National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

FRONT OFFICE FOOD AND PRODUCT SAFETY

Literature overview on the safety of the use of probiotica in infant formula

Risk assessment requested by:	Office for Risk Assessment & Research (BuRO)
Risk assessment performed by:	RIVM
Date of request:	21-09-2021
Date of risk assessment:	17-12-2021 (1st draft version) 10-03-2022 (2nd draft version) 05-04-2022 (3rd draft version) 08-04-2022 (final version)
Project number:	V/093130

Nederlandse samenvatting

Aan zuigelingenvoeding worden in toenemende mate probiotica toegevoegd. In 2014 concludeerde de Europese voedselveiligheidsautoriteit (EFSA) dat er geen bewijs was dat zuigelingenvoeding met probiotica onveilig was. Echter, er was ook geen overtuigend bewijs van gunstige gezondheidseffecten van probiotica in zuigelingenvoeding. Ook de European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) kwam tot deze conclusie in 2011. Sinds 2014 zijn er nieuwe studies gepubliceerd die de veiligheid en gunstige effecten van probiotica in zuigelingenvoeding hebben onderzocht bij zowel gezonde zuigelingen als bij zuigelingen met een verhoogd risico op negatieve gezondheidseffecten.

Het Bureau Risicobeoordeling & Onderzoek (BuRO) heeft het Front Office Voedsel- en Productveiligheid (FO) gevraagd om de bevindingen van EFSA (2014) en ESPGHAN (2011) samen te vatten en om een systematisch literatuuronderzoek te doen naar studies die de veiligheid van de toevoeging van probiotica aan zuigelingenvoeding hebben onderzocht en zijn gepubliceerd sinds 2014. De wettelijke kaders over het gebruik van probiotica zijn buiten beschouwing gelaten.

In totaal zijn 17 review- en meta-data analyse artikelen geanalyseerd door het FO. Deze artikelen laten zien dat de aanwezigheid van probiotica in zuigelingenvoeding geen negatieve of positieve effecten lijkt te hebben op de gezondheid van zuigelingen. Echter, in verscheidene reviewartikelen wordt geconstateerd dat door (1) het bestaan van grote onzekerheden rond de onderzoekresultaten, (2) het niet systematisch rapporteren van negatieve bijwerkingen en (3) het gebrek aan onderzoek naar de langetermijneffecten, het niet mogelijk is om algemene conclusies te trekken over eventueel negatieve en positieve effecten van probiotica in zuigelingenvoeding.

Een uitzondering is de toevoeging van probiotica aan zuigelingenvoeding voor premature baby's die lijden aan acute gastro-enteritis en antibiotica-gerelateerde diarree. In 2020 heeft het ESPGHAN panel een update gepubliceerd (van de Akker et al., 2020) met conditionele aanbevelingen voor gebruik van *L. rhamnosus* GG ATCC 53103 (dosis: $1x10^9$ – 6×10^9 kolonie vormende eenheden (kve) en een combinatie van *B. infantis* Bb-02, *B.*

lactis bB-12 en *S. thermophilus* TH-4 (dosis: 3,0-3,5 x 10^8 kve per stam) in premature baby's om necrotiserende enterocolitis (NEC) te verminderen.

Subject
Infant formulae are increasingly supplemented with probiotics, despite uncertainties regarding their efficacy. In 2014, the European Food Safety Authority (EFSA) concluded that there was no evidence for a concern about the safety of infant formula supplemented with probiotics (EFSA, 2014). This conclusion was the same as that of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) based on a review paper about probiotics in infant formula in 2011 (Braegger et al., 2011). Since 2014, new studies have been published that have investigated the safety of probiotics in infant formula in both healthy and high-risk infants. In these studies, the beneficial effects of probiotics have also been examined.

Question
The Office for Risk Assessment & Research (BuRO) has asked the Front Office Food and Consumer Product Safety (FO) to summarise the conclusions of the 2014 EFSA opinion on the addition of probiotics to infant formula for (young) infants and of the 2011 literature review of ESPGHAN. Furthermore, FO was asked to conduct a systematic literature search into the safety of the addition of probiotics to infant formula for (young) infants examined in studies published since 2014. Apart from safety, also information on the health benefits of adding probiotics in infant formula was included in this assessment. The legislation on the use of probiotics was not part of this assessment.

Conclusion
The European Food Safety Authority (EFSA) and the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) looked into the safety and beneficial effects of probiotics added to infant formula in 2014 and 2011, respectively. Both organisations concluded that there was no evidence for a concern about the safety of infant formula supplemented with probiotics, as well as no evidence of beneficial effects of adding probiotics to infant formula.

