Original Article

Effects of fructooligosaccharides on cecum polyamine concentration and gut maturation in early-weaned piglets

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(Received 2 August, 2010; Accepted 21 September, 2010; Published online 26 April, 2011)

Polyamines are molecules involved in cell growth and differentiation and are produced by bacterial metabolism. However, their production and effects by the microbiota selected by fructooligosaccharides consumption are controversial. In this study, we investigated the influence of supplementation of fructooligosaccharides on the cecal polyamine production by the microflora selected, and its effect on gut maturation in newborn piglets. Twenty piglets were fed a control formula (n = 10) or a formula supplemented with fructooligosaccharides (8 g/l) (n = 10) for 13 days. Colony-forming unit's count of cecal content was done in different media. Several intestinal development parameters were measured as well as the polyamine concentration in the cecal mucosa and cecal content. A dose-dependent study on *in vitro* polyamine production by fructooligosaccharides addition to the isolated cecal content was performed. Bifidogenic activity of fructooligosaccharides increased polyamine concentration in the cecal content, mainly putrescine, with no beneficial effect on gut maturation. *Bifidobacterium* spp. were able to produce polyamines, but they were not the most significant bacterial producer of polyamines in the cecum of piglets fed fructooligosaccharides. Bifidogenic activity of fructooligosaccharides did not lead to an increase in gut maturation in piglets of 15 days of age although polyamines were increased in the cecal content.

***Key Words*:** **fructooligosaccharides, polyamines, gut, cecal microorganisms, development**

Oligosaccharides present in human milk affect gastrointestinal flora of infants.[1,2] The composition and structure of the oligosaccharides of human milk can not be accurately reproduced by the food industry since cow's milk is used as the basis. For this reason, other groups of oligosaccharides of vegetable origin, such as fructooligosaccharides (FOS), are used in infant foods, in an attempt to mimic the beneficial effects of the oligosaccharides in human milk.[3] FOS escape hydrolysis by mammalian digestive enzymes, but are largely fermented by colonic bacteria to produce a wide variety of compounds that may affect the gut as well as systemic physiology.[4,5] FOS increase the gut populations of potentially beneficial bacteria such as bifidobacteria accompanied by a significant reduction in the number of pathogenic potential bacteria.[6–8] In addition, a great number of properties have been attributed to these compounds such as decreasing levels of serum cholesterol, phospholipids and triglycerides, the inhibition of colonic carcinogenesis, stimulation of the immune system or enhanced vitamin synthesis.[9] Since the effects of FOS are of major importance for immature gut systems such as preterm babies or nursing children, their study during the first days of life is of major interest.

The polyamines, putrescine, spermidine and spermine, are molecules involved in cellular proliferation and apoptosis, and have been also related to gut maturation.[10–12] In addition to the endogenous sources of polyamines inside the cell, exogenous sources of polyamines are constituted by dietary intake and by the polyamines produced by gut bacteria metabolism.[11,12]

Recent studies have suggested that the polyamine production in cecal tissue by bacterial fermentation of FOS and non-digestible polysaccharides might be involved in their beneficial effects in the gut.[13,14] Nevertheless, other effects inside the gut related to the extensive fermentation of fructans by endogenous bacteria, such as the decrease in pH due to the production of short-chain fatty acids (SCFA), increased calcium and magnesium absorption and displacement of nitrogen excretion could be also involved.[15,16] However, unexpectedly, it has been reported that FOS may also impair the intestinal barrier in rats as well as having significant effects on infection-induced growth impairment, gut inflammation and diarrhea.[17,18] Because there is little information available on the influence of FOS on gut maturation during the neonatal period, as well as the potential effects of the polyamines produced by the intestinal microflora modified by the FOS, more studies on the physiological mechanism related with dietary FOS consumption would be of interest.

In this study, we analyze the influence of FOS dietary supplementation in newborn piglets on bacterial polyamine concentrations in cecum and its effect on gut maturation of cecum mucosa. In addition, various bacterial strains of the FOS modified cecal microbial flora were isolated to evaluate their ability to produce polyamines. The possible existence of a dose-dependent relationship between FOS addition and bacterial polyamine production was also investigated.

