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Transition milk stimulates intestinal development of neonatal Holstein calves

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ABSTRACT

Colostrum stimulates gastrointestinal development. Similar to colostrum, transition milk (TM; the first few milkings after colostrum) contains elevated nutrient levels and bioactive components not found in milk replacer (MR), albeit at lower levels than the first colostrum. We hypothesized that feeding neonatal calves TM, compared with MR, for 4 d following colostrum at birth would further stimulate intestinal development. Holstein bull calves were fed 2.8 L of colostrum within 20 min of birth, allocated to 1 of 11 blocks based on birth date and body weight (BW), randomly assigned to MR (n = 12) or TM (n = 11) treatments within block, and fed treatments 3 times per day. Milk from milkings 2, 3, and 4 (TM) of cows milked 2 times daily was pooled by milking number and fed at 1.89 L per feeding; milking 2 was fed at feedings 2 through 5, milking 3 at feedings 6 through 8, and milking 4 at feedings 9 through 12. TM was not pasteurized and contained 17% solids, 5% fat, 7% protein, 4% lactose, and 20 g of IgG per liter on average, whereas MR (as fed) contained 15% solids, 4% protein, 3% fat, 6% carbohydrate, and no IgG. Refusals were similar, so calves fed TM consumed 1.0 Mcal of metabolizable energy per day more than those fed MR. On the morning of d 5, calves were injected i.v. with 5 mg of bromodeoxyuridine per kg of BW and slaughtered 130 min later; then, intestinal sections were excised. Feeding TM, instead of MR, doubled villus length, villus width, villus to crypt ratio, and mucosal length in all intestinal sections, increased submucosal thickness 70% in the proximal and mid jejunum, and tended to increase submucosal thickness in duodenum and ileum. Mucosal surface area was also increased in both the ileum and mid jejunum when feeding TM by 19 and 36%, respectively. Treatment did not alter crypt depth. Bromodeoxyuridine labeling was increased 50% by TM compared with MR in the cells along the epithelium of the crypts and within the villi of all sections, indicating that TM increased cell proliferation compared with MR. Calves fed TM gained more BW than calves fed MR and had improved cough, fecal, nose, and ear scores. We conclude that feeding TM for 4 d following an initial feeding of colostrum stimulates villus, mucosal, and submucosal development in all sections of the small intestine in the first few days of life and improves health and growth.
Key words: calf, transition milk, intestinal development

INTRODUCTION

Transition milk (**TM**) is defined as milkings 2 to 6 after calving (Godden, 2008). Colostrum contains a high immunoglobulin content and is thus essential for providing passive immunity to the neonate. Colostrum also contains bioactive compounds and nutrients at elevated concentrations compared with milk. Transition milk also has elevated concentrations of IgG and other bioactive compounds such as IGF-1, growth hormone, and insulin; moreover, these concentrations gradually decrease as mammary secretions transition from colostrum to regular milk (Blum and Hammon, 2000; Blättler et al., 2001; Kühne et al., 2000). Blum (2006) suggested neonatal calves' gastrointestinal tract is relatively mature at birth but requires morphological and functional changes from colostrum's nutrient and nonnutrient components. The hormones and growth factors within colostrum stimulate growth of the gastrointestinal tract (**GIT**) and regeneration after inflammatory damage (Uruakpa et al., 2002).

Roffler et al. (2003) demonstrated that calves fed bovine colostrum extract for the first 5 d of life had larger intestinal villi than those fed milk replacer (**MR**). Benefits from early life colostrum supplementation have also been observed in swine (van Barneveld et al., 2011); for instance, piglets who consumed colostrum protein isolate had increased growth, liquid feed consumption, feed efficiency, and GIT development compared with piglets consuming a whey protein concentrate for the first 28 d of life. When comparing calves fed colostrum with those fed high-density MR, Kühne et al. (2000) concluded the elevated nutrients and bioactive and growth-promoting compounds in colostrum increased metabolic rate, growth rate, and decreased incidence

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of loose feces. Blättler et al. (2001) demonstrated that feeding large quantities of colostrum, compared with MR, reduced apoptosis of mucosal epithelial cells, enhanced crypt fission, increased number of epithelial cells from crypt to villus, and increased cell proliferation in parts of the small intestine. They observed no differences in crypt growth in the first 7 d of life, but overall, their data clearly show that colostrum enhanced GIT development.

We recently found that feeding TM to calves following colostrum improved growth rates and decreased serum haptoglobin concentration (Van Soest et al., 2020). This supported an earlier study in which feeding TM following colostrum tended to increase weight gain and improve eye, ear, and nasal health scores (Conneely et al., 2014). Because the composition of TM has similarities to colostrum, we expected that TM might also enhance intestinal development, and in turn, explain the improved health and growth seen in earlier studies. We hypothesized that feeding TM in the first 4 d of life following colostrum would promote intestinal development when compared with feeding MR. Our objective was to test this hypothesis in newborn Holstein bull calves. We determined whether morphology and cell proliferation in the duodenum, jejunum, and ileum, immune cell counts in the ileum, health scores, and growth were different for calves fed TM compared with MR.

MATERIALS AND METHODS

Animals and Treatments

The Institutional Animal Care and Use Committee of Michigan State University approved all experimental procedures. Twenty-three neonatal Holstein bull calves were purchased from a large commercial Michigan dairy farm 1 h from campus. Four calves were selected each weekend at about 1100 h that had been born within the previous 12 h and had received 2.84 L of stored colostrum by esophageal tube within 20 min of birth, administered by the farm's maternity ward caretaker. Colostrum was from a previous dam that had tested above 23 on a Brix refractometer. The 4 calves were transferred to the Michigan State University Beef Cow and Calf Research facility (East Lansing). Upon arrival, calves were housed in calf hutches, bedded with wheat straw, and provided water ad libitum. Calves were blocked by BW to 2 blocks per weekend and randomly assigned within the block to either TM or MR. On the last weekend of the study, only 3 calves were available, so all calves stayed in 1 block; thus, we had 11 calves fed TM and 12 fed MR. The TM was collected from 16 cows (6 primiparous and 10 multiparous) milked 2 times/d at the Michigan State University Dairy Research and Teaching Center (East Lansing), with milkings 2, 3, and 4 collected and managed separately. Upon collection, TM was frozen at −20°C, and subsequently thawed, pooled by milking, and refrozen in 1.89-L batches at −20°C. The TM was sent to a commercial laboratory for analysis by near-infrared spectroscopy (Table 1; CentralStar Cooperative Inc.). The MR consisted of dried whey and milk products, animal fat, vegetable oil, and supplements (Table 1; Purina Cold Front BOV MOS Medicated Milk Replacer); furthermore, MR was mixed to contain 14.6% DM and fed at 1.89 L (275 g of DM) per feeding. At time of feeding, all diets were warmed and fed at 40°C. All calves were bottle-fed 1.89 L of their respective diet 3 times/d: 0400, 1130, and 1800 h. Calves in the TM group were fed milking 2 at feedings 2 to 5, milking 3 at feedings 6 to 8, and milking 4 at feedings 9 to 12; this resulted in a decreased feeding rate of DM each successive day, as the solids content in TM decreased with each milking. Despite the decrease each day, calves fed TM consumed greater DM, ME, and CP overall than the calves fed MR.

