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Prebiotics in infant nutrition: a critical appraisal

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Abstract: Supplementation of infant formula with ingredients potentially able to manage, in a way positive for the host, the gut microbiota of infants, is nowadays widely used by infant food producers.

The impact of this class of compounds, named prebiotics, on gut microbiota composition, was initially measured on the enrichment of *Bifidobacterium* and *Lactobacillus* spp., but the use of culture-independent analytical techniques has allowed to establish that a larger number of intestinal microorganisms are affected by the ingestion of prebiotics.

However, despite a relevant number of scientific publications, actually there is no consensus on the amount of prebiotics to be administered daily, the potentially different actions exerted by prebiotics which differ in biochemical structures, as well as the impact of different percentages of the same prebiotics when used in mixtures and/or combination.

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This paper is aimed to critically review the available data on the use of prebiotics in infant formula, with special attention paid to identify a link between, the dosage used, the mixture composition and the observed outcomes, keeping in mind the influences in nutrition quality and growth influence.

Keywords: Prebiotics; Infant Nutrition; Infant formula; Gut microbiota; *Lactobacillus*; *Bifidobacterium*; Fructo-oligosaccharides; Galacto-oligosaccharides; Polidextrose; Acidic oligosaccharides.

1. Introduction

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The concept of prebiotic as a "nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacterial species already resident in the colon, and thus improves host health" dates back to 1995 [1]. Prebiotics are then considered a specific fuel that indigenous probiotic bacteria can utilize to grow. Most commonly known and characterized prebiotics are fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), inulin, lactulose, and breast milk oligosaccharides [2]. FOS are able to cross the digestive lumen, undigested and unabsorbed, to reach the ascending colon unmodified, where they will be metabolized by the resident probiotic component of the microbiota. Overall, oligosaccharides have a long history of use as food ingredients able to provide health benefits.

On this scenario, however, from the initial 1995 definition, several attempts to modify and/or improve the prebiotic definition have been done. In 2003, Reid et al. [3] defined prebiotics "non-digestible substances that provide a beneficial physiological effect on the host by selectively stimulating the favorable growth or activity of a limited number of indigenous bacteria". In 2004, the definition of prebiotics was further modified "selectively fermented ingredients that allow specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health" [4]. In 2007 the Food and Agricultural Organization (FAO) of the United Nations organized a Technical Meeting to update the definition of prebiotics. The outcome of this meeting was the following definition: "a non-viable food component that confers a health benefit on the host associated with modulation of the microbiota" [5]. In 2010 Gibson et al. [6] defined the narrower category of "dietary prebiotics" as "a selectively fermented ingredient that results in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health". In 2015, Bindels et al. [7] proposed that specificity requirements should be removed on the basis of reports showing that multiple taxa, rather than particular species, were enriched by prebiotics. This proposal led to another definition of a prebiotic as "a non-digestible compound that, through its metabolization by

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68 microorganisms in the gut, modulates the composition and/or activity of the gut microbiota, thus,
69 conferring a beneficial physiological effect on the host". In 2017 Gibson et al. [8] defined prebiotic
70 a substrate that is selectively utilized by host microorganisms conferring a health benefit. This
71 definition expands the concept of prebiotics to possible applications to body sites other than the
72 gastrointestinal tract, and diverse categories other than food, while the requirement for selective
73 microbiota-mediated mechanisms was retained.

74 A common point of all definitions is that prebiotics differ from dietary fibers, which promote
75 the growth of a wide variety of gut microorganisms, as they selectively serve as nutrients for
76 beneficial microorganisms harbored by the host, including administered probiotic strains and
77 indigenous (resident) microorganisms. Therefore a prebiotic should not be broadly metabolized but
78 elicit a metabolism biased towards health-promoting microorganisms within the indigenous
79 ecosystem. Due to the fact that the concept of prebiotics is not so clearly defined and remains a
80 controversy, Table 1 summarized all the definitions in order to give the insights of definition and
81 have the ambitious to derive at the end a unique and accepted definition.

82

83 **Table 1.** The concept of prebiotic over time

Definition	Authors	Reference
A nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacterial species already resident in the colon, and thus improves host health	Gibson & Roberfroid, 1995	[1]
Nondigestible substances that provide a beneficial physiological effect on the host by selectively stimulating the favourable growth or activity of a limited number of indigenous bacteria	Reid et al., 2003	[3]
A selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host wellbeing and health	Gibson et al., 2004	[4]
A nonviable food component that confers a health benefit on the host associated with modulation of the microbiota	Pineiro et al., 2008	[5]
A selectively fermented ingredient that results in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s)	Gibson et al., 2010	[6]

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upon host health		
A nondigestible compound that, through its metabolization by microorganisms in the gut, modulates composition and/or activity of the gut microbiota, thus conferring a beneficial physiological effect on the host	Bindels et al., 2015	[7]
A substrate that is selectively utilized by host microorganisms conferring a health benefit	Gibson et al., 2017	[8]

As regards gut health and microbiota management, the other side of the coin are probiotics whose definition seems to have reached, after more than half century from its first usage [9], a consensus among the scientific community as " live microorganisms that, when administrated in adequate amounts, confer a health benefit on the host" to exert a wide range of effects [10]. The Panel confirmed that, although some mechanisms might be shared between strains belonging to the same species/genus, health effects are strain-specific and claims of specific benefits must be determined at the strain level. Moreover, to exert the desired effect, any probiotic must be delivered at a functional dose, and the Panel stressed that to establish a clear association between any substance, including probiotics, and a desired effect it is important to examine dose response, among other criteria. It is thus interesting to note that, while probiotics are well described in term of adequate amount and specific role/effect as well as type of microorganisms, the definitions of prebiotics proposed to date have not considered either the specificity of the chemical composition of the prebiotic or its dosage. This is reflected by the heterogeneity of the studies available on prebiotics which differ a lot in terms of amount/dose and type of prebiotics administrated, making direct comparison among results very difficult and not really focused also in meta-analysis and systematic reviews [11].

Because there are on the market a wide range of prebiotics, the aim of this paper is to offer a critical appraisal of the existing literature about the clinical outcomes of these gut-directed ingredients in the age 0-12 months, when they are used as a supplement to formula milk.

2. Methods

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Only randomized controlled trials (RCT) were eligible for inclusion. Enrolled subjects have to be aged 0-12 months; studies on preterm and unhealthy subject were included. Only full paper written in English were retained for analysis.

Studies in which prebiotics were not administered as an ingredient of formula, for example in capsules, were discarded. Studies in which synthetic human milk oligosaccharides were used were excluded.

A number of RCTs described the same study population but focused on different outcome measures; they were considered as separate reports and treated individually.

A search with the keywords “infants” and prebiotic(s)” and “formula” filtered for “clinical trials” in the databases PubMed, Scopus, Web Of Science, retrieved 145 items, after filtering for duplications, papers not in English, letters or not full papers..

Among them a further selection of 71 was done, discarding those dealing with probiotics synbiotics , those enrolling subjects older than 12 months of age , subjects in severe pathological conditions , or those out of scope of this paper (18) for specific reasons (see Supplementary Table.1)

3. Results

3.1. *Studies assessing the effects of a single prebiotic ingredient*

Table S2 is devoted to the outcomes obtained when a single probiotic ingredient has been tested in human trials.

Results obtained when GOS are used as only prebiotic ingredient, at the dosage of 0.4 g/100 mL [12–14] suggested that that at this dosage this prebiotic could have an impact on the microbiota composition; fecal parameters such stool consistency and volume are also positively affected, while GOS-supplemented formulas do not seem to exert any effect on the frequency of defecation. Parameters related to health, such as the incidence of infections or allergic manifestations [14,15],

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are not influenced by GOS supplementation, even when a slightly higher dose (0.5 g/100 mL) is used [15].

GOS dosed at 0.8 g/100 mL in the very first weeks of life seem to cause watery stools [13] and this observation could be used as starting point to establish a safety upper level of supplementation; this note is also supported by data provided in 2014 by Williams et al. [13]. These authors, in a trial conducted in United States in 2007-2008 with a formula supplemented with 0.8 g/100 mL of GOS, reported a higher percentage of very soft stools in treated infants.

Positive results additional to alteration of microbiota and stool composition are reported by Giovannini et al. [12] as they recorded a lower incidence of colic in the supplemented group of infants with respect to the control group.

Additionally, Paganini et al. [16] found a lower abundance of virulence and toxin genes in GOS-treated group when compared with the control and Fe only groups. Underwood et al. [17], on the other hand, did not detect significant increases in bifidobacteria compared with baseline in preterm infants dosed with GOS.

Turning to fructan prebiotics, among the 5 papers which reported the use of FOS as single prebiotic ingredient [18–22], only Kapiki et al. [20] showed significant, consistent effects on microbiota, in particular on the abundance of bifidobacteria, using 0.4 g/100 mL; a dose of 0.3 g/100 mL [18,20,21], is most likely enough for FOS to have a positive influence on stool characteristics.

The use of kestose, a fructo-oligosaccharide, has been reported by Shibata et al. [23] to have a positive influence on SCORAD scores of atopic infants. Two papers [24,25] reported the use of fructans derived from agave (Agave tequilana Weber cv. Azul). After a safety assessment in healthy, full-term babies [24] two ingredients were assessed for impact on immune response, serum ferritin, C-reactive protein, bone metabolism, and gut bacteria changes. Analysis were performed at 20 – 27 days, and three months of age. The authors state that there were statistically significant

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differences for the groups of infants fed only with infant formula and with formula enriched with these fructans.

As far as inulin is concerned, Kim et al. [26] assessed the effects of native inulin in formula-fed babies on gut microbiota, pH, consistency and amount of feces, and on frequency of defecation at a daily dosage of about 5 g/d. The consumption of inulin increased significantly the content of *Bifidobacterium* in the feces of formula-fed babies compared to control ,but neither the frequency of defecation nor stool consistency were affected by the consumption of inulin.

