

## Research Article

# Gene Expression Analysis of the IPEC-J2 Cell Line: A Simple Model for the Inflammation-Sensitive Preterm Intestine

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The IPEC-J2 cell line was studied as a simple model for investigating responses of the newborn intestinal epithelium to diets. Especially, the small intestine of immature newborns is sensitive to diet-induced inflammation. We investigated gene expression of epithelial- and immune response-related genes in IPEC-J2 cells stimulated for 2 h with milk formula (CELL-FORM), colostrum (CELL-COLOS), or growth medium (CELL-CONTR) and in distal small intestinal tissue samples from preterm pigs fed milk formula (PIG-FORM) or colostrum (PIG-COLOS). High throughput quantitative PCR analysis of 48 genes revealed the expression of 22 genes in IPEC-J2 cells and 31 genes in intestinal samples. Principal component analysis (PCA) discriminated the gene expression profile of IPEC-J2 cells from that of intestinal samples. The expression profile of intestinal tissue was separated by PCA into 2 groups according to diet, whereas no diet-dependent grouping was seen for IPEC-J2 cells. Expression differences between PIG-FORM and PIG-COLOS were found for *DEFB1*, *CXCL10*, *IL1RN*, and *ALPI*, while *IL8* was upregulated in CELL-FORM compared with CELL-CONTR. These differences, between IPEC-J2 cells and intestinal tissue from preterm pigs, both used as models for the newborn intestine, underline that caution must be exercised prior to analysis and interpretation of diet-induced effects on gene expression.

## 1. Introduction

The intestine is the site for nutrient digestion and absorption, but also a major immunological defense barrier that recognizes and responds to external antigens. In addition to the gut mucosal immune system, intestinal epithelial cells (IECs) are involved in the initiation and coordination of the intestinal immune response by the production of signaling molecules including cytokines and chemokines [1]. The IPEC-J2 cell line originates from the jejunum of an unsuckled neonatal pig [2] and is morphologically and functionally similar to IECs with microvilli and tight junctions as well as expression and production of cytokines, defensins, toll-like receptors, and mucins [2–4]. It has previously been used to investigate host-pathogen interactions and immune responses with relevance for human and swine intestinal diseases [2, 3, 5–8] and could

be a simple *in vitro* model to investigate the immune response of IECs in newborns.

Necrotizing enterocolitis (NEC) is a serious gastrointestinal disease in preterm infants arising from the combined effect of prematurity, abnormal bacterial colonization, and enteral feeding. In particular, feeding with milk formula predisposes to NEC, whereas human milk is protective [9, 10]. We set out to evaluate the IPEC-J2 cell line as an *in vitro* model for the preterm neonatal intestine and as a supporting model for the well-established preterm pig model of NEC, which is a valuable model for investigation of diet-induced effects [11–14]. In the preterm pig model of NEC, milk formula-fed preterm pigs have more NEC lesions compared with preterm pigs fed porcine or bovine colostrum, rich in growth- and immuno-modulatory factors [11, 15, 16].

Compared with animal models, cell line studies are less cost intensive, associated with no ethical concerns, and provide a highly-controlled simple model to investigate isolated factors, for example diet, on the IECs response. The IPEC-J2 cell line has already been shown to be a valuable model for the investigation of host-pathogen interactions and could also be a promising model for *in vitro* studies of innate immune functions of neonatal IECs in response to dietary stimuli. In this study, we evaluated the potential of the IPEC-J2 cell line as an *in vitro* model to study diet-induced effects on the preterm neonatal intestine. We analyzed and compared the expression of epithelial- and immune response-related genes in the IPEC-J2 cell line and in preterm pig intestinal tissue.

