Maternal short-chain fructo-oligosaccharide supplementation increases intestinal cytokine secretion, goblet cell number, butyrate concentration and *Lawsonia intracellularis* humoral vaccine response in weaned pigs

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Abstract

Prebiotic supplementation modulates immune system development and function. However, less is known about the effects of maternal prebiotic consumption on offspring intestinal defences and immune system responsiveness. We investigated the effects of maternal short-chain fructo-oligosaccharide (scFOS) supplementation on mucin-secreting cells, ileal secretory IgA and cytokine secretion of weaned offspring and their humoral response to an oral vaccine against obligate intracellular *Lawsonia intracellularis*. Sows were fed a control diet (CTRL) or scFOS-supplemented diet during the last third of gestation and throughout lactation. At weaning, each litter was divided into two groups receiving a post-weaning CTRL or scFOS diet for a month. Pigs from the four groups were either non-vaccinated (*n* 16) or vaccinated (*n* 117) at day 33. Biomarkers related to intestinal defences and immune parameters were analysed 3 weeks later. SCFA production was assessed over time in suckling and weaned pigs. Maternal scFOS supplementation improved ileal cytokine secretions (interferon (IFN)-γ, $P < 0.05$; IL-4, $P = 0.07$) and tended to increase caecal goblet cell number ($P = 0.06$). It increased IgA vaccine response in the serum ($P < 0.01$) and ileal mucosa ($P = 0.08$). Higher bacterial fermentative activity was observed during lactation (total faecal SCFA, $P < 0.001$) and after weaning (colonic butyrate, $P = 0.10$) in pigs from scFOS-supplemented mothers. No synergistic effect between maternal and post-weaning scFOS supplementation was observed. Therefore, maternal scFOS supplementation has long-lasting consequences by strengthening gut defences and immune response to a vaccine against an intestinal obligate intracellular pathogen. Prebiotic consumption by gestating and lactating mothers is decisive in modulating offspring intestinal immunity.

Key words: Intestinal immune system: Maternal nutrition: Prebiotics: Short-chain fructo-oligosaccharides: Vaccination

The physical intestinal defences (peristaltism and mucus layer) cooperatively with the mucosal immune system confer appropriate protection from harmful pathogens along with tolerance to ubiquitous dietary antigens and microbiota[1]. The mucus layer is an important player of intestinal defence by protecting against invasion of pathogens. The luminal release of secretory IgA (sIgA) further contributes to this barrier function by preventing the passage of potentially harmful dietary and microbial antigens through the epithelial barrier. The mucosal immune system actively participates in the intestinal defences through modulated release of cytokines and expressions of immune surface molecules by epithelial cells in response to luminal stimulation[2]. At birth, the reduced pro-inflammatory type 1 helper T (Th1) cell-polarising function results in high susceptibility to infectious diseases and impairs immune response to most vaccines in neonates[3]. Fortunately, breast-feeding

provides primary protection to newborns against pathogenic species, owing to the presence of specific maternal antibodies (IgG and IgA) and immune cells[4,5], whereas the neonatal immune system continues to develop in order to become fully functional.

Gut microbial stimulation provides the strongest environmental signal for postnatal maturation of both the non-specific intestinal defences and the mucosal immune system responsiveness[6–8] with potential effects on health later in life[9]. Prebiotics are selectively fermented ingredients that allow specific changes, both in the composition and activity of the intestinal microbiota, conferring benefits upon host well-being and health[10]. Short-chain fructo-oligosaccharides (scFOS) obtained from sucrose and consisting of two to four fructose units linked to one glucose molecule belong to prebiotics. Several studies have clearly demonstrated that scFOS consumption by infants or adults

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influenced intestinal physiology and immune system. In fact, scFOS are not digestible and resist absorption in the upper gastrointestinal tract, reaching the colon intact before undergoing microbial fermentation. Produced SCFA are promptly absorbed in the colon, and are either used by enterocytes as fuel or enter the bloodstream where they affect the function and metabolism of peripheral organs and tissues such as the liver, the pancreas, adipocytes, immune cells and skeletal muscle tissue[11,12]. Therefore, SCFA could interact with immune cells throughout the small intestine[13]. Besides these SCFA-dependent mechanisms, it has been demonstrated that *in vitro* prebiotics could cross the epithelial barrier[14] and bind to pathogen-recognition receptors such as TLR, NOD, C-type lectin receptors and galectins, expressed on dendritic cells[15,16]. Indeed, dietary supplementation with scFOS resulted in increased intestinal villi height, crypt depth and number of mucin-producing goblet cells in neonatal[17] and weaned[18,19] pigs. It stimulated intestinal IgA secretion in infant mice[20] and in adult mice and dogs[21,22], as well as cytokine secretion by Peyer's patch cells in adult mice[21]. Moreover, we recently demonstrated that maternal scFOS supplementation during gestation and lactation stimulated the development of the intestinal immune system in suckling piglets through the polarisation of mesenteric lymph node and Peyer's patch cells secretory activity towards more Th1-type cytokines and higher levels of sIgA[23]. Overall, early prebiotic scFOS supplementation is a promising dietary strategy to favour intestinal immune system maturation. However, longer-lasting functional consequences of such supplementation on intestinal functions have never been studied. Therefore, our objective was to determine whether maternal scFOS supplementation associated or not with post-weaning scFOS supplementation would improve intestinal defences (mucin-secreting cells, ileal sIgA and cytokine secretion) and immune response to an oral vaccine challenge against *Lawsonia intracellularis* in weaned pigs. As expected for obligate intracellular bacteria, *L. intracellularis* infection induced both specific humoral (IgA and IgM) and Th1 (IFN-γ) cell-mediated immune responses in serum (systemic) and intestinal mucosa.