Materials and Methods

Animals and diets. Twenty newborn piglets (Landrace × Large White) were provided by the veterinary farm of the University of Murcia, Spain. All piglets were nursed by sows until 2 days of age, after which they were randomly allocated into one of two groups (10 animals/group): control formula group (n = 10) and FOS-supplemented formula group (n = 10). These piglets were housed in groups of three or four animals, in cages provided with attached spot heat lamps and fed every three hours for 13 days.

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doi: 10.3164/jcbn.10-100

The milk formula was designed to resemble sow milk in its macronutrient composition,[19] and to meet the National Research Council (NRC) nutrient requirements[20] for growing piglets: crude protein 270.5 g/kg diet (lysine 21.4 g/kg diet), fat 332.2 g/kg, lactose 299.6 g/kg, minerals 47.6 g/kg (calcium 6.9 g/kg diet and phosphorus 4.9 g/kg), and vitamin formula 50 g/kg. The formulas were dissolved in warm water at a concentration of 200 g/l. The FOS-supplemented formula contained 8 g/l of Raftilose P95 provided by DANONE S.A. (Barcelona, Spain), reflecting the oligosaccharide levels detected in human milk.[21] Raftilose P95 was composed by 95% FOS (polymerization degree of 3 to 10).

Dissection protocol. At 15 days of age, the piglets were deprived of food overnight (about 8 h) and anesthetized via retroocular injection with a 50:50 mixture of Ketamine:propofol (1 ml/kg). The abdominal wall was opened and the entire gastrointestinal tract was removed. The cecal content was removed and immediately placed in a sterile plastic container under anaerobic conditions ($CO_2 + H_2$ atmosphere). The cecal content samples were transported and microbiologically processed within 2 to 4 h after collection. Two cecal tissue samples of 1 cm length were removed from the cecum and kept in Bouin fixative until analysis. Additionally, mucosa samples from the cecum were removed by scraping the entire luminal surface with a glass coverslip over an ice-cold Petri dish, frozen immediately in liquid nitrogen and stored at –80°C. Finally, the piglets were euthanized by an intracardial injection of sodium pentobarbital (1 ml/kg).

Analytical methods. The microscopical morphology measurements of the gut were performed with the two intestinal tissue samples collected from the cecum. After fixation of the tissue samples in Bouin liquid (75 ml picric acid water saturated solution, 25 ml formaldehyde and 5 ml acetic acid), a portion of each sample was embedded in paraffin wax using standard techniques. Two cross sections were selected from the samples, stained with hematoxylin and eosin, and examined with an optical microscope, Axioskop (Zeiss, Germany). Crypt depth was measured using an image processing program (Microm Image Processing, software 4.5). The depths of at least 20 crypts were measured and the mean crypt depth was calculated.[22]

Alkaline phosphatase and γ-glutamyl transferase are membrane enzymes which are going to corroborate changes in the biological membranes functionality. Mucosal samples were used to determine alkaline phosphatase and gamma glutamyl transferase activities and polyamine levels. After homogenisation of the cecal mucosa samples in saline solution (1:10, w:v) and centrifugation at 1500 g for 20 min, the supernatants were analysed. Total protein content was analyzed according to Bradford,[23] alkaline phosphatase and gamma glutamyl transferase activities by Biosystems diagnostic kits (Barcelona, Spain).[24,25]

The polyamine content in cecal mucosa and cecal content samples was quantified following a modified version of Seiler's method.[26] Briefly, mucosa samples were homogenised (1:10, w:v) in 5-sulfosalicylic acid (0.2 g/ml) and kept frozen overnight at –80°C; the cecal content was dispersed in saline buffer (1:10, w:v) and after centrifugation at 6700 g for 20 min, 500 µl 5-sulfosalicylic acid (0.2 g/ml) were added to 500 µl of the supernatant and then kept frozen overnight at –80°C. After centrifugation of these samples at 6700 g for 20 min, 50 µl of 1,6-diaminohexane (internal standard), 200 µl of saturated sodium carbonate and 400 µl of dansyl chloride (10 mg/ml in acetone) were successively added to 100 µl of the supernatant which was previously filtered through a nylon filter (0.45 µm of pore diameter). This dansylation mixture was incubated overnight at room temperature. Dansylated derivatives were then extracted with 1.5 ml of cyclohexene and subsequently dried prior to being dissolved in 100 µl of the injection medium (i.e. acetonitrile:methanol 3:2, v:v). Dansylated polyamines were quantified by high-performance liquid chromatography using a reverse-phase column (Nova-Pak C18, Waters, Madrid, Spain). We used a two phase gradient, starting with 57% phase A (phase A: acetonitrile:methanol 3:2; phase B: Milli Q water), reaching 100% phase A after a 53 min run. Polyamine standards (putrescine dihydrochloride; spermidine trihydrochloride and spermine tetrahydrochloride) were purchased from Sigma Chemical Co, St. Louis, MO.