Additionally, 17 reviews and meta-data analysis papers published in the last 5 years about the use of probiotics in infant formula were evaluated. The data described in these papers did not raise concerns about the safety of probiotics in infant formula in healthy and high-risk infants. However, although it was noted in the review papers that many studies report no adverse effects, most studies were not designed to monitor these effects and did not systematically screen for or report on the incidence of relevant safety outcomes. Furthermore, several review papers concluded that many studies had methodological limitations and contained too many uncertainties to draw conclusions on negative or positive health effects of probiotics in infant formula for healthy or high-risk infants.

An exception was the use of probiotics in infant formula for preterm infants suffering from acute gastroenteritis and antibiotic-associated diarrhoea. The use of probiotics in this group may result in a beneficial health effect. In 2020, the ESPGHAN panel formulated a positive conditional recommendation for *L. rhamnosus* GG ATCC 53103 (dose: $1x10^9$ – 6 x 10^9 colony-forming units (cfu) and a conditional recommendation for the combination of *B. infantis* Bb-02, *B. lactis* bB-12 and *S. thermophilus* TH-4 (dose: 3.0-3.5 x 10^8 cfu per strain) in preterm infants to reduce necrotizing enterocolitis (NEC) stage 2 or 3.

1. Introduction

Infant formulae are increasingly supplemented with probiotics, prebiotics or synbiotics despite uncertainties regarding their efficacy. Probiotics are defined as live organisms that have a beneficial effect on the health of the host when administered in adequate amounts. Prebiotics are defined as a substrate that is selectively utilized by host microorganisms to confer a health benefit. Probiotics are often administered in combination with prebiotics. This combination of a probiotic with prebiotics that support the chosen probiotic is called synbiotics. The use of prebiotics or synbiotics are not part of this literature study.

The gut microbiota of the foetus are established in early pregnancy and vary depending on maternal nutritional habits, maternal infections, and gestational age (Navarro-Tapia et al., 2020). Furthermore, the delivery mode as well as breastfeeding or formula feeding strongly influences the abundance and diversity of infant microbiota. The primary mode of action of probiotics is through competitive exclusion. The probiotic microorganisms prevent the colonization of the mucosa by potentially pathogenic microorganisms through competition for adhesion and nutrient sites, and through the production of antimicrobial compounds. Strains often used as probiotics are *Bifidobacteria* and *Lactobacilli*. These strains are present in the gastro-intestinal tract of healthy infants but in reduced numbers in preterm infants (Almeida et al., 2021). Only strains that have a Quality Presumption of Safety (QPS) status can be used as a supplement in food (Herman et al., 2019).

In this assessment, FO first provides a summary of the 2014 opinion on probiotics in infant formula of the European Food Safety Authority (EFSA) and the review paper on this subject of the European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) published in 2011 (see section 2). Additionally, a systematic literature research was performed to evaluate the safety of the use of probiotics as a supplement in infant formula based on relevant studies published since 2014 (see section 3). Apart from safety, also information on the health benefits of adding probiotics in infant formula was included.

2. Summary conclusions EFSA and ESPGHAN about probiotics in infant formula

In 2014, EFSA published an opinion on the essential components of infant formula including the use of probiotics (EFSA, 2014). EFSA concluded that the evidence for beneficial effects of infant formula supplemented with probiotics (or synbiotics) on infant health was mainly based on single studies and studies with methodological limitations and were found to be inconsistent across the few studies that were comparable. No evidence was found for safety concerns of the tested probiotics and the available information was also insufficient to draw conclusions on the beneficial effects on infant health. The overall conclusion was that there was no necessity for routine use of probiotics (and/or synbiotics) in infant formula (EFSA 2014).

In 2011, ESPGHAN published a review paper containing recommendations on the use of probiotics in infant formula (Braegger et al., 2011). In this systematic review, the safety and health effects of infant formula supplemented with probiotics on healthy infants were compared with unsupplemented formula. Selected studies were evaluated using the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) system, which was developed by the GRADE working group of ESPGHAN. This system can be used to grade the strength of evidence and recommendations (Guyatt et al., 2008). ESPGHAN concluded that the available scientific data suggest that the administration of the evaluated probiotic supplemented infant formula to healthy infants did not raise safety concerns regarding growth and adverse health effects.

Also, the administration of probiotic supplemented infant formula during early life (≤ 4 months of age) did not result in any consistent beneficial effects. Although normal growth seemed to be supported by probiotics, conclusions on a beneficial effect on growth were difficult to draw due to a limited number of studies and small number of participants or because the follow-up period was too short. ESPGHAN found only limited evidence for reduction of the risk of gastrointestinal infections. The limited evidence showed no reduction of the risk of respiratory infections and no association was found with a reduced use of antibiotics. For colic, irritability or allergies, also no significant difference was found between the treated infants and the control group. A modest statistically significant effect was found on stool frequency and stool consistency; however, the clinical significance of this effect was unclear.