In the study by Closa-Monasterolo et al. [27], oligofructose-enriched inulin at a dose of 0.8 g/100 mL was tested, and the authors found a tendency towards higher *Bifidobacterium* cell counts, with a significant higher frequency of depositions and a softer consistency of stools [28].

3.2.Studies assessing the effects of combinations of prebiotic ingredient: not strictly gut-related clinical outcomes of GOS/FOS administration

A group of papers, all of them with a very similar clinical design and a dosage of GOS/FOS of 0.8 g/100 mL, was devoted to establish a link between the use of this ingredient and immune response (Table S3). Moro et al. [29] reported that 102 infants in the prebiotic group and 104 infants in the placebo group (all of them at risk for atopy) completed the study (duration: 6 months). Ten infants in the intervention group and 24 infants in the control group developed atopic dermatitis. Prebiotic supplementation was also producing a significantly higher number of fecal bifidobacteria compared with placebo (maltodextrin) group. An hydrolyzed protein formula was used in both groups.

The same design has been used by van Hoffen et al. [30] to assess the effect of GOS/FOS on the immune response in infants at risk for allergy. An hypoallergenic whey formula with either 0.8 g/100 mL or 0.8 g/100 mL maltodextrin (placebo) were used for 6 months. At 6 months of age, plasma samples were collected from the 41 treated infants and the 43 subjects of the placebo group.

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GOS/FOS supplementation resulted into a significant reduction of total IgE, IgG1, IgG2 and IgG3, but not of IgG4 amount. The authors concluded that GOS/FOS supplementation could be a method to limit the atopic development.

An additional study on the effect of supplementation of GOS/FOS on plasma markers of allergic disease in infants at risk for allergy was published by Schouten et al. [31]; also in this study hypoallergenic whey formula containing 0.8 g/mL of the GOS/FOS mixture was administered for 6 months to 34 infants, while the control group (n=40) received maltodextrin; all of them at risk for atopy. In this paper immunoglobulin free light-chain (Ig-fLC) was checked as a marker of allergic disease. Results supported a positive role of GOS/FOS as in infants receiving the prebiotic mixture, the Ig-fLC levels were significantly lower compared to the placebo-fed infants.

MacDonald et al. [32] by means of an 8-week open-label, single-arm, pilot intervention study in 9 infants (8-week median age) tried to establish a role for prebiotics (GOS/FOS 0.8 g/100mL) added to a protein substitute suitable for infants with Phenylketonuria. All infants exhibited microbiota dominated by bifidobacteria, although no statistically significant change from baseline was observed at study endpoint.

Arslanoglu et al. [33] showed a preventive effect of the prebiotic mixture GOS/FOS against infections during the first 6 months of life. In this study the cumulative incidence of all types of recurring infections was significantly lower in the GOS/FOS group; prebiotic mixture was used as an ingredient of a hypoallergenic formula. The dose used was 0.8 g/100 mL.

Bisceglia et al.[34] published a paper aimed to investigate the effect of dietary supplementation with prebiotics on moderate hyperbilirubinaemia in healthy, term infants. Seventy-six consecutive newborns were randomly assigned to receive a formula containing 0.8 g/100 mL GOS/FOS supplement or maltodextrins as placebo for 28 days. Bilirubin levels and daily defecation frequency was also recorded. A statistically significant lower transcutaneous bilirubin was detected

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in neonates receiving prebiotics, from 72 h of life, till the end of the dietary intervention (day 28). Treated infants also showed a higher frequency of defecation, when compared to neonates on placebo, over all the duration of dietary intervention.

The dose of 0.4 g/100 mL has been used by Bruzzese et al. [35] in order to reduce the incidence of intestinal and respiratory infections in healthy infants. In this massive study 352 subjects were enrolled and fed with the prebiotic formula or the control one for a period of one year. The incidence of gastroenteritis and the number of children with multiple antibiotic courses/year was lower in the supplemented group than in the controls.

A few other studies tested the effects of mixtures of GOS/FOS with atypical dosages, i. e. different from the concentrations 0.4-0.8 g/100 mL (Table S4).

Brunser et al. [36] evaluated the effects on intestinal microbiota composition of infants but after an amoxicillin treatment for upper respiratory tract infections. This study is of particular relevance for the purpose of this review, in fact the mixture used was oligofructose and inulin in 70/30 proportion by weight (and not 9:1), at a dosage of 0.45 g/100 mL after formula reconstitution. This dosage showed that infants fed a milk formula supplemented with prebiotics had higher counts of bifidobacteria and lactobacilli than those fed the same formula but without the prebiotics The total fecal bacterial count population returned to the levels before amoxicillin administration without differences between the groups, confirming that prebiotics do not influence the recovery of the total flora.

A dose different from the 0.4-0.8 g/100 mL was also used by Alliet et al. [37]: these authors used GOS/FOS in a 9:1 ratio but a dose of 0.6 g/100 mL. Aim of this study was the evaluation of prebiotics consumption related to cholesterol and triacylglycerol levels in infants. The study was organized into 3 arms: breast fed, standard formula and the same formula supplemented with GOS/FOS. Among the 187 infants which completed the study the total cholesterol and LDL levels

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were significantly lower in the formula-fed groups than in the breast-fed infants but there were no significant differences between the formula-fed groups. Authors concluded that there are no differences in total cholesterol and LDL cholesterol in infants receiving an infant formula with GOS/lcFOS (with this dosage) from infants receiving a control infant formula.

3.3. Studies assessing the effects of combinations of prebiotic ingredient: intestinal effects of GOS/FOS

The majority of papers reporting the use of GOS/FOS in a 9:1 ratio, were aimed to assess the impact of this prebiotic mixture on microbiota composition of full term infants, with special emphasis on lactobacilli, bifidobacteria, enterobacteriaceae and clostridia as well as on defecation frequency and stool parameters (Table S5).

Moro et al., in two different clinical trials [38,39] used two levels of prebiotics (0.4 and 0.8 g/100 mL) to supplement infant formula in 180 infants in order to identify the most active dose in promoting a microbiota composition close to that one on breast fed babies. After these preliminary steps, and since the paper of Knol et al. [40], the selected dosage seemed to be the 0.8 g/100 mL.and used in the majority of papers. Knol et al. [40] analysed by means of plate counts and fluorescent in vivo hybridization (FISH) the stools of 19 infants treated with the GOS/FOS mixture in comparison with 19 infants fed breast milk and 15 fed the control formula. In addition to bacterial counts several stool parameters were assessed such as lactate, short-chain fatty acids (acetate, propionate), pH, stool consistency. All of them were more close to parameters detected in breast-fed babies mainly when the 0.8 g/100 mL was used. An increase in frequency of defecation was noticed by Moro et al. [38] only in infants receiving the 0.8 g/100 mL dose.

Haarman & Knol in two following papers used a quantitative PCR approach to evaluate species-specific changes in *Bifidobacterium* [41] and *Lactobacillu*s [42] in full-term babied fed with

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a formula containing 0.8 g/100 mL of the GOS/FOS mixture. In both articles this analytical approach showed that the prebiotic mixture supports species that are dominant in breast-fed babies.

Bakker-Zierikzee et al. [43] compared the effects of GOS/FOS 0.8 g/100 mL in 19 infants to the impact of the use of a probiotic *Bifidobacterium* in the early stage of life (5 to 16 weeks). The only significant difference was higher fecal acetate ratio and lactate concentration and a lower pH at 16 weeks.

Costalos et al. [44],using a dose of dose of 0.4 g/100 mL, found a significantly higher stool frequency and softer consistency in the prebiotic group while these outcomes were noticed only in the group treated with 0.8 g/mL in the paper by Moro et al. [38]. On the other hand, this paper [44] confirms that, at this dosage, no statistically significant differences are detectable in the bacterial counts. In addition, Holscher et al. [45] also using the low dose of 0.4 g/100 mL reported that feces from infants fed with prebiotics had a higher absolute number and proportion of bifidobacteria when compared to control formula and they did not differ from breast-fed infants.

On the contrary, Vivatvakin et al. [46] using the low dosage reported better scores of gastrointestinal comfort in infants fed a whey-predominant formula containing long chain polyunsaturated fatty acids and GOS/FOS, when compared to infants fed a control casein-predominant formula without additional ingredients. Healthy, full-term infants were randomized to receive exclusively either experimental (n=67) or control formula (n=69) from 30 days to 4 months of age; exclusively breast-fed infants (n=67) served as reference. Compared to the control, the experimental group had less hard stools as well as a microbiota composition closer to that of the breast-fed group.

Veereman-Wauters et al. [47] reported results of a mixture of two types of fructose-based oligosaccharides (long and short chain 50:50) administered at a dose of either 0.4 or 0.8 g/100 mL and the GOS/FOS mixture administered at 0.8 g/100 mL. The study lasted for 28 days and subjects enrolled were 81, divided into 3 treatments groups and one control, plus a reference group of 29 breast-fed infants. The authors reported that all the 3 prebiotic groups maintained soft stools, only

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slightly harder than those of breast-fed infants but the control group (formula without prebiotics) had significantly harder stools at weeks 2 and 4. The total number of fecal bacteria increased in all of the prebiotic groups. In the groups supplemented with 8 g/100 mL, *Bifidobacterium* counts were significantly higher and were comparable with the breast-fed group.

Salvini et al. [48] recruited 20 newborns of hepatitis C virus-infected mothers who decided not to breast-feed. These neonates were randomly assigned to either a formula with 0.8 g/100 mL of a GOS/FOS or a placebo formula (maltodextrin). Measured outcomes were anthropometric data, microbiological analysis of fecal samples, and blood leukocyte population. Prebiotic supplementation resulted in more fecal bifidobacteria and lactobacilli compared with the placebo group. These differences between the groups were maintained during the second half of the first year without any prebiotic supplementation, supporting a long lasting effect of the prebiotic treatment.

