Probiotics, Prebiotics and Synbiotics use in Neonates: A critical appraisal of the evidence and evaluation of its application by the food industry.

by
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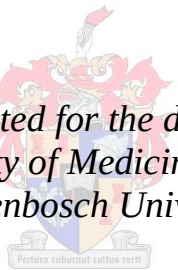

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DECLARATION

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Abstract

Background:

Synbiotics, probiotics and prebiotics are being added to infant formula. This study was an in-depth evaluation of research on infants fed infant formula containing synbiotics, probiotics or prebiotics and was carried out in two phases. Phase one included two systematic reviews that assessed if synbiotics, probiotics or prebiotics led to improved growth and clinical outcomes in formula fed full term and preterm infants. Phase two included two studies: A systematic review compared the methodological quality and outcomes of industry and non-industry sponsored randomized controlled trials (RCTs) and a descriptive study evaluated how the food industry applies the knowledge and evidence gained from probiotics, prebiotics or synbiotics research in infants.

The research questions were: Does the consumption of probiotics, prebiotics or synbiotics supplemented infant formula lead to improved clinical outcomes in infants? Is there an association between source of funding and methodological quality, clinical outcomes and author's conclusions in trials using probiotics, prebiotics or synbiotics supplemented formula in infants? Does the food industry use the evidence gained through probiotics, prebiotics and synbiotics research trials on infants for the benefit of the general paediatric population?

The hypotheses were: Consumption of probiotics, prebiotics or synbiotics by infants leads to improved clinical outcomes; The source of funding in research trials using probiotics, prebiotics or synbiotics supplemented formula in infants is associated with outcomes in favour of the sponsor's products and authors' conclusions; Methodological qualities of non-industry sponsored trials are equivalent to industry sponsored trials; Evidence gathered through probiotics, prebiotics and synbiotics research is implemented by the food industry.

Methods:

Phase one:
Both systematic reviews on preterm and full term infants: Cochrane methodology was followed using RCTs which compared preterm or full term formula containing probiotics, prebiotics or synbiotics to conventional infant formula with / without placebo among healthy preterm or full term infants. The mean difference (MD) and corresponding 95% confidence intervals (CI) were reported for continuous outcomes, risk ratio (RR) and corresponding 95% CI for dichotomous outcomes.

Phase two:
In the systematic review, Cochrane methodology was used to assess the risk of bias of included RCTs. Association between source of funding and risk of bias, clinical outcomes and conclusions were assessed. In the descriptive study, all listed companies that manufacture infant food products with added synbiotics, probiotics or prebiotics for infants were identified and invited to participate. A letter of invitation was sent and if they expressed willingness to take part in the study, a questionnaire with a written consent form was sent. Descriptive statistics and associations between categorical variables were to be tested using a Chi-square test.

Results:

Phase one:
Review on preterm infants: 8 studies were included. Probiotics increased stool frequency with no effect on other clinical outcomes. Prebiotics increased stool frequency and bifidobacteria counts only.

Review on full term infants: 25 studies were included. Synbiotics improved stool frequency but had no effect on other clinical outcomes. Probiotics did not have an effect on any clinical outcome. Prebiotics increased weight gain and stool frequency with no effect on other outcomes.

Phase two:
Systematic review: 67 studies were included, majority were funded by food industry. There was no significant association between the source of funding and four domains (sequence generation, allocation concealment, blinding, selective reporting), majority of reported clinical outcomes or authors' conclusions. Source of funding was significantly associated with two domains (incomplete outcome data, free of other bias), antibiotic use and conclusions on weight gain.

Descriptive study: 25 companies were identified and invited to participate. No company agreed to participate in the survey for different reasons.

Conclusions

Phase one:
Review on preterm infants: There is not enough evidence to state that supplementation with probiotics or prebiotics results in improved growth and clinical outcomes in exclusively formula fed preterm infants.

Review on full term infants: There is not enough evidence to state that supplementation of term infant formula with synbiotics, probiotics or prebiotics does result in improved growth or clinical outcomes in term infants. There is no data available to establish if synbiotics are superior to probiotics or prebiotics.

Phase two:

Systematic review: In RCTs on infants fed infant formula containing probiotics, prebiotics or synbiotics, the source of funding does not influence majority of outcomes in favour of the sponsors' products. More non-industry funded research is needed to further assess the impact of funding on reported clinical outcomes and authors' conclusions.

Descriptive study: Due to companies refusing to participate in this study, no conclusion could be drawn on how the food industry applies evidence gained through probiotics, prebiotics or synbiotics research on infants. More transparency is needed from the infant formula manufactures on how they apply the evidence gained from probiotic, prebiotic or synbiotic research on infants.

Abstrakt

Agtergrond

Synbiotika, probiotika en prebiotika word gereeld by baba formule gevoeg. Hierdie studie was 'n in-diepte evaluering van navorsing oor babas gevoed met formule melk wat synbiotika, probiotika of prebiotika bevat en is uitgevoer in twee fases. Fase een het twee sistematiese oorsigte ingesluit wat die rol van synbiotika, probiotika en prebiotika op verbeterde groei en kliniese uitkomste van formule gevoede volterm babas en vroeg gebore babas evalueer het. Fase twee het bestaan uit twee studies: 'n sistematiese oorsig wat die metodologiese kwaliteit en uitkomste van die bedryf en nie-bedryf geborgde ewekansige gekontroleerde proewe (RCTs) evalueer het, asook 'n beskrywende studie wat die kennis en toepassing van bewyse oor die effektiewiteit van probiotika, prebiotika of synbiotika in die voedsel industrie bestudeer het.

Die hipotese stellings was: verbruik van probiotika, prebiotika of synbiotika by babas lei tot verbeterde kliniese uitkomste; die bron van befondsing vir synbiotics, probiotika of prebiotika navorsing beïnvloed uitkomste ten gunste van die borg se produkte; bewyse ingesamel deur middel van probiotika, prebiotika en synbiotika navorsing word geïmplementeer deur die voedselindustrie.

Metodes

Fase een:
Beide sistematiese oorsigte op volterm en premature babas: Cochrane metodes is gevolg deur ewekansige, gekontroleerde studies wat vol termyn of premature formule met probiotika, prebiotika of synbiotika met konvensionele baba formule met / sonder plasebo onder gesonde volterm of premature babas bestudeer. Die gemiddelde verskil (MD) en die ooreenstemmende 95% vertrouensintervalle is gebruik vir deurlopende uitkomste, risiko verhouding (RR) en die ooreenstemmende 95% CI vir tweeledige uitkomste.

Fase twee:
In die sistematiese oorsig is Cochrane metodiek gebruik om die risiko van vooroordeel van ingesluite ewekansige, gekontroleerde studies te evalueer. Assosiasie tussen bron van befondsing en die risiko van vooroordeel, asook kliniese uitkomste en gevolgtrekkings was beoordeel. In die beskrywende studie, is alle genoteerde maatskappye wat babavoeding produkte vervaardig met bygevoegde synbiotika, probiotika of prebiotika vir babas geïdentifiseer en uitgenooi om deel te neem. 'n Uitnodigingsbrief is vir die relevante maatskappye gestuur om hul bereidwilligheid om deel te neem te bevestig. Indien hulle wel bereid was om

deel te neem was 'n vraelys met 'n skriftelike toestemming vorm gestuur. Beskrywende statistiek en assosiasies tussen kategoriese veranderlikes was getoets met behulp van 'n Chi-kwadraat toets.

Resultate

Fase een:
Oorsig oor premature babas: 8 studies was ingesluit. Probiotika verhoog stoelgang frekwensie met geen effek op ander kliniese uitkomste. Prebiotika verhoog ook stoelgang frekwensie en slegs bifidobakteriële tellings. Oorsig oor die vol termyn babas: 25 studies was ingesluit. Synbiotika verbeter stoelgang frekwensie, maar het geen effek op ander kliniese uitkomste gehad nie. Probiotika het nie 'n effek op enige kliniese uitkoms gehad nie. Prebiotika verhoog gewigstoename en stoelgang frekwensie met geen effek op ander uitkomste.

Fase twee:
Sistematiese oorsig: 67 studies was ingesluit, en die meerderheid was befonds deur die voedsel bedryf. Daar was geen beduidende assosiasie tussen die bron van befondsing en vier gebiede (toekenningsvolgorde, toekenningsverberging, studie verblinding, selektiewe verslaggewing), en die meerderheid van gerapporteerde kliniese uitkomste of skrywers se gevolgtrekkings. Die bron van befondsing was beduidend verbind met twee gebiede (onvolledige uitslag data, vry van ander vooroordeel), antibiotika gebruik en gevolgtrekkings op gewigstoename.

Beskrywende studie: 25 maatskappye is geïdentifiseer en genooi om deel te neem. Geen maatskappy het ingestem om deel te neem aan die studie om verskillende redes.

Gevolgtrekkings

Fase een:
Oorsig oor premature babas: Daar is nie genoeg bewyse dat die aanvulling met probiotika of prebiotika resultate in verbeterde groei en kliniese uitkomste in uitsluitlik formule gevoede premature babas tot gevolg het nie.
Oorsig oor die volle termyn babas: Daar is nie genoeg bewyse om te sê dat die aanvulling van term baba formule met synbiotika, probiotika of prebiotika lei tot verbeterde groei of kliniese uitkomste in termyn babas. Daar is geen inligting beskikbaar om te stel of synbiotika beter is as probiotika of prebiotika nie.

Fase twee:
Sistematiese oorsig: In studies op babas gevoed met formule melk wat probiotika, prebiotika of synbiotika bevat het, het die bron van befondsing nie meerderheid van die uitkomste in die guns van die borge se

produkte beïnvloed nie. Meer nie-industrie befondsde navorsing is nodig om verder die impak van befondsing op kliniese uitkomste en skrywers se gevolgtrekkings te evalueer.

Beskrywende studie: Aangesien al die maatskappy deelname geweier het, kon geen gevolgtrekking gemaak word of die voedsel bedryf bewyse oor die gebruik van probiotika, prebiotika of synbiotika toepas nie. Meer deursigtigheid is nodig van die formule vervaardigers oor hoe hulle die bewyse oor die gebruik van probiotika, prebiotika of synbiotika toepas.

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Mary Letizia Nkatha Mugambi

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List of Abbreviations

%: percent

^{0}C: Degrees centigrade

AFSSA: French Food Safety Agency

cfu: Colony Forming Units

CI: Confidence Interval

cm: Centimetres;

DHA: Docosahexaenoic acid

ELBW: Extremely low birth weight

ESPGHAN: European Society for Paediatric, Gastroenterology, Hepatology and Nutrition

FAO: Food and Agriculture Organisation

FDA: Food and Drug administration OR Federal Drug Administration

FOS: Fructooligosaccharide

g / day: grams per day

g / kg / day: grams / kilogram / day

g: grams

GI: Gastrointestinal

GOS: Galactooligosaccharide;

GRAS: Generally regarded as safe

IFN-γ: Interferon – gamma

IgA: Immunoglobulin A

IL-10: Interleukin - 10

IL-12: Interleukin - 12

IL-1β: Interleukin – 1beta

IL-6: Interleukin – 6

IQR: Inter Quartile Range

Kcal / kg / day: Kilocalories / kilogram (body weight) / day

Kcal / ml: Kilocalories / millilitre

Kcal: Kilocalories

Kg: Kilogram

kJ: Kilojoules

L/M: Lactulose Mannitol

LBW: Low birth weight

LoE: Levels of evidence

MCTs: Medium Chain Triglycerides

MD: Mean Difference

Mg / dl: Milligrams / decilitre

mls: Millilitre

mm: millimetres;

NEC: Necrotising Enterocolitis

NK cells: Natural killer cells

NPO: Nil by mouth

PN: Parenteral nutrition

RCT: Randomized Control Trial

RR: Risk Ratio;

SA: South Africa

SCFAs: Short chain fatty acids

SD: Standard Deviation

SPSS: Statistical Program for Social Sciences

TNF-α: Tumour Necrosis Factor – alpha

UNICEF: United Nations Children's Fund

URTI: Upper respiratory tract infections

UTI: Urinary tract infections

VLBW: Very low birth weight

WHO: World Health Organisation

Definition of terms

In this study, specific terms were used which had several meanings. They include:

- Infants referred to preterm, low birth weight, very low birth weight or extremely low birth weight and full term infants
- Infant formula referred to preterm formula, full term formula (standard formula, protein hydrolysates, soy based formula, amino acid based formula), follow-on formula.
- Review referred to Systematic review
- Probiotics was all live bacteria added to infant formula and included strains that are normally found in the gastrointestinal system. Examples include bifidobacteria, lactobacillus among others.
- Prebiotics were fructooligosaccharide, inulin, galactooligosaccharides, oligosaccharides and other prebiotics such as Arabica gum.
- Synbiotics were a combination of any live probiotic bacteria with any prebiotic added simultaneously to infant formula.

Chapter 1. Introduction

1.0 Introduction

1.0.1 Overview of study

Twenty five years of research confirms that breastfeeding is the optimal way to feed infants, since it contains all the essential nutrients babies need, and antibodies that fight off infection. [1 - 5] The World Health Organisation (WHO) estimates that 1.4 million infant deaths could be prevented annually if women breastfed their infants. [6] Despite the well documented benefits of breastfeeding, more women are choosing to formula feed instead of breastfeeding. As a result, global breastfeeding rates have decreased and formula feeding has increased. The high demand for formula has resulted in stiff competition among companies to manufacture new and innovative infant formula. [6] To identify how infant formula can be adapted to more closely resemble the composition and function of human milk, rigorous research has been done. This has resulted in different components being added to infant formula, such as docosahexaenoic acid (DHA), arachidonic acid, probiotics and prebiotics or synbiotics. [7]

This two phase research project was an in-depth evaluation of research done on infants, given infant formula containing probiotics and prebiotics or synbiotics.

Phase one was a critical appraisal of the evidence on probiotics, prebiotics and synbiotics in infants. It included two systematic reviews. The first review explored the effects of probiotics, prebiotics and synbiotics on growth and clinical outcomes in formula fed preterm infants and low birth weight infants. The second review explored the effects of synbiotics, probiotics or prebiotics on growth and clinical outcomes on formula fed full-term infants.

Phase two was an evaluation of the application of evidence by the food industry on probiotics, prebiotics and synbiotics in infants. This phase included both a systematic review and a descriptive study. The systematic review explored the association between the source of funding, methodological quality and research outcomes in randomized clinical trials of probiotics, prebiotics and synbiotics studies in infants. The descriptive study explored how the food industry implements research evidence on probiotics, prebiotics and synbiotics in infants for the benefit of the general paediatric population.

1.1 Background

1.1.1 Growth

The health status of newborn infants is assessed using various indicators of growth and development. Indicators usually include weight, length and head circumference for gestational age. Tracking growth in an infant over a time period helps to identify health problems and prevent or minimize a slow growth rate. A steady weight and length gain over time is a sign that enough calories and nutrients are being provided. Therefore, a nutritional goal for infants is to provide enough calories and nutrients to maintain adequate growth. [6, 8, 9] Below is a brief description of growth in preterm and full term infants.

1.1.1a Premature infants

In premature and low birth weight infants, growth is a major challenge. By definition, premature infants are infants born before 37 weeks gestation. Low birth weight infants (LBW) weigh less than 2500 g at birth, very low birth weight infants (VLBW) weigh less than 1500 g and extremely low birth weight infants (ELBW) weigh less than 1000 g. [10 - 12] They have several risk factors that may result in nutritional deficiencies and poor growth including: poor nutrient stores of glycogen, fat, protein, fat soluble vitamins, calcium, phosphorus, magnesium and trace minerals [11 - 14]; immature gastrointestinal tracts (GI) and intestines; and villi and microvilli are short and fewer in number, decreasing overall absorptive capacity and utilization of nutrients. [11, 12, 14] In addition, they have a lengthy nil by mouth (NPO) status due to prolonged illnesses such as NEC (Necrotising Enterocolitis) or bowel obstruction which delays the introduction of enteral feeds. [3, 11 - 13] They also have increased nutritional demands due to a rapid growth phase, tissue development, stress from surgery and poor temperature control. [11, 15]

Therefore, for premature infants, the goals are to provide adequate nutrition to foster: intra-uterine growth rate (in an extra-uterine environment); adequate weight gain without metabolic complications; foster catch-up growth and nutrient accumulation, during the post discharge period. [3, 11, 12, 15, 16 – 18] For preterm infants, this is important because infants lose weight after birth (up to 6% to 8% for extreme low birth weight infants) and they often do not regain the weight for up to 1 to 2 weeks. [15, 16, 19] Daily growth monitoring (weight gain, linear and head circumference) then becomes vital. [3]

To achieve optimum growth, a weight gain of 15 to 20 g / kg /day; length of 0.75 to 1.0 cm / week; and head circumference 0.75 cm / week is required. This is difficult to achieve and requires between 130 – 135 kcal / kg / day to maintain this growth rate. [3, 15, 18, 20] For optimum growth, full feeds of 150 mls / kg / day are

needed to meet the nutritional goals of: 120 to 130 kcals / kg / day; 3.0 to 4.0 g protein / kg / day (to promote weight gain similar to in foetuses in utero); 10.8 to 16.8 g carbohydrate / kg / day; 4.4 g to 6 g fat / 100 kcals (to promote fat deposition similar to foetuses in utero which is 3g/kg/day); 120 to 230 mg calcium / kg / day; and 60 to 140 mg phosphorus / kg / day (to decrease risk of fractures and osteopenia). [3, 11, 12, 14, 15, 20, 21]

Constant surveillance is required to detect early signs of feed intolerance including: increased gastric residuals, abdominal distension, vomiting, heme- positive stools, change in bowel sounds, apnea or bradycardia with feeds and unstable body temperature. Depending on the findings, enteral feeds are either withdrawn, decreased, diluted, discontinued or feed frequencies are changed. [11, 12, 15]

1.1.2b Full term infants

Healthy full term infants do not have the complex challenges of the preterm infants. However, for full term infants, the first year of life is also characterized by very rapid growth. Weight increases by 115%, body length 34% and head circumference 22%. [8, 22, 23] Initially, infants lose weight after birth and take 8-10 days to regain it. Weight gain increases by approximately 1.1 to 1.2 kg / month during the first 6 months, slowing down to 0.4 to 0.5 kg / month during the second 6 months. Length increases by 3.5 to 3.9 cm / month during the first 4 months, slowing down to 1.8 cm / month at 6 month of age. [8, 22] At birth, average head circumference is 35 cm and increases by an estimated 12 cm during the first year of life, to approximately 47 cm. A faltering head circumference has serious implications for neural growth, maturation and is an indicator for possible problems with brain growth. [23] Monitoring growth (weight, length and head circumference) evaluates the overall health of the infant and determines adequacy of nutritional intake. [8, 22, 24]

For optimum growth, the formula fed infant at birth must consume 6 to 10 feeds of 30 to 90 mls / day of formula, gradually increasing to 3 feeds of 210 to 240 mls / day at 10 to 12 months of age. [25] To maintain optimum growth during the first 6 months of life, infants must consume formula providing more than 500 kcals / day (males 570 kcals, females 520 kcals); 9.1 g / day (or 1.5g / kg / day) protein; and 31 g / day (0.98 g / kg / day) of fat. From 6 months to 1 year, infants must consume more than 670 kcals / day (males 743 kcals / day, females 676 kcals / day); 11g / day protein; and 30 g /day of fat. [26, 27]

1.2 Infant feeding

Infant feeding choices for mothers of newborn infants are either to breastfeed, formula feed or to give both (mixed feeds). WHO and UNICEF's guidelines for optimal infant feeding state that infants should receive exclusive breastfeeding for their first 6 months, then nutritionally adequate and safe complementary feeds should be initiated from the 6 months of age, with continued breastfeeding up to 2 years of age or beyond. [1, 2, 6] Breastfeeding has several documented advantages over formula including: superior nutritional composition; immunologic and enzymatic components; health benefits for mothers; lower cost and convenience; enhanced maternal-infant bonding; decreased incidence of respiratory, gastrointestinal infections; leaner body composition of infants at 1 year of age and improved cognitive development. [25, 28, 29]

Despite these documented benefits of breastfeeding, more mothers are choosing not to breast feed but to formula feed their infants. There are health conditions in which formula feeding is indicated. These include:

Infant conditions

- Rare medical conditions such as galactosemia, maple syrup urine disease and phenylketonuria all require specialized infant formula. [6]
- Premature infants with less than 32 weeks gestation and very low birth weights of of less than 1500 g require specialized infant formula as a supplement to breastmilk for a limited time period. [6, 28, 29]
- Inadequate weight gain requires infant formula to be given as a supplement to breastmilk. [28, 29]

Maternal conditions

- HIV infection requires formula feeding if it is feasible, affordable, sustainable and safe. [6]
- Severe illness that diminishes maternal capacity to care for her infant requires infant formula. [6]
- Herpes simplex [6]
- Maternal medication [6]

In the absence of breast milk, commercial infant formulas are available for preterm and full term infants. The different types of preterm, full term formulas given to infants are also used as a vehicle to deliver probiotics, prebiotics and synbiotics to infants. These are briefly described below.