The available studies reviewed by ESPGHAN varied in methodological quality, specific probiotic studied, duration of interventions and doses used. ESPGHAN concluded that there was too much uncertainty in the available data to draw conclusions on the health benefits of probiotic use in infant formulae and that more research was needed to prove the potential benefits of probiotic use in infant formula, in particular addressing long term effects. In general, ESPGHAN did not recommend the routine use of probiotic supplemented formula in infants.

3. Literature search

3.1. Selection of relevant papers

The systematic literature research conducted by the FO focused on literature published since the outcome of the EFSA opinion in 2014. The search for relevant references was conducted in Pubmed, Google Scholar, Scopus and Embase using the following search terms: probiotic and infant formula combined with safety or adverse effect or healthy. The different combinations of search terms resulted in many references. Because of this, it was decided to restrict the assessment to review and meta-data analysis articles published during the last five years (from 2017 onwards).

Using Pubmed, Scopus and Embase, this restriction resulted in a total of 45, 44 and 14 review or meta-data analysis references, respectively. The number of Google Scholar references were still very high when selecting for reviews only (no other options available) and were therefore not included. It was estimated that the probability of missing relevant references due to this was small, ratified by just seven extra references when the results of Scopus and Embase were combined with those of Pubmed. In Appendix I, the number of references per combination of search terms and search machine are explained in more detail.

All references from Pubmed, Scopus and Embase (n = 103) were exported to EndNote and undoubled. References in languages other than English, Dutch and German were excluded as well as those on the effect of probiotics in adults and the presence of probiotics in foodstuffs other than infant formula. This resulted in 52 unique references. Relevant articles referred to in the selected references were also included in this systematic review.

3.2. Results of the literature search

Examination of the 52 references showed that part of them referred to studies of poor quality, e.g., including only a limited number of studies or examining the effect of only one strain or only in children with a specific disease. These studies were excluded from the analysis, resulting in 17 papers that were evaluated in this assessment.

In most reviews, results were evaluated using the GRADE system (see section 2). Some reviews also used the criterium of the Food and Drug Administration (US) that at least two adequate and well-controlled studies, each convincing on its own, are needed to establish the effectiveness of an intervention (Szajewska, 2011).

Probiotics studied included amongst others the following strains:

- *Bacillus causii, Bacillus coagulans* and *Bacillus subtilis*
- *Bifidobacterium animalis* subsp. *lactis* (also named *B. bifidum* and *B. lactis* Bb12), *Bifidobacterium breve, Bifidobacterium infantis* and *Bifidobacterium longum*
- *Enterococcus faecium*
- *Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus fermentum, Lactobacillus gasseri, Lactobacillus helveticus*, *Lactobacillus johnsonii, Lactobacillus paracasei, Lactobacillus plantarum, Lactobacillus rhamnosus, Lactobacillus reuteri* and *Lactobacillus salivarius*
- *Saccharomyces boulardii*,
- *Streptococcus thermophilus*,

3.2.1 Adverse effects of probiotics in infant formula

All reviews in this literature search conclude that in general the evaluated trials do not show adverse effects or do not report at all on adverse effects of administration of probiotics in infants. However, most trials were not designed to monitor these effects and did not systematically screen for or report on the incidence of relevant safety outcomes. Adverse event cases are usually withdrawn from the trials or adverse events could not be related to the use of probiotics. Most trials report that probiotics are often well tolerated without providing a definition of well tolerated. Cabana et al. (2019) and Davis et al. (2020) concluded in their reviews that administration of probiotics to healthy infants does not raise any safety concerns: no association with adverse effects was found. Almeida et al. (2021) concluded that scientific data explored in their review suggest that the consumption of infant formula supplemented with probiotics clinically evaluated in healthy term infants did not raise concerns about microbiological safety, child development or adverse effects. Most reviews found that the adverse event rate was low, and no serious adverse effects could be attributed to probiotics (Guo et al., 2019; Ong et al., 2019; Seghesio et al., 2021).

However, adverse events in infants and older individuals are usually poorly documented and it is seldom described what parameters were monitored. Some reports have described occurrence of probiotic related infections in infants such as sepsis, pneumonia and meningitis (Aceti et al., 2018). Common adverse effects reported were rash, nausea, gas, flatulence, abdominal bloating and constipation. There are a few case studies that described fungemia and bacteraemia potentially associated with administered probiotics especially in immune compromised individuals (Cohen, 2018).

