a positive effect because butyrate plays an important role in the regulation of cell growth and differentiation, the regulation of energy metabolism and the promotion of intestinal barrier function due to its anti-inflammatory activity or decreasing epithelial permeability in Caco-cell lines (Macfarlane

and Macfarlane, 2012; Magne et al., 2020). Although, unfortunately, neither fecal pH was measured nor fecal microbiota was characterized in the present study, the decrease of propionate proportion in C10 calves might be also linked to oligosaccharides fermentation in the colon. Literature, based on human studies, has reported a decrease of fecal pH when oligosaccharides were supplemented in term infant formulas (Günther and Guido, 2008), and this acidic environment, due to the presence of acid lactic bacteria, might have been detrimental for propionate-producing bacteria, which many of them belong to Bacteroidetes spp, which are less tolerant to acidic conditions (Louis and Flint, 2017). Although we did not observe differences in the F/B ratio and *Fecalis prausnitzii*, we are aware that to study the effects of BC and TM on microbiota, we would need more statistical power to draw accurate conclusions about microbiota populations. However, our preliminary results suggest that neither of the feeding treatment caused a severe dysbiosis to calves to alter the F/B ratio, and BC feeding might promote butyrate-producing bacteria.

In summary, the results of the present investigation showed that BC and TM supplementation after a FRF period influenced calf recovery. On one hand, differences in nutrient composition (fat and lactose) changed the recovery of energy status (higher BHB concentrations) of BC and TM fed calves. In contrast, although no performance differences among treatments were observed, results from serum citrulline, and the molar proportion of butyrate in feces, especially in calves fed BC, support the hypothesis that bioactive molecules present in BC and TM may help to restore the intestinal absorptive function and provide gut immune protection after the FRF period. Although extended BC and TM feeding maintained enterocyte mass and increased the IgA detected at d 11, BC supplementation had an undesirable effect on calves scouring. Overall, we would recommend the use of BC for 4 d or TM for 10 d to cope with the negative effects of the FRF period on calf health, but fecal scours should be monitored. However, research increasing the statistical power, number of calves per treatment, is needed to further evaluate the effects of BC and TM after a FRF period on health status and microbiota.

CONCLUSIONS

Feeding either BC or TM after an episode of FRF helps to recover intestinal barrier functionality and provide gut immune protection. The feeding treatments with the most desirable health outputs were feeding colostrum for 4 d or TM for 10 d. However, further research is needed to confirm the undesirable effects on fecal consistency when colostrum was fed for 10 d.

ACKNOWLEDGMENTS

This research was supported by the Spanish Ministry of Science and Innovation under the project number PGC2018-098847-B-I00 and by the predoctoral grant PRE2019-087640 of the Spanish Ministry of Science and Innovation and the European Regional Development Fund. The authors are also indebted to AGAUR (Barcelona, Spain) for project 2021 SGR 01552. We also show our gratitude to CERCA Programme (Generalitat de Catalunya). This work has been carried out within the framework of the Doctorate in Animal Production of the Autonomous University of Barcelona (Bellaterra, Spain). The authors have not stated any conflicts of interest.

REFERENCES

Adewuyi, A. A., E. Gruys, and F. J. C. M. Van Eerdenburg. 2005. Non esterified fatty acids (NEFA) in dairy cattle. A review. Vet. Q. 27:117–126. https://doi.org/10.1080/01652176.2005.9695192.

Amado, L., H. Berends, L. N. Leal, J. Wilms, H. Van Laar, W. J. J. Gerrits, and J. Martín-Tereso. 2019. Effect of energy source in calf milk replacer on performance, digestibility, and gut permeability in rearing calves. J. Dairy Sci. 102:3994–4001. https://doi.org/10.3168/jds.2018-15847.

AOAC International. 1995. Official Methods of Analysis. 16th ed. AOAC International.

AOAC International. 1996. Official Methods of Analysis. 16th ed. AOAC International.

Berge, A. C. B., T. E. Besser, D. A. Moore, and W. M. Sischo. 2009. Evaluation of the effects of oral colostrum supplementation during the first fourteen days on the health and performance of preweaned calves. J. Dairy Sci. 92:286–295. https://doi.org/10.3168/jds.2008-1433.