1.2.1 Preterm infant formula

Preterm infant formulas are designed to meet the nutritional needs of preterm infants without exceeding volume requirements or tolerance. [3, 11, 15, 21] Preterm formulas are fed to preterm infants of less than 36

weeks gestation when breastmilk is not available. [3] Preterm formulas have higher concentrations of vitamins, minerals and electrolytes than standard formulas. [11 - 13, 15] In general, in preterm formulas, glucose polymers make up 50 to 60 % of carbohydrate calories, decreasing the lactose load to the infant, because lactose contibues 40 to 50 % of carbohydrate calories to facilitate calcium absorption. Medium chain triglycerides (MCT) are 40 to 50 % of fat calories, aiding fat absorption and weight gain, because preterm infants have limited pancreatic lipase secretion and small bile acid pools. [11 - 13, 15] The protein has a 60 / 40 whey / casein ratio compared to 80 / 20 ratio of colostrum; 55 / 45 ratio of mature breast milk; and 18 / 82 whey / casein ratio of cow milk. Since the preterm formulas are predominately whey based, this promotes gastric emptying and digestion. Preterm formulas also contain amino acids cysteine and taurine, because preterm infants are usually deficient in them. [3] The age and weight at which a preterm formula is terminated varies among neonatal intensive care units, the vitamin concentration in the formula and the volume of intake. [12, 21] Premature formulas can be used until the infant reaches a weight of 2.5 to 3.5 kg. [12]

Transition formulas (also known as post discharge formula) are designed for premature infants at discharge, or when the infant should have reached a weight of at least 2.0 to 2.5 kg. [3, 12, 21] The aim of giving transition formulas is to provide enough nutrients to address any nutritional deficits; and to promote normal growth and neurodevelopment without over feeding the preterm infant. Transition formulas are given at least 2 days before discharge to document tolerance and weight gain. These formulas have a nutrient composition that is between premature formula and standard infant formulas. [3] Glucose polymers contain 50 to 60 % of carbohydrate calories and lactose comprises 40 to 50 %. MCTs are 20 to 25 % of the fat calories; and protein is either 60 / 40, or 50 / 50 whey to casein ratio. [12] Compared to term formula, transition formulas have higher levels of protein (1.8 to 1.9 g / 100 ml) than standard preterm formulas, more energy (72 to 74 kcal / 100 ml), additional calcium, phosphorus, zinc, trace minerals and vitamins. [3]

1.2.2 Full term infant formula

Standard infant formulas are suitable for infants from birth to 12 months. In general, for every 100 mls, standard formulas provide approximately 65 kcals (272 kJ), 1.5 g protein, 7.0 g carbohydrate and 3.8 g fat. [21, 25, 27 - 29] Follow-on formulas are used after 6 months. Standard formulas are made from cow's milk that is changed by removing the butterfat; adding vegetable oils and carbohydrates; and decreasing the protein. [25, 27, 29] In addition to standard formula, there are other formulas which were used by study participants:

- Soy protein formula: For infants with cow milk protein allergy, soy protein-based formulas are used. They are free of lactose, with the carbohydrate source being a glucose polymer. [21] These formulas are

fortified with methionine, carnitine, taurine and iron. They also contain a soy protein isolate and vegetable oils. [21, 25, 27- 29]

- Protein hydrolysate formulas: These formulas are given in cases of severe cow milk allergy or soy intolerance. The protein (such as casein, whey) is denatured using heat and hydrolysed using proteolytic enzymes, resulting in small peptides and free amino acids. The enzymes are then denatured by heat. [21, 25, 27 - 29]
- Amino acid based formulas contain only pure amino acid mixtures. They are indicated for infants who are extremely sensitive to protein and do not tolerate protein hydrolysate formulas. [21, 25, 29]

1.3 Intervention

1.3.1 Definitions of probiotics, prebiotics, synbiotics

Probiotics have been defined as "live microorganisms which when administered in adequate amounts may confer a health benefit to the host." [30, 31, 32, 33] A new definition by the American Academy of Pediatric Committee on Nutrition states that probiotics are "microbes that generate small molecular metabolic by-products that exert beneficial regulatory influence on host biological functions and may function as immunomodulators." [34] A number of genera of bacteria and yeast are used as probiotics, including leuconostoc, pediococcus, enterococcus, lactobacillus and bifidobacteria. The main probiotic organisms currently used worldwide belong to the genera lactobacillus and bifidobacteria and are found in the gastrointestinal microflora. [32]

Prebiotics are "non- digestible food ingredients that may benefit the host by selectively stimulating the growth and / or activity of one or a limited number of bacteria in the colon and improving the host's health." [30, 31, 35] This definition was refined to "prebiotics are selectively fermented ingredients that allows specific changes in the composition and / or activity in the gastrointestinal microbiota thus conferring benefits to the host's health." [36, 37, 38] The most widely studied prebiotics are inulin and fructooligosaccharide (FOS), which are plant storage carbohydrates in vegetables, cereals and fruit. [30, 31]

When probiotics and prebiotics are administered simultaneously, the combination is termed Synbiotics. The prebiotic in the synbiotic mixture improves the survival of the probiotic bacteria, and stimulates the activity of the host's endogenous bacteria. [30, 31, 39]

1.4 Probiotics

Probiotics are consumed in the form of fermented food, dairy products and more recently, infant and toddler formula. [33, 40] Probiotics can also be added to other foods such as cereals, biscuits, soy milk and sausages. [41 - 44]

Not all bacteria qualify as probiotics. There are minimum requirements for bacteria to be classified as a probiotic for human use. The bacteria must be: of human origin and completely identified to determine the genus, species and strain. They must also undergo: *in vitro* tests to screen for activity and safety; and *in vivo* studies to substantiate health effects in the target host. [30] Guidelines from the Food and Agriculture Organisation (FAO) and World Health Organisation (WHO) state that to qualify as a probiotic, a strain of bacteria must: be resistant to gastric acidity; be resistant to bile; exhibit bile salt hydrolase activity; adhere to mucus and / or human epithelia cells; have antimicrobial activity against potential pathogenic bacteria; and have the ability to reduce pathogenic adhesion surfaces. For vaginal probiotics, the bacteria must be able to resist spermicides. [45, 46] Up to 56 species of lactobacillus and 29 species of bifidobacteria have been identified (Shah 2007) Examples include: *Lactobacillus acidophilus* NCFM, *Lactobacillus acidophilus Johsonii* La1, *Lactobacillus casei* Shirota, *Bifidobacteria lactis* Bb-02, *Bifidobacteria infantis* Shirota, *Bifidobacteria longum* BB536

Most probiotics are registered as food supplements, therefore these do not have to meet the quality requirements for medicines. It is now mandatory to do research on the mechanisms of action of specific strains. Clinical trials with commercial products, with added probiotics are also mandatory. [30] This is because the effects conferred by probiotic bacteria are strain specific. The *in vivo* effects of one type of strain may be opposite to those shown *in vitro*. Therefore, effects demonstrated by one strain cannot be extrapolated to other strains. [30, 32, 40]

1.4.1 Mechanism of action of probiotics

Probiotics use several ways to exert their effects. However, no single strain of probiotics will use all the mentioned mechanisms of action to exert its effects on the host. [30, 40, 47] A few of the mechanisms of action of probiotics relating to its effects on the gastrointestinal tract are briefly summarised below.

Strengthening the epithelial tight junctions

The epithelial tight junctions are a major component of the intestinal barrier function. They act as a physical and functional barrier against the penetration of bacteria and macromolecules from the lumen. This prevents bacteria from gaining access to the sub-mucosa or even the circulation and causing illnesses such as sepsis.

The tight junctions consist of four types of proteins: occludins, claudens, tri-cellulin and junction adhesion molecules. A disruption of tight junctions is usually the beginning of many diseases. [48] Probiotics (such as *L. plantarum*) act on tight junctions by inducing the production of occludins and actinins. As a result, intestinal permeability is reduced. [31, 34]

Mucus production

The gut epithelium is covered by a gel-like mucus layer composed of mucins. Mucins are heavy molecular weight proteins secreted by goblet cells located throughout the entire length of the intestines. The mucus layer provides protection from pathogens, enzymes, toxins, dehydration and abrasion. [49] Certain strains of probiotics (such as *L. plantarum, L. rhamnosus*) stimulate the production of MUC2, MUC3 intestinal mucins. This decreases adherence of pathogens to intestinal epithelial cells, preventing bacteria translocation from the lumen. [34, 50]

Enhancing gut immune system

Immunoglobulin-A (IgA), resistant to protease plays a crucial role in binding pathogens in the mucus layer, because IgA binds to mucins. This decreases the pathogen access to epithelial cells. [31, 34, 47, 51, 52] Probiotics increase IgA production and secretion by altering the cytokine environment in the gut mucosa. Probiotics induce the production of interleukin-10 (IL-10) and interleukin-6 (IL-6), enhancing IgA production. [49] Inducing the production of IL-10 results in an anti-inflammatory or suppressive response and IL-6 elicits a pro-inflammatory response.

Regulation of appropriate bacterial colonisation

Probiotics produce antibacterial substances, such as bacteriocins and microcins with bacteriocidal (kills bacteria) and bacteriostatic (inhibits growth) activity against pathogens. Bacteriocins and microcins are produced in a strain-specific manner by probiotics and commensal bacteria. Bacteriocins also act as signalling molecules to other bacteria and to the immune system. The presence of bacteriocins ensures the sustained presence of beneficial bacteria in the gut. Examples of bacteriocins include lactocidin, acidolin, acidophilin, lactacium-B produced by *L. acidopohillus*, bifidolin and bifilong produced by bifidobacteria. [49, 51, 53] Both bacteriocins and microcins ensure there is rapid bacteria death, maintaining an intestinal barrier free of pathogens. [53] Probiotics also produce defensins, which are antibacterial peptides rich in cysteine. Defensins disrupt the cytoplasm in susceptible pathogens. Probiotics such as Escherichia coli Nissle 1917 stimulate the production of human beta-defensins. [49]

Probiotics also produce short chain fatty acids (SCFAs) including lactic acid, acetic acid, propionic acid and butyrate, lowering the pH of the gut, thus disrupting pathogen growth. SCFAs also disrupt the outer membranes of gram negative pathogens, inhibiting their growth. [34, 51, 53]

Probiotics also have the ability to adhere to the intestinal epithelial cells. They compete with pathogens for attachment sites and nutrients. This decreases the availability of attachment sites, decreasing the adhesion of pathogens and their toxins to the gastrointestinal tract. Ultimately this disrupts colonisation of the gut by pathogens. [31, 34, 47, 51]

Modulation of the immune system

The effects of probiotics on the immune system are well documented. The effects are strain specific and vary widely with complex chemical pathways; these are beyond the scope of this document. In general, probiotics stimulate a decrease in the production of pro–inflammatory cytokines, such as tumour necrosis factor – alpha (TNF-α), interferon – gamma (IFN-γ) and interleukin – 12 (IL-12). Probiotics also increase the production of anti-inflammatory cytokines, such as interleukin -10 (IL-10), which is produced by many cell types, such as monocytes, Th2 cells, B cells or Treg cells. Probiotics have antimicrobial properties; they induce an increase in the phagocytosis capacity of macrophages and natural killer cells (NK cells). [34]

1.4.2 Minimum dose of probiotics required for an effect

It is not possible to accurately state a minimum dose of probiotic needed for an effect. The required dose varies for different strains and for the specific health condition under investigation. A daily dose of probiotic is needed for any measurable effect. There is consensus that doses between 10^6 and 10^9 colony forming units per day (cfu/ day) are required. Some probiotics show positive effects at 10^8 cfu / day, while others are more effective at 10^{12} cfu /day. [33, 34, 54]

1.4.3 Safety of probiotics

Probiotics have been granted GRAS (generally regarded as safe) status by the Food and Drug Administration (FDA) due to the long history of safe use, and the bacteria used in the probiotic preparations are identical to those found in the gastrointestinal and vaginal flora. [30, 55, 56]

In healthy people, probiotics rarely cause disease. Systemic infections such as endocarditis, fungaemia have been reported but are extremely rare. Bacteraemia from ingested lactobacilli occurs in fewer than one in one

million users; and fungaemia (from Saccharomyces Boulardii) occurs in fewer than one in 5.6 million users. [57, 58] There are reported cases of bacteraemia, sepsis or endocarditis, occurring in immune-compromised or severely debilitated people.. Bacteraemia (from *lactobacillus*) and fungaemia (from *saccharomyces Boulardii*) have occurred in children with underlying risk factors such as immunodeficiency, short gut syndrome and antibiotic related diarrhoea. [59, 60] In these cases, the probiotic causing the disease is often from other sources, such as contaminated catheters and not from the consumed probiotic. [59, 60] In infants, risk factors associated with sepsis from probiotics include: prematurity, presence of central venous catheters, impairment of the intestinal epithelial barrier and concurrent administration of broad spectrum antibiotics to which the probiotic is resistant. [30, 61] There is consensus that caution should be exercised when using probiotics in individuals with these risk factors. [62, 63]

In a recent review, the ESPGHAN Committee on Nutrition reviewed the evidence on probiotic use in infants. The committee concluded that "the available data on probiotic supplemented infant formula for infants, does not raise any safety concerns on growth and adverse events in healthy infants." [64, 65] The safety and efficacy of each probiotic strain, however must be tested separately. [60] This is because the health effects and safety profile of one probiotic strain cannot be extrapolated to another strain. [60, 64]

1.5 Prebiotics

Fructooligosaccharide (FOS) and inulin are added to different foods as fat and sugar replacements to improve texture, or for their functional benefits. [30, 31] Inulin occurs naturally in food such as onions, garlic, leeks, artichokes, asparagus, wheat, oats and soybeans. [37] Formula companies also add prebiotics to infant formula. [35, 66] For an ingredient to be classified as a prebiotic, it must be resistant to gastric acidity, hydrolysis by mammalian enzymes and absorption in the upper gastrointestinal tract. It must also be fermented by beneficial bacteria in the intestine, selectively stimulating the growth and or activity of colonic microflora, resulting in a healthier composition. [36, 37, 38]

1.5.1 Mechanism of action of prebiotics

Most RCTs on prebiotics have used fructans, such as fructooligosaccharide, inulin and oligosaccharides. [67] The mechanism of action of these fructans on the GI are briefly described below.

Short chain fatty acids (SCFAs)

Prebiotics are not digested or absorbed in the upper gastro intestinal tract. Once they reach the colon, prebiotics are selectively fermented by the residential bacteria into SCFAs, such as acetic acid, propionic acid and butyrate. The SCFAs are not toxic to the host but are an essential fuel for the epithelial cells. Prebiotics are selectively fermented, because each bacteria genus or strain has a preferred substrate. Most strains of bifidobacteria and lactobacillus ferment fructans (such as fructooligosaccharides) more efficiently than glucose. [31, 36] An increase in short chain fatty acids results in a decrease in pH of the lumen, inhibiting pathogen growth. . [36] Prebiotics selectively stimulate the growth and / or activity of colon microflora towards a healthier composition by increasing bifidobacteria and lactobacillus levels. [36, 68]

Immune modulation

There are many complex mechanisms by which prebiotics modulate the adoptive and innate immune system. However, the human gastrointestinal (GI) immune system is inaccessible, resulting in human studies relying on *ex-vivo* systemic immune markers. [38] In general, consumption of prebiotics, such as galactooligosaccharides(GOS), fructooligosaccharide (FOS) and inulin, significantly increases phagocytosis, natural killer cells activity, increasing production of anti-inflammatory cytokines, such as IL-10 and reducing pro-inflammatory cytokines, such as IL-1β, IL-6, TNF-α. [38, 68]

Disruption of attachment by pathogens and improved intestinal architecture

Prebiotics inhibit the adherence of pathogens to the epithelial cells preventing colonisation. [36] For infants, prebiotics stimulate the growth of only beneficial bacteria, in the gastrointestinal tract, to the levels found in breastfed infants. [69 - 71] As these beneficial bacteria increase, they exclude pathogens; the gut mucosal barrier improves preventing infections with enteric pathogens or trans-located gut bacteria. [31, 66, 72] Prebiotics also improve the intestinal architecture by increasing villi height, thicker mucus layer, deeper crypts and increased globlet cells, which improves intestinal permeability. [73, 74]

Enhanced mineral absorption

Prebiotics enhance the absorption of minerals, including calcium, magnesium and iron. [67, 68] An increase in SCFAs and the corresponding decrease in lumen pH, calcium solubility is improved, resulting in its increased bioavailability. Ultimately, this leads to improved bone health (bone calcium content and bone mineral density). [37, 38, 67, 68] In addition, with the improved intestinal architecture (increased villi height, deeper crypts), the surface for mineral absorption is greatly improved and increased. [38]

Stool effects

Consumption of prebiotics increases stool bulk, by 1.5 to 2 grams for each gram of non-digestible oligosaccharides. Prebiotics also normalize or increase stooling frequency (non –diarrhoea). [68]

1.5.2 Minimum dose of prebiotics required for an effect

A dose of 4 – 20 g / day of FOS or inulin, significantly increases bifidobacteria counts. [75] The stool bulking index for FOS or inulin is 1.5 to 2 grams of stool per gram consumed. Stool bulking index is an expression of increase in daily faecal mass. [66]

1.5.3 Safety of prebiotics

Prebiotics have a good safety record at levels found in existing food components. Cases of flatulence or abdominal bloating are reported at doses greater than 20 g / day. Abdominal cramps or diarrhoea are reported at doses greater than 50 g / day. [73, 74] In a recent review, the ESPGHAN Committee on Nutrition reviewed the evidence on prebiotic use in infants. The committee concluded that "the available data on prebiotic supplemented formula given to healthy infants indicated that prebiotics do not raise any safety concerns on growth and adverse events." However, the clinical effects of one prebiotic cannot be extrapolated to another prebiotic. [64]

1.6 Evidence of probiotics, prebiotics and synbiotics effects on gastrointestinal tract in infants

1.6.1 Description of evidence

Most randomized controlled trials (RCTs) on probiotics, prebiotics and synbiotics use in infants published from 1980 to 2010 varied in: intervention (RCTs used different strains and doses of probiotics, used GOS, FOS, inulin or different combinations of these prebiotics); duration (most studies had short treatment durations), methodological quality and small sample sizes. There were very few studies using synbiotics on infants, most RCTs used probiotics or prebiotics. [64, 76]

Numerous RCTs on probiotics and prebiotics were conducted on infants from 1980 to the 2010. Similarly, there are numerous systematic reviews using these published (and unpublished) RCTs. The Oxford Centre for Evidence- based Medicine guidelines state that systematic reviews of randomized clinical trials offer the

highest level of evidence for information on the effectiveness of an intervention, followed by RCT(s). [77 - 79] The results from the RCTs and the systematic reviews on probiotics, prebiotics and synbiotics use in infants can be conflicting and confusing.

Several systematic reviews report that probiotics and prebiotics have had a significant effect on clinical outcomes. Systematic reviews on full term infants given probiotics show different strains of probiotics improve stool consistency, frequency and support normal growth [64]. While other recent systematic reviews (published from 2007 to 2011) focused only on the prevention of allergic disease, food hypersensitivity, upper respiratory tract infections, antibiotic associated diarrhoea and acute infectious diarrhoea. Some conclude there is insufficient evidence that probiotics or prebiotics prevent these conditions [80 - 82], whereas others report positive effects [83 - 84]. [Table 1]

Reviews on preterm infants are equally conflicting and confusing. One review reports that administration of probiotics **did** result in reduced risk of Necrotising Enterocolitis and mortality [85], whereas a recent systematic review reported that supplementation **did not** result in decreased incidence of NEC, late onset sepsis or time to full enteral feeds. [86] A third review reported supplementation with probiotics or prebiotics **had no** significant advantage over standard formula or placebo. [87]

1.6.2 Routine use of probiotics and prebiotics in infants

Some systematic reviews and research groups support routine supplementation with probiotics and prebiotics in infants, whereas others do not support routine supplementation. This also causes confusion. For example, the Cochrane Neonatal Review Group states that "the current evidence on probiotics to prevent Necrotising Enterocolitis (NEC) supports the routine administration of probiotics in preterm infants." [34] Other systematic reviews do not support routine supplementation with probiotics or prebiotics in infants. (Table 1) For full term infants there is a lack of: consistent clinical effects in early infancy (less than 4 months of age); heterogeneity of studies (methodological quality, types of probiotics used, duration and doses of intervention used); improved growth and clinical outcomes. [64] For preterm infants, there is no conclusive evidence to support routine use since the effects of probiotics on NEC and mortality needs to be reconfirmed, using large adequately powered RCTs. For example, Mihatsch proposes an RCT consisting of at least 714 infants per study group is required to show that an intervention reduces the incidence of NEC by at least 50%. [87]. An RCT of this size on preterm infants given probiotics has not been conducted to date. In addition, there is still no convincing evidence that probiotics prevent sepsis in preterm infants. [87]

Despite three decades of research using probiotics, prebiotics and recently synbiotics in infants, there are no probiotic products approved for routine use in preterm infants by regulatory agencies such as the Federal Drug Administration (FDA) in United States, or the Therapeutic Goods Administration of Australia. [34]

However, the FDA has approved a probiotic supplemented formula for full term infants. [88] In Southern Africa, infant formula with probiotics, prebiotics and synbiotics are sold directly to the public in retail outlets.

