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Systematic review of the safety and suitability of dietary supplementation with short-chain fructo-oligosaccharides in infants and young children

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Abstract
This review summarizes the latest knowledge related to the safety and suitability of short-chain fructo-oligosaccharides (scFOS, DP 3-5) in infant and young children formulae. PubMed/Medline and CAB abstract databases were searched from 1997 onwards, evaluating scFOS effects in children below 3 years old and in newborn animals. On 252 studies identified, only 6 RCTs and 6 studies on neonate animals were included, based on their quality. The supplementation of scFOS is well tolerated, leading to normal growth during early life. At 4 or 5 g/L, they increased Bifidobacteria count after one month of supplementation. Kestose (DP3), at 1-2 g/day, alleviated the clinical symptoms of atopic dermatitis in infants and toddlers. ScFOS are safe up to 4 g/L in infant formulae, and 5 g/L in formulae for young children. Evidences exist to consider that scFOS supplementation of infant and follow-on formulae bring infants health benefits that represents a good nutritional strategy when breastfeeding is impossible by mothers.

Keywords: prebiotic, infant and growing-up formulae, digestive tolerance, gut microbiota, immune response

1. Introduction
Breast feeding is universally considered the best nutrition for the newborn infant, having a lot of beneficial effects [1]. Compared to bottle-fed infants, breastfed infants are less prone to infections [2], may experience allergic conditions less frequently [3] and exhibit improved cognitive development [4]. These effects are probably partly mediated through intestinal microbiota modulation: compared to formula-fed infants, breast-fed infants exhibit a higher proportion of Bifidobacteria and Lactobacilli [5, 6]. Oligosaccharides in human milk are likely to be involved in positive modulation of microbiota in breast-fed infants [7, 8].
Oligosaccharides represent a large proportion of the total solid component of human breast milk but are absent from traditional formulae. Indeed, mature human milk contains 12 13 g/L of oligosaccharides which generally constitutes the third largest solid component after lactose and lipids [9] and is about 20-fold higher than that of bovine milk. For these reasons, new infant formulae containing prebiotic oligosaccharides have been developed over the past fifteen years to mimic the beneficial effects of human milk on infant health. In Europe, some infant formulae are supplemented with a mix of short-chain galacto-oligosaccharides (GOS) and long-chain fructo-oligosaccharides (FOS) to a 9:1 ratio but also with GOS or FOS alone. Along with GOS and lactulose, fructans are considered to be the most well-described prebiotic compounds [10]. Fructans are linear chains of fructose units linked to an initial glucose by β (2-1) bonds, constituting energy storage polymers in numerous plants.
Fructans as inulin or fructo-oligosaccharides are a normal component of food products in the standard Western diet. Their classification depends on their origin and degree of polymerisation [11]. The degree of polymerisation (DP) of native inulin, as extracted from chicory, varies from 2 to 60. Oligofructose, with DP 2 to 8, is produced from native inulin by partial enzymatic hydrolysis. Short-chain fructo-oligosaccharides (scFOS), with a DP of 3 to 5 (average DP 4, hence "short-chain" or sc), are produced from the sugar extracted from sugar beet. Through a fermentation process sugar is converted by an enzyme produced by the Aspergillus fijiensis fungus. The scFOS have been extensively studied in healthy adults, showing dose-response bifidogenic effects for doses ranging from 2.5 to 10 g/day and positive effects on the colonic environment and digestive comfort [12, 13].
In a recent review, Skorka and colleagues [14] addressed evidences on effects of the administration of prebiotic supplemented infant formulae compared to formulae not supplemented. They took in consideration supplementation with short-chain GOS, long-chain FOS, acidic oligosaccharides, oligofructose plus inulin, polydextrose, lactulose and mixtures of those prebiotics, but have hardly considered infant formulas supplemented with short-chain FOS. The objective of this review was to evaluate the safety and suitability of scFOS in formulae dedicated to infants and young children, and in the diet of newborn animals.

2. Materials and Methods
Animal studies and prospective studies recognised as randomized controlled trials (RCTs) were considered for inclusion. The present review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [15].

