

# Abstract Submission

## Nutrition

### Clinical Nutrition

ESPGHAN2015-1805

## NEOMUNE-NEONUTRINET: NUTRITION FOR PRETERM INFANTS AROUND THE WORLD DURING THE FIRST WEEKS AFTER BIRTH

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**Presentation Preference:** Oral or Poster

**ESPGHAN Membership:** Non Member

**Has the abstract been previously presented?:** No

**Objectives and Study:** Feeding very low birth weight (VLBW) infants is a challenge and the optimal timing, volume and diet remain controversial. The NeoNutriNet database gives an overview of differences in feeding practice for preterm infants around the world. This will help to identify optimal feeding regimens and design appropriate intervention studies.

**Methods:** Fourteen hospitals in 'Western' (USA, Denmark, Netherlands, UK, Australia, New Zealand) and 'non-Western' (China, India, Taiwan) regions participated. Infants with a birth weight <1500g born between Jan 2011 and Sept 2014 were included. Collected data include timing and composition of (par)enteral nutrition and anti-/probiotics, anthropometrics and clinical complications from birth until a gestational age (GA) 37 weeks or discharge from hospital. Here we present preliminary results from seven hospitals, four non-Western (A-D) and three Western (E-G).

**Results:** Birth weight, GA, gender distribution and mortality rates differed significantly among hospitals (Table). Nutritional regimes and outcomes (time to full enteral feeding (TFF; 150 ml/kg/day), incidence of necrotizing enterocolitis (NEC), weight gain, anti-/probiotics use) also differed markedly. Infants from Western hospitals had lowest birth weight, but reached full enteral feeding earlier (median 13 vs. 27 d).

### Image:

	A	B	C	D	E	F	G	p-value
N <sup>2</sup>	100	54	126	209	63	284	102	
GA (wk)	30.1	30.2	29.9	30.3	29.0	27.6	29.3	<0.0001
Birth weight (g)	1327	1350	1300	1300	1100	958	1120	<0.0001
Male (%)	54.0	59.3	65.1	63.6	34.9	45.8	54.5	<0.0001
TFF (d)	29	31	29	34	13	13	13	<0.0001
NEC (%)	0.0	5.8	5.6	10.1	4.8	7.1	7.9	0.019
Death (%)	16.3	1.9	7.9	3.9	0.0	13.4	8.9	<0.0001
Growth velocity <sup>4</sup>	8.1	8.6	10.1	4.9	12.5	12.1	10.9	<0.0001
Z-score weight <sup>5</sup>	-1.28	-1.11	-1.03	-1.48	-0.66	-0.81	-0.72	<0.0001
Antibiotics <sup>3</sup> (%)	40.8	27.2	52.3	37.5	26.8	22.9	45.8	<0.0001
Probiotics (%)	23.5	0.0	37.9	34.3	64.5	80.8	0.0	<0.0001

<sup>1</sup>In median or percentages. <sup>2</sup>Number of infants in hospital cohort; up to 50% missing data for individual items due to discharge. <sup>3</sup>Relative to total admission days. <sup>4</sup>In g/kg/day, day 1-28. <sup>5</sup>day 28-1.

**Conclusion:** Nutritional practices and associated clinical outcomes in VLBW infants show marked differences among hospitals. The variations may relate to differences in infant biology, culture or clinical practice between Western and non-Western regions. This is important to clarify because it is suggested that early enteral feeding influences later outcomes. Results from the NeoNutriNet database will be a valuable tool to help design future intervention studies in this field.