## 2. Materials and Methods

**2.1. IPEC-J2 Cell Line Study.** IPEC-J2 cells [2] were maintained in Dulbecco's modified eagle medium (DMEM)/Ham's F-12 (1:1) supplemented with 10% fetal calf serum, penicillin (100 U/mL), streptomycin (100 µg/mL), pyruvate (1 mM), and L-glutamine (2 mM) (growth medium, Sigma-Aldrich, Brøndby, Denmark) in a humidified atmosphere of 5% CO<sub>2</sub> at 37°C. Passage was performed approximately once a week, and cells between passages 82–85 were used in the experiment. Prior to the experiment, the cell culture was found to be free of mycoplasma.

For the experiment, cells were transferred to Corning Transwell-COL collagen coated-membrane (0.4 µm pore size; Sigma-Aldrich) and grown in growth medium supplemented with epidermal growth factor (5 mg/mL; Sigma Aldrich) and insulin-transferrin-selenium (5 µg/mL of each; Sigma-Aldrich) until stable transepithelial electric resistance values, measured with an EVOM—Epithelial VoltOhmmeter (World Precision Instruments, Berlin, Germany), were reached after approximately 12 d. The cells were stimulated for 2 h in three different diet solutions: growth medium alone (CELL-CONTR,  $n = 4$ ), growth medium containing 1% gamma-irradiated bovine colostrum (CELL-COLOS,  $n = 4$ ), or growth medium containing 1% milk formula (CELL-FORM,  $n = 4$ ). These conditions were selected based on results from preexperiments testing cell viability under different concentration of colostrum and periods of time. The colostrum and milk formula were identical to those used in the pig study (see later). The lipid fraction and cellular debris were removed by centrifugation at 3500 rpm for 20 min at 4°C. After stimulation, the cells were gently washed twice with Dulbecco's phosphate buffered saline (D8537, Sigma-Aldrich), harvested, and stored at –80°C.

**2.2. Preterm Pig Study.** Nineteen preterm pigs were delivered from four sows by Caesarean section (Large White × Danish Landrace × Duroc, Askelygaard Farm, Roskilde, Denmark) at 105–107 d of gestation (90%–92% gestation). Procedures for Caesarean section and nursing of the preterm pigs followed a standard protocol previously described in details [11, 12]. The pigs were given total parenteral nutrition through a vascular catheter (4 mL/kg/h advancing to 6 mL/kg/h) for 2 d based on Nutriflex Lipid Plus (Braun, Melsungen, Germany) and

adjusted in nutrient composition to meet the requirements of pigs as described by [11]. Hereafter, pigs were randomized according to birth weight into two enteral nutrition groups: gamma-irradiated (1 × 10 kGy, Sterigenics, Espergærde, Denmark) bovine colostrum (PIG-COLOS,  $n = 6$ ; kindly donated by Biofiber-Damino A/S, Gesten, Denmark) or milk formula (PIG-FORM,  $n = 13$ ; 80 g Pepdite, 70 g Maxipro, and 75 g Liquigen per L of water, all products kindly donated by Nutricia, Allerød, Denmark). Bovine colostrum was collected from the first milking after parturition. The products were stored at –20°C and warmed to body temperature in a water bath before feeding to the pigs (feeding dose interval: 15 mL/kg body weight/3 h). Colostrum was diluted in tap water to obtain the same dry matter content as in the milk formula before use. All pigs were euthanized within the first 50 h after initiation of enteral nutrition according to earlier protocols [16], and tissue samples from the distal small intestinal region were immediately snap-frozen in liquid nitrogen and stored at –80°C. All animal protocols and procedures were approved by the Danish National Committee on Animal Experimentation.

**2.3. Gene Expression Analysis.** Total RNA from IPEC-J2 cells was extracted using RNeasy Mini kit (Qiagen, Ballerup, Denmark) and on-column DNase treated using RNase-free DNase set (Qiagen) according to manufacturer's protocol. Distal intestinal tissue was homogenized, and total RNA was extracted using RNeasy Lipid Tissue Midi kit (Qiagen) and on-column DNase treated using RNase-free DNase set (Qiagen) according to the manufacturer's protocol. Purity of extracted total RNA was assessed using UV absorption spectrums including OD 260/280 and OD 260/230 ratios on a Nanodrop ND-1000 spectrophotometer (Saveen Biotech, Aarhus, Denmark), and total RNA was quantified at OD 260. Quality (integrity) of extracted total RNA was determined using on-chip electrophoresis on an Agilent 2100 Bioanalyzer (Agilent Technologies, Nærum, Denmark). An RNA integrity number was assigned to each sample using the 2100 Expert software (Agilent Technologies, v.B.02.01).