2.1. Eligibility criteria
Interventions were considered eligible for the systematic review if the following criteria were met: (1) original in vivo studies conducted in neonate or infant animals (<3 weeks old in rats) [16] or in healthy infants and young children (<3 years old); (2) evaluation of short-chain fructo-oligosaccharides (fructose units linking with β(2-1)

glycosidic bonds with a terminal D-glucose unit) with a degree of polymerisation between 3 and 5, respectively called kestose (GF2), nystose (GF3) and fructofuranosyl nystose (GF4); (3) publication written in the English language. Short-chain FOS take different names depending on geographical area. Those known as scFOS are Actilight®, Meioligo®, Neosugar®, Nutraflora® for human nutrition and Profeed®, Fortifeed® for animal nutrition.

2.2. Literature search and study selection

A full electronic search was carried out in September 2017. Electronic databases (CAB abstract and PubMed/Medline) were identified and searched for articles published from 1997 onwards. This was chosen as a cut-off as an opinion about "Actilight – Fructo-oligosaccharides" from the Scientific Committee on Food, reviewing all safety issues related to this ingredient was published in 1997 [17]. Full search terms are provided in Box 1 (in supplementary data). Reference lists of articles identified using this strategy and of currently published systematic review were scanned to identify potential studies for inclusion in the review (manual addition). Figure 1 shows the flow diagram of the review process. Two independent reviewers screened the titles and abstracts for relevance to the systematic review.

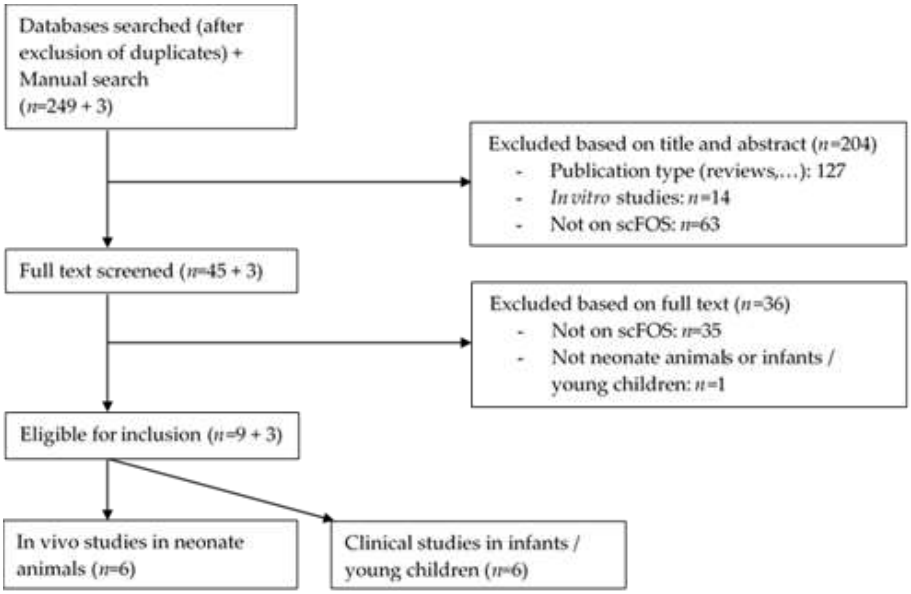

Fig 1: Flow diagram for retrieval of the relevant published studies with reasons for excluding studies after title/abstract and full text filtering

2.3. Data extraction

Two reviewers extracted the data from the studies included. The following general study information was extracted: first author, year, country of the study, treatment groups, dose, mean age at enrolment, duration of intervention, number of subjects (females and males), and main outcomes.

2.4. Risk of bias

Publication bias was assessed using Cochrane Collaboration's tool [18] including following criteria: sequence generation, allocation of sequence concealment, blinding, incomplete outcome data, selective outcome reporting, and other potential sources of bias (table 2).

Table 2: Risk of bias assessments for selected clinical studies. +: low risk of bias? unclear risk of bias.