Of particular concern is that probiotics are considered food supplements and thus lack the strict quality regulation of other pharmaceuticals. This has led to differences between the label and actual content of the probiotic product. In addition, there is the risk of product contamination during production or preparation of the probiotic supplement or supplemented infant formula which can have serious consequences especially when administered to vulnerable individuals such as preterm infants. This was shown by the death of an 8-day-old premature infant due to fungal contamination of a probiotic supplement used to treat the child (Aceti et al., 2018; Cohen, 2018). Probiotic sepsis is the most feared side effect and has been described in single strain use and combination studies (for instance: *B. Infantis* and *L. rhamnosus* GG bacteraemia in premature neonates, *L. reuteri, S. boulardii* and *B. breve* BBG-001 and *Escherichia coli* Nissle 1917

(van den Akker et al, 2020)). However probiotic sepsis can also be caused by administration mode or by contamination of the central line after preparation of the probiotic. In 56 trials investigated by Dani et al. (2016), no probiotic related sepsis was found.

Accumulation of D-Lactate is also theoretically possible when consuming probiotics. Preterm infants tend to be acidotic and are more prone to suffer from conditions that make them more acidotic such as sepsis and renal insufficiency. Some *Lactobacillus* strains produce mainly L-lactate, many strains produce a mixture, but some predominantly produce D-lactate. Although quantities may be low, D-lactate is difficult to dispose of after enteral uptake, which could be especially problematic in premature infants (van den Akker et al, 2020). Two documented cases of paediatric D-lactic acidosis occurred in children with short bowel syndrome (Lukasik et al., 2018). It was suggested that acidosis might have been provoked by an intestinal microbiota disturbance due to probiotic intake, but there were no data to support the conclusion that it may occur in otherwise healthy infants (Lukasik et al., 2018). To avoid any risks, the Codex Alimentarius states that probiotics added to infant formula may only contain L-lactate-producing cultures. On the other hand, *L. reuteri* DSM 17938 (known for its ability to produce D-lactate) has been reviewed as generally recognized as safe (GRAS) for the use in infant formula by the FDA (van den Akker et al, 2020).

Some trials conducted in an intensive care unit and a neonatal unit reported serious adverse events in severely debilitated or immune compromised children with underlying risk factors. Some reviews also identified the potential of horizontal gene transfer as a risk, particularly of antibiotic resistance genes, between probiotics and opportunistic (gut) infection causing micro-organisms (Aceti et al., 2018; van den Akker et al, 2020).

Overall, despite the above mentioned potential risks, reviews do not indicate a statistically significant increased risk of adverse events in healthy, medium risk or critically ill infants due to the administration of probiotics compared to control groups (Almeida et al., 2021). Probiotics seem to be well tolerated, with no significant differences between intervention and control groups of infants regarding adverse effects occur (Li et al., 2019). However, there is a lack of systematic reporting of adverse events. Furthermore, studies looking into the long-term health effects of the consumption of infant formula supplemented with probiotics are not available.

3.2.2 Possible beneficial health effects of probiotics in infant formula
Several studies have reported on a beneficial effect of probiotics on health of infants. Research is mainly directed to the following health issues: growth, eczema, gastrointestinal infections, diarrhoea, respiratory tract infections, colic and irritability, allergic manifestations, stool frequency and -consistency, and antibody production. In 2014, the working group of ESPGHAN published positive conditional recommendations for the use of some probiotics in the treatment of acute gastroenteritis in addition to rehydration therapy (Szajewska et al., 2014). *L. rhamnosus* GG (dose: $\geq 10^{10}$ colony-forming units (cfu) per day (typically 5-7 days)) and *S. boulardii* (dose 250-750 mg per day (typically 5-7 days)) (both low quality of evidence, strong conditional recommendation), and *L. reuteri* DSM 17938 (dose: 10^8 to $4x10^8$ cfu per day (typically 5-7 days)) (very low quality of evidence, weak conditional recommendation) reduced diarrhoea with approximately 1 day in healthy children. However, *Enterococcus faecium* received a negative recommendation due to risks because of the presence of vancomycin resistance genes in this strain.