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 B-35-275-32). A total of twelve sows (Large White × Landrace, 244·9 (SEM 7·2) kg) and their offspring ((Large White × Landrace) × Pietrain) from the INRA experimental herd were used. Sows were fed a control diet (CTRL, *n* 6) or a diet supplemented with scFOS for the last 4 weeks of gestation and the first 4 weeks of lactation (scFOS, *n* 6) (Fig. 1). At weaning (postnatal day (PND) 28), pigs (*n* 134) were divided into two groups of weight-matched and sex-matched littermates (Fig. 1). They were fed *ad libitum* either a standard post-weaning diet (with 0·15 % of maltodextrin, equal to approximately 1·2 g/d; CTRL group) or a scFOS-supplemented diet (0·15 % of scFOS, equal to approximately 1·2 g/d) from day 29 to day 56. Thus, we had four different groups of pigs according to the maternal diet and the post-weaning diet: CTRL/CTRL, CTRL/scFOS, scFOS/CTRL and scFOS/scFOS (Fig. 1).

All diets were formulated to meet sow and weaned pig nutritional requirements (online Supplementary Table S1). Sow diets were regular gestation and lactation diets (Cooperl) supplemented with either maltodextrin (MALDEX; Tereos Syral; CTRL group; *n* 6) or scFOS (95 % of scFOS with molecular chain length between three and five monomeric units, Profeed® P95; Beghin-Meiji;
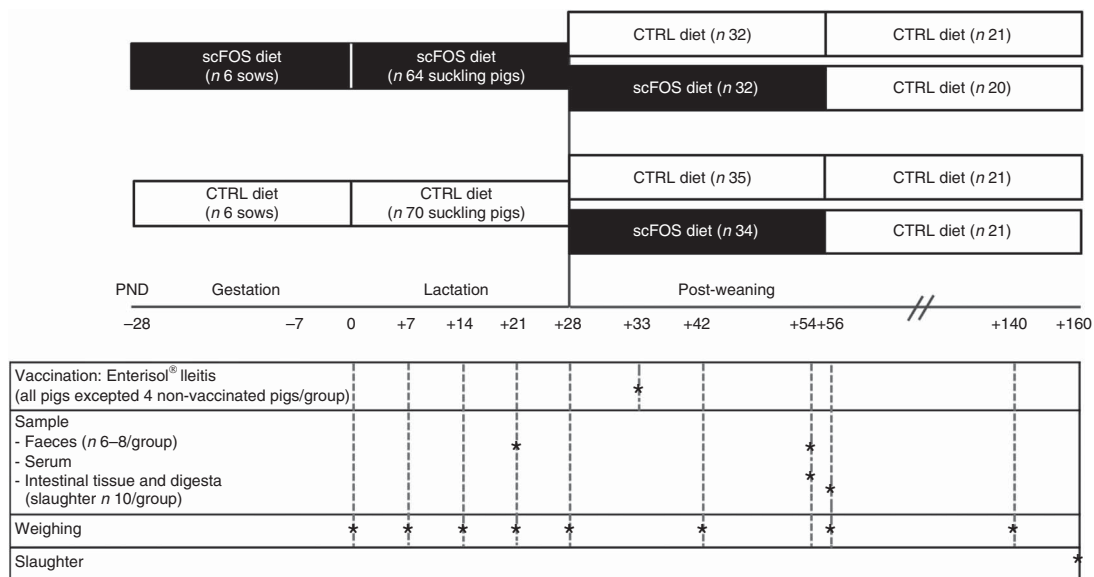

Fig. 1. Experimental design: twelve sows and their offspring (*n* 134) were used, and 28 d before the expected farrowing sows were fed either a control (CTRL) diet (*n* 6) or a short-chain fructo-oligosaccharide (scFOS)-supplemented diet (*n* 6) until the end of lactation. At weaning, each litter was divided into two groups of piglets receiving either a CTRL diet or a scFOS-supplemented diet until day 56. At day 33, 117 pigs were challenged with *Lawsonia intracellularis* vaccine (Enterisol® Ileitis). Four non-vaccinated pigs per group were used as negative controls and were housed separately from vaccinated pigs in order to validate the specificity of the vaccine response. PND, postnatal day.

scFOS group; n 6). Sows were given 3 kg/d of feed during gestation and were fed *ad libitum* during lactation, resulting in an approximately daily intake of 10 g of scFOS over the experimental gestation and lactation periods, as detailed previously[23]. Within 12 h following farrowing, litter size and the individual piglet birth weight were measured. When possible the litter was adjusted to eleven piglets by adding or removing piglets within each sow's dietary group. This was carried out on the 2nd day after parturition, without changing the mean litter birth weight. Before weaning, sow-reared piglets had no access to creep feed or to maternal feed, due to the shape and size of the maternal basket. From PND 28 to PND 56, weaned pigs were fed *ad libitum* a commercial starter diet (1st phase and 2nd phase; Cooperl) supplemented with 0·15 % of maltodextrin (CTRL; n 67) or scFOS (scFOS; n 66). From PND 28 to PND 56, they were monitored daily for food intake and for fever or diarrhoea. Thereafter, pigs were fed a growing diet (Cooperl) up to an average commercial weight of 116·2 (SEM 5·1) kg until they were killed in a slaughterhouse (n 71). Body weight of offspring was measured weekly until weaning, then every 2 weeks until the end of scFOS supplementation and at PND 140. Body composition of the carcass was evaluated using the CSB-Image-Meater® technology consisting of software that captures carcass images, identifies structures and provides visualised evaluations of different areas of muscles and back fat (Cooperl). No medication or antibiotic treatment was administered throughout the experimental period.