**Disclosure of Interest:** None Declared

# Abstract Submission

## *Nutrition*

### *Neonatal Nutrition*

ESPGHAN2015-1342

## **BOVINE COLOSTRUM AS NUTRITION FOR PRETERM INFANTS IN THE FIRST DAYS OF LIFE: A PILOT FEASIBILITY STUDY (PRECOLOS-NEOMUNE)**

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**Presentation Preference:** Oral or Poster

**ESPGHAN Membership:** Non Member

**Has the abstract been previously presented?:** No

**Objectives and Study:** The optimal feeding regimen for preterm infants is not clear, especially when mother's own milk (MM) is not available. Infant formula (IF) and donor milk (DM) are potentially inferior to MM in promoting feeding tolerance, growth and intestinal maturation. Bovine colostrum (BC) contains large amounts of protein, growth factors and immuno-regulatory components (IGFs, IgG, lactoferrin) which may be beneficial. We investigated whether feeding BC to preterm infants during the first days of life is safe, well tolerated and may promote nutrient uptake and gut maturation when MM is limited. PreColos is a three-phase (A, B, C), dual-site (Rigshospitalet, RH and Foshan Women and Children's Hospital, FWCH), pilot feasibility study (ClinicalTrials.gov NCT02054091). The protocol and preliminary results of phases A and B are summarized here.

**Methods:** In phases A and B, 12 infants delivered with gestational age (GA) between 27+0 and 32+6 weeks (RH) or birth weights (BW) 1000-1800 g (FWCH) were recruited before the first feeding. BC was administered as a supplement to MM for up to 10 d with maximum total protein intake of 4.5 g/kg/d. In phase C, a randomized controlled trial, 40 infants will be recruited to BC intervention or control feeding (DM at RH and IF at FWCH). Outcomes are feeding intolerance (FI), time to enteral feeding at 120 ml/kg/d (TTF120), growth, combined incidence of serious infections/NEC, plasma amino acids, plasma bovine IgG, intestinal functions, and faecal microbiota. Data are presented as median (interquartile range).

**Results:** The BW and gestational age (GA) for the 12 infants (7/5, female/male) were 1499 (1231-1650) g and 30.4 (29.8-31.9) week. Infants received BC for 7.5 (5.8-9.0) d at a dose of 17.7 (12.2-25.3) ml/kg/d and 1.4 (1.0-2.0) g/kg/d protein from BC. At 37 weeks or discharge, body weight reached 2280 (2112-2510) g and average growth velocity was 12.3 (10.3-13.8) g/kg/d. TTF120 and days on PN were 10.0 (6.0-14.5) and 11.0 (0.0-15.5) d. Seven infants showed FI in the first week and 1 infant in the second. Total volume of gastric residual was 39 (6-79) ml in the first week and 4 (1-12) ml in the second. On day 7, 5 infants showed a transient hypertyrosinemia, which disappeared on day 14 for all infants. Plasma bovine IgG was below the detection limit (5µg/ml, n=7). No adverse reactions to BC were observed.

**Conclusion:** Feeding BC as a supplement to MM during the first 10 d of life was well tolerated in preterm infants with GA between 27+0 and 32+6 weeks or BW 1000-1800 g. Phase C will proceed as planned and plasma tyrosine will be closely monitored.

**Disclosure of Interest:** Y. Li: None Declared, S. M. Petersen: None Declared, X. Ye: None Declared, R. L. Shen: None Declared, P. T. Sangild Conflict with: Univ of Copenhagen has filed a patent regarding the use of bovine colostrum for pediatric patients, G. O. Greisen: None Declared

# Abstract Submission

*Gastroenterology*

*Basic Science*

ESPGHAN2015-1847

## ORAL ANTIBIOTICS PREVENT NECROTIZING ENTEROCOLITIS IN PRETERM PIGS

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**Presentation Preference:** Poster

**ESPGHAN Membership:** Non Member

**Has the abstract been previously presented?:** No

**Objectives and Study:** Prematurity, enteral nutrition and gut colonization are dominating risk factors for necrotizing enterocolitis (NEC). Neonatal antibiotic (AB) treatment is common for preterm infants but it is not known whether oral vs. systemic AB provide different effects on gut colonization and NEC sensitivity. We hypothesized that prophylactic oral AB would be superior to systemic AB in suppressing gut colonization and NEC development.

