Bovine colostrum improves intestinal function following formula-induced gut inflammation in preterm pigs

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*Non-standard abbreviations: NEC, necrotizing enterocolitis; TPN, total parenteral nutrition; FORM, treatment group receiving infant formula; FCOLOS, treatment group receiving colostrum; FISH, fluorescence in situ hybridization; TLR4, toll-like receptor 4; TNN, tumor necrosis factor alpha; DEFB4A, defensin, beta 4A; SAA, serum amyloid A; B2M, beta-2-microglobulin; ACTB, beta-actin; HPRT1, hypoxanthine phosphoribosyltransferase 1; IL, interleukin; CFU, colony forming units; OA, organic acids.

Methods: After receiving TPN for 2 days, preterm pigs were fed formula (FORM, n = 14), bovine colostrum (COLOS, n = 6), or formula (6 h) followed by bovine colostrum (FCOLOS, n = 14). Intestinal lesions, function, and structure, and abundance and location of bacteria, and inflammation markers were investigated.

Results: NEC severity and interleukins (IL)-1ß and -8 protein concentrations were lower, while villus height, galactose absorption, and brush-border enzyme activities were increased in the distal small intestine in COLOS and FCOLOS pigs, relative to FORM pigs. Intestinal gene expression of serum amyloid A, IL-1ß, -6 and -8, and bacterial abundance, correlated positively with NEC severity of the distal small intestine.

Conclusions: Bovine colostrum restores intestinal function after initial formula-induced inflammation in preterm pigs. Further studies are required to test if bovine colostrum may also benefit preterm infants during the challenging transition from total parenteral nutrition to enteral nutrition, when human milk is unavailable.

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associated with a more difficult and less tolerant transition to enteral nutrition. An abrupt transition to milk formula reduces villus height, mucosa percentage, digestive enzyme activities and nutrient absorption, and increases bacterial adherence, mucosal atrophy and inflammatory cytokine levels within just 8 h of feeding in preterm pigs. In contrast, preterm pigs fed porcine or bovine colostrum are less affected by NEC. This may be due to the high content of bioactive compounds such as growth factors, antioxidants, antimicrobial and immune-modulatory factors in colostrum, which may support intestinal maturation, balance and priming of the immune system and establishment of a beneficial gut microbiota. It is known that colostrum given as minimal enteral nutrition may prevent the inflammatory cascade leading to NEC lesions in preterm pigs, while human milk may reduce NEC in preterm infants compared with milk formula. However, the ability of colostrum to regenerate an already compromised gut exposed to a short period of formula feeding is unknown. Knowledge about the possible therapeutic effect of colostrum on an intestine initially fed formula is important, because lack of mother’s milk following preterm birth leads to variable periods of formula feeding. Thus, there is a need to know to which extent mother’s own milk, or a possible substitute bioactive product like bovine colostrum, may help to suppress the pro-inflammatory state of the immature intestine resulting from a few days of TPN followed by a period of formula feeding.

We hypothesized that the dysfunction, induced by the combination of TPN with an abrupt transition to milk formula, is reduced by feeding bovine colostrum just after an initial formula feeding period. We used a preterm pig model of NEC to investigate the effects of bovine colostrum on intestinal structure, digestive and absorptive functions, microbiota, and plasma and tissue proteins and tissue mRNA levels of inflammatory markers. A group of preterm pigs was fed formula followed by colostrum and was compared with a group of preterm pigs fed only formula (negative control) after the TPN period. Values from these groups were also compared with a third group of preterm pigs fed only colostrum which according to our previous studies protects against NEC.