Extracted RNA was converted into cDNA by reverse transcription of 500 ng total RNA using the QuantiTect Reverse Transcription kit (Qiagen), containing a mix of random primers and oligo-dT, according to the manufacturer's instructions. cDNA was diluted 1:7 in low EDTA TE-buffer (VWR—Bie & Berntsen, Herlev, Denmark) prior to pre amplification. Pre amplification was performed using TaqMan PreAmp Master Mix (Applied Biosystems, Nærum, Denmark). Stocks of 200 nM primer mix were prepared combining equal concentration of all primers used in the present study (Table 1), and in the following the genes will be denoted by their gene symbol (Table 1). TaqMan PreAmp Master Mix (5 µL) was mixed with 2.5 µL 200 nM stock primer mix and 2.5 µL diluted cDNA and incubated at 95°C in 10 min followed by 16 cycles of 95°C in 15 sec and 60°C in 4 min. Pre amplified cDNA was diluted at least 1:4 in low EDTA TE-buffer (VWR—Bie & Berntsen) before qPCR.

Primers were designed using Primer3 (<http://frodo.wi.mit.edu/>) as described in [17] and synthesized at TAG

TABLE 1: Gene symbol, forward and reverse primer sequences, and amplicon length.

Gene symbol	Sequence (5'-3')	Amplicon length
<i>ACTB</i>	F: CTACGTCGCCCTGGACTTC R: GCAGCTCGTAGCTCTTCTCC	76
<i>ALPI</i>	F: TCCCAGACATAACAACGTGGA R: GGTCTGGTAGTTGGCCTTGA	90
<i>AOAH</i>	F: GTAATGGCATTGTTGGGGTGTC R: TCTCCCAGCAAAATGATTCC	97
<i>APOA1</i>	F: GTTCTGGGACAACCTGGAAA R: GCTGCACCTTCTTCTTCACC	86
<i>CCL2</i>	F: GCAAGTGTCTAAAGAAGCAGTG R: TCCAGGTGGCTTATGGAGTC	103
<i>CCL3</i>	F: CCAGGTCTTCTCTGCACCAC R: GCTACGAATTGCGAGGAAG	90
<i>CCL5</i>	F: CTCCATGGCAGCAGTCGT R: AAGGCTTCTCCATCCTAGC	121
<i>CD14</i>	F: GGGTTCCTGCTCAGATTCTG R: CCCACGACACATTACGGAGT	164
<i>CD40</i>	F: TGAGAGCCCTGGTGGTTATC R: GCTCCTTGGTCACCTTTCTG	90
<i>CD163</i>	F: CACATGTGCCAACAAAATAAGAC R: CACCACCTGAGCATCTTCAA	130
<i>CD200</i>	F: TCCCCAGGAAGTTTTGATTG R: CCATGGTTCTTGCTGAAGGT	84
<i>CLDN3</i>	F: ATCGGCAGCAGCATTATCAC R: ACACTTTGCACTGCATCTGG	94
<i>CRP</i>	F: GGTGGGAGACATTGGAGATG R: GAAGGTCCCACCAGCATAGA	85
<i>CXCL10</i>	F: CCCACATGTTGAGATCATTGC R: GCTTCTCTCTGTGTTTCGAGGA	141
<i>C3</i>	F: ATCAAATCAGGCTCCGATGA R: GGGCTTCTCTGCATTGATG	76
<i>DEFB1</i>	F: ACCTGTGCCAGGTCTACTAAAAA R: GGTGCCGATCTGTTTCATCT	109
<i>DEFB4A</i>	F: CAGGATTGAAGGGACCTGTT R: CTTCACTTGGCCTGTGTGTC	99
<i>FGG</i>	F: GAATTTTGGCTGGGAAATGA R: CAGTCCTCCAGCTGCACTCT	86
<i>HP</i>	F: ACAGATGCCACAGATGACAGC R: CGTGCGCAGTTTGTAGTAGG	105
<i>HPRT1</i>	F: ACACTGGCAAAACAATGCAA R: TGCAACCTTGACCATCTTTG	71
<i>IL1B</i>	F: CCAAAGAGGGACATGGAGAA R: GGGCTTTTGTCTGCTTGAG	123
<i>IL1RN</i>	F: TGCCTGTCCTGTGTCAAGTC R: GTCCTGCTCGCTGTTCTTTC	90