Holscher et al. [45] enrolled full-term, formula-fed (FF) infants compared to a breast-fed group (BF). FF infants were randomized to consume a partially hydrolyzed whey formula with or without 0.4 g/100 mL GOS/FOS. Fecal bacteria, pH, and SCFA were assessed at baseline, 3 weeks, and 6 weeks. Feces from infants fed prebiotics had a higher absolute number and proportion of bifidobacteria which did not differ from that of BF infants. Notably, the established bifidogenic effect of GOS/FOS did not result in any differences in infant growth parameters compared to control infant formula without prebiotics and/or probiotics [49].

Dasopoulou et al. [50] published a study similar to those of Indrio et al. [51,52] testing the hypothesis that prebiotics could alter motilin and gastrin secretion and reduce lipids in healthy preterms. Outcomes were positive as mean motilin increase compared with the control group while gastrin remained high in both groups.

The most recent development of the use of GOS/FOS at 0.8 g/100 mL is their addition to a formula milk fermented with *Bifidobacterium breve* and *Streptococcus thermophilus* [53,54]. Huet et al. [53] studied safety and tolerance of the combination of partly fermented infant milk formula

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and GOS/FOS in healthy term infants enrolled before 28 days of age and followed up to 17 weeks of age. Four hundred and thirty infants were divided into 4 groups: (1) formula with scGOS/lcFOS, (2) formula + GOS/FOS +15% fermented formula, (3) formula + GOS/FOS +50% fermented formula, (4) formula + 50% fermented formula, in order to check the daily weight gain during intervention and to monitor infants' anthropometrics data, formula intake, number, and safety. Physiological and bacterial data were obtained from stool samples collected at baseline and at the end of the trial. Results showed equivalence of weight gain while no differences were observed in other controlled parameters. Supplementation with GOS/FOS provided beneficial effects such as a lower pH, lower *Clostridium difficile* levels, and higher secretory immunoglobulin A levels.

Vandeplas et al. [54] using the same sample size than above assessed the impact of this combination among formula, fermented formula and prebiotic on colic. After 4 weeks of treatment the research group demonstrated a reduced overall incidence of infantile colic in group 3 GOS/FOS +50% FERM, better than in group 1 and 4, suggesting a dose response for the fermented formula.

The mixture GOS/FOS 9:1 has been also used also in feeding of preterm infants (Table S6).

Boehm et al. [55] used a dosage (i.e. 1 g/100 mL) higher than those used in full term infants (0.4 or 0.8 g 100/mL); not surprisingly, they demonstrated a strong bifidogenic effect of prebiotic-supplemented formula as well as a positive impact on stool frequency.

Mihatsch, et al. [56] used the same dosage to evaluate tolerability and the capacity of prebiotics to reduce stool viscosity and accelerate gastrointestinal transport, in order to improve feeding tolerance. Both these parameters were positively affected by the prebiotic supplementation in the 10 treated infants compared to the same number of control subjects.

Indrio et al. [52] measured gastric electrical activity and the gastric emptying time in 10 preterm neonates fed with a formula containing 0.8 mg/100 mL GOS/FOS. After a feeding period of 15 days, both parameters were significantly improved when compared to the 10 infants of the placebo group. These results were confirmed in a larger study of the same research group [51]

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involving 49 preterm newborns. After an intervention period of 30 days, infants administered 0.8 mg/100 mL GOS/FOS showed a percentage of EGG slow wave propagation and a gastric half emptying time similar to those of breast-fed infants.

Modi et al. [57] performed a large study in preterm infants (79 control and 71 useful for the primary outcome) using formula plus prebiotics to augment insufficient maternal milk volume; the prebiotic milk was dosed at 0.8/100 mL. The primary outcome was set in a total milk intake of 150 mL/kg/d; no significant differences were found in this outcome from trial entry to 28 days, while some benefits were recorded for secondary outcomes.

Armanian et al. [58] evaluated if prebiotics have benefits for the management of hyperbilirubinemia in preterm neonates. The prebiotic group displayed lower bilirubin level and peak bilirubin level than the placebo group over the study period. In addition, stool frequency was significantly increased in the prebiotic group.

3.4. Studies assessing the effects of combinations of prebiotic ingredients: effects of prebiotic mixtures based on polydextrose (PDX)

Considerable research has been devoted to investigate the potential effects of polydextrose (PDX) in association with galacto-oligosaccharides (Table S7).

The first paper reporting data on the use of polydextrose as a prebiotic for infant was published in 2007 by Ziegler and coworkers [59]. The study evaluated 2 different combinations of prebiotic ingredients, PDX, GOS and lactulose (LOS), at 2 different intake levels on growth and tolerance in healthy term infants up to 120 days of age. Dosage was 0.4 g/100 mL (PG4) of a prebiotic blend containing PDX and GOS in a 50:50 ratio (74 subjects) or 0.8 g/100 mL (PG8) of a prebiotic blend containing PDX, GOS, and LOS, 50:33:17 ratio (76 subjects); a control group (76 subjects) fed with the same formula without any prebiotic supplementation was also enrolled. The authors reported that : “There were no statistically significant differences among the 3 formula

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353 groups for weight growth rate or length growth rate at any time point. Significant differences in
354 stool consistency were detected among the 3 formula groups at 30, 60, and 90 days of age with the
355 supplemented formula groups having looser stools than the control group. A statistical difference
356 was detected among the formula groups in 3 categories of adverse events: diarrhea (control vs
357 PG4,), eczema (PG4 vs control; PG4 vs PGL8), and irritability (control vs PGL8)".

358 Nakamura et al. [60] using the same trial design with the addition of a breast-fed reference
359 group and focusing on the bacterial composition of gut microbiota, reported the lack of adverse
360 effects in treated infants, and a stool consistency significantly softer or looser in the breast fed
361 group than in all of the groups that received formula. As regards the bacterial communities they
362 noticed that individual profiles tended to cluster by subject rather than by group.

363 The following five papers all used the PDX/GOS dose of 0.4 g/100 mL in a 1:1 ratio of
364 PDX/GOS.

365 Scalabrin et al.[61], in a study in which 230 infants completed the protocol, showed that
366 consuming PDX/GOS resulted in softer stools than control at all times . Using qPCR, bacterial
367 counts in PDX/GOS were closer to those of the breast-fed group.

368 Ashley et al.[62] published a paper in which the PDX/GOS mixture was compared to the
369 same amount (0.4 g/100 mL) of GOS only. The noticed softer stools for infants in the PDX/GOS
370 and GOS groups versus control till 90 days but this positive remark remained 120 days for the
371 PDX/GOS group only.

372 Hicks et al. [63] investigated the effect of PDX/GOS supplementation on calcium absorption
373 in infants fed a formula containing or not prebiotics and in comparison with a group of human
374 milk-fed (HM) infants. The authors did not observe any significant effect of prebiotics on calcium
375 absorption or other markers of bone mineral metabolism.

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Pärtty et al. [64] used the PDX/GOS supplementation in preterm infants in comparison with the supplementation of a probiotic *Lactobacillus*. Both supplementation lasted for the first 2 months of life while infants were followed up for 1 year. The major result reported was that infants classified as excessive criers were significantly less in the prebiotic and the probiotic groups (no significant differences between the two treatments) than in the placebo group.

Finally, Luoto et al. [65] also using a cohort of preterm infants and the same prebiotic/probiotic comparison, hypothesized that early prebiotic or probiotic supplementation could reduce the risk of virus-associated respiratory tract infections (RTIs) during the first year of life. A significantly lower incidence of RTIs, including those due to rhinovirus, was detected in infants receiving prebiotics compared with those receiving placebo.

3.5. Studies assessing the effects of combinations of prebiotic ingredients: effects of GOS/FOS in association with pectin hydrolysate-derived acidic oligosaccharides (pAOS)

The addition to formula of with 0.2 g/100 mL acidic oligosaccharides (pAOS), derived from citrus pectin by enzymatic hydrolysis, was studied for the first time by Fanaro et al. [66] (Table S8). Three groups were enrolled: a placebo group (maltodextrins, 16 subiects), a pAOS group (16 subjects) and a reference group (15 subjects) with pAOS supplementation together with 0.6 g/100mL GOS/FOS 9:1. The only positive outcome detected in the pAOS treated infants was a stool consistency significantly softer in comparison with the group fed the standard formula. In infants fed the three oligosaccharides, bifidobacteria and lactobacilli counts were increased, the stool frequency was significantly higher while stool consistency scores were the lowest of the 3 groups.

Also Magne et al. [67] used the 0.6 g/100 mL dose of GOS/FOS; these authors checked the impact of the 3 oligo-containing prebiotics on microbiota composition and also in this case, while the presence of GOS/FOS positively altered gut microbiota composition, the further addition of pAOS does not seem to have any effects.

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A further evaluation of this 3 faceted prebiotic supplementation has been published in 2001 by Piemontese et al. [68]; in this work, also aimed to established safety and gut impact of this new prebiotic formulation, the dose of 0.8 g /100 mL of GOS/FOS was used. Growth, tolerance and adverse events were assessed at 8, 16, 24 and 52 weeks of age in three groups of infants. The prebiotic and control groups showed similar anthropometric parameters at each study point. The stool consistency in the prebiotic group was softer than in the control group at 8, 16 and 24 weeks and closer to that of the breast-feeding group. There was no difference in the incidence of adverse events between the two formula groups.

After these preliminary papers a full range of studies (6 out of the total 15 selected for our analysis) has been published on the potential use of this 3 components prebiotic formula in preterm infants.

Focus on the potential role of this mixture in preterm infants was put by Westerbeek and coworkers in 5 following papers [69–73] .