2. Materials and methods

2.1. Animals and their treatment

Thirty-four preterm pigs were delivered from four sows by caesarean section (Large White × Danish Landrace × Duroc, Askelygaard Farm, Roskilde, Denmark) at 105–107 days gestation (90–92% gestation). The procedures for caesarean section, passive immunization (maternal serum given three times: 4, 5, and 7 ml/kg body weight during the first 24 h after birth) and nursing of the preterm pigs followed a standard protocol. During the first 48 h, pigs were given TPN through a vascular catheter (advancing from 4 to 6 ml/kg/h). The TPN solution was based on Nutriflex Lipid Plus (Braun, Melsungen, Germany) and adjusted in nutrient composition to meet the requirements of pigs. After the TPN period, the pigs were stratified according to birth weight into three total enteral nutrition groups fed either milk formula (FORM, n = 14), 6% milk formula followed by bovine colostrum (FCOLOS, n = 14), or bovine colostrum (COLOS, n = 6) until euthanasia. The COLOS group was included as a reference group confirming the protective effects of exclusive feeding with bovine colostrum shown in a previous study. The feeding dose for all groups was 15 ml/kg body weight/3 h. The milk formula contained 80 g Peptide, 70 g Maxipro, and 75 g Liquigen/l of water, resulting in a dry matter content of 18.8% (all products kindly donated by Nutricia, Allerød, Denmark). Bovine colostrum was obtained from the first milking after parturition (kindly donated by Biofibr-Damino, Gesten, Denmark), sterilized by gamma-irradiation (1 × 10 kGy; Sterigenics, Espetgårde, Denmark) and stored at −20°C. Before use, the colostrum was diluted in tap water to a dry matter content of 14.2%. Both products were warmed to body temperature in a water bath. All animal protocols and procedures were approved by the Danish National Committee on Animal Experimentation (Licence: 2004/561-910).

2.2. Clinical evaluation and tissue collection

Pigs were monitored closely for clinical symptoms of NEC such as abdominal distension, lethargy, cyanosis and bloody diarrhea. Pigs suffering from NEC before the end of the study protocol were immediately euthanized and the tissue collected according to earlier protocols. Pigs not showing clinical signs of NEC were euthanized at day 2 of enteral feeding and the tissue collected. All pigs were given a NEC severity score ranging from 1 (no or minimal focal hyperemic gastroneterolitis) to 6 (severe extensive hemorrhagic and necrotic gastroenterolitis) in the proximal, middle and distal small intestine, stomach and colon as previously described. Pigs with a severity score of 3 or more in any gastrointestinal region were considered suffering from NEC. A mean NEC severity score was calculated as the mean of the NEC severity score across the intestinal regions. To determine small intestinal enzyme activities, gene expression, and cytokine concentrations, full thickness tissue samples from the small intestinal regions were immediately snap-frozen in liquid nitrogen and stored at −80°C. A 10 cm segment of each small intestinal region was used to measure intestinal circumference and to determine the proportion represented by mucosa according to Bjørnsvad et al. Samples from the distal small intestine were collected and fixed in 4% neutral buffered paraformaldehyde for 24 h and transferred to 70% ethanol before preparation for fluorescence in situ hybridization (FISH) and evaluation of gut morphology.

Blood collected at euthanasia was used for later determination of inflammatory factors (see below). All blood samples were collected in EDTA- or heparin coated tubes, placed on ice, centrifuged for 10 min at 4°C and 2500 g and the plasma was stored at −20°C until further analyses.

2.3. Gut morphology and intestinal function

Distal small intestinal villus height and crypt depths were evaluated on scanning pictures obtained from FISH analysis using the morphometric software SoftWoRx Explorer version 1.2.0 (Applied Precision, Issaquah, WA, USA). One representative cross-section was selected from each pig and 10 representative villi and crypts were measured. Intestinal function was evaluated by measuring enzyme activities of dipeptidylpeptidase IV, aminopeptidase N, aminopeptidase A, lactase and maltase according to Sangild et al. Finally, trehalase activity was measured as described for lactase and maltase using 0.6 M D-(-)trehalose dihydrate (EC 202-739-6, Sigma-Aldrich, Brandby, Denmark) as a substrate. In vivo plasma galactose concentrations were measured according to a previous study before enteral nutrition (0 h) and at 6 and 30 h after enteral food introduction by collection of a blood sample 20 min after an oral bolus (15 ml/kg, 5% galactose) via an oro-gastric feeding tube. The results from each treatment group at 6 h and 30 h were compared with the baseline level at 0 h. The urinary ratio of lactulose to mannitol, to estimate gut permeability, was determined by giving an oral bolus of 5% lactulose and 2% mannitol (15 ml/kg) 4–6 h prior to euthanasia as previously described.