**Methods:** Preterm caesarean-delivered pigs were fed increasing doses of infant formula after birth. The pigs were given either oral broad-spectrum AB (metronidazole/ampicillin/gentamycin) (PO, n=16), an equivalent dose of systemic AB (SYS, n=17) or saline (CON, n=16). Pigs were euthanized on d 5 and scored for macroscopic NEC lesions (range 1-6). Intestinal samples were collected for analyses of digestive enzyme activity. Bacterial abundance in the small intestine was evaluated with fluorescence in situ hybridization (FISH) on tissue sections using a semi-quantitative score (range 1-7) and by qPCR on luminal colon content. Short-chain fatty acids (SCFA) in colon content were analysed by gas chromatography. A sugar absorptive capacity test was done on d 4 and intestinal permeability was estimated as urine lactulose/mannitol ratios following oral administration 4 h before euthanasia.

**Results:** The NEC incidence was lower (0%) for PO than for SYS and CON pigs (59-63%,  $P<0.001$ ), with the most severe lesions in CON pigs. PO showed reduced small intestinal bacterial abundance, relative to the other groups (1.1 vs. 1.9-2.4 FISH scores,  $P<0.05$ ). SYS showed reduced bacterial abundance relative to CON only in the proximal part (1.7 vs. 2.9,  $P<0.05$ ). Bacterial translocation into the intestinal wall was only seen in CON and SYS. Relative to SYS and CON pigs, PO pigs had lower numbers of 16S rRNA gene copies in colon contents ( $\log_{10}$ (16S rRNA gene copies/g) 6.05 vs. 7.88-7.94,  $P<0.001$ ). Total SCFA concentration was lower in AB treated pigs compared to CON ( $13.4\pm 17.5$  vs.  $61.1\pm 25.6$   $\mu\text{mol/g}$ ,  $P<0.001$ ) and lower in PO compared to SYS ( $4.3\pm 6.9$  vs.  $21.5\pm 20$ ,  $P<0.05$ ). Total SCFA was higher in NEC pigs compared to healthy pigs ( $38.0\pm 28.3$  vs.  $21.4\pm 29.5$ ,  $P<0.01$ ). No differences were found for digestive enzyme activities, galactose absorptive capacity and intestinal permeability.

**Conclusion:** Oral AB is superior to systemic AB treatment with regard to NEC prevention. Oral AB more profoundly suppressed bacterial colonization and fermentation in the intestine and colon, without marked effects on small intestinal function. Further studies are required to elucidate the possible long term effect of oral AB on gut function and NEC, and the development of antibiotics-associated microbial resistance.

**Disclosure of Interest:** None Declared

# Abstract Submission

*Nutrition*

*Clinical Nutrition*

ESPGHAN2015-1306

## BOVINE COLOSTRUM REDUCES DOXORUBICIN-INDUCED INTESTINAL TOXICITY IN PIGLETS RELATIVE TO FORMULA

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**Presentation Preference:** Oral or Poster

**ESPGHAN Membership:** Non Member

**Has the abstract been previously presented?:** No

**Objectives and Study:** Chemotherapy-induced gastrointestinal (GI) toxicity is a common adverse effect of cancer treatment. Using piglets as models for infants, we hypothesized that the immunomodulatory and gastrointestinal tropic effects of bovine colostrum would reduce the severity of GI complications after doxorubicin treatment.

**Methods:** Thirty-two 5-day-old piglets were given an intravenous infusion of doxorubicin (DOX, 1 x 100 mg/m<sup>2</sup>, n=18) or an equivalent infusion of saline (SAL, n=14) and subjected to formula feeding (DOX-Form, n=9, SAL-Form, n=7) or feeding with bovine colostrum (DOX-Colos, n=9, SAL-Colos, n=7). Pigs were euthanized five days after initiation of chemotherapy to assess markers of intestinal function and inflammation.

**Results:** DOX-treated animals developed diarrhea, growth deficits, and had reduced intestine, colon and spleen weights (all p<0.05). DOX-Colos pigs had higher lactose digestion and absorption capacity than DOX-Form (37 vs. 8 uM plasma galactose after lactose bolus, p<0.01), corresponding with longer intestinal villi and higher activities of brush border enzymes (lactase, maltase, DPP-IV, all p<0.05). Intestinal permeability was reduced (0.03 vs. 0.30 for urinary lactulose/mannitol ratio, p<0.01) with reduced intestinal tissue IL-8 levels (33 vs. 76 ng/g, p < 0.05) and reduced levels of plasma C-reactive protein (CRP), relative to DOX-Form (118 vs. 170 mg/L, p<0.05).