Berge, A. C. B., P. Lindeque, D. A. Moore, and W. M. Sischo. 2005. A clinical trial evaluating prophylactic and therapeutic antibiotic use on health and performance of preweaned calves. J. Dairy Sci. 88:2166–2177. https://doi.org/10.3168/jds.S0022-0302(05)72892-7.

Blättler, U., H. M. Hammon, C. Morel, C. Philipona, A. Rauprich, V. Romé, I. Le Huërou-Luron, P. Guilloteau, and J. W. Blum. 2001. Feeding colostrum, its composition and feeding duration variably modify proliferation and morphology of the intestine and digestive enzyme activities of neonatal calves. J. Nutr. 131:1256–1263. https://doi.org/10.1093/jn/131.4.1256.

Blum, J., and H. Hammon. 2000. Colostrum effects on the gastrointestinal tract, and on nutritional, endocrine, and metabolic parameters in neonatal calves. Livest. Prod. Sci. 66:151–159. https://doi.org/10.1016/S0301-6226(00)00222-0.

Blum, J. W. 2006. Nutritional physiology of neonatal calves. J. Anim. Physiol. Anim. Nutr. (Berl.) 90:1–11. https://doi.org/10.1111/j.1439-0396.2005.00614.x.

Celi, P., V. Verlhac, E. Pérez Calvo, J. Schmeisser, and A. M. Kluenter. 2019. Biomarkers of gastrointestinal functionality in animal nutrition and health. Anim. Feed Sci. Technol. 250:9–31. https://doi.org/10.1016/j.anifeedsci.2018.07.012.

Chamorro, M. F., N. Cernicchiaro, and D. M. Haines. 2017. Evaluation of the effects of colostrum replacer supplementation of the milk replacer ration on the occurrence of disease, antibiotic therapy, and performance of pre-weaned dairy calves. J. Dairy Sci. 100:1378–1387. https://doi.org/10.3168/jds.2016-11652.

Conneely, M., D. P. Berry, J. P. Murphy, I. Lorenz, M. L. Doherty, and E. Kennedy. 2014. Effect of feeding colostrum at different volumes and subsequent number of transition milk feeds on the serum immunoglobulin G concentration and health status of dairy calves. J. Dairy Sci. 97:6991–7000. https://doi.org/10.3168/jds.2013-7494.

Cooke, A. S., K. A. Watt, G. F. Albery, E. R. Morgan, and J. A. J. Dungait. 2020. Lactoferrin quantification in cattle faeces by ELISA. PeerJ 8:e8631. https://doi.org/10.7717/peerj.8631.

Curis, E., I. Nicolis, C. Moinard, S. Osowska, N. Zerrouk, S. Bénazeth, and L. Cynober. 2005. Almost all about citrulline in mammals. Amino Acids 29:177. https://doi.org/10.1007/s00726-005-0235-4.

Dietert, R. R., and E. K. Silbergeld. 2015. Biomarkers for the 21st century: Listening to the microbiome. Toxicol. Sci. 144:208–216. https://doi.org/10.1093/toxsci/kfv013.

Earley, B., K. Buckham Sporer, and S. Gupta. 2017. Invited review: Relationship between cattle transport, immunity, and respiratory disease. Animal 11:486–492. https://doi.org/10.1017/S1751731116001622.

Echeverry-Munera, J., L. Amado, H. Berends, L. N. Leal, M. A. Steele, and J. Martín-Tereso. 2023. Effect of partial exchange of lactose with fat in milk replacer on performance and blood metabolites of Holstein calves. JDS Commun. 4:19–24. https://doi.org/10.3168/jdsc.2022-0231.

Echeverry-Munera, J., L. N. Leal, J. N. Wilms, H. Berends, J. H. C. Costa, M. Steele, and J. Martín-Tereso. 2021. Effect of partial exchange of lactose with fat in milk replacer on ad libitum feed intake and performance in dairy calves. J. Dairy Sci. 104:5432–5444. https://doi.org/10.3168/jds.2020-19485.