Ong et al. (2019) evaluated 6 studies (out of 3284 literature hits) meeting their criteria on the effect of probiotics on infantile colic compared with placebo. Studies varied in probiotics (*L. reuteri, L. rhamnosus, L. paracasei* and *B. animalis*) and administration (during pregnancy and via infant formula, dose varied $1x10^7$- $9x10^9$ cfu, duration treatment 30 days to 6 months). This evaluation did not find differences in serious adverse effects between probiotic and placebo, as well as clear evidence that probiotics may prevent infantile colic, however, the authors noted that they were limited in drawing conclusions by the low certainty of the evidence (Ong et al., 2019). Qamer et al. (2019) concluded in their review that there was only limited low-quality evidence indicating that probiotics in young children may be associated with the acquisition of tolerance to cow milk protein allergy at the end of 3 years compared with placebo group, although this was highly biased since only 1 study reported outcomes beyond 12 months (probiotic and dosage used: *L. rhamnosus* 1,47 x 10^7- 3 x 10^9 cfu, *L. casei* and *B. lactis* 10^7 cfu per gram, *B. breve* 1,47 $x10^9$ cfu per 100ml formula). Furthermore, the data were inadequate to assess the effect of probiotics on symptoms of allergy and growth. Other limitations were small sample size, high statistical heterogeneity, difference in probiotic (type, dose and duration) used, variations in follow-up period and high risk of bias in the included trials.

In 2020, the ESPGHAN working group published a position paper on the use of probiotics specifically in preterm infants (van den Akker et al., 2020). Articles on probiotic administration to at least 247 preterm infants per group were reviewed and included the following strains: *B. breve* BBG-001 (YIT4010), *L. reuteri* DSM 17938, *L. rhamnosus* GG ATCC 53103*, S. boulardii* CNCM I-745, the combination of *B. bifidum* NCDO 1453 with *L. acidophilus* NCDO 1748 (ATCC 4356, LA37, or NCIMB 30316), and the combination of *B. infantis* Bb-02, *B. lactis* Bb-12, and *S. thermophilus* TH-4. A large number of trials on probiotic strains were excluded for reasons of too low number of infants or not specified at strain level.[1] The panel formulated a positive conditional recommendation for *L. rhamnosus* GG ATCC 53103 (dose: $1x10^9$ – 6 x 10^9 cfu) in preterm infants to reduce necrotizing enterocolitis (NEC) stage 2 or 3 (low certainty of evidence) and a conditional recommendation for the combination of *B. infantis* Bb-02, *B. lactis* bB-12 and *S. thermophilus* TH-4 at a dose of 3.0-3.5 x 10^8 cfu (each strain) to reduce NEC stage 2 or 3 (low certainty of evidence). No recommendations could be made in either direction regarding the use of *L. reuteri* DSM 17938 in preterm infants to reduced mortality, NEC stage 2 or 3 or sepsis (very low certainty of evidence) and because of *L. reuteri* being a partially D-lactate producing strain for which there is insufficient safety data available in preterm infants. Also no recommendations could be made in either direction for the combination of *B. bifidum* NCDO 1453 (currently reclassified as *B. longum*) with *L. acidophilus* NCDO 1748 in preterm infants to reduce the risk of mortality, NES stage 2 or 3 or sepsis (very low to moderate certainty of evidence) and *L. acidophilus* being a partially D-lactate producer. The authors recommended against use of *B. breve* BBG-001 to reduce mortality, NEC stage 2 or 3 or sepsis (low to moderate certainty of evidence). They did not recommend routine use of *S. boulardii* for safety reasons in infants with a central venous catheter, critically ill infants or immunocompromised infants because of risk of fungaemia as well as lack of efficacy (very low to low certainty of evidence).

[1] Strains excluded were four strains of *B. clausii; B. coagulans* (previously *Lactobacillus sporogenes*); combination of *B. subtilis* R0179 and *E. faecium* R0026; *B. bifidum* OLB6378; combination of *B. bifidum*, *B. infantis*, *B. longum*, and *L. acidophilus*; combination of *B. bifidum*, *B. lactis*, *B. longum*, and *L. acidophilus*; *B. breve* M-16 V, combination of *B. breve* and *L. casei*; combination of *B. infantis* ATCC 15697 and *L. acidophilus* ATCC 4356; combination of *B. infantis*, *L. acidophilus*, *L. casei*, *L. plantarum*, *L. rhamnosus*, and *S. thermophilus*; combination of *B. infantis* PTA-5843, *E. faecium* PTA-5844, and *L. gasseri* PTA-5845; combination of *B. lactis* Bb-12 and *B. longum* BB536; *B. longum* BB536; combination of *B. longum* BB536 and *L. rhamnosus* GG; combination of *B. longum* 35624 and *L. rhamnosus* GG; combination of *B. longum* R00175, *L. helveticus* R0052, *L. rhamnosus* R0011, and *S. boulardii* CNCM I-1079; *L. acidophilus* Lb; *L. acidophilus* LA-5 (DSM 13241); and *S. boulardii* CNCM I-3799).