2.4. Gene expression analyses of inflammatory factors

The expression of proinflammatory markers IL1B, IL6, IL8, toll-like receptor 4 (TLR4), tumor necrosis factor alpha (TNF), anti-inflammatory marker IL10, defensin beta 4A (DEFB4A), acute
phase protein serum amyloid A (SAA), and reference genes beta-2-microglobulin (B2M), beta-actin (ACTB) and hypoxanthine phosphoribosyltransferase 1 (HPRT1) in distal small intestinal tissue was determined using quantitative real-time PCR. Total RNA was extracted using RNeasy Lipid Tissue Midi kit (Qiagen, Ballerup, Denmark) and on-column DNAse treated using RNase-free DNase set (Qiagen) according to manufacturer’s protocol. Quantity and quality of extracted total RNA were measured as described previously.18 One μg of total extracted RNA was converted into cDNA using QuantiTect Reverse Transcription Kit (Qiagen) according to manufacturer’s instructions. Quantitative PCR was performed using diluted cDNA (1:5 or more) in a total reaction volume of 25 μl containing gene-specific primers (300 nM), SYBR Green PCR Master Mix (Sigma–Aldrich) and MgCl2 (Table 1). PCR cycling was performed on a RotorGene 3000 Detection System (Corbett Research, Sydney, Australia) using the following conditions: 2 min at 50 °C, 10 min at 95 °C, followed by 40 cycles with 15 s at 95 °C and 1 min at 59–62 °C (Table 1). Primer design and validation were performed as described by Skovgaard et al.,18 and primers were synthesized at TAGC Copenhagen, Copenhagen, Denmark. All PCR reactions were performed in triplicate and outliers excluded using Grubb’s test.19 Non-template controls were included in each run to control for contamination and nonspecific amplification problems. Standard curves of samples expressing the gene of interest at a high level were included to assign primer efficiency and dynamic range. The primer efficiency for the standard curves ranged between 0.85 and 1.10 with a correlation coefficient >0.99. Interrun calibration was performed based on several samples. Expression levels of all samples were normalized to the normalization factor calculated in geNorm20 based on the geometric mean of three most stably expressed reference genes: B2M, ACTB, and HPRT1 (Table 1). To assess differential gene expression between the three treatment groups the mean for the COLOS group was set to one, and the mean of the gene in question from the three other groups was displayed as relative expression compared to the COLOS group.

2.5. Levels of circulating and tissue inflammatory factors

A multiplex bead based assay was used to determine the concentrations of interleukin (IL)-6, IL-8 and IL-10 in distal small intestinal samples and in plasma samples, as previously described,21 with analysis of IL-1β added to the assay. Briefly, magnetic beads coupled with the specific antibodies were incubated with plasma samples or intestinal homogenates (homogenized in 1% Triton X-100; 6 ml/g wet tissue). For plasma analysis, standards, controls and samples were diluted in assay buffer, to which was added 10% filtered and heat inactivated (56 °C for 30 min) normal porcine plasma. Biotinylated antibodies were added followed by wash and incubation with phycoerythrin-labeled streptavidin, the samples were analyzed on a Luminex100 (Bio-Rad, Hercules, CA, USA) and the data was analyzed using BioPlex software Manager 4.1.1 (Bio-Rad Laboratories, Copenhagen, Denmark). The results were expressed relative to total protein concentration in each sample determined by spectrophotometry (E280).