	Random sequence generation (selection bias)	**Allocation concealment** (selection bias)	**Blinding of participants and personnel** (performance bias)	**Blinding of outcome assessment** (detection bias)	**Incomplete outcome data** (attrition bias)	**Selective reporting** (reporting bias)
Xia, 2012	?	?	?	+	+	?
Lasekan, 2015	?	?	?	?	+	+
Paineau, 2014	+	?	+	?	?	+
Ripoll, 2015	+	+	+	+	+	+
Shibata, 2009	+	?	+	+	+	+
Koga, 2016	?	?	?	?	+	+

2.5. Data analysis

Data analysis consists of a descriptive synthesis of selected studies. The main outcomes are the digestive tolerance, the growth, the gut microbiota composition, the response to vaccination and the score for atopic dermatitis.

3. Results

3.1. Study selection and characteristics

The electronic search identified 249 titles, and 3 further studies were identified through other searches. Two reviewers screened the titles and abstracts and concluded that 204 did not meet the eligibility criteria. The 48 full-text remaining studies were then assessed by the two reviewers. Finally, 6 randomized, controlled clinical studies were identified and selected. Two studies focused only on kestose [19, 20]; among the studies on scFOS, one study was published in 2012 [21] and the 3 others between 2014 and 2015 [22-24], thus after publication of the review by Tijhuis *et al.* as preparatory work for evaluation of the essential composition of infant formula [25]. Details of the main findings from these 6 studies are described in Table 1.

Table 1: Description of included published randomized clinical trials in infants evaluating scFOS in infant and follow-on formulae.

Reference (country)	Treatment groups	Mean age at enrolment	Duration of intervention	N (baseline) Gender M/F %	Main outcomes
Xia, 2012 (USA)	Controls: cows' milk-based formula scFOS: same formula with 2g/L of scFOS scFOS: same formula with 3g/L of scFOS	2.5 days 2.2 days 2.3 days	28 days	101 similar in all groups	Microbiota Digestive tolerance
Lasekan, 2015 (USA)	Controls: soy-based formula scFOS: same formula with scFOS 2.5g/L (digestible CHO: 20% sucrose, 80% corn syrup) scFOS: same formula with scFOS 2.5g/L (digestible CHO: 0% sucrose, 100% corn syrup)		35 days	195 49/51	Digestive tolerance
Paineau, 2014 (France)	Controls: cows' milk-based formula scFOS: same formula with partial replacement of maltodextrin by scFOS – 5g/L	4.2 days 4.0 days	4 months	61 50/50	Bifidobacteria Digestive tolerance Growth Response to vaccination
Ripoll, 2015 (Spain)	Controls: cow's milk-based formula scFOS; same formula with partial replacement of maltodextrin by scFOS – 4g/L	17.8 weeks 17.8 weeks	6 months	75 47/53	Bifidobacteria Digestive tolerance Growth Response to vaccination
Shibata, 2009 (Japan)	Control: maltose (1-2g/d) Kestose (1-2g/d)	17.2 months	3 months	30 63/37	Score for atopic dermatitis Bifidobacteria
Koga, 2016, (Japan)	Control: maltose (1g/d for < 1-yr-old, 2g/d for 1 to 3-yr-old or 3g for 4 to 5-yr-old) Kestose (1g/d for < 1-yr-old, 2g/d for 1 to 3-yr-old or 3g for 4 to 5-yr-old)	17 (<1 yr) 13 (1-2 yr) 28 (2-5 yr)	4.5 months	65 57/43	Score for atopic dermatitis Bifidobacteria *Faecalibacterium praustnizii*

In addition, six studies with healthy neonate animals were also selected: 2 were conducted in piglets [26, 27], 3 in rats [28-30] and 1 in mice [31] (details of the main findings summarized in Table 2).

3.2. Safety of scFOS and animal studies

Animal models are relevant to evaluate the safety dosage of prebiotics and involved mechanism to health benefits. Safety information is provided by two recent studies which used scFOS as a reference high dose control to evaluate, in juvenile rats, the safety of two synthetic oligosaccharides structurally similar to human milk oligosaccharides (HMO), Lacto-N-neotetraose [30] and 2' O Fucosyllactose [29]. The two new synthetic HMO and the comparative reference scFOS were administered to the animals for 90 days at the dose of 5,000 or 6,000 mg/kg BW/day, versus a control with no synthetic HMO. Based on behavioral observations, body weight gain, food consumption, ophthalmoscopy, clinical pathology, organ weight and histopathology findings, no adverse effect was observed for these dosages, which are considered as being around 95 times higher than exposure in the human target population, i.e. infants and young children.