As regards infectious morbidity [69] the authors reported that enteral supplementation of GOS/FOS/pAOS does not significantly reduce the risk of serious infectious morbidity in preterm infants even if they noticed a tendency toward a lower incidence of infections, especially for those caused by endogenous bacteria. The two papers of 2011 were centered on the effects on intestinal inflammation, as measured by fecal IL-8 and calprotectin [70] as well as on intestinal permeability of preterm infants [71]. Conclusions of both paper were not positive: the use of this mixture of prebiotics does not enhance the reduction of intestinal permeability in the first week of life [71] and the levels of fecal IL-8 and calprotectin are not positively affected.

A more recent paper on the use of this prebiotic mixture in preterm infants has been published by the same group [72]; this paper was aimed to investigate the fecal microbiota and intestinal microenvironment in preterm infants. In this case authors noticed an increase in the postnatal intestinal colonization, even if the extensive use of broad-spectrum antibiotics in preterm infants

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has a negative impact on of all component of the intestinal microbiota, then hampering and delaying the normal microbiota development.

In the same year the same group [73] tested 113 preterm infants to establish a potential link between the use of these prebiotics and the vaccine antibody response. The selected vaccination was a diphtheria, tetanus, acellullar pertussis, polio and *Haemophilus influenzae* type b combination vaccine. The outcomes were negative as geometric mean titers were not different after prebiotic supplementation at 5 months of life and therefore authors' conclusions were that enteral supplementation of GOS/FOS/pAOS does not improve the immunization response in preterm infants.

These outcomes confirmed those reported two years before by Stam et al.[74] with a similar vaccine as they conclude that " no effect of prebiotics supplementation on vaccination specific antibody levels was found in children up to the age of 12 months; the vaccine specific antibody levels in infants fed the study prebiotics or a control diet were similar during the first year of life".

The mixture GOS/FOS/pAOS was, on the contrary, positively tested in allergic infants; Gruber et al. [75] assessed the impact of formula with this supplementation on the occurrence of atopic dermatitis in the first year of life. In this case healthy term infants with low atopy risk were recruited before the age of 8 weeks. A total of 414 infants were randomized to the prebiotic group, 416 infants to the control group (no prebiotics) and 300 infants were the reference breast-fed group. At the first birthday, atopic dermatitis occurred in significantly fewer infants from the prebiotic group than from the control group and the cumulative incidence of atopic dermatitis in the prebiotic group was in the low range of the breast-feeding group. Authors conclude that the number needed to prevent 1 case of atopic dermatitis by supplementation of prebiotics was 25 infants.

Opposite results were obtained by Boyle et al. [76] as regards eczema incidence. Infants with a family history of allergic disease were randomized to be fed with an active (n = 432) or control (n = 431) formula until 6 months of age and the primary outcome was cumulative incidence of eczema by 12 months. Eczema occurred by 12 months at 28.7% in both groups. Prebiotic formula did not

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change most immune markers but reduced cow's milk-specific IgG1 and increased regulatory T-cell and plasmacytoid dendritic cell percentages.

Also a third paper published by Niele et al. [77] reported no effects when the 3 prebiotic mixture is administered to potentially allergic infants. A short-term supplementation of GOS/FOS/pAOS was administered during the first postnatal weeks. Incidence of allergic and infectious diseases was assessed by validated questionnaires. The incidence of atopic dermatitis, bronchial hyper-reactivity and infections of the upper and lower respiratory tract as well as gastrointestinal diseases was not different between the groups.

Infections occurrence in prebiotic treated groups was investigated by van Stuijvenberg et al. [78] The objective of the study was to assess the number of fever episodes in the first year of life. No effect of adding specific prebiotics to standard formula feeding was found in reducing the number of fever episodes..

4. Discussion

In 2012 the European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) underlined that the available scientific data suggest that formula supplemented with prebiotics and administered to healthy infants do not raise safety concerns with regard to growth and adverse effects.

The same evaluation has been recently presented by Skorka et al [11] as regards growth and tolerance parameters, so confirming the same statements of all most recent reviews [79–85]. Then the following considerations will be focused only on the efficacy of prebiotics as functionally active ingredients of infant formula.

Effects on stool: consistency.

Softer stool consistency is reported in substantially all papers that have checked this outcome, even in papers in which components more likely to have a bulking, fiber-like effects are absent [13,14,18,20,62]. Quite significantly, for the evaluation of a dose-related effect, FOS seems to have

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effect on consistency when dosed at 0.3 g/100 mL and not at a dose of 0.15 g/100 mL [18] while GOS, even if dosed at 0.4 g 100/mL seem to reduce consistency of stools in two trials [13,14] but not in another [12].

However, a more significant difference about the incidence of hard stool has been reported when more than one prebiotic is used and at a higher dose: GOS/FOS are more active when dosed at 0.8 g/100 mL when compared to 0.4 g/mL [39,40,44].

The addition of pAOS to GOS/FOS does not seem to have an additional impact on stool consistency when compared to results for GOS/FOS [66]; the addition to PDX to GOS resulted in stool consistency very close to those reported for GOS/FOS [59,62].

Effects on stool: frequency.

Reports on a positive impact on frequency are less frequent than the previous ones regarding consistency. Higher frequency were reported for FOS [18,20] GOS [14] GOS/FOS/pAOS [66] and in preterm [55,56].

Gut –related effects: microbiota composition.

Substantially all papers reported some positive effects on the counts of bifidobacteria and lactobacilli, regardless of the kind of prebiotic, the daily dose and the length of treatment. The few pares reporting no increase in bifidobateria used a low dosage [43] or pAOS alone [66]. However, this general observation is in contrast with the reported outcomes “outside the gut” : as it will be showed below, outcomes related to immune system, allergy, infections are definitely more dose related. This could suggest that prebiotics have an impact on gut microbiota components different from those generally analyzed in all the published papers and that these still undetermined components of the intestinal bacterial ecosystems are the real responsible for the positive effects of prebiotics.

Gut –related effects: intestinal discomfort.

Colic is one of the most common intestinal discomfort for infants; a potential role of the use of prebiotic substances in reducing colics has been reported by authors using GOS only at a low

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dosage [12] but also with GOS/FOS used in a fermented formula [54]. Reduction of excessive crying was also reported by using PDX/GOS [64] or GOS/FOS at 0.8 g/100 mL supplemented to a fermented formula [54] but also with the simple addition of 0.4 g /100 mL GOS [12]. Incidence of diarrhea was reduced also when the 0.4 g/100 mL dose was used [35].

Effects on the immune system and infections.

The effects on the immune systems seem those in which a marked effect of the dose could be observed.

Effects on the immune system and infections: allergy.

Improvements of the SCORAD evaluations have been reported for kestose [86] administered at 2 g/day but, quite interestingly for the scope of this paper, authors were unable to establish a link with the bifidobacteria counts. Quite interestingly, no changes in the incidence of allergy was noticed when 0.4 g/100 mL were used [14] as well as 0.6 g/100 mL [87]. Trials with GOS/FOS reported positive outcomes for atopy when the dose of 0.8 mg/ 100 mL was used [29] as well as for higher secretory immunoglobulin A levels [53], and serum immunoglobulin levels [30]. The addition of pAOS to GOS/FOS was reported to have no [74,77] or marginal effects [76].

Effects on the immune system and infections: infections.

The dose of 0.8 mg/100 mL has been shown to be active in reduction of all types of infections [33]. No effect on infections incidence for pAOS/GOS/FOS [69,77,88]. No effect on infections incidence for GOS/FOS dosed lower that 0.8 g/100 mL [14].

Effects on preterm infants.

A number of papers reported the outcomes of prebiotic supplementation in preterm infants [20,50–52,56–58,69–73,77,88]. No problems of tolerance and safety were reported, regardless of the prebiotic mixture and dose used, even when a very high dose of GOS/FOS [56], higher than those used in full term infants (0.4 or 0.8 g 100/mL) was aimed to evaluate tolerability and reduce stool viscosity and accelerate gastrointestinal transport, in order to improve feeding tolerance. Both

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these parameters were positively affected by the prebiotic supplementation in the 10 treated infants compared to the same number of control subjects.

As regards efficacy, there are conflicting reports about the use of prebiotic ingredients in this specific population group. Absence of results were reported by Modi et al. [57] when GOS/FOS at the usual dose of 0.8 g/100 mL was fed to a large (79 subjects) cohort of preterm infants, with the aim to obtain a total milk intake of 150 mL/kg; on the contrary, the same prebiotic preparation [58] provided an increase of stool frequency, an improvement of feeding tolerance and reduction of bilirubin level; Indrio et al. [51,52] reported positive outcomes for gastric motility, results confirmed by Dasopoulou et al. [50]. Absence of positive outcomes was also reported for the use of the pAOS/GOS/FOS mixture formulation in a series of papers by Westerbeek and coworkers [69–71,88], by van den Berg et al. [73] and Niele et al. [77], with the exception of positive outcomes recorded for stool gut microbiota characteristics [66,68,89]..

5. Conclusions

In this paper we have tried to critically evaluate the outcomes, as reported by authors, of the use of prebiotic ingredients in feeding infants aged 0-12 months.

The overall appraisal on a potential dose effect on stool consistency and frequency is that a 0.3 g/100 mL for fructans and 0.8 g/100 mL for GOS/FOS seem to be the amount of choice in providing significant improvements of these two parameters, but lower doses and different composition of the prebiotic mixture have been shown to have positive effects. It could be possible, in a way similar to that describe for probiotics [10] to define these effects as “widespread”.

More “rare” or “specific” are the effects on the immune system, allergy and the incidence of infections. The core information for this statement derives from the worth of papers dealing with the use of GOS/FOS at 0.8 g/100 mL, while the use of additionally components such as pAOS or PDX does not seem to have any significant effect.

Prebiotics in preterm infants seem to have little or no impact; however this could be due to a lack of a significant number of studies in large cohorts.