Microbial studies.

Bacterial enumeration. 1.5 g of cecal content was placed into pre-weighed tubes with 13.5 ml of cysteine (0.5 g/l) reduced phosphate buffered saline (PBS-Cys). After homogenization, the specimens were subjected to a series of 10-fold dilutions (10^{-2} to 10^{-8}) in PBS-Cys, and triplicate aliquots of each dilution were plated on non-selective and selective media. The culture media, microorganisms investigated, atmosphere composition and time of incubation are detailed in Table 1. Results were expressed as log10 of colony-forming units (CFU)/g cecal content. Pure strains were isolated from single colonies from the cecal contents of piglets and identified using the API 20A, and API CL50 kits (bioMerieux, Lyon, France), according to the manufacturer's recommendations.

In vitro bacterial polyamine study. To study the production capacity of bacterial polyamines, representative strains of bifidobacteria and lactobacilli favoured by FOS were grown anaerobically in Falkow's medium and Falkow's supplemented with either arginine, lysine or ornithine (0.5% w/v) and cultured at 37°C for 72 h. Falkow's medium is used in microbiology to study decarboxylating capacity of aminoacids in anaerobic conditions.[27]

In vitro dose-depentent study. To study the possible dose-dependent relationship between FOS and bacterial polyamine production, the microorganisms from the cecal content of piglets fed control ($n = 10$) and FOS-supplemented milk formula ($n = 9$) was inoculated in Falkow's medium and incubated at 37°C for 72 h supplemented with ornithine (0.5% w/v) and different concentrations of Raftilose P95: 5, 10 and 15 g/l.

The study was approved by the Animal Care Committee at the University of Murcia and conforms to the European Union Regulation of Animal Care for the care and use of animals for research.

Statistical analyses. The results were expressed as mean ±

Table 1. Selective and non-selective culture media, times, and conditions of incubation for the groups of microorganisms studied

Microorganisms	Culture media	Atmosphere	Time (h)
Total aerobes	Columbia blood agar base	O_2	24
Total anaerobes	Wilkins-chalgren anaerobe broth	$CO_2 + H_2$	48
Clostridia	Reinforced clostridial agar	$CO_2 + H_2$	48
Coliforms and other enterobacteria	MacConkey agar	O_2	24
Fungi and yeast	Modified Sabouraud dextrose agar†	O_2	24
Bifidobacteria	Modified Berens‡	$CO_2 + H_2$	48
Lactobacilli	Rogose agar	Microaerophilia	48

†Sabouraud dextrose agar was modified adding 5 g/l of chloramphenicol.
‡Modified Berens contained 47 g Brain heart infusion agar, 5 g glucose, 0.5 g ferric citrate, 0.5 g cysteine, and distilled water up to 1000 ml.

SEM. To determine the effect of diet as source of variation, we used the Mann-Whitney non-parametric tests when the data did not show a normal distribution (assayed by Kolmogorov-Smirnov test and Levene statistical test). T-student test were performed when the data showed a normal distribution. The correlations between histological parameters, enzymatic activities and polyamine concentrations were evaluated by Pearson's correlation test. Two-way ANOVA was used to determine the FOS dose-dependent study in the two groups of animals (control and FOS). Differences were considered significant at $p<0.05$. All statistical analyses were carried out using SPSS for Windows (release 12.0; SPSS Inc., Chicago, IL).