In addition to rat studies evaluating the safety of scFOS intake, two studies have been conducted in piglets to evaluate the impact of scFOS supplementation of the severity of symptoms induced by a Salmonella typhimurium infection and some markers of the immune response. Indeed, as dietary fibre (soy polysaccharide) was previously reported to reduce the duration of diarrhoea symptoms in children and infants, Correa-Matos and colleagues tested the hypothesis that scFOS recognised as fermentable fibres reduce infection-associated symptoms and enhance intestinal structure and function in neonates [26]. Oral gavage with Salmonella typhimurium of 7-day-old piglets produced diarrhoea in control group, but not in the group receiving formula (15 mL/kg/h) supplemented with 7.5 g/L scFOS (completely fermentable). Recovery time and infection-associated symptoms (physical activity, lethargy) were also improved and short-chain fatty acids (SCFA) concentration in the colonic contents significantly increased in the scFOS group compared to the control [26].

Milo and colleagues also investigated the effects of dietary fibres in young animals fed formulas with varying degrees of fermentable prebiotics, scFOS or soy polysaccharides (SPS), in 7-day-old piglets infected with S. typhimurium [27]. They studied small intestinal lymphocyte and neutrophil migration, jejunal and ileal proinflammatory cytokine messenger RNA abundance, and interleukin-6 (IL-6) secretion in response to a lipopolysaccharide challenge using an in vitro whole blood model. Infection did not reduce small intestine lymphocyte migration in animals consuming either the SPS or the scFOS diet, suggesting a protective effect from fermentable fibres after infection. Fermentable fibres in the formulae did not influence cytokine messenger RNA abundance (TNF-α, IL-1β and IL-6). However, 2 hours after the lipopolysaccharide challenge, plasma samples from the piglets consuming scFOS-supplemented diets contained a significantly lower ($p<0.015$) concentration of IL-6 compared with samples from the piglets consuming the control or SPS diet, regardless of infection with *S. typhimurium* [27].

Table 2: Description of included published in-vivo studies evaluating scFOS in animal neonates.

Reference	Animal model, *n*	Age at enrolment	Treatment groups	Duration of intervention	Main outcomes
Correa-Matos, 2003	Piglets, 12/group	2 days	Control (sows milk replacer formula) FOS (Control containing 7.5g/L FOS)	14 days	Diarrhoea score after *Salmonella typhimurium* infection Digestive enzyme activity Physical activity
Milo, 2004	Piglets, 12/group	2 days	Control (sows milk replacer formula) FOS (Control containing 7.5g/L FOS)	14 days	Immune response to *Salmonella typhimurium* infection
Nakamura, 2004	Mice, 6/group	21 days	Control diet FOS (5%)	23 days	Intestinal immune system development
Lee, 2008	Rats, 9/group	21 days	Control diet Sn-2 palmitic acid-fortified oil Sn-2 palmitic acid-fortified oil + FOS (5% in diet)	21 days	Body weight, food intake Lipid absorption Calcium absorption
Coulet, 2013	Rats, 20/group	7 days	Control (diet A04C-10) FOS (14d: up to 7,500mg/kg/BW/day and 90d: 6,000mg/kg BW/day)	92 days	Safety evaluation Haematology Body weight, food intake
Coulet, 2014	Rats, 20/group	7 days	Control (diet A04C-10) FOS (up to 5,000mg/kg/BW/day)	14 days 90 days	Safety evaluation Body weight, food intake

3.3. Human studies

3.3.1. Digestive tolerance and somatic growth

Gastrointestinal disturbances, such as an increased prevalence of spit-ups, flatulence, and loose stools have been identified as a possible concern associated with the administration of oligosaccharides in infant formulas [32]. Information regarding the digestive tolerance to scFOS in infants was assessed in four human studies out of the six identified. The incidence of the following digestive symptoms was especially reported: abdominal pain evaluated by counting the number of episodes of crying, constipation versus soft stools, nausea, vomiting and regurgitation. The number of serious adverse effects was also occasionally reported. In all 4 studies, digestive symptoms showed similar frequencies in control and scFOS groups, regardless of the symptom in question.