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From the microbial ecology point of view what could be concluded by the vast amount of data collected is that, while it is likely that any addition of prebiotic could induce some changes in the composition of the gut microbiota, a clinical significance could be achieved only with some specific prebiotic formulation and doses, and this specificity could be worthwhile to be addressed in the definition of the word "prebiotic".

Moreover, studies using the most updated techniques of Next Generation Sequencing, and the analysis of the microbiome composition and not of the microbiota only, are necessary to really understand the role of these ingredients in infant feeding.

The most recent studies [90–94] clearly supported the concept that gut microbiota of infants is more complex than a *Bifidobacterium* /*Lactobacillus* ecosystem. Evaluation of the impact of prebiotics on the strictly anaerobic population, such as Ruminococci, Lachnospiraceae, *Blautia*, could be the relevant target for new research and clinical trials.

Supplementary Materials

Table S1: List of all the references considered for the review.

Table S2: Human trials assessing the effects of a single prebiotic ingredient.

Table S3: The link between GOS/FOS administration and infant immune system.

Table S4: Effects of GOS/FOS with atypical dosages.

Table S5: Intestinal effects of GOS/FOS in term infants.

Table S6: Intestinal effects of GOS/FOS in preterm infants.

Table S6: Effects of polydextrose in association with other prebiotic ingredients.

Table S8: Trials assessing the effects of pectin hydrolysate-derived acidic oligosaccharides (pAOS) combined with GOS/FOS.

Author Contributions

M.L. developed the concept for the manuscript and wrote the first draft. V.P. and A.P. provided critical feedback and revised the text. All the authors approved the final version of the manuscript.

Conflicts of Interest

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581 The authors declare no conflict of interest.

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586 **References**

587 1. Gibson, G. R.; Roberfroid, M. B. Dietary modulation of the human colonic microbiota:
588 introducing the concept of prebiotics. *J. Nutr.* **1995**, *125*, 1401–12,
589 doi:10.1093/jn/125.6.1401.

590 2. Roberfroid, M. Prebiotics: the concept revisited. *J. Nutr.* **2007**, *137*, 830S–7S,
591 doi:10.1093/jn/137.3.830S.

592 3. Reid, G.; Sanders, M. E.; Gaskins, H. R.; Gibson, G. R.; Mercenier, A.; Rastall, R.;
593 Roberfroid, M.; Rowland, I.; Cherbut, C.; Klaenhammer, T. R. New scientific paradigms for
594 probiotics and prebiotics. *J. Clin. Gastroenterol.* **2003**, *37*, 105–18.

595 4. Gibson, G. R.; Probert, H. M.; Loo, J. Van; Rastall, R. A.; Roberfroid, M. B. Dietary
596 modulation of the human colonic microbiota: updating the concept of prebiotics. *Nutr. Res.*
597 *Rev.* **2004**, *17*, 259–75, doi:10.1079/NRR200479.

598 5. Pineiro, M.; Asp, N.-G.; Reid, G.; Macfarlane, S.; Morelli, L.; Brunser, O.; Tuohy, K. FAO
599 Technical Meeting on Prebiotics. *J. Clin. Gastroenterol.* **2008**, *42*, S156–S159,
600 doi:10.1097/MCG.0b013e31817f184e.

601 6. Gibson, G. R.; Scott, K. P.; Rastall, R. A.; Tuohy, K. M.; Hotchkiss, A.; Dubert-Ferrandon,
602 A.; Gareau, M.; Murphy, E. F.; Saulnier, D.; Loh, G.; Macfarlane, S.; Delzenne, N.; Ringel,
603 Y.; Kozianowski, G.; Dickmann, R.; Lenoir-Wijnkoop, I.; Walker, C.; Buddington, R.
604 Dietary prebiotics: current status and new definition. *Food Sci. Technol. Bull. Funct. Foods*

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 30 September 2018 doi:10.20944/preprints201809.0597.v1

2010, *7*, 1–19, doi:10.1616/1476-2137.15880.

7. Bindels, L. B.; Delzenne, N. M.; Cani, P. D.; Walter, J. Towards a more comprehensive concept for prebiotics. *Nat. Rev. Gastroenterol. Hepatol.* **2015**, *12*, 303–10, doi:10.1038/nrgastro.2015.47.

8. Gibson, G. R.; Hutkins, R.; Sanders, M. E.; Prescott, S. L.; Reimer, R. A.; Salminen, S. J.; Scott, K.; Stanton, C.; Swanson, K. S.; Cani, P. D.; Verbeke, K.; Reid, G. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nat. Rev. Gastroenterol. Hepatol.* **2017**, *14*, 491–502, doi:10.1038/nrgastro.2017.75.

9. Fuller, R. Probiotics in man and animals. *J. Appl. Bacteriol.* **1989**, *66*, 365–78.

10. Hill, C.; Guarner, F.; Reid, G.; Gibson, G. R.; Merenstein, D. J.; Pot, B.; Morelli, L.; Canani, R. B.; Flint, H. J.; Salminen, S.; Calder, P. C.; Sanders, M. E. Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat. Rev. Gastroenterol. Hepatol.* **2014**, *11*, 506–14, doi:10.1038/nrgastro.2014.66.

11. Skórka, A.; Pieścik-Lech, M.; Kołodziej, M.; Szajewska, H. Infant formulae supplemented with prebiotics: Are they better than unsupplemented formulae? An updated systematic review. *Br. J. Nutr.* **2018**, *119*, 810–825, doi:10.1017/S0007114518000120.

12. Giovannini, M.; Verduci, E.; Gregori, D.; Ballali, S.; Soldi, S.; Ghisleni, D.; Riva, E.; PLAGOS Trial Study Group Prebiotic effect of an infant formula supplemented with galacto-oligosaccharides: randomized multicenter trial. *J. Am. Coll. Nutr.* **2014**, *33*, 385–93, doi:10.1080/07315724.2013.878232.

13. Williams, T.; Choe, Y.; Price, P.; Katz, G.; Suarez, F.; Paule, C.; Mackey, A. Tolerance of

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 30 September 2018 doi:10.20944/preprints201809.0597.v1

formulas containing prebiotics in healthy, term infants. *J. Pediatr. Gastroenterol. Nutr.* **2014**, *59*, 653–8, doi:10.1097/MPG.0000000000000513.

14. Sierra, C.; Bernal, M.-J.; Blasco, J.; Martínez, R.; Dalmau, J.; Ortuño, I.; Espín, B.; Vasallo, M.-I.; Gil, D.; Vidal, M.-L.; Infante, D.; Leis, R.; Maldonado, J.; Moreno, J.-M.; Román, E. Prebiotic effect during the first year of life in healthy infants fed formula containing GOS as the only prebiotic: a multicentre, randomised, double-blind and placebo-controlled trial. *Eur. J. Nutr.* **2015**, *54*, 89–99, doi:10.1007/s00394-014-0689-9.

15. Boženský, J.; Hill, M.; Zelenka, R.; Skýba, T. Prebiotics do not influence the severity of atopic dermatitis in infants: A randomised controlled trial. *PLoS One* **2015**, *10*, 1–8, doi:10.1371/journal.pone.0142897.

16. Paganini, D.; Uyoga, M. A.; Kortman, G. A. M.; Cercamondi, C. I.; Moretti, D.; Barth-Jaeggi, T.; Schwab, C.; Boekhorst, J.; Timmerman, H. M.; Lacroix, C.; Karanja, S.; Zimmermann, M. B. Prebiotic galacto-oligosaccharides mitigate the adverse effects of iron fortification on the gut microbiome: a randomised controlled study in Kenyan infants. *Gut* **2017**, *66*, 1956–1967, doi:10.1136/gutjnl-2017-314418.

17. Underwood, M. A.; Kalanetra, K. M.; Bokulich, N. A.; Mirmiran, M.; Barile, D.; Tancredi, D. J.; German, J. B.; Lebrilla, C. B.; Mills, D. A. Prebiotic oligosaccharides in premature infants. *J. Pediatr. Gastroenterol. Nutr.* **2014**, *58*, 352–60, doi:10.1097/MPG.0000000000000211.

18. Euler, A. R.; Mitchell, D. K.; Kline, R.; Pickering, L. K. Prebiotic effect of fructo-oligosaccharide supplemented term infant formula at two concentrations compared with unsupplemented formula and human milk. *J. Pediatr. Gastroenterol. Nutr.* **2005**, *40*, 157–64.

19. Brunser, O.; Figueroa, G.; Gotteland, M.; Haschke-Becher, E.; Magliola, C.; Rochat, F.; Cruchet, S.; Palframan, R.; Gibson, G.; Chauffard, F.; Haschke, F. Effects of probiotic or

652 prebiotic supplemented milk formulas on fecal microbiota composition of infants. *Asia Pac.*
653 *J. Clin. Nutr.* **2006**, *15*, 368–376.

654 20. Kapiki, A.; Costalos, C.; Oikonomidou, C.; Triantafyllidou, A.; Loukatou, E.; Pertrohilou, V.
655 The effect of a fructo-oligosaccharide supplemented formula on gut flora of preterm infants.
656 *Early Hum. Dev.* **2007**, *83*, 335–9, doi:10.1016/j.earlhumdev.2006.07.003.

657 21. Khoshoo, V.; Sun, S. S.; Storm, H. Tolerance of an enteral formula with insoluble and
658 prebiotic fiber in children with compromised gastrointestinal function. *J. Am. Diet. Assoc.*
659 **2010**, *110*, 1728–1733, doi:10.1016/j.jada.2010.08.011.

660 22. Xia, Q.; Williams, T.; Hustead, D.; Price, P.; Morrison, M.; Yu, Z. Quantitative analysis of
661 intestinal bacterial populations from term infants fed formula supplemented with fructo-
662 oligosaccharides. *J. Pediatr. Gastroenterol. Nutr.* **2012**, *55*, 314–320,
663 doi:10.1097/MPG.0b013e3182523254.