Results

Study *in vivo*. There were not significant differences in body weight changes between the piglets fed the control formula and those fed the FOS-supplemented formula (control group: 1.17 ± 0.54 kg, FOS group: 0.94 ± 0.29 kg). Bifidobacteria and Lactobacilli CFU counts were significantly higher in the cecal content of piglets fed FOS-supplemented milk formula (FOS group) than in the control group (Table 2). The CFU counts of the rest of microorganisms (total anaerobes, clostridia, enterobacteria, coliform and total aerobes) did not show any variations between the two groups of animals fed formulas, only fungi and yeast counts were significantly lower in the cecal content of the FOS group than in the control group (Table 2). There were no differences in the cecal pH of the piglets fed the control formula and those fed the FOS-supplemented formula (control group 6.32 ± 0.34, FOS group 6.47 ± 0.21).

Piglets fed FOS-supplemented formula had significantly lower crypt depth in the cecum than those fed control milk formula (Table 3). Similarly, the alkaline phosphatase and gamma glutamyl transferase activities in the cecum showed the same pattern (Table 3); these enzymatic activities were positively correlated with the crypt depth in the cecum ($R = 0.419$, $p = 0.021$ and $R = 0.402$, $p = 0.028$, respectively) indicating their relationship with the degree of gut maturation. However, it is important to consider that, these values were similar to those obtained in piglets of 15d of age kept with their mothers and fed with sow milk (crypt depth 287.6 ± 14.04 μm; alkaline phosphatase 94.6 ± 27.0 nmol*min/mg prot; gamma GT 28.4 ± 5.60 nmol*min/mg prot). Nevertheless, we do not report data on microbiology or polyamine concentration in piglets fed sow milk because these variables can be mainly affected by the farm conditions of these animals respect to the laboratory conditions of the animals fed the formulas.

Putrescine was by far the predominant polyamine in the cecal content (Fig. 1A), while spermidine and spermine were the main polyamines in cecal mucosa (Fig. 1B). Total polyamine concentrations were significantly higher in the cecal content of piglets fed FOS than in controls (Fig. 1A). In contrast, dietary FOS significantly decreased the mucosal concentration of total polyamines, spermine and spermidine, and even tended to lower putrescine concentration, compared with controls (Fig. 1B).

Study of bacterial polyamine production *in vitro*. The strains included in this study were three of *Bifidobacterium sp.*, five *Lactobacillus fermentum* and two *Lactobacillus acidophilus*. They were the most predominant strains after FOS supplementation. All these isolated strains were able to produce polyamines in the Falkow's medium (Table 4). Since the production of total polyamine was significantly higher in Falkow's medium supplemented with ornithine respect to the other aminoacids (Table 4), the medium supplemented with ornithine was therefore used to evaluate the polyamine production *in vitro* using different doses of FOS to the cecal content of the piglets fed either control or FOS formulas.

In Falkow's medium with ornithine the putrescine and spermine production by *Bifidobacterium sp* was significantly higher than that of *L. fermentum* and *L. acidophilus* (Table 4), while spermidine production was higher in *L. acidophilus* than by the rest of strains. In any case, putrescine was the main polyamine produced by these strains (Table 4).

Dose-dependent study *in vitro*. No significant differences were detected in polyamine concentrations in tubes containing cecal microbiota from FOS or control group in Falcow's medium supplemented with ornithine (Fig. 2). In addition, FOS addition to the medium in different concentrations did not produce a dose-

Table 2. Mean logarithmic microbial counts per gram of dry cecal content of early-weaned piglets fed control milk formula and FOS-supplemented milk formula

Microbial type	Dietary treatment		
	Control (n = 10)	FOS (n = 10)	$p^{\dagger}$
	(Mean ± SD of Log ufc/g dry cecal content)		
Bifidobacterium	9.1 ± 0.5*	10.0 ± 0.5*	0.00
Lactobacilli	9.5 ± 0.3*	9.8 ± 0.3*	0.021
Clostridia	6.7 ± 1.4	6.1 ± 0.8	NS
Coliforms and other enterobacteria	8.6 ± 0.6	8.2 ± 1.4	NS
Total anaerobes	10.5 ± 0.2	11.0 ± 0.6	NS
Total aerobes	9.1 ± 0.5	9.1 ± 0.9	NS
Fungi and yeasts	4.0 ± 0.3*	2.8 ± 1.2*	0.003

Samples were collected at 15 days of life. Mann-Whitney nonparametric test was used to determine the effects of diet. Asterisks into the same row indicate statistical differences ($p<0.05$).
$^{\dagger}p$ values; NS: no significant differences.