In one RCT, the number of days with vomiting was lower ($p=0.05$) in the group of infants receiving, for 6 months from the age of 4 months, a formula supplemented with 5 g/L of scFOS [24]. At a concentration of 2 to 3 g/L, no difference in the frequency of spit-up or vomiting milk was observed among the supplemented and control groups, including a group fed with human milk [21, 22]. A significantly lower rate of oral candidiasis infection ($p<0.01$) was reported in infants fed for 4 weeks with a soy-based formula with an added 2.5 g/L of scFOS [22]. Another study reported a similar frequency in abdominal pain (with crying), diarrhoea (liquid stools) and nausea in the scFOS group (at 4 g/L) and control group [23].

In the 2 studies with longer scFOS administration (4-month duration for [23] and 6-month duration for [24]), body weight and body length remained similar between the groups and close to the median range of the WHO standards for child growth [33].

3.3.2. Stool frequency and consistency

Compared to control children, the number of days with soft stools was higher ($p=0.03$) in infants receiving a follow-on formula with 5 g/L of scFOS [24]. Mean stool consistency tended to be softer ($p=0.08$) in a group of newborn infants receiving a standard formula supplemented with 3 g/L of scFOS for ~4 weeks compared to group on the standard formula without scFOS, but without a significant difference in the average daily number of stools [21]. ScFOS at 2.5 g/L did not induce significant differences in the average daily number of stools and in predominant stool consistency, colour, odour between the groups of infants fed a soy-based infant formula or the same formula with scFOS and various [22].

3.3.3. Modulation of faecal microbiota

Five studies addressed the effect of scFOS added to infant formula on the intestinal microbiota. In a study by Shibata and colleagues, faecal bifidobacteria count tended to increase in a group of infants receiving a formula containing 1-2 g of kestose per day (scFOS, DP3) and to decrease in the placebo group. However, globally, no significant difference was found between groups after 12 weeks of supplementation, except in the infants whose basal count was low (<9.0*log10 cell count per gram of faeces) [19]. In a more recent study, Koga and colleagues reported, in addition to a significant higher *Bifidobacterium* count, a significant 10-fold increase of the number of *Faecalibacterium prausnitzii* in the faeces of infants (0 to 1-year-old) in whom the formulae had been supplemented with 1 g of kestose per day for 12 weeks [20]. In another study, in which newborn infants were fed cow's milk formulae supplemented with scFOS at a slightly higher dosage (2 or 3 g/L) for 4 weeks, supplementation did not suppress *Escherichia coli* or *Clostridium difficile* as effectively as human milk, but resulted in bifidobacteria and lactobacilli faecal populations comparable to those in infants fed with human milk [21]. In addition, the mean absolute number of faecal bifidobacteria (expressed as colony-forming-unit per g of dry matter) in 15 healthy full-term infants receiving a formula enriched with 4 g/L of scFOS, and who completed the study (i.e. per protocol subjects), increased after 2 months and even further after 3 months. In contrast, it decreased in the 18 per protocol infants in the control group receiving the same formula without scFOS, resulting in a considerably significant difference in the change in bifidobacteria count between the two groups ($p=0.03$ at 2 months and $p=0.003$ at 3 months) [23]. Finally, a significant increase in bifidobacteria was observed after one month in the group supplemented with 5 g/L of scFOS compared to baseline ($p=0.03$) and this effect was higher in the subgroup of infants who were never breastfed. However, the effects were no longer significant after two months of supplementation [24].