TABLE 1: Continued.

Gene symbol	Sequence (5'-3')	Amplicon length
<i>IL6</i>	F: TGGGTTCAATCAGGAGACCT R: CAGCCTCGACATTTCCCTTA	116
<i>IL8</i>	F: TTGCCAGAGAAATCACAGGA R: TGCATGGGACACTGGAAATA	78
<i>IL10</i>	F: CTGCCTCCCCTTTCTCTTG R: TCAAAGGGGCTCCCTAGTTT	95
<i>IL18</i>	F: CTGCTGAACCGGAAGACAAT R: TCCGATTCCAGGTCTTCATC	100
<i>IFNG</i>	F: CCATTCAAAGGAGCATGGAT R: TTCAGTTTCCCAGAGCTACCA	76
<i>ITIH41</i>	F: ATGACAGCAAGCGAACAGTG R: GGGGATCCCTCTTGGAATC	85
<i>ITIH42</i>	F: AGGCCCTCACCATATCACAG R: GTTGCCATCCAGGACTGTTT	110
<i>LBP</i>	F: CCCAAGGTCAATGATAAGTTGG R: ATCTGGAGAACAGGGTCGTG	83
<i>LCT</i>	F: CACTCAAAGCTGTGCAGGAC R: GGATCCTTGGCAGAGAAAGTG	144
<i>MUC1</i>	F: GGATTTCTGAATTGTTTTGTCAG R: ACTGTCTTGGAAAGGCCAGAA	116
<i>MUC2</i>	F: GCACGTCTGCAACAAGGAC R: CAAAGCCCTCCAGGCAGT	125
<i>NFKBIA</i>	F: GAGGATGAGCTGCCCTATGAC R: CCATGGTCTTTTAGACACTTTCC	85
<i>NFKBI</i>	F: CTCGCACAAGGAGACATGAA R: GGGTAGCCAGTTTTTGTCA	97
<i>OCLN</i>	F: CGGTGAGAAGATTGGCTGAT R: TTTCAAAGGCCTGGATGAC	100
<i>PAFAH1B1</i>	F: GCAAAGTGGCTACTGTGTGAAG R: GCACAGTCTGGTCATTGGAA	113
<i>PTGS2</i>	F: AGGCTGATACTGATAGGAGAAACG R: GCAGCTCTGGGTCAAACCTC	100
<i>RPL13A</i>	F: ATTGTGGCCAAGCAGGTACT R: AATTGCCAGAAATGTTGATGC	76
<i>SAA</i>	F: TAAAGTGATCAGCAATGCCAAA R: TCAACCCTTGAGTCCTCCAC	96
<i>SFTPA1</i>	F: CATGGGTGTCCTCAGTTTCC R: CATCAAAAGCGACTGACTGC	86
<i>SLC5A1</i>	F: CTGCAAGAGAGTCAATGAGGAG R: CCGGTTCCATAGGCAAACCT	99
<i>TF</i>	F: CTCAACCTCAAACCTCCTGGAA R: CCGTCTCCATCAGGTGGTA	82
<i>TGFB1</i>	F: GCAAGGTCTGCTCTGTGTA R: TAGTACACGATGGGCAGTGG	97

TABLE 1: Continued.

