Perinatal short-chain fructooligosaccharides program intestinal microbiota and improve enteroinsular axis function and inflammatory status in high-fat diet-fed adult pigs

Cindy Le Bourgot, Stéphanie Ferret-Bernard, Emmanuelle Apper, Bernard Taminiau, Armelle Cahu, Laurence Le Normand, Frédérique Respondek, Isabelle Le Huërou-Luron, Sophie Blat

Perinatal short-chain fructooligosaccharides program intestinal microbiota and improve entero-insular axis function and inflammatory status in high-fat diet fed adult pigs

Cindy Le Bourgot[1,2], Stéphanie Ferret-Bernard[2], Emmanuelle Apper[1], Bernard Taminiau[3], Armelle Cahu[2], Laurence Le Normand[2], Frédérique Respondek[1], Isabelle Le Huërou-Luron[2], Sophie Blat[2]

[1] Tereos, Marckolsheim, France

[2] INRA, INSERM, Univ Rennes, Nutrition Metabolisms and Cancer, NuMeCan, Rennes, France

[3] University of Liege, Belgium

[*]Corresponding author: Sophie Blat, INRA, NuMeCan, 16 Le Clos, F-35590 Saint-Gilles, France. Tel: +33 223 485 754. Fax: +33 223 485 080. E-mail: sophie.blat@inra.fr

Running title: scFOS program gut microbiota and metabolic health

Abbreviations: AUC, area under the curve; CTRL, control; DOHaD, developmental origins of health and diseases; FFAR, free fatty acid receptor; GLP-1, glucagon-like peptide-1; HF, high-fat; IAP, intestinal alkaline phosphatase; IFN, interferon; IL, interleukin; IVGTT, intravenous glucose tolerance test; LPS, lipopolysaccharide; MLN, mesenteric lymph node; OF, oligofructose; OTU, operational taxonomic unit; PND, postnatal day; SCFA, short-chain fatty acid; scFOS, short-chain fructooligosaccharides; sIgA, secretory immunoglobulin A; TNF, tumour necrosis factor.

ABSTRACT

Perinatal nutrition programs physiological and metabolic functions, with consequences on the susceptibility to develop metabolic diseases in adulthood. The microbiota represents a key factor of such programming. We investigated whether perinatal prebiotic (short-chain fructooligosaccharides, scFOS) supplementation improved adult metabolic health in association with microbiota changes, in pigs used as human model. Sows were supplemented with scFOS or not during the end of gestation and the entire lactation, and offspring received scFOS accordingly during one month after weaning. Pigs were then fed a standard diet for five months followed by a high-fat diet for three months once adults. Perinatal scFOS supplementation induced a persistent modulation of the composition of the faecal microbiota in adulthood, notably by increasing *Prevotella* genus. Meanwhile, scFOS animals displayed improved capacity to secrete GLP-1 and improved pancreas sensitivity to glucose without any changes in peripheral insulin sensitivity. Perinatal scFOS supplementation also increased ileal sIgA secretion and alkaline phosphatase activity, and decreased TNFα expression in adipose tissue. In conclusion, perinatal scFOS supplementation induced long-lasting modulation of intestinal microbiota, and had beneficial consequences on the host physiology in adulthood. Our results highlight the key role of perinatal nutrition on later microbiota and host metabolic adaptation to an unbalanced diet.

Keywords: nutritional programming, prebiotic, GLP-1, *Prevotella*, pig

INTRODUCTION

The developmental origins of health and disease (DOHaD) concept, based on substantial epidemiological evidences and experimental data (1, 2), stipulates that adult metabolic health may be programmed by environmental factors, including nutrition, during perinatal life. The early stages of life, from the foetal life to the end of early childhood in humans (two years old), are a critical window of sensitivity to such programming.

The role of the microbiota in programming physiology and metabolism has recently emerged. Exposure to antibiotics in infancy, for example, has long-lasting consequences on the intestinal microbiota composition (3, 4) and on overweight and metabolic status (5-7). The underlying mechanisms are not fully understood but may involve the known cross-talk between the microbiota and host physiology. For instance, bacterial metabolites such as short-chain fatty acids (SCFA), namely acetate and propionate, enhance the release of glucagon-like peptide-1 (GLP-1) by L-cells of the distal small intestine and proximal colon through the activation of the G-protein-coupled free fatty acid receptor (FFAR) 2 (8). GLP-1, also termed incretin, is an intestinal hormone known to potentiate the glucose-induced insulin secretion and interestingly, it also stimulates β-cell neogenesis and proliferation and inhibits their apoptosis, thereby increasing β-cell mass and insulin secretion capacity (9). This specific communication between the gut and the pancreas, that is mandatory to maintain glucose homeostasis, has been integrated under the concept of the entero-insular axis. Intestinal microbiota, influencing this entero-insular communication, can therefore modulate host glucose homeostasis.

The infant microbiota is shaped by early environment as delivery mode and maternal dietary habits, in humans and animal models (10-12). In fact, it is very influenced by the microbiota of its mother, from passage through the placenta and from the birth canal, to breastfeeding and skin contact (13). Favouring the colonization of a beneficial microbiota in infancy, generating for instance beneficial metabolites for the host, could be a good way to promote health later in life.

Prebiotic fibres are good candidates to positively modulate microbiota. They are selectively fermented ingredients that allow specific changes of the intestinal microbiota, both in composition and metabolism, conferring benefits upon the host well-being and health (14). The impact of consuming a diet supplemented with prebiotics during pregnancy and lactation on offspring health has not been extensively studied but deserves attention due to the influence of prebiotics on microbiota composition and activity. Short-chain fructooligosaccharides (scFOS) are highly interesting prebiotic fibres, obtained from sucrose

and consisting of two to four fructose units linked to one glucose molecule, which are selectively fermented by the gut microbiota, promoting growth of *Lactobacillus*, *Bifidobacterium*, *Akkermensia* and *Blautia coccoides* mainly, as well as SCFA production (15-18). The high degree of similarity between the gut microbiota of mothers supplemented with scFOS and that of their offspring in mice two weeks after birth (19) suggests that improving the profile of maternal gut microbiota does improve early colonization of the offspring gut through bacterial transfer *in utero*, at birth, and during suckling. We previously demonstrated in pigs, that maternal scFOS supplementation induced an increased fermentative activity of the offspring microbiota during lactation with a higher butyrate production after weaning (20), compared to offspring of non-supplemented sows. We also observed a long-lasting effect of the perinatal scFOS supplementation on microbiota metabolite production, more than two months after the end of supplementation (21), suggesting a persistent effect of early scFOS supplementation on microbiota composition. Consequently, it seems possible to modulate the early colonization of the gut microbiota by a maternal prebiotic supply with persistent effect on the microbiota composition and SCFA distal gut content. However, to our knowledge, the consequences of such a microbiota modulation on the physiology and the metabolism of the host have not been investigated. Our hypothesis is that the establishment of a favourable microbiota in neonates by perinatal consumption of prebiotics could have a positive impact on the developmental trajectory of the intestine and pancreas, enabling individuals to better cope metabolically an unbalanced diet as adults. We therefore investigated whether perinatal scFOS supplementation could impact the gut microbiota and the host physiology, entero-insular axis and glucose homeostasis in adult offspring.

For such purpose, we supplemented sows with scFOS during the last third of gestation and the entire lactation, and went on the supplementation of their offspring for one month after weaning. Given evidence that the developmental programming effects of early nutrition can be latent, adult pigs received an unbalanced diet during three months to reveal the potential effects of early scFOS supplementation on programming of their entero-insular axis and metabolic health.

MATERIALS AND METHODS

Animals, diets and experimental design

The experimental protocol was designed in compliance with legislations of the European Union (directive 86/609/EEC) and France (decree 2001-464 29/05/01) for the care and use of

laboratory animals (agreement for animal housing number C-35-275-32). The regional Ethics Committee in Animal Experiments approved the procedure described herein (2016020217308570). Twenty-nine sows (Large White x Landrace) and their piglets ((Large White x Landrace) x Pietrain) from the INRA experimental herd (Saint-Gilles, France) were used in two replicates (n = 17 in the 1st replicate (R1) and n = 12 in the 2nd replicate (R2)). From 28 days before the presumed day of farrowing, sows were fed a standard diet (Cooperl, Lamballe, France) supplemented with either scFOS (95% of scFOS with molecular chain length between 3 and 5 monomeric units, Profeed® P95, Beghin-Meiji, Marckolsheim, France; scFOS group; n = 14) or maltodextrin as a control (MALDEX, Tereos Starch & Sweeteners Europe, Marckolsheim, France; CTRL group; n = 15) (Figure 1). Sows were given 3 $kg.day^{-1}$ of feed during gestation and were fed *ad libitum* during lactation, resulting in an approximately daily intake of 10 g scFOS over the experimental gestation and lactation periods, as detailed previously (21). Before weaning, sow-reared piglets had no access to creep feed. At weaning (postnatal day (PND) 28), piglets were fed *ad libitum* a commercial starter diet supplemented with 0.15 % scFOS (scFOS; n = 37) or maltodextrin (CTRL; n = 42) according to the maternal diet during 4 to 7 weeks. This early post-weaning period corresponds to the onset of food diversification in humans, this is why we decided to cover this period with scFOS supplementation too, in addition to maternal supplementation. Then, pigs were randomly selected to be further used in the experiment (n = 17 CTRL (10 from R1 and 7 from R2) and n = 18 scFOS (11 from R1 and 7 from R2)) and reared on a commercial growing diet until 6 months of age followed by a high-fat high-energetic (HF) diet, formulated at INRA to provide 22.6% of energy from lipids, until 9 months of age (PND 274) (Figure 1). Offspring body weight was measured weekly until weaning and then every two weeks during the supplementation period. From PND 155 (5 weeks before the introduction of the HF diet), pigs were weighed weekly until the end of the experiment. Pigs were monitored daily for food intake, as well as for fever or diarrhoea. No medication or antibiotic treatment was administered throughout the experimental protocol. Composition of sow diets and pig diets is provided in the supplemental Table 1.

Faecal and blood sample collection

Faecal and blood samples were collected at different stages of development during the experiment. Faecal samples were collected from a subset of piglets at PND 21, 50, 190, 211 and 253 to analyse microbiota composition (PND 21, n = 6 per group; PND 190, n = 6 per group; PND 253, n = 6 per group) and to determine the evolution of SCFA production (n = 7

per group and per age) (Figure 1). For microbiota analysis, subsamples of faeces were stored immediately at -80°C after collection without any treatment whereas for SCFA analysis, 1 ml of 0.5% ortho-phosphoric acid solution per g of faeces was added and samples were centrifuged at 1,700 g for 15 min at 4°C. Supernatants were then stored at -20°C for later analysis. Blood was collected in a subset of piglets at PND 21 (n = 91 CTRL and n = 79 scFOS) and after weaning, at PND 77 (n = 42 CTRL and n = 37 scFOS), PND 190 (n = 17 CTRL and n = 18 scFOS) and PND 253 (n = 17 CTRL and n = 18 scFOS) in tubes containing EDTA. After centrifugation at 2,500g for 10 min at 4°C, plasma samples were stored at -20°C for further analysis of glucose, insulin and inflammatory marker concentrations.

16S rRNA high throughput sequencing of faecal microbiota

Genomic DNA was extracted from faeces using a QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions, including a bead-beating step. PCR-amplification of the V1-V3 region of the 16S rDNA and library preparation were performed as previously described (22). Briefly, PCR-amplification of the V1-V3 region of the 16S rDNA and library preparation were performed with the following primers (with Illumina overhand adapters), forward (5'-GAGAGTTTGATYMTGGCTCAG-3') and reverse (5'-ACCGCGGCTGCTGGCAC-3'). Each PCR product was purified with the Agencourt AMPure XP beads kit (Beckman Coulter, Pasadena, USA) and submitted to a second PCR round for indexing, using the Nextera XT index primers 1 and 2. After purification, PCR products were quantified using the Quant-IT PicoGreen (ThermoFisher Scientific, Waltham, USA) and diluted to 10 $ng.\mu l^{-1}$. A final quantification, by qPCR, of each sample in the library was performed using the KAPA SYBR® FAST qPCR Kit (KapaBiosystems, Wilmington, USA) before normalization, pooling and sequencing on a MiSeq sequencer using v3 reagents (ILLUMINA, USA).