1.6.3 Rationale for addition of probiotics, prebiotics or synbiotics to infant formula

There is evidence that to achieve optimal health and growth, a healthy intestinal micro-flora in infants (preterm, low birth weight and full term infants) is necessary. [89] For infants who are not breastfed, there is a rationale to adapt infant formulas to promote an intestinal microbiota resembling that of breastfed infants. It has a greater concentration of bifidobacteria and fewer potentially pathogenic bacteria than formula fed infants. Strategies to achieve this goal include the addition of probiotics, prebiotics or synbiotics to infant formula for full term and preterm infants to improve growth, development and decreasing infection. [33, 76] Adding these ingredients to infant formula changes the intestinal microbiota.[39, 90] Adding prebiotics to formula stimulates the growth of only beneficial bacteria in the gastrointestinal tract, to levels found in breastfed infants. [69, 70, 71]

Table 1. Summary of systematic reviews of probiotics, prebiotics in infants and children

Author, Year of publication	Aim /objective of review	Methodology, Inclusion criteria, N =	Conclusion(s)
AlFaleh 2011 [85]	To compare the efficacy and safety of prophylactic enteral probiotics administration versus placebo or no treatment in the prevention of severe NEC and/or sepsis in preterm infants.	**Methodology** Review followed guidelines from the Cochrane Collaboration and its neonatal group. **Inclusion criteria** Only randomized or quasi-randomized controlled trials that enrolled preterm infants < 37 weeks gestational age and / or < 2500 g birth weight were considered. **Intervention** Enteral administration of any live microbial supplement (probiotics) at any dose for more than seven days compared to placebo or no treatment. **Participants:** Preterm infants < 37 weeks and / or birth weight < 2500 g. **Results** • N=16 trials. • Enteral probiotics supplementation: • Significant reduction in the incidence of severe NEC typical RR 0.35, 95% CI 0.24 to 0.52 and mortality RR 0.40, 95% CI 0.27 to 0.60. • No significant reduction of nosocomial sepsis RR 0.90, 95% CI 0.76 to 1.07 • No reported systemic infection with the probiotics supplemental organism. Statistical test of heterogeneity for NEC, mortality and sepsis was insignificant.	• Enteral supplementation of probiotics prevents severe NEC and all-cause mortality in preterm infants. • This updated review of available evidence supports a change in practice. • More studies are needed to assess efficacy in ELBW infants and assess the most effective formulation and dose to be utilized.
Allen 2010 [84]	To assess the effects of probiotics in proven or presumed acute infectious	**Methodology** Review followed guidelines from the Cochrane	• Used alongside rehydration therapy,

	diarrhoea.	Collaboration. **Inclusion criteria and intervention** Randomized and quasi-randomized controlled trials comparing a specified probiotic agent with a placebo or no probiotic in infants, children and adults with acute diarrhoea that is proven or presumed to be caused by an infectious agent. **Participants:** Infants, children, adults **Results** N= 56 • No adverse events were attributed to the probiotic intervention. • Probiotics reduced the duration of diarrhoea, although the size of the effect varied considerably between studies. • Mean duration of diarrhoea (MD 24.76 hours; 95% CI 15.9 to 33.6 hours; n=4555, trials=35) diarrhoea lasting 4 days (RR 0.41; 0.32 to 0.53; n=2853, trials=29) and stool frequency on day 2 (MD 0.80; 0.45 to 1.14; n=2751, trials=20). • Differences in effect size between studies was not explained by study quality, probiotic strain, the number of different strains, the viability of the organisms, dosage of organisms, the causes of diarrhoea, or the severity of the diarrhoea, or whether the studies were done in developed or developing countries.	probiotics appear to be safe and have clear beneficial effects in shortening the duration and reducing stool frequency in acute infectious diarrhoea. However, more research is needed to guide the use of particular probiotic regimens in specific patient groups.
Bragger 2011 [64]	Systematically reviews published evidence related to safety and health effects of administration of formula supplemented with probiotics and / or prebiotics compared to un-supplemented formula	Review followed guidelines from the Cochrane Collaboration. **Inclusion criteria and intervention** Studies that compared use of infant formula or follow-on formula supplemented with probiotics and or prebiotics	**Probiotics** • Probiotics do not raise safety concerns with regard to growth and adverse effects. • Safety of 1 probiotic cannot be extrapolated to others. • There is lack of data on long term

		during manufacture process. **Participants:** Healthy Term infants **Results** Probiotics: N = 20 studies Prebiotics: N = 23 studies Synbiotics: N = 3 studies **Probiotics** Summary and interpretation of data effects of probiotic supplementation of infant formula on: Growth: Interpreting studies on the effects of probiotic on growth is difficult due to the limited number of studies, which were with insufficient power to identify relevant effects on growth, follow-up periods were short. In general, for a few probiotic strains that were used support normal growth in healthy term infants GI infections: Limited evidence shows (*B lactis;BL999* and *LPR*) does not reduce the risk of GI infections. Respiratory infections: Limited available evidence shows *B lactis* does not reduce the risk of respiratory infections. Antibiotic use Limited available evidence suggests that BL999 and LPR is not associated with a reduced use of antibiotics. Colic / irritability The administration of *B lactis, BL999* and *LPR, L reuteri,* or *LGG* was not associated with a lower frequency of colic,	effects of probiotic supplementation. Therefore ESPHGAN committee does not recommend probiotics for routine use in infants **Prebiotics** • Prebiotics do not raise safety concerns. • Effect of 1 prebiotic cannot be extrapolated to others. • There are some benefits (increased stool frequency, stool softening). • There is a lack of data on long term effects of prebiotic supplementation. • Therefore ESPHGAN committee does not recommend prebiotics for routine use in infants. **Synbiotics** • Only a few synbiotics preparations have been studied**,** there are no associated adverse effects. • Efficacy and safety of synbiotics need to be established. • Therefore ESPHGAN committee does not recommend prebiotics for routine use in infants.

		crying, or irritability. Allergy Limited data available suggest no effect of the probiotics studied (*BL999* and *LPR*) on allergy. Stool consistency LGG, but not *B lactis* or *L reuteri ATCC 55730* or *BL999* and *LPR*, administration had a modest, statistically significant effect on stool consistency. Clinical significance of this effect is unclear. There is too much uncertainty to draw reliable conclusions from the available data on the effects of probiotics on GI infections, respiratory infections, antibiotic use, colic / irritability and allergy **Prebiotics** Summary and interpretation of data effects of prebiotic supplementation of infant formula on: Growth: Interpreting studies on the effects of prebiotics on growth can be difficult because few studies have analysed the effects on growth, studies were small with insufficient power, the follow-up periods were short. Prebiotic supplementation with a mixture of GOS/FOS, has no adverse effects on growth in healthy term infants, but the effect on improved growth is modest. Stool pH Prebiotic supplementation has the potential to reduce faecal pH, whether this reduction in faecal pH per se is of benefit to the infants is currently not established.	

		Stool frequency Prebiotic supplementation has the potential to increase stool frequency but the clinical significance of this is unclear. Stool consistency Prebiotic supplementation has the potential to soften stools but clinical significance of this finding is unclear. **Synbiotics** A limited number of synbiotic preparations in infant formulae in context of a formal RCT The available data suggest that the products are safe, but caution in over interpretation of the results. The efficacy and safety of each synbiotic product should be established.	
Hao 2011 [83]	To assess the effectiveness and safety of probiotics for preventing acute URTIs.	**Methodology** Review followed guidelines from the Cochrane Collaboration. **Inclusion criteria** Randomised controlled trials (RCTs) to prevent acute URTIs. **Intervention** Any probiotic (single or mixture of strains, any dosage regimen and any route of administration) for more than seven days compared to placebo, or no treatment. **Participants** Children and adults of all ages. **Results**	• Available evidence shows that probiotics are better than placebo in reducing the number of participants experiencing acute episodes of upper respiratory tract Infection. (URTI), the rate ratio of episodes of acute URTI and reducing antibiotic use, although there were no data concerning older people in the review.

		• N=14 • Probiotics were better than placebo in the number of participants experiencing episodes of acute URTI: • One episode URTI: odds ratio (OR) 0.58; 95% CI 0.36 to 0.92; • Three episodes URTI: OR 0.53; 95% CI 0.36 to 0.80; • Rate ratio of episodes of acute URTI: rate ratio 0.88; 95% CI 0.81 to 0.96; • Reduced antibiotic prescription rates for acute URTIs: OR 0.67; 95% CI 0.45 to 0.98. • Probiotics and placebo were similar when measuring the mean duration (MD) of an episode of acute URTI: MD -0.29; 95% CI -3.71 to 3.13 and adverse events: OR 0.92; 95% CI 0.37 to 2.28. • Side effects of probiotics were minor and GI symptoms were the most common. • Some subgroups had a high level of heterogeneity in the pooled analyses.	
Mihatsch 2012 [87]	Systematically analyse the level of evidence of published RCTs on probiotics in preterm infants	Review used the levels of evidence-based single or meta analyses scored following the Oxford Centre for Evidence based Medicine guidelines. Methodological quality of RCTs assessed using the Cochrane Collaboration's risk of bias tool. **Inclusion criteria** RCTs that had studied clinical outcomes: NEC, mortality, sepsis, feeding advancement or neurodevelopmental long term follow-up. **Intervention:** RCTs using terms: Infants, neonates, probiotic(s), prebiotic(s), lactobacillus(i), bifidobacterium(a), saccharomyces, Ecoli Nissle **Participants:** Preterm infants, **Results:**	• No conclusive level of evidence on which to base a general recommendation for routine use of probiotics in preterm or very low birth weight infants. • Available strains do not permit a decision to be made on optimum strains, dosing or protocol. • It is beyond the available evidence to decide if a single strain, or multiple strain products are more effective. • Data on one strain cannot be applied to another strain. • Safety and efficacy of each probiotic strain has to be proven separately. • Large adequately powered, multi-centre RCTs are required to reconfirm available results,

		• N = 15 studie: Two 1b Levels of Evidence (LoE) trials and thirteen 2b LoE trials. • Methodological assessment revealed considerable heterogeneity. • Some probiotics may be beneficial in relation to reduction of severe NEC (2b LoE) and reduction of mortality (2b LoE). • Probiotics do not accelerate feeding advancement (1b and 2b LoE). • There was no convincing benefit with regard to prevention of sepsis (1b and 2b LoE).	especially RCTs with severe NEC (Bell stage≥ 2) or mortality as primary outcomes.
Osborn 2007 [81]	To determine the effect of **probiotics** given to infants for the prevention of allergic disease or food hypersensitivity.	**Methodology** Review followed guidelines from the Cochrane Collaboration. **Inclusion criteria** Randomised and quasi-randomised controlled trials comparing the use of a probiotic to no probiotic; or the use a specific probiotic compared to a different probiotic; or a probiotic with added prebiotic to control. **Intervention** Probiotics added to human milk or infant formula, added in the manufacturing process or given separately compared to control (placebo or no treatment) **Participants** Enterally fed infants in the first six months of life, without clinical evidence of allergic disease or food hypersensitivity, both with and without risk factors for allergy and food hypersensitivity **Results** • N= 12 • Studies had adequate randomisation, allocation	• Insufficient evidence to recommend the addition of probiotics to infant feeds for prevention of allergic disease or food hypersensitivity. • There was a reduction in clinical eczema in infants but this effect was not consistent among studies.

		concealment and blinding of treatment. • Treat finding with caution due to excess losses in patient follow-up (17% to 61%). • Significant reduction in infant eczema (typical RR 0.82, 95%CI 0.70, 0.95) but there was significant heterogeneity between studies. • One study reported the difference in eczema between groups persisted to 4 years age. When analysis was restricted to trials reporting atopic eczema (confirmed by skin prick test or specific IgE), the findings were no longer significant (typical RR 0.80, 95% CI 0.62, 1.02). • Studies reporting significant benefits used *L. rhamnosus* and enrolled infants at high risk of allergy. • No other benefits were reported for any other allergic disease or food hypersensitivity outcome.	
Osborn 2013 [80]	To determine the effect of **prebiotics** given to infants for the prevention of allergic disease or food hypersensitivity.	**Methodology** Review followed guidelines from the Cochrane Collaboration. **Inclusion criteria** Randomised and quasi-randomised controlled trials that compared the use of a prebiotic to a control (placebo or no treatment); or used a specific prebiotic compared to a different prebiotic. **Intervention** Prebiotics added to human milk or infant formula, whether added in the manufacturing process or given separately compared to control (placebo or no treatment), or a different prebiotic. **Participants:** Infants in the first six months of life without clinical evidence of allergic disease or food hypersensitivity, both with and without risk factors for allergic disease.	• Further research is needed before routine use of prebiotics can be recommended for prevention of allergy in formula fed infants. • There is some evidence that a prebiotic supplement added to infant feeds may prevent eczema. • It is unclear whether the use of prebiotic should be restricted to infants at high risk of allergy or may have an effect in low risk populations; or whether it may have an effect on other allergic diseases including asthma.

		Results • N=4 studies • No significant difference in infant asthma but significant heterogeneity was found between studies. • Significant reduction in eczema: typical risk ratio 0.68, 95% CI 0.48 to 0.97; typical risk difference -0.04, 95% CI -0.07 to -0.00; number needed to treat to benefit (NNTB) 25, 95% CI 14 to > 100; P = 0.03). No significant heterogeneity was found between studies. • One study reported no significant difference in urticaria. • No significant subgroup differences in to infants at risk of allergy or type of infant feed. • Individual studies reported a significant reduction in asthma and eczema from supplementation with a mixture of GOS/FOS 9:1 ratio, 8 g/L in infants at high risk of allergy • Significant reduction in eczema from supplementation with GOS/FOS (9:1) (6.8 g/L) and acidic oligosacccharide (1.2 g/L) in infants not selected for allergy risk.	
Srinivasjois 2013 [86]	To systematically review randomized controlled trials, evaluating the safety and efficacy of prebiotic oligosaccharide supplementation in preterm infants, with less than 37 weeks of gestation.	**Methodology** Guidelines from the Cochrane Neonatal Review group, PRISMA Statement, Centre for Reviews and Dissemination group were followed for conducting and reporting this systematic review. **Inclusion criteria** Randomised controlled trials and quasi-randomised trials published in any language were eligible for inclusion. **Intervention** Trials comparing formula milk supplemented with prebiotic OS vs placebo or un-supplemented formula milk. The prebiotic OS could be GOS, FOS, acidic	• Supplementation with prebiotic oligosaccharides was safe and did not result in decreased incidence of NEC, late onset sepsis and time to full enteral feeds but resulted in a significantly higher growth of beneficial microbes.

		oligosaccharide (AOS), inulin or lactulose. The supplementation should be for a minimum of 2 weeks. **Participants** Preterm infants with gestation < 37 weeks at birth. **Results** • N=7 • NEC: pooled RR (95% CI) of 1.24 (0.56 to 2.72), p 0.23 • Late onset sepsis: RR (95% CI) 0.81 (0.57 to 1.15) • 3 trials did not observe any improvement in time to enteral feeds post intervention. • Statistically significant difference in the growth of bifidobacteria in the oligosaccharide group with a weighted mean difference of 0.53 (95% CI: 0.33, 0.73) *106 colonies/g, p < 0.00001. • A reduction in stool viscosity and pH was also observed. • None reported life threatening adverse effects.	

1.7 Rationale for study

1.7.1 Phase 1: Both systematic reviews on preterm and full term infants

A search of electronic databases, such as Medline, Embase, Scopus, using key term such as "probiotics, prebiotics, synbiotics, infants, preterm, premature, full term" reveals numerous studies on infants, using these ingredients. As a result, consumers, health care policy makers and providers are overwhelmed with large amounts of information on the topic of probiotics and prebiotics. Often, they do not have the time and resources to find, assess, interpret this evidence and incorporate it into health care decisions. A systematic review helps identify relevant research, assess the evidence, synthesize and present it in an accessible format. [91]

The effects of probiotics on premature intestinal microbiota have been varied, and the effects on weight gain are mixed. Prebiotic effects on the growth of premature infants also vary; the effects on feed tolerance and the risk of NEC in very low birth weight infants are yet to be assessed more closely. With premature infants, issues around safety, the risk of invasive infections with probiotic bacteria, optimal strains and dose regimens are yet to be examined closely. [40, 90, 92]

Past systematic reviews (published from 2005 to 2009) on the use of probiotics or prebiotics in preterm infants focused on the prevention of Necrotizing Enterocolitis and / or sepsis and the impact on diarrhoea. [82, 85, 93, 94] These published reviews were on studies that used breastmilk and mixed feeds (formula combined with breastmilk). The current reviews on preterm and full term infants focused on infants given only infant formula, with a focus on growth and clinical outcomes. The review on preterm infants further examined outcomes inadequately addressed by previous reviews, such as the impact of probiotics or prebiotics on intestinal permeability and gastrointestinal microbiota.

The combination of probiotics and prebiotics (known as synbiotics) in infant formula is said to ensure the survival of probiotics in the synbiotic mixture and stimulate the growth of selected indigenous bacteria. [92, 95, 96] The superiority of synbiotics compared to either probiotic or prebiotic has not been established. However, there is emerging data that the use of synbiotic mixtures leads to favourable health outcomes. The current review on full term infants examined the impact of synbiotics on growth, immune and gastrointestinal function compared to probiotics and prebiotics. A search of several databases, such as the Cochrane library revealed there were no systematic reviews listed on the use of synbiotics in full term infants. None of the reviews listed in other electronic databases such as PubMed compared the impact of

synbiotics infant formula to either probiotic or prebiotic containing infant formula in full term infants. In addition there were no reviews comparing the impact of probiotics to prebiotics in full term infants who were not breastfed.

1.7.2 Phase 2: Systematic review: Association between funding source, methodological quality and research outcomes

The relationship between financial sponsorship of biomedical research and published outcomes has been explored in numerous publications. [97] There is evidence that trials sponsored by industry are more likely to report results in favour of the sponsor's products. This is especially true for the phamaceutical industry. [98 - 102] A few reviews have explored the impact of funding by the food industry on outcomes of research studies. [103, 104] Two nutrition-related, systematic reviews found that industry-sponsored trials were more likely to report outcomes favourable to the sponsor. [103, 105]

A sponsors' financial risk is reduced significantly when only positive outcomes in a research trial are reported. This may create biases in design (methodology) and reporting of outcomes in research. In nutrition research, this type of bias could adversely affect public health; influence policy formulation; professional dietary guidelines; public health interventions design; and regulation of food product health claims. [103]

There was little information available on the impact of funding by the food industry on outcomes and methodological quality of probiotic research in infants. This review explored whether financial sponsorship by the food industry affected outcomes and methodological quality of published studies on synbiotics, probiotics or prebiotics in infants. Methodological quality of studies may be compromised when insufficient information is provided regarding sequence generation, allocation concealment, blinding, bias introduced from other sources and incomplete outcome reporting.

1.7.3 Phase 2: Descriptive study: Application of evidence by food industry

There was little information about how the food industry applied the knowledge and evidence gained from research on probiotics, prebiotics or synbiotics on the general paediatric population. This study explored what happens after the research trials using infant formula were completed, and the data published or unpublished. Based on the new scientific evidence: did the companies develop and market new probiotic, prebiotic or synbiotic containing infant formula, or improve on ones that are already sold on the market? There is little or no information on the differences between the study formula and the retailed product. This

study tried to ascertain the differences between the two, such as genera of probiotic used with product viability at the end of shelf life.

The consumer does not know or understand the meaning of scientific terms such as probiotic, lactobacillus, fructooligosaccharide, or inulin. Thus, there is a great need for clear information in a format the consumer can understand. It is not clear how the manufacturers of probiotics, prebiotics or synbiotics containing infant formula educate the consumers. This study explored the effectiveness and type of medium the infant formula manufacturers use to educate the consumers.

World Health Organization(WHO) guidelines on infant formula preparations recommends that water with a minimum temperature of 70^0 C should be used to minimize the risk of infections caused by *Enterobacter Sakazakii*, bacteria found in infant formula. [106, 107] However, two probiotic infant formula brands available in the Western Cape, South Africa retail outlets state that using water with temperatures above 40^0 C will compromise the natural cultures. This contradiction makes safety an area of concern. In addition, there is a lack of published evidence on clinical benefits from long term use of probiotic containing infant formula. [63] This study explored how infant formula companies address the contradiction to the WHO guidelines on formula preparation and safety issues of long term usage of probiotic infant formula.

1.8 Ethics

Ethics approval for the research project was sought from the Human Research Ethics Committee at the University of Stellenbosch, Faculty of Medicine and Health Sciences.

Information for the systematic reviews in Phase 1 and 2 consisted of completed RCTs reports that were published (unpublished RCTs were provided by the companies). This information was already in the public domain, therefore the systematic reviews were exempt from ethical review.

For the descriptive study (survey) in phase 2, the ethical issues considered were:

- Adequate written communication

  Each study participant was to receive a letter of invitation to take part in the study. The letter of invitation (in addition to the informed consent form) explained to all aspects of the study to study participants: objective, rationale for study, method of data collection, the requirements of a study participant and anonymity issues.

- Informed consent

Each study participant was expected to sign an informed consent form. The standard informed consent form for the University of Stellenbosch was adopted for this study. The informed consent form also had information on all aspects of the study.

- Participant confidentiality
  Participant confidentiality was ensured during all stages of the study. During data processing, only product and company name were to be used. In the final published report, only a list of participating companies was given; and all study participants were to be anonymously acknowledged for contributing to the success of the study. The following clause was to be inserted in the published study report to thank the participants:
  "We wish to thank all the people who participated in the study. Your insights contributed to the successful completion of this study."

Ethics approval was granted by the Human Research Ethics Committee at the University of Stellenbosch, Faculty of Medicine and Health Sciences. Reference numbers: N11/02/135 (for the entire research project) and N11/07/203 (for the descriptive study). [See Appendix 1]

1.9 Research questions, objectives, hypotheses.

The research questions, objectives, null hypotheses and study hypotheses for phase 1 and 2 are listed in tables 2 to 4 below:

Table 2. Phase 1: Research questions, objectives and hypotheses for systematic reviews

Phase 1: Systematic Reviews
General research question
Does the consumption of probiotics, prebiotics or synbiotics supplemented infant formula lead to improved clinical outcomes in infants?
Objectives
1. To assess if addition of probiotics, prebiotics and synbiotics to pre- term infant formula led to improved growth and clinical outcomes in preterm or low birth weight infants 2. To determine the effects of infant formula containing probiotics, prebiotics or synbiotics on clinical outcomes in full term infants 3. To explore if synbiotics are superior over probiotics or prebiotics

Null Hypothesis
Consumption of probiotics, prebiotics or synbiotics supplemented formula by infants has no distinct advantage over conventional infant formula.
Study Hypothesis
Consumption of probiotics, prebiotics or synbiotics supplemented formula leads to improved clinical outcomes in infants.

Table 3. Phase 2: Research questions, objectives and hypotheses for systematic review

Phase 2: Systematic review
General research question
Is there an association between source of funding and methodological quality, clinical outcomes and author's conclusions in trials using probiotics, prebiotics or synbiotics supplemented formula in infants?
Objectives
To compare the methodological quality and outcomes of industry sponsored trials versus non industry sponsored trials with regards to supplementation of synbiotics, probiotics and prebiotics in infant formula.
Null Hypothesis
The source of funding has no influence over methodological quality, study outcomes or authors' conclusions in research trials using probiotics, prebiotics or synbiotics supplemented formula in infants.
Study Hypotheses
1. The source of funding in research trials using probiotics, prebiotics or synbiotics supplemented formula in infants is associated with outcomes and authors' conclusions in favour of the sponsor's products. 2. Methodological qualities of non-industry sponsored trials are equivalent to industry sponsored trials.