Ellis, J. A. 2017. How efficacious are vaccines against bovine respiratory syncytial virus in cattle? Vet. Microbiol. 206:59–68. https://doi.org/10.1016/j.vetmic.2016.11.030.

Council of the European Union. 2005. Council Regulation (EC) No 1/2005 of 22 December 2004 on the protection of animals during transport and related operations and amending Directives 64/432/EEC and 93/119/EC and Regulation (EC) No 1255/97OJ L 3, 5.1.2005. Off. J. Eur. Union L 3:1–44.

European Commission. 2009. Comission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed. Off. J. Eur. Union L 54:1–130.

European Directorate for the Quality of Medicines & HealthCare. 2012. European Pharmacopoeia. 7th ed. Osmolarity. Council of Europe.

Fischer-Tlustos, A. J., K. Hertogs, J. K. van Niekerk, M. Nagorske, D. M. Haines, and M. A. Steele. 2020. Oligosaccharide concentrations in colostrum, transition milk, and mature milk of primi- and multiparous Holstein cows during the first week of lactation. J. Dairy Sci. 103:3683–3695. https://doi.org/10.3168/jds.2019-17357.

Foley, J. A., and D. E. Otterby. 1978. Availability, storage, treatment, composition, and feeding value of surplus colostrum: A review. J. Dairy Sci. 61:1033–1060. https://doi.org/10.3168/jds.S0022-0302(78)83686-8.

Fu, X., Z. Liu, C. Zhu, H. Mou, and Q. Kong. 2019. Nondigestible carbohydrates, butyrate, and butyrate-producing bacteria. Crit. Rev. Food Sci. Nutr. 59(Suppl.):S130–S152. https://doi.org/10.1080/10408398.2018.1542587.

García, A., J. A. Ruiz-Santa-Quiteria, J. A. Orden, D. Cid, R. Sanz, M. Gómez-Bautista, and R. De La Fuente. 2000. Rotavirus and concurrent infections with other enteropathogens in neonatal diarrheic dairy calves in Spain. Comp. Immunol. Microbiol. Infect. Dis. 23:175–183. https://doi.org/10.1016/S0147-9571(99)00071-5.

Guan, L. L. 2022. The role of gut microbiome in metabolic health of preweaned calves. J. Dairy Sci. 105(Suppl.):135. (Abstr.)

Gultekin, M., H. Voyvoda, K. Ural, H. Erdogan, C. Balikci, and G. Gultekin. 2019. Plasma citrulline, arginine, nitric oxide, and blood ammonia levels in neonatal calves with acute diarrhea. J. Vet. Intern. Med. 33:987–998. https://doi.org/10.1111/jvim.15459.

Günther, B., and M. Guido. 2008. Structural and functional aspects of prebiotics used in infant nutrition. J. Nutr. 138:1818S–1828S. https://doi.org/10.1093/jn/138.9.1818S.

Hagiwara, K., S. Kataoka, H. Yamanaka, R. Kirisawa, and H. Iwai. 2000. Detection of cytokines in bovine colostrum. Vet. Immunol. Immunopathol. 76:183–190. https://doi.org/10.1016/S0165-2427(00)00213-0.

Kargar, S., M. Bahadori-Moghaddam, S. M. Ghoreishi, A. Akhlaghi, M. Kanani, A. Pazoki, and M. H. Ghaffari. 2021. Extended transition milk feeding for 3 weeks improves growth performance and reduces the susceptibility to diarrhea in newborn female Holstein calves. Animal 15:100151. https://doi.org/10.1016/j.animal.2020.100151.

Knowles, T. G., S. N. Brown, J. E. Edwards, A. J. Phillips, and P. D. Warriss. 1999. Effect on young calves of a one-hour feeding stop during a 19-hour road journey. Vet. Rec. 144:687–692. https://doi.org/10.1136/vr.144.25.687.

Knowles, T. G., J. E. Edwards, K. J. Bazeley, S. N. Brown, A. Butterworth, and P. D. Warriss. 2000. Changes in the blood biochemical and haematological profile of neonatal calves with age. Vet. Rec. 147:593–598. https://doi.org/10.1136/vr.147.21.593.