Table 3. Crypt depth and enzymatic activities in the cecum of early-weaned piglets fed two different diets

	Control (n = 10)	FOS (n = 10)	p
	(Mean ± SEM)		
Crypt depth (μm)	305.8 ± 12.81*	261.2 ± 7.09*	0.007
Alkaline phosphatase (nmol*min/mg prot)	146.6 ± 18.7*	84.4 ± 14.0*	0.016
Gamma GT (nmol*min/mg prot)	33.5 ± 4.01*	19.2 ± 4.3*	0.024

Asterisks indicate differences among the two groups of animals by T-Student test ($p<0.05$).

doi: 10.3164/jcbn.10-100

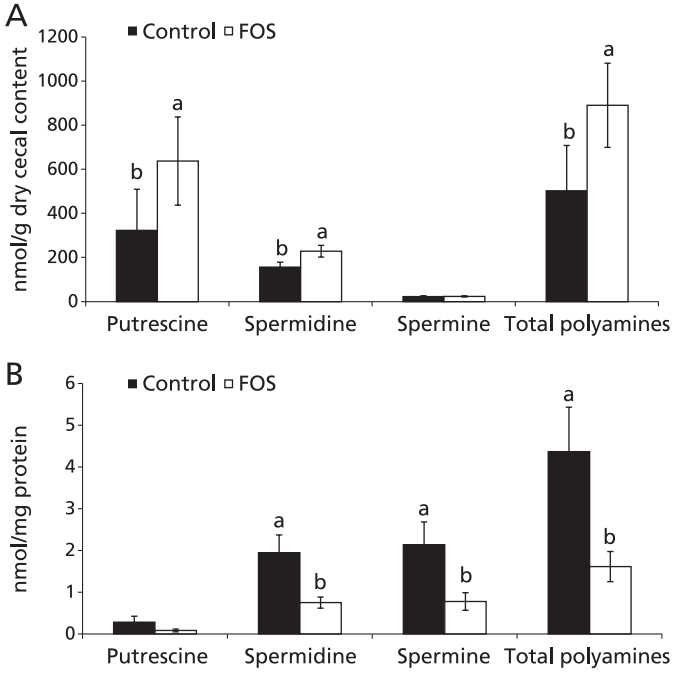

Fig. 1. Polyamine concentration in cecal content (A) and cecum mucosa (B) in piglets of 15 days of age fed two different diets (control and FOS). Results are means ± SEM. Kruskal-Wallis test ($p<0.05$).

dependent relationship between FOS and bacterial polyamine production (Fig. 2). As described in cecal content, putrescine was the main polyamine produced by cecal bacteria cultured in the different media.

Discussion

Milk formulas added by FOS at the milk physiological levels of oligosaccharides were found to induce higher concentrations of Bifidobacteria, Lactobacilli and higher polyamine levels in the cecal content of newborn piglets than control formulas, but had no beneficial effect on gut maturation. Putrescine was the main polyamine detected in cecal content but not in cecal tissue, pointing towards low effect of this polyamine on cell proliferation in the cecum.

Our results confirmed the bifidogenic activity of FOS.[7,28,29] The intake of FOS (8 g/l) also increases the gut populations of *Lactobacillus* and decrease the number of potentially pathogenic bacteria such as fungi and yeasts population, while the rest of microorganisms (total anaerobe, total aerobes, enterobacteria, coliforms and clostridia) did not show variation. These results are the basis for FOS using as prebiotics in infant formulas.

The higher polyamine concentrations found in the cecal content of the piglets fed FOS formula than in the control group, confirmed previous observation that the polyamine content in the gut is highly influenced by the metabolic activity of intestinal bacteria.[13,14] However, in the present study, the amount of polyamines produced by the microbiota had no effect in increasing the polyamine concentration in the mucosa of the neonatal piglets that even was lower in the FOS than in the control group. The mucosa of neonatal piglets itself could have used them for its own metabolism, since polyamine absorption by the gut was reported to be very fast and complet.[30,31] Nevertheless, Delzenne *et al.*[14] reported that in adult rats, fed diets containing oligofructose (10%) for 4 weeks, the concentration of putrescine in the cecal content

Table 4. Polyamine concentrations (nmol/ml) in bacterial strains isolated of the intestinal microflora cultivated in Falkow's medium supplemented with different type of aminoacids (0.5% w/v)