Plasma concentrations of SAA were determined using a sandwich enzyme-linked immunosorbent assay kit according to the manufacturer’s instructions (Tridelta Developments Ltd. Maynooth, County Kildare, Ireland).

2.6. Microbiology and organic acids

The luminal contents of the distal small intestine were immediately kept on ice and plated on C-calves blood agar (SSI Diagnostica, Hillerød, Denmark), and incubated under anaerobic and aerobic conditions at 37 °C for 24 h to determine the number of colony forming units (CFU)/g luminal content. The abundance and location of bacteria in distal small intestinal tissue were evaluated using FISH. For each pig, one SuperFrost/plus slide (Menzel-Gläser, Braunschweig, Germany) with cross-sections of 3 μm formalin-fixed and paraffin-embedded distal small intestinal tissue samples was deparaffinized in xylene, and dehydrated in 99% ethanol. Hybridization was performed using an Alex-Fluor 555-oligonucleotide probe targeting 16S rRNA in most bacteria (5’-GCT GCC TCC CGT AGT-3’). Eurofins MWG Operon AG, Eberswalde, Germany) according to Mølbak et al.23 with minor modifications. The slides were scanned on an ArrayWoRx® microarray scanner (Applied Precision, Issaquah, WA, USA) and one representative cross-section was selected from each pig and evaluated using Adobe Photoshop version C4 (Adobe Systems, San Jose, CA, USA). All slides were evaluated using the two following

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a Annealing temperature.

b MgCl2 is the total concentration of MgCl2 in the reaction mix.
semi-quantitative scores: Bacterial abundance: 1 = No or very few microcolonies; 2 = Few microcolonies; 3 = Intermediate number of microcolonies; 4 = Abundant number of microcolonies; 5 = Extensive colonization. Bacterial location was scored as follows: 1 = Present only in the lumen; 2 = Present in the lumen and in association with the tip of the villus; 3 = Present in the lumen and between villi; 4 = Present in the lumen and between villi and translocation to muscularis mucosae.

As a marker of bacterial metabolic activity, the concentrations of 15 organic acids (OA) were measured in stomach and colon contents as described by Canibe et al., however only one gram gastrointestinal sample was used in the extraction procedure.

2.7. Statistical analysis

Group differences in NEC incidences were evaluated pair wise using Fisher’s exact test. All other statistical analyses were based on parametric ANOVA using the MIXED model of SAS with treatment and region as fixed variables and litter as random variable (SAS Enterprise Guide 3.0, SAS Institute), or non-parametric Kruskal–Wallis or Mann–Whitney test (SAS or GraphPad Prism 5, Version 5.02, La Jolla, CA, USA). Correlation analyses were performed in GraphPad Prism 5 using Spearman correlation according to Zar. Results are presented as means with standard error of the mean (SEM) and p < 0.05 was used as the critical level of significance.

3. Results

3.1. Clinical outcome, organ dimensions and intestinal function

The NEC severity score was lower in COLOS pigs and tended to be lower in FCOLOS pigs (p = 0.07, Fig. 1a), relative to FORM pigs. The incidence of NEC did not differ significantly among groups (FORM: 71%, 10/14; FCOLOS: 64%, 9/14; COLOS: 50%, 3/6). Mean daily weight gain was higher in FCOLOS pigs (21 ± 6 g/d, p < 0.01) than in FORM pigs (6 ± 3 g/d), with COLOS pigs being intermediate (17 ± 2 g/d).

Intestinal villus height was lower in FORM relative to FCOLOS pigs, with intermediate values in COLOS pigs (Fig. 1b), and small intestinal circumference was higher in FORM (10.3 ± 0.2 mm) relative to FCOLOS pigs (9.6 ± 0.13 mm, p < 0.01), with values in COLOS pigs being in-between (9.7 ± 0.2 mm). The two groups fed colostrum had a higher proportion of dry mucosa in the small intestine (0.70 ± 0.01, pooled values), relative to the FORM group (0.65 ± 0.02, p < 0.05).