3.3.4. Modulation of immune response

Vaccine-specific secretory IgA (sIgA) collected in saliva or in faeces are considered as highly suitable markers of immune response as they correlate with clinical endpoints [34]. In infants vaccinated again poliomyelitis and fed from birth to 4 months with a formula enriched with 4g/L of scFOS, or not enriched, a marginally significant difference in the faecal levels of poliovirus-specific IgA per mg of humid faeces (p=0.08) was observed at 4 months between the two groups [23]. As the gut bifidobacteria content in the scFOS-fed children was also significantly increased, the improved immune response was likely linked to modification of the intestinal microbiota.

The primary outcome of another study was also to investigate the change in faecal-specific poliovirus sIgA level, associated with faecal bifidobacteria concentration, following vaccination [24]. The effects of scFOS on the immune response were evaluated in 75 healthy infants fed for 6 months, from the age of 4 months, either with a standard formula, or with a scFOS enriched formula. No significant difference in sIgA concentration between the scFOS group and the control group was observed after one and two months with the scFOS-supplemented follow-on formula at 5 g/L while a significant increase in bifidobacterial after one month of scFOS supplementation was observed. However, in previously breastfed infants, an unsignificant increase in specific sIgA in the scFOS group and a decrease in the control group was noticed after one month of supplementation (4.63 ± 4.63 vs. -12.35 ± 9.31 µg/mg; p=0.13). Also, this study showed that a follow-on formula supplemented with 5 g/L scFOS is safe and well-tolerated, leading to a normal growth in infants after the age of 4 months [24].

In addition, Japanese studies addressed the ability of scFOS to alleviate the clinical symptoms of atopic dermatitis in infants and toddlers. The diagnostic criteria were those of the Japanese Dermatologist Association for typical dermatitis eczematous [35], i.e. pruritus, erythema, papules, scaling, excoriation, lichenification and itching evaluated according to the Intensity Score of Atopic Dermatitis (ISAD) and the Severity Scoring of Atopic Dermatitis Index (SCORAD). In a pilot study, followed by a randomized controlled trial, kestose, a DP3 scFOS (GF2), was orally administered for 12 weeks to children under 3 years old at a dose of 1 g/day (under 1 year) or 2 g/day (1-3 years) [19]. In addition, SCFA and bifidobacteria count (by real-time PCR assay) were evaluated in faecal samples. Compared to the placebo group, the ISAD score in the scFOS group was significantly decreased in week 6 (p=0.004) and week 12 (p<0.001). A significant increase in bifidobacteria count was observed in the pilot study, but in the RCT it was only observed in subjects whose basal counts were low. Improvement was maintained until 6 weeks after the end of kestose administration. No significant difference was found in SCFA concentration in the faeces between the groups [19]. In a more recent study, Koga and colleagues reported a significant positive correlation between an increase in *F. praunsnitzii* count and an improvement in the SCORAD index of toddlers (2-5-year-old) with atopic dermatitis when 2-3 g/day of kestose was added to their diet for 6 weeks [20].

A modulation of the immune response following scFOS supplementation has also been demonstrated in animal models. In mice, feeding dams and their mouse pups a diet enriched with scFOS (5% W/W, scFOS(+) group) from weaning (at 21 days old) results in a beneficial effect on the mucosal immune system compared to control mice without scFOS (scFOS(–) group) [31]. At 36 days of age, faecal IgA level was significantly increased in the scFOS(+) diet group compared to the scFOS(–) group. In the scFOS(+) group, total IgA level was also enhanced in tissue extracts isolated from the colon and from the jejunum and ileum. Of note, intestinal polymeric immunoglobulin receptor, which plays a critical role in transepithelial transport of intestinal IgA onto the mucosal surface, was significantly higher in the colon and ileum of the mice fed with scFOS [31]. Thus, it was suggested that dietary scFOS positively affect the mucosal immune system in the colon as well as in the small intestine of infant mice. In addition, butyric acid concentration in the caecal contents of the dietary scFOS(+) group was significantly enhanced at 36 days of age compared to the scFOS(–) diet group [31]. This study suggested that FOS was fermented by intestinal flora such as bifidobacteria in the colon and formed some types of organic acid such as acetic acid, propionic acid and butyric acid.