Culture media	*Polyamine type (nmol/ml medium)*		
	Bifidobacterium spp. (*n* = 3 strains)	*Lactobacillus fermentum* (*n* = 5 strains)	*Lactobacillus acidophilus* (*n* = 2 strains)
	Putrescine (mean ± SEM)		
Falkow	3.83 ± 0.74^{b}	4.40 ± 0.51^{ab}	7.52 ± 1.14^{b}
Falkow + Arg	6.30 ± 4.32^{b}	2.95 ± 0.42^{b}	2.58 ± 0.90^{b}
Falkow + Lys	3.48 ± 0.53^{b}	5.75 ± 2.03^{ab}	5.75 ± 0.14^{b}
Falkow + Orn	$25.86 \pm 8.89^{a,1}$	$12.72 \pm 1.85^{a,2}$	$22.66 \pm 3.92^{a,12}$
	Spermidine (mean ± SEM)		
Falkow	$2.47 \pm 0.32^{a,2}$	$1.86 \pm 0.47^{a,2}$	$9.94 \pm 4.51^{a,1}$
Falkow + Arg	1.15 ± 1.15^{b}	0.45 ± 0.45^{a}	0.36 ± 0.36^{b}
Falkow + Lys	3.09 ± 0.28^{ab}	2.02 ± 0.77^{a}	1.86 ± 0.59^{b}
Falkow + Orn	8.62 ± 3.75^{a}	4.33 ± 0.49^{a}	3.23 ± 2.72^{ab}
	Spermine (mean ± SEM)		
Falkow	1.89 ± 0.05^{b}	1.35 ± 0.31^{a}	2.29 ± 0.09^{a}
Falkow + Arg	0.95 ± 0.83^{b}	0.12 ± 0.00^{a}	0.12 ± 0.00^{a}
Falkow + Lys	2.15 ± 0.20^{ab}	1.64 ± 0.44^{a}	2.36 ± 0.6^{a}
Falkow + Orn	$5.66 \pm 3.09^{a,1}$	$2.15 \pm 0.29^{a,2}$	$1.64 \pm 0.86^{a,2}$
	Total polyamines (mean ± SEM)		
Falkow	8.19 ± 0.84^{b}	7.61 ± 1.04^{ab}	19.75 ± 5.57^{ab}
Falkow + Arg	8.40 ± 6.30^{b}	3.52 ± 0.83^{b}	3.06 ± 1.26^{b}
Falkow + Lys	8.71 ± 0.82^{b}	9.41 ± 2.22^{ab}	9.97 ± 0.04^{ab}
Falkow + Orn	$40.14 \pm 15.67^{a,1}$	$19.20 \pm 1.84^{a,2}$	$27.52 \pm 0.36^{a,12}$

Polyamine production was analyzed by two-way ANOVA with a posteriori Bonferroni test. Different letters represent statistically significant differences by culture media, and different numbers represent differences by bacterial strains. Interaction (strain × medium) was statistically significant for spermidine, ($p = 0.015$).
Arg, arginine; Lys, lysine; Orn, Ornithine.