The galactose absorption capacity (Fig. 2) decreased in the FCOLOS group after the initiation of enteral feeding. With subsequent colostrum feeding in the FCOLOS group, the values increased to reach the levels seen at 0 h and in the COLOS group at 30 h of enteral feeding, while the value in FORM decreased further. The digestive capacity, indicated by small intestinal enzyme activities across the three small intestinal regions, were increased for both colostrum groups (COLOS, FCOLOS) relative to the FORM group for
four of the six enzymes measured (Fig. 1c–f), while FCOLOS had a higher maltase activity than COLOS and FORM (Fig. 1g). Only trehalase activity did not show any difference among the groups (0.30 ± 0.02 U/g of wet tissue, pooled values). Intestinal permeability measured as the ratio of lactulose to mannitol did not differ among the three groups (0.12 ± 0.04). No differences were observed between the groups in the following parameters (pooled values): body weight at birth (919 ± 28 g) and at euthanasia (973 ± 28 g), total life span (89 ± 2 h), relative length of the small intestine (308 ± 9 cm/kg body weight), distal small intestinal crypt depth (93 ± 4 μm), total small intestinal area (303 ± 11 cm²), or relative weights (to body weight, g/kg) of stomach (7.0 ± 1.5), lungs (20.3 ± 1.0), heart (79 ± 0.2), kidneys (10.1 ± 0.3), and spleen (2.1 ± 0.1).

3.2. Tissue and circulating cytokine mRNA and protein levels

In distal small intestinal tissue, the expression of IL6, IL8, IL10, DEFB4A, TLR4, TNF, and SAA did not differ between the three groups, while IL1B were lower in FORM compared with FCOLOS (Table 2). Positive correlations were observed between the NEC severity score of the distal small intestine and the expression of IL1B (r = 0.532, p < 0.01), IL6 (r = 0.361, p < 0.05), IL8 (r = 0.521, p < 0.01), and SAA (r = 0.398, p < 0.05), whereas no correlation were observed with IL10, DEFB4A, TLR4, and TNF (not shown).

The protein concentrations of IL-1β in the distal small intestine were higher in FORM pigs than in COLOS and FCOLOS pigs (Fig. 1h). Furthermore, the IL-1β tissue concentration correlated positively with the NEC severity score of the distal small intestine (r = 0.450, p < 0.01) and with the expression of IL1B in tissue (r = 0.656, p < 0.001). The protein concentrations of IL-8 were higher in FORM than FCOLOS, while the difference between FORM and COLOS was p = 0.062 (Fig. 1i). No significant correlations were found for the protein concentration of IL-8 and the NEC severity score of the distal small intestine or the expression of IL8 (not shown). The distal small intestinal concentrations of IL-6 and IL-10 were generally below detection limit and hence no results are shown for these cytokines. Plasma levels of IL-10 (58.1 ± 6.2 pg/ml, pooled values) and SAA (81 ± 12 μg/ml, pooled values) showed similar values across the three groups, and no correlation was observed between either IL-10 or SAA, and the NEC severity score of the distal small intestine (not shown). The plasma levels of IL-1β, IL-6, and IL-8 were generally below the detection limit (not shown).