3.3.5. Hydration effect

Due to the propensity of formula supplemented with oligosaccharides and/or probiotics to produce watery stools [32, 36, 37], in a recent study Lasekan and colleagues specifically addressed the safety concern of the water balance in infants fed such formulas [22]. They compared gastrointestinal tolerance in healthy term new-born infants (0-8 days old) fed a commercial soy-based formula with history of safe use, versus the same formula supplemented with 2.5 g/L scFOS (and various carotenoids). The primary assessed outcomes were mean rank stool consistency and infant' hydration status deducted from urine specific gravity. The study demonstrated good tolerance for formulae supplemented with scFOS, and hydration comparable to the control. Indeed, results indicated no significant differences in formula intake, growth, mean rank stool consistency, stool frequency, spit-up/vomiting, adverse events and in urine specific gravity between groups.

4. Discussion

4.1. Safety and hydration

According to what was reported by several European scientific bodies [25, 38, 39] and an expert consensus [40], no safety concerns have been raised by studies having evaluated the effect of non-digestible oligosaccharides in infants at adequate dosage. If they are osmotically active, their dosage should not carry a risk as to the water balance in infants as suggested by the experts.

More particularly, use of scFOS in infant and follow-on formulae has also been confirmed to be generally safe by several health authorities, such as in Australia / New Zealand at up to 3g/L in infant and follow-on formulae [41], and in the USA for a dosage up to 4g/L in infant formulae and up to 5g/L in follow-on formulae [42]. These recent opinions confirm a history of safe use of scFOS in infant formulae from the late eighties in Japan, among 20,742 healthy infants surveyed from birth to 4.5 months of age (up to 4.2g/day of scFOS) [43]. In Europe, the recognition of fructo-oligosaccharides as food ingredient was acknowledged by the Scientific Committee for Food in 1997 on the basis of toxicological studies [44] in animal models and human clinical studies. They mentioned that there was

no concern about the safety of scFOS, keeping in mind their laxative potential at high dosage. Indeed, the ED50 for diarrhoea was estimated at 0.8 g/kg BW, i.e. approximately 50-60 g/day in adults [17].

4.2. Impact of scFOS on gut microbiota and digestive tolerance

Prebiotics are fibres able to selectively stimulate growth and/or activity of specific microbial species in the gut microbiota that confer health benefits to the host [10]. An approach to improve infant formulae in order to mimic the benefits of human milk, is supplementation by the addition of specific prebiotics such as scFOS since they are fermentable [45] and bifidogenic in different animal models, as well as in adult humans [12, 46-48].

The development of the microbiota in early life and its relationship with the immune system and later health parameters, notably obesity and allergy, has been recently reviewed [11, 49]. There is growing scientific evidence that breastfed neonates are better protected than formula-fed neonates against upper or lower respiratory and gastrointestinal tract infections [50]. It has been suggested that improved gut microbiota composition and activity contributed to these health benefits. The microbiota of breastfed infants is typically dominated by bifidobacteria and lactobacilli [5, 6], as human milk provides a continuous inoculum of lactic acid bacteria and Bifidobacterium species [51]. In addition, human breast milk contains large amounts of prebiotics which are complex fermentable oligosaccharides (HMO) that putatively modulate the intestinal microbiota, mainly favouring *Bifidobacterium* increase [52, 53]. However, most of them are absent from cow's milk, and therefore from conventional infant formulae. Since, in addition to animal studies, scFOS have been extensively studied in healthy adults showing dose-response bifidogenic effects for doses ranging from 2.5 to 10 g/d [12, 47] after only 7 days, supplementation of infant formula with such prebiotics may come close to HMO health benefits.

Five out of 6 of the in-human studies presently reviewed addressed the ability of scFOS to improve infant microbiota. Results are clearly in favour of a positive dose-effect relationship on bifidobacteria development, a significant increase in this bacterium being observed from a dosage of 4 g/L in formula, mainly in infants not previously breastfed or with low initial bifidobacteria levels. Of note, in adults, scFOS begins to increase faecal bifidobacteria from 2.5 g/day and was recognized as safe up to 4 g/L in infant formulae and 5 g/L in follow-on formulae [42]. Based on a daily formula intake ranging from 0.5 to 0.8 L for infants before 4 months of age, 4 g/L of formula corresponds to a scFOS intake of 2 to 3 g/day. Pure kestose (DP3) may be effective at lower dosage (1 to 2 g per day). Previous studies using other type of prebiotic compounds in formula-fed infants, like GOS or high-molecular weight FOS, found similar results but at a higher daily intake level, with a dose-dependent bifidogenic effect from 4 to 8 g/L [54].