**Conclusion:** A single dose of doxorubicin induces intestinal toxicity in suckling pigs. The toxic response is diet dependent with beneficial effects of bovine colostrum compared with a suboptimal formula. Systemic inflammatory responses may result partly from damage to intestinal structure and functions. It is important during chemotherapy to provide an enteral diet that adequately supports intestinal growth, function and defense mechanisms. Bovine colostrum may be considered as an interventional diet for infants and children subjected to chemotherapy.

**Disclosure of Interest:** None Declared

**Keywords:** None

# Abstract Submission

*Gastroenterology*

*Basic Science*

ESPGHAN2015-1154

## ORAL ANTIBIOTICS MODULATE IMMUNE CELL DEVELOPMENT AND PREVENT NECROTIZING ENTEROCOLITIS IN NEONATAL PRETERM PIGS

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**Presentation Preference:** Oral or Poster

**ESPGHAN Membership:** Non Member

**Has the abstract been previously presented?:** No

**Objectives and Study:** The systemic and mucosal immune systems are immature in newborns, particularly those born preterm, leading to higher susceptibility to infections and necrotizing enterocolitis (NEC). Following birth, a few days of systemic treatment with antibiotics is often applied for preterm infants to prevent sepsis and infections but the effects on immune system development and NEC are not clear. Likewise, it is not clear whether the route of administration, oral or systemic, is important.

**Methods:** Preterm pigs received increasing amounts of infant formula for 5 days after birth (0-120 mL/kg/day). During this period, groups of pigs (n = 17-18) were administered saline (CON) or broad spectrum antibiotics orally (ORA) or systemically (SYS). Temporal changes of blood cell parameters were analyzed by flow cytometry. Bacteria in the intestine and the blood were characterized by sequencing and mass spectrometry, and quantified by quantitative PCR and culturing.

**Results:** At birth, preterm pigs were immunologically immature as evidenced by lower counts of neutrophils, thrombocytes and erythrocytes, and higher frequency of progenitor cells, relative to term pigs. Regardless of treatment, blood neutrophils and monocytes gradually matured postnatally with increased CD14 expression and decreased CD172a expression, whereas TLR2 expression was unchanged. ORA pigs had more mature neutrophils with lower cell size and CD172a expression whereas monocytes in CON pigs were activated to higher degree with greater CD14 expression and cell granularity. None of the ORA pigs developed NEC within the first 5 days after birth and on day 5 they had lower number and granularity of monocytes, and negligible amount of bacteria in the blood and intestinal lumen. In contrast, CON and SYS pigs showed high NEC incidence (59-63%), abundant Gram-positive bacteria in the blood and the gut lumen. NEC was associated with low counts of total leukocytes and lymphocytes, high monocyte granularity, and excessive intensity of CD14 in monocytes and neutrophils.

**Conclusion:** Oral antibiotics induce maturation of neutrophils and maintain the gut microbiota at low density, thereby preventing bacterial translocation and NEC in preterm pigs. The impaired TLR2 development in neutrophils and monocytes suggests a low clearance capability for blood Gram-positive bacteria. This may justify the use of prophylactic oral antibiotics during the first few days after preterm birth. However, the risk of selection of microbial resistance remains to be explored.

**Disclosure of Interest:** None Declared

**Keywords:** None

# Abstract Submission

*Nutrition*

*Basic Science*

ESPGHAN2015-1333

## INTRODUCTION OF FORMULA FEEDING INDUCES SUBCLINICAL INFLAMMATION AND ALTERED CHROMATIN STRUCTURE IN THE INTESTINE OF PRETERM PIGS PREDISPOSING TO NECROTIZING ENTEROCOLITIS

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**Presentation Preference:** Oral or Poster

**ESPGHAN Membership:** Full ESPGHAN Member

**Has the abstract been previously presented?:** No

**Objectives and Study:** To analyze how enteral food introduction affects intestinal gene regulation and chromatin structure in preterm neonates, potentially predisposing to necrotizing enterocolitis (NEC).