Sampling and Measurements

All calves were weighed upon arrival at Michigan State University on d 1 (at ~1200 h), just before their 1130 h feeding on d 2 and 3, and just before the 0400 h feeding on d 5. At each weighing, heart girth, withers height, and hip height were measured with a measuring tape and adjustable ruler. Health was scored and recorded for fecal consistency, ear disposition, eye

Table 1. Composition of experimental diets

	Diet			
	TM[1]			
Item	Milking 2	Milking 3	Milking 4	MR[2]
Feedings[3]	2–5	6–8	9–12	2–12
IgG (g/L)	36.8	16.0	7.9	0
Solids (g/L)	190	164	158	146
Protein (g/L)	89.1	59.1	50.5	39.1
Fat (g/L)	48.0	51.1	54.5	30.1
Lactose (g/L)	38.8	42.9	43.5	63.2
ME[4] (Mcal/L)	1.03	0.91	0.89	0.70

[1]The composition of transition milk (TM) changed with each milking.
[2]Industry standard milk replacer (MR; 97% DM, 26% protein, 20% fat).
[3]Feeding 1 was colostrum.
[4]ME was calculated using NRC (2001) equations. Enthalpies of 9.2, 5.7, and 3.95 kcal/g were used for fat, protein, and lactose, respectively. Gross energy was multiplied by 0.97 to calculate digestible energy and then by 0.96 to calculate ME. The carbohydrate fraction of the milk replacer was ~87% lactose, but all carbohydrates were given a value of 3.95 kcal/g.

discharge, nasal discharge, and cough prevalence by 2 researchers at each feeding using the Wisconsin Calf Health Scoring Chart (University of Madison–Wisconsin School of Veterinary Medicine, 2011; https://www.vetmed.wisc.edu/fapm/svm-dairy-apps/calf-health-scorer-chs/) on a scale of 0 to 3 with 0 being healthy and 3 being severely ill.

Blood samples were collected via jugular venipuncture into either BD Vacutainer Venous Blood Collection Tubes: Vacutainer Plus Glass Serum Tubes, K2 EDTA, or sodium fluoride and potassium oxalate tubes (Becton, Dickinson and Company). We collected blood on d 1 about 4 h after arrival and before treatments began (1800 h), before feeding on d 2 and 3 (1100 h), once 2 h postfeeding on d 3 (1330 h), and before feeding on d 5 (0400 h). Blood was centrifuged at 1,700 × *g* for 15 min at 4°C. The respective serum or plasma was harvested and stored at −20°C. Concentrations of IgG in TM and concentrations of IgG and total protein in serum from prefeeding blood samples were measured via radial immunodiffusion (Shivley et al., 2018) and digital refractometer, respectively, by Saskatoon Colostrum Company. Glucose (from sodium fluoride and potassium oxalate tubes), insulin, and nonesterified fatty acids (**NEFA**) were measured in plasma (the rest used K2 EDTA tubes; PGO Enzyme Product No. P7119; Sigma Chemical Co.; Mercodia Bovine Insulin ELISA; and HR Series NEFA-HR, Wako Pure Chemical Co., respectively). Haptoglobin and the binding protein for lipopolysaccharide (**LBP**) were analyzed in blood serum samples from d 3 and 5 (Bovine Haptoglobin ELISA Kit, Immunology Consultants Laboratory Inc.; and LBP Various Species Kit, Hycult Biotech, HK503 Edition, respectively).

Measurements After Euthanasia

On d 5, calves received an injection via the jugular vein of bromodeoxyuridine (**BrdU**) starting at 0720 h at a rate of 5 mg/kg of BW using 10 mg/mL BrdU (Sigma-Aldrich) solution to label cells in the S phase of the cell cycle. Calves were killed 130 ± 4 min after BrdU by i.v. injection of 10 mL of pentobarbital sodium (SomnaSol Euthanasia-III Solution, Henry Schein Animal Health). The abdomen was opened and 4 samples were collected. Duodenum was collected starting at 5 cm caudal from the pylorus and working downstream. Proximal jejunum (**ProxJj**) was collected starting at 100 cm caudal from the pylorus. Middle jejunum (**MidJj**) was collected starting approximately 30 cm proximal from the collateral branch of the cranial mesenteric artery. Ileum was collected starting 10 cm proximal to the cecum. From each section, we removed 4 cm for morphological measurements and immunohistochemistry and 20 cm for collection of digesta. Tissues were flushed with ice-cold 1× PBS buffer at pH 7.4 and placed in 10% formalin solution to fix for histology.

Tissues were sectioned (5 μm) and stained with hematoxylin and eosin staining. Slides were visualized at 4× magnification with a light microscope (Leica Microsystems). A minimum of 20 well-oriented and intact crypt-villus units per intestinal section per calf were selected and morphological dimensions were measured by one observer blinded to treatment using ImageJ software (Rasband, W.S., ImageJ, US National Institutes of Health). Villus height and width, crypt width and depth, and thickness of the mucosa and submucosa were measured, and the villus height/crypt depth (**V/C**) ratio and mucosal surface area index were calculated. As in Pyo et al. (2020), mucosal surface area index = $[(a \times b) + (a/2 + c/2) - (a/2)^2]/(b/2 + c/2)^2$, where a = villi width (μm), b = villi height (μm), and c = crypt width (μm).