A positive effect of colonic environment and digestive comfort has also been observed in adults [13]. In children, fructo-oligosaccharides have been shown to reduce the duration of diarrhoea episodes when consumed at around 2.5 to 5 g per day [55, 56]. In infants, the bifidogenic effect of scFOS supplementation was not associated with adverse digestive events in the reviewed studies, it was even shown a decrease in the number of days with vomiting, with improved digestive tolerance and bowel habits [22, 23].

4.3. scFOS and immunity

The effect of prebiotics on the performance of the immune system has been investigated in infants by their impact on immune parameters measuring faecal secretory IgA (sIgA) levels in response to vaccination [57, 58], as well as intestinal or systemic infections and atopy.

Experimental studies in animal models demonstrated that dietary scFOS increases IgA response in the small intestine as well as in the colon [31, 59]. More particularly it was shown that scFOS increased the relative expression of polymeric immunoglobulin receptor at the ileum and colonic level in infant mice. However, in the studies reviewed here, the lack of increase in faecal poliovirus-sIgA levels after repeated vaccination associated with 4 months of scFOS supplementation at 4 g/L [23], or the poor response with 5 g/L for 6 months, contrasts with previous animal studies. It has been suggested that faecal IgA levels may not reflect intestinal IgA secretion accurately as secretory IgA may be digested in the intestinal lumen by some bacterial species that possess proteases capable of degrading IgA [31]. On the other hand, two studies have shown that long term kestose (the shortest scFOS) supplementation at 1 or 2 g/day alleviates clinical symptoms in infants and toddlers with atopic dermatitis [19, 20], and are in keeping with modulation of immune response correlated to specific changes in intestinal microbiota in infants.

4.4. Perspectives

It should be noted that high concentrations of fucosylated HMOs in breast milk positively influenced survival of uninfected children born to HIV-infected mothers in Lusaka, Zambia [60]. A recent study in pig models demonstrated that maternal scFOS supplementation led to an improved immune quality of the colostrum with higher levels of IgA and Transforming Growth Factor β1 and accelerated the development of intestinal immunity in offspring [61]. Thus, that could enhance their intestinal defence mechanisms and their response to an immune challenge (e.g. vaccination) later in life [62]. In NC/Nga mice, a mouse model for human atopic dermatitis, maternal and offspring consumption of scFOS lowered the severity of skin lesions in the offspring, accompanied by lower serum concentrations of total IgG1 and lower levels of expression of TNF-α in the damaged tissue [63]. In addition, dietary supplementation with 5% 1-kestose during pregnancy and lactation in BALB/c mice resulted in a significant increase in total IgA concentrations and anti-Bacteroides IgA levels in the milk [64]. Other research demonstrated that the consumption of scFOS by 84 pregnant and lactating women, at 8 g/day from week 26 of pregnancy to 4 weeks after birth, increases expression of interleukin-27 in breast milk with possible physiological consequences on the baby's intestinal tract and immune system [65]. These studies suggest that supplementation of pregnant and lactating mothers can impact the immune quality of colostrum and breast milk. However, it is not clear whether this effect is directly linked to greater development of intestinal bifidobacteria in neonates or in the mother who received scFOS supplementation during gestation and showed higher faecal concentration than in the control group [48].

5. Conclusions

In conclusion, studies conducted in neonate animals and non-breastfed young children during the last 20 years indicate that supplementation of the diet with scFOS, in addition to being safe, may promote health benefits on gut microbiota, digestive tolerance, intestinal transit, and also immune response. Therefore, addition of scFOS to infant formulae or to formulae for young children, at a dose of 1-5 g/day, may be a useful means of improving their advantages with respect to those of human breast milk.

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