**Methods:** Preterm piglets were fed total parenteral nutrition (TPN) or PN plus slowly increasing volumes of enteral nutrition (0-64 mL/kg/d formula or bovine colostrum). Intestinal gene expression profiles, CpG signaling and chromatin structure were analyzed five days after birth. Chromatin structure changes and inflammatory response genes were investigated in CaCo-2 cells.

**Results:** Enteral feeding led to differential up-regulation of several inflammatory and pattern recognition receptor genes, including *IL8* and *TLR4*. This correlated with mild mucosal lesions and more open chromatin configurations particularly in formula-fed pigs. Colostrum-fed pigs were only minimally affected and also showed elevated *IL10* mRNA levels. In CaCo-2 cells, treatment with a histone deacetylase inhibitor led to marked increase in *TLR4* mRNA and more pronounced *IL8* mRNA expression upon stimulation with lipopolysaccharide.

**Conclusion:** Initiation of enteral feeding, particularly with formula, induces subclinical inflammatory lesions in the premature intestine and a more open chromatin structure in key inflammatory genes. This may predispose to later intestinal dysfunction and NEC. Consequently, it is critical to optimize the time, amount and diet of the first enteral feeds for preterm infants.

**Disclosure of Interest:** None Declared

**Keywords:** None

# Abstract Submission

*Gastroenterology*

*Enteropathy (other than Coeliac Disease)*

ESPGHAN2015-1133

## CHEMOTHERAPY EFFECTS ON INTESTINAL GENE EXPRESSION PROFILES IN PIGLETS

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**Presentation Preference:** Poster

**ESPGHAN Membership:** Non Member

**Has the abstract been previously presented?:** No

**Objectives and Study:** While limited knowledge is available from children, studies in animals indicate that cytotoxic therapy leads to marked changes in intestinal structure, function, immunity and of the microbiota. More detailed knowledge about chemotherapy-related expression patterns of intestinal genes may provide further insights into the mechanisms underlying chemotherapy-induced gut toxicity and help to identify biomarkers and targets for intervention.

**Methods:** Jejunal tissue samples were obtained from piglets, used as preclinical models of chemotherapy-induced gastrointestinal toxicity in children. Prior to tissue collection, the piglets were treated with either busulfan and cyclophosphamide (BuCy) (n=10), or a single dose of doxorubicin (Dox) (n=12) and compared to saline controls. Pigs were euthanized 9-11 days after chemotherapy in the Dox and BuCy experiment, respectively. Expression profiles were measured using the Agilent 4 x 44K porcine expression microarray (Design id: 026440) and global pathway analysis was done using Gene Set Enrichment Analysis.

**Results:** Gene expression analysis identified 1163 differentially expressed genes (570 down-regulated, 593 up-regulated) in the groups receiving chemotherapy. In the doxorubicin treated piglets, 594 genes were differentially expressed (396 down, 198 up). In piglets treated with busulfan and cyclophosphamide, 1328 genes were differentially expressed (657 down, 671 up). Bioinformatics analysis demonstrated repression of Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes pathways for treated vs. untreated animals related to cellular immunity and the adaptive immune system, including the intestinal network related to IgA production. Examination of the 137 genes sharing differential expression across the two chemotherapy regimens showed similarly repression of cellular immunity and the adaptive immune system. Several up-regulated genes were related to innate immune defense, suggesting a compensatory up-regulation of such genes after chemotherapy. These included surfactant protein D (SP-D), deleted in malignant brain tumors 1 (DMBT 1) and peptidoglycan recognition protein 2 (PGLYRP2), all related to primary defense against invading bacteria and vira on mucosal surfaces and important for epithelial growth and differentiation.

**Conclusion:** Innate immune factors, including SP-D, DMBT1 and PGLYR2, were differentially up-regulated in the intestinal tissue after chemotherapy. Further investigation into such genes will establish whether their corresponding proteins could be markers of gastrointestinal toxicity or have possible functional, prognostic or treatment-related implications.