Gene symbol	Sequence (5'-3')	Amplicon length
<i>TLR2</i>	F: GTTTTACGGAAATTGTGAAACTG	136
	R: TCCACATTACCGAGGGATTT	
<i>TLR4</i>	F: TTTCCACAAAAGTCGGAAGG	145
	R: CAACTTCTGCAGGACGATGA	
<i>TNF</i>	F: CCCCCAGAAGGAAGAGTTTC	92
	R: CGGGCTTATCTGAGGTTTGA	
<i>TNFAIP3</i>	F: CCCAGCTTTCTCTCATGGAC	113
	R: TTGGTTCTTCTGCCGTCTCT	

Copenhagen (Copenhagen, Denmark). Primer sequences and amplicon length are shown in Table 1. The 48 genes were chosen based on a previous study showing expression of the selected reference-, epithelial-, and immune-related genes in neonatal pig tissue [18]. Primer amplification efficiencies and dynamic range were acquired from standard curves constructed from dilution series of highly responding samples. Melting curves were inspected for all primer assays and agarose gel electrophoresis, and sequencing of most amplicons were performed to ensure primer specificity.

Quantitative PCR (qPCR) was performed in 48.48 Dynamic Array integrated fluidic circuits (Fluidigm Corporation, San Francisco, CA, USA), combining 48 samples with 48 primer sets for 2304 simultaneous qPCR reactions. Reaction mix was prepared using the following components for each of the 48 samples: 3  $\mu$ L ABI TaqMan Gene Expression Master Mix (Applied Biosystems), 0.3  $\mu$ L 20X DNA binding dye sample loading reagent (Fluidigm Corporation), 0.3  $\mu$ L 20X EvaGreen (Biotium, VWR—Bie & Berntsen), and 0.9  $\mu$ L low EDTA TE buffer (VWR—Bie & Berntsen). Reaction mix (4.5  $\mu$ L) was mixed with 1.5  $\mu$ L preamplified cDNA. Primer mix for each of the 48 primer sets was prepared using 2.3  $\mu$ L 20  $\mu$ M specific primer (forward and reverse), 2.5  $\mu$ L 2X assay loading reagent (Fluidigm Corporation), and 0.2  $\mu$ L low EDTA TE buffer (VWR—Bie & Berntsen). Reaction mix, including cDNA (6  $\mu$ L) and primer mix (5  $\mu$ L), was dispensed and loaded into the integrated fluidic circuit of the Dynamic Array using the IFC Controller (Fluidigm Corporation). After loading, the Dynamic Array was placed in the BioMark real-time PCR instrument (Fluidigm Corporation), and the following cycle parameter was used: 2 min at 50°C, 10 min at 95°C, followed by 35 cycles with denaturing for 15 s at 95°C, and annealing/elongation for 1 min at 60°C. Melting curves were generated to confirm a single-PCR product for each reaction (from 60°C to 95°C, increasing 1°C/3 s). Reactions were performed in duplicates (cDNA replicates). Non-template controls were included to indicate potential problems with nonspecific amplification or sample contaminations. Quantification cycle (*C<sub>q</sub>*) was acquired using the fluidigm real-time PCR analysis software 3.0.2 (Fluidigm Corporation) and exported to GenEx5 (MultiD Analyses AB, Göteborg, Sweden).