The 16S rRNA gene reads were processed with the MOTHUR package (23). The quality of all sequence reads were denoised using the Pyronoise algorithm implemented in MOTHUR and filtered with the following criteria: minimal length of 425 bp, an exact match to the barcode and 1 mismatch allowed to the proximal primer. The sequences were checked for the presence of chimeric amplifications using Uchime. The resultant read sets were compared to a reference dataset of aligned sequences of the corresponding region derived from the SILVA database (v1.19) of full-length rRNA gene sequences implemented in MOTHUR (24). The final reads were clustered into operational taxonomic units (OTUs) using the nearest neighbor algorithm using MOTHUR with a 0.03 distance unit cutoff. A taxonomic identity was

attributed to each OTU by comparison with the SILVA database (80% homogeneity cutoff). As MOTHUR is not dedicated to the taxonomic assignment beyond the genus level, all unique sequences for each OTU were compared to the SILVA dataset 111 using BLASTN algorithm. For each OTU, a consensus detailed taxonomic identification has been given based upon the identity (less than 1% of mismatch with the aligned sequence) and the metadata associated with the best hit (validated bacterial species or not).

SCFA measurement in faeces

SCFA assay was performed by gas chromatography in supernatant of ortho-phosphoric acid-treated faeces as previously described (25).

Tissue sample collection

At PND 21 and 274, 17 (n = 9 CTRL and n = 8 scFOS) and 24 (n = 11 CTRL and n = 13 scFOS) pigs, respectively, were euthanized in our experimental slaughterhouse by electrical stunning and exsanguination (Figure 1). After opening the intestinal cavity, portal blood was collected (PND 21) in tubes containing dipeptidylpeptidase-IV (DPP-IV) inhibitor (10 µl/ml blood, Millipore, Billeria, USA) and EDTA. Mesenteric lymph nodes (MLN) were removed and placed immediately in Hank's balanced saline solution (Sigma-Aldrich, Saint-Quentin Fallavier, France) supplemented with 2% foetal calf serum (FCS), 100 IU/ml penicillin and 100 µg/ml streptomycin (Sigma-Aldrich) for mononuclear immune cell isolation. Ileal biopsies (with and without the Peyer's patch) were rinsed with PBS containing 1% dithiothréitol (DTT, Sigma-Aldrich) and 1% FCS and then placed in medium made with 74% PBS, 25% DMEM (Dulbecco's Modified Eagle Medium, Sigma-Aldrich) and 1% FCS supplemented with 5 g/ml gentamicin (Sigma-Aldrich) and 1.2 mg/ml amphotericin B (Sigma-Aldrich) for immediate explant culture. Intestinal tissues from distal ileum (10 cm proximal to the ileo-caecal junction), caecum (distal part, that is bottom of the organ) and proximal colon (at the end of the first third of colon) (PDN 21 and 274) were collected, rinsed with cold PBS and stored at -80°C until GLP-1 extraction and assay. For later immunohistochemical analyses, samples of 1 cm^3 were dissected from caecum and from the pancreas body and fixed in 4% paraformaldehyde. Another sample of the pancreas body was collected and stored immediately at -80°C for insulin extraction. In addition, at PND 274, a 5 cm ileal segment was rinsed with cold PBS and collected ileal lavage was stored at -20°C until sIgA analysis. Mucosa was scrapped from a 10 cm ileal segment for further intestinal alkaline phosphatase assay. Samples of dorsal longissimus muscle, visceral and dorsal

subcutaneous adipose tissue were frozen in liquid nitrogen and stored at -80°C until further molecular biology analysis.

Intravenous glucose tolerance test (IVGTT)

At PND 253, under general anaesthesia, a catheter was inserted into one external jugular vein of the animals (26) (n = 11 CTRL and n = 13 scFOS). One week after surgery and an overnight fast, the intravenous glucose tolerance test (IVGTT) was performed, consisting in multiple blood samplings: two basal samples were taken at 30 and 15 min before the IV glucose injection (0.5 g/kg body weight) and at times 0 (just after the end of glucose injection), 3, 6, 10, 15, 20, 25, 30, 35, 40, 50, 60 and 75 min after glucose injection. Blood was collected in tubes containing EDTA (or EDTA plus a DPP-IV inhibitor as described above in the tissue sample collection section). After centrifugation at 2,500g for 10 min at 4°C, plasma samples were stored at -20°C (or -80°C for GLP-1 assay dedicated samples) for later analyses. Insulin and glucose assays were performed on all plasma samples. GLP-1 assays were only performed on basal samples. The incremental area under the curve (AUC) was calculated over 75 and 30 min for glucose and insulin. The acute insulin response (AIR = mean insulin concentration above basal values for the first 6 min) and the rate of glucose disappearance (K_G = the negative slope of the regression line obtained with the log-transformed plasma glucose values from 3 to 30 min, in % per min) were also calculated. Insulin sensitivity was evaluated from fasting basal values of glucose (G_b) and insulin (I_b) using the homeostasis model assessment insulin resistance index (HOMA-IR= (G_bxI_b)/22.5), and by calculating S_2 index described to be well-correlated to the insulin sensitivity index obtained by the gold standard euglycemic hyperinsulinemic clamp (27) and taking into account $AUC_{0\text{-}30min}$ of insulin and K_G. The glucose and insulin responses were also integrated using the minimal model described by Bergman (28) to obtain the insulin sensitivity index S_I as well as the glucose efficiency index S_G.

Hormone, glucose, lipid and inflammatory marker assays

Insulin content was extracted from the pancreas in 20 ml of ethanol acid solution (1.5% HCl 12M, 75% absolute ethanol, 23.5% H_2O) (Polytron 3100, Kinematica, 24,000 rpm, 2 x 20 s). Insulin concentrations were measured on plasma and pancreas extracts (dilution 1:3000) by a commercially available RIA assay kit using iodinated porcine insulin (INSULIN-CT, CisbioBioassays, Codolet, France). GLP-1 content was extracted from ileal mucosa, caecum and colon by homogenisation of 1 g of tissue in 5 ml of ethanol acid solution (1% HCl 12M,

74% absolute ethanol, 25% H_2O) (Polytron 3100, Kinematica, 24,000 rpm, 2 x 20 s). Then, GLP-1 concentration was measured in plasma and intestinal samples (dilutions 1:1000, 1:200 and 1:250 for ileal mucosa, caecum and colon, respectively) using commercially available GLP-1 (active) ELISA kit (Millipore).

Plasma glucose, non-esterified fatty acids (NEFA), triglycerides, cholesterol and haptoglobin were assessed by an automated spectrophotometric method (Konelab 20i, Fisher Scientific, Illkirsh, France) using specific commercial kits for each marker (Biomérieux, Bruz, France). Plasma LPS was measured using porcine LPS ELISA kit (MyBiosource, San Diego, USA). The concentration of bioactive phosphatase alkaline was determined in ileal mucosa with a commercial kit (Sensolyte®, Anaspec, San Jose, USA) using para-nitrophenyl phosphate as the substrate. Alkaline phosphatase activity concentrations were expressed per mg of soluble protein.

Secretory IgA were measured in ileal washes. Once collected, samples of ileal washes were homogenized and centrifuged for 10 min at 4°C at 500 g and supernatants were collected and stored at -20°C until analysis of total sIgA level using swine IgA ELISA Quantitation Kit (Bethyl Laboratories, Montgomery, USA).

Finally, MLN cells were isolated by mechanical dissociation before purification over a density gradient (Histopaque, density of 1.077 g/ml, Sigma-Aldrich) and cultured for 72 h at 37°C under an atmosphere containing 5% CO_2, as we previously described (21). Culture of both types of ileal biopsies (with and without the Peyer's patch) were processed as already described (29) and incubated for 20 h. Three different conditions of culture were performed for MLN cells and biopsies: unstimulated condition, in the presence of 5 µg/ml of concanavalin A (ConA, from *Canavalia ensiformis* (Jack bean), Sigma-Aldrich), or in the presence of 10 µg/ml LPS (ultra-pure LPS, from *Escherichia coli* 0111:B4 strain, InvivoGen, Toulouse, France) for MLN cells and 25 µg/ml LPS (from *Escherichia coli* 055:B5 strain, Sigma-Aldrich) for biopsies. Supernatants were collected and stored at -20°C for later cytokine analysis by ELISA (R&D Systems Europe, Lille, France). Concentrations were expressed in pg/ml of supernatants for MLN cells (IFNγ, TNFα, IL-10) and pg/mg of tissue for explant cultures (IFNγ, TNFα, IL-10, IL-8).

Immunohistochemical analyses

After fixation for 24 h in 4% paraformaldehyde, pancreas and caecum samples were cryoprotected overnight at 4°C in PBS containing 30% sucrose, embedded in the OCT compound (TissueTek, Sakura Finetek Europe B. V., Zoeterwoude, The Netherlands), frozen

in isopentane and sectioned (10 µm) using a cryostat-microtome . Immunohistochemical analysis of pancreas was processed as previously described (30) to obtain the number, area of islets and percentage of endocrine tissue. Caecum sections were incubated with PBS containing 4% normal horse serum and 0.5% Triton X-100 for 30 min. Sections were then exposed overnight to a rabbit anti-GLP-1 antibody (1:500; Abcam, Cambridge, UK). After washing with PBS, they were incubated with an anti-rabbit antibody conjugated with FITC (1:200; Jackson Immuno Research, Baltimore, USA) for 3 h. Sections were washed again with PBS, cover-slipped with Vectashield (Vector Labs, Burlingame, USA) and examined with a fluorescence microscope (Eclipse E400, Nikon Instruments France, Champigny-Sur-Marne, France) attached to a digital camera (Digital Still DXM 1200, Nikon Instruments France). The whole section of the tissue was scanned using digital slide scanner (Nanozoomer 2.0-RS, Hamamatsu Photonics France SARL, Massy, France). Recorded files were analysed using the software NDPI (Hamamatsu Photonics) for determination of the number of GLP-1-secreting cells per area of mucosa.

RNA extraction and quantitative real-time PCR (qPCR)

To characterize sensitivity of peripheral tissues to insulin, we analysed mRNA levels of GLUT-4 and INSR in long back muscle, subcutaneous and visceral adipose tissues, plus SREBP1C in both adipose tissues, the level of these mRNA being well correlated to protein expression in these tissues (31, 32). Total RNA from adipose tissue and muscle samples was extracted via the TRIzol method (Fisher Scientific). Extracted RNA samples were quantified using a NanoDrop ND-1000 spectrophotometer (Fisher Scientific). The RNA quality was verified using the Agilent RNA 6000 Nano kit and an Agilent 2100 Bioanalyzer (Agilent Technologies France, Massy, France). All samples met quality criteria. All extracted RNA exhibited RIN (RNA Integrity Number) greater than 7. Total RNA (2 µg) was used for reverse transcription performed according to manufacturer protocol (High Capacity Complementary DNA Reverse Transcription Kit; Fischer Scientific). Real-time PCR was performed with the StepOnePlus real-time PCR machine using SyberGreen master mix (Fisher Scientific) for detection. For each primer pairs, efficiencies of PCR were measured by the slope of a standard curve using serial dilutions of a pool of cDNA from the present experiment. In this experiment, they ranged from 95% to 100% with R^2>0.99. The relative quantity of target gene transcripts was normalized against the geometric mean of three or two housekeeping genes and analysed using the ΔΔ Ct method (33). In this experiment, *RPL4*, *GAPDH* and *YWHAZ* were used

as housekeeping genes for muscle and visceral adipose tissue samples while *GAPDH* and *HPRT1* were identified as stable reference genes for subcutaneous adipose tissue samples since they exhibit high stability values between samples and were not significantly affected by dietary treatments (Supplemental Table 2). Relative quantification of genes of interest was obtained comparing the treated group (scFOS) to the untreated group (CTRL).