Salminen et al. (2020) concluded that health benefits of probiotics are both strain and disease specific and they formulated similar conclusions as formulated by van den Akker et al. (2020) for preterm infants to reduce NEC. No effect on mortality or sepsis was found in these infants. Seghesio et al. (2021) also reviewed the effect of probiotics on NEC in preterm infants, and showed that some trials found a protective effect while others did not. The combination of *B. infantis*, *S. thermophilus* and *B. lactis* (dose 10^6 cfu each) administered until discharge was found to be effective in reducing NEC in preterm infants. As was a combination of *B. infantis* and *L. acidophilus* (dose: 2.5 x 10^8 cfu each). In addition, supplementation with *B. breve* (dose: 6,7 x 10^7-10^9 cfu) did not reduce NEC. The most effective were combinations of *Bifidobacterium* with *Lactobacillus* spp. or with *Streptococcus* spp. and combinations of *Bifidobacterium* spp. with *Lactobacillus* spp. and *Streptococcus* spp.. the single genus *Bifidobacterium* or *Lactobacillus* spp. were also effective in reducing NEC but less effective than their combination. Studies comparing the efficacy of different strains with each other are lacking. There are only a few trials that report on infants with low birth weight or gestational age. Dosage of probiotics used in the different trials vary from 1 - 6 x 10^9 cfu per day, showing mixed results and leading to the conclusion that the dose-dependent effects are in fact strain dependent. Adverse effects were not reported although Seghesio et al. (2021) mentioned one trial in which probiotic administration was associated with a decrease in NEC and death, but with an increase in Candida (yeast) infections.

All systematic reviews were not able to draw conclusions about possible beneficial health effects of probiotics in healthy infants, because of inconsistent effects of probiotic administered to these infants (Davis et al., 2020). While encouraging data on efficacy of probiotics on disease prevention exist, no broad consensus could be found to recommend the use of probiotics in infant formula with the exception of two conditionally recommendations for preterm infants suffering from NEC (van den Akker et al., 2020).

3.2.3 Factors influencing the conclusions described in the papers

Drawing conclusions on the safety of adding probiotics to infant formula is difficult. There is a general lack of consistency across the different trials. All reviews included in this literature overview report methodological limitations in the evaluated trials. For instance, the definition of infant is unclear, such as differences in the age of participating infants, the delivery mode, high risk infants versus healthy infants and the different medical conditions of the participating infants are not reported. The different types of countries (developing vs. industrialized) is also not reported, while this influences the initial health status of the infants. All reviews state that there are large variations in the reporting of effects in absence of a clear definition of negative or positive effects and the studied trials do not specify inclusion criteria. Most trials evaluated only a short-term time period and there is a lack of follow-up studies (Cohen, 2018; Davis et al., 2020; Guo et al., 2019; Seghesio et al., 2021; Szajewska et al., 2014).

In addition, the working group of ESPGHAN identified uncertainty in the naming of the strains used, manufacturing processes, combinations of probiotic strains, concentrations/doses used, method of administration, and duration of administration (Szajewska et al., 2014). This working group also raised questions on the pooling of different probiotics strains together in a meta-analysis (Szajewska et al., 2014). Moreover, rarely more than two clinical trials were conducted in infants with the same probiotic strain against the same medical condition (Brüssow, 2019), and it is difficult to clarify the long-term negative or positive effects of probiotic intake by infants because of lack of long-term trials (Seghesio et al., 2021).

Aceti et al. (2018) formulated the gaps directing future research based on more well-designed trials, and to clarify the impact of type of feeding and the relation between probiotic use and clinical outcome in infants. They suggest that more research is needed to provide evidence about the potential greater efficacy of multi-strains probiotics compared to single strain products, and to investigate the risk of antibiotic resistance gene transfer in preterm infants and the long-term effects.

4. Conclusion

EFSA and ESPGHAN looked into the safety and beneficial effects of probiotics added to infant formula in 2014 and 2011, respectively. Both organisations concluded that there was no evidence for a concern about the safety of infant formula supplemented with probiotics, as well as no evidence of beneficial effects of adding probiotics to infant formula.

Additionally, 17 reviews and meta-data analysis papers published in the last 5 years about the use of probiotics in infant formula were evaluated. The data described in these papers did not raise concerns about the safety of probiotics in infant formula in healthy and high-risk infants. However, although it was noted in the review papers that many studies report no adverse effects, most studies were not designed to monitor these effects and did not systematically screen for or report on the incidence of relevant safety outcomes. Furthermore, several review papers concluded that many studies had methodological limitations and contained too many uncertainties to draw conclusions on negative or positive health effects of probiotics in infant formula for healthy or high-risk infants.