For immunohistochemistry, samples were processed, embedded in paraffin, and sectioned on a rotary microtome at 4 μm. Incorporation of BrdU was measured in all 4 intestinal sections; however, B and T cell numbers were only examined in the ileal sample. Slides were fully prepared and made by the Michigan State University Human Pathology Histology Laboratory. Slides were dried at 56°C, deparaffinized in xylene, and rehydrated using a series of washes in descending grades of ethanol. Endogenous peroxidase was blocked by bathing slides for 30 min in 3% hydrogen peroxide in methanol and rinsed with tap and distilled water. Sections were denatured using 4.0 *M* hydrochloric acid, rinsed, and placed in Tris-buffered saline pH 7.4 (**TBS**; Scytek Labs) to adjust the pH. Sections were retrieved utilizing 0.4% pepsin (Sigma-Aldrich) in 0.2 *N* aqueous hydrochloric acid for 15 min at 37°C, followed by rinses with tap water, distilled water, and TBS plus Tween 20 solution. Following pretreatments, standard micropolymer complex staining steps were performed at room temperature on the IntelliPath Flex Auto Stainer, followed by rinses in TBS (Biocare Medical). Sections were blocked for nonspecific proteins and incubated for 30 min with the respective primary antibody for each staining procedure. Antibodies were monoclonal mouse anti-BrdU (#347580, Becton Dickinson), rabbit anti-B cell (#ab78237 Abcam; Epitomics), and polyclonal rabbit anti-T cell (#ab5690 Abcam). Micro-polymer (ProMark Mouse on Farma HRP Polymer, Biocare) reagents were incubated for 30 min, followed by reaction development with Romulin AEC (Biocare) and counterstained with Cathe Hematoxylin (1:10 antibody for BrdU and 1:5 for T and B cells). The BrdU-labeled cells were visualized at 20× magnification with light microscopy (Leica Microsystems) of 20 fully intact crypt-

villus units containing no artifacts that were clearly and completely visible in the duodenum, ProxJj, MidJj, and ileum. Cells were quantified by one observer blinded to treatment as cells/mm of crypt epithelium and cells/mm^2 of lamina propria extending from the crypt to the villi. Slides for B and T cells were analyzed at 40× and 20× magnification, respectively, on a light microscope (Leica Microsystems). The B cells were identified within 10 Peyer's patches per slide and counted by one observer blinded to treatment and expressed as B cells/mm^2 of Peyer's patches. The T cells were located within 20 fully intact crypt-villus units containing no artifacts that were clearly and completely visible for counting by one observer blinded to treatment as T cells/mm of epithelium and as T cells/mm^2 of lamina propria.

Digesta were collected from abomasum, duodenum, ProxJj, MidJj, ileum, and cecum. The pH of each sample was determined using a calibrated pH probe (Thermo Fisher Scientific). The digesta in the abomasum was weighed, filtered (1.5-cm filter) to remove curds, and the curds were weighed.

Statistical Analysis

The MIXED Procedure in SAS version 9.4 (SAS Institute Inc.) was used to analyze statistical differences. For all blood measurements, we used a repeated measures model with fixed effects of treatment and time (or day) and random effect of block with one analysis of only prefeeding samples and one analysis of only d 3 samples before and after feeding to examine the effect of feeding. The 10 health scores after treatments began were averaged for each calf and analyzed using Friedman's test for nonparametric analysis; because this analysis requires balanced data, block 11 was not used. Our model for all nonrepeated measures included the fixed effect of treatment and random effect of block. For BrdU labeling, time between injection and euthanasia was tested as a covariate, but it had no effect. Residuals were tested for normality using the Shapiro-Wilk test, and homogeneity of variance was tested with Bartlett's test. Insulin and haptoglobin were log-transformed to satisfy homoscedasticity and then back-transformed. Significance was denoted at P-value ≤ 0.05 and tendencies were denoted as P-values ≤ 0.1 and >0.05 on all main effects.

RESULTS

Calves fed TM and MR refused about 20% of the milk offered in the first 2 d (Table 2). Calves fed TM consumed 13% more solids, 30% more ME, and 29% more protein than those fed MR and gained twice as much BW over the 4-d study than those fed MR (Table 2; $P = 0.005$). TM tended to increase gain in heart girth and hip height compared with MR, whereas no differences were observed for gain in withers height.

Table 2. Intake of treatments and growth

Variable	Treatment[1]		P-value
	TM	MR	
Milk fed (L/d)	5.67	5.67	—
% refused overall	12%	11%	0.6
% refused (feedings 2–6)	24%	20%	0.6
Solids consumed (g/d)	841	741	<0.008
ME consumed[2] (Mcal/d)	4.66	3.55	<0.001
Protein consumed (g/d)	321	248	<0.001
Fat consumed (g/d)	256	153	<0.001
Birth weight (kg)	41.6	41.9	0.8
Initial heart girth (cm)	79.5	81.3	0.12
Initial hip height (cm)	80.0	80.8	0.5
Initial withers height (cm)	76.8	76.8	0.9
Final weight (kg)	43.5	43.8	0.8
BW gain (kg/d)	0.64	0.34	0.06
Heart girth gain (cm)	2.77	1.12	0.07
Hip height gain (cm)	1.45	0.15	0.09
Withers height gain (cm)	0.00	0.71	0.28

[1]Calves fed transition milk (TM) or milk replacer (MR).
[2]ME consumed postcolostrum during trial (feedings 2–12).

Compared with MR, TM increased villus length of the duodenum by 63%, 96% in ProxJj, 76% in MidJj, and 52% in ileum ($P \leq 0.003$ for all; Figure 1; Table 3). Transition milk also increased villus width 43% in duodenum, 63% in ProxJj, 60% in MidJj, and 40% in ileum ($P \leq 0.008$ for all) compared with MR. The V/C (ratio of villus length to crypt depth) was increased by TM compared with MR 54% in duodenum, 101% in ProxJj, 81% in MidJj, and 48% in ileum (Table 3; $P \leq 0.01$ for all). Compared with MR, TM increased mucosa thickness 37% in duodenum, 58% in ProxJj, 47% in MidJj, and 33% in ileum ($P \leq 0.013$ for all). Submucosa thickness was significantly increased for both the MidJj and ProxJj while tending to increase in the ileum and duodenum for TM compared with MR. No differences were observed for crypt depth in any section. Compared with MR, TM increased the calculated mucosal surface area index in both the MidJj by 36% and the ileum by 19%, whereas no differences were detected for the ProxJj and duodenum.