Statistical analysis

Considering microbiota analysis, statistical differences of population abundance between groups were assessed with ANOVA, corrected for multi-testing (Benjamini-Hochberg False Discovery Rate) using STAMP software (34). Statistical differences of specific bacterial populations between same-age groups were assessed by two-way ANOVA and Tukey-Kramer post-hoc test using GraphPad Prism® software (version 6.04 for Windows, GraphPad Software, La Jolla California USA, www.graphpad.com). All other data, not relating to the composition of the microbiota, were subjected to ANOVA using R Core Team (2013; R Foundation for Statistical Computing, Vienna, Austria; URL http://www.R-project.org/). Two-way ANOVA with diet (CTRL *vs*. scFOS), replicate (R1 *vs.* R2) and their interaction as factors was performed for body weight, body composition, metabolic and inflammatory parameters at each age. Adult pig body weight and energy intake during the HF diet period was subjected to ANOVA with repeated measurements including diet (CTRL *vs*. scFOS), time (weeks), replicate (R1 *vs.* R2) and the interactions between diet and time, and diet and replication. When diet effect or interaction between diet and time were significant, differences between dietary groups at each time were further measured by Tukey post-hoc test. Insulin and glucose responses to IVGTT were analysed by ANOVA with repeated measurements including diet (CTRL *vs*. scFOS), time (minutes after glucose injection), replication (R1 *vs.* R2) and the interactions between diet and time, and diet and replication, followed by Tukey post-hoc test. Correlations between all parameters were evaluating using Spearman's r-test. All data are presented as means ± SEM. Statistical significance was defined as a P-value $\leq$ 0.05, and trend was reported as a P-value $\leq$ 0.10.

RESULTS

Early scFOS supplementation did not modify body weight at any stage of development (until PND 190) (Table 1). The HF diet did not induce any further growth differences between scFOS and CTRL pigs (Figure 2A). The energy intake of the HF diet decreased continuously

with time, except during the 1st week, with a peak of energy intake (Figure 2B), being higher for the scFOS group than for CTRL pigs (P = 0.02), but without differences on growth between both groups (Figure 2A). Moreover, scFOS pigs tended to ingest more energy during the 8th week (P = 0.07) of HF diet (Figure 2B).

Early scFOS intake modified faecal microbiota composition and faecal SCFA content, and impacted the glucose metabolism of suckling piglets

In 21-day-old piglets nursed by sows supplemented with scFOS, the abundance of bacteria belonging to Bacteroidetes phylum was increased (P < 0.001), while that of bacteria from Firmicutes phylum was reduced (P < 0.001) (Figure 3A). At the genus level, maternal scFOS supplementation induced a higher relative proportion of *Prevotella* (P < 0.001), *Bacteroidales_unclassifed* (P < 0.01) and *Treponema* (P < 0.001), and a reduction of *Bacteroides* (P < 0.001) and *Ruminococcaceae_unclassified* (P < 0.001) (Figure 3A). The highest proportion of *Treponema* was specifically linked to one piglet. The faecal SCFA content was also modified with an increase in total SCFA concentration in faeces of suckling piglets whose mothers received scFOS supplementation (P = 0.001; Table 2), more particularly acetate, propionate, valerate and caproate (P < 0.05). Yet, these differences in faecal SCFA concentrations were not maintained after weaning at the end of the scFOS supplementation (PND 50; Table 2).

Plasma insulin concentration of scFOS piglets at PND 21 was reduced (P = 0.02) with no difference in glycaemia, resulting in a lower insulin/glucose ratio (P = 0.02) (Table 3). In addition, plasma GLP-1 concentration tended to decrease at PND 21 in scFOS piglets (P = 0.09) without modulation of the density of GLP-1 secreting L-cells in the caecal part of the intestine (Table 3). The proportion of pancreatic endocrine tissue was significantly decreased in scFOS piglets (P = 0.01) as well as the pancreatic content of insulin (P = 0.05) (Table 3). In addition, the macronutrient composition of sow's milk measured three weeks after farrowing indicated that scFOS supplemented sows produced milk with higher dry matter (P = 0.004), lipid (P = 0.01) and protein (P = 0.09) contents resulting in a higher energy value (P = 0.005) (Supplemental Table 3).

The early changes in faecal microbiota were maintained in older pigs independently of the diet

At PND 190, while they were fed a standard diet, pigs from the scFOS group displayed a higher proportion of Bacteroidetes phylum (P < 0.001), as a consequence of the increase of

Prevotella genus abundance ($P < 0.001$) (Figure 3B). A higher proportion of *Lachnospiraceae_unclassified* genus ($P < 0.001$) was also observed in scFOS group. In addition, the early scFOS supplementation induced a decrease in the proportion of *Ruminococcaceae_unclassified* and *Bacteroidales_unclassified* genus ($P < 0.05$) (Figure 3B). However, the faecal SCFA concentrations was not different between groups ($P = 0.76$, Table 2).

Three weeks after the beginning of the HF diet (at PND 211), scFOS pigs tended to display a higher acetate concentration in faeces ($P = 0.09$) but it did not persist 6 weeks later (at PND 253, Table 2). However, the microbiota composition differed between CTRL and scFOS groups at PND 253. Adult scFOS pigs under a HF diet displayed an increased proportion of Proteobacteria phylum ($P < 0.01$) without any changes in the two main phyla (Firmicutes and Bacteroidetes) (Figure 3C). At the genus level, the proportion of *Prevotella* was still higher in scFOS group ($P < 0.001$) (Figure 3C). Interestingly, *Prevotella* genus abundance was positively correlated to total SCFA concentration in faecal content ($P < 0.05$; $R = 0.48$).

Early scFOS supplementation programmed the entero-insular axis and glucose metabolism in adult pigs

At PND 253, the GLP-1 secreting L-cell density in the caecum was higher (+33%) in scFOS group as compared to CTRL pigs ($P = 0.03$; Figure 4A). Concomitantly, fasting plasma GLP-1 concentration tended to increase about twice in the scFOS group as compared to the CTRL group ($P = 0.09$; Figure 4B). Interestingly, these two parameters were positively correlated ($P = 0.05$, $R = 0.47$). In pancreas, no difference was measured on the proportion of endocrine tissue ($0.97 \pm 0.16\%$ for CTRL and $0.96 \pm 0.09\%$ for scFOS group) nor on the insulin content (6.70 ± 1.61 µUI/g for CTRL and 7.20 ± 1.57 µUI/g for scFOS group). However, the pancreatic insulin content was positively correlated to the density of caecal GLP-1 L-cells ($P = 0.001$, $R = 0.65$).

Plasma fasting glucose and insulin concentrations were not different between groups neither before (PND 190) nor after the HF diet period (PND 253) (Table 4), nor were the lipid concentrations (triglycerides, free fatty acids and total cholesterol; data not shown). When the glucose homeostasis was challenged by an intravenous injection of glucose (IVGTT), scFOS animals secreted more insulin than CTRL animals with no difference in glucose profile (Figure 5). Calculated indexes from insulin and glucose profiles resulted in a tendency to a higher insulin AUC between 0 and 30 min after glucose injection in scFOS pigs ($P = 0.07$), as well as a reduction in S_2 ($P = 0.09$) and in S_I ($P = 0.02$) indices (Table 4). A negative

correlation was established between glucose $AUC_{0\text{-}30min}$ and the caecal GLP-1 producing L-cells (P = 0.001, R = -0.62).

Interestingly, HOMA-IR index was negatively correlated to the relative abundance of *Prevotella* (R = -0.64), but positively to *Ruminococcaceae_unclassified* genus (R = 0.66). Conversely, plasma GLP-1 concentration was negatively correlated to the proportion of *Ruminococcaceae_unclassified* genus (R = -0.60) but positively to total SCFA (R = 0.66), acetate (R = 0.67), propionate (R = 0.53) and butyrate (R = 0.54) in faeces.

Early scFOS intake did not modify insulin sensitivity but reduced pro-inflammatory cytokine expression in visceral adipose tissue

No difference in subcutaneous adiposity but an increase in visceral adipose tissue weight was noticed in scFOS pigs (P = 0.02). No other difference in relative organ weight was recorded between groups at slaughter (Table 1). None of the studied genes representative of insulin sensitivity in muscle and adipose tissues displayed differences in mRNA levels between CTRL and scFOS groups at PND 273 (Table 5). *TNFα* and *IL-10* expression was not different between scFOS and CTRL animals in the subcutaneous adipose tissue, but *TNFα* expression significantly decreased in the visceral adipose tissue of scFOS pigs as compared to CTRL pigs (P = 0.02; Table 5). Plasma haptoglobin and LPS concentrations were not modified by early scFOS supplementation (Table 6).

Early scFOS intake improved intestinal protection in adult pigs

Adult scFOS pigs displayed a tendency for an increased intestinal alkaline phosphatase (IAP) level in the ileal mucosa (P = 0.098) and a significant increase in sIgA concentration in ileal washes (P = 0.03) compared to CTRL pigs (Table 6). The concentration of sIgA in ileal washes was positively correlated to the IAP level (P = 0.03, R = 0.46) and negatively to the plasmatic LPS (P = 0.02, R = -0.48). *In vitro* stimulation of MLN cells from scFOS animals with ConA mitogen secreted more IFNγ than those from CTRL animals (P = 0.003), but there was no difference when MLN cells were stimulated with LPS (Table 6). The secretion of the other measured cytokines (TNFα and IL-10) was not different between groups (Table 6) in both conditions (except LPS-induced IL-10 secretion which tended to be lower in scFOS, P = 0.06). Cytokine secretions (TNFα, IL-10, IFNγ and IL-8) after *in vitro* culture of ileal

explants, with and without the Peyer's patch, were not modified by early scFOS supplementation (Supplemental Table 4).

DISCUSSION

Accumulating evidences suggest that nutrition during foetal and early postnatal life programs microbial, metabolic and immune development in offspring, with later consequences on their susceptibility to metabolic diseases. We showed that scFOS supplementation, known to well-balance the gut microbiota, during perinatal life, programs the intestinal endocrine function with increased pancreas sensitivity to glucose in a GLP-1 dependent manner through microbiota changes, as well as the resistance to inflammation. This scFOS-induced programming may be beneficial to cope with an unbalanced diet in adulthood.

We first analysed the microbiota and metabolic profile of suckling piglets whose mothers were supplemented with scFOS or not. Our results demonstrated that it is possible to modulate microbiota establishment in suckling piglets by maternal scFOS supplementation during the perinatal period. Indeed, the bacterial composition of scFOS offspring displayed a higher proportion of Bacteroidetes phylum, and more particularly an increase in *Prevotella* genus compared to the non-supplemented control group. Total SCFA content, notably acetate, was increased in faeces of suckling scFOS piglets. This is consistent with the increase of *Prevotella*, known to ferment complex carbohydrates and to produce high amounts of SCFA (35). In connection with these early microbiota modifications, the maternal scFOS supplementation induced an improvement of the glucose tolerance in suckling piglets, as the amount of insulin required to return to the basal glycaemia after suckling was diminished. Maternal scFOS supplementation did not modify the intestinal endocrine function, with the same L-cell density and GLP-1 tissue content between both groups, but reduced the development of the endocrine pancreas, objectified by a reduction in endocrine tissue percentage and insulin secretion by the pancreas, in association with a tendency to a decreased plasma GLP-1 concentration. The difference of maternal milk composition, with an increase in lipid content, energy value and a tendency for more proteins with scFOS supplementation, could explain such effects. In fact, a modulation of the diet during the perinatal life (*via* the maternal diet or directly by the postnatal diet) can induce significant effects on endocrine pancreas development and glucose tolerance (36-38). In addition, the lower development of endocrine pancreas, a potential consequence of a lower request of its endocrine function, could be due to an improved insulin sensitivity of the peripheral tissues, as observed in rats

fed by mothers supplemented with 21.6% of OF/inulin with an over-expression of *UCP-1* and *PGC-1α* in adipose tissue (39).