Vaccination

The vaccine challenge was performed using the vaccine against *L. intracellularis* with an intestinal tropism (Enterisol® Ileitis; Boehringer Ingelheim Vetmedica GmbH). This vaccine is a live attenuated vaccine composed of *L. intracellularis* as active substance from $10^{4·9}$ to $10^{6·1}$ Tissue Culture Infective Dose$_{50}$. At PND 33, 117 weaned pigs were vaccinated (n 31 CTRL/CTRL, n 30 CTRL/scFOS, n 28 scFOS/CTRL and n 28 scFOS/scFOS) with a ten-times dose (10✕) of vaccine in 2-ml sterile water by oral drenching. The 10✕ dose of vaccine was used to induce detectable levels of *L. intracellularis*-specific Ig. Indeed, the administration of a 1✕ dose of vaccine induced moderate or even undetectable levels of specific Ig in the serum and ileal mucosa[24]. In each litter, one or two pigs assigned to either a standard post-weaning diet or a scFOS-supplemented diet were not vaccinated (four pigs per dietary group). This reference group was used to detect differences between the effect of dietary treatments and the effect of vaccination on serum and intestinal parameters. Non-vaccinated pigs were housed separately (in another room) from the vaccinated ones. Vaccine response was evaluated 3 weeks after oral immunisation (PND 54–56). No other vaccine was administered during the experiment.

Sample collection

At PND 21 and PND 50, faecal samples were randomly collected from suckling piglets (n 14) and weaned pigs (n 31), at the rate of one or two pigs per litter, for later SCFA analysis.

At PND 54 (3 weeks after oral vaccination), serum samples were collected from the jugular vein of all pigs and stored at −20°C until further analysis of specific IgA and IgG against *L. intracellularis*.

At PND 56, forty pigs (two non-vaccinated pigs plus eight vaccinated pigs per dietary group) were stunned by electronarcosis and killed by exsanguination by a qualified staff member. The caecum was excised, weighed and stored for histological analysis, and the caecal and colon contents were collected for SCFA analysis (intestinal contents). A 5-cm ileal segment was excised, rinsed with PBS and the scraped mucosa was stored for further mucosal cytokine and sIgA assays.

Histology

Once rinsed with PBS and fixed in 4 % paraformaldehyde for 24 h at 4°C, caecum samples were cryoprotected at 4°C in PBS containing 30 % sucrose (Sigma), frozen with carbonic ice and sectioned (10 μm) using a cryostat microtome (Leica). Sections were stained with alcian blue (Sigma) and periodic acid Schiff (VWR) and examined under a light microscope (Nikon Eclipse E40; Nikon Instruments) using an image analysis software (NIS-Elements AR 3.0; Nikon Instruments). Crypt depth, crypt area and the number of mucin-producing goblet cells were measured in at least fifteen well-oriented crypt units per pig.

Secretory IgA measurement in ileal mucosa

Once collected, samples of ileal mucosa were homogenised in extraction buffer (0·5 mM-EDTA, 250 mg/l protease inhibitor cocktail in PBS; Sigma) for 30 min. After centrifugation (30 min at 4°C, 18 000 $\boldsymbol{g}$), supernatants were collected and stored at −20°C until analysis of total sIgA levels using swine IgA ELISA Quantitation Kit (Bethyl Laboratories). Samples were diluted in TRIS buffer with 1 % bovine serum albumin and 0·05 % Tween-20 according to preliminary assays.

Ileal cytokine concentration assay

To extract cytokines from the ileal mucosa, 1 ml of lysis buffer composed of RIPA buffer with 1 % protease inhibitor solution (Sigma) was added to 100 mg of scraped mucosa (Leica), and mixed three times for 15 s at 6000 rpm. After centrifugation at 10 000 $\boldsymbol{g}$ for 15 min at 4°C, the supernatant was collected and stored at −80°C. Concentrations of IL-4, IFN-γ, TNF-α and IL-6 were measured using capture sandwich ELISA (porcine Duoset® ELISA kit; R&D Systems) according to the manufacturer's instructions.

Analysis of specific Ig against Lawsonia intracellularis in serum and ileal mucosa

Blocking immunoenzymatic technique was used to detect *L. intracellularis* vaccine-specific IgA in serum (PND 54) and ileal mucosa (PND 56) as well as specific IgG in serum (BioScreen Ileitis Antibody ELISA; BioScreen). Serum was used at 1:40 and 1:100 dilutions for specific IgA and IgG, respectively, and mucosa samples were adjusted to 10 mg/ml of

protein and further used at 1:20 dilution. Samples were added onto the plate coated with *L. intracellularis* antigen. Following incubation, goat anti-pig IgA or IgG conjugated to peroxidase (AbD Serotec) was added at a dilution of 1:10 000 during 1 h at 37°C. Tetramethylbenzidine substrate reagent was added for 6 min before spectrophotometer analysis at 450 and 630 nm as recommended. *L. intracellularis*-specific IgA and IgG were expressed in arbitrary units with levels of specific Ig of vaccinated pigs being compared with the reference group (i.e. non-vaccinated pigs).