Compared with MR, TM increased the number of cells in the lamina propria labeled with BrdU 54% in the duodenum, 75% in ProxJj, 121% in MidJj, and 64% in ileum ($P \leq 0.01$ for all; Figure 2; Table 4). Compared with MR, TM also increased the number of BrdU-labeled cells in the crypt epithelium layer of the duodenum by 40%, 72% in ProxJj, 78% in MidJj, and 56% in ileum (Table 4; $P \leq 0.0001$ for all). Treatment did not alter number of B cells in Peyer's patches in the ileum or number of T cells within the lamina propria

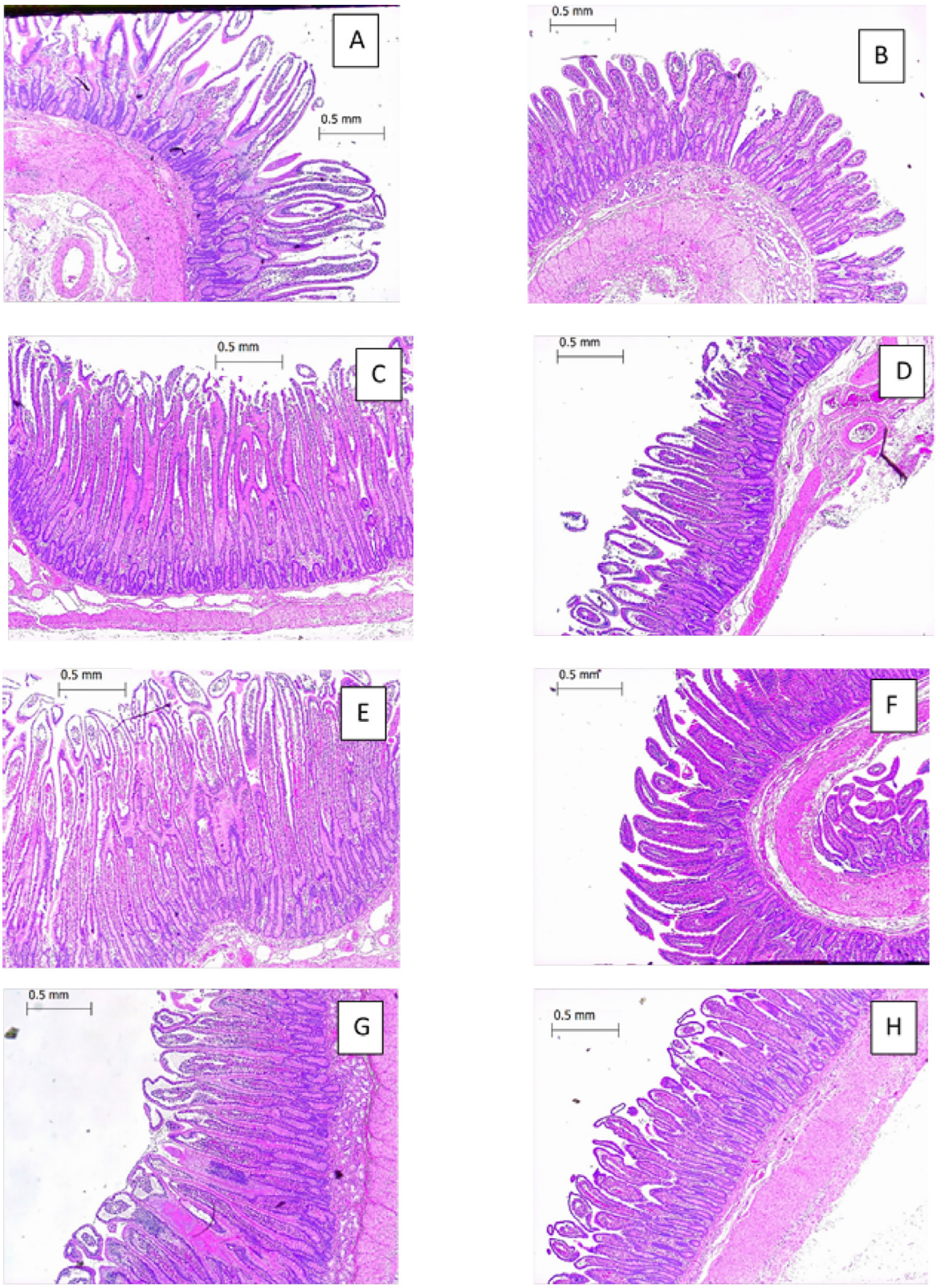

Figure 1. Example views for morphological measures of the small intestine. Examples of light micrographs of hematoxylin and eosin-stained duodenum (TM: A and MR: B), proximal jejunum (TM: C and MR: D), mid jejunum (TM: E and MR: F), and ileum (TM: G and MR: H) sections of neonatal calves fed either transition milk (TM) or milk replacer (MR) for the first 4 d of life. Observation was done at 4× magnification. The TM calves had increased morphological measures other than crypt length.

Table 3. Small intestine morphology (mean ± SE)