Table 4: Phase 2 Continued: Research questions, objectives and hypotheses for descriptive study

Phase 2 continued: Descriptive study
General research question
How does the food industry apply the evidence gained through probiotics, prebiotics and synbiotics research trials on infants for the benefit of the general paediatric population?
Objective
To investigate how the infant food industry applies evidence gained through probiotic, prebiotic and synbiotic research trials on infants.
Null Hypothesis
The food industry does not implement evidence gathered through research trials that use probiotics, prebiotics or synbiotics in infants for the benefit of the general paediatric population.
Study Hypothesis
Evidence gathered through research trials that use probiotics, prebiotics and synbiotics in infants is implemented by the food industry for the benefit of the general paediatric population.

1.10 Scope of work:

1.10.1 Phase 1: Two systematic reviews and meta-analysis were conducted.

The first systematic review was on pre-term infants given probiotics, prebiotics containing formula. The review explored effects of probiotics and prebiotics, on growth and clinical outcomes. All randomized controlled trials (RCTs), irrespective of language, comparing pre-term infant formula containing probiotic(s) OR prebiotic(s) to conventional pre-term infant formula, with or without placebo amongst preterm infants born <37 weeks gestation or low birth weight infants weighing <2.5 kg at birth. All infants were hospitalized and receiving formula feeds and / or parenteral feed were considered.

The second systematic review was on full term infants given probiotics, prebiotics and synbiotics containing formula. The review explored the effects of probiotics, prebiotics and synbiotics on growth, and clinical outcomes in full term infants The review considered all randomized controlled trials (RCTs), irrespective of language, which compared the use of term infant formula containing synbiotics, probiotics or prebiotics to conventional infant formula, with or without placebo amongst healthy full term infants (>37 weeks gestation

or ≥ 2.5 kg birth weight, age: 0-12 months, with no disease, congenital abnormality, allergy or eczema) receiving formula feeds only.

Systematic reviews were conducted using Cochrane methodology. The two review processes were identical and began with literature searches in all languages using different electronic databases. RCTs published in non-English language journals were translated by independent translators familiar with the subject matter. A hand search was conducted on abstracts of major conference proceedings, cross checked references cited in RCTs and in recent reviews (published from 2005 to 2009) for additional studies not identified by electronic searches and specialty journals not included in any database. Two reviewers independently reviewed all abstracts and citations to identify potentially eligible studies. The full reports of eligible studies were retrieved and the pre-specified selection criteria applied independently, by two reviewers, using a study eligibility form. (See Appendix 2, 4) If more than one publication of a study existed, all reports of the study were grouped together under one study name. Any disagreements between the reviewers were resolved through discussion, or a third party was consulted. A third party was consulted regarding the eligibility of 8 studies (4 studies on the systematic review for preterm infants and 4 on the systematic review on full term infants).

The inclusion criteria for the systematic reviews included the following:

1. Study participants: Infants (preterm, low birth weight, full term infants) who were fed formula containing synbiotics, probiotics or prebiotics. The infants were: Preterm infants: less than 37 weeks gestation or less than 2500 g birth weight. Healthy full term infants: > 37 weeks gestation, age: birth to12 months, birth weight > 2.5 kg.

2. Type of studies: Randomized controlled trials (in all languages) comparing infant formula with probiotics, prebiotics or synbiotics to conventional formula, with or without placebo were included

Data was independently extracted by two reviewers using a pre tested data extraction form. (See Appendix 3, 5) The risk of bias of included studies was assessed according to the Cochrane Handbook for Systematic Reviews for Interventions in the following 6 components. 1) sequence generation; 2) allocation concealment; 3) blinding; 4) incomplete outcome data; 5) selective outcome reporting; and 6) other sources of bias. The extracted data were cross-checked and any differences resolved through discussion, or third party consultations. If necessary, trial authors were contacted for missing data, or for clarification on the methodology of their studies. Results for probiotic and prebiotic and synbiotic studies were analysed separately.

For continuous outcomes, the mean difference (MD) and corresponding 95% confidence intervals (CI) were calculated. For dichotomous outcomes, the risk ratio (RR) and corresponding 95% CI were calculated. Heterogeneity of the trials used in the review was assessed by visually inspecting the forest plots to detect overlapping confidence intervals and by performing a chi^2 test. A $p<0.1$ was considered statistically significant. An I-square test (I^2) was used to test for inconsistencies across studies. A statistician was consulted at every step of the review process. One reviewer drafted the manuscript and other reviewers critically reviewed the manuscript before publication.

1.10.2 Phase 2: Two studies were conducted: a systematic review and a descriptive (survey) study

Systematic review

All randomized controlled trials (RCTs) conducted from 1980 to 2012 (irrespective of language) on synbiotics, probiotics, or prebiotics added to infant formula were included. Study participants were healthy full term infants (>37 weeks gestation or > 2.5 kg birth weight, 0-12 months old); preterm infants (born < 37 weeks gestation); low birth weight (<2.5 kg at birth); and extreme low birth weight infants (<1000 g at birth). Infants were fed either infant formula (preterm or full term formula); and mixed feeds (breast milk with infant formula) with added synbiotics, probiotics or prebiotics, or conventional infant formula, with or without placebo.

A literature search regardless of language was conducted on different electronic databases. The search strategy was modified for each electronic database. A hand search was conducted on: major conference proceeding abstracts; and cross-checked references cited in RCTs and in recent reviews (published from 2003 to 2012) for additional RCTs. These additional RCTs had not been identified by electronic searches and speciality journals which were not included in any database. To identify ongoing and unpublished trials, experts in the field and manufacturers of infant formula containing probiotics and prebiotics were contacted; web sites of companies that have conducted or were conducting RCTs on probiotics and prebiotics were searched. Prospective trial registries were searched. Two reviewers independently reviewed all abstracts and citations to identify potentially eligible studies. The full reports of eligible studies were retrieved and the pre-specified selection criteria applied independently by two reviewers using a study eligibility form. (See Appendix 6) If more than one publication of a study existed, all reports of the study were grouped together under one study name. Any disagreements between the reviewers were resolved through discussion or a third party was consulted. A third party was consulted regarding the eligibility and data presentation of 4 studies.

The inclusion criteria for the systematic review included the following:

1. Types of studies: Randomized controlled trials (irrespective of language) on synbiotics, probiotics, or prebiotics added to infant formula published from 1980 – 2012. Study participants: Healthy full term infants,

pre-term infants (born < 37 weeks gestation); low birth weight (<2.5 kg at birth); and extreme low birth weight infants (<1000 g at birth)

3. Intervention: The study group was fed infant formula; and mixed feeds (breast milk with infant formula) with added synbiotics, probiotics or prebiotics. The control group was fed conventional infant formula with or without placebo and without synbiotics, probiotics or prebiotics.

Data was independently extracted by two reviewers using a pre-tested data extraction form designed for this review. (See Appendix 7) The risk of bias of included studies was assessed, as described in the Cochrane Handbook for Systematic Reviews for Interventions, according to the following 6 components. 1) sequence generation; 2) allocation concealment; 3) blinding; 4) incomplete outcome data; 5) selective outcome reporting; and 6) other sources of bias. The extracted data, cross checked data and any differences were resolved through discussion, or a third party was consulted. Trial authors were contacted for missing data or for clarification. A total of 16 authors were contacted, only 3 replied with the requested data: Indrio 2008 [84], Indrio 2009 [85] and Mihatsch 2006 [90].

The source of funding or support of the RCTs was defined and categorized as industry, non-industry and none. The primary and secondary outcomes from each study report were independently evaluated and categorized by two reviewers as positive, negative and neutral. The authors' overall study conclusion and conclusions on reported clinical outcomes were evaluated and categorized as positive, negative, neutral and no clear conclusion. All the review outcomes were dichotomous and are described in frequencies and percentages. The association between funding source and methodological quality, clinical outcomes and author's conclusions were assessed using both the Pearson's Chi-square test and the Fisher's exact test. A statistician was consulted at every step of the review process. One reviewer drafted the manuscript and other reviewers critically reviewed the manuscript before publication.

Descriptive study

In the descriptive study (a survey), a structured questionnaire developed for the study was used. Companies that manufacture and / or market food products with added probiotics, prebiotics or synbiotics for infants and children were identified through several electronic databases, such as EBSCOhost, Business Source Premier and DATAMONITOR360. Company websites were searched to obtain their contact information. People responsible for research and development in the infant food companies were invited to participate in the survey, including clinical research managers and individual researchers. The inclusion criteria included only companies that manufacture infant formula and baby food that contained probiotics, prebiotics or synbiotics. There are few formula manufacturers and infant food companies, so all listed companies were invited to participate in the study.

A letter of invitation was sent to selected study participants, explaining all aspects of the study. (See Appendix 8) If they expressed willingness to take part in the study, a questionnaire with a written consent form was sent to them via post, email or fax. (See Appendix 9, 10) A maximum of four reminders were given to the participants to complete the questionnaire. Due to the expected small sample size, maintaining anonymity of study participants with the corresponding company name was difficult. Therefore, data processing was done according to product and company name. During report writing, all identifying details (name of study participant, product and company name) were excluded. Only the researcher and statistician had access to the data.

The questionnaire sent to companies focused on product specific questions, research-based questions, consumer education and safety issues. The questionnaire was validated for content by sending it to experts in the field of probiotics, prebiotics, infant nutrition and experience in research. The experts reviewed the study protocol, consent form and questionnaire. They then gave feedback on how to adopt the questionnaire in order to meet the study objectives. These experts did not participate in the study nor were they associated with the infant food industry. Data was entered into SPSS (Statistical Program for Social Sciences) for analysis. Descriptive statistics were used and associations between categorical variables tested using a Chi-square test. A $p<0.05$ was considered statistically significant. A statistician was consulted at every step of the study process. One author drafted the manuscript and other authors critically reviewed the manuscript before publication.

1.10.3 Exclusion criteria of research project

This research project excluded (both in Phase 1 and 2) the following:

1. Publications that were not RCTs such as commentaries, editorials, letters to the editor and studies that were not RCTs

2. Randomized controlled trials which included:

a. Infants with cardiac defects, pulmonary diseases, gastrointestinal diseases, major congenital abnormalities, chromosomal abnormalities or received other types of milk such as cow, buffalo, goat milk

b. Children above 1 year of age

c. Comparisons of synbiotic containing preterm infant formula to conventional preterm formula. At the drafting of protocol for this study and conducting of systematic reviews, there were no trials that had tested the use of synbiotics in preterm infants. Therefore the trials could not be included in the reviews, nor the effects of synbiotics on preterm infants be evaluated.

1.11 Chapter overviews

1.11.1 Chapter 2

Probiotics, prebiotics infant formula use in preterm or low birth weight infants: a systematic review

Chapter 2 is a systematic review and meta-analysis of preterm infants given probiotics and prebiotics containing formula; and was conducted using Cochrane methodology. The review explored the effects of probiotics and prebiotics on growth; and clinical outcomes not adequately addressed by previous reviews. The RCTs which were used compared preterm formula containing probiotic(s) OR prebiotic(s) to conventional preterm formula, in preterm infants. A total of eight studies were included and 27 studies were excluded.

Primary outcomes included short term growth parameters: weight gain (grams/day or grams/week); linear growth (centimeters/week) and head growth (cm/week). Secondary outcomes included complications: Incidence of NEC, sepsis, other infections and mortality / death. Adverse events during entire study duration: number of days on parenteral; number of days to full enteral nutrition; and maximal enteral feed (millilitres/day, millilitres/kilogram/day, millilitres /kilogram). Feed intolerance: of vomiting, gastric aspirates and abdominal distension incidences. Stool characteristics: stooling frequency and stool consistency as firm, loose or watery. Changes in intestinal permeability as measured by ratio of lactulose / mannitol in urine or other sugar absorption tests (such as lactulose / L – rhamnose ratio, D- xylose, 3-O- methyl-D-glucose tests). Gastrointestinal (GI) micro flora: number of colony forming units (cfu) of bifidobacteria, lactobacillus and pathogens post intervention.

This systematic review has been published in Nutrition Journal and is cited as:

Mugambi MN, Musekiwa A, Lombard M, Young T, Blaauw R. **Probiotics, prebiotics infant formula use in preterm or low birth weight infants: A systematic review.** *Nutr J* 2012, **11**:58 doi:10.1186/1475-2891-11-58

Involvement of the PhD candidate:

For this systematic review, the PhD candidate:

- developed and edited the protocol
- sought ethics approval before commencement of study
- developed study materials (study eligibility and data extraction form)
- identified studies by: conducting literature searches using electronic databases; hand search of conference proceedings; crossed-checked references cited in RCTs and systematic reviews
- contacted experts in the field for unpublished studies
- screened and selected RCTs using a study eligibility form
- extracted data using a data extraction form
- assessed risk of bias in included studies, as described in the Cochrane Handbook for Systematic Reviews for Interventions
- analysed data using Review Manager (RevMan 5) and interpreted results (later verified by statistician)
- developed, edited and critically reviewed the study manuscript for publication.

For this systematic review, the second reviewer:

- identified studies by: conducting literature searches using electronic databases; hand search of conference proceedings; crossed-checked references cited in RCTs and systematic reviews
- screened and selected RCTs using a study eligibility form
- extracted data using a data extraction form
- assessed risk of bias in included studies, as described in the Cochrane Handbook for Systematic Reviews for Interventions
- critically reviewed the study manuscript for publication.

1.11.2 Chapter 3

Synbiotics, probiotics or prebiotics in infant formula for full term infants: a systematic review.

Chapter 3 is a systematic review and meta-analysis of full term infants given probiotics, prebiotics or synbiotics containing infant formula; and focused on growth and clinical outcomes. The Cochrane methodology was also followed, using randomized controlled trials (RCTs), which compared term infant

formula containing probiotics, prebiotics or synbiotics to conventional infant formula with / without placebo, among healthy full term infants. A total of 25 studies were included.

Primary outcomes were: growth changes (assessed for entire study duration): weight gain (g/day); linear growth (cm/week, mm/month); and head growth (cm/week, mm/month).
Secondary outcomes were: tolerance to formula.
Stool characteristics were: frequency, consistency, diarrhoea.
Gastrointestinal symptoms were: colic incidences; spitting up/regurgitation, vomiting, crying and average formula intake (mls/day).
Infections: frequency and type of infections; use of medication (antibiotic intake);
Hospitalization: Number of days in hospital.
Changes in GI microflora: Changes in colony forming units (cfu/g of stool) of bifidobacteria, lactobacillus post intervention, colony forming units (cfu/g of stool) of pathogens post intervention.
Immune response: C- reactive protein levels (mg / dl), Interleukin 6 (IL-6) levels (mg/dl).

This systematic review has been published in Nutrition Journal and is cited as:

Mugambi MN, 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

Involvement of the PhD candidate:

For this systematic review, the PhD candidate:

- developed and edited the protocol
- sought ethics approval before commencement of study
- developed study materials (study eligibility and data extraction form)
- identified studies by: conducting literature search using electronic databases, hand search of conference proceedings, crossed-checked references cited in RCTs and systematic reviews
- contacted experts in the field for unpublished studies
- screened and selected RCTs using a study eligibility form
- extracted data using a data extraction form
- assessed risk of bias in included studies, as described in the Cochrane Handbook for Systematic Reviews for Interventions

- analysed data using Review Manager (RevMan 5) and interpretated results (later verified by statistician)
- developed, edited and critically reviewed the study manuscript for publication.

For this systematic review, the second reviewer:

- identified studies by: conducting literature searches using electronic databases; hand search of conference proceedings; crossed-checked references cited in RCTs and systematic reviews
- screened and selected RCTs using a study eligibility form
- extracted data using a data extraction form
- assessed risk of bias in included studies, as described in the Cochrane Handbook for Systematic Reviews for Interventions
- critically reviewed the study manuscript for publication.

1.11.3 Chapter 4

Associations between funding source, methodological quality and research outcomes in randomized controlled trials of synbiotics, probiotics and prebiotics added to infant formula: A Systematic Review

Chapter 4 is a systematic review exploring whether financial sponsorship by the food industry affected outcomes and methodological quality of published studies on synbiotics, probiotics or prebiotics used in infants. Cochrane methodology was used to assess the risk of bias of included studies in sequence generation, allocation concealment, blinding, incomplete outcome data, selective outcome-reporting and other sources of bias. From each trial, the primary and secondary outcomes, overall study conclusion and conclusions on outcomes were evaluated and categorized as positive, negative or neutral. Associations among funding source, bias risk, clinical outcomes and outcomes conclusions were evaluated.

Outcomes included funding source, methodological quality (risk of bias), clinical RCTs outcomes, author's conclusions (overall study conclusion) and conclusions on clinical outcomes. In addition, outcomes included the associations among funding source and methodological quality, clinical outcomes, author's conclusions and conclusions on clinical outcomes. A total of 67 studies were included.

This systematic review has been published in journal called BMC Medical Research Methodology and is cited as:

Mugambi MN, Musekiwa A, Lombard M, Young T, Blaauw R. **Association between funding source, methodological quality and research outcomes in randomized controlled trials of synbiotics, probiotics and prebiotics added to infant formula: A systematic review.** *BMC Med Res Methodol*. 2013, **13** (1) Article number 137

Involvement of the PhD candidate:

For this systematic review, the PhD candidate:

- developed and edited the protocol
- sought ethics approval before commencement of study
- developed study materials (study eligibility and data extraction form)
- identified studies by: conducting literature search using electronic databases, hand search of conference proceedings, crossed-checked references cited in RCTs and systematic reviews
- contacted experts in the field for unpublished studies,
- screened and selected RCTs
- extracted data using a pretested data extraction form
- assessed bias risk in included studies, as described in the Cochrane Handbook for Systematic Reviews for Interventions
- Analysed data using the SPSS version 19 and interpretation of results (later verified by statistician)
- developed, edited and critically reviewed the study manuscript for publication.

For this systematic review, the second reviewer:

- identified studies by: conducting literature search using electronic databases, hand search of conference proceedings, crossed-checked references cited in RCTs and systematic reviews
- screened and selected RCTs
- extracted data using a pretested data extraction form
- assessed bias risk in included studies, as described in the Cochrane Handbook for Systematic Reviews for Interventions
- critically reviewed the study manuscript for publication.

1.11.4 Chapter 5

Application of evidence on probiotics, prebiotics and synbiotics by food industry: A descriptive study.

Chapter 5 is a descriptive study (survey).The aim was to determine how the food industry applied the evidence gained through probiotic(s), prebiotic(s) and synbiotic(s) research on infants. A survey was conducted on companies manufacturing and / or marketing food products with added probiotics, prebiotics or synbiotics for infants.

The survey aimed to determine the following:

Application of evidence: Did new research evidence result in new infant formula products? What are the differences in study and retailed infant formula, what is the frequency of research using probiotics, prebiotics or synbiotics containing infant formula?

Publication of results: Did companies intentionally NOT publish study results viewed as: negative, or having no clinical benefit for infants? Was new research conducted to confirm the perceived negative results?

Medium for consumer education: What type and how effective was the medium used to educate the consumer? Was there a presence of bias in promoting formula feeding more than breastfeeding?

Compliance to WHO guidelines: How do companies comply with WHO guidelines on formula preparation with a focus on high water temperature and its effect on prebiotic, synbiotic containing infant formula?

Safety of long term use of probiotic or synbiotic containing infant formula: How do companies address safety in the context of there being little published evidence on the clinical benefits of long term consumption of probiotic containing formula (longer than 1 year)?

Product viability: Does formula remain viable throughout storage, or are there substantial changes in the number of colony forming units at the end of shelf life?

How companies keep abreast of the latest research on probiotics, prebiotics and synbiotics in infant formula and weaning foods: Do the formula companies have staff designated to track research, or is this on "*ad hoc*" basis?

A total of 25 major infant formula and baby food manufacturers were identified and invited to participate in this survey. No company agreed to participate in the survey for various reasons.

This descriptive study was submitted for publication to the journal titled BMC Research Notes on October 11th 2013 and is currently under peer review and is titled:

Mugambi MN, Young T, Blaauw R. **Application of evidence on probiotics, prebiotics and synbiotics by food industry: A descriptive study**

Involvement of the PhD candidate:

For this descriptive study, the PhD candidate did the following:

- developed and edited the protocol
- sought ethics approval before commencement of study
- developed study materials (letters of invitation to participate in study, questionnaire, informed consent form)
- identified formula companies through electronic databases
- acquired contact information through company websites
- extended invitations to study participants in all listed companies to participate in study
- dialogued with study participants and sent them study materials after they expressed willingness to participate
- developed, edited and critically reviewed the study manuscript for publication.

1.11.5 Chapter 6

Conclusion and recommendations chapter

Chapter 6 is a conclusion chapter which includes: a summary of findings of all the individual studies, limitations, conclusions from the different studies, an overall thesis conclusion, the summary of contributions, implications for practice and recommendations for further research in the field of probiotics, prebiotics and synbiotics in formula fed infants.

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Chapter 2: Probiotics, prebiotics infant formula use in preterm or low birth weight infants: A systematic review

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NUTRITION JOURNAL

REVIEW Open Access

Probiotics, prebiotics infant formula use in preterm or low birth weight infants: a systematic review

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Abstract

Background: Previous reviews (2005 to 2009) on preterm infants given probiotics or prebiotics with breast milk or mixed feeds focused on prevention of Necrotizing Enterocolitis, sepsis and diarrhea. This review assessed if probiotics, prebiotics led to improved growth and clinical outcomes in formula fed preterm infants.

Methods: Cochrane methodology was followed using randomized controlled trials (RCTs) which compared preterm formula containing probiotic(s) or prebiotic(s) to conventional preterm formula in preterm infants. The mean difference (MD) and corresponding 95% confidence intervals (CI) were reported for continuous outcomes, risk ratio (RR) and corresponding 95% CI for dichotomous outcomes. Heterogeneity was assessed by visual inspection of forest plots and a chi^2 test. An I^2 test assessed inconsistencies across studies. I^2> 50% represented substantial heterogeneity.