Kráčmar, S., F. Buňka, I. Hoza, L. Čechová, and P. Valášek. 2007. Changes in amino acids composition of cows colostrum (durring first 72 hours after parturition). Acta Univ. Agric. Silvic. Mendel. Brun. 55:81–94. https://doi.org/10.11118/actaun200755010081.

Kuehn, C. S., D. E. Otterby, J. G. Linn, W. G. Olson, H. Chester-Jones, G. D. Marx, and J. A. Barmore. 1994. The effect of dietary energy concentratiion on calf performance. J. Dairy Sci. 77:2621–2629. https://doi.org/10.3168/jds.S0022-0302(94)77203-9.

Louis, P., and H. J. Flint. 2017. Formation of propionate and butyrate by the human colonic microbiota. Envrion. Microbiol. 19:29–41. https://doi.org/10.1111/1462-2920.13589.

Lührs, H., T. Gerke, J. G. Müller, R. Melcher, J. Schauber, F. Boxberger, W. Scheppach, and T. Menzel. 2002. Butyrate inhibits NF-κB activation in *Lamina Propria* macrophages of patients with ulcerative colitis. Scand. J. Gastroenterol. 37:458–466. https://doi.org/10.1080/003655202317316105.

Macfarlane, G. T., and S. Macfarlane. 2012. Bacteria, colonic fermentation, and gastrointestinal health. J. AOAC Int. 95:50–60. https://doi.org/10.5740/jaoacint.SGE_Macfarlane.

Magne, F., M. Gotteland, L. Gauthier, A. Zazueta, S. Pesoa, P. Navarrete, and R. Balamurugan. 2020. The firmicutes/bacteroidetes ratio: A relevant marker of gut dysbiosis in obese patients? Nutrients 12:1474. https://doi.org/10.3390/nu12051474.

Marcato, F., H. van den Brand, B. Kemp, B. Engel, M. Wolthuis-Fillerup, and K. van Reenen. 2020. Effects of pretransport diet, transport duration, and type of vehicle on physiological status of young veal calves. J. Dairy Sci. 103:3505–3520. https://doi.org/10.3168/jds.2019-17445.

Mohri, M., K. Sharifi, and S. Eidi. 2007. Hematology and serum biochemistry of Holstein dairy calves: Age related changes and comparison with blood composition in adults. Res. Vet. Sci. 83:30–39. https://doi.org/10.1016/j.rvsc.2006.10.017.

Pakkanen, R., and J. Aalto. 1997. Growth factors and antimicrobial factors of bovine colostrum. Int. Dairy J. 7:285–297. https://doi.org/10.1016/S0958-6946(97)00022-8.

Pardon, B., B. Catry, R. Boone, H. Theys, K. De Bleecker, J. Dewulf, and P. Deprez. 2014. Characteristics and challenges of the modern Belgian veal industry. Vlaams Diergeneeskd. Tijdschr. 83:155–163. https://doi.org/10.21825/vdt.v83i4.16641.

Parkin, D. M., D. B. L. Mcclelland, R. R. Samson, M. M. Lees, and D. J. C. Shearman. 1973. The effect of acid, pepsin and trypsin on human colostral IgA agglutinins. Eur. J. Clin. Invest. 3:66–71. https://doi.org/10.1111/j.1365-2362.1973.tb00331.x.

Pederzolli, R. A., A. G. Van Kessel, J. Campbell, S. Hendrick, K. M. Wood, and G. B. Penner. 2018. Effect of ruminal acidosis and short-term low feed intake on indicators of gastrointestinal barrier function in Holstein steers. J. Anim. Sci. 96:108–125. https://doi.org/10.1093/jas/skx049.

Pisoni, L., M. Devant, M. Blanch, J. J. Pastor, and S. Marti. 2022. Simulation of feed restriction and fasting: Effects on animal recovery and gastrointestinal permeability in unweaned Angus-Holstein calves. J. Dairy Sci. 105:2572–2586. https://doi.org/10.3168/jds.2021-20878.