Item	Treatment[1]		*P*-value
	TM	MR	
Villus length (mm)			
Duodenum	0.824 ± 0.060	0.504 ± 0.058	0.003
Proximal jejunum	1.190 ± 0.048	0.609 ± 0.047	<0.001
Mid jejunum	1.004 ± 0.059	0.568 ± 0.058	<0.001
Ileum	0.812 ± 0.045	0.536 ± 0.044	0.001
Villus width (mm)			
Duodenum	0.140 ± 0.0099	0.0977 ± 0.0096	0.008
Proximal jejunum	0.146 ± 0.0089	0.0891 ± 0.0086	<0.001
Mid jejunum	0.150 ± 0.0092	0.0942 ± 0.0090	<0.001
Ileum	0.146 ± 0.0096	0.105 ± 0.0093	0.006
Crypt depth (mm)			
Duodenum	0.336 ± 0.023	0.338 ± 0.023	0.9
Proximal jejunum	0.346 ± 0.020	0.356 ± 0.020	0.7
Mid jejunum	0.324 ± 0.019	0.332 ± 0.019	0.8
Ileum	0.313 ± 0.023	0.310 ± 0.022	0.9
Villus/crypt ratio			
Duodenum	2.52 ± 0.14	1.63 ± 0.13	<0.001
Proximal jejunum	3.55 ± 0.11	1.76 ± 0.10	<0.001
Mid jejunum	3.27 ± 0.20	1.80 ± 0.20	<0.001
Ileum	2.81 ± 0.21	1.90 ± 0.20	0.01
Mucosal thickness (mm)			
Duodenum	1.159 ± 0.078	0.848 ± 0.074	0.01
Proximal jejunum	1.522 ± 0.063	0.964 ± 0.062	<0.001
Mid jejunum	1.323 ± 0.067	0.899 ± 0.065	<0.001
Ileum	1.121 ± 0.055	0.841 ± 0.053	0.004
Submucosal thickness (mm)			
Duodenum	0.232 ± 0.023	0.171 ± 0.022	0.08
Proximal jejunum	0.218 ± 0.012	0.110 ± 0.012	<0.001
Mid jejunum	0.196 ± 0.012	0.119 ± 0.011	<0.001
Ileum	0.159 ± 0.013	0.133 ± 0.013	0.09
Mucosal surface area index[2]			
Duodenum	0.716 ± 0.042	0.648 ± 0.040	0.26
Proximal jejunum	0.566 ± 0.037	0.516 ± 0.035	0.35
Mid jejunum	0.731 ± 0.048	0.539 ± 0.046	0.015
Ileum	0.776 ± 0.042	0.650 ± 0.042	0.01

[1]Calves fed transition milk (TM, n = 11) or milk replacer (MR, n = 12).

[2]Calculated using the equation of Pyo et al. (2020); mucosal surface area = $[(a \times b) + (a/2 + c/2) - (a/2)^2]/(b/2 + c/2)^2$, where a = villi width (μm), b = villi height (μm), and c = crypt width (μm).

in the ileum (Figure 3; Table 4). Compared with MR, TM tended to increase number of T cells within the epithelial layer of the ileum (30.6 vs. 24.1 T cells/mm, $P = 0.06$).

Compared with MR, TM tended to reduce pH of the digesta in the abomasum (2.71 vs. 3.52, $P = 0.06$) and MidJj (6.08 vs. 6.35, $P = 0.09$). No differences in pH were observed for the digesta of the duodenum, ProxJj, ileum and colon. Treatments did not alter mass of the abomasal contents (TM 1,280 vs. MR 800 g, $P > 0.1$), but TM dramatically increased the mass of curds and concentration of curds within the abomasum compared with MR [524 vs. 0 g, $P < 0.001$ and 45 vs. 0% (wt/wt), $P = 0.001$, respectively].

Compared with MR, TM improved average health scores in the first 4 d after colostrum (Table 5); in fact, TM reduced fecal score 46% ($P = 0.02$) and nasal score 95% ($P = 0.01$) and tended to reduce cough score (62%, $P = 0.06$) and ear score (60%, $P = 0.06$). Eye score was not altered.

No significant differences ($P > 0.2$) of treatment groups for the concentrations of most blood variables were detected before the first milk treatment was consumed. However, compared with MR calves, those fed TM tended to have higher serum total protein (6.1 vs. 5.7 g/dL, $P = 0.08$) and serum haptoglobin (3.8 vs. 3.0 μg/mL, $P = 0.10$) before treatments started. Compared with MR, TM increased average concentrations of serum IgG 20% ($P < 0.001$; Table 6) and serum total protein 11% ($P < 0.001$). Serum IgG decreased 20% from d 2 to d 5 with no interaction of treatment with day. Neither day nor treatment by day was significant for total protein. For blood serum collected on d 3 and 5, the concentrations of LBP and haptoglobin were 38% less ($P < 0.001$) and 46% less ($P = 0.04$) in TM than MR calves.