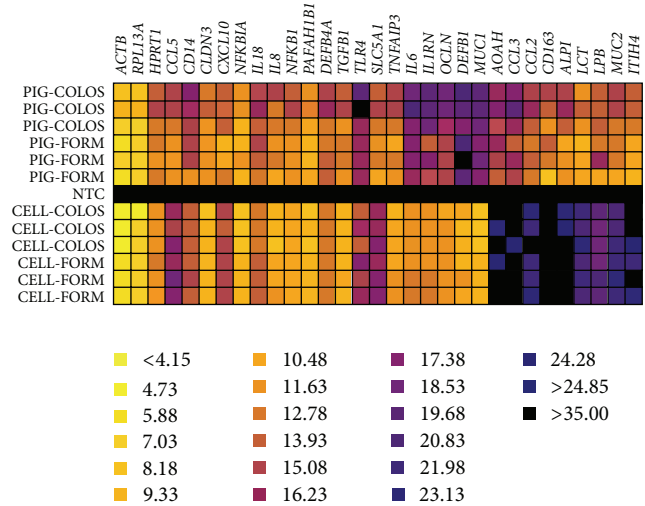


FIGURE 1: Heat map showing the *C<sub>q</sub>* value of non-normalized raw data from representative distal small intestinal samples of preterm pigs and IPEC-J2 cells, in addition to a non-template control (NTC). A high gene expression corresponds to a low *C<sub>q</sub>* value (yellow), while a low gene expression corresponds to a high *C<sub>q</sub>* value (purple/black). Relative quantities of differentially expressed genes can be seen in Figure 2.

**2.4. Data Analysis and Statistics.** Data preprocessing, normalization, relative quantification, and statistics were performed using GenEx5 (MultiD Analyses AB). Data were preprocessed as follows. (1) Data were corrected for PCR efficiency for each primer assay individually. (2) Interdynamic array variation was compensated by using several highly stable samples as interdynamic array calibrators. (3) Hypoxanthine phosphoribosyltransferase 1 (*HPRT1*) and beta-actin (*ACTB*) were found to be the most stably expressed reference genes in the present study using both GeNorm [19] and NormFinder [20]; therefore, the geometric mean of these two genes was used to normalize all samples in GenEx5. (4) The average of cDNA technical repeats was performed after reference gene normalization but before *C<sub>q</sub>* was transformed to linear scale (relative quantities). In rare cases of high standard deviation between the two cDNA replicates, one or both of the samples or the primer assay were excluded from the analysis based on visual inspection of fluorescence and melting curves. To visualize differential gene expression, relative expression for all samples was calculated relative to the sample with the lowest expression for each primer set in the group of samples tested. Data were log<sub>2</sub> transformed prior to *t*-test, ANOVA, and principal component analysis (PCA). Gene expression was considered significantly different if the *P* value <0.05 and the relative expression >2.0.

### 3. Results

An overview of raw data for genes expressed in intestinal tissue from preterm pigs and in IPEC-J2 cells is presented in the heat map (Figure 1), based on color coding of the expression level before preprocessing. After preprocessing,