Playford, R. J., and M. J. Weiser. 2021. Bovine colostrum: Its constituents and uses. Nutrients 13:265. https://doi.org/10.3390/nu13010265.

Porter, P., D. E. Noakes, and W. D. Allen. 1972. Intestinal secretion of immunoglobulins in the preruminant calf. Immunology 23:299–312.

Quigley, J. D., T. A. Wolfe, and T. H. Elsasser. 2006. Effects of additional milk replacer feeding on calf health, growth, and selected

blood metabolites in calves. J. Dairy Sci. 89:207–216. https://doi.org/10.3168/jds.S0022-0302(06)72085-9.
Radostits, O. M., D. C. Blood, and C. C. Gay. 1994. Veterinary Medicine: A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses. 8th ed. Baillière Tindall, London.
Renaud, D. L., T. F. Duffield, S. J. LeBlanc, D. B. Haley, and D. F. Kelton. 2018. Clinical and metabolic indicators associated with early mortality at a milk-fed veal facility: A prospective case-control study. J. Dairy Sci. 101:2669–2678. https://doi.org/10.3168/jds.2017-14042.
Roland, L., M. Drillich, and M. Iwersen. 2014. Hematology as a diagnostic tool in bovine medicine. J. Vet. Diagn. Invest. 26:592–598. https://doi.org/10.1177/1040638714546490.
Rossi, R. 2020. EU Trade and Transport of Live Animals. Accessed Mar. 7, 2022. https://epthinktank.eu/2020/02/10/eu-trade-and-transport-of-live-animals/.
Schaut, R. G., P. M. Boggiatto, C. L. Loving, and V. K. Sharma. 2019. Cellular and mucosal immune responses following vaccination with inactivated mutant of *Escherichia coli* O157:H7. Sci. Rep. 9:6401. https://doi.org/10.1038/s41598-019-42861-z.
Schwiertz, A., M. Jacobi, J.-S. Frick, M. Richter, K. Rusch, and H. Köhler. 2010. Microbiota in pediatric inflammatory bowel disease. J. Pediatr. 157:240–244.e1. https://doi.org/10.1016/j.jpeds.2010.02.046.
Song, Y. 2018. Early Life Gut Microbiome in Dairy Calves and Its Responses to Colostrum Feeding Strategies. University of Alberta.
Staley, T. E., L. D. Corley, L. J. Bush, and E. Wynn Jones. 1972. The ultrastructure of neonatal calf intestine and absorption of heterologous proteins. Anat. Rec. 172:559–579. https://doi.org/10.1002/ar.1091720310.
Step, D. L., C. R. Krehbiel, H. A. DePra, J. J. Cranston, R. W. Fulton, J. G. Kirkpatrick, D. R. Gill, M. E. Payton, M. A. Montelongo, and A. W. Confer. 2008. Effects of commingling beef calves from different sources and weaning protocols during a forty-two-day receiving period on performance and bovine respiratory disease. J. Anim. Sci. 86:3146–3158. https://doi.org/10.2527/jas.2008-0883.
Stojanov, S., A. Berlec, and B. Štrukelj. 2020. The influence of probiotics on the firmicutes/bacteroidetes ratio in the treatment of obesity and inflammatory bowel disease. Microorganisms 8:1715. https://doi.org/10.3390/microorganisms8111715.
Stott, G. H., D. B. Marx, B. E. Menefee, and G. T. Nightengale. 1979. Colostral immunoglobulin transfer in calves I. Period of absorption. J. Dairy Sci. 62:1632–1638. https://doi.org/10.3168/jds.S0022-0302(79)83472-4.
Takai, K., and K. Horikoshi. 2000. Rapid detection and quantification of members of the archaeal community by quantitative PCR using fluorogenic probes. Appl. Environ. Microbiol. 66:5066–5072. https://doi.org/10.1128/AEM.66.11.5066-5072.2000.