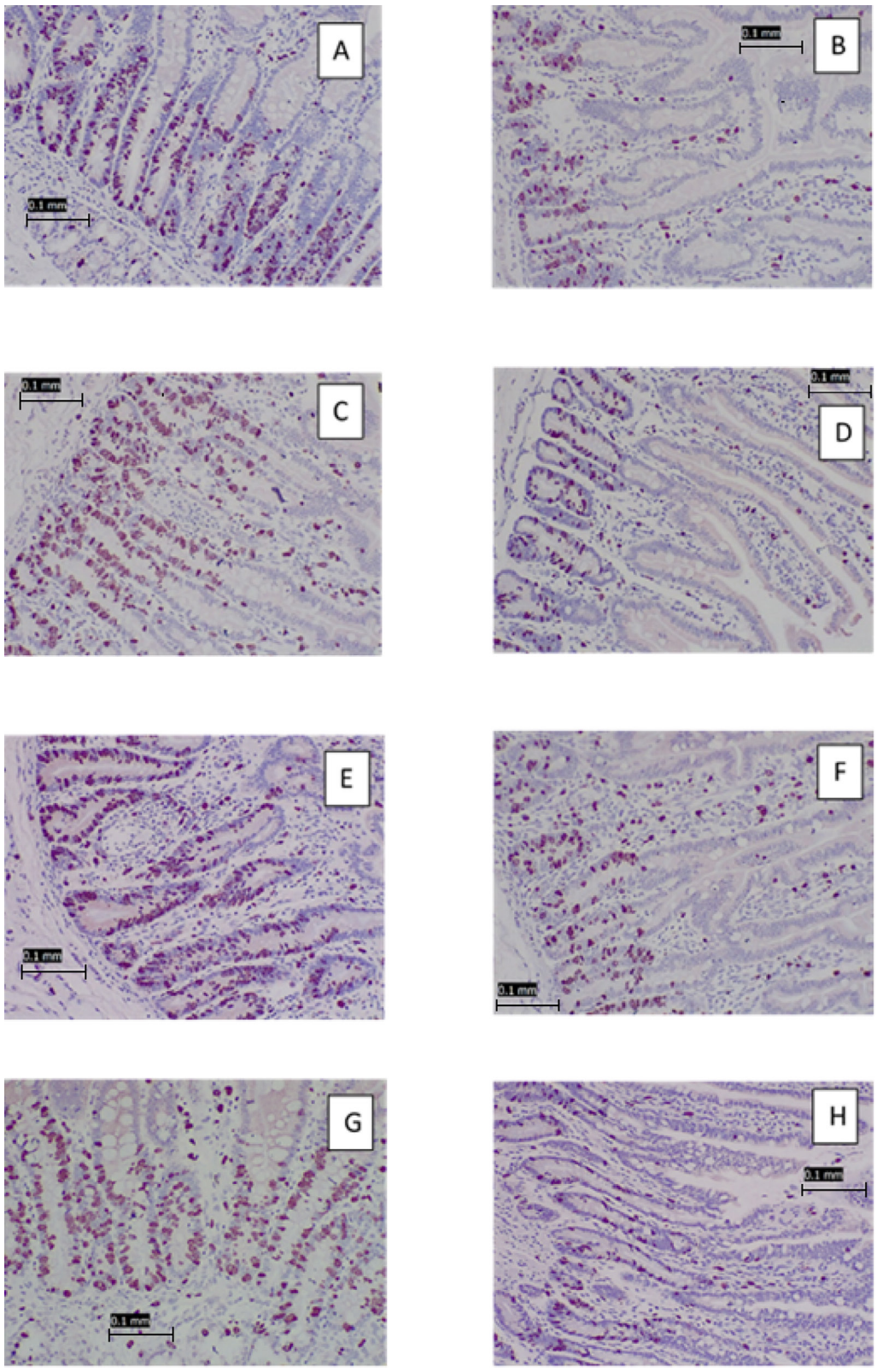

Figure 2. Example views of bromodeoxyuridine (BrdU)-labeled cells in the small intestine. Examples of light micrographs of BrdU-stained duodenum (TM: A and MR: B), proximal jejunum (TM: C and MR: D), mid jejunum (TM: E and MR: F), and ileum (TM: G and MR: H) sections of neonatal calves fed either transition milk (TM) or milk replacer (MR) for the first 4 d of life. Observation was done at 20× magnification. The TM had larger inclusion of BrdU-stained cells in all sections.

Table 4. Bromodeoxyuridine (BrdU)-labeled cells, B cell, and T cell counts in the small intestine (mean ± SE)

Item	Treatment[1]		*P*-value
	TM	MR	
BrdU-labeled cells in the lamina propria[2] (cells/mm^2)			
Duodenum	1,011 ± 83	653 ± 80	0.01
Proximal jejunum	969 ± 63	555 ± 61	<0.001
Mid jejunum	1,122 ± 60	507 ± 57	<0.001
Ileum	901 ± 59	548 ± 58	<0.001
BrdU-labeled cells in the crypt epithelium[3] (cells/mm)			
Duodenum	71.1 ± 2.3	50.9 ± 2.2	<0.001
Proximal jejunum	85.3 ± 2.2	49.7 ± 2.1	<0.001
Mid jejunum	85.0 ± 1.5	47.8 ± 1.5	<0.001
Ileum	70.1 ± 3.3	44.9 ± 3.1	<0.001
Immune cells in the ileum[4]			
B cells (cells/mm^2 Peyer's patches)	4,700 ± 602	3,430 ± 574	0.16
T cells (cells/mm^2 lamina propria)	996 ± 85	811 ± 81	0.15
T cells (cells/mm epithelium)	30.6 ± 2.5	24.1 ± 2.4	0.06

[1]Calves fed transition milk (TM, n = 11) or milk replacer (MR, n = 12).

[2]Cell count of cells/mm^2 of lamina propria in the respective section of the small intestine within the cross section of the microscope slide.

[3]Cell count of cells/mm of crypt epithelium in the respective section of the small intestine within the cross section of the microscope slide.

[4]Cell count of either cells/mm or cells/mm^2 within the ileum epithelium or lamina propria, respectively, within the cross section of the microscope slide.

Treatment did not alter the average concentration of glucose or insulin in prefeeding blood plasma samples from d 2, 3, and 5, but TM tended ($P = 0.08$) to increase the average prefeeeding concentration of NEFA by 16%. The prefeeding concentrations of glucose, insulin, and NEFA averaged for all calves decreased from d 2 to d 5 by 9% ($P = 0.08$), 50% ($P = 0.003$), and 18% ($P = 0.01$), respectively. The interaction of treatment with day was significant ($P < 0.001$) for glucose and insulin but not for NEFA. Glucose concentration before feeding on d 2, 3, and 5 decreased with day for calves fed MR (114, 93, and 85 mg/dL, respectively) but increased for calves fed TM (96, 101, and 105 mg/dL, respectively). Likewise, plasma insulin concentration before feeding on d 2, 3, and 5 decreased with day for calves fed MR (1.05, 0.31, and 0.18 U/L, respectively) but increased for calves fed TM (0.34, 0.48, and 0.52 U/L, respectively). The concentration of glucose in plasma was not different before compared with after feeding, and treatment did not alter the effect of feeding ($P > 0.7$). The concentration of plasma insulin was 66% greater ($P = 0.006$) at 2 h postfeeding compared with 0.5 h prefeeding, and treatment did not alter this effect ($P = 0.3$). The concentration of plasma NEFA averaged for both treatments was not altered by feeding ($P = 0.3$), but NEFA decreased 6% in response to consumption of MR but increased 26% after consumption of TM ($P = 0.12$ for treatment × time interaction).

DISCUSSION

This study definitively showed that intestinal development is increased by feeding calves TM at an equal volume compared with a standard MR, and that this increase occurs in calves that have already consumed 2.8 L of high-quality colostrum within 20 min of birth. Consumption of TM over the next 4 d increases most measures of intestinal development by at least 50%. We found that TM increased width and length of villi and thickness of mucosa and submucosa in all 4 sections of the small intestine but did not alter crypt depth compared with MR. These results are nearly identical to studies showing that feeding colostrum for the first 3 d of life compared with MR or whole milk also increases length and width of villi, thickness of mucosa and submucosa, but not crypt depth, in the small intestine (Blättler et al., 2001; Blum, 2006; Pyo et al., 2020). We also observed increased epithelial cell proliferation in all sections of the small intestine in response to TM, whereas colostrum had mixed effects on proliferation in Blättler et al. (2001) and no effect on proliferation in Pyo et al. (2020), compared with MR. Reasons for this difference are not clear. In our study, TM calves consumed 13% more solids, 30% more ME, 67% more fat, and 29% more protein than MR calves and were slaughtered on d 5. In Blättler et al. (2001), calves fed increasing amounts of colostrum consumed about the