664 23. Shibata, R.; Kimura, M.; Takahashi, H.; Mikami, K.; Aiba, Y.; Takeda, H.; Koga, Y. Clinical
665 effects of kestose, a prebiotic oligosaccharide, on the treatment of atopic dermatitis in infants.
666 *Clin. Exp. Allergy* **2009**, *39*, 1397–1403, doi:10.1111/j.1365-2222.2009.03295.x.

667 24. López-Velázquez, G.; Díaz-García, L.; Anzo, A.; Parra-Ortiz, M.; Llamosas-Gallardo, B.;
668 Ortiz-Hernández, A. A.; Mancilla-Ramírez, J.; Cruz-Rubio, J. M.; Gutiérrez-Castrellón, P.
669 Safety of a dual potential prebiotic system from Mexican agave "Metlin® and Metlos®",
670 incorporated to an infant formula for term newborn babies: a randomized controlled trial.
671 *Rev. Invest. Clin. 65*, 483–90.

672 25. López-Velázquez, G.; Parra-Ortiz, M.; Mora, I. D. la M.-D. la; García-Torres, I.; Enríquez-
673 Flores, S.; Alcántara-Ortigoza, M. A.; Angel, A. G.-D.; Velázquez-Aragón, J.; Ortiz-
674 Hernández, R.; Cruz-Rubio, J. M.; Villa-Barragán, P.; Jiménez-Gutiérrez, C.; Gutiérrez-
675 Castrellón, P. Effects of Fructans from Mexican Agave in Newborns Fed with Infant

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 30 September 2018 doi:10.20944/preprints201809.0597.v1

676 Formula: A Randomized Controlled Trial. *Nutrients* **2015**, *7*, 8939–51,
677 doi:10.3390/nu7115442.

678 26. Kim, S. H.; Da, H. L.; Meyer, D. Supplementation of infant formula with native inulin has a
679 prebiotic effect in formula-fed babies. *Asia Pac. J. Clin. Nutr.* **2007**, *16*, 172–177.

680 27. Closa-Monasterolo, R.; Gispert-Llaurado, M.; Luque, V.; Ferre, N.; Rubio-Torrents, C.;
681 Zaragoza-Jordana, M.; Escribano, J. Safety and efficacy of inulin and oligofructose
682 supplementation in infant formula: Results from a randomized clinical trial. *Clin. Nutr.* **2013**,
683 *32*, 918–927, doi:10.1016/j.clnu.2013.02.009.

684 28. Nowacki, J.; Lee, H.-C.; Lien, R.; Cheng, S.-W.; Li, S.-T.; Yao, M.; Northington, R.; Jan, I.;
685 Mutungi, G. Stool fatty acid soaps, stool consistency and gastrointestinal tolerance in term
686 infants fed infant formulas containing high sn-2 palmitate with or without oligofructose: a
687 double-blind, randomized clinical trial. *Nutr. J.* **2014**, *13*, 105, doi:10.1186/1475-2891-13-
688 105.

689 29. Moro, G.; Arslanoglu, S.; Stahl, B.; Jelinek, J.; Wahn, U.; Boehm, G. A mixture of prebiotic
690 oligosaccharides reduces the incidence of atopic dermatitis during the first six months of age.
691 *Arch. Dis. Child.* **2006**, *91*, 814–9, doi:10.1136/adc.2006.098251.

692 30. van Hoffen, E.; Ruiter, B.; Faber, J.; M'Rabet, L.; Knol, E. F.; Stahl, B.; Arslanoglu, S.;
693 Moro, G.; Boehm, G.; Garssen, J. A specific mixture of short-chain galacto-oligosaccharides
694 and long-chain fructo-oligosaccharides induces a beneficial immunoglobulin profile in
695 infants at high risk for allergy. *Allergy* **2009**, *64*, 484–7, doi:10.1111/j.1398-
696 9995.2008.01765.x.

697 31. Schouten, B.; Van Esch, B. C. A. M.; Kormelink, T. G.; Moro, G. E.; Arslanoglu, S.; Boehm,
698 G.; Knippels, L. M. J.; Redegeld, F. A.; Willemsen, L. E. M.; Garssen, J. Non-digestible
699 oligosaccharides reduce immunoglobulin free light-chain concentrations in infants at risk for

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 30 September 2018 doi:10.20944/preprints201809.0597.v1

allergy. *Pediatr. Allergy Immunol.* **2011**, *22*, 537–42, doi:10.1111/j.1399-3038.2010.01132.x.

32. MacDonald, A.; Cochrane, B.; Wopereis, H.; Loveridge, N. Specific prebiotics in a formula for infants with Phenylketonuria. *Mol. Genet. Metab.* **2011**, *104 Suppl*, S55-9, doi:10.1016/j.ymgme.2011.09.015.

33. Arslanoglu, S.; Moro, G. E.; Boehm, G. Early supplementation of prebiotic oligosaccharides protects formula-fed infants against infections during the first 6 months of life. *J. Nutr.* **2007**, *137*, 2420–4, doi:10.1093/jn/137.11.2420.

34. Bisceglia, M.; Indrio, F.; Riezzo, G.; Poerio, V.; Corapi, U.; Raimondi, F. The effect of prebiotics in the management of neonatal hyperbilirubinaemia. *Acta Paediatr.* **2009**, *98*, 1579–81, doi:10.1111/j.1651-2227.2009.01387.x.

35. Bruzzese, E.; Volpicelli, M.; Squeglia, V.; Bruzzese, D.; Salvini, F.; Bisceglia, M.; Lionetti, P.; Cinquetti, M.; Iacono, G.; Amarri, S.; Guarino, A. A formula containing galacto- and fructo-oligosaccharides prevents intestinal and extra-intestinal infections: an observational study. *Clin. Nutr.* **2009**, *28*, 156–61, doi:10.1016/j.clnu.2009.01.008.

36. Brunser, O.; Gotteland, M.; Cruchet, S.; Figueroa, G.; Garrido, D.; Steenhout, P. Effect of a milk formula with prebiotics on the intestinal microbiota of infants after an antibiotic treatment. *Pediatr. Res.* **2006**, *59*, 451–6, doi:10.1203/01.pdr.0000198773.40937.61.

37. Alliet, P.; Scholtens, P.; Raes, M.; Hensen, K.; Jongen, H.; Rummens, J.-L.; Boehm, G.; Vandenplas, Y. Effect of prebiotic galacto-oligosaccharide, long-chain fructo-oligosaccharide infant formula on serum cholesterol and triacylglycerol levels. *Nutrition* **2007**, *23*, 719–23, doi:10.1016/j.nut.2007.06.011.

38. Moro, G.; Minoli, I.; Mosca, M.; Fanaro, S.; Jelinek, J.; Stahl, B.; Boehm, G. Dosage-related bifidogenic effects of galacto- and fructooligosaccharides in formula-fed term infants. *J.*

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 30 September 2018 doi:10.20944/preprints201809.0597.v1

Pediatr. Gastroenterol. Nutr. **2002**, *34*, 291–5.

39. Moro, G. E.; Mosca, F.; Miniello, V.; Fanaro, S.; Jelinek, J.; Stahl, B.; Boehm, G. Effects of a new mixture of prebiotics on faecal flora and stools in term infants. *Acta Paediatr. Suppl.* **2003**, *91*, 77–9.

40. Knol, J.; Scholtens, P.; Kafka, C.; Steenbakkers, J.; Gro, S.; Helm, K.; Klarczyk, M.; Schöpfer, H.; Böckler, H.-M.; Wells, J. Colon microflora in infants fed formula with galacto- and fructo-oligosaccharides: more like breast-fed infants. *J. Pediatr. Gastroenterol. Nutr.* **2005**, *40*, 36–42.

41. Haarman, M.; Knol, J. Quantitative real-time PCR assays to identify and quantify fecal Bifidobacterium species in infants receiving a prebiotic infant formula. *Appl. Environ. Microbiol.* **2005**, *71*, 2318–24, doi:10.1128/AEM.71.5.2318-2324.2005.

42. Haarman, M.; Knol, J. Quantitative real-time PCR analysis of fecal Lactobacillus species in infants receiving a prebiotic infant formula. *Appl. Environ. Microbiol.* **2006**, *72*, 2359–65, doi:10.1128/AEM.72.4.2359-2365.2006.

43. Bakker-Zierikzee, A. M.; Alles, M. S.; Knol, J.; Kok, F. J.; Tolboom, J. J. M.; Bindels, J. G. Effects of infant formula containing a mixture of galacto- and fructo-oligosaccharides or viable Bifidobacterium animalis on the intestinal microflora during the first 4 months of life. *Br. J. Nutr.* **2005**, *94*, 783–90.

44. Costalos, C.; Kapiki, A.; Apostolou, M.; Papathoma, E. The effect of a prebiotic supplemented formula on growth and stool microbiology of term infants. *Early Hum. Dev.* **2008**, *84*, 45–9, doi:10.1016/j.earlhumdev.2007.03.001.

45. Holscher, H. D.; Faust, K. L.; Czerkies, L. A.; Litov, R.; Ziegler, E. E.; Lessin, H.; Hatch, T.; Sun, S.; Tappenden, K. A. Effects of prebiotic-containing infant formula on gastrointestinal

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 30 September 2018 doi:10.20944/preprints201809.0597.v1

tolerance and fecal microbiota in a randomized controlled trial. *JPEN. J. Parenter. Enteral Nutr.* **2012**, *36*, 95S–105S, doi:10.1177/0148607111430087.

46. Vivatvakin, B.; Mahayosnond, A.; Theamboonlers, A.; Steenhout, P. G.; Conus, N. J. Effect of a whey-predominant starter formula containing LCPUFAs and oligosaccharides (FOS/GOS) on gastrointestinal comfort in infants. *Asia Pac. J. Clin. Nutr.* **2010**, *19*, 473–80.