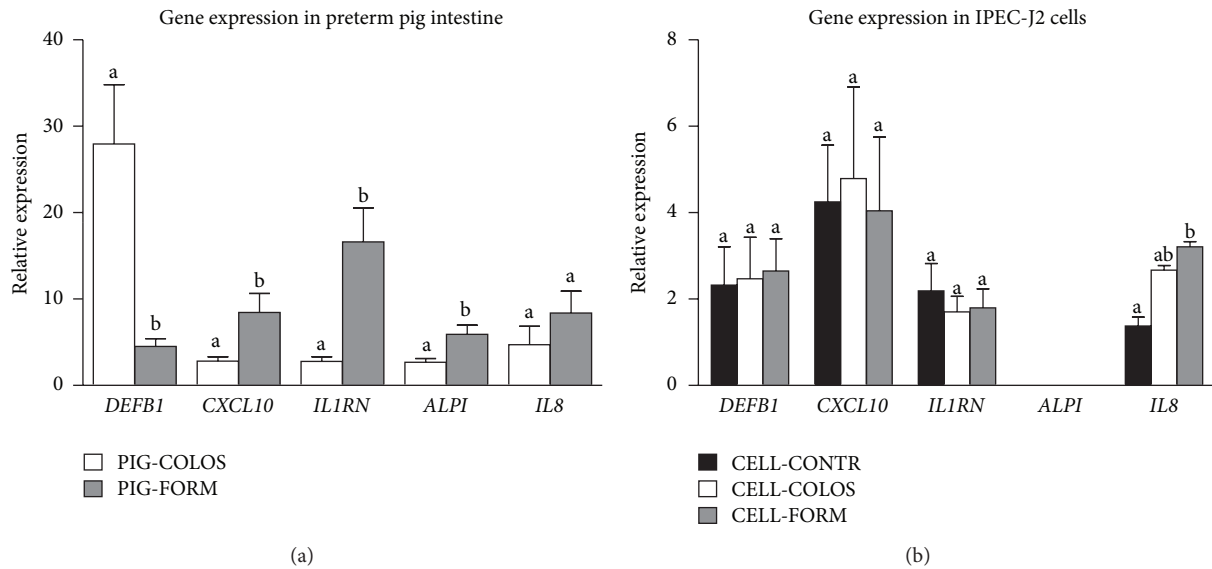


FIGURE 2: Relative expression (mean  $\pm$  SEM) of genes expressed significantly different in intestinal tissue of PIG-COLOS versus PIG-FORM (a) and between different IPEC-J2 cell treatment groups: CELL-CONTR, CELL-COLOS, and CELL-FORM (b). Relative expression for all samples was calculated relative to the sample with the lowest expression within each gene in the group of samples tested. Different superscript letters within each gene indicate significant difference ( $P < 0.05$ ).

including visual inspection of melting curves, 31 reference- and epithelial- and immune response-related genes were expressed within the dynamic range in pig intestinal tissue, and of these, 22 genes were expressed in IPEC-J2 cells. No genes were expressed only in IPEC-J2 cells.

In PIG-COLOS, *DEFBI* was more highly expressed, while *CXCL10*, *IL1RN*, and *ALPI* were less expressed than in PIG-FORM intestinal tissue (Figure 2(a)). In IPEC-J2 cells, no differences were seen in the expression of *DEFBI*, *CXCL10*, and *IL1RN* between the three groups, and *ALPI* was not expressed in IPEC-J2 cells. In contrast, *IL8* expression was significantly higher in CELL-FORM compared with CELL-CONTR with intermediate expression in CELL-COLOS (Figure 2(b)). When comparing CELL-FORM and CELL-COLOS with PIG-FORM and PIG-COLOS in a PCA, two major groups were identified (Figure 3), discriminating gene expression profiles of pig intestinal tissue from that of IPEC-J2 cells. Furthermore, discrimination between PIG-COLOS and PIG-FORM was achieved by PCA, while no discrimination between CELL-COLOS and CELL-FORM was observed. As expected, major differences in gene expression were seen between IPEC-J2 cells and pig intestinal tissue, which accounted for the clear clustering in the PCA.

Transcript coding for *CXCL10*, *CCL5*, and *SLC5A1* was found at a higher level in pig intestinal tissue compared with IPEC-J2 cells; in contrast, the expression of *CLDN3*, *DEFBI*, *IL1RN*, *IL6*, *MUC1*, and *OCN* was lower in intestinal tissue compared with IPEC-J2 cells (data not shown).

#### 4. Discussion

In this study, the IPEC-J2 cell line was evaluated as a possible *in vitro* model for investigation of the gene expression of

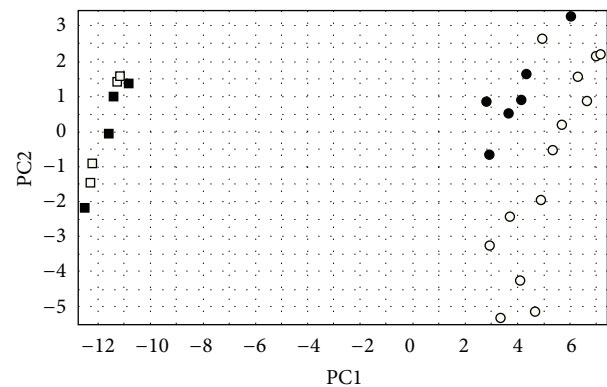


FIGURE 3: Principal component analysis of expression data from distal small intestinal tissue from preterm pigs: PIG-FORM (white circles) and PIG-COLOS (black circles) and IPEC-J2 cells: CELL-FORM (white squares) and CELL-COLOS (black squares).