3.3. Microbiology and organic acid concentrations

The luminal concentration of bacteria in the distal small intestine was in the range of 10⁹ CFU per g contents and did not differ among the three groups neither for anaerobic bacteria (6.3 × 10⁸ ± 2.0 × 10⁸ CFU/ml luminal content, pooled values) nor aerobic bacteria (4.4 × 10⁸ ± 1.5 × 10⁸ CFU/ml luminal content, pooled values). Likewise, FISH analyses of the distal small intestinal mucosa-associated microflora revealed no differences in the score for bacterial abundance (1.9 ± 0.2, pooled values) or location (2.2 ± 0.2, pooled values) among the three groups (Fig. 3a, b). However, a positive correlation between the NEC severity score of the distal small intestine and the bacterial abundance score (r = 0.524, p < 0.01) was observed. The total concentration of OA in stomach contents (Fig. 4a) did not differ between groups. Of the individual organic acids, octanoic acid was lower in the COLOS group than in the FORM group, with FCOLOS group being intermediate. Furthermore, the concentration of butyric acid in the stomach was lower in FORM compared with FCOLOS and COLOS, which were similar.

The concentration of OA in the colon contents (Fig. 4b) was higher in the FORM group compared with values of the two colostrum groups combined (pooled values, p < 0.01). Furthermore, a significantly higher concentration of lactic acid was observed in the FORM pigs compared with COLOS and FCOLOS pigs.

4. Discussion

We have previously shown that an abrupt transition from parenteral to moderate amounts of an enteral diet, especially formula, induces an intestinal dysfunction that may predispose to development of NEC, at least in preterm pigs. Changes to intestinal structure and function occur within just 8 h of enteral feeding and are much more pronounced with formula, relative to sow’s colostrum, and we now document that bovine colostrum feeding reverses the immediate induction of intestinal dysfunction caused by a short term (6 h) feeding with moderate amounts of formula to preterm TPN-fed pigs. This was seen by the observed tendency toward lower NEC severity, lower intestinal circumference (indicating better muscular tone), increased villus height, enzyme activities and hexaside absorption and lower tissue IL-1β and IL-8 levels and colonic lactic acid production in the FCOLOS group, relative to the FORM group. The colostrum diet had this effect even when it was obtained from another species (bovine) and after sterilization by gamma-irradiation. Further studies will show whether bovine colostrum could be considered as a supplemental diet also for preterm infants during the challenging parenteral-enteral nutritional transition.

As in previous studies, formula-fed pigs had an increased severity of NEC, compared with pigs fed bovine colostrum.
Colostrum feeding did not fully protect against NEC, as a mild degree of NEC was observed in both colostrum groups. This was not surprising, as it has previously been documented in preterm pigs that bovine colostrum only protects 100% against NEC when provided already from the time of birth, and in slowly increasing volumes. An abrupt transition to enteral colostrum feeding after TPN may also be associated with a higher incidence of NEC-like lesions in the stomach region and indeed, two of the three NEC-diagnosed COLOS pigs had high NEC scores only in the stomach region. This contributed to a higher rating of these on the severity score, and thus increased the severity and incidence of NEC in the COLOS group. If stomach lesions were not included in the severity score the incidence of NEC in the COLOS group would decrease to 17% (1/6) in contrast to 50% (3/6) resulting in a significantly lower incidence of NEC than in the FORM group (p < 0.05). Across all three groups, there was a tendency toward higher amounts of the cytotoxic agent, butyric acid, in the stomach contents from pigs suffering from NEC (p = 0.06, data not shown) and in the COLOS group, only pigs suffering from lesion in the stomach had significantly higher amount of butyric acid in the stomach than healthy colostrum pigs (p < 0.01, data not shown).

The overall higher NEC severity in the FORM group was accompanied by a reduced activity of five brush border enzymes relative to the colostrum groups. While the direct diet effects have been demonstrated previously, the present study proves for the first time that colostrum feeding can reverse the diet-induced effects on enzyme activities known to take place already within the first hours of formula feeding. Results that provided further support for the therapeutic effect of colostrum feeding, were the galactose absorption tests. As expected, the absorption capacity fell in the FCOLOS and FORM groups during the first 6 h after initiation of enteral feeding, whereas the absorption capacity of galactose in COLOS pigs remained at the 0-h level during this period. Thus, as demonstrated previously, the initial formula feeding appears to induce a very rapid decrease in the function of the sodium-glucose linked transporter. The decrease was reversed by colostrum feeding since the galactose level in FCOLOS pigs at 30 h after start of feeding was similar to that in COLOS pigs, while the level decreased further in FORM pigs at 30 h. The improved digestive and absorptive capacity not only removes more nutrients from the intestinal lumen but also reduces the amount of substrate available for bacterial fermentation. This can explain that the concentration of colonic lactic acid was reduced in both colostrum groups, relative to formula. Colostrum feeding resulted in a decrease of the formula-induced increases in both IL-1β and IL-8 production in the distal small intestine. A previous study showed that feeding sow’s