47. Veereman-Wauters, G.; Staelens, S.; Van de Broek, H.; Plaskie, K.; Wesling, F.; Roger, L. C.; McCartney, A. L.; Assam, P. Physiological and bifidogenic effects of prebiotic supplements in infant formulae. *J. Pediatr. Gastroenterol. Nutr.* **2011**, *52*, 763–71, doi:10.1097/MPG.0b013e3182139f39.

48. Salvini, F.; Riva, E.; Salvatici, E.; Boehm, G.; Jelinek, J.; Banderali, G.; Giovannini, M. A specific prebiotic mixture added to starting infant formula has long-lasting bifidogenic effects. *J. Nutr.* **2011**, *141*, 1335–9, doi:10.3945/jn.110.136747.

49. Szajewska, H.; Ruszczyński, M.; Szymański, H.; Sadowska-Krawczenko, I.; Piwowarczyk, A.; Rasmussen, P. B.; Kristensen, M. B.; West, C. E.; Hernell, O. Effects of infant formula supplemented with prebiotics compared with synbiotics on growth up to the age of 12 mo: a randomized controlled trial. *Pediatr. Res.* **2017**, *81*, 752–758, doi:10.1038/pr.2017.5.

50. Dasopoulou, M.; Briana, D. D.; Boutsikou, T.; Karakasidou, E.; Roma, E.; Costalos, C.; Malamitsi-Puchner, A. Motilin and gastrin secretion and lipid profile in preterm neonates following prebiotics supplementation: a double-blind randomized controlled study. *JPEN. J. Parenter. Enteral Nutr.* **2015**, *39*, 359–68, doi:10.1177/0148607113510182.

51. Indrio, F.; Riezzo, G.; Raimondi, F.; Bisceglia, M.; Cavallo, L.; Francavilla, R. Effects of probiotic and prebiotic on gastrointestinal motility in newborns. *J. Physiol. Pharmacol.* **2009**, *60*, 27–31, doi:10.1016/j.iccn.2010.07.001.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 30 September 2018 doi:10.20944/preprints201809.0597.v1

769 52. Indrio, F.; Riezzo, G.; Raimondi, F.; Francavilla, R.; Montagna, O.; Valenzano, M. L.;
770 Cavallo, L.; Boehm, G. Prebiotics improve gastric motility and gastric electrical activity in
771 preterm newborns. *J. Pediatr. Gastroenterol. Nutr.* **2009**, *49*, 258–61,
772 doi:10.1097/MPG.0b013e3181926aec.

773 53. Huet, F.; Abrahamse-Berkeveld, M.; Tims, S.; Simeoni, U.; Beley, G.; Savagner, C.;
774 Vandenplas, Y.; Hourihane, J. O. Partly Fermented Infant Formulae With Specific
775 Oligosaccharides Support Adequate Infant Growth and Are Well-Tolerated. *J. Pediatr.*
776 *Gastroenterol. Nutr.* **2016**, *63*, e43-53, doi:10.1097/MPG.0000000000001360.

777 54. Vandenplas, Y.; Ludwig, T.; Bouritius, H.; Alliet, P.; Forde, D.; Peeters, S.; Huet, F.;
778 Hourihane, J. Randomised controlled trial demonstrates that fermented infant formula with
779 short-chain galacto-oligosaccharides and long-chain fructo-oligosaccharides reduces the
780 incidence of infantile colic. *Acta Paediatr.* **2017**, *106*, 1150–1158, doi:10.1111/apa.13844.

781 55. Boehm, G.; Lidestri, M.; Casetta, P.; Jelinek, J.; Negretti, F.; Stahl, B.; Marini, A.
782 Supplementation of a bovine milk formula with an oligosaccharide mixture increases counts
783 of faecal bifidobacteria in preterm infants. *Arch. Dis. Child. Fetal Neonatal Ed.* **2002**, *86*,
784 F178-81.

785 56. Mihatsch, W. A.; Hoegel, J.; Pohlandt, F. Prebiotic oligosaccharides reduce stool viscosity
786 and accelerate gastrointestinal transport in preterm infants. *Acta Paediatr.* **2006**, *95*, 843–8,
787 doi:10.1080/08035250500486652.

788 57. Modi, N.; Uthaya, S.; Fell, J.; Kulinskaya, E. A randomized, double-blind, controlled trial of
789 the effect of prebiotic oligosaccharides on enteral tolerance in preterm infants
790 (ISRCTN77444690). *Pediatr. Res.* **2010**, *68*, 440–5, doi:10.1203/PDR.0b013e3181f1cd59.

791 58. Armanian, A. M.; Barekatain, B.; Hoseinzadeh, M.; Salehimehr, N. Prebiotics for the
792 management of hyperbilirubinemia in preterm neonates. *J. Matern. Fetal. Neonatal Med.*

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 30 September 2018 doi:10.20944/preprints201809.0597.v1

2016, *29*, 3009–13, doi:10.3109/14767058.2015.1113520.

59. Ziegler, E.; Vanderhoof, J. A.; Petschow, B.; Mitmesser, S. H.; Stolz, S. I.; Harris, C. L.; Berseth, C. L. Term infants fed formula supplemented with selected blends of prebiotics grow normally and have soft stools similar to those reported for breast-fed infants. *J. Pediatr. Gastroenterol. Nutr.* **2007**, *44*, 359–64, doi:10.1097/MPG.0b013e31802fca8c.

60. Nakamura, N.; Gaskins, H. R.; Collier, C. T.; Nava, G. M.; Rai, D.; Petschow, B.; Russell, W. M.; Harris, C.; Mackie, R. I.; Wampler, J. L.; Walker, D. C. Molecular ecological analysis of fecal bacterial populations from term infants fed formula supplemented with selected blends of prebiotics. *Appl. Environ. Microbiol.* **2009**, *75*, 1121–8, doi:10.1128/AEM.02359-07.

61. Scalabrin, D. M. F.; Mitmesser, S. H.; Welling, G. W.; Harris, C. L.; Marunycz, J. D.; Walker, D. C.; Bos, N. A.; Tölkkö, S.; Salminen, S.; Vanderhoof, J. A. New prebiotic blend of polydextrose and galacto-oligosaccharides has a bifidogenic effect in young infants. *J. Pediatr. Gastroenterol. Nutr.* **2012**, *54*, 343–52, doi:10.1097/MPG.0b013e318237ed95.

62. Ashley, C.; Johnston, W. H.; Harris, C. L.; Stolz, S. I.; Wampler, J. L.; Berseth, C. L. Growth and tolerance of infants fed formula supplemented with polydextrose (PDX) and/or galactooligosaccharides (GOS): double-blind, randomized, controlled trial. *Nutr. J.* **2012**, *11*, 38, doi:10.1186/1475-2891-11-38.

63. Hicks, P. D.; Hawthorne, K. M.; Berseth, C. L.; Marunycz, J. D.; Heubi, J. E.; Abrams, S. A. Total calcium absorption is similar from infant formulas with and without prebiotics and exceeds that in human milk-fed infants. *BMC Pediatr.* **2012**, *12*, 118, doi:10.1186/1471-2431-12-118.

64. Pärtty, A.; Luoto, R.; Kalliomäki, M.; Salminen, S.; Isolauri, E. Effects of early prebiotic and probiotic supplementation on development of gut microbiota and fussing and crying in

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 30 September 2018 doi:10.20944/preprints201809.0597.v1

preterm infants: a randomized, double-blind, placebo-controlled trial. *J. Pediatr.* **2013**, *163*, 1272-7.e1–2, doi:10.1016/j.jpeds.2013.05.035.

65. Luoto, R.; Ruuskanen, O.; Waris, M.; Kalliomäki, M.; Salminen, S.; Isolauri, E. Prebiotic and probiotic supplementation prevents rhinovirus infections in preterm infants: a randomized, placebo-controlled trial. *J. Allergy Clin. Immunol.* **2014**, *133*, 405–13, doi:10.1016/j.jaci.2013.08.020.

66. Fanaro, S.; Jelinek, J.; Stahl, B.; Boehm, G.; Kock, R.; Vigi, V. Acidic Oligosaccharides from Pectin Hydrolysate as New Component for Infant Formulae: Effect on Intestinal Flora, Stool Characteristics, and pH. *J. Pediatr. Gastroenterol. Nutr.* **2005**, *41*, 186–190, doi:10.1097/01.mpg.0000172747.64103.d7.

67. Magne, F.; Hachelaf, W.; Suau, A.; Boudraa, G.; Bouziane-Nedjadi, K.; Rigottier-Gois, L.; Touhami, M.; Desjeux, J.-F.; Pochart, P. Effects on faecal microbiota of dietary and acidic oligosaccharides in children during partial formula feeding. *J. Pediatr. Gastroenterol. Nutr.* **2008**, *46*, 580–8, doi:10.1097/MPG.0b013e318164d920.

68. Piemontese, P.; Giannì, M. L.; Braegger, C. P.; Chirico, G.; Grüber, C.; Riedler, J.; Arslanoglu, S.; van Stuijvenberg, M.; Boehm, G.; Jelinek, J.; Roggero, P.; MIPS 1 Working Group Tolerance and safety evaluation in a large cohort of healthy infants fed an innovative prebiotic formula: a randomized controlled trial. *PLoS One* **2011**, *6*, e28010, doi:10.1371/journal.pone.0028010.

69. Westerbeek, E. A.; van den Berg, J. P.; Lafeber, H. N.; Fetter, W. P.; Boehm, G.; Twisk, J. W.; van Elburg, R. M. Neutral and acidic oligosaccharides in preterm infants: a randomized, double-blind, placebo-controlled trial. *Am. J. Clin. Nutr.* **2010**, *91*, 679–86, doi:10.3945/ajcn.2009.28625.