**Disclosure of Interest:** None Declared

**Keywords:** None

# Abstract Submission

*Nutrition*

*Basic Science*

ESPGHAN2015-1155

## BOVINE LACTOFERRIN REGULATES CELL SURVIVAL, CELL DEATH AND ENERGY METABOLISM IN INTESTINAL EPITHELIAL CELLS

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**Presentation Preference:** Oral or Poster

**ESPGHAN Membership:** Non Member

**Has the abstract been previously presented?:** No

**Objectives and Study:** Lactoferrin is a multifunctional protein present in both human (1-7 g/L) and bovine milk (0.1-2 g/L). It has a potential to attenuate intestinal inflammatory diseases in early life, such as necrotizing enterocolitis (NEC). Previous studies have shown that low doses (0.01-1 g/L) of bovine lactoferrin (bLF) increased intestinal epithelial cell (IEC) proliferation whereas high doses (10 g/L) triggered inflammation and exacerbated intestinal inflammation in preterm pigs.

**Methods:** To further elucidate the cellular mechanisms of these effects, we profiled the porcine IEC proteome stimulated with bLF at 0, 0.1, 1 and 10 g/L (0, 1.25, 12.5 and 125 µM) by iTRAQ-LC-MS-based proteomics.

**Results:** bLF was internalized into the IECs with the uptake correlating with increasing doses of bLF from 0 to 1 g/L, but without further uptake at 10 g/L. Among 122 differentially expressed porcine proteins, we focused on and grouped 31 proteins according to their biological functions: a) cell survival and cell death, b) energy metabolism, c) hypoxia inducible factor 1 (HIF-1) pathway, and d) aminoacyl-tRNA ligase activity. At 0.1-1 g/L, bLF up-regulated some proteins involved in cell survival and energy metabolism (cathepsin D, pyruvate dehydrogenase). At 10 g/L, bLF increased three proteins involved in cell death (apoptosis inducing factor, annexin 1, cyclophilin), two proteins of the HIF-1 pathway (ubiquitin carboxyl-terminal hydrolase, DNA lyase), and six aminoacyl-tRNA ligases. The high dose also down-regulated two anti-apoptotic proteins (catalase, huntingtin-interacting protein 1), three proteins involved in proliferation (CD63, granulins, 7-dehydrocholesterol reductase), and ten proteins involved in energy metabolism. Most differentially expressed proteins were regulated to a greater extent by 10 g/L of bLF than by lower doses.

**Conclusion:** Low bLF doses increase bLF uptake and signaling to facilitate cell survival, protection against stress and energy metabolism. Conversely, high doses may inhibit proliferation, stimulate apoptosis and cell death, and trigger inflammation. Careful selection of bLF dose is therefore crucial for its supplementation to infant formula with the aims to stimulate intestinal maturation and defense in preterm neonates.

**Disclosure of Interest:** None Declared

**Keywords:** None



# Abstract Submission

*Nutrition*

*Nutrition and Metabolism*

ESPGHAN2015-1192

## BIOACTIVE WHEY PROTEIN CONCENTRATE AND LACTOSE STIMULATE GUT FUNCTION IN PRETERM PIGS

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**Presentation Preference:** Oral or Poster

**ESPGHAN Membership:** Non Member

**Has the abstract been previously presented?:** No

**Objectives and Study:** Formula feeding is associated with feeding intolerance, necrotizing enterocolitis (NEC) and compromised intestinal health in preterm neonates. Reduced level of bioactive proteins in commercial whey protein concentrate (WPC) and replacement of lactose with maltodextrin in formulas may play a role. The effect of two WPCs, with either high or low levels of bioactive proteins (WPC H or L), were investigated in preterm pigs (Exp 1). Levels of lactoferrin, IgG and IGF-I were higher in WPC H than L (5-, 3-, and 3-fold). Each WPC was included in formulas based on either lactose or maltodextrin to test the interaction between protein and carbohydrate fractions. In Exp 2 we investigated whether bioactive proteins in WPC can be preserved by reduced thermal processing and whether this bioactive WPC (Bio) improves intestinal health, relative to a conventionally produced WPC (Con).