Afterward, we analysed the programming effects of such early modifications in adulthood. There was no difference in faecal SCFA concentration between scFOS and CTRL groups after weaning or in adulthood, contrary to our previous results showing an increase butyrate content in the distal intestine in two- (20) and three-month-old pigs (21). However, early scFOS-induced differences in microbiota composition persisted until adulthood, particularly the higher relative proportion of *Prevotella* genus. Accordingly, in another study in rats, microbiota modulation in early life by maternal oligofructose (OF)/inulin intake (21.6% from conception to weaning) induced persistent modifications with an increase of *Bifidobacterium spp.* and *Clostridium coccoides* in adult rat offspring (40).

In association with these microbiota changes, metabolic parameters of the host were modulated by early scFOS intake. Actually, a higher fasting GLP-1 concentration in adult pigs, that received early scFOS supplementation, correlated to a significant increased GLP-1 secreting L-cell density in the caecum indicating an increased capacity of the scFOS adult offspring to secrete GLP-1. Adult rats, following a perinatal OF supplementation, did not display changes in postprandial GLP-1 level but showed an increase in gastric inhibitory peptide concentration, another incretin hormone, secreted by more proximal K-cells (41). Moreover, when ingested directly by healthy adults or adults displaying deleterious metabolic conditions (due to a HF diet or obesity), prebiotics increased the number of intestinal GLP-1 producing L-cells, GLP-1 content and circulating levels of GLP-1 in humans, rats and mice (42-49), which might, in turn, improved their metabolic status (50). SCFA produced by the microbiota after scFOS intake increase the production and release of GLP-1 by entero-endocrine L-cells in the intestine and favour L-cells differentiation in crypts (47). In our study, perinatal scFOS consumption displayed similar effects on L-cells seven months after the end of supplementation. One explanation could be the maintenance over time of a favourable microbiota, notably with high fermentative activity, established during early life. This is supported by the positive correlation observed between the SCFA production and plasmatic GLP-1 concentration. Therefore, our data demonstrated a programming of the intestinal endocrine function probably mediated by the early establishment of a beneficial microbiota composition and fermentative activity, which persisted into adulthood.

Once released from intestinal L-cells, GLP-1 stimulates insulin secretion and has a trophic effect on pancreas by regulating islet cell proliferation and differentiation (9). No difference was observed in pancreatic anatomy and insulin content between scFOS and CTRL adult pigs

but scFOS group tended to secrete more insulin in response to an intravenous glucose stimulus with no modification of the glucose profile. This increased insulin release highlighted a higher pancreas sensitivity to glucose in scFOS pigs, as they displayed no insulin resistance in their peripheral tissues. This could be due to their higher basal GLP-1 concentration, as suggested by Chan *et al.* who demonstrated that increasing doses of GLP-1 stimulated insulin release during IVGTT in mice (51). The positive correlation between GLP-1 secreting L-cell density and pancreatic insulin content underlies such role of GLP-1 on pancreas insulin secretion capacity in our study. This was in accordance with data in HF fed diabetic mice, in which OF supplementation during four weeks was efficient to reduce fasting and postprandial glycaemia in association with increased levels of plasma and pancreatic insulin. These results were obtained in a GLP-1-dependent manner through an increased plasma GLP-1 concentration and total GLP-1 content in the proximal colon (44). In our study, we did not observe changes of glucose profile during the IVGTT, but the negative correlation seen between $AUC_{Glucose}$ and the GLP-1-producing L-cells supported the role of intestinal endocrine function in the regulation of glucose homeostasis. Interestingly, the observed negative correlation between *Prevotella* abundance and HOMA-IR index may suggest that the increased *Prevotella* proportion could be a key factor to prevent development of insulin resistance.

The programming of the intestinal immune function, which is also dependent of the microbiota composition and its fermentative activity, was investigated in adult pigs. Perinatal scFOS supplementation induced a beneficial long-lasting effect on intestinal sIgA secretion and IAP concentration (higher values of both parameters in scFOS pigs) with no significant difference in plasma LPS. The expression of IAP has been shown to be controlled by the gut microbiota (52), as well as the sIgA release in the intestinal lumen (53), in accordance with the persistent microbiota modulation induced by perinatal scFOS supplementation in adulthood. The increased sIgA secretion could be linked to the modification of bacteria populations, and more particularly to the higher relative abundance of *Prevotella*, as suggested by Mach *et al.* showing a positive correlation between *Prevotella* and sIgA luminal concentration in weaned pigs (54). Intestinal sIgA secretion is essential to prevent inflammatory responses by down-regulating the expression of inflammatory cytokines such as TNFα and IL-6 (55). Another regulator of intestinal inflammation is IAP that controls several gut biological processes such as LPS detoxification, reducing its toxicity for epithelial cells, inflammation and bacterial translocation to the lymphoid organs (56). An increased IAP activity may help to chronically decrease plasma LPS concentration, and thereby may reduce

metabolic endotoxemia which contributes to HF diet-induced metabolic disorders (50). Although there was no significant difference in plasma LPS, numeric values were 37% lower in scFOS pigs, and once again, *TNFα* expression in their visceral adipose tissue was significantly decreased as compared to CTRL pigs. Accordingly, Hallam *et al.* showed a reduction of plasma LPS in adult rats supplemented with prebiotics in early life, linked to a higher abundance of *Bifidobacterium spp.*, suggested to be mediated by milk oligosaccharides (40). In our study, maternal scFOS supplementation induced macronutrient composition differences in sow milk, that could contribute to modulate the composition and the metabolism of the microbiota colonizing the neonatal gut and consequently their long-lasting effects (40). Finally, there was a positive correlation between sIgA and IAP activity, and a negative one between sIgA and plasma LPS concentration, underlying a possible link between these different inflammatory parameters. Overall, our data suggested that scFOS adult offspring may be more resistant to inflammation.

The pattern of growth was not affected by perinatal scFOS supplementation nor was the body weight gain during the HF challenge, even if scFOS pigs tended to ingest more energy. Both groups regulated their energy intake after the first week of HF diet transition and did not develop hyperphagia. scFOS pigs displayed an increased visceral adiposity at PND 274, but as adiposity is predominantly located in subcutaneous adipose tissue in pigs (around 65%) whereas internal adiposity represents only 5% of total body fat (57), this reflected only a very slight quantitative effect on global adiposity. It had indeed no effect on the systemic inflammatory status and on the insulin sensitivity of peripheral tissues. Interestingly, even if heavier, the visceral tissue of scFOS pigs expressed less *TNFα* compared to CTRL group. Hallam *et al.* showed a reduction in the percentage of total body fat in adult rats fed a high-fat high-sucrose diet whose mothers had been supplemented with prebiotics (41). This discrepancy could be explained by a species variability, but also by the higher dose of prebiotic and the different period of supplementation used in their study (21.6% during the whole gestation) compared to ours (0.33% during the last third of gestation and 0.15% for lactation and post-weaning) as well as the different diet composition in adulthood (41).

In conclusion, our study demonstrated that the intestinal endocrine function of adult pigs and their susceptibility to inflammation is submitted to a nutritional programming that may participate to the metabolic imprint. This programming is highly connected to the microbiota. These results are of high interest to understand nutritional programming in humans and thus to develop nutritional strategies to prevent metabolic disorders. Indeed, pig used as model for humans is considered to be more relevant than rodents, especially because there is higher

similarity in anatomy, physiology, metabolism, and microbiota between humans and pigs (58). Among nutritional strategies, perinatal scFOS supplementation, known to well-balance the microbiota durably, seems to be an interesting way to prevent metabolic disorders and susceptibility to inflammation later in life, notably when confronted to a deleterious nutritional environment.

ACKNOWLEDGMENTS

The authors wish to thank the technical staff of NuMeCan for their expert assistance during the course of this project. They also acknowledge all the staff of Rennes porcine experimental facilities (UEPR) for animal care and feeding.

FINANCIAL SUPPORT

This study received funding from Tereos, a company producing short-chain fructooligosaccharides. FR and EA are employed by Tereos. A French patent application has been filed (FR 17/0035).

CONFLICT OF INTEREST

Author disclosures: C. Le Bourgot, S. Ferret-Bernard, E. Apper, B. Taminiau, A. Cahu, L. Le Normand, F. Respondek, I. Le Huërou-Luron and S. Blat have declared no conflict of interest and funding disclosure.

AUTHORSHIP

C.L.B., I.L.H.-L and S.B. designed research; C.L.B., S.F.-B., B.T., A.C., L.L.N., I.L.H.-L. and S.B. conducted research and analysed data; F.R. and E.A. contributed reagents/materials/analysis; C.L.B., S.F.-B., E.A., I.L.H.-L. and S.B. wrote the manuscript. All authors read and approved the final manuscript, agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

REFERENCES

1. Gluckman, P. D., Hanson, M. A., and Beedle, A. S. (2007) Early life events and their consequences for later disease: a life history and evolutionary perspective. *Am J Hum Biol* **19**, 1-19
2. Godfrey, K. M., Gluckman, P. D., and Hanson, M. A. (2010) Developmental origins of metabolic disease: life course and intergenerational perspectives. *Trends Endocrinol Metab* **21**, 199-205
3. Bedford Russell, A. R., and Murch, S. H. (2006) Could peripartum antibiotics have delayed health consequences for the infant? *Bjog* **113**, 758-765
4. Schokker, D., Zhang, J., Vastenhouw, S. A., Heilig, H. G., Smidt, H., Rebel, J. M., and Smits, M. A. (2015) Long-lasting effects of early-life antibiotic treatment and routine animal handling on gut microbiota composition and immune system in pigs. *PLoS One* **10**, e0116523
5. Ajslev, T. A., Andersen, C. S., Gamborg, M., Sorensen, T. I., and Jess, T. (2011) Childhood overweight after establishment of the gut microbiota: the role of delivery mode, pre-pregnancy weight and early administration of antibiotics. *Int J Obes (Lond)* **35**, 522-529
6. Cox, L. M., Yamanishi, S., Sohn, J., Alekseyenko, A. V., Leung, J. M., Cho, I., Kim, S. G., Li, H., Gao, Z., Mahana, D., Zarate Rodriguez, J. G., Rogers, A. B., Robine, N., Loke, P., and Blaser, M. J. (2014) Altering the intestinal microbiota during a critical developmental window has lasting metabolic consequences. *Cell* **158**, 705-721
7. Trasande, L., Blustein, J., Liu, M., Corwin, E., Cox, L. M., and Blaser, M. J. (2013) Infant antibiotic exposures and early-life body mass. *Int J Obes (Lond)* **37**, 16-23
8. Tolhurst, G., Heffron, H., Lam, Y. S., Parker, H. E., Habib, A. M., Diakogiannaki, E., Cameron, J., Grosse, J., Reimann, F., and Gribble, F. M. (2012) Short-chain fatty acids stimulate glucagon-like peptide-1 secretion via the G-protein-coupled receptor FFAR2. *Diabetes* **61**, 364-371
9. Baggio, L. L., and Drucker, D. J. (2007) Biology of incretins: GLP-1 and GIP. *Gastroenterology* **132**, 2131-2157
10. De Filippo, C., Cavalieri, D., Di Paola, M., Ramazzotti, M., Poullet, J. B., Massart, S., Collini, S., Pieraccini, G., and Lionetti, P. (2010) Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proc Natl Acad Sci U S A* **107**, 14691-14696
11. Dominguez-Bello, M. G., Costello, E. K., Contreras, M., Magris, M., Hidalgo, G., Fierer, N., and Knight, R. (2010) Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proc Natl Acad Sci U S A* **107**, 11971-11975
12. Penders, J., Thijs, C., Vink, C., Stelma, F. F., Snijders, B., Kummeling, I., van den Brandt, P. A., and Stobberingh, E. E. (2006) Factors influencing the composition of the intestinal microbiota in early infancy. *Pediatrics* **118**, 511-521
13. Pannaraj, P. S., Li, F., Cerini, C., Bender, J. M., Yang, S., Rollie, A., Adisetiyo, H., Zabih, S., Lincez, P. J., Bittinger, K., Bailey, A., Bushman, F. D., Sleasman, J. W., and Aldrovandi, G. M. (2017) Association Between Breast Milk Bacterial Communities and Establishment and Development of the Infant Gut Microbiome. *JAMA Pediatr*
14. Roberfroid, M., Gibson, G. R., Hoyles, L., McCartney, A. L., Rastall, R., Rowland, I., Wolvers, D., Watzl, B., Szajewska, H., Stahl, B., Guarner, F., Respondek, F., Whelan, K., Coxam, V., Davicco, M. J., Leotoing, L., Wittrant, Y., Delzenne, N. M., Cani, P. D., Neyrinck, A. M., and Meheust, A. (2010) Prebiotic effects: metabolic and health benefits. *Br J Nutr* **104 Suppl 2**, S1-63
15. Pan, X. D., Chen, F. Q., Wu, T. X., Tang, H. G., and Zhao, Z. Y. (2009) Prebiotic oligosaccharides change the concentrations of short-chain fatty acids and the microbial population of mouse bowel. *J Zhejiang Univ Sci B* **10**, 258-263
16. Respondek, F., Gerard, P., Bossis, M., Boschat, L., Bruneau, A., Rabot, S., Wagner, A., and Martin, J. C. (2013) Short-chain fructo-oligosaccharides modulate intestinal microbiota and metabolic parameters of humanized gnotobiotic diet induced obesity mice. *PLoS One* **8**, e71026