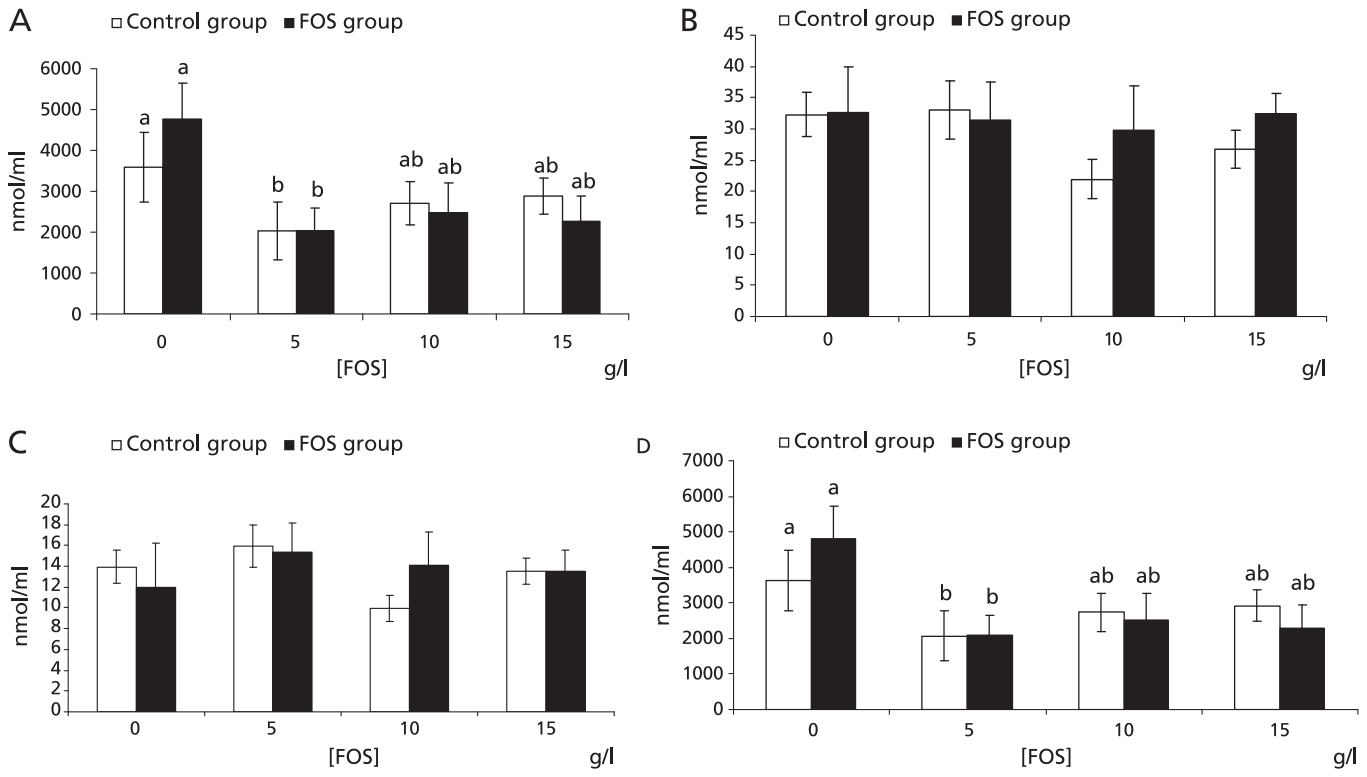

Fig. 2. Polyamine concentrations (A, putrescine; B, spermidine; C, spermine; and D, total polyamines) by the microorganisms from the cecal content of piglets fed control and FOS-supplemented milk formula cultivated in medium of Falkow supplemented with ornithine (0.5% w/v) and different FOS concentrations (0, 5, 10 and 15 g/l). Values are means ± SEM. (n = 9). Two-way ANOVA with a posteriori Bonferroni test was used (animal group × FOS concentration). Significant differences $p<0.05$.

had almost doubled and increased polyamine concentration in cecal tissue.

In our study, the enzymatic activities and crypt depth measurements were significantly lower and smaller in piglets fed FOS than in those fed the control formula (Table 3), although there were no differences with piglets fed maternal sow milk (data not shown). Hedemann *et al.*[22] reported that crypt depth in the colon of pigs fed diets containing fiber of various physico-chemical properties and concentrations (73, 104, or 145 g of dietary fiber/kg of DM) was not affected by diet; moreover, when pectin alone was used, crypt depth was even smaller in the pigs. Delzenne *et al.*[14] also showed no difference in the histological pattern (cell proliferation, crypt depth, villous height) of Wistar rats fed oligofructose (10%) despite the levels of all three polyamines in cecal tissue were significantly higher than in controls. Benamouzig *et al.*[32] observed also no changes in cell proliferation in the colonic mucosa of pigs despite higher levels of polyamines in the tissue. In contrast, Howard *et al.*[6] reported increased proximal colonic mucosal crypt depth with FOS consumption (3 g/l of formula) in pigs, suggesting that SCFA were made available for proliferative activity when FOS were supplied; surprisingly, they did not report differences in SCFA levels with FOS consumption. As regards to the production of bacterial polyamines as a possible action mechanism of fructooligosaccharides, it is important to consider the increase in the concentration of polyamines in the cecal contents of animals fed FOS, as well as, the significant reduction of the gut maturation parameters in these same animals.