**Methods:** 92 caesarean-delivered preterm pigs were administered increasing doses of formulas for 4 days (16-120 mL/kg/d). In Exp 1, pigs were fed WPC H or WPC L contained in lactose- (lactose/maltodextrin: 3/1) or maltodextrin-dominant (maltodextrin/lactose: 3/1) formulas (4 groups, n =15-16). In Exp 2, pigs were fed Bio or Con contained in lactose-dominant formulas (2 groups, n=15-16). NEC, feeding intolerance, and intestinal indices, including morphology, permeability, hexose absorptive capacity, and brush border enzymes were evaluated.

**Results:** A reduction in thermal processing preserved lactoferrin and TGF- $\beta$ 2 in Bio (3- and 10-fold vs. Con). Weight gain, NEC incidence (49% across groups) and haematology were similar among groups. In Exp 1, regardless of the carbohydrate fraction, pigs fed WPC H showed or tended to show increased mucosal mass ( $P<0.05$ ) and villus height ( $P=0.09$ ), relative to WPC L pigs. Only in lactose-dominant formulas did WPC H stimulate hexose absorptive capacity and lactase activity relative to WPC L ( $P<0.05$ ). In Exp 2, no Bio pigs had feeding intolerance, compared with 7/16 Con pigs ( $P<0.01$ ). Bio pigs also tended to show higher hexose absorptive capacity ( $P=0.09$ ) and lower gut permeability ( $P=0.07$ ).

**Conclusion:** WPC with higher levels of bioactive proteins improves gut maturation, but mainly when contained in lactose-based formulas. Reduced thermal processing preserves bioactive proteins in WPC and improves feeding tolerance and intestinal functions. Lactose-based formulas containing WPC with maximal preservation of bioactive proteins could be important to support intestinal maturation and health in sensitive newborn infants.

**Disclosure of Interest:** Y. Li Conflict with: Was employee of Arla Foods Ingredients when part of the study was performed , D. N. Nguyen: None Declared, T. Thymann: None Declared, D. E. W. Chatterton: None Declared, A. S. Kvistgaard Conflict with: Arla Foods Ingredients, S. B. Bering: None Declared, P. T. Sangild: None Declared

**Keywords:** None

# Abstract Submission

## *Nutrition*

### *Clinical Nutrition*

ESPGHAN2015-1821

## **MALNOURISHED PARENTERALLY-FED PIGLETS SHOW ALTERED ACUTE PHASE REACTION FOLLOWING ENDOTOXIN EXPOSURE**

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**Presentation Preference:** Oral or Poster

**ESPGHAN Membership:** Non Member

**Has the abstract been previously presented?:** No

**Objectives and Study:** Malnutrition has been associated with adverse outcomes in both hospitalized paediatric patients and in children experiencing severe acute malnutrition (SAM) in low income countries. Small bowel bacterial overgrowth and release of endotoxin to the systemic circulation may play a role for disease in both groups of children. Endotoxemia induces multiple hemodynamic and acute phase reactions associated with septic shock. Altered fluid homeostasis including formation of oedema in the interstitial compartment is also known to be associated with endotoxemia. Using parenterally-fed piglets as a model for malnourished children, we hypothesized that endotoxin-induced acute phase response would be altered following malnutrition.

**Methods:** Three-day old piglets were given total parenteral nutrition (TPN) with optimal (OPT) or suboptimal (SUB) nutritional composition. Relative to OPT, the SUB group was given TPN with lower amino acid (5.5 vs 47 g/L) but higher fat (34 vs 10 g/L) and dextrose (97 vs 86 g/L) contents, and all micronutrients were lower. After seven days, half of the pigs from each group were co-infused with endotoxin (LPS, given  $10 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  for 9 h; OPT-LPS, n=10 and SUB-LPS, n=8), whereas the rest were co-infused with saline (OPT-SAL, n=10 and SUB-SAL, n=8). Oedema formation was assessed with abdominal ultrasonography before and after LPS infusion.