Results: Four probiotics studies (N=212), 4 prebiotics studies (N=126) were included. Probiotics: There were no significant differences in weight gain (MD 1.96, 95% CI: -2.64 to 6.56, 2 studies, n=34) or in maximal enteral feed (MD 35.20, 95% CI: -7.61 to 78.02, 2 studies, n=34), number of stools per day increased significantly in probiotic group (MD 1.60, 95% CI: 1.20 to 2.00, 1 study, n=20). Prebiotics: Galacto-oligosaccharide / Fructo-oligosaccharide (GOS/FOS) yielded no significant difference in weight gain (MD 0.04, 95% CI: -2.65 to 2.73, 2 studies, n=50), GOS/FOS yielded no significant differences in length gain (MD 0.01, 95% CI: -0.03 to 0.04, 2 studies, n=50). There were no significant differences in head growth (MD −0.01, 95% CI: -0.02 to 0.00, 2 studies, n=76) or age at full enteral feed (MD −0.79, 95% CI: -2.20 to 0.61, 2 studies, n=86). Stool frequency increased significantly in prebiotic group (MD 0.80, 95% CI: 0.48 to 1.1, 2 studies, n=86). GOS/FOS and FOS yielded higher bifidobacteria counts in prebiotics group (MD 2.10, 95% CI: 0.96 to 3.24, n=27) and (MD 0.48, 95% CI: 0.28 to 0.68, n=56).

Conclusions: There is not enough evidence to state that supplementation with probiotics or prebiotics results in improved growth and clinical outcomes in exclusively formula fed preterm infants.

Keywords: Probiotic, Prebiotic, Preterm infant, Low birth weight infant

Background

Growth is a major challenge for premature and low birth weight infants (born < 37 weeks gestation or with a birth weight of < 2500 g). They have several factors that put them at risk for nutritional deficiencies resulting in poor growth. Decreased nutrient stores result in low body stores of glycogen, fat, protein, fat soluble vitamins, calcium, phosphorus, magnesium and trace minerals. Preterm infants require increased energy and nutrients for rapid growth and may need a 10 fold increase in weight gain in order to achieve optimum catch up growth [1,2]. To achieve optimum growth for the preterm infant, the goals are to continue the process of intra-uterine growth in an extra-uterine environment until 40 weeks post conception, foster catch-up growth and nutrient accumulation in the post discharge period [3-6]. A weight gain of 15 to 20 g/ kg/day, length of 0.75 to 1.0 cm/week and head circumference 0.75 cm/week is required. This is difficult to achieve and requires between 130 – 135 kcal / kg /day to maintain this growth

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rate [3]. Furthermore, infants lose weight after birth (up to 6% to 8% for extreme low birth weight infants) and they often do not regain the weight for up to 1 to 2 weeks [5]. Daily growth monitoring (weight gain, linear and head circumference) then becomes vital.

Preterm infants have immature physiological systems due to an underdeveloped gastrointestinal barrier function as reflected by increased intestinal permeability. As a result, potentially pathogenic bacteria translocate from the intestinal lumen and cause systemic infections [7]. Reducing intestinal permeability is associated with gut maturation which promotes growth and avoids severe infections [4]. In addition, digestive and absorptive capabilities are decreased due to low concentration of lactase, pancreatic lipase and bile salts. Gastrointestinal motility and stomach capacity are decreased which limits feeding volume and gastric emptying. A coordinated suck and swallow is not developed until 32 to 34 weeks gestation. Introduction of enteral feeding maybe delayed due to increased risk of aspiration [1,2,8,9]. Preterm infants in neonatal intensive care units (NICUs) develop a different intestinal microbiota compared to healthy breast fed infants. This is due to decreased exposure to the maternal microbiota, increased exposure to organ- isms that colonize NICUs, multiple courses of antibiotics and delays in feeding [8,9].

Humans have consumed probiotics in the form of fermented food, dairy products and more recently infant and toddler formula. Probiotics are defined as "live microorganisms" which when administered in adequate amounts confer a health benefit to the host [10]. The main probiotic organisms used worldwide belong to the genera Lactobacillus and Bifidobacteria and are found in the gastrointestinal micro flora [10,11]. Prebiotics are found in fruit and vegetable components, they are non-digestible food ingredients that benefit the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon and improving the host's health [12,13]. The most widely studied prebiotics are inulin, fructo-oligosaccharide (FOS) and galacto-oligosaccharide (GOS) which are plant storage carbohydrates in vegetables, cereals and fruit. FOS and inulin are added to different foods as fat and sugar replacements to improve texture or for their functional benefits [12,14-16]. Probiotics and prebiotics are added to infant formula to promote an intestinal microbiota resembling that of breastfed infants which have a greater concentration of bifidobacteria and less pathogenic bacteria than formula fed infants [10,17].

There are a number of ways in which probiotics improve health. Health benefits conferred by probiotic bacteria are strain specific and not species or genus specific [10]. Probiotic bacteria improve health by affecting the immune system in different ways. They increase cytokine production such as Interleukin-6 (IL-6), Interferon- gamma (IFN-γ), Tissue Necrosis Factor – alpha (TNF-α), Interleukin-1beta (IL-1β) and Interleukin-10 (IL-10) [18]. Some strains increase phago- cytic activity of peripheral blood leukocytes (monocytes, polymorphonuclear cells). Other strains strengthen the mucosal barrier function by promoting the production of mucosal antibodies and reducing the trans mucosal transfer of antigens. This reduces the intestinal perme- ability which in turn promotes growth [19-22]. Probio- tics bacteria also enhance production of low molecular weight antibacterial substances produced by epithelial cells and production of short chain fatty acids, the main energy source for colonocytes. This maintains the integrity of colon mucosa [19,23-26].

Prebiotics are resistant to digestive enzymes and pH extremes found in the human gastrointestinal tract. They transit through the upper gastrointestinal tract and reach the colon intact where they are selectively fermented by indigenous bacteria, especially bifidobacteria and lactobacilli [12,15,26,27]. Beneficial bacteria (including bifidobacteria and lactobacilli) possess enzymes needed to metabolize prebiotics, while other bacteria (such as E coli, clostridia and salmonella) do not [15,27]. Consumption of prebiotics by preterm formula fed infants results in an increase of beneficial microorganisms in the colon, decreasing harmful bacteria to the levels found in breastfed infants. This improves the gastrointestinal mucosal barrier (decreasing intestinal permeability) which prevents infections and eventually results in improved growth [27,28]. In general the aim of adding probiotics and prebiotics to preterm infant formula is to improve growth, development and decrease infections by promoting an intestinal microbiota resembling that of breastfed infants [9,29,30].

The effects of probiotics on the intestinal microbiota of premature infants have been varied due to differences on gestational age and products administered. Effects of probiotics on weight gain have also been varied. Administration of *Bifidobacteria breve* led to improved weight gain while *Saccharomyces bourladii* did not [9]. With premature infants optimal strains and dose regimens are yet to be examined closely [8]. The effects of prebiotics on the growth of premature infants are not clear. If prebiotic supplementation reduces the risk of Necrotizing Enterocolitis (NEC) or improves feed tolerance in very low birth weight infants is yet to be established [8,9]. Recent systematic reviews (published from 2005 to 2009) on the use of probiotics or prebiotics in preterm infants have focused on prevention of NEC and / or sepsis, impact on diarrhea [31-34]. These reviews focused on studies that used breast milk and mixed feeds (formula combined with breast milk). This review included infants given only infant formula and focused on growth with

clinical outcomes that were not adequately addressed by previous reviews.

The Human Research Ethics Committee at the University of Stellenbosch, South Africa reviewed the review protocol (unpublished), ruled that all data to be collected for this review was from the public domain and was therefore exempt from ethical approval.

Objective

To assess if addition of probiotics or prebiotics to preterm infant formula led to improved growth and clinical outcomes in preterm or low birth weight infants.

Methods

Eligibility criteria

All randomized controlled trials (RCTs), irrespective of language, which compared the use of preterm infant formula containing probiotic(s) or prebiotic(s) to conventional preterm infant formula without or with placebo amongst preterm infants born <37 weeks gestation, low birth weight infants with <2.5 kg at birth and hospitalized, receiving formula feeds and / or parenteral feed were considered. Studies published as abstracts were included if sufficient information could be obtained to assess study quality and obtain relevant study findings.

Outcome measurements

Primary outcomes included: Short term growth parameters (assessed for entire study duration approximately 4 weeks): weight gain (grams/day or grams/week), linear growth (centimeters/week), head growth (cm/week). Secondary outcomes included: Complications: Incidence of NEC (defined as suspected or confirmed positive Bell stage II or more), Sepsis (defined as signs or symptoms of infection and positive blood culture), Other infections (example bacteraemia defined as blood cultured positive for bacteria), Mortality / death. Adverse events during entire study duration: Number of days on parenteral, number of days to full enteral nutrition, maximal enteral feed (millilitres/day, millilitres/kilogram/day, millilitres /kilogram). Feed intolerance: Incidence of vomiting, gastric aspirates, abdominal distension. Stool characteristics: Stooling frequency and stool consistency as firm, loose or watery. Changes in intestinal permeability as measured by ratio of Lactulose / mannitol in urine or other sugar absorption tests (such as lactulose / L – rhamnose ratio, D- xylose, 3-*O2*- methyl-D- glucose tests). Gastrointestinal (GI) micro flora: number of colony forming units (cfu) of bifidobacteria, lactobacillus and pathogens post intervention).

Search method for identification of studies

A literature search in all languages was conducted on electronic databases which included The Cochrane Central Register for Controlled Trials 2009, Scopus (1990 to 19/01/2010), EBSCO host (1960 to 15/11/2009), OVID (1950 to 01/12/2009), SPORT Discus (1960 to 19/01/2010), Web of Science (1970 to 19/01/2010), Science Direct (1950 to 30/11/2009), EMBASE (1980 to 01/12/2009), CINAHL (1981 to 19/01/2010), PUBMED / MEDLINE (1966 to 10/04/2010), Latin American Caribbean Health Sciences literature (LILACS), (1965 to 19/01/2010), NLM Gateway (1950–1966). RCTs published in non-English language journals were translated by independent translators who were familiar with the subject matter. The search strategy used to search PUBMED is shown on Table 1. This search strategy was modified to search other electronic databases.

Table 1 Search strategy used in PUBMED

1)	Search (probiotic* OR prebiotic*) AND (infant formula* OR infant feeding OR formula OR formula milk) AND (preterm or premature or low birth weight babies) AND (randomized controlled trial* OR controlled clinical trial* OR random allocation*) Limits: Human
2)	Search (probiotic* infant formula* OR prebiotic* infant formula* OR prebiotic* OR probiotic*) AND (infant formula* OR infant feeding) AND (premature OR preterm) AND (randomized controlled trial* OR controlled clinical trial OR random allocation* OR double blind method OR single-blind method OR clinical trial OR placebo* OR random* OR research design OR comparative study OR follow-up studies OR prospectiv* OR volunteer* OR control* (singl* OR doubl* OR trebl* OR tripl*) NEAR (blind* OR mask*) Limits: Human

We conducted a hand search on abstracts of major conference proceedings such as the Pediatric Academic Society meetings (www.pas-meetings.org, www.abstracts2view.com), cross checked references cited in RCTs and in recent reviews (published from 2005 to 2009) for additional studies not identified by electronic searches and specialty journals which were not included in any database such as Pediatrika, Chinese Journal of Microecology and International Journal of Probiotics and Prebiotics.

To identify on-going and unpublished trials, we contacted experts in the field, manufacturers of infant formula containing probiotics and prebiotics, we searched web sites of companies that have conducted or were conducting RCTs on probiotics and prebiotics e.g. Pfizer (www.pfizerpro.com/clinicaltrials), Chris Hansen Laboratory (www.chr-hansen.com/research_development/documentation.html). We also searched prospective trial registries such as World Health Organisation (WHO) International Clinical Trials Registry Platform Search Portal (www.who.int/trialsearch), Clinical Trials.gov register (www.clinicaltrials.gov), Current Controlled Trials *meta* *R*egister of Controlled Trials [*mRCT*] (www.controlled-trials.com/mrct) and www.clinicaltrialresults.org.

Selection of studies

Two reviewers (MM, ML) independently reviewed all abstracts, citations and identified potentially eligible

studies. The full reports of eligible studies were retrieved by one reviewer (MM) and the pre-specified selection criteria applied independently by two reviewers (MM, ML) using a study eligibility form. (Figure 1) If more than one publication of a study existed, all reports of the study were grouped together under one study name. Any disagreements between the reviewers were resolved through discussion. If disagreements could not be resolved a third party was consulted. Trial authors were contacted if eligibility was unclear.

STUDY ELIGIBILITY FORM

Review title:	**Probiotic, prebiotics infant formula use in preterm or low birth weight infants: A systematic review**		
Study ID (Author last name, initials)			
Refworks ID number			
Date of review for eligibility (DD-MM-YYYY)			
Journal title			
Title of study/article			
Year/volume/issue/page			
Extractor (Last name, initials)			
Type of study	**Put a check (√) mark in appropriate box.**		
Is this study a Randomized controlled study?	YES	UNCLEAR	NO
	Go to next question		**Exclude**
Trial intervention			
Preterm infant formula containing probiotic(s)	YES	UNCLEAR	NO
Preterm infant formula containing prebiotic(s)	YES	UNCLEAR	NO
Conventional preterm formula / placebo			
	Go to next question		**Exclude**
Study Participants			
Premature infants <37 weeks gestation	YES	UNCLEAR	NO
Low birth weight infants ≤ 2.5 kg at birth	YES	UNCLEAR	NO
	Go to next question		**Exclude**
Study Outcomes (≥1 outcomes below)			
Short term growth parameters (Wt, Ht, Hd Circum)	YES	UNCLEAR	NO
Adverse events (# days on parenteral, full enteral nutrition, maximal enteral feed, vomiting, GI aspirates, abdomen distension, stool characteristics- consistency, frequency)	YES	UNCLEAR	NO
Complications (NEC, Sepsis, other infection, death)	YES	UNCLEAR	NO
Intestinal permeability	YES	UNCLEAR	NO
GI Microflora (Bifidobacteria, Lactobacillus, pathogen cfu)	YES	UNCLEAR	NO
		Clarify missing information	
Other reasons for excluding study	**NO**		**Yes**
Final decision	**Include**	**Unclear**	**Exclude**
		For discussion	
Comments			

Figure 1 Study eligibility form.

Assessment of quality of evidence

Two reviewers (MM, ML) independently assessed the risk of bias of included studies as described in the Cochrane Handbook for Systematic Reviews for Inter- ventions according to the following 6 components. 1) se- quence generation; 2) allocation concealment; 3) blinding; 4) incomplete outcome data; 5) selective out- come reporting; and 6) other sources of bias [35]. Where necessary, trial authors were contacted for clarification on the methodology of their studies. Any disagreements regarding risk of bias were resolved through discussion between MM, ML and RB.

Data extraction and management

Two reviewers (MM, ML) independently extracted data using a pre tested data extraction form. The reviewers (MM, ML) cross checked data and resolved any differ- ences through discussion. One reviewer (MM) entered the data in Review Manager (RevMan 5) and the other reviewer (ML) validated the data. Trial authors were contacted for missing data or for clarification.

Data synthesis and management

Results for probiotic and prebiotic studies were analysed separately. For continuous outcomes the mean differ- ence (MD) and corresponding 95% confidence intervals (CI) were calculated. For dichotomous outcomes, the risk ratio (RR) and corresponding 95% CI were calcu- lated. Trial authors were contacted if there was missing data in their reports. Available case analysis was used where there was missing data. The potential impact of the missing data on the results of the review is addressed in the discussion section. Heterogeneity of the trials used in the review was assessed by visually inspecting the for- est plots to detect overlapping confidence intervals and by performing a chi^2 test. A $p<0.1$ was considered statistically significant. An I-square test (I^2) was used to test for inconsistencies across studies. If the I^2 exceeded 50% and visual inspection of the forest plot supported these results, this represented substantial heterogeneity.

If the included studies were not clinically diverse and had similar outcome measures, a Meta - analysis was carried out in Review Manager software (RevMan 5) by one review author (AM). For continuous data, if heterogeneity was low, an inverse variance fixed-effect method was used. If heterogeneity was high, an inverse variance random-effects method was used. For dichotomous data, if heterogeneity was low, a Mantel-Haenszel fixed-effects method was used. If heterogeneity was high, a Mantel-Haenszel random-effects method was used. The source of heterogeneity was explored through subgroup analysis with respect to the type of intervention. If studies were too diverse, no Meta-analysis was conducted and a narrative synthesis was provided. We had intended to perform sensitivity analysis with respect to study quality in order to investigate the robustness of our findings but this could not be done mainly because most of the meta-analysis had too few studies (mostly two) to warrant sensitivity analysis. In some cases, all the studies in the meta-analysis had similar study quality thus rendering sensitivity analysis inappropriate.

Results

Results of the search and description of studies

Electronic search of available databases yielded 151 citations. After reading titles, abstracts, the duplicate reports were removed and 35 potentially relevant articles were identified. A hand search yielded 4 more articles. The full text reports were retrieved and reviewed for eligibility. One study was published in two other reports. The three studies were considered as one study since they reported the same identical study and are referred to as Boehm 2002 in this review [36-38]. Eight published

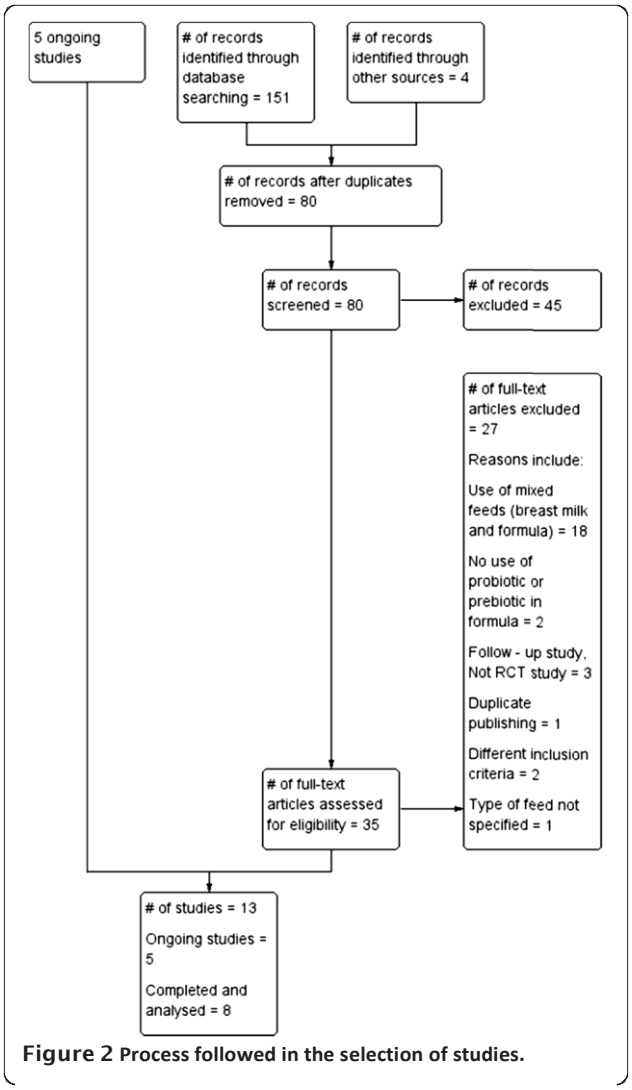

Figure 2 Process followed in the selection of studies.

Table 2 Excluded studies with reasons for exclusion

Reasons for exclusion of studies								
Use of breast milk or mixed feeds (breast milk and formula)			**No use of probiotic, prebiotic**	**Follow up - study, Not RCT**		**Duplicate publishing**	**Different inclusion criteria and outcomes**	**Type of feed unspecified**
Agarwal 2003 [52]	Lin H-C 2008 [53]	Riskin 2009 [54]	Andrews 1969 [55]	Chou I-C 2009 [56]	Stansbridge 1993 [57]		Cukrowska 2002 [58]	Karvonen 2002 [51]
Bin-Nun 2005 [59]	Manzoni 2006 [60]	Rouge 2009 [61]	Taylor 2009 [62]	Hoyos 1999 [63]			Wang 2007 [64]	
Dani 2002 [65]	Millar 1993 [66]	Samanta 2005 [67]		Lidesteri 2003 [68]				
Kitajima 1997 [69]	Mohan 2006 [70]	Westerbeek 2008 [71]						
Lee 2007 [72]	Mohan 2008 [73]	Westerbeek 2010 [74]						
Lin H-C 2005 [75]	Patole 2005 [76]	Yong Gu 2009 [77]						

studies (four probiotic and four prebiotic studies) [36,39-45] and five on-going studies were included in this review [46-51]. The process followed is shown in Figure 2. Table 2 gives a list of 27 studies which were excluded for: use of breast milk or mixed feeds (18 studies), no use of probiotic or prebiotic (2 studies), being a follow −up study, not RCT (3 studies), duplicate publishing (1 study); using different inclusion criteria with

Table 3 A summary of four included probiotic studies

	Costalos 2003 [39]	**Indrio 2008 [42]**	**Reuman 1986 [41]**	**Stratiki 2007 [40]**
Location of study	Athens, Greece	University of Bari, Policinico, Italy	Gainesville, Florida, USA	Alexandra Regional Hospital, Greece
Participants-inclusion criteria	28 - 32 weeks gestation	3- 5 days old, appropriate for gestational age, preterm infants with normal agpar scores	Premature infants, <2000g at birth, less than 72 hours old (>24 old to <72 hours old)	27 to 37 weeks gestation, in stable state
Number of study participants	Study group=51 , Placebo = 36	Study group = 10 , Placebo = 10	Study group = 15, Placebo = 15	Study group = 41, Placebo = 34
Probiotic bacteria used	*Saccharomyces Bourlardii*	*Lactobacillus Reuteri ATCC 55730*	*Lactobacillus acidophilus*	*Bifidobacteriumlactis*
Dose of probiotic	10^9cfu at 50mg/kg every 12 hours	1 X 10^8cfu/day	9 X 10^6cfu/ml formula	2 X 10^7cfu/g milk powder
Placebo	Maltodextrin	Indistinguishable placebo	Conventional preterm formula	Conventional preterm formula
Dose of placebo	50 mg /kg / 12 hours	Not reported		
Treatment initiation	1st week of life as soon as enteral feed was tolerated	At 3−5 days of life	1st 72 hours of life	1st 2 days of life
Treatment duration	30 days	30 days	Not specified	30 days
Reported Outcomes				
Growth parameters	Weight gain	Weight gain	Weight gain	Weight gain, Linear growth, Head circumference
Timing and duration of measurement of growth parameters	Measured daily for 30 days	Measured daily for 30 days	Measured daily, duration not specified	Weight gain: measured daily, Lineargrowth (measured weekly), Head circumference (measured weekly)
Feed tolerance	Number of days to full enteral feed, Maximal enteral feed, vomiting	Number of days to full enteral feed, Maximal enteral feed, vomiting	Maximal enteral feed	Number of days to full enteral feed, Maximal enteral feed
Stool characteristics		Stooling frequency		
Complications	NEC, Sepsis		Mortality / death	NEC, Sepsis
Intestinal permeability	Changes in Intestinal permeability			Changes in Intestinal permeability
Changes in gastrointestinal microflora	cfu of bifidobacteria, lactobacillus, pathogens			cfu of bifidobacteria

different outcomes (2 studies) and type of feed was unspecified (1 study). No eligible studies were excluded for failure to report the review's pre-specified outcomes.