An exception was the use of probiotics in infant formula for preterm infants suffering from acute gastroenteritis and antibiotic-associated diarrhoea. The use of probiotics in this group may result in a beneficial health effect. In 2020, the ESPGHAN panel formulated a positive conditional recommendation for *L. rhamnosus* GG ATCC 53103 (dose: $1x10^9$ – 6×10^9 colony-forming units (cfu) and a conditional recommendation for the combination of *B. infantis* Bb-02, *B. lactis* bB-12 and *S. thermophilus* TH-4 (dose: $3.0\text{-}3.5 \times 10^8$ cfu per strain) in preterm infants to reduce necrotizing enterocolitis (NEC) stage 2 or 3.

References

Aceti, A., Beghetti, I., Maggio, L., Martini, S., Faldella, G., & Corvaglia, L. (2018). Filling the Gaps: Current Research Directions for a Rational Use of Probiotics in Preterm Infants. *Nutrients*, *10*(10). https://doi.org/10.3390/nu10101472.

Almeida, C. C., Mendonça Pereira, B. F., Leandro, K. C., Costa, M. P., Spisso, B. F., & Conte-Junior, C. A. (2021). Bioactive Compounds in Infant Formula and Their Effects on Infant Nutrition and Health: A Systematic Literature Review. *Int J Food Sci*, 8850080. https://doi.org/10.1155/2021/8850080.

Braegger, C., Chmielewska, A., Decsi, T., Kolacek, S., Mihatsch, W., Moreno, L., Piescik, M., Puntis, J., Shamir, R., Szajewska, H., Turck, D., van Goudoever, J., & Nutrition, E. C. o. (2011). Supplementation of infant formula with probiotics and/or prebiotics: a systematic review and comment by the ESPGHAN committee on nutrition. *J Pediatr Gastroenterol Nutr*, *52*(2), 238-250. https://doi.org/10.1097/MPG.0b013e3181fb9e80.

Brüssow, H. (2019). Probiotics and prebiotics in clinical tests: an update. *F1000Res*, *8*. https://doi.org/10.12688/f1000research.19043.1.

Cabana, M. D., Salminen, S., & Sanders, M. E. (2019). Probiotic Safety-Reasonable Certainty of No Harm. *JAMA Intern Med*, *179*(2), 276. https://doi.org/10.1001/jamainternmed.2018.7498.

Cohen, P. A. (2018). Probiotic Safety-No Guarantees. *JAMA Intern Med*, *178*(12), 1577-1578. https://doi.org/10.1001/jamainternmed.2018.5403.

Dani, C., Coviello, C. C., Corsini, I. I., Arena, F., Antonelli, A., & Rossolini, G. M. (2016). Lactobacillus Sepsis and Probiotic Therapy in Newborns: Two New Cases and Literature Review. *AJP Rep*, *6*(1), e25-29. https://doi.org/10.1055/s-0035-1566312.

Davis, E. C., Dinsmoor, A. M., Wang, M., & Donovan, S. M. (2020). Microbiome Composition in Pediatric Populations from Birth to Adolescence: Impact of Diet and Prebiotic and Probiotic Interventions. *Dig Dis Sci*, *65*(3), 706-722. https://doi.org/10.1007/s10620-020-06092-x.

EFSA (2014). Scientific opinion on the essential composition of infant and follow-on formulae. *EFSA Journal 12*(7):3760. https://doi.org/10.2903/j.efsa.2014.3760.

Guo, Q., Goldenberg, J. Z., Humphrey, C., El Dib, R., & Johnston, B. C. (2019). Probiotics for the prevention of pediatric antibiotic-associated diarrhea. *Cochrane Database Syst Rev*, *4*(4), Cd004827. https://doi.org/10.1002/14651858.CD004827.pub5.

Guyatt, G. H., Oxman, A. D., Vist, G. E., Kunz, R., Falck-Ytter, Y., Alonso-Coello, P., & Schünemann, H. J. (2008). GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. *Bmj*, *336*(7650), 924-926. https://doi.org/10.1136/bmj.39489.470347.AD.

Herman, L., Chemaly, M., Cocconcelli, P. S., Fernandez, P., Klein, G., Peixe, L., Prieto, M., Querol, A., Suarez, J. E., Sundh, I., Vlak, J., & Correia, S. (2019). The qualified presumption of safety assessment and its role in EFSA risk evaluations: 15 years past. *FEMS Microbiol Lett*, *366*(1). https://doi.org/10.1093/femsle/fny260.

Li, L., Han, Z., Niu, X., Zhang, G., Jia, Y., Zhang, S., & He, C. (2019). Probiotic Supplementation for Prevention of Atopic Dermatitis in Infants and Children: A Systematic Review and Meta-analysis. *Am J Clin Dermatol*, *20*(3), 367-377. https://doi.org/10.1007/s40257-018-0404-3.