SCFA assay

Upon collection, faecal samples as well as intestinal contents were diluted with 0·5 % ortho-phosphoric acid solution (1 ml/g faeces or digesta). After centrifugation at 1700 *g* for 15 min at 4°C, supernatants were stored at −20°C until SCFA analysis by GC[25].

Statistical analysis

Data were analysed using R Core Team (2013; R Foundation for Statistical Computing; http://www.R-project.org/). Two-way ANOVA was used to test the effect of maternal diet, post-weaning diet, sex, vaccination and the interaction between maternal diet and post-weaning diet, maternal diet and vaccination and post-weaning diet and vaccination for all parameters. The effect of vaccination, but not the interactions between vaccination and maternal or post-weaning diets, was only significant ($P < 0.05$) for the vaccine-specific Ig levels in serum and mucosa. In this case, only vaccinated pigs were further included in ANOVA analysis to test the effect of maternal diet, post-weaning diet, sex and the interaction between maternal diet and post-weaning diet, with *post hoc* analysis. For the other parameters, the effect of vaccination was not significant ($P > 0.05$). Therefore, ANOVA analysis (ten pigs per group) testing the effect of maternal diet, post-weaning diet, sex and the interaction between maternal diet and post-weaning diet, with *post hoc* analysis, was applied taking into account all pigs. Finally, correlations were evaluated using Pearson's *R* test. Statistical significance was defined as a *P* value ≤0·05, and trends were reported as a *P* value ≤0·10. Data are represented as means with their standard errors.

Results

Caecum and colon morphometry

At PND 56, empty caecum weight was higher in weaned pigs whose mothers were supplemented with scFOS ($P < 0.05$; Table 1) compared with pigs whose mothers were fed the CTRL diet. Empty colon weight tended to be higher in the scFOS/CTRL group compared with the CTRL/CTRL group ($P = 0.06$; Table 1). The number of goblet cells per crypt in the caecum tended to increase with maternal scFOS supplementation ($P = 0.06$; Table 1).

Ileal cytokine and secretory IgA production

Maternal scFOS supplementation increased IFN-γ concentrations ($P < 0.05$) and tended to increase that of IL-4 ($P = 0.07$) in the ileal mucosa of weaned pigs at PND 56 (Fig. 2(a) and (b)). Direct scFOS supplementation in the post-weaning diet tended to decrease ileal TNF-α concentrations ($P = 0.10$, Fig. 2(c)). Concentration of ileal sIgA was significantly reduced in weaned pigs directly fed the scFOS diet whatever the maternal diet ($P < 0.05$; Fig. 2(d)).

Specific IgG and IgA response to Lawsonia intracellularis vaccination

Vaccine-specific IgA levels were significantly increased in the vaccinated groups compared with the non-vaccinated group, whatever the diet, in the serum (PND 54, $P < 0.01$) and in the ileal mucosa (PND 56, $P < 0.05$). Maternal scFOS diet increased vaccine-specific IgA levels in the serum ($P < 0.01$; Fig. 3(a)) and tended to increase the levels in the ileal mucosa ($P = 0.08$; Fig. 3(b)). A positive correlation was established between total sIgA and vaccine-specific IgA in the ileal mucosa (R 0·84, $P < 0.001$). In contrast, vaccination did not induce any specific IgG response in the serum (data not shown).

SCFA concentration in faeces, caecum and colon contents

Faeces from suckling piglets (PND 21) whose mothers were fed a scFOS-supplemented diet displayed a higher level of total SCFA ($P < 0.001$), resulting from an increase in acetate ($P < 0.01$), propionate ($P < 0.01$), valerate ($P < 0.05$) and caproate ($P < 0.01$)

Table 1. Caecum and colon morphometry in postnatal day (PND) fifty-six pigs
(Mean values with their standard errors of the four groups of weaned pigs; *n* 10/group)

	CTRL/CTRL		CTRL/scFOS		scFOS/CTRL		scFOS/scFOS		*P*		
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Maternal diet	Weaning diet	Interaction
Empty caecum (g/kg BW)	1·86	0·08	1·90	0·12	2·31*	0·14	2·0	0·09	0·01	NS	0·07
Caecal content (g/kg BW)	4·62	0·92	5·05	0·79	5·61	0·95	5·40	0·60	NS	NS	NS
Caecal crypt depth (μm)	445·8	21·2	430·0	19·6	418·2	21·9	412·9	19·7	NS	NS	NS
Caecal goblet cell/crypt (*n*)	38·6	2·6	43·1	2·5	42·6	3·5	46·7	2·9	0·06	NS	NS
Empty colon (g/kg BW)	17·9	0·7	18·7	0·5	19·5†	0·5	18·7	0·4	NS	NS	0·04
Colonic content (g/kg BW)	25·0	2·4	29·0	2·7	28·5	1·4	28·5	2·6	NS	NS	NS