A summary of the included probiotic, prebiotic and ongoing studies are shown in Tables 3, 4 and 5. The included probiotic studies (N=212) were conducted in Greece, Italy and United States of America (USA). Treatment duration was 30 days using different probiotics. All four probiotic studies reported short term growth parameters (weight gain) which were recorded daily during the entire study duration [Table 3]. None of the probiotic studies reported data on: other types of infections, use of parenteral nutrition, feed intolerance (gastric aspirate [ml], abdominal distension) and stool consistency. The included prebiotic studies (N=126) were conducted in conducted in Greece, Italy, and Germany. Treatment duration ranged from 14 days to 28 days. All four prebiotic studies reported short term growth parameters (weight gain, length, head growth) which were recorded at different intervals during the en- tire study duration [Table 4]. None of the prebiotic stud- ies reported data on: complications (NEC, sepsis, other types of infections, death / mortality), use of parenteral nutrition, feed intolerance (vomiting, gastric aspirate [ml], abdominal distension) and changes in intestinal permeability.

Risk of bias

The quality of the included studies was assessed across six domains using guidelines from the Cochrane Handbook for Systematic Reviews of Interventions [35] (Figure 3).

Random sequence generation: Three trials described clearly the methods used for random sequence generation [40,41,44]. Mihatsch used computer generated random lists with variable block sizes [44]. Stratiki used balance block randomization using random numbers [40] and Reuman used random numbers list combined with the last digit of the patients' medical record [41]. The method used for random sequence generation was not clearly described 5 studies [36,39,42,43,45].

Allocation Concealment: In two trials treatment allocation was adequately concealed [33,40]. In the Stratiki trial, treatment allocation was conducted by a third party who was not involved in the study (Nutritional service) [40]. Mihatsch used precoded sachets in sealed envelopes [44]. In one study treatment allocation was not adequately concealed because the method used was

Table 4 A summary of four included prebiotic studies

	Boehm 2002 [36]	Indrio 2009 [43]	Kapiki 2007 [45]	Mihatsch 2006 [44]
Location of study	Milan, Italy	University of Bari, Policinico, Italy	Athens, Greece	Ulm University, Germany
Participants - entry criteria	<32 weeks gestation	Healthy preterm newborns	≤ 36 weeks gestation	< 1500 g birth weight
Number of study participants	Study group = 15, Placebo = 15	Study group = 10 , Placebo = 10	Study group = 36, Placebo = 20	Study group = 10, Placebo = 10
Prebiotic used	GOS 90%, FOS 10%	scGOS, lcFOS at ratio 9:1	FOS	GOS, FOS
Dose of prebiotic	1g/dl	0.8 g/dl	0.4g/100ml	1g/dl
Placebo	Maltodextrin	Maltodextrin	Maltodextrin	Maltodextrin
Dose of placebo	1 g/dl	0.8 g/dl	0.4 g	1.8 / 90 ml
Treatment initiation	When enteral feed ≥ 80 mls /kg/day was tolerated	Not clear	Exclusively formula fed at start of study	At full enteral feed at start of study
Treatment duration	28 days	15 days	14 days	15 days
Reported Outcomes				
Growth parameters	Weight gain, linear growth	Weight gain, linear growth, head growth	Weight gain, linear growth, head growth	Weight gain
Timing and duration of measurement of growth parameters	Measured on days 1, 7, 14, 28	Measured before start of study, days 3, 5, 15	Measured on days 1, 7, 14	Weight gain: reported as "Average weight gain during study."
Feed tolerance	Number of days to full enteral feed, maximal enteral feed	Number of days to full enteral feed, maximal enteral feed	Number of days to full enteral feed	Number of days to full enteral feed, maximal enteral feed
Stool characteristics	Stooling frequency, consistency		Stooling frequency, consistency	Stool viscosity, Stooling frequency, consistency
Changes in gastrointestinal microflora	cfu bifidobacteria		cfu bifidobacteria, pathogens	

Table 5 A summary of five on-going studies

	Jacobs 2007 [46]	Lozano 2008 [47]	Al-Hosni 2010 [48]	Patole 2009 [49]	Underwood 2009 [50]
Location of study	Australia	Colombia	USA	Australia	USA
Participants - inclusion criteria	<32 weeks gestation, <1500 g birth weight, 1–3 days old	Birth weight <2000 grams, < 48 hours of age, admission in NICU, Hemodynamic-ally stable	Extremely Low Birth weight infants: < 1000 grams, 1 to 14 old, intention to start enteral feeds	32 weeks Gestation and 6 days, <1500g birth weight, ready to commence on enteral feeds for up to 12 hours	< 500grams birth weight, age less than 33 weeks gestation, exclusively formula fed
Probiotic bacteria used	*Bifidobacteriuminfantis, BifidobacteriumBifidus, Streptococcus thermophilus*	*Lactobacillus reuteri DSM 17938*	*Lactobacillus rhamnosus GG, Bifidobacteriuminfantis*	*Lactobacillus acidophilus 375 million, bifidobacteriumbifidum, bifidobacteria longus*	1. ProlactPlus 2..GOS 3. *Bifidobacteriuminfantis* 4. *Bifidobacteriumanimalis*
Dose	$1X10^9$	1X108 CFU in 5 drops of oil suspension 1/ day until discharge.	*L rhamnosus*: 500 million cfu, *B.infantis*: 500 million cfu	*L. acidophilus*:375 m organisms, *B bifidum, B. longus*: 125 million organisms	1. week 1 95:5 to week 5 75:25 2. week: 0.25g/dL, to week 5: 2.0 g/dL 3. week 1: $5X10^7$, to week 5: 4.2 $X10^9$ 4. week 1: $5X10^7$, to week 5: 4.2 $X10^9$
Start date of study	July- 2007	August 2008	February 2008	June 2009	June 2009
Reported Outcomes	Sepsis,	Sepsis	Average weight gain	Sepsis	Fecal microflora
	NEC	NEC	Growth velocity	NEC	
	Death	Death	Feed tolerance	All-cause mortality	
	Frequency of events (150 mls/kg/day)		Volume of feed/day	Time to reach full feeds	
Length of hospital admission				Gut colonisation by probiotic	
Number of antibiotic courses					
Days to full enteral feeds					

alternation, matching of infants by birth weight and gestational age [41]. In the rest of the studies, allocation concealment was not clearly demonstrated or described [36,39,42,43].

Blinding: Blinding of study participants, care providers and assessors was clearly done in 4 trials [39-41,44]. In the other 4 trials, there was not enough information given on the blinding method to make a judgement [36,42,43,45].

Incomplete outcome data: Reported outcome data was satisfactory for all the eight included studies. Five studies had no missing outcome data [36,41-44]. In other three studies, the missing outcome data was balanced across the intervention groups with similar reasons reported [39,40,45].

Selective reporting (reporting bias): In all eight studies, the pre-specified outcomes in the methods section were reported in the results section [36,39-45].

Other potential sources of bias: Only one trial had a baseline imbalance which was a potential source of bias. Costalos had 51 infants enrolled in the treatment group and 36 infants in the placebo group. No explanation was presented whether the imbalance was due to a problem at randomization stage [39]. All other studies appeared to be free from other potential sources of bias.

Effects of interventions

Probiotics versus control

Four studies investigated the effect of probiotic administration versus no probiotic (control group) [39-42].

Primary outcomes: short term growth parameters

Weight gain All four studies reported on weight gain [39-42]. Results from two studies (n=34) were pooled in a meta-analysis [41,42]. There was no statistically

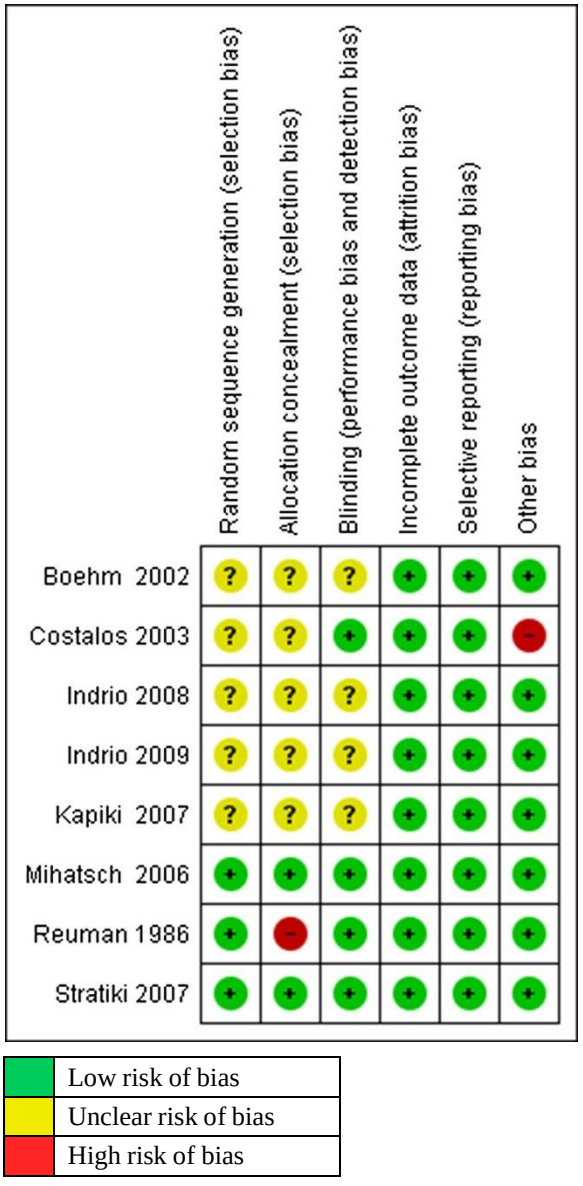

Figure 3 Methodological quality of included studies.

significant difference in weight gain (g/day) between the probiotic and control groups (MD 1.96, 95% CI: -2.64 to 6.56). No statistically significant heterogeneity was observed (Chi^2=0.18, p=0.67, I^2=0%) (Figure 4)

Two studies [39,40] reported their results using medians and could not be pooled in a meta - analysis. Costalos 2003 reported no statistically significant difference in weight gain (g/week) between the probiotic and control groups (p>0.05) [median (Interquartile range) of 163.5 (17.7) for the probiotic group (n=51) compared to 155.8 (16.5) for the control group (n=36)] [39]. Stratiki 2007 also reported no statistically significant difference in weight gain (g/day) between the probiotic and control groups (p=0.144) [median (range) of 28.3 (12 to 38) for the probiotic group (n=41) compared to 30 (10 to 40) for the control group (n=34)] [40].

Linear growth Only one study reported this outcome but found no statistically significant difference in length gain (cm/week) between the probiotic and control groups (p=0.124) [median (range) of 1.4 (0 to 3) for the probiotic group (n=41) compared to 1.5 (0 to 3.5) for the control group (n=34)] [40].

Head growth Only one study reported this outcome but found no statistically significant difference in head growth (cm/week) between the probiotic and control groups (p=0.124) [median (range) of 1.1 (0.45 to 1.9) for the probiotic group (n=41) compared to 0.9 (0 to 2) for the control group (n=34)] [40].

Secondary outcomes

Complications

Necrotizing enterocolitis [NEC] Two studies (n=162) reported on NEC and their results were pooled in a meta-analysis [39,40]. Administration of probiotics failed to significantly reduce the risk of NEC compared to controls (RR 0.42, 95% CI: 0.15 to 1.16). No significant het- erogeneity was observed (Chi^2=1.06, p=0.30, I^2=6%) (Figure 5).

Sepsis Two studies (n=162) reported on sepsis and their results were pooled in a meta-analysis [39,40]. Adminis- tration of probiotics failed to significantly reduce the risk of sepsis compared to controls (RR 0.40, 95% CI: 0.11 to 1.45. No significant heterogeneity was observed (Chi^2=1.18, p=0.28, I^2=15%). (Figure 6)

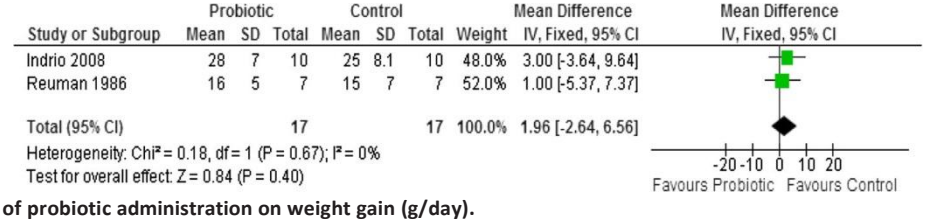

Figure 4 Effect of probiotic administration on weight gain (g/day).

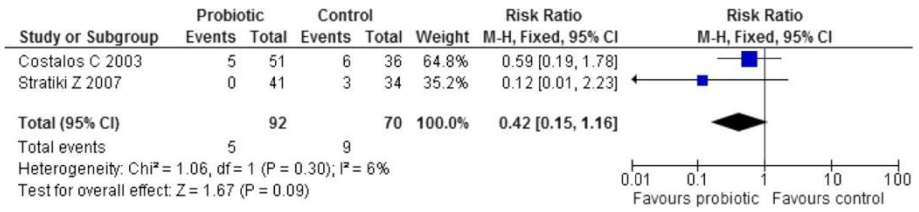

Figure 5 Effect of probiotic administration on NEC.

Other infections No study reported on this outcome.

Mortality Only one study [42] reported on mortality. The risk ratio for this one study (n=30) was calculated and it showed that the probiotics failed to significantly reduce the risk of death compared to the control (RR 0.33, 95% CI: 0.04 to 2.85).

Number of days on parenteral nutrition No study reported on this outcome.

Number of days to full enteral feed Two studies reported this outcome but their results could not be pooled in a meta-analysis because they reported the outcome in terms of medians and ranges [39,40]. Costalos 2003 reported no statistically significant difference in the number of days to full enteral feeding between the two groups (p>0.1) [median (IQR) of 9.3 (2.7) for the probiotic group (n=51) and 9.9 (4.5) for the control group (n=36)] [32]. Stratiki 2007 also reported no statistically significant difference in the number of days to full enteral feeding [median (range) of 10 (0 to 52) for the probiotic group (n=41) and 10 (0 to 30) for the control group (n=34)] [40].

Maximal enteral feed All four studies reported on this outcome [39-42]. Results from two studies (n=34) were pooled in a meta-analysis as they both reported the aver- age amount of feeding (ml/day) in terms of mean (SD) [41,42]. There was no statistically significant difference in the mean amount of feeding (ml/day) between the pro- biotic and control groups (MD 35.20, 95% CI: -7.61 to 78.02) No statistically significant heterogeneity was observed between the studies (Chi^2=1.65, p=0.20, I^2=39%).

Costalos 2003 reported no statistically significant difference in the milk intake (ml/kg/day) at maximal enteral feeding (p>0.1) [median (IQR) of 155 (15) for the probiotic group (n=51) versus 148 (13) for the control group (n=36)] [39]. Stratiki 2007 also reported no statistically significant difference in the maximal milk intake (ml/kg/day) (p=0.624) [median (range) of 210 (165 to 250) for the probiotic (n=41) group versus 192 (120 to 250) for the control group (n=34)] [40].

Feed tolerance: vomiting, gastric aspirate, abdominal distension Two studies (n=107) reported on vomiting and were pooled in a meta-analysis [39,42]. There was no statistically significant difference in the frequency of vomiting between the probiotic and control groups (RR 0.78, 95% CI: 0.18 to 3.37). No statistically significant heterogeneity was observed (Chi^2=0.41, p=0.52, I^2=0%).In all four probiotic studies, there were no reported incidences of gastric aspirates, abdominal distension or diarrhea. Authors were further contacted for clarification and one responded [42] and stated categorically that none of these symptoms were observed.

Stool characteristics

Stool frequency Only one study (n=20) reported stool frequency as the number of episodes of evacuations per day in terms of mean (SD) [42]. The mean difference for this one study was calculated and it showed that pro- biotic consumption resulted in a statistically significant larger number of stools per day compared to the control group (MD 1.60, 95% CI: 1.20 to 2.00).

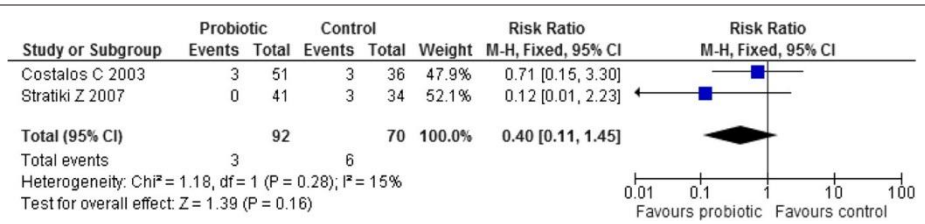

Figure 6 Effect of probiotic administration on sepsis.

Stool consistency No study reported on the effects of probiotics on stool consistency.

Changes in intestinal permeability Two studies reported this outcome but their results could not be pooled in a meta-analysis [39,40]. The studies used two different tests to test for intestinal permeability. Costalos 2003 used a 1-hour D-Xylose blood test and reported no statistically significant difference between the two groups (p>0.1) [median (IQR) of 1.5 (0.4) millimols/L for the probiotics (n=51) and 1.35 (0.3) mmol/L for the control (n=36)] [39]. Stratiki 2007 used a lactulose/mannitol (L/M) urine test and reported no statistically significant difference in the L/M ratios between the probiotic and control groups (p=0.073) but the values for median (range) were presented in a figure from which they could not be accurately extracted [40].

Changes in gastrointestinal micro flora

Bifidobacteria Two studies reported on bifidobacteria but their results could not be pooled in a meta-analysis [39,40]. Costalos 2003 reported a significantly higher log viable Bifidobacteria counts per gram of positive infants in the probiotics group compared to the controls (p<0.001) [median (IQR) of 2.65 (0.083) for the probiotics group (n=51) and 2.27 (0.075) for the control group (n=36)] [39]. Stratiki 2007 reported bifidobacteria in terms of log 10 cfu/g wet feces but found no statistically significant difference between the two groups (p=0.075) [median (range) of 9.7 (7.5-10.3) for the probiotics group (n=41) and 8.9 (7.2-10.2) for the control group (n=34)] [40].

Lactobacillus Only one study reported on lactobacillus [39]. This study reported no statistically significant dif- ference in the log viable bacterial lactobacillus counts per gram of positive infants between the two groups (p>0.05) [median (IQR) of 1.57 (0.285) for the probiotics group (n=51) and 1.42 (0.287) for the control group (n=36)].

Pathogens Only one study reported this outcome (enterococci, bacteroides, and staphylococci) in terms of the median (IQR) of log viable bacterial counts per gram of positive infants [39] (Table 6). The study reported significantly higher counts of Enterococci (p<0.05) and Staphylococci (p<0.001) in the probiotic group compared to the controls. However, the study found no statistically significant difference in the counts of bacteroides between the two groups (p>0.05).

Prebiotic versus control

Four studies investigated the effect of prebiotics administration versus no prebiotics (control group) [36,43-45].

Table 6 Log viable bacteria counts per gram of stool in positive infants fed probiotics

Costalos 2003 [39]	Median (IQR)	
Pathogens	Probiotic	Control
n= 51	n=36	
Enterococci	2.14 (0.359)	2.19 (0.138)
Bacteriodes	2.17 (0.164)	2.25 (0.363)
Staphylococci	1.23 (0.869)	0.6 (0.281)

Primary outcomes: short-term growth parameters

Weight gain All four studies reported on weight gain [36,43-45]. Results from three studies (n=106) were pooled in a meta-analysis [36,43,45]. Moderate heterogeneity was observed between the studies (Chi^2=4.04, p=0.13, I^2=51%). An investigation of heterogeneity by subgroup analysis with respect to the prebiotic type used (GOS/ FOS versus FOS only) yielded statistically significant subgroup differences (Chi^2=4.04, df=1, p=0.04, I^2=75.2%) implying that prebiotic type may be the source of heterogeneity. There was no statistically significant heterogeneity between the two studies in the GOS/ FOS subgroup (Chi^2=0.01, df=1, p=0.94, I^2=0%) [36,43]. The results for the GOS/FOS subgroup yielded no significant difference in weight gain (g/ day) between the two groups (MD 0.04, 95% CI: -2.65 to 2.73, n=50, 2 studies) while the other FOS subgroup yielded a significantly higher weight gain in controls compared to the prebiotics (MD −4.60, 95% CI: -8.24 to −0.96, n=56, 1 study). (Figure 7) Sensitivity analysis with respect to study quality could not be done because all three studies were of poor quality since the methods used for sequence generation, allocation concealment and blinding were all not clear.

Mihatsch 2006 reported no statistically significant difference in weight gain (g/kg/day) between the two groups (p=0.4) [median (range) of 17.6 (8.1 to 23.4) for the prebiotic group (n=10) compared to 13 (9.3 to 21.9) for the control group (n=10)] [44].