17. Swanson, K. S., Grieshop, C. M., Flickinger, E. A., Bauer, L. L., Chow, J., Wolf, B. W., Garleb, K. A., and Fahey, G. C., Jr. (2002) Fructooligosaccharides and Lactobacillus acidophilus modify gut microbial populations, total tract nutrient digestibilities and fecal protein catabolite concentrations in healthy adult dogs. *J Nutr* **132**, 3721-3731
18. Liu, T. W., Cephas, K. D., Holscher, H. D., Kerr, K. R., Mangian, H. F., Tappenden, K. A., and Swanson, K. S. (2016) Nondigestible Fructans Alter Gastrointestinal Barrier Function, Gene Expression, Histomorphology, and the Microbiota Profiles of Diet-Induced Obese C57BL/6J Mice. *J Nutr* **146**, 949-956
19. Fujiwara, R., Watanabe, J., and Sonoyama, K. (2008) Assessing changes in composition of intestinal microbiota in neonatal BALB/c mice through cluster analysis of molecular markers. *Br J Nutr* **99**, 1174-1177
20. Le Bourgot, C., Le Normand, L., Formal, M., Respondek, F., Blat, S., Apper, E., Ferret-Bernard, S., and Le Huerou-Luron, I. (2017) Maternal short-chain fructo-oligosaccharide supplementation increases intestinal cytokine secretion, goblet cell number, butyrate concentration and Lawsonia intracellularis humoral vaccine response in weaned pigs. *Br J Nutr* **117**, 83-92
21. Le Bourgot, C., Ferret-Bernard, S., Le Normand, L., Savary, G., Menendez-Aparicio, E., Blat, S., Appert-Bossard, E., Respondek, F., and Le Huerou-Luron, I. (2014) Maternal short-chain fructooligosaccharide supplementation influences intestinal immune system maturation in piglets. *PLoS One* **9**, e107508
22. Bindels, L. B., Neyrinck, A. M., Salazar, N., Taminiau, B., Druart, C., Muccioli, G. G., Francois, E., Blecker, C., Richel, A., Daube, G., Mahillon, J., de los Reyes-Gavilan, C. G., Cani, P. D., and Delzenne, N. M. (2015) Non Digestible Oligosaccharides Modulate the Gut Microbiota to Control the Development of Leukemia and Associated Cachexia in Mice. *PLoS One* **10**, e0131009
23. Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., Lesniewski, R. A., Oakley, B. B., Parks, D. H., Robinson, C. J., Sahl, J. W., Stres, B., Thallinger, G. G., Van Horn, D. J., and Weber, C. F. (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl Environ Microbiol* **75**, 7537-7541
24. Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., and Glockner, F. O. (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res* **41**, D590-596
25. Jouany, J. P., Zainab, B., Senaud, J., Groliere, C. A., Grain, J., and Thivend, P. (1981) Role of the rumen ciliate protozoa polyplastron-multivesiculatum, entodinium sp and isotricha-prostoma in the digestion of a mixed diet in sheep. *Reproduction Nutrition Development* **21**, 871-884
26. Mosnier, E., Le Floc'h, N., Etienne, M., Ramaekers, P., Seve, B., and Pere, M. C. (2010) Reduced feed intake of lactating primiparous sows is associated with increased insulin resistance during the peripartum period and is not modified through supplementation with dietary tryptophan. *J Anim Sci* **88**, 612-625
27. Christoffersen, B., Ribel, U., Raun, K., Golozoubova, V., and Pacini, G. (2009) Evaluation of different methods for assessment of insulin sensitivity in Gottingen minipigs: introduction of a new, simpler method. *Am J Physiol Regul Integr Comp Physiol* **297**, R1195-1201
28. Bergman, R. N. (2005) Minimal model: perspective from 2005. *Horm Res* **64 Suppl 3**, 8-15
29. Chatelais, L., Jamin, A., Gras-Le Guen, C., Lalles, J. P., Le Huerou-Luron, I., and Boudry, G. (2011) The level of protein in milk formula modifies ileal sensitivity to LPS later in life in a piglet model. *PLoS One* **6**, e19594
30. Blat, S., Morise, A., Sauret, A., Louveau, I., Mace, K., Le Huerou-Luron, I., and Seve, B. (2012) The protein level of isoenergetic formulae does not modulate postprandial insulin secretion in piglets and has no consequences on later glucose tolerance. *Br J Nutr* **108**, 102-112

31. Atkinson, B. J., Griesel, B. A., King, C. D., Josey, M. A., and Olson, A. L. (2013) Moderate GLUT4 overexpression improves insulin sensitivity and fasting triglyceridemia in high-fat diet-fed transgenic mice. *Diabetes* **62**, 2249-2258
32. Kampmann, U., Christensen, B., Nielsen, T. S., Pedersen, S. B., Orskov, L., Lund, S., Moller, N., and Jessen, N. (2011) GLUT4 and UBC9 protein expression is reduced in muscle from type 2 diabetic patients with severe insulin resistance. *PLoS One* **6**, e27854
33. Livak, K. J., and Schmittgen, T. D. (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* **25**, 402-408
34. Parks, D. H., and Beiko, R. G. (2010) Identifying biologically relevant differences between metagenomic communities. *Bioinformatics* **26**, 715-721
35. Chen, T., Long, W., Zhang, C., Liu, S., Zhao, L., and Hamaker, B. R. (2017) Fiber-utilizing capacity varies in Prevotella- versus Bacteroides-dominated gut microbiota. *Sci Rep* **7**, 2594
36. Delamaire, E., Parnet, P., Coupe, B., Hoebler, C., Blat, S., Poupeau, G., Boquien, C. Y., Champ, M., and Darmaun, D. (2012) Long term metabolic impact of high protein neonatal feeding: a preliminary study in male rat pups born with a low birth weight. *Clin Nutr* **31**, 741-748
37. Guo, F., and Jen, K. L. (1995) High-fat feeding during pregnancy and lactation affects offspring metabolism in rats. *Physiol Behav* **57**, 681-686
38. Petrik, J., Reusens, B., Arany, E., Remacle, C., Coelho, C., Hoet, J. J., and Hill, D. J. (1999) A low protein diet alters the balance of islet cell replication and apoptosis in the fetal and neonatal rat and is associated with a reduced pancreatic expression of insulin-like growth factor-II. *Endocrinology* **140**, 4861-4873
39. Maurer, A. D., and Reimer, R. A. (2011) Maternal consumption of high-prebiotic fibre or -protein diets during pregnancy and lactation differentially influences satiety hormones and expression of genes involved in glucose and lipid metabolism in offspring in rats. *Br J Nutr* **105**, 329-338
40. Hallam, M. C., Barile, D., Meyrand, M., German, J. B., and Reimer, R. A. (2014) Maternal high-protein or high-prebiotic-fiber diets affect maternal milk composition and gut microbiota in rat dams and their offspring. *Obesity (Silver Spring)* **22**, 2344-2351
41. Hallam, M. C., and Reimer, R. A. (2013) A maternal high-protein diet predisposes female offspring to increased fat mass in adulthood whereas a prebiotic fibre diet decreases fat mass in rats. *Br J Nutr* **110**, 1732-1741
42. Cani, P. D., Dewever, C., and Delzenne, N. M. (2004) Inulin-type fructans modulate gastrointestinal peptides involved in appetite regulation (glucagon-like peptide-1 and ghrelin) in rats. *Br J Nutr* **92**, 521-526
43. Cani, P. D., Hoste, S., Guiot, Y., and Delzenne, N. M. (2007) Dietary non-digestible carbohydrates promote L-cell differentiation in the proximal colon of rats. *Br J Nutr* **98**, 32-37
44. Cani, P. D., Knauf, C., Iglesias, M. A., Drucker, D. J., Delzenne, N. M., and Burcelin, R. (2006) Improvement of glucose tolerance and hepatic insulin sensitivity by oligofructose requires a functional glucagon-like peptide 1 receptor. *Diabetes* **55**, 1484-1490
45. Cani, P. D., Possemiers, S., Van de Wiele, T., Guiot, Y., Everard, A., Rottier, O., Geurts, L., Naslain, D., Neyrinck, A., Lambert, D. M., Muccioli, G. G., and Delzenne, N. M. (2009) Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. *Gut* **58**, 1091-1103
46. Delmee, E., Cani, P. D., Gual, G., Knauf, C., Burcelin, R., Maton, N., and Delzenne, N. M. (2006) Relation between colonic proglucagon expression and metabolic response to oligofructose in high fat diet-fed mice. *Life Sci* **79**, 1007-1013
47. Kaji, I., Karaki, S., Tanaka, R., and Kuwahara, A. (2011) Density distribution of free fatty acid receptor 2 (FFA2)-expressing and GLP-1-producing enteroendocrine L cells in human and rat lower intestine, and increased cell numbers after ingestion of fructo-oligosaccharide. *J Mol Histol* **42**, 27-38

48. Parnell, J. A., and Reimer, R. A. (2012) Prebiotic fibres dose-dependently increase satiety hormones and alter Bacteroidetes and Firmicutes in lean and obese JCR:LA-cp rats. *Br J Nutr* **107**, 601-613
49. Everard, A., Lazarevic, V., Derrien, M., Girard, M., Muccioli, G. G., Neyrinck, A. M., Possemiers, S., Van Holle, A., Francois, P., de Vos, W. M., Delzenne, N. M., Schrenzel, J., and Cani, P. D. (2011) Responses of gut microbiota and glucose and lipid metabolism to prebiotics in genetic obese and diet-induced leptin-resistant mice. *Diabetes* **60**, 2775-2786
50. Everard, A., and Cani, P. D. (2013) Diabetes, obesity and gut microbiota. *Best Pract Res Clin Gastroenterol* **27**, 73-83
51. Chan, H. M., Jain, R., Ahren, B., Pacini, G., and D'Argenio, D. Z. (2011) Effects of increasing doses of glucagon-like peptide-1 on insulin-releasing phases during intravenous glucose administration in mice. *Am J Physiol Regul Integr Comp Physiol* **300**, R1126-1133
52. Bates, J. M., Akerlund, J., Mittge, E., and Guillemin, K. (2007) Intestinal alkaline phosphatase detoxifies lipopolysaccharide and prevents inflammation in zebrafish in response to the gut microbiota. *Cell Host Microbe* **2**, 371-382
53. Ohland, C. L., and Jobin, C. (2015) Microbial activities and intestinal homeostasis: A delicate balance between health and disease. *Cell Mol Gastroenterol Hepatol* **1**, 28-40
54. Mach, N., Berri, M., Estelle, J., Levenez, F., Lemonnier, G., Denis, C., Leplat, J. J., Chevaleyre, C., Billon, Y., Dore, J., Rogel-Gaillard, C., and Lepage, P. (2015) Early-life establishment of the swine gut microbiome and impact on host phenotypes. *Environ Microbiol Rep* **7**, 554-569
55. Boullier, S., Tanguy, M., Kadaoui, K. A., Caubet, C., Sansonetti, P., Corthesy, B., and Phalipon, A. (2009) Secretory IgA-mediated neutralization of Shigella flexneri prevents intestinal tissue destruction by down-regulating inflammatory circuits. *J Immunol* **183**, 5879-5885
56. Lalles, J. P. (2010) Intestinal alkaline phosphatase: multiple biological roles in maintenance of intestinal homeostasis and modulation by diet. *Nutr Rev* **68**, 323-332
57. Mourot, J. (2004) Control of the adiposity in breeding animals. *Bulletin de l'Académie Vétérinaire de France* **157**, 29-34
58. Roura, E., Koopmans, S. J., Lalles, J. P., Le Huerou-Luron, I., de Jager, N., Schuurman, T., and Val-Laillet, D. (2016) Critical review evaluating the pig as a model for human nutritional physiology. *Nutr Res Rev* **29**, 60-90