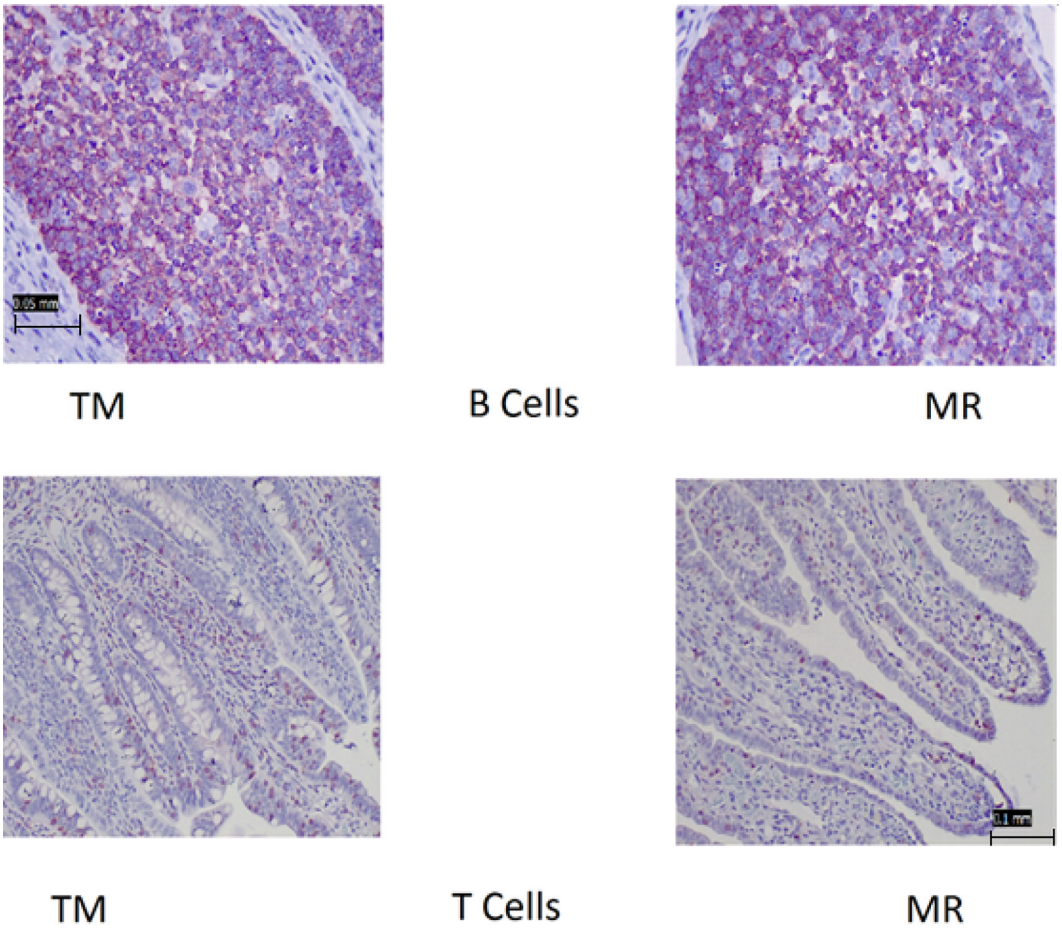

Figure 3. Example view of B-cell and T-cell staining in the ileum. Examples of light micrographs of ileal sections stained for B and T cells in neonatal calves fed transition milk (TM) or milk replacer (MR) for the first 4 d of life. Observation was done at 40× magnification for B cells and 20× magnification for T cells. No difference was observed in the number of B cells in Peyer's patches or the number of T cells in the lamina propria. Transition milk increased the number of T cells in the epithelium of villi.

same amount of energy and nutrients as calves fed MR and were killed on d 7; additionally, in Pyo et al. (2020), calves fed increasing amounts of colostrum compared with MR also consumed more energy and protein and were killed on d 3. Blättler et al. (2001) suggested the increased villi length and unchanged crypt depth are the result of epithelial cell migration to the villus tips from the crypt coupled with reduced apoptosis. We did not measure apoptosis, but almost all of the proliferating epithelial cells in our study were in the crypt. As these cells migrate toward the tips of villi, they would likely be functional epithelial cells that are no longer proliferating.

From our study, we could not determine whether the increased intestinal development of TM calves was due to the higher solids, protein, fat, and energy content of TM compared with MR, to the nonnutritive, bioactive components of TM, or to both. We speculate that we would have seen enhanced intestinal development even if we had fed MR mixed to match the solids or energy content of TM, given that Blättler et al. (2001) also saw enhanced gut development with smaller differences

Table 5. Average health scores from d 2 until slaughter on d 5

	Treatment[2]			
Health variable[1]	TM	MR	SE[3]	*P*-value
Cough	0.13	0.34	0.080	0.06
Fecal	0.55	1.02	0.158	0.02
Nose	0.01	0.19	0.066	0.01
Ear	0.25	0.63	0.106	0.06
Eye	0.04	0.08	0.039	0.56

[1]Scores on a scale of 0 to 3 (0 being healthy, 3 being ill). Scores were taken 3 times a day on d 2, 3, and 4 and once on d 5, and the 10 scores for each variable were averaged for each calf.

[2]Calves fed transition milk (TM) or milk replacer (MR).

[3]SE and *P*-value are according to Friedman's test for nonparametric analysis; this analysis requires balanced data, so block 11 was not used.

Table 6. Blood measurements for d 2 to 5 before feeding

	Treatment[2]			
Variable[1]	TM	MR	SE	*P*-value
IgG (mg/mL)	30.3	25.1	1.5	<0.001
Serum total protein (g/dL)	6.60	5.94	0.12	<0.001
LPS binding protein	1.37	2.07	0.17	<0.001
Haptoglobin[3] (μg/mL)	2.72	5.68	1.24	0.04
Glucose (mg/dL)	101	97	3	0.36
Insulin[3] (U/L)	0.44	0.38	0.05	0.41
Nonesterified fatty acids (μEq/L)	300	259	17	0.08

[1]Average concentrations before feeding on d 2 (1100 h), d 3 (1100 h), and d 5 (0400 h).

[2]Calves fed transition milk (TM) or milk replacer (MR).