Linear growth Three studies reported on length gain [36,43,45]. Meta-analysis of the results from these three studies (n=106) revealed significant heterogeneity between the three studies (Chi^2 = 139.41, df = 2, p < 0.00001, I^2 = 99%). An investigation of heterogeneity by subgroup analysis with respect to the prebiotic type used (GOS/ FOS versus FOS only) yielded statistically significant subgroup differences (Chi^2=139.41, df=1, p<0.00001, I^2=0%) implying that prebiotic type may be the source of heterogeneity. There was no statistically significant heterogeneity between the two studies in the GOS/ FOS subgroup (Chi^2=0.17, df=1, p=0.68, I^2=0%). [36,43]. The results for the GOS/FOS subgroup yielded

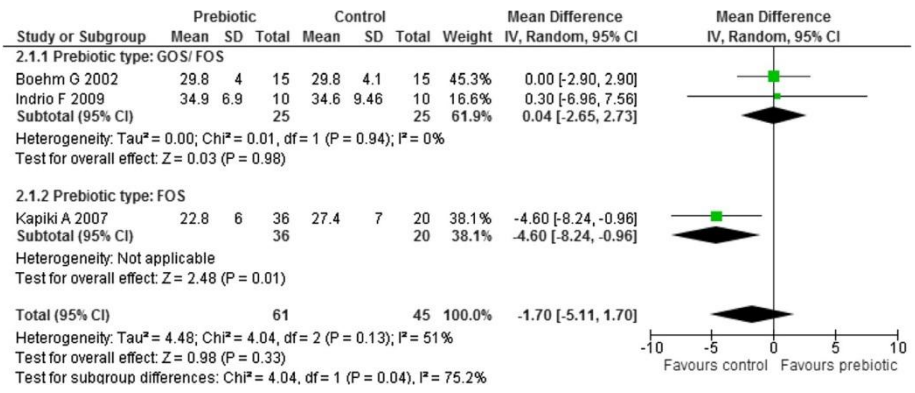

Figure 7 Effect of prebiotic administration of weight gain (g/day).

no statistically significant difference in length gain (cm/ week) between the two groups (MD 0.01, 95% CI: -0.03 to 0.04, n=50, 2 studies) while the other FOS subgroup yielded a significantly higher length gain (cm/ week) in prebiotics compared to the controls (MD 0.30, 95% CI: 0.27 to 0.33, n=56, 1 study). (Figure 8) Sensitivity analysis with respect to study quality could not be done because all three studies were of poor quality since the methods used for sequence generation, allocation concealment and blinding were all not clear.

Head growth Two studies reported on head growth (cm/week) [43,45]. Meta-analysis of the results from these two studies (n=76) failed to yield statistically significant difference in head growth (MD −0.01, 95% CI: -0.02 to 0.00). No significant heterogeneity was detected between the two studies (Chi^2 = 0.10, p =0.75, I^2 = 0%).

Secondary outcomes

Complications No prebiotic study reported on Necrotizing Enterocolitis (NEC), Sepsis, other infections and mortality.

Feeding tolerance

Number of days on parenteral nutrition No study reported on parenteral nutrition.

Age at full enteral feed Two studies reported on age at full enteral feeds [36,45]. Meta-analysis of the results from these two studies (n=86) did not find statistically significant difference in the age at full enteral feed (MD −0.79, 95% CI: -2.20 to 0.61). No significant heterogeneity was detected between the two studies (Chi^2 =1.16, p =0.28, I^2 = 14%).

Maximal enteral feed Two studies reported on this outcome but their results could not be pooled in a meta-analysis [36,44]. Boehm 2002 reported the feeding volume (ml/kg/day) in terms of the mean (SD) and therefore a mean difference was calculated. There was no statistically significant difference in feeding volume between the prebiotics group (n=15) and control groups (n=15) (MD −4.10, 95% CI: -18.16 to 9.96) [36].
Mihatsch 2006 reported no statistically significant difference in the average formula intake within the study period (ml/kg/d) between the two groups (p=0.35) [median (range) of 156 (127 to 165) for the prebiotic group

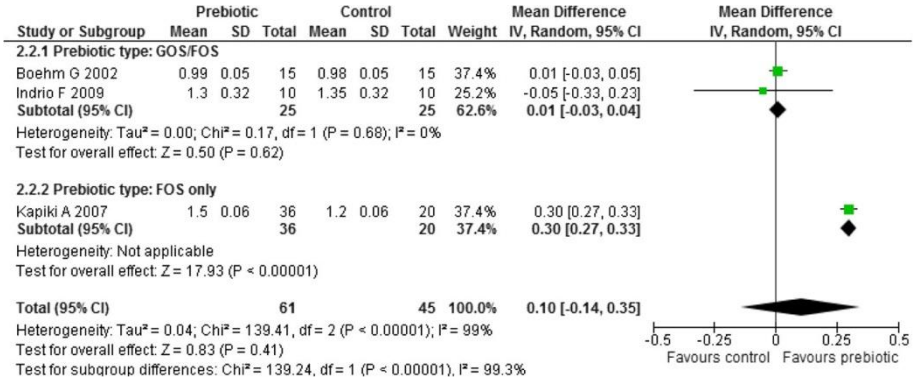

Figure 8 Effect of prebiotic administration of linear growth (cm/week).

(n=10) compared to 151 (117 to 169) for the control group (n=10)] [44].

Feed tolerance: vomiting, gastric aspirate, abdominal distension, diarrhea All four studies reported this outcome [36,43-45]. In all 4 studies (n=126), there were no observed incidences of feed intolerance. There was no vomiting, gastric aspirate removed, no abdominal distension or diarrhea reported. All infants tolerated the preterm formula with prebiotic or control. From further communication with study authors, 2 study authors [43,44] responded that none of these outcomes were observed.

Stool characteristics

Stool frequency Three studies reported on stool frequency [36,44,45]. Two studies reported the results in form of mean (SD) of the number of stools per day (number/ day) [36,45]. Meta-analysis of results from these two studies (n=86) showed a significantly higher stool frequency in the prebiotic group compared to the control group (MD 0.80, 95% CI: 0.48 to 1.1). No significant heterogeneity was detected between the two studies (Chi^2 =0.13, p =0.72, I^2 = 0%) (Figure 9).
Mihatsch 2006 reported no statistically significant difference in stool frequency between the two groups (p=0.059) [median (range) of 3.6(1.7 to 6.9) stools/day in prebiotic group (n=10) compared to 2.6 (2 to 4.9) stools/ day in control group (n=10)] [44].

Stool consistency Three studies reported on stool consistency but using three different scales of measurement [36,44,45]. Although two studies [36,45] both measured consistency in form of a scale ranging from 1 to 5 and reported their results as mean (SD), they could not be pooled in a meta-analysis because their scales were going in opposite directions; Boehm 2002 (1=watery, 2=soft, 3=seedy, 4=formed, 5=hard) [36]. Kapiki 2007 (5=watery, 4=loose, 3=soft, 2=firm, hard=1) [45]. The mean differences for these two studies were therefore calculated separately.
In Boehm 2002, the stools from the prebiotic group (n=15) were significantly more watery as compared to the control group (n=15). (MD −0.91, 95% CI: -1.41 to −0.37) [36]. In Kapiki 2007, the stools from the prebiotic group (n=36) were significantly harder as compared to the control group (n=20). (MD −0.34, 95% CI: -0.66 to −0.02) [45].
Mihatsch 2006 reported a statistically significantly lower stool viscosity at day 14 (Newtons) for the prebio- tics compared to controls (p=0.006) [median (range) of 31.8 (1.9 to 67.3) in the prebiotic group (n=10) compared to 157.5 (24.1 to 314.0) in the control group (n=10)] [44].

Changes in intestinal permeability

No prebiotic study reported on changes in intestinal permeability.

Changes in gastrointestinal micro flora

Bifidobacteria Two studies reported on this outcome [36,45]. Meta-analysis of these two studies (n=84) revealed statistically significant heterogeneity between the two studies (Chi^2 =7.63, p =0.006, I^2 = 87%). An investigation of heterogeneity by subgroup analysis with respect to the prebiotic type used (GOS/ FOS versus FOS only) yielded statistically significant subgroup differences (Chi^2 =7.63, p =0.006, I^2 = 86.7%) implying that prebiotic type may be the source of heterogeneity. The results for the GOS/FOS subgroup yielded significantly higher bifidobacteria counts in prebiotics compared to controls (MD 2.10, 95% CI: 0.96 to 3.24) [36]. The other FOS subgroup also yielded significantly higher bifidobacteria counts in prebiotics compared to controls (MD 0.48, 95% CI: 0.28 to 0.68) [45] (Figure 10).

Lactobacilli Only one study [36] reported this outcome but the actual values were not given.

Pathogens [Post-intervention] Two studies reported on this but their results could not be pooled in a meta- analysis [36,45]. Boehm 2002 reported the sum of clinic- ally relevant pathogens at the end of the intervention period in the form of mean (SD) log cfu/g stool. The values were used to calculate the mean difference which showed that the sum of the studied pathogens was sig- nificantly lower in the prebiotic group (n=12) compared to the control group (n=13). (MD −0.43, 95% CI: -0.79 to −0.07) [36].

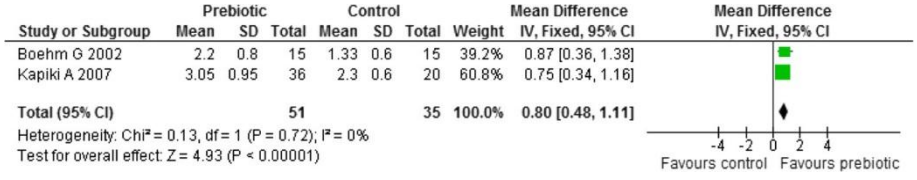

Figure 9 **Effect of prebiotic administration on stool frequency.**

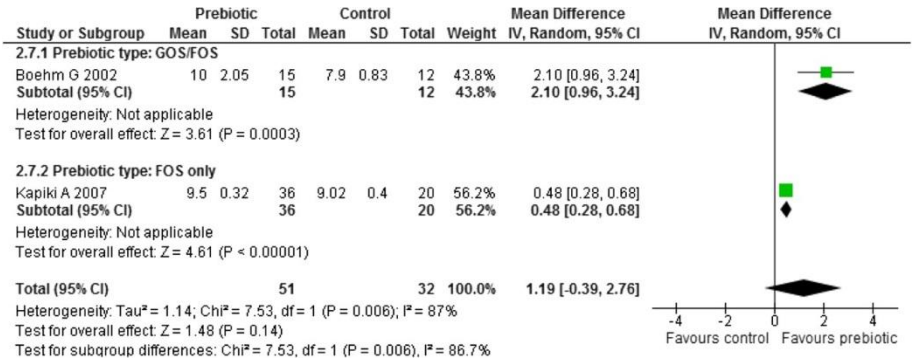

Figure 10 Effect of prebiotic administration on total counts of Bifidobacteria.

Kapiki 2007 reported this outcome (staphylococci, E. coli, bacteroides, and enterococci) in terms of mean (SD) log 10 CFU/g wet feces [45]. Mean differences for each of these pathogens were calculated. There was no statistically significant difference in the number of staphylococci (MD 0.00, 95% CI: -0.17 to 0.17) between the two groups but there were significantly fewer E. coli (MD −1.69, 95% CI: -1.85 to −1.53) and enterococci (MD −0.80, 95% CI: -0.99 to −0.61) in the prebiotic group (n=36) compared to the control group (n=20). With regards to bacteroides, there were significantly more bacteroides in the prebiotic group (n=36) com- pared to the control group (n=20) (MD 0.50, 95% CI: 0.36 to 0.64) [45].

Discussion

The objective of this review was to assess if addition of probiotics or prebiotics to preterm infant formula led to improved growth and clinical outcomes in preterm or low birth weight infants. Studies that used breast milk or mixed feeds (breast milk and infant formula) were excluded. All RCTs evaluated probiotics or prebiotic use in preterm infants, were of small sample size, varied in enrolment criteria, intervention, treatment initiation and duration.

Summary of main findings

Probiotics

This review was under powered to detect clinically important differences in primary outcomes (weight gain, linear growth, head growth) because of the few number of studies, small sample size (n=34) and poor methodological quality of studies. This review found no significant effect on weight gain from use of pro- biotics added to infant formula. There was also no significant probiotic effect on linear and head growth from the one study measuring these two outcomes. Probiotic supplementation failed to significantly reduce the risk of complications such as NEC, sepsis and death compared to control group. Outcomes such as number of days on parenteral nutrition and other infections were not reported. There was no significant difference in the amount of feed volume (ml/day) and frequency of vomiting between study groups. Preterm infant formula with probiotics was well tolerated as no gastric aspirates, abdominal distension or diarrhea was reported. Effects of probiotics on stool character- istics were under reported. Results from one study showed probiotics supplementation did result in a lar- ger number of stools per day.

Effects on intestinal permeability could not be evalu- ated since two different laboratory tests (lactulose / mannitol ratio and D- xylose tests) were reported and the results could not be pooled. Sugar absorption tests (such as lactulose / mannitol ratio) are a direct measure of intestine integrity which reflects gut maturation and in research; they demonstrate the effects of experimental therapy [78,79]. Monitoring changes in intestinal perme- ability in preterm infants is essential since there is evi- dence that initiation of enteral feeds decreases intestinal permeability [78,80]. However, this could not be estab- lished in this review. Other outcomes such as age at full enteral feeds and intestinal micro flora (pathogens) could not be evaluated as medians (inter quartile ranges) were reported. No probiotic study reported any data on low birth weight infants therefore no conclusions could be made on this population.

The included probiotic studies had short treatment duration of 30 days. This confirms the European Society for Pediatric, Gastroenterology, Hepatology and Nutri- tion (ESPGHAN) statement that there is a "lack of pub- lished evidence on clinical benefits from long term use of probiotic containing infant formula" [81]. This review confirms that there is a need for long term follow-up RCTs on preterm infants. Live probiotic bacteria were used in the trials. There have been few reports of bacter- aemia from probiotic use in the biomedical literature [82-84]. There were no cases of sepsis reported as a re- sult of probiotic consumption in the included studies. In recent reviews, the time to reach full enteral feeds was

earlier in the preterm infants given probiotics with breast milk or mixed feeds. This review could not evaluate this outcome. Well-designed RCTs with similar feeding regimes are needed to evaluate this outcome.

Prebiotics

This review was under powered to detect clinically important differences in primary outcomes (weight gain, linear growth, head growth) because of few number of studies, small sample size (n=106) and poor methodological quality of studies. Addition of prebiotic combinations of GOS /FOS or FOS alone to preterm infant formula did not have any significant effect on weight gain. Addition of GOS / FOS to preterm infant formula did not have any effect on linear growth. However, addition of FOS alone did have a significant effect on linear growth. Neither GOS / FOS combination nor FOS alone had any effect on head growth.

None of the prebiotic studies reported on NEC, sepsis, other infections, mortality (death), parenteral nutrition or changes in intestinal permeability; therefore these outcomes could not be evaluated. Prebiotics did not have any significant effect on the age at which infants reached full enteral feeds or volume of feed tolerated. Prebiotic preterm formula was well tolerated because there were no reports of vomiting, gastric aspirates, abdominal distension or diarrhea. Prebiotic supplementa- tion did result in a higher stooling frequency compared to control. Effects on stool consistency were inconclusive as results from one study resulted in more watery stools in the prebiotic study group compared to control group, in a second study, the prebiotic group experienced harder stools compared to control group. The third study results were presented in medians (range) there- fore no conclusions could be made. In preterm infants, frequent watery stools may signify intolerance, a transient lactase deficiency or another pathological state which always require further investigation [6].

Prebiotics did have a significant effect on intestinal micro flora. Addition of GOS / FOS combination or FOS alone significantly increased counts of bifidobac- teria. Effects on lactobacillus counts could not be evalu- ated as actual figures were not available. The sum of studied pathogens and some selected pathogens (E- coli, enterococci) were significantly fewer in the prebiotic group compared to control group. There was no effect on staphylococci levels while bacteroides were signifi- cantly higher in the probiotic group compared to control group. No prebiotic study reported any data on low birth weight infants; therefore no evaluations could be made.

The prebiotic studies were of short duration ranging from 14 to 28 days. The dose of the prebiotic used (GOS, FOS) varied from 0.4 g/dl o 1g/dl. The European Committee on Food recommends that prebiotics added to formula milk do not exceed 0.8 g/100 ml. The rationale for prebiotic doses not exceeding 1g/ml in clinical trials is an attempt to maximize the bifidogenic effect with minimal intolerance as exhibited by, abdominal distension [85]. The preterm infants tolerated the prebiotic formula as there were no symptoms of feed intolerance reported.

Prebiotic supplementation did have some short term benefits: increased stooling frequency and bifidobacteria counts, fewer pathogens in the prebiotic group compared to control group. However, large RCTS with long term follow -up are needed to find out if these short term benefits translate into improved general health and reduced morbidities in preterm infants. Due to the short duration of prebiotic studies, routine supplemen- tation with prebiotics in preterm infants cannot be recommended.

Quality of the evidence and potential biases

In this review, the quality of the evidence was compromised by several factors: Sample size: included studies were of small individual sample size, number of study participants ranged from 20 to 87 in the probiotic studies, 20 to 56 in prebiotic studies. Intervention: Different types of probiotic and prebiotics, doses and treatment duration were used. Methodological quality: Inadequate information was published to assess methodological quality of the studies. Information was missing on sequence generation, allocation concealment, blinding, incomplete outcome data, selective reporting and free of other bias domains. The significance of any relationship between methodological quality and study outcomes could not be verified since no subgroup analysis with respect to study quality could be done as a result of either too few studies in a meta-analysis or having all studies with similar quality in a meta-analysis. Not all the reviews pre- specified outcomes were addressed by the included studies.

At the conclusion of the review process and preparation of the manuscript (for this review), one on- going study was terminated due to being under powered [47]. One study was completed and data analysis commenced. The results from this study could not be included in this review [48]. The other three studies were still on-going [46,49,50]. The reviewers used thorough comprehensive search strategies adopted for the available databases. All attempts were made to minimize publication bias. All steps of this review were conducted independently by the reviewers.

Agreements and disagreements with other reviews

No significant difference was found in contrast with past reviews and that the potential reasons are lack of power, poor quality of studies or a lack of effect in formula fed

infants. This review did agree with some aspects of past reviews. Prebiotics did have an impact on GI micro flora (increased bifidobacteria counts, reduction in certain pathogens); feed tolerance (no reported gastric aspirates, abdominal distension).

Conclusion

There is not enough evidence to state that supplementation of preterm infant formula with probiotics or prebiotics does result in improved growth and clinical outcomes in preterm infants. Therefore this review does not support the routine supplementation of preterm for- mula with probiotics or prebiotics.

Implications for research

For clear recommendations to be made, long term large RCTs on exclusively formula fed preterm and low birth weight infants are required to investigate the effects of probiotics and prebiotics supplementation in preventing NEC, sepsis, death/mortality; changes in intestinal micro flora and intestinal permeability; explore the efficacy of different doses of the same probiotic on clinical outcomes because available studies used different probiotic doses; similarly, explore the efficacy of different doses of the same prebiotic on clinical outcomes because available studies used similar prebiotics with different doses and treatment duration.

Abbreviations

Cfu: Colony forming units; CI: Confidence interval; cm: Centimetres; ESPGHAN: European society for pediatric gastroenterology, hepatology and nutrition; FOS: Fructo-oligosaccharide; GI: Gastrointestinal; GOS: Galacto-oligosaccharide; IQR: Inter quartile range; IFN-γ: Interferon – gamma; IL-6: Interleukin – 6; IL-10: Interleukin – 10; IL-1β: Interleukin – 1beta; kg: Kilogram; L/M: Lactulose mannitol; MD: Mean difference mmol: millimols; ml: Millilitres; NEC: Necrotizing enterocolitis; TNF-α: Tissue necrosis factor – alpha; RCTs: Randomized controlled trials; RR: Risk ratio; SD: Standard deviation; USA: United States of America; WHO: World Health Organisation.

Competing interests

The authors declared that they have no competing interests.

Authors' contributions

The authors contributed the following: MM: Developed review protocol, selected RCTs, carried out data extraction; assessment of risk of bias in included studies, developed, edited and critically reviewed the manuscript. ML: Selected RCTs, carried out data extraction, assessment of risk of bias in included studies, critically reviewed the manuscript. AM: Carried out the statistical analysis, interpretation of results and critically reviewed the manuscript. TY: Assisted in designing the review and critically reviewed the manuscript. RB: Assisted in designing the review and critically reviewed the manuscript. All authors read and approved the final manuscript.

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Chapter 3: Synbiotics, Probiotics or prebiotics in infant formula for full term infants: A systematic review

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NUTRITION JOURNAL

REVIEW Open Access

Synbiotics, probiotics or prebiotics in infant formula for full term infants: a systematic review

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Abstract

Background: Synbiotics, probiotics or prebiotics are being added to infant formula to promote growth and development in infants. Previous reviews (2007 to 2011) on term infants given probiotics or prebiotics focused on prevention of allergic disease and food hypersensitivity. This review focused on growth and clinical outcomes in term infants fed only infant formula containing synbiotics, probiotics or prebiotics.

Methods: Cochrane methodology was followed using randomized controlled trials (RCTs) which compared term infant formula containing probiotics, prebiotics or synbiotics to conventional infant formula with / without placebo among healthy full term infants. The mean difference (MD) and corresponding 95% confidence intervals (CI) were reported for continuous outcomes, risk ratio (RR) and corresponding 95% CI for dichotomous outcomes. Where appropriate, meta-analysis was performed; heterogeneity was explored using subgroup and sensitivity analyses. If studies were too diverse a narrative synthesis was provided.

Results: Three synbiotic studies (N = 475), 10 probiotics studies (N = 933) and 12 prebiotics studies (N = 1563) were included. **Synbiotics** failed to significantly increase growth in boys and girls. Use of synbiotics increased stool frequency, had no impact on stool consistency, colic, spitting up / regurgitation, crying, restlessness or vomiting. **Probiotics** in formula also failed to have any significant effect on growth, stool frequency or consistency. Probiotics did not lower the incidence of diarrhoea, colic, spitting up / regurgitation, crying, restlessness or vomiting. **Prebiotics** in formula did increase weight gain but had no impact on length or head circumference gain. Prebiotics increased stool frequency but had no impact on stool consistency, the incidence of colic, spitting up / regurgitation, crying, restlessness or vomiting. There was no impact of prebiotics on the volume of formula tolerated, infections and gastrointestinal microflora. The quality of evidence was compromised by imprecision, inconsistency of results, use of different study preparations and publication bias.