FIGURE LEGENDS

Figure 1: Overview of the study design

Twenty-nine sows and their piglets were used in two replicates (n = 17 in the 1st replicate (R1) and n=12 in the 2nd replicate (R2)). From 28 days before the presumed day of farrowing, sows were fed either a control diet (n = 15, CTRL group) or short-chain fructooligosaccharide (scFOS) supplemented diet (n = 14, scFOS group) until the end of the lactation. Plasma was collected in suckled piglets (n = 170) at postnatal day (PND) 21 and in weaned pigs at PND 77 (n = 79), PND 190 (n = 35) and PND 253 (n = 35) in order to analyse glucose homeostasis at different stages. An intravenous glucose tolerance test (IVGTT) was performed at the end of the experiment at PND 267 (n = 24) to evaluate the glucose homeostasis. Faecal contents were collected at PND 21, PND 190, PND 211 and PND 253 (n = 14/age of collect) to determine the SCFA production, reflective of the fermentative activity of the microbiota. Seventeen piglets were sacrificed at PND 21 to investigate the development of the entero-pancreatic axis and 24 pigs were sacrificed at the adult stage to evaluate the adaptation of the entero-pancreatic axis to 3 months of HF diet.

Figure 2: Body weight and food intake in adult pigs

A) Growth of adult pigs (n = 17 CTRL and n = 18 scFOS). Growth increased significantly with time ($P < 0.001$) whatever the perinatal diet.

B) Energy intake expressed in MJ/d/kg of body weight (BW) (n = 17 CTRL and n = 18 scFOS). Energy intake decreased with time ($P < 0.001$) and a tendency to an interaction between perinatal diet and time ($P = 0.09$) was observed.

Mean values ± SEM. *: $P < 0.05$ vs CTRL. #: $P < 0.10$ vs CTRL.

CTRL, perinatal control diet; scFOS, perinatal short-chain fructooligosaccharide supplemented diet; HF, high-fat diet, W, weeks after the beginning of the HF diet.

Figure 3: Faecal microbiota composition analysis by 16S profiling

Mean phylotype distribution (phylum and genus levels) expressed as cumulated relative abundance mean (n = 6 per group) at PND 21 during suckling (A), PND 190 after standard diet feeding (B) and at PND 253 after a 3 month period of HF diet feeding (C).

*↑↓: $P < 0.05$ between both groups at the same age.

CTRL, perinatal control diet; scFOS, perinatal short-chain fructooligosaccharide supplemented diet; HF, high-fat diet.

Figure 4: GLP-1 secreting L-cell density in caecum and plasma GLP-1 concentration at PND 273

A) GLP-1 secreting L-cell density in caecum (n = 11 CTRL and n = 13 scFOS).

B) Plasma GLP-1 concentration after an overnight fast (n = 11 CTRL and n = 13 scFOS). Mean values ± SEM; *: P < 0.05; #: P < 0.10.

CTRL, perinatal control diet; scFOS, perinatal short-chain fructooligosaccharide supplemented diet.

Figure 5: Glucose and insulin responses to an IVGTT at PND 267

A) Insulin profile (n = 11 CTRL and n = 13 scFOS).

B) Glucose profile (n = 11 CTRL and n = 13 scFOS).

Mean values ± SEM; *: P < 0.05 *vs* CTRL; #: P < 0.10 *vs* CTRL.

CTRL, perinatal control diet; scFOS, perinatal short-chain fructooligosaccharide supplemented diet.

Table 1: Growth performance from birth to PND 274 and body composition (PND 253-274) of pigs born from sows supplemented in scFOS or not during the perinatal life

	CTRL	**scFOS**	**Diet p-value**
Body weight at birth, *kg*	1.46 ± 0.03	1.40 ± 0.02	0.11
Body weight PND 28, *kg*	9.15 ± 0.14	9.02 ± 0.16	0.54
Body weight PND 56, *kg*	19.48 ± 0.41	19.74 ± 0.49	0.66
Body weight PND 190, *kg*	146.7 ± 3.4	147.3 ± 3.6	0.56
HF transition	**CTRL**	**scFOS**	**Diet p-value**
Body weight PND 274, *kg*	214.7 ± 3.3	217.6 ± 4.4	0.80
Subcutaneous fat thickness PND 253, *mm*	24.4 ± 1.3	24.6 ± 1.7	0.86
Visceral adipose tissue PND 274, *g/kg*	15.83 ± 0.92	20.31 ± 1.47	**0.02**
Relative caecum weight PND 274, *g/kg*	0.98 ± 0.06	0.99 ± 0.07	0.65
Relative colon weight PND 274, *g/kg*	10.66 ± 0.52	11.11 ± 0.72	0.41

Mean values ± SEM; PND, postnatal day; CTRL, perinatal control diet; scFOS, perinatal short-chain fructooligosaccharide supplemented diet; HF, high-fat diet.

Table 2: Short-chain fatty acid concentration in faeces (mmol/kg) of pigs supplemented perinatally with scFOS (scFOS) or not (CTRL)

	CTRL	**scFOS**	**Diet p-value**
PND 21			
Total SCFA	16.5 ± 4.6	33.4 ± 2.8	**0.001**
Acetate	10.0 ± 2.7	20.5 ± 1.5	**0.003**
Propionate	1.9 ± 0.6	4.9 ± 0.6	**0.005**
Butyrate	1.7 ± 0.6	3.1 ± 1.0	0.37
Valerate	0.37 ± 0.12	1.03 ± 0.28	**0.04**
Caproate	0.008 ± 0.007	0.187 ± 0.091	**0.004**
PND 50			
Total SCFA	138.7 ± 24.2	127.3 ± 19.1	0.70
PND 190			
Total SCFA	101.3 ± 12.4	106.0 ± 8.3	0.76
HF transition	**CTRL**	**scFOS**	**Diet p-value**
PND 211			
Total SCFA	90.1 ± 6.1	114.1 ± 13.2	0.12
Acetate	52.3 ± 3.3	67.2 ± 7.5	*0.09*
Propionate	18.9 ± 1.3	23.0 ± 2.4	0.16
Butyrate	10.7 ± 1.3	13.7 ± 2.4	0.28
PND 253			
Total SCFA	91.4 ± 16.1	87.5 ± 7.1	0.83

Mean values ± SEM. CTRL, perinatal control diet; scFOS, perinatal short-chain fructooligosaccharide supplemented diet; SCFA, short-chain fatty acids; PND, postnatal day; HF, high-fat diet.

Table 3: Glucose homeostasis and entero-insular axis in suckling piglets (PND 21) born from sows supplemented in scFOS or not during the perinatal life

	CTRL	scFOS	Diet p-value
Plasma			
Glucose, *mmol/l*	7.55 ± 0.12 (n=90)	7.29 ± 0.16 (n=79)	0.50
Insulin, *µIU/ml*	9.54 ± 0.58 (n=91)	8.24 ± 0.61 (n=79)	**0.02**
Insulin/glucose	1.26 ± 0.08 (n=90)	1.08 ± 0.08 (n=78)	**0.02**
GLP-1, *pmol/l*	7.18 ± 1.74 (n=8)	3.40 ± 0.43 (n=7)	*0.09*
Caecum			
GLP-1-secreting L-cell, *nb/mm^2 mucosa*	1.73 ± 0.20 (n=5)	1.37 ± 0.41 (n=5)	0.46
Pancreas			
Endocrine tissue proportion, *%*	2.87 ± 0.20 (n=6)	1.99 ± 0.15 (n=5)	**0.01**
Insulin content, *IU/g*	13.62 ± 0.60 (n=8)	11.43 ± 0.84 (n=6)	**0.05**

Mean values ± SEM. Parameters were measured 1 hour after breastfeeding.
CTRL, maternal control diet; scFOS, maternal short-chain fructooligosaccharide supplemented diet.

Table 4: Glycaemia, insulinemia and IVGTT indices before (PND 190) and after the 3 month (PND 253) of high-fat diet in adult pigs supplemented perinatally with scFOS (scFOS) or not (CTRL)

	CTRL	scFOS	Diet p-value
PND 190[1]			
Fasting glucose, *mmol/l*	4.49 ± 0.12	4.46 ± 0.15	0.629
Fasting insulin, *µIU/ml*	18.70 ± 1.29	18.17 ± 2.08	0.436
HOMA-IR	3.80 ± 0.32	3.80 ± 0.64	0.440
PND 253[2]			
Glucose, *mmol/l*	4.75 ± 0.12	4.84 ± 0.12	0.872
Insulin, *µIU/ml*	17.54 ± 2.47	18.15 ± 1.97	0.878
HOMA-IR	3.77 ± 0.55	4.03 ± 0.53	0.956
IVGTT indices			
$AUC_{Glucose\ 0\text{-}30min}$	503.5 ± 21.8	521.8 ± 14.8	0.483
$AUC_{Insulin\ 0\text{-}30min}$	4235.1 ± 339.5	5316.5 ± 430.8	*0.068*
$AIR_{Insulin\ 0\text{-}6min}$	114.5 ± 9.5	136.6 ± 11.6	0.164
K_G (%/min)	4.19 ± 0.42	4.0 ± 0.27	0.695
S_2 ($min^{-1}.(\mu IU.\ ml^{-1})^{-1}$)	3.72 ± 0.61	2.66 ± 0.23	*0.085*
S_i ($10^4.min^{-1}.(\mu IU.ml^{-1})^{-1}$)	6.89 ± 0.81	5.31 ± 0.84	**0.019**
S_G ($10^2.min^{-1}$)	2.51 ± 0.24	2.04 ± 0.21	0.109

Mean values ± SEM.

[1] Standard diet feeding; [2] HF diet feeding.

CTRL, perinatal control diet; scFOS, perinatal short-chain fructooligosaccharide supplemented diet; PND, postnatal day; HOMA-IR, homeostasis model assessment of insulin resistance; HF, high-fat diet; AUC, area under the curve; AIR, acute insulin response; K_G, rate of glucose disappearance; S_2 and S_i, insulin sensitivity indices; S_G, glucose efficiency index.

Table 5: Expression of genes related to insulin sensitivity and inflammation in muscle and adipose tissues in adult pigs supplemented perinatally with scFOS (scFOS) or not (CTRL)

	CTRL	**scFOS**	**Diet p-value**
Muscle			
GLUT4	1.01 ± 0.04	1.00 ± 0.08	0.67
INSR	1.02 ± 0.06	1.11 ± 0.06	0.28
Subcutaneous adipose tissue			
GLUT4	1.11 ± 0.16	0.83 ± 0.08	0.12
INSR	1.05 ± 1.00	0.86 ± 0.08	0.14
SREBP1C	1.03 ± 0.09	0.83 ± 0.08	*0.10*
IL-10	1.12 ± 0.15	1.45 ± 0.24	0.66
TNFα	1.10 ± 0.13	1.22 ± 0.18	0.59
Visceral adipose tissue			
GLUT4	1.06 ± 0.23	0.76 ± 0.09	0.23
INSR	1.02 ± 0.17	0.85 ± 0.06	0.27
SREBP1C	1.01 ± 0.12	0.76 ± 0.09	0.20
TNFα	1.03 ± 0.20	0.50 ± 0.09	**0.02**

Values are expressed as the ratio of the relative expression of the scFOS group to the control group.

CTRL, perinatal control diet; scFOS, perinatal short-chain fructooligosaccharide supplemented diet.