CTRL, control diet; scFOS, supplemented diet with short-chain fructo-oligosaccharide; BW, body weight; NS, no significant effect ($P > 0.10$).
† Tendency to be different to CTRL/CTRL ($P < 0.10$).
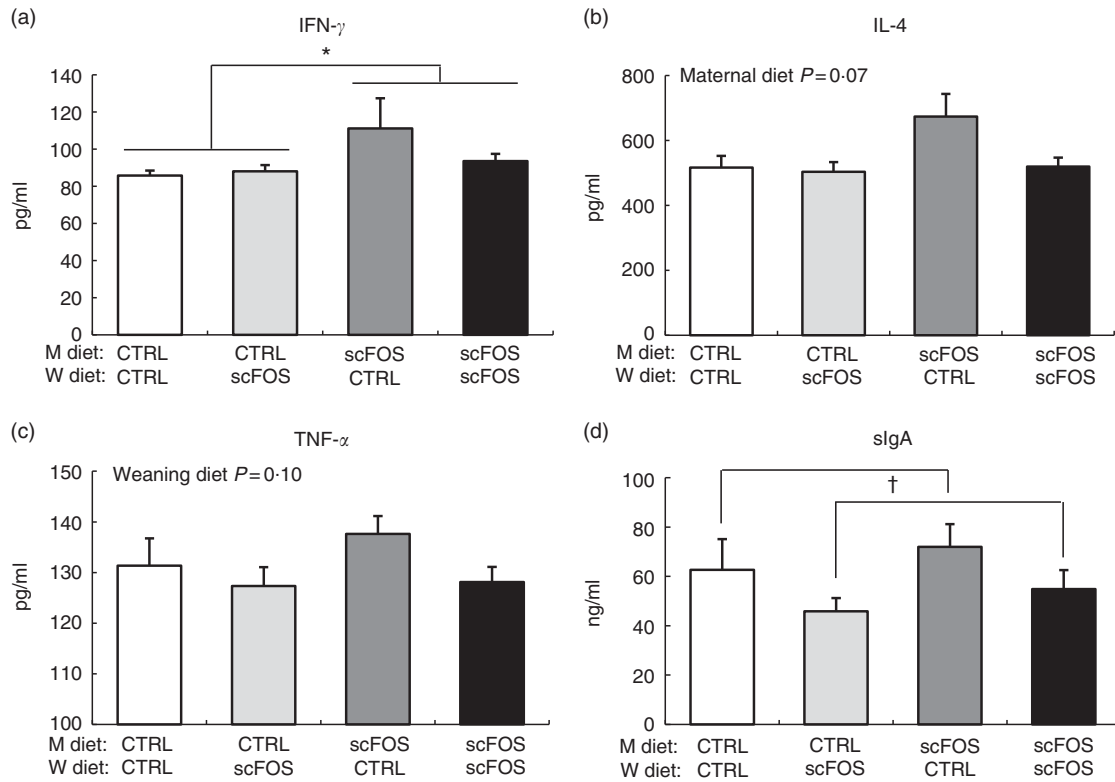* Significantly different to CTRL/CTRL ($P < 0.05$).

Fig. 2. IFN-γ (a), IL-4 (b), TNF-α (c) and secretory IgA (sIgA) (d) concentrations in the ileal mucosa of PND 56 pigs. Values are means (n 10/group), with their standard errors of the four groups of weaned pigs, the maternal diet (M diet) and the weaning diet (W diet): CTRL/CTRL, CTRL/scFOS, scFOS/CTRL and scFOS/scFOS (ANOVA with maternal diet, post-weaning pig diet, interaction between both diets and sex factors). * Significant effect of the maternal diet ($P < 0.05$). †, significant effect of the post-weaning pig diet ($P < 0.05$). CTRL, control diet; scFOS, supplemented diet with short-chain fructo-oligosaccharide; PND, postnatal day.
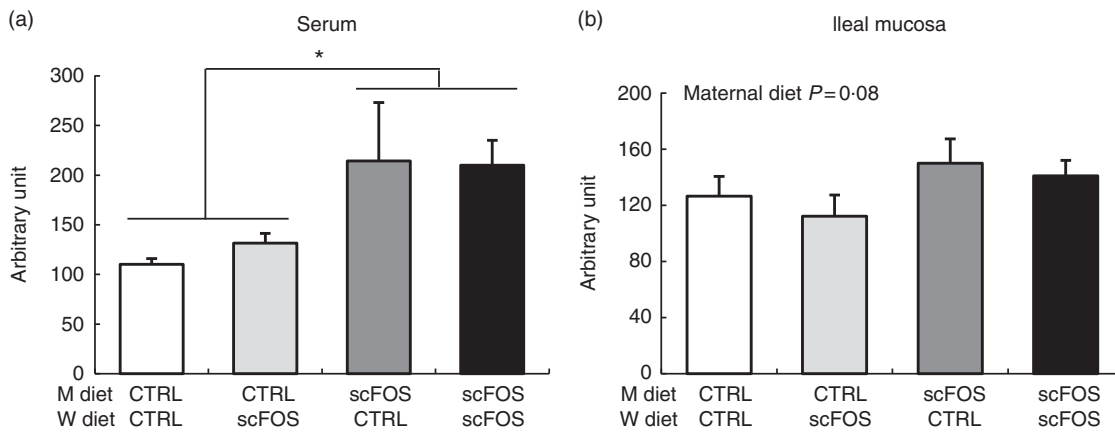