IECs in relation to dietary effects on the neonatal intestine. To our knowledge, this is the first demonstration of *CCL5*, *CD14*, *CXCL10*, *IL1RN*, *PFAH1B1*, and *SLC5A1* expression in IPEC-J2 cells. The expression of *DEFBI*, *DEFB4A*, *IL6*, *IL8*, *IL18*, *MUC1*, *NFKB1A*, *NFKB1*, *OCN*, *TLR4*, *TGFB1*, and *TNFAIP3* has been reported previously [2–6, 21]. On the other hand, a lack of *TGFB1* expression has also been reported in infection studies [2]. Finally, we confirmed the lack of expression of *CCL2* [4] and *MUC2* [2] in IPEC-J2 cells.

We found that the IPEC-J2 cells and intestinal tissue clustered in two distinct groups in the PCA of gene expression patterns. Furthermore, it was possible by PCA to discriminate between the gene expression profiles of intestinal tissue from

preterm pigs in different diet groups; however, discrimination by PCA between the IPEC-J2 cell diet groups, CELL-FORM and CELL-COLOS, was not possible. Four genes were differentially expressed between pigs fed milk formula or colostrum. *DEFB1*, encoding the antimicrobial protein defensin betal [22], was upregulated in PIG-COLOS compared with PIG-FORM, which suggests that colostrum may stimulate the host antimicrobial response. The expression of *ALPI*, coding for the enzyme intestinal alkaline phosphatase, in addition to *CXCL10* and *IL1RN* was downregulated in PIG-COLOS compared with PIG-FORM. *CXCL10* is involved in T-lymphocyte activation and induction of chemotaxis toward infected tissues [23]; and *IL1RN*, coding for the IL-1 receptor antagonist, has previously been found to show similar expression patterns as *CXCL10* in viral lung infection of pigs [24]. None of these four genes were differentially expressed in IPEC-J2 cells. However, the expression of *IL8*, coding for the potent neutrophil chemoattractant IL-8, was higher in CELL-FORM relative to CELL-CONTR. Thus, milk formula might induce specific parts of a proinflammatory response in IPEC-J2 cells. Of the genes up-regulated in IPEC-J2 cells relative to pig intestinal tissue, *MUC1* and *DEFB1* stood out as highly differentially expressed, with a relative expression of more than 1000 (data not shown). These genes are coding for proteins secreted by IECs and involved in the first line of defense, and thus expected to be highly expressed in the IPEC-J2 cell line, which consist of IECs only, in contrast to the intestinal tissue consisting of many different cell types.

The IPEC-J2 cell line may, under the conditions used in this study, not be an optimal model to investigate diet-related effects, since the gene expression differences found in intestinal tissue could not be demonstrated in the IPEC-J2 cell line. The expression differences between IPEC-J2 cells and preterm pig intestinal tissue are possibly due to the heterogeneous population of cells in intestinal tissue, in addition to the intestinal microbiota, which may affect the expression level of certain genes. On the other hand, the IPEC-J2 cell line is a homogenous cell population consisting of IECs only. *In vivo* digestion of the diet may also change its effect on IECs, and exposure to artificially digested diets of the IPEC-J2 cells may provide a situation that more closely resembles that of the intestine. Furthermore, studies could include gene expression analysis of intestinal samples from term born pigs to further determine if the observed differences in gene expression could be due to the difference in maturational state.

## 5. Conclusion

The observed differences in the diet-dependent gene expression patterns between IPEC-J2 cells and intestinal tissue from preterm, newborn pigs underline that responses to environmental stimuli (e.g., diet) may differ markedly between isolated enterocyte cell systems and intact tissue responses, both acting as models for the sensitive newborn intestine. However, this *in vitro* cell model still provides the opportunity to investigate the interaction between a limited number of factors in a standardized setting, although caution must be

exercised in the interpretation of diet-induced effects on gene expression in this model.

## Conflict of Interests

The authors claim to have no conflict of interests in the context of this work.

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