Table 6: Plasma inflammatory, ileal defence markers and stimulated MLN cell cytokine secretions in adult pigs supplemented perinatally with scFOS (scFOS) or not (CTRL)

	CTRL	**scFOS**	**Diet p-value**
PND 260			
Plasma haptoglobin, *mg/ml*	2.17 ± 0.21	2.50 ± 0.31	0.45
Plasma LPS, *ng/ml*	17.00 ± 3.73	10.63 ± 2.31	0.64
PND 274			
Ileal alkaline phosphatase, *mg/g protein*	0.36 ± 0.04	0.48 ± 0.05	*0.10*
Ileal washes sIgA, *mg/g protein*	25.8 ± 10.0	61.1 ± 24.0	**0.03**
ConA-induced MLN cell IFNγ secretion, *pg/ml*	271.3 ± 93.1	502.1 ± 178.5	**0.003**
LPS-induced MLN cell IFNγ secretion, *pg/ml*	47.7 ± 23.9	25.5 ± 12.9	0.78
ConA-induced MLN cell TNFα secretion, *pg/ml*	439.7 ± 84.5	394.1 ± 76.9	0.69
LPS-induced MLN cell TNFα secretion, *pg/ml*	119.2 ± 31.4	86.2 ± 28.9	0.40
ConA-induced MLN cell IL-10 secretion, *pg/ml*	1167.2 ± 117.5	868.2 ± 103.0	0.14
LPS-induced MLN cell IL-10 secretion, *pg/ml*	74.8 ± 14.3	44.1 ± 13.8	*0.06*

Mean values ± SEM; CTRL, perinatal control diet; scFOS, perinatal short-chain fructooligosaccharide supplemented diet; PND, postnatal day; LPS, lipopolysaccharide; ConA, concanavalin A; MLN, mesenteric lymph node.

Figure 1

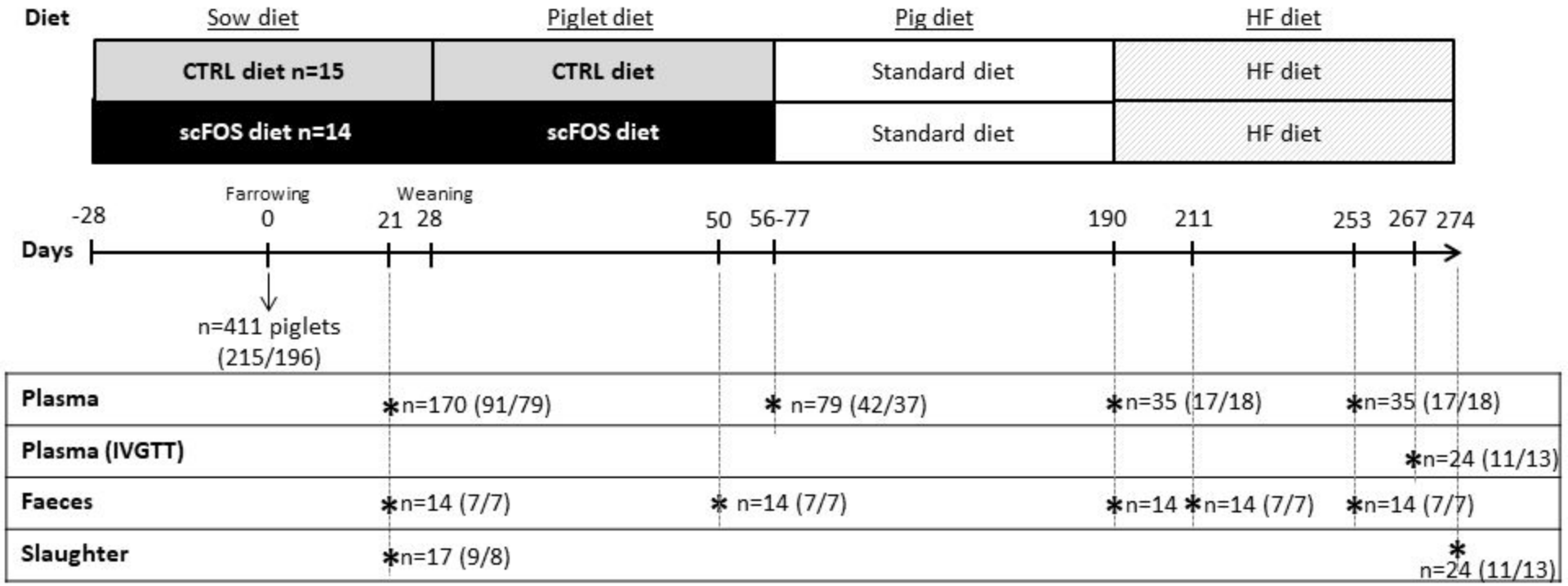


Figure 1
Diet
Sow diet
Piglet diet
Pig diet
HF diet
CTRL diet n=15
CTRL diet
Standard diet
HF diet
scFOS diet n=14
scFOS diet
Standard diet
HF diet
Farrowing
Weaning
-28
0
21
28
50
56-77
190
211
253
267
274
Days
n=411 piglets
(215/196)
Plasma
n=170 (91/79)
n=79 (42/37)
n=35 (17/18)
n=35 (17/18)
Plasma (IVGTT)
n=24 (11/13)
Faeces
n=14 (7/7)
n=14 (7/7)
n=14
n=14 (7/7)
n=14 (7/7)
Slaughter
n=17 (9/8)
n=24 (11/13)