Fig. 3. Vaccine-specific IgA response in the serum (a) and ileal mucosa (b). Values are means (n 8/group), with their standard errors of the four groups of weaned pigs, with the maternal diet (M diet) and weaning diet (W diet): CTRL/CTRL, CTRL/scFOS, scFOS/CTRL and scFOS/scFOS, in the serum (PND 54) and ileal mucosa (PND 56) (ANOVA with maternal diet, post-weaning pig diet, interaction between both diets and sex factors). * Significant effect of the maternal diet ($P < 0.05$). CTRL, control diet; scFOS, supplemented diet with short-chain fructo-oligosaccharide; PND, postnatal day.

concentrations (Fig. 4(A)). At 3 weeks after weaning, no dietary effect was observed on faecal or caecal SCFA concentrations (data not shown), whereas colonic butyrate concentration tended to increase ($P = 0.10$; Fig. 4(B)) in maternal scFOS dietary groups (scFOS/CTRL and scFOS/scFOS) compared with maternal CTRL groups (CTRL/CTRL and CTRL/scFOS). Interestingly, Pearson's analysis revealed positive correlations between colonic butyrate concentration and colon weight ($R = 0.39$, $P < 0.05$), caecum weight ($R = 0.44$, $P < 0.05$), caecum

butyrate ($R = 0.35$, $P = 0.05$) and total SCFA in the caecum ($R = 0.43$, $P < 0.05$). In addition, colonic butyrate concentration tended to be positively correlated with the number of caecal goblet cells ($R = 0.34$, $P = 0.06$).

Pig body weight and body composition

Piglet growth during lactation was not impacted by maternal diet supplementation (Table 2). Similarly, no effects of maternal
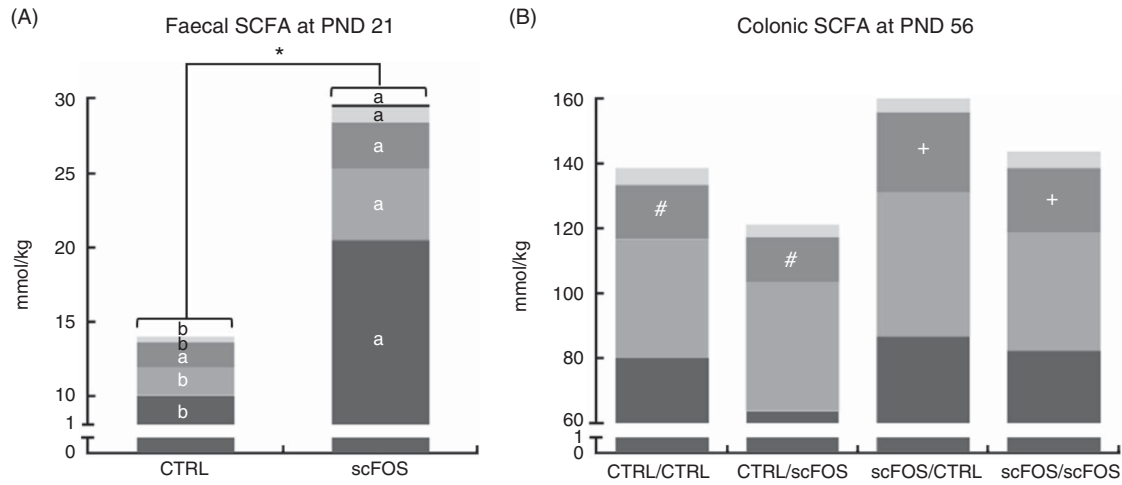
Fig. 4. SCFA concentration in faeces (A) and colonic content (B). Values are means. (A) Faecal SCFA concentration was measured in CTRL (n 6) and scFOS (n 8) pigs at PND 21 (ANOVA with maternal diet and sex factors), (B) colonic SCFA concentration was measured in the four groups of weaned pigs, the maternal diet (M diet) and the weaning diet (W diet): CTRL/CTRL (n 8), CTRL/scFOS (n 8), scFOS/CTRL (n 8) and scFOS/scFOS (n 7) at PND 56 (ANOVA with maternal diet, post-weaning pig diet, interaction between both diets and sex factors). * Significant effect of the maternal diet on total SCFA concentration ($P < 0.05$). [a,b] Mean values with unlike letters indicate a significant M diet effect for each metabolite level ($P < 0.05$). [+,#] Different signs indicate a tendency for a maternal diet effect for the metabolite C4 ($P < 0.10$). [x] Very low values of C6 to be readable on the graph: at PND 21; C6 values are 0.008 mmol/kg for CTRL and 0.190 mmol/kg for scFOS; at PND 56, C6 values <0.160 mmol/kg for all groups. CTRL, control diet; scFOS, supplemented diet with short-chain fructo-oligosaccharide; PND, postnatal day; C2 (■), acetate; C3 (■), propionate; C4 (■), butyrate; C5 (□), valerate; C6[x] (■), caproate.