Breast-fed infants typically have lower pH values in the cecum and colon (ranging between 5.0 and 7.0) than infants fed formulas.[29,33] It has been described, that FOS fermentation by intestinal microflora results in the production of SCFA, which have several functions including acting as an energy source for colonocytes, regulating cell growth and lowering intestinal pH, which may contribute to inhibiting the growth of pathogens[34] and selecting microbiota which may promote polyamine production.[35] Nevertheless, conflicting results on the effect of FOS on cecal pH have been reported in pigs, rats and humans.[6,7,13,36–38] In our study, faecal pH values did not differ between the dietary treatments and were only slightly below 7; a possible explanation is the release of polyamines to the cecal content by microorganisms could have increased the pH values. In contrast, other authors have reported that FOS-supplemented infant formulas (8 g/l) significantly decrease the faecal pH in infants indicating a relevant shift in the metabolic activity of the cecal microflora.[36,37]

Our *in vitro* studies have shown for the first time the capacity of various strains of *Bifidobacterium* to produce polyamines. The isolated strains of *L. fermentum* and *L. acidophilus* were also able to produce polyamines. These microbial types were specially favoured by FOS consumption, and this fact could explain the effect of the FOS to change the cecal polyamine levels. It should be noted, however, that Noack *et al.*[13] reported the inability of some strains of bifidobacteria to produce intracellular polyamines. They suggested that bifidobacteria depend on exogenous polyamines for cell growth and maintenance. Bacteria synthesize polyamines by descarboxylation of the aminoacids ornithine, arginine and lysine.[39] In fact, the above strains produced the highest levels of polyamine when the Falkow's media were supplemented with ornithine (0.5% w/v) as opposed to the other amino acids. The predominant polyamine produced was putrescine, which is directly synthesized from ornithine by a reaction catalyzed by the enzyme ornithine decarboxilase.

Our results also showed that putrescine was the main polyamine produced in the cecal content of the FOS group; in contrast, the predominant polyamines in the cecal tissue were spermidine and

doi: 10.3164/jcbn.10-100

spermine. Thus, the concentration of polyamines in the cecal content could be attributed to the intestinal microbiota which predominantly forms putrescine. On the other hand, cells can synthesize their own polyamine from ornithine, which may explain the different polyamine pattern in the cecal tissue. In any case, it appears that the putrescine effect is different than the proliferative effect produced at intestinal trophic level by spermidine or spermine intake.[40,41] In fact, we reported significantly higher crypt depth in newborn piglets fed a milk formula supplemented with spermine and spermidine at physiological doses.[42]

It was previously reported that supplementation of term infants with FOS has shown a dose-dependent stimulating effect on the growth of bifidobacteria and lactobacilli in the intestine.[36] Our results did not show a dose-dependent relation between FOS and bacterial polyamine production *in vitro*, which suggests that although *Bifidobacterium* and *Lactobacilli* are able to produce polyamines they may not be the main microbial producers of polyamines in the gut. It is known that polyamines concentration is strongly influenced by growing conditions such as pH of the medium, atmosphere conditions, presence of amino acids, among others. So, it suggested that polyamine formation by various bacterial species in the gut is stimulated by the supply of suitably metabolizable substrate.[35] It is possible that *in vitro* bifidogenic activity of FOS also decreases the growth of bacteria which produce high levels of polyamines such as *Fusobacterium*, *Bacteroides* and Gram-positive anaerobic cocci that have been reported to increase polyamine concentration in rats.[13]

Conclusion

The intake of FOS at physiological doses increased proportion of bifidobacteria, lactobacilli, and polyamine concentrations in the cecal content, but not in cecal tissue of neonatal piglets. Our results did not show that higher polyamine levels in the cecal content resulting from FOS administration may improve gut maturation. Bifidobacterium and lactobacilli are able to produce polyamines but they seem not be the main microbial producers of polyamines in the cecum of piglets fed FOS.

Acknowledgments

We acknowledge the expert technical assistance of Drs. Antonio Muñoz and José Salvador Martínez Martínez and the collaboration of Almudena Haro Revenga. DANONE S.A. Spain is acknowledged for financial support.

Abbreviations

CFU	colony forming units
FOS	fructooligosaccharides
NRC	National Research Council
PBS	Phosphate buffered saline
SCFA	short-chain fatty acids

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doi: 10.3164/jcbn.10-100