Terré, M., I. Ortuzar, J. Graffelman, A. Bassols, M. Vidal, and A. Bach. 2021. Using compositional mixed-effects models to evaluate responses to amino acid supplementation in milk replacers for calves. J. Dairy Sci. 104:7808–7819. https://doi.org/10.3168/jds.2020-20035.
Tolhurst, G., H. Heffron, Y. S. Lam, H. E. Parker, A. M. Habib, E. Diakogiannaki, J. Cameron, J. Grosse, F. Reimann, and F. M. Gribble. 2012. Short-chain fatty acids stimulate glucagon-like peptide-1 secretion via the G-protein-coupled receptor FFAR2. Diabetes 61:364–371. https://doi.org/10.2337/db11-1019.
Ulfman, L. H., J. H. W. Leusen, H. F. J. Savelkoul, J. O. Warner, and R. J. J. van Neerven. 2018. Effects of bovine immunoglobulins on immune function, allergy, and infection. Front. Nutr. 5:52. https://doi.org/10.3389/fnut.2018.00052.
Van Soest, B., M. Weber Nielsen, A. J. Moeser, A. Abuelo, and M. J. VandeHaar. 2022. Transition milk stimulates intestinal development of neonatal Holstein calves. J. Dairy Sci. 105:7011–7022. https://doi.org/10.3168/jds.2021-21723.
Vidali, S., S. Aminzadeh, B. Lambert, T. Rutherford, W. Sperl, B. Kofler, and R. G. Feichtinger. 2015. Mitochondria: The ketogenic diet—A metabolism-based therapy. Int. J. Biochem. Cell Biol. 63:55–59. https://doi.org/10.1016/j.biocel.2015.01.022.
Wang, H.-B., P.-Y. Wang, X. Wang, Y.-L. Wan, and Y.-C. Liu. 2012. Butyrate enhances intestinal epithelial barrier function via up-regulation of tight junction protein claudin-1 transcription. Dig. Dis. Sci. 57:3126–3135. https://doi.org/10.1007/s10620-012-2259-4.
Welboren, A. C., B. Hatew, O. López-Campos, J. P. Cant, L. N. Leal, J. Martín-Tereso, and M. A. Steele. 2021. Effects of energy source in milk replacer on glucose metabolism of neonatal dairy calves. J. Dairy Sci. 104:5009–5020. https://doi.org/10.3168/jds.2020-19405.
Wilson, D. J., J. Stojkov, D. L. Renaud, and D. Fraser. 2020. Risk factors for poor health outcomes for male dairy calves undergoing transportation in western Canada. Can. Vet. J. 61:1265–1272.
Wulff Helge, J. 2002. Long-term fat diet adaptation effects on performance, training capacity, and fat utilization. Med. Sci. Sports Exerc. 34:1499–1504. https://doi.org/10.1097/00005768-200209000-00016.
Yang, Q., Q. Liang, B. Balakrishnan, D. P. Belobrajdic, Q. J. Feng, and W. Zhang. 2020. Role of dietary nutrients in the modulation of gut microbiota: A narrative review. Nutrients 12:381. https://doi.org/10.3390/nu12020381.
Yang, Y. W., M. K. Chen, B. Y. Yang, X. J. Huang, X. R. Zhang, L. Q. He, J. Zhang, and Z. C. Hua. 2015. Use of 16S rRNA gene-targeted group-specific primers for real-time PCR analysis of predominant bacteria in mouse feces. Appl. Environ. Microbiol. 81:6749–6756. https://doi.org/10.1128/AEM.01906-15.
Zhang, S., J. R. Aschenbach, D. R. Barreda, and G. B. Penner. 2013. Recovery of absorptive function of the reticulo-rumen and total tract barrier function in beef cattle after short-term feed restriction. J. Anim. Sci. 91:1696–1706. https://doi.org/10.2527/jas.2012-5774.

ORCIDS

M. Tortadès https://orcid.org/0000-0001-8538-1823
S. Marti https://orcid.org/0000-0002-8115-0582
M. Devant https://orcid.org/0000-0001-8474-5058
F. Fàbregas https://orcid.org/0000-0002-4062-5246
M. Terré https://orcid.org/0000-0002-2815-9035