[3]Concentrations of haptoglobin and insulin were transformed for analysis to ln scale and back-transformed for reported means.

in milk nutrient density. The MR in our study was fed at the manufacturer recommended density and feeding rate and calves fed either diet refused ~20% of milk in the first 5 feedings; whether they would have consumed more solids if the MR had had a higher percentage of solids is not known.

We did not measure nonnutritive components of TM in our study, but previous research has shown that TM has greater concentrations of hormones, growth factors, and cytokines, as expected for the transition from colostrum to regular milk (Blättler et al., 2001). These bioactive compounds alter proliferation, migration, differentiation, and longevity of epithelial cells, as well as digestion, absorption, and development and function of the immune system (Blum and Baumrucker, 2008). Some peptide hormones, such as IGF-1, are capable of surviving and remaining active in the GIT for at least 20 min, as has been shown in neonatal pigs (Xu et al., 2002); therefore, this supports the idea that the bioactive factors in TM could have localized effects mediated by specific receptors in epithelial cells, endothelial cells, fibroblasts, and smooth muscle cells of the GIT (Howarth, 2003). This localized stimulus from different components of TM could partially explain the increased intestinal development of calves fed TM compared with MR in the present study. Whether this increased intestinal development would benefit the calf is not clear. Calves fed TM had decreased LBP and haptoglobin concentrations compared with calves fed MR; therefore, we suggest this indicates more effective functioning of the intestinal barrier. In addition, the digestibility of liquid feeds increases during the first month of a calf's life (Quigley et al., 2021). Maturation of the gut is likely responsible for this increase, and we suggest that feeding TM instead of MR in the first few days would increase the ability of calves to absorb a greater percent of their diet sooner and grow more efficiently.

For both plasma glucose and insulin, we found that concentrations before feeding were less on d 2 but greater on d 5 for calves fed TM compared with calves fed MR. The explanation for this interaction is likely based on the lower lactose but greater fat and casein concentrations of TM compared with MR. On d 2, both groups refused about 20% of their milk, and TM calves consumed less lactose than MR calves and had less glucose and insulin in blood. On d 3, insulin and glucose concentrations before feeding were not different for the treatments and both groups had similar increases in insulin concentration after feeding with no change in glucose concentration. On d 4 and 5, all calves consumed all of their milk allocation and the lactose content of TM was slightly higher than on d 2. Even though calves fed TM still consumed less lactose than those fed MR on d 5, they perhaps had greater glycogen reserves, better functioning digestive tracts, and systems that were more adaptable to use available nutrients most efficiently.

In this study, we found that calves fed TM grew 0.3 kg/d faster than calves fed MR. Based on NRC (2001) calf growth predictions, almost all of this increase could be accounted for by the increase in estimated ME intake. In addition, calves fed TM had ~0.5 kg more abomasal contents at slaughter (5 h after feeding), so differences in GIT contents also might have accounted for some of the increased gain in TM calves. Based on our previous study, calves fed TM grew 10% faster for the entire preweaning period (Van Soest et al., 2020). Similar increases in gains for the preweaning period were observed for calves fed colostrum twice instead of only once in the first day of life (Abuelo et al., 2021). Thus, we expect the TM calves in this study also would have continued to grow faster throughout the preweaning period had they not been killed.

Calves fed TM had more IgG in serum over the 5 d of study than calves fed MR, as previously observed

for calves fed colostrum once versus 6 times by Hammon and Blum (1998). All calves received high-quality colostrum within the first 20 min of life at the commercial farm, ensuring successful transfer of passive immunity of >10 mg/mL IgG (Jaster, 2005). The first feeding of treatment was offered between 6 and 24 h of birth. Because TM also contains IgG, the calves fed TM therefore received more IgG in the first 24 h of life than those fed MR. Although the IgG of the first feeding of TM would have been less efficiently absorbed, absorption extends out to 24 h after birth (Chigerwe et al., 2008) with essentially no absorption of IgG occurring after 24 h (Weaver et al., 2000). Thus, the greater serum IgG concentrations of calves fed TM was likely the result of the increased IgG consumed on d 1. This benefit of feeding TM likely would have been minimal if all calves had been fed colostrum twice on d 1 instead of only once.

In the current study, calves fed TM had improved health scores, which was somewhat surprising given that all calves were killed on d 5 and were all from a farm with mortality of <2%. Perhaps the hour-long transport to the campus farm within hours of birth stressed the calves. Conneely et al. (2014) also found that eye, ears, and nasal health scores were improved with increased feedings of TM compared with feeding MR, although this effect was for more than just the first 5 d. In our study, TM also increased the number of T cells in the ileal epithelium. This increase in epithelial T cells might prevent illness at the level of the GIT. Norrman et al. (2003) saw a reduction in the number of B cells in Peyer's patches in calves fed colostrum versus formula, but not in the number of T cells in the ileum. In contrast, we observed no difference in the number of B cell within Peyer's patches. Our results agree with David et al. (2003) where the number of B cells in Peyer's patches was similar for calves fed colostrum and calves fed MR. David et al. (2003) speculated that even with the reduced apoptosis and increase proliferation observed in Peyer's patches of calves fed colostrum compared with MR, the number of B cells did not increase due to migration out of the Peyer's patches. Because the size of the villi and thickness of the mucosa and submucosa were all increased by TM compared with MR in this study, the number of immune cells was increased even if no change in the density of immune cells was detected.

CONCLUSIONS

Feeding TM compared with MR in the first 4 d following colostrum immediately after birth improved intestinal development. Calves fed TM, compared with MR, had greater villi length, villi width, mucosal thickness, submucosal thickness, V/C ratio, and epithelial cell proliferation in duodenum, jejunum, and ileum. Compared with MR, TM also improved health scores, elevated IgG concentrations in blood, decreased haptoglobin and LBP concentrations in serum, and increased the number of T cells in the ileal epithelium. Calves fed TM gained 0.64 kg/d compared with 0.34 kg/d for calves fed MR. Although some of the increased gut development might be attributed to the greater energy, fat, and protein intake of TM calves, we suggest that these improvements were at least partly caused by the presence of the bioactive compounds found in TM. We further speculate that improved intestinal development and health would have carry-over benefits and enhance health and growth for most of the critical first 3 wk in the life of a calf, as supported by previous research. These data confirm the recent recommendation of NASEM (2021, p. 232) that "transition milk should be fed during days 2 and 3 if possible."

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