70. Westerbeek, E. A. M.; Mørch, E.; Lafeber, H. N.; Fetter, W. P. F.; Twisk, J. W. R.; Van

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 30 September 2018 doi:10.20944/preprints201809.0597.v1

Elburg, R. M. Effect of neutral and acidic oligosaccharides on fecal IL-8 and fecal calprotectin in preterm infants. *Pediatr. Res.* **2011**, *69*, 255–8, doi:10.1203/PDR.0b013e318206fd25.

71. Westerbeek, E. A. M.; van den Berg, A.; Lafeber, H. N.; Fetter, W. P. F.; van Elburg, R. M. The effect of enteral supplementation of a prebiotic mixture of non-human milk galacto-, fructo- and acidic oligosaccharides on intestinal permeability in preterm infants. *Br. J. Nutr.* **2011**, *105*, 268–74, doi:10.1017/S0007114510003405.

72. Westerbeek, E. A. M.; Slump, R. A.; Lafeber, H. N.; Knol, J.; Georgi, G.; Fetter, W. P. F.; van Elburg, R. M. The effect of enteral supplementation of specific neutral and acidic oligosaccharides on the faecal microbiota and intestinal microenvironment in preterm infants. *Eur. J. Clin. Microbiol. Infect. Dis.* **2013**, *32*, 269–76, doi:10.1007/s10096-012-1739-y.

73. van den Berg, J. P.; Westerbeek, E. A. M.; van der Klis, F. R. M.; Berbers, G. A. M.; Lafeber, H. N.; van Elburg, R. M. Neutral and acidic oligosaccharides supplementation does not increase the vaccine antibody response in preterm infants in a randomized clinical trial. *PLoS One* **2013**, *8*, e70904, doi:10.1371/journal.pone.0070904.

74. Stam, J.; van Stuijvenberg, M.; Garssen, J.; Knipping, K.; Sauer, P. J. J. A mixture of three prebiotics does not affect vaccine specific antibody responses in healthy term infants in the first year of life. *Vaccine* **2011**, *29*, 7766–72, doi:10.1016/j.vaccine.2011.07.110.

75. Grüber, C.; van Stuijvenberg, M.; Mosca, F.; Moro, G.; Chirico, G.; Braegger, C. P.; Riedler, J.; Boehm, G.; Wahn, U.; MIPS 1 Working Group Reduced occurrence of early atopic dermatitis because of immunoactive prebiotics among low-atopy-risk infants. *J. Allergy Clin. Immunol.* **2010**, *126*, 791–7, doi:10.1016/j.jaci.2010.07.022.

76. Boyle, R. J.; Tang, M. L.-K.; Chiang, W. C.; Chua, M. C.; Ismail, I.; Nauta, A.; Hourihane, J. O. B.; Smith, P.; Gold, M.; Ziegler, J.; Peake, J.; Quinn, P.; Rao, R.; Brown, N.; Rijnierse,

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 30 September 2018 doi:10.20944/preprints201809.0597.v1

A.; Garssen, J.; Warner, J. O.; PATCH study investigators Prebiotic-supplemented partially hydrolysed cow's milk formula for the prevention of eczema in high-risk infants: a randomized controlled trial. *Allergy* **2016**, *71*, 701–10, doi:10.1111/all.12848.

77. Niele, N.; van Zwol, A.; Westerbeek, E. A. M.; Lafeber, H. N.; van Elburg, R. M. Effect of non-human neutral and acidic oligosaccharides on allergic and infectious diseases in preterm infants. *Eur. J. Pediatr.* **2013**, *172*, 317–23, doi:10.1007/s00431-012-1886-2.

78. van Stuijvenberg, M.; Eisses, A. M.; Grüber, C.; Mosca, F.; Arslanoglu, S.; Chirico, G.; Braegger, C. P.; Riedler, J.; Boehm, G.; Sauer, P. J. J. Do prebiotics reduce the number of fever episodes in healthy children in their first year of life: a randomised controlled trial. *Br. J. Nutr.* **2011**, *106*, 1740–8, doi:10.1017/S0007114511004053.

79. Rao, S.; Srinivasjois, R.; Patole, S. Prebiotic supplementation in full-term neonates: a systematic review of randomized controlled trials. *Arch. Pediatr. Adolesc. Med.* **2009**, *163*, 755–64, doi:10.1001/archpediatrics.2009.94.

80. Mugambi, M. N.; Musekiwa, A.; Lombard, M.; Young, T.; Blaauw, R. Synbiotics, probiotics or prebiotics in infant formula for full term infants: a systematic review. *Nutr. J.* **2012**, *11*, 81, doi:10.1186/1475-2891-11-81.

81. Osborn, D. A.; Sinn, J. K. H. Prebiotics in infants for prevention of allergy. *Cochrane database Syst. Rev.* **2013**, CD006474, doi:10.1002/14651858.CD006474.pub3.

82. Vandenplas, Y.; De Greef, E.; Veereman, G. Prebiotics in infant formula. *Gut Microbes* **2014**, *5*, 681–7, doi:10.4161/19490976.2014.972237.

83. Cuello-Garcia, C.; Fiocchi, A.; Pawankar, R.; Yepes-Nuñez, J. J.; Morgano, G. P.; Zhang, Y.; Agarwal, A.; Gandhi, S.; Terracciano, L.; Schünemann, H. J.; Brozek, J. L. Prebiotics for the prevention of allergies: A systematic review and meta-analysis of randomized controlled

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 30 September 2018 doi:10.20944/preprints201809.0597.v1

trials. *Clin. Exp. Allergy* **2017**, *47*, 1468–1477, doi:10.1111/cea.13042.

84. La Fata, G.; Rastall, R. A.; Lacroix, C.; Harmsen, H. J. M.; Mohajeri, M. H.; Weber, P.; Steinert, R. E. Recent Development of Prebiotic Research-Statement from an Expert Workshop. *Nutrients* **2017**, *9*, doi:10.3390/nu9121376.

85. Lei, W.-T.; Shih, P.-C.; Liu, S.-J.; Lin, C.-Y.; Yeh, T.-L. Effect of Probiotics and Prebiotics on Immune Response to Influenza Vaccination in Adults: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Nutrients* **2017**, *9*, 1175, doi:10.3390/nu9111175.

86. Shibata, R.; Kimura, M.; Takahashi, H.; Mikami, K.; Aiba, Y.; Takeda, H.; Koga, Y. Clinical effects of kestose, a prebiotic oligosaccharide, on the treatment of atopic dermatitis in infants. *Clin. Exp. Allergy* **2009**, *39*, 1397–403, doi:10.1111/j.1365-2222.2009.03295.x.

87. Raes, M.; Scholtens, P. A. M. J.; Alliet, P.; Hensen, K.; Jongen, H.; Boehm, G.; Vandenplas, Y.; Rummens, J. L. Exploration of basal immune parameters in healthy infants receiving an infant milk formula supplemented with prebiotics. *Pediatr. Allergy Immunol.* **2010**, *21*, e377-85, doi:10.1111/j.1399-3038.2009.00957.x.

88. Westerbeek, E. A. M.; van Elburg, R. M.; van den Berg, A.; van den Berg, J.; Twisk, J. W. R.; Fetter, W. P. F.; Lafeber, H. N. Design of a randomised controlled trial on immune effects of acidic and neutral oligosaccharides in the nutrition of preterm infants: carrot study. *BMC Pediatr.* **2008**, *8*, 46, doi:10.1186/1471-2431-8-46.

89. Westerbeek, E. A. M.; Hensgens, R. L.; Mihatsch, W. A.; Boehm, G.; Lafeber, H. N.; van Elburg, R. M. The effect of neutral and acidic oligosaccharides on stool viscosity, stool frequency and stool pH in preterm infants. *Acta Paediatr.* **2011**, *100*, 1426–31, doi:10.1111/j.1651-2227.2011.02295.x.

90. Sagheddu, V.; Patrone, V.; Miragoli, F.; Puglisi, E.; Morelli, L. Infant Early Gut

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 30 September 2018 doi:10.20944/preprints201809.0597.v1

911 Colonization by Lachnospiraceae: High Frequency of Ruminococcus gnavus. *Front. Pediatr.*
912 **2016**, *4*, 57, doi:10.3389/fped.2016.00057.

913 91. Sagheddu, V.; Patrone, V.; Miragoli, F.; Morelli, L. Abundance and Diversity of
914 Hydrogenotrophic Microorganisms in the Infant Gut before the Weaning Period Assessed by
915 Denaturing Gradient Gel Electrophoresis and Quantitative PCR. *Front. Nutr.* **2017**, *4*, 29,
916 doi:10.3389/fnut.2017.00029.

917 92. Piacentini, G.; Peroni, D.; Bessi, E.; Morelli, L. Molecular characterization of intestinal
918 microbiota in infants fed with soymilk. *J. Pediatr. Gastroenterol. Nutr.* **2010**, *51*, 71–6,
919 doi:10.1097/MPG.0b013e3181dc8b02.

920 93. Timmerman, H. M.; Rutten, N. B. M. M.; Boekhorst, J.; Saulnier, D. M.; Kortman, G. A. M.;
921 Contractor, N.; Kullen, M.; Floris, E.; Harmsen, H. J. M.; Vlieger, A. M.; Kleerebezem, M.;
922 Rijkers, G. T. Intestinal colonisation patterns in breastfed and formula-fed infants during the
923 first 12 weeks of life reveal sequential microbiota signatures. *Sci. Rep.* **2017**, *7*, 8327,
924 doi:10.1038/s41598-017-08268-4.

925 94. Chang, J. Y.; Shin, S. M.; Chun, J.; Lee, J.-H.; Seo, J.-K. Pyrosequencing-based molecular
926 monitoring of the intestinal bacterial colonization in preterm infants. *J. Pediatr.*
927 *Gastroenterol. Nutr.* **2011**, *53*, 512–9, doi:10.1097/MPG.0b013e318227e518.

928