Table 2. Pig growth throughout the experiment and carcass composition at slaughter†
(Mean values with their standard errors)

	CTRL		scFOS		P	
	Mean	SEM	Mean	SEM	Maternal diet	
Body weight of suckling piglets						
At birth (kg)	1·54 (n 70)	0·04	1·5 (n 64)	0·03	NS	
At PND 27 (kg)	8·74 (n 70)	0·18	8·42 (n 64)	0·23	NS	

	CTRL/CTRL		CTRL/scFOS		scFOS/CTRL		scFOS/scFOS		P		
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Maternal diet	Weaning diet	Interaction
Body weight of weaned pigs											
At PND 56 (kg)	17·7 (n 35)	0·6	18·2 (n 34)	0·4	18·1 (n 32)	0·5	18 (n 32)	0·6	NS	NS	NS
At PND 140 (kg)	97·7 (n 21)	3·0	94·2 (n 21)	2·3	93·7 (n 20)	3·3	90·5 (n 21)	3·0	0·05	NS	NS
Body composition at slaughter											
Muscle proportion (%)	58·7 (n 12)	0·8	60·1 (n 13)	0·6	61·1* (n 18)	0·4	60·4 (n 10)	0·4	0·02	NS	0·06
Subcutaneous fat thickness (mm)	17·3 (n 11)	1·4	14·6 (n 12)	1	13·6 (n 16)	0·6	14·4 (n 7)	0·8	0·06	NS	NS

PND, postnatal day; CTRL, control diet; scFOS, supplemented diet with short-chain fructo-oligosaccharide; M diet, maternal diet; W diet, weaning diet.
NS, no significant effect ($P > 0.10$).
* Significantly different to CTRL/CTRL group ($P < 0.05$).
† Piglets were weaned at PND 28.

diet and post-weaning diet were observed on body weight at PND 56 (Table 2), and neither on food intake during the month after weaning from PND 28 to PND 56 (data not shown). However, at PND 140, body weight was lower in pigs whose mothers were fed a scFOS-supplemented diet ($P < 0.05$; Table 2). This change was associated with modifications in carcass composition at slaughter. Indeed, the muscle proportion was higher ($P < 0.05$) and the subcutaneous fat thickness tended to be lower ($P = 0.07$) in pigs whose mothers were supplemented with scFOS (Table 2).

Discussion

Our aim was to evaluate the effect of maternal dietary prebiotic supplementation, at a daily low dose of 10 g scFOS, on offspring intestinal defences and immune response to a vaccine against intestinal bacteria, after weaning. The possible synergy between maternal and post-weaning scFOS supplementation was also evaluated. We demonstrated that maternal scFOS supplementation reinforced non-specific intestinal defences in weaned pigs by increasing caecal mucin-secreting goblet cells in association with a

greater ileal cytokine production and a higher fermentative activity of the microbiota. In addition, consumption of scFOS by the sow markedly enhanced the offspring-specific IgA response (+75 % in serum) to *L. intracellularis* vaccine challenge. Finally, maternal scFOS supplementation improved body composition by enhancing muscle proportion and reducing subcutaneous fat thickness in young adults. It is worth noticing that no synergistic effect between maternal and post-weaning scFOS supplementation was observed on immune and body composition parameters.

L. intracellularis infection causes proliferative enteropathy associated with thickening of the intestinal mucosa by abnormal proliferation of immature crypt enterocytes, more specifically termed 'adenomatosis'. This is important because it is only this type of cells that proliferates, that is, not the goblet cells that could even decrease[26]. Contrarily, maternal scFOS supplementation during the last month of gestation and the whole lactation period modified intestinal defences in weaned (PND 56) offspring by increasing the number of goblet cells in the caecum. Goblet cells are specialised epithelial cells that secrete mucin glycoproteins involved in the maintenance of intestinal mucosal surface integrity[27]. MUC2 is the major gel-forming mucin synthesised and secreted by intestinal goblet cells[28]. Gourbeyre *et al.*[29] observed that exposure to prebiotics during both perinatal and post-weaning periods increased MUC2 expression in the jejunum. In our study, the trophic effect of maternal scFOS supplementation on caecal goblet cell number and on the weight of the caecum and colon was observed in weaned PDN 56 animals, that is, 1 month after maternal supplementation was ended. The higher proportion of goblet cells may be related to an increased proliferation and cell density in the intestinal mucosa together with a higher fermentative activity of the microbiota[18,19]. Colonic infusion of butyrate or SCFA resulted in enhanced epithelial proliferation in distant intestinal segments[30,31], suggesting that the production of SCFA in the colon induces physiological changes throughout the intestinal tract. These results are in contrast with the effect of oligofructose supplementation in gestating and lactating rats (at a dose of 216 g oligofructose/kg diet compared with 1·5–3 g scFOS/kg diet in our study) that increased the weights of the small intestine and colon in suckling offspring without any modifications 2 weeks after weaning[32]. The intestinal trophic effect of the maternal scFOS supplementation may be due to a modulation of the fermentative activity of the microbiota. Indeed, SCFA produced by the microbiota are known to induce a proliferative effect on the gut, and several studies have shown positive associations between the release of SCFA induced by the consumption of scFOS and the trophic effect on intestinal mucosa[33–35]. In our study, we observed a higher production of total SCFA, particularly acetate, propionate, valerate and caproate, in faeces of suckling piglets whose mothers were fed scFOS diets, suggesting that the presumably modified microbiota of scFOS-supplemented sows was transmitted to the neonates during parturition and lactation as previously demonstrated in a mouse model, where maternal scFOS-induced changes in the gut microbiota of suckling mice were maintained several weeks after weaning[36]. Similarly, a higher colonic butyrate production was observed in our study 3 weeks after weaning in pigs whose mothers were fed a scFOS-supplemented diet. Butyrate is a well-known fuel used by colonic cells for proliferation. This was

confirmed by positive correlations between colonic butyrate concentration and caecum and colon weights as well as the number of mucin-secreting cells. The increased butyrate production may result from either an increased production of lactate by lactobacilli and bifidobacteria genera that can be used by other bacteria to produce butyrate, or by scFOS stimulation of *Clostridium coccoides* and *Eubacterium rectale* proliferation[37,38], described as the main butyrate producers in humans and pigs[39]. The trophic effect of maternal scFOS supplementation may also be related to the higher secretion of ileal IFN-γ and IL-4. IL-4 is an important inducer of mucin gene expression by up-regulating MUC2 gene expression, whereas IFN-γ can promote a transmembrane mucin, MUC1, gene expression[40].