Lukasik, J., Salminen, S., & Szajewska, H. (2018). Rapid review shows that probiotics and fermented infant formulas do not cause d-lactic acidosis in healthy children. *Acta Paediatr*, *107*(8), 1322-1326. https://doi.org/10.1111/apa.14338.

Navarro-Tapia, E., Sebastiani, G., Sailer, S., Toledano, L. A., Serra-Delgado, M., Garcia-Algar, O., & Andreu-Fernandez, V. (2020). Probiotic Supplementation During the Perinatal and Infant Period: Effects on Gut Dysbiosis and Disease. *Nutrients*, *12*(8). https://doi.org/10.3390/nu12082243.

Ong, T. G., Gordon, M., Banks, S. S., Thomas, M. R., & Akobeng, A. K. (2019). Probiotics to prevent infantile colic. *Cochrane Database Syst Rev*, *3*(3), Cd012473. https://doi.org/10.1002/14651858.CD012473.pub2.

Qamer, S., Deshmukh, M., & Patole, S. (2019). Probiotics for cow's milk protein allergy: a systematic review of randomized controlled trials. *Eur J Pediatr*, *178*(8), 1139-1149. https://doi.org/10.1007/s00431-019-03397-6.

Salminen, S., Stahl, B., Vinderola, G., & Szajewska, H. (2020). Infant Formula Supplemented with Biotics: Current Knowledge and Future Perspectives. *Nutrients*, *12*(7). https://doi.org/10.3390/nu12071952.

Seghesio, E., De Geyter, C., & Vandenplas, Y. (2021). Probiotics in the Prevention and Treatment of Necrotizing Enterocolitis. *Pediatr Gastroenterol Hepatol Nutr*, *24*(3), 245-255. https://doi.org/10.5223/pghn.2021.24.3.245.

Szajewska, H. (2011). Short- and long-term effects of probiotics administered early in life. *Nestle Nutr Workshop Ser Pediatr Program*, *68*, 65-78; discussion 78-82. https://doi.org/10.1159/000325665.

Szajewska, H., Guarino, A., Hojsak, I., Indrio, F., Kolacek, S., Shamir, R., Vandenplas, Y., & Weizman, Z. (2014). Use of probiotics for management of acute gastroenteritis: a position paper by the ESPGHAN Working Group for Probiotics and Prebiotics. *J Pediatr Gastroenterol Nutr*, *58*(4), 531-539. https://doi.org/10.1097/mpg.0000000000000320.

Van den Akker, C., Van Goudoever, J., Shamir, R., Domellof, M., Embleton, N.,Hojsak, I., Lapillonne, A., Mihatsch, W., Canani, R., Bronksy, J., Campoy, C., Fewtrell, M., Fidler Mis, N., Guarino, A., Hulst, J., Indrio, F., Kolacek, S., Orel, R., Vandenplas, Y., Weizman, Z and Szajewska, H. (2020). Probiotics and Preterm Infants: a position paper by the European Society for Paediatric Gastroenterology Hepatology and Nutrition Committee on Nutrition and the European Society for Paediatric Gastroenterology Hepatology and Nutrition Working group for Probiotics and Prebiotics. *J Pediatr Gastronenterol Nutr*, 70(5), 664-680. https://doi.org/10.1097/MPG.0000000000002655

Appendix I. Number of references per search machine and search term, October 2021

Search machine	Search terms in title, abstract and keyword	Number of references	Reviews and meta-data analyses[1,2]	Last 5 years (≥ 2017)
Pubmed				
1	Probiotic infant formula	517	176	-
2	Probiotic infant formula safety	81	32	10
3	Probiotic infant formula healthy	123	35	14
4	Probiotic infant formula adverse effect	117	44	21
	Total			45
Scopus				
1	Probiotic infant formula	658	176	-
2	Probiotic infant formula safety	138	42	12
3	Probiotic infant formula healthy	171	51	20
4	Probiotic infant formula adverse effect	75	22	12
	Total			44
Embased				
1	Probiotic infant formula	573	130	-
2	Probiotic infant formula safety	100	27	6
3	Probiotic infant formula healthy	142	26	6
4	Probiotic infant formula adverse effect	62	11	2
	Total			14
Google Scholar				
1	Probiotic infant formula	25500	8370	-
2	Probiotic infant formula safety	16300	5610	2890
3	Probiotic infant formula healthy	17400	8200	4150
4	Probiotic infant formula adverse effect	18400	15200	8700
	Total			15740

[1] Scopus did not have an option to select meta-data analysis references. In addition, one book chapter reference was selected with the search terms 'probiotic infant formula healthy'.
[2] Google Scholar only had an option to select review references.