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**Fig. 3.** Representative scanning photos from FISH analysis applied to cross-sections of the distal small intestine of preterm pigs: Bacterial abundance score = 1, and distal small intestinal NEC severity score = 1 (a) and bacterial abundance score = 5 (red color, white arrows), and distal small intestinal NEC severity score = 5 (b). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

**Fig. 4.** Total concentration and the concentration of the dominating OAs in stomach contents (a) and colon contents (b) in FORM pigs (white), COLOS pigs (gray), and FCOLOS pigs (black). All data are presented as means ± SEM, and different superscript symbols indicate significant differences (p < 0.05).
colostrum to preterm pigs down-regulates IL-1β and the initial feeding-induced intestinal lesions relative to formula,10 and this study shows that bovine colostrum has a similar effect. The group differences seen at the protein level for IL-1β and IL-8 were not consistently observed also at the mRNA level, and for IL-1β a higher tissue expression level in the FCOLOS group was observed, compared with the FORM group. However, this higher expression of IL-1β in the FCOLOS group was caused by two pigs suffering from severe NEC (relative gene expression >150). The lack of consistent correlations between protein- and mRNA levels of cytokines and inflammatory markers may be due to differences in the time-related onset of the expression of the mRNA and the following production of the protein.27 The positive correlation between the expression of IL-1β, IL6, IL8 and SAA and the NEC severity score of the distal small intestine suggests that the expression of these inflammatory factors may be more correlated to disease progression than to the type and amount of nutrition (colostrum, formula). No correlation was observed between the IL-10 and SAA levels in plasma and the NEC severity score of the distal small intestine. Plasma SAA levels have been found to correlate with NEC disease progression in infants.28,29 However, acute phase proteins like SAA may behave very differently in different species.30 Furthermore, protein levels of IL-1β, IL6 and IL-8 in plasma were below detection limits and therefore could not be used as biomarkers for NEC in preterm pigs. The results demonstrate that the use of cytokines and other inflammatory mediators as markers for diet-related effects on gut disease progression must be interpreted with caution, and that the results depend on the type of sample (plasma or tissue) and the level of analyses (mRNA or protein).

The density of the gut microbiota in the distal small intestine, as well as the bacterial attachment to the mucosa, was not affected by diet. However the NEC severity score of the distal small intestine correlated positively with the bacterial abundance score. Thus, bacterial density at the tissue surface may therefore be more associated with the progression of NEC than with the nature and amount of the enteral diet as also indicated by a previous study.12 The trend toward higher total amount of colonic OA in the FORM group suggests that formula feeding resulted in a higher microbial activity in the colon. Thus, colostrum does not suppress bacterial density and mucosal attachment, but rather support tissue responses toward feeding and bacteria. Our study underlines the importance of diet composition in the first enteral feedings to preterm neonates to promote intestinal maturation and resistance against NEC. It remains to be investigated whether bovine colostrum could be used as a safe and effective substitute product to preterm neonates to promote intestinal maturation and resistance against NEC. The study was partly supported by Biofiber-Damino, which also donated the colostrum used in the study. This commercial partner had no influence on any decisions regarding study design, interpretation of results and conclusion of the study. University of Copenhagen has submitted a patent application for the use of bovine colostrum in paediatric patients.

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References