The increased secretion of mucosal cytokines may result from the modification of fermentative activity and/or composition of microbiota. Indeed, in addition to their role on epithelial cells, SCFA have been reported to regulate T cells. Butyrate displayed regulatory effects on lymphocyte cytokine production such as IL-4 and IL-10[41]. Moreover, *in vitro* studies demonstrated that commensal bacteria regulated cytokine secretion. Strains from lactobacilli promoted pro-inflammatory IFN-γ secretion, whereas bifidobacteria strains produced a more anti-inflammatory profile[42,43]. The enhanced maturation of the mucosal immune system by maternal scFOS during the suckling period[23] could explain such stimulating effects through a higher potential to produce immune mediators, and thus to promote intestinal defences.

Inconsistent with previous studies in adults fed scFOS-enriched diets[18,19,35,44], no direct effect of post-weaning scFOS supplementation on intestinal architecture or on SCFA production in intestinal segments was observed. This lack of obvious effects may be explained by differences in the levels of supplementation. In our study, 1·5 g scFOS/kg post-weaning diet was low compared with 4–100 g scFOS/kg diet in other studies on weaned pigs[18,19] and with 50–100 g oligofructose/kg diet frequently used in experimental studies with rodents[35,44].

The specific IgA response to *L. intracellularis* vaccine challenge was stimulated by maternal scFOS supplementation. In order to evaluate the intestinal adaptive immunity, the vaccine challenge against the intestinal *L. intracellularis* was used as a broad indicator of immune responsiveness to a model of intestinal infection with standardised dose of pathogens, timing and exposure modalities. Vaccine-specific Ig production in biological fluids is classified among markers with high suitability to assay immune function[45]. We demonstrated the effectiveness of an indirect nutritional intervention, that is, maternal scFOS supplementation, to improve responsiveness of offspring to an intestinal bacteria vaccine challenge unveiled by increased vaccine-specific IgA levels in serum and ileal mucosa of weaned pigs. In response to such an intestinal vaccine challenge, post-weaning scFOS consumption was proven ineffective. This contrasts with our previous results on the humoral response to influenza vaccination[46]. Indeed, we previously observed an improved humoral immune response to a vaccine challenge against *influenza* virus in pigs directly supplemented with scFOS for 7 weeks after weaning, whereas maternal scFOS consumption had no effect[46]. In infants, convergent results on the enhancement of specific immune responses to intestinal infection or

vaccine challenge with direct prebiotic supplementation were reported[47–49]. This suggests that scFOS supplementation enhanced specific vaccine responses in different ways, depending on the period of supplementation (via the mother or directly after weaning) and also on the type of vaccine/pathogen used. We demonstrated that maternal scFOS supplementation is more effective than direct intake of scFOS to induce a mucosal immune reaction in response to an oral vaccine against intestinal bacteria.

In our study, the increased specific IgA response may be related to the improved mucosal IL-4 secretion. IL-4 is a type 2 helper T cell (Th2) cytokine known to up-regulate IgA production[50]. IL-4 and IFN-γ have also been shown to synergistically favour the delivery of sIgA in mucosal secretions by increasing total polymeric Ig receptor levels in human intestinal epithelial cells[51,52]. We did not observe significant differences in total sIgA concentrations in the ileal mucosa following maternal scFOS supplementation, but we established a significant positive correlation between total sIgA and specific IgA in the ileal mucosa. We can propose that the enhanced mucosal immune maturation in suckling piglets whose mothers were supplemented with scFOS[23] promoted sustainable local immune modifications that led to a further positive reinforcement of the humoral immune response to a bacterial challenge with an intestinal tropism.

Moreover, maternal scFOS supplementation improved body composition of the young adult pig. Indeed, reduced body weight in 5-month-old pigs whose mothers were supplemented with scFOS was associated with changes in body composition (higher muscle proportion associated with lower subcutaneous fat thickness). Similarly, Hallam & Reimer[53]. reported a long-term fat mass reduction in adult rats fed a high-fat/high-sucrose diet for 8 weeks and whose mothers were supplemented with prebiotics. Several studies have shown that prebiotic consumption changes glucose and lipid metabolism in young and adult animal models[32,44,53,54]. Long-lasting modifications of body composition following maternal scFOS supplementation reveal modifications of the host metabolism, the mechanisms of which remain to be explored.

In conclusion, our results underline the impact of maternal scFOS supplementation on both physical intestinal defences and mucosal immune response of the offspring. The maternal scFOS-induced stimulation of development and maturation of the intestinal immune system[23] is demonstrated as a determinant of the intestinal immune responsiveness in later life, supporting the concept of nutritional programming of the immune system. Our promising results confirm the role played by prebiotic supplementation on the early immune system development of the intestine in achieving a beneficial maturation of intestinal defences and immunity. Further investigations on the specific interaction between maternal scFOS and early microbiota are warranted.

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Supplementary material

For supplementary material/s referred to in this article, please visit https://doi.org/10.1017/S0007114516004268

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