**Results:** There was a marked reduction in body weight gain in SUB vs OPT pigs (120 vs. 567 g,  $P < 0.001$ ), and biochemical and haematological profiling showed higher CRP, bilirubin, cholesterol, globulin, gamma-tocopherol and lactate, whereas malondialdehyde, albumin, haptoglobin, glutathione, ALP, ASAT, CK, urea, Fe, Mg, P, Na, platelets and lymphocytes were lower (all  $P < 0.05$ ) in SUB, relative to OPT. Following LPS infusion, perirenal oedema formation was higher in SUB-LPS than OPT-LPS ( $P < 0.01$ ), whereas no oedema was detected before LPS infusion or in the saline-infused control groups. Among the measured biochemical and haematological markers, TNF- $\alpha$  increased in both OPT-LPS and SUB-LPS, relative to saline-controls, but with the highest increase in OPT-LPS at 2h after start of LPS infusion (all  $P < 0.01$ ).

**Conclusion:** The combination of endotoxemia and insufficient nutrition may play an important role for altered acute phase responses and excessive oedema formation as seen in malnourished children.

**Disclosure of Interest:** None Declared

# Abstract Submission

*Nutrition*

*Neonatal Nutrition*

ESPGHAN2015-1823

## EFFECTS OF HUMAN MILK OLIGOSACCHARIDES ON INTESTINAL FUNCTION AND NECROTISING ENTEROCOLITIS IN PRETERM PIGS

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**Presentation Preference:** Oral or Poster

**ESPGHAN Membership:** Non Member

**Has the abstract been previously presented?:** No

**Objectives and Study:** Human milk oligosaccharides (HMOs) may mediate prebiotic and anti-inflammatory effects of milk in the infant intestine. This is of particular importance for preterm infants born with an immature gut and therefore being highly susceptible to dysfunction and necrotizing enterocolitis (NEC). We hypothesized that an HMO-supplemented infant formula (IF) improves intestinal function and NEC resistance after birth, and tested this in a pig model of preterm infants.

**Methods:** Preterm pigs (n = 112) were fed IF (Lacprodan, Arla FI; Peptide and Liguigen-MCT, SHS International) supplemented with a blend of either 5 HMOs including free sialic acid (5-HMO) or >25 different HMOs including free sialic acid, fucose and lactose (25-HMO), respectively (Glycom). In short-term experiments, 5-HMO (5 g/L) or 25-HMO (6 g/L) IF were compared to controls for 5 days after birth. In a longer-term experiment, the 5-HMO (10 g/L day 1-4; 5 g/L day 5-11) in IF (Alprem, Nestlé Nutrition) was compared to control for 11 days after preterm birth. The 5-HMO effects on intestinal cell proliferation and IL-8 secretion was investigated in IPEC-J2 cells.

**Results:** All added HMOs were found in urine and faeces of HMO pigs, and the formation of short chain fatty acids in the colon was higher in the HMO pigs, relative to controls (P<0.05). Development of NEC during the first 5 days was similar between control pigs and the 5-HMO (38 vs. 39%) and 25-HMO groups (63 vs. 74%), respectively, and only colon weights increased in the 25-HMO vs. control pigs (15%, P<0.01). In the 11-day experiment, NEC incidence tended to be lower in the 5-HMO vs. control group (56 vs. 79%), whereas dehydration and diarrhoea was more pronounced at day 7 and onwards, dehydration being higher for HMO pigs (P<0.05). Expression of *MUC1*, *MUC2*, *IAP*, *TNF $\alpha$* , *IFN $\gamma$* , *IL10*, *TLR4*, *TGF $\beta$* , *IL12*, and *IL17* was higher in the 5-HMO pigs after 11 days relative to controls. The 5-HMO blend decreased enterocyte proliferation (-30%) and LPS-induced IL-8 secretion (-10%) in IPEC-J2 cells (P<0.05).

**Conclusion:** Supplementation of IF with HMOs had limited effects after 5 days, but tended to improve NEC resistance within 11 days after preterm birth, yet diarrhoea and dehydration were more pronounced. Together, we conclude that 5-10 g/L HMOs induce limited acute effects on the immature new-born intestine. Longer exposure to HMOs (e.g. several weeks) may be required to induce beneficial effects to support immune and microbial defence and homeostasis in the immature intestine.

**Disclosure of Interest:** None Declared