Figure 2

Figure 2

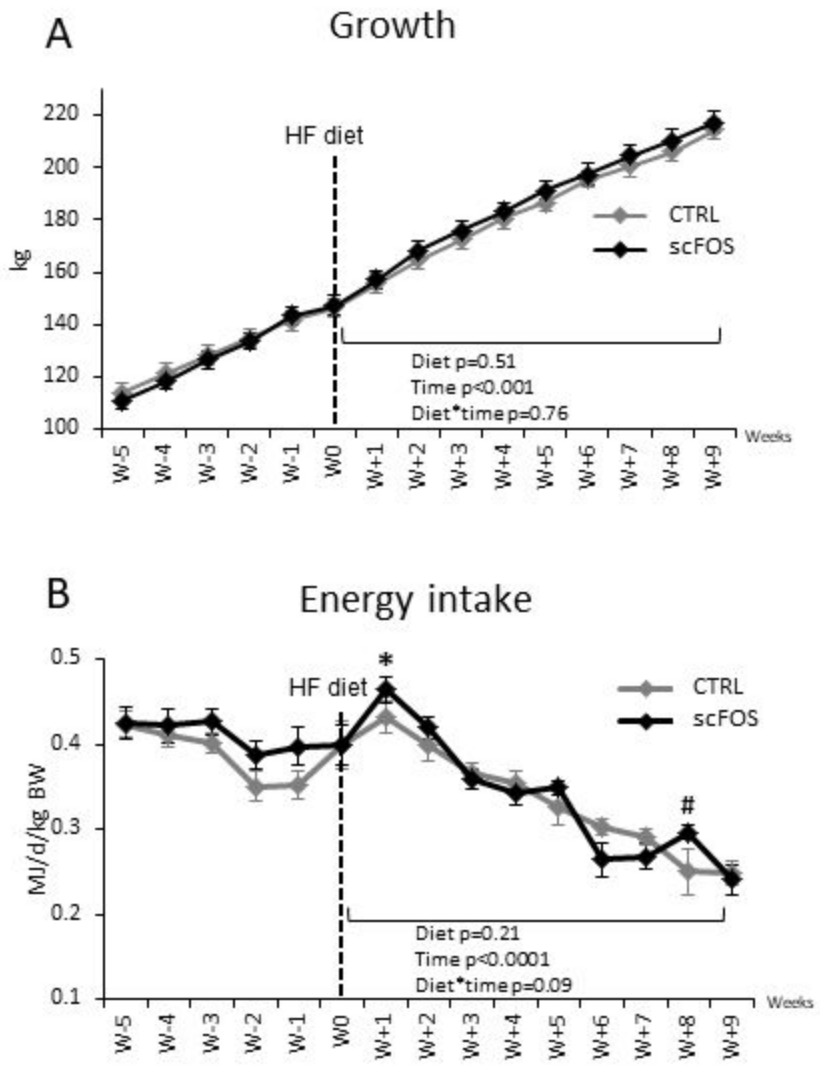


Figure 3

Figure 3

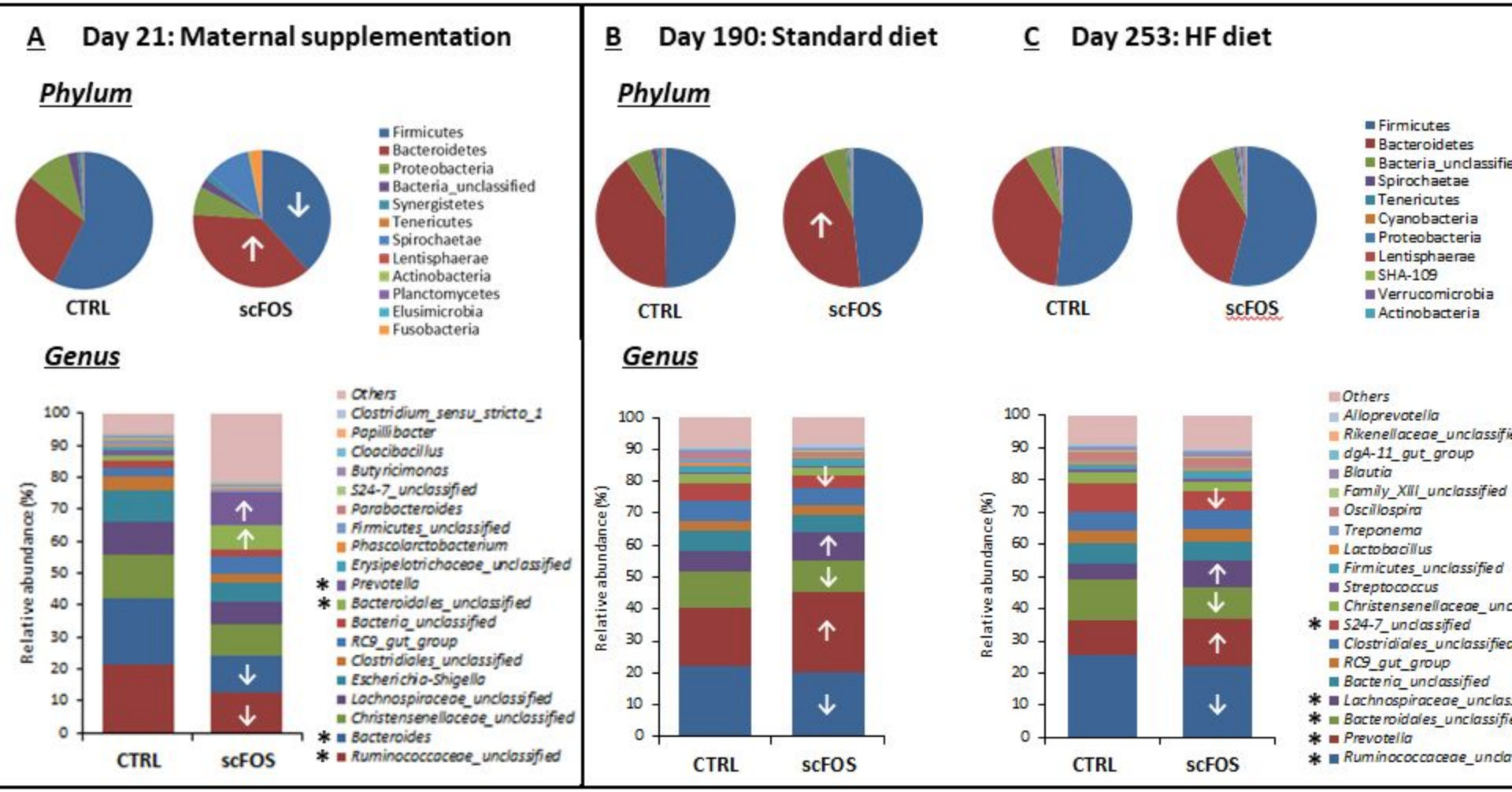


Figure 4

Figure 4

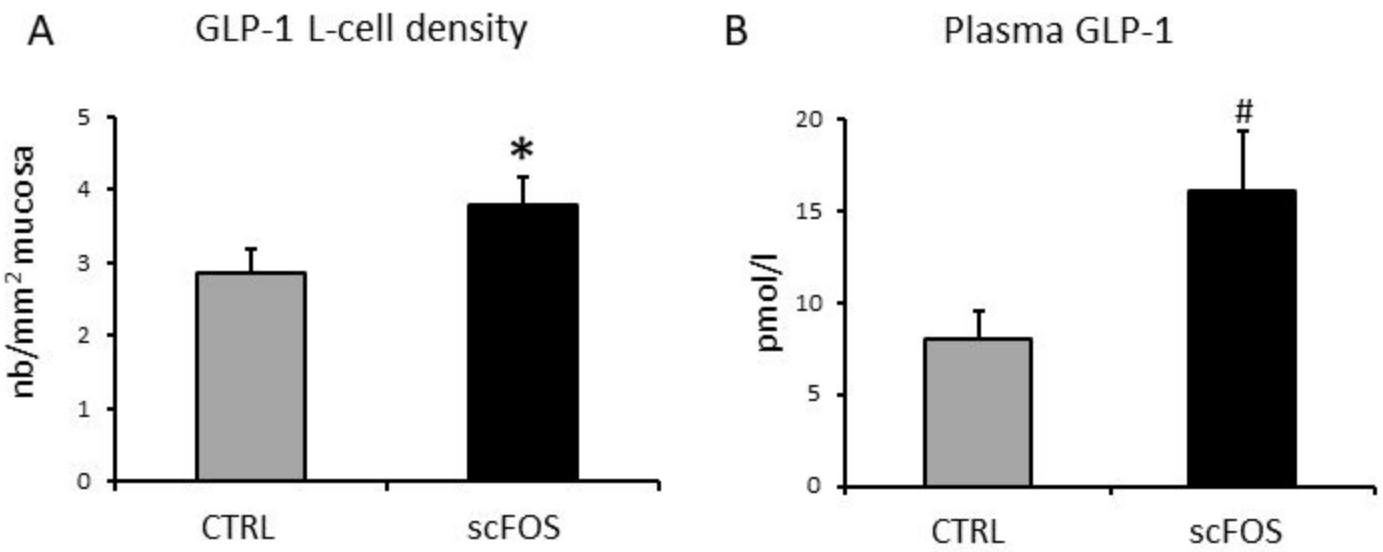
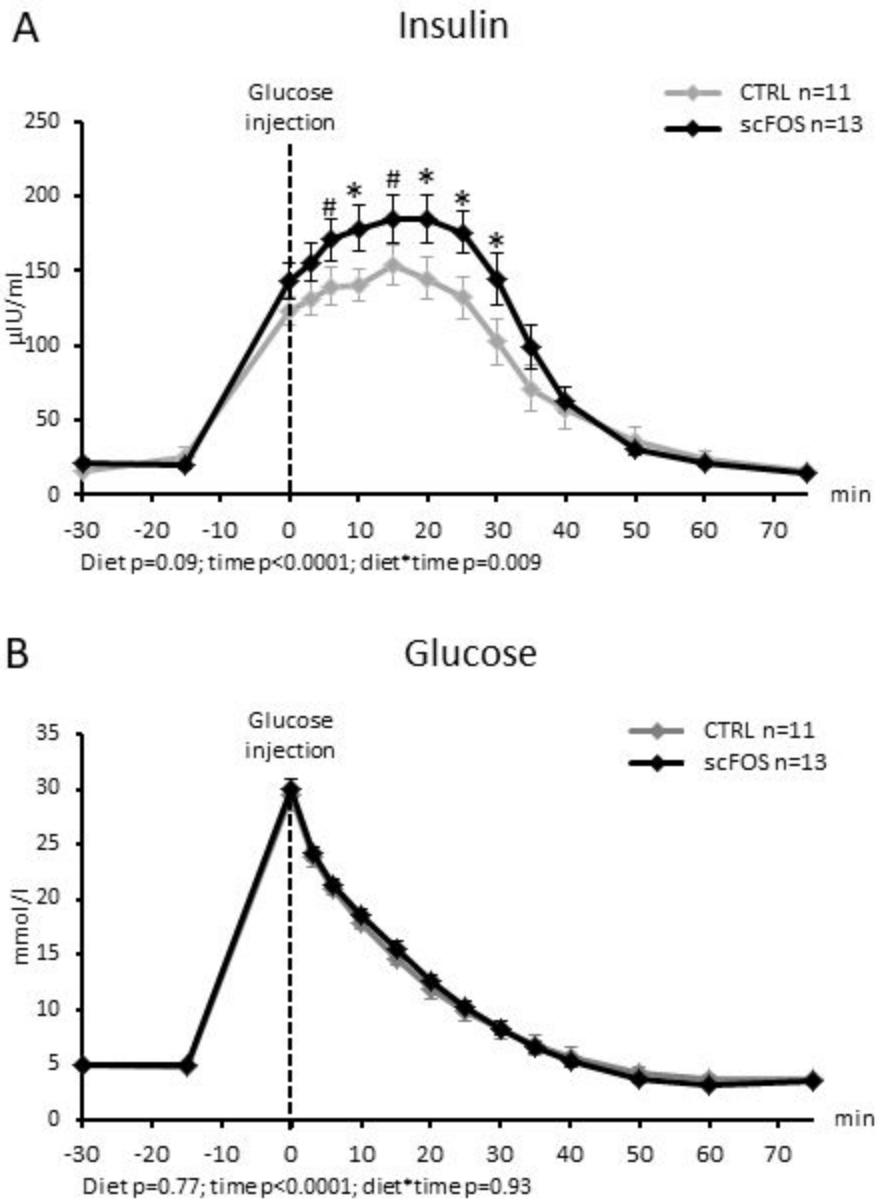


Figure 5

Figure 5



Supplemental Table 1: Diet composition

	Gestation	Lactation	Starter (1st phase)	Starter (2nd phase)	STD grower	HF grower
Ingredients (g.kg^{-1} fresh matter)						
Wheat	229	256	-	232	262	420.3
Corn	110	120	-	250	160	-
Barley	348	257	453	241	255.5	150.0
Wheat bran	159	100	-	-	50	80
Soybean meal	100	180	175	226	190	150
Lard	-	-	-	-	-	100
Soybean protein	-	-	25	-	-	-
Dehydrated whey	-	-	200	-	-	-
Refatted skim-milk	-	-	80	-	-	-
Molasse	-	30	-	-	30	20
Palm oil	20	20	23	5.0	20	-
Maltodextrin or scFOS	3.3	1.5	1.5	1.5	-	-
Calcium carbonate	17.3	12.0	14.1	11.3	12.9	8
Bicalcium phosphate	3.0	10.2	-	-	5	10
Sodium chloride	4.5	4.5	-	4.0	4.5	5
Trace element and vitamin mix[1]	5.0	5.0	5.0	5.0	6.1	6

[1]The premix (Cooperl, Lamballe, France) supplied per kg of diet: retinol 10,000 UI, cholecalciferol 1,500 UI, α-tocopherol 45 mg, menadione 2 mg, thiamine 2 mg, riboflavin 4 mg, niacin 20 mg, D-pantothenic acid 10.9 mg, pyridoxine 3 mg, D-biotin 0.2 mg, folic acid 3 mg, vitamin B12 20 µg, choline 500 mg, Fe 81.5 mg as iron carbonate and sulphate, Cu 10 mg as copper sulphate, Mn 40 mg as manganese oxide, Zn 99.2 mg as zinc oxide, Co 0.1 mg as cobalt carbonate, I 0.6 mg and Se 0.3 mg. scFOS: short-chain fructooligosaccharides.

Supplemental Table 2: Primers used for quantitative RT-PCR

Gene	Forward (5' – 3')	Reverse (5' – 3')
Housekeeping genes		
RPL4	CAAGAGTAACTACAACCTTC	GAACTCTACGATGAATCTTC
GAPDH	CATCCATGACAACTTCGGCA	GCATGGACTGTGGTCATGAGTC
YWHAZ	ATGCAACCAACACATCCTATC	GCATTATTAGCGTGCTGTCTT
HPRT1	CCGAGGATTTGGAAAAGGT	CTATTTCTGTTCAGTGCTTTGAT
Target genes		
INSR	TTCTTCGAACCCCGAGTACCT	CGATGTCCCTGGCGTTTC
SREBP1C	CTTCCTTCAGCAGAGCAACCA	GTCCTTCAGAGACTTGCTTTTGTG
GLUT4	GGCAGCCCCTCATCATTG	TCGAAGATGCTGGTTGAATAGTAGAA
IL-10	TGAGAACAGCTGCATCCACTTC	TCTGGTCCTTCGTTTGAAAGAAA
TNFα	GGTTATCGGCCCCCAGAA	TGGGCGACGGGCTTATC

Supplemental Table 3: Nutritional composition of sows' milk at PND 21

	CTRL (n=9)	**scFOS (n=9)**	**Diet p-value**
Total dry matter, *g/100g*	18.80 ± 0.34	20.03 ± 0.73	**0.004**
Ashes, *g/100g*	0.71 ± 0.04	0.69 ± 0.02	0.70
Carbohydrates, *g/100g* *	5.76 ± 0.18	5.61 ± 0.29	0.57
Lipids, *g/100g*	7.79 ± 0.32	8.81 ± 0.86	**0.013**
Proteins, *g/100g*	4.54 ± 0.16	4.92 ± 0.16	*0.091*
Energy value, *kcal/100g*	111.2 ± 2.7	121.5 ± 7.1	**0.005**

Mean values ± SEM (n = 9 per group). CTRL, control diet; scFOS, short chain fructooligosaccharide supplemented diet. * Estimated values for carbohydrate content.

Supplemental Table 4: Stimulated biopsies cytokine secretions at PND 274 in pigs supplemented perinatally with scFOS (scFOS) or not (CTRL)

	CTRL	**scFOS**
Ileal biopsy without PP		
ConA-induced IFNγ secretion, *pg/ml*	450.1 ± 194.3	631.3 ± 193.7
LPS-induced IFNγ secretion, *pg/ml*	0.80 ± 0.12	1.04 ± 0.22
ConA-induced TNFα secretion, *pg/ml*	13.98 ± 5.85	11.16 ± 4.24
LPS-induced TNFα secretion, *pg/ml*	1.24 ± 0.24	2.77 ± 1.88
ConA-induced IL-10 secretion, *pg/ml*	20.48 ± 8.93	40.29 ± 11.83
LPS-induced IL-10 secretion, *pg/ml*	1.65 ± 0.53	2.40 ± 0.60
ConA-induced IL-8 secretion, *pg/ml*	1.10 ± 0.09	1.00 ± 0.10
LPS-induced IL-8 secretion, *pg/ml*	1.57 ± 0.43	1.31 ± 0.23
Ileal biopsy with PP		
ConA-induced IFNγ secretion, *pg/ml*	138.8 ± 26.8	360.8 ± 95.0
LPS-induced IFNγ secretion, *pg/ml*	0.98 ± 0.21	0.96 ± 0.13
ConA-induced TNFα secretion, *pg/ml*	8.62 ± 2.68	7.56 ± 1.32
LPS-induced TNFα secretion, *pg/ml*	1.48 ± 0.49	1.12 ± 0.13
ConA-induced IL-10 secretion, *pg/ml*	12.11 ± 4.54	24.15 ± 10.56
LPS-induced IL-10 secretion, *pg/ml*	1.27 ± 0.23	3.20 ± 1.24
ConA-induced IL-8 secretion, *pg/ml*	0.96 ± 0.06	1.01 ± 0.10
LPS-induced IL-8 secretion, *pg/ml*	1.17 ± 0.13	1.13 ± 0.10

Values are differences between stimulated secretion and unstimulated secretion. Mean values ± SEM; CTRL, perinatal control diet; scFOS, perinatal short chain fructooligosaccharide supplemented diet; PND, postnatal day; LPS, lipopolysaccharide; ConA, concanavalin A; PP, Peyer's Patch.