Authors' conclusions: There is not enough evidence to state that supplementation of term infant formula with synbiotics, probiotics or prebiotics does result in improved growth or clinical outcomes in term infants. There is no data available to establish if synbiotics are superior to probiotics or prebiotics.

Keywords: Synbiotic, Probiotic, Prebiotic, Full term infant

Background

The first year of life is characterized by very rapid growth. Weight increases by 115%, body length 34% and head circumference 22% [1,2]. Many full term infants lose weight after birth and take 8–10 days to regain it back. The average infant achieves a weight gain of approximately 1.1 to 1.2 kg/month during the first 6 months, slowing down to 0.4 to 0.5 kg/month during the second 6 months. Length increases by 3.5 to 3.9 cm/month during the first 4 months, slowing down to 1.8 cm/month at 6 month of age [1]. At birth average head circumference is 35 cm and increases by an estimated 12 cm during the first year of life to approximately 47 cm. A faltering head circumference has serious implications for neural growth, maturation and is diagnostic for possible problems of brain growth [2]. Monitoring growth (weight, length and head circumference) evaluates the overall health of the infant and determines adequacy of nutritional intake [1].

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To promote optimum growth, development and de- crease infections, probiotics, prebiotics are added to infant formula to promote an intestinal micro flora resembling that of breastfed infants [3]. The intestinal micro flora of breastfed infants have a greater concentra- tion of bifidobacteria and fewer potentially pathogenic bacteria compared to formula fed infants. Probiotics are "live microorganisms" which when administered in adequate amounts confer a health benefit to the host [3]. The main probiotic organisms used worldwide belong to the genera Lactobacillus and Bifidobacteria and are found in the gastrointestinal micro flora [3,4]. Probiotics are consumed in the form of fermented food, dairy pro- ducts, infant and toddler formula. Prebiotics are non- digestible food ingredients that benefit the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon and thereby improving the host's health [4,5]. The most widely stud- ied prebiotics are inulin, fructooligosaccharide (FOS) and galactooligosaccharide (GOS) which are plant stor- age carbohydrates in vegetables, cereals and fruit. Fruc- tooligosaccharide and inulin are added to different foods as fat and sugar replacements to improve texture or for their functional benefits [5-8].

Probiotics improve health in different ways [3,9]. The health benefits conferred by probiotic bacteria are strain specific [3,9]. Some strains increase phagocytic activity of peripheral blood leukocytes, others strains promote production of mucosal antibodies reducing the trans- mucosal transfer of antigens. This strengthens the mucosal barrier function [10-12]. Other probiotic strains increase cytokine production such as interleukin 6 (IL-6) [13]. In healthy people probiotics rarely cause disease. The risk of developing bacteraemia from ingested lacto- bacilli is less than 1 per 1 million users; risk of develop- ing fungaemia (from Saccharomyces Boulardii) is less than1 per 5.6 million users [14-16]. In many studies on infants, C- reactive protein (CRP) and IL-6 have been used to diagnose the early onset of infection [17,18]. CRP is an acute phase protein, blood levels begin to rise to 10 − 1000 fold from 1 ug/ml within 4−6 hours at the onset of an infective or inflammatory process. C- reactive protein has a relatively short half-life making it useful in monitoring infection, inflammation and response to treatment [19]. IL-6 is a pro-inflammatory cytokine which stimulates the production of acute phase pro- teins (such as CRP) [20]. It is readily detected in serum during inflammation and indicates the presence of infection [18,19].

Adding prebiotics to formula stimulates the growth of beneficial bacteria (such as bifidobacteria, lactobacilli) in the gastrointestinal tract to levels found in breastfed infants [9,21]. As these beneficial bacteria increase, they occupy more of the "microbiological niches" in the intestine excluding pathogens. This improves the gut mucosal barrier, prevents infections with enteric patho- gens or trans-located gut bacteria [22,23]. Prebiotics have a good safety record at levels found in existing food components. Flatulence or abdominal bloating is reported at doses greater than 20g / day. Abdominal cramps or diarrhoea are reported at doses greater than 50 g / day [23].

When probiotics and prebiotics are administered sim- ultaneously, the combination is termed Synbiotics. The prebiotic in the synbiotic mixture improves the survival of the probiotic bacteria and stimulates the activity of the host's endogenous bacteria [9,21,24,25]. The superiority of synbiotics compared to either probiotics or prebiotics have not been demonstrated. No review has examined the impact of synbiotics on clinical out- comes in formula fed term infants. Recent systematic reviews (published from 2007 to 2011) on the use of probiotics or prebiotics in term infants have focused on prevention of allergic disease and food hypersensitivity [26,27]. Reviews on children and adults focused on upper respiratory tract infections, antibiotic associated diarrhoea and acute infectious diarrhoea [28-30]. This review focused on full term infants given only infant for- mula with synbiotics, probiotics or prebiotics.

The Human Research Ethics Committee at the Univer- sity of Stellenbosch, South Africa reviewed the protocol, ruled that all data to be collected for this review was from the public domain and was therefore exempt from ethical approval.

Objectives

The objectives of this systematic review were:

1) To determine the effects of infant formula containing synbiotics, probiotics or prebiotics on clinical outcomes in full term infants.
2) To explore if synbiotics are superior over probiotics or prebiotics.

Methods

Criteria for considering studies for this review

All randomized controlled trials (RCTs), irrespective of language, which compared the use of term infant formula containing synbiotics, probiotics or prebiotics to conventional infant formula with or without placebo amongst healthy full term infants (>37 weeks gesta- tion or ≥ 2.5 kg birth weight, age: 0−12 months, with no disease, congenital abnormality, allergy or eczema) receiving formula feeds only. Studies published as abstracts were included if sufficient information could be obtained to assess study quality and obtain relevant study findings.

Types of outcome measures

Primary outcomes

Growth changes (assessed for entire study duration): weight gain (g/day), linear growth (cm/week, mm/ month), head growth (cm/week, mm/month). *Secondary outcomes*: Tolerance to formula: Stool characteristics: frequency, consistency, diarrhoea; Gastrointestinal symptoms (incidence of colic, spitting up/ regurgitation, vomiting, crying), average formula intake (mls/day). Infections: frequency and type of infections, use of medication (antibiotic intake); Hospitalization: Number of days in hospital. Changes in GI microflora: Changes in colony forming units (cfu/g of stool) of bifidobacteria, lactobacillus post intervention, colony forming units (cfu/g of stool) of pathogens post intervention. Immune response: C- reactive protein levels (mg/dl), Interleukin 6 (IL-6) levels (mg/dl).

Search methods for identification of studies

A literature search regardless of language was conducted on electronic databases including The Cochrane CENTRAL Register for Controlled Trials (2010), EMBASE (1980+), Scopus (1990 present), EBSCO host (1960 to 2010), PUBMED / MEDLINE (1966 to 2010), OVID (1950 to 2010), SPORTDiscus (1960 to 2010), Web of Science (1970 to 2010), Science Direct (1950 to 2010), CINAHL (1981 to 2010), Science citation index (1970 to 2010), Latin American Caribbean Health Sciences literature (LILACS) (1965 to 2010), NLMGateway (1950–1966). RCTs published in non-English language journals were translated by independent translators who were familiar with the subject matter. The search strategy used to search PUBMED is shown below. This search strategy was modified to search other electronic databases.

(synbiotic* and probiotic* OR prebiotic*) AND (FOS or fructooligosaccharide or inulin or GOS or galactooligosaccharide) AND (infant formula* OR infant feeding OR formula OR formula milk) AND (infant* or baby or babies) NOT (preterm or premature or low birth weight babies or allergy or eczema) AND (randomized controlled trial* OR controlled clinical trial* Or random allocation*) Limits: Humans.

We also conducted a hand search on abstracts of major conference proceedings such as the Pediatric Aca- demic Society meetings from 1990 (www.pas-meetings. org, www.abstracts2view.com), cross checked references cited in RCTs and in recent reviews (published from 2005 to 2009) for additional studies not identified by electronic searches and specialty journals which were not included in any database such as Pediatrika and Chinese Journal of Microecology.

To identify on-going and unpublished trials, we contacted experts in the field, manufacturers of infant formula containing probiotics and prebiotics, we searched web sites of companies that have conducted or were conducting RCTs on probiotics and prebiotics e.g. Pfizer (www.pfizerpro. com/clinicaltrials), Chris Hansen Laboratory (www.chr-hansen.com/research_development/documentation.html). We also searched prospective trial registries such as World Health Organization (WHO) International Clinical Trials Registry Platform Search Portal (www.who.int/ trialsearch), Clinical Trials.gov register (www.clinicaltrials.gov), Current Controlled Trials *meta*Register of Controlled Trials [*mRCT*] (www.controlled-trials.com/ mrct) and www.clinicaltrialresults.org.

Selection of studies

One reviewer (MM) independently reviewed all abstracts, citations and identified potentially eligible studies. The full reports of eligible studies were retrieved by one reviewer (MM) and the pre-specified selection criteria applied independently by two reviewers (MM, ML) using a study eligibility form (Figure 1). If more than one publication of a study existed, all reports of the study were grouped together under one study name. Any disagreements between the reviewers were resolved through discussion. Unresolved disagreements were resolved by a third party. Trial authors were contacted if eligibility was unclear.

Assessment of quality of evidence

Two reviewers (MM, ML) independently assessed the risk of bias of included studies as described in the Cochrane Handbook for Systematic Reviews for Interventions according to the following 6 components: 1) allocation sequence generation; 2) allocation concealment; 3) blinding; 4) incomplete outcome data; 5) selective outcome reporting; and 6) other sources of bias [31]. Where necessary, trial authors were contacted for clarification on the methodology of their studies. Any disagreements regarding risk of bias were resolved through discussion between MM, ML and RB. The quality of evidence was assessed using guidelines from the Grading of Recommendations Assessment, Development and Evaluation Working Group (GRADE), www.gradeworkinggroup. org (accessed 2012-06-07).

Data extraction and management

Two reviewers (MM, ML) independently extracted data using a pretested data extraction form. The reviewers (MM, ML) cross checked data and resolved any differences through discussion. One reviewer (MM) entered the data in Review Manager (RevMan 5) and the other reviewers (AM, ML) validated the data. Trial authors were contacted for missing data or for clarification.

STUDY ELIGIBILITY FORM

Review title:	**Effects of probiotic, prebiotic and, synbiotic containing infant formula on clinical outcomes in term infants**			
Study ID (Author last name, initials)				
Refworks ID number				
Date of review for eligibility (DDMMYYYY):				
Title of study/article				
Journal title				
Year/volume/issue/page				
Extractor (Last name, initials)				
Type of study	**Put a check (√) mark in appropriate box.**			
Is this study a Randomized controlled study?	YES	UNCLEAR	NO	
	Go to next question		**Exclude**	**Intervention used in study:**
Trial intervention				Circle below
Experimental group: Term infant formula containing either probiotic(s) or prebiotic(s) or synbiotic(s)	YES	UNCLEAR	NO	Probiotic
Control group: Conventional term formula with or without placebo	YES	UNCLEAR	NO	Prebiotic
Note: All formulas are Cow based milk formula, no soy based formula. Breastmilk when used as reference only	YES	UNCLEAR	NO	Synbiotic
Study Participants	Go to next question		**Exclude**	
Healthy Full term infants (≥37 weeks gestation or ≥ 2.5 kg birth weight), Age: 0-12 months. Strictly formula fed infants only.	YES	UNCLEAR	NO	
Exclude: If infants have Congenital malformations, chromosomal abnormalities. Breastfed infants (unless they are used as a reference group)	**NO**	UNCLEAR	**YES**	
Study Outcomes. Does the study have one or more of the following outcomes:	Go to next question		**Exclude**	
Growth parameters: (weight, Height/length, Head Circum)	YES	UNCLEAR	NO	
Tolerance to feed: stool pattern (frequency, consistency), vomiting, diarrhoea, volume of feed tolerated	YES	UNCLEAR	NO	
Infections: frequency, type, use of meds	YES	UNCLEAR	NO	
Immune respone: CRP, IL-6, other immune system parameters	YES	UNCLEAR	NO	
Stool Microbiology: levels of bifidobacteria, lactobacilli, pathogens	YES	UNCLEAR	NO	
Hospitalization: # days in hospital, ICU (if any)	YES	UNCLEAR	NO	
		Clarify missing information		
Other reasons for excluding study	**NO**		**Yes**	
Final decision	**Include**	**Unclear**	**Exclude**	
		For discussion		
Comments				

Figure 1 Study Eligibility form

Data synthesis and management

Results for probiotic, prebiotic and synbiotics studies were analysed separately. No imputation measures for missing data were applied. Trial authors were contacted if there was missing data. Available case analysis was used where there was missing data. The potential impact of missing data on results is addressed in the discussion section.

Heterogeneity of the trials used in the review was assessed by visually inspecting the forest plots to detect overlapping confidence intervals and by performing a Chi^2 test ($p<0.1$ was considered statistically significant because of the low statistical power of this test). An I-square test (I^2) was also used to test for inconsistencies across studies. If the I^2 exceeded 50% and visual inspection of the forest plot supported these results, this represented substantial heterogeneity. Since all of our meta-analyses had less than ten studies, the assessment of publication bias using funnel plots could not be done [31]. If the included studies were not clinically diverse and had similar outcome measures, a meta-analysis was carried out in Review Manager (RevMan 5) by two reviewers (AM, MM). The random effects meta-analysis model was applied to all meta-analyses since the studies were clinically heterogeneous in terms of different settings (countries), doses and strains of synbiotics, probiotics or type of prebiotics, different treatment durations, and other unforeseen factors. The inverse-variance method was used for continuous data and the Mantel-Haenszel method was used for dichotomous data. For continuous outcomes the mean difference (MD) and corresponding 95% confidence intervals (CI) were calculated. For dichotomous outcomes, the risk ratio (RR) and corresponding 95% CI were calculated. The source of statistical heterogeneity was explored using subgroup and sensitivity analyses. If studies were too diverse, no meta-analysis was conducted and a narrative synthesis was provided.

Results

Results of the search and description of studies

Electronic search of available databases yielded 142 citations. After reading titles and abstracts, duplicate reports were removed, 118 articles were screened and 55 articles were excluded. A hand search yielded 2 more articles. Potentially relevant full text reports were retrieved, reviewed for eligibility and a further 38 studies were excluded. One study was published in two other reports [32-34]. The three studies were considered as one study and are referred to as Moro 2006 [32]. Another study was also published in two reports; and is referred as Moro 2002 [35,36]. Twenty five studies (3 synbiotic, 10 probiotic and 12 prebiotic studies) and three on-going studies were included in this review [21,24,25,37-56]. The selection process is shown in Figure 2. Table 1 gives a list of 38 studies which were excluded for: use of breast milk or mixed feeds (12 studies), no use of probiotic or prebiotic (2 studies), being a cross over study, not RCT (5 studies), type of feed was unspecified (3 studies), different inclusion criteria or outcomes (12 studies), no data available for end of treatment period (1 study) and data presentation inappropriate for meta- analysis (3 studies) [57-94]. No eligible studies were excluded for failure to report the review's pre-specified outcomes.

Included studies

Summary of the included synbiotics, probiotics, prebiotics, and on-going studies are shown in Tables 2 3, 4, 5. All studies were conducted on healthy infants and used standard infant formula.

Synbiotic studies: Three studies (N = 475) used various synbiotic (probiotic and prebiotic) combinations [21,24,25]. Two studies [21,24] used a probiotic combination of *Bifidobacterium longum BL999* with *Lactobacillus rhamnosus; Bifidobacterium animalis ssp lactis* with *Lactobacillus paracasei.*One study [25] used *Bifidobacterium longum* alone. Dosage varied from 1×10^7 to 2×10^7 cfu/g powder to 1.28×10^8 to 2.5×10^8 cfu/100 ml. The prebiotics used were a combination of 90% GOS 10% FOS [24,25] or GOS alone [21]. The prebiotic doses ranged from 0.24 g to 0.4 g/100ml. Treatment duration varied from 4 months to 6 months. The synbiotic studies were conducted in France, Italy and Netherlands. None of the synbiotic studies reported data on volume of feed tolerated, hospitalization, changes in GI microflora and immune response.

Probiotic studies: Ten probiotic studies (N = 933) were included. One study [55] used a reduced protein infant formula and one study [50] used an acidified formula given to healthy infants born to HIV positive mothers. The most widely studied probiotics were *Bifidobacterium lactis (BB-12)* which was administered alone [40,44,46,50-52]. Other probiotic strains used were *Lactobacillus reuteri* and *Bifidobacterium bifidum*. Doses ranged widely. For Bifidobacteria: 1.5×10^6 to 3.85×10^8 cfu/g powder and Lactobacillus: 1×10^6 to 1×10^8 cfu/g powder. Treatment duration varied from 14 days to 7 months. The probiotic studies were conducted in Australia (Adelaide), Belgium, Chile (Santiago), France, Israel (Beersheva), South Africa (Johannesburg) and USA (Iowa). None of the probiotic studies reported data on immune response.

Prebiotic studies: Twelve prebiotic studies (N = 1563) were included. The studied prebiotics were FOS [37], GOS [43,47,53,54], acidic oligosaccharide [42] or a mixture of GOS and FOS [32,35,39,41,49]. Two studies used long chain FOS [32,41]. One study used poly dextrose with GOS [56]. The doses ranged from 0.15 g to 0.8 g/ 100 ml. Treatment duration ranged from 28 days to 12

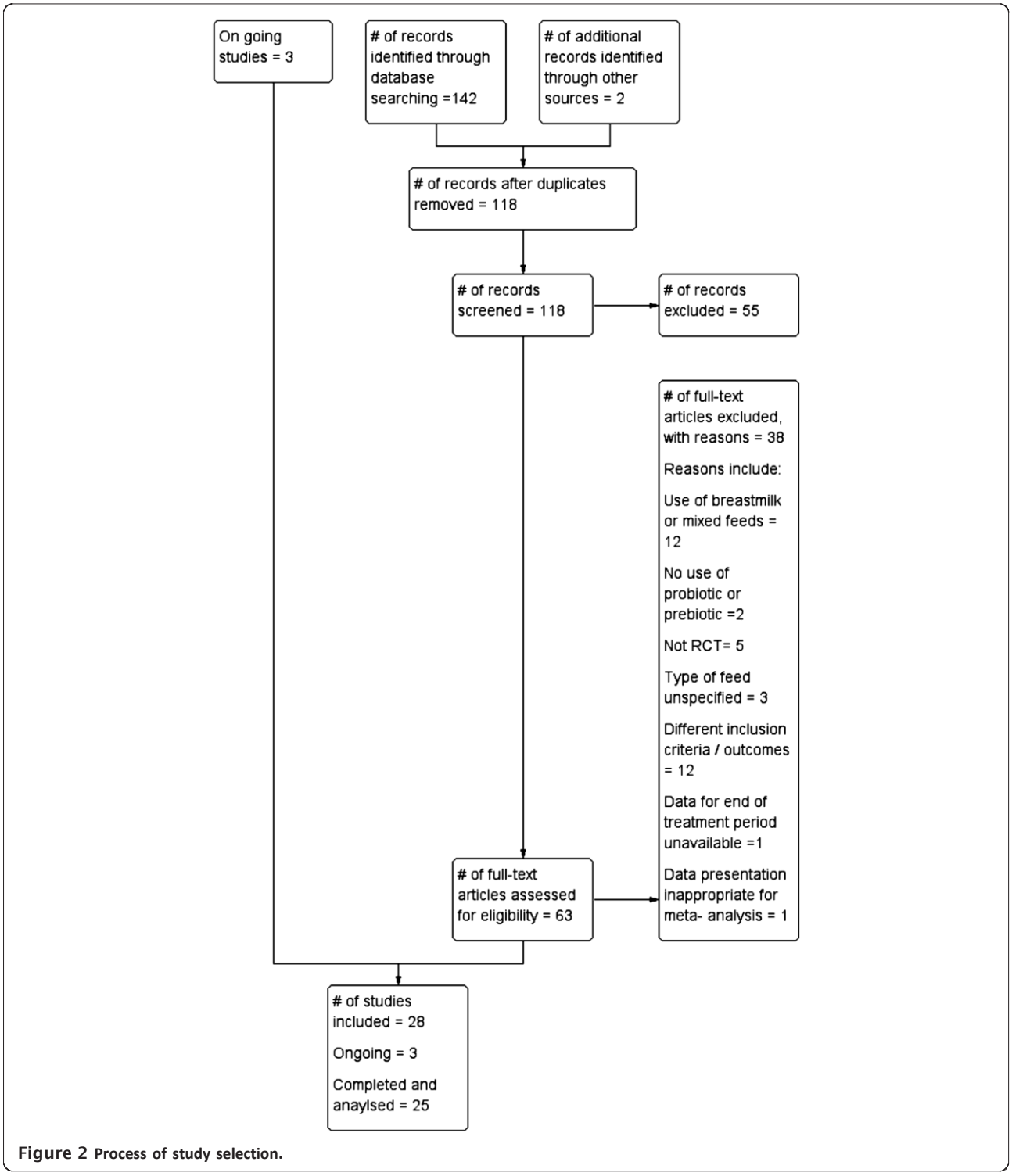

Figure 2 Process of study selection.

months. The prebiotic studies were conducted in China (Nanjing), Greece, Germany (Griefswald), Italy (Ferrara, Milan, Turin, Verona), Spain (Los Palmas, Seville) and USA (Iowa). None of the prebiotic studies reported data on hospitalisation and immune response.

Risk of bias

The risk of bias of the included studies was assessed across six domains using guidelines from the Cochrane Handbook for Systematic Reviews of Interventions (Higgins 2008). See Figure 3.

Table 1 Excluded studies, with reasons for exclusion

Reasons for exclusion of studies								
Use of breast milk or mixed feeds (breast milk, formula, other milk – cow, buffalo, goat milk)		No use of probiotic, prebiotic	Cross over trial / study, Not RCT	Type of feed not clear / specified	Different inclusion criteria or outcomes treatment period not available		Data for end of	Data presentation inappropriate for Meta -analysis
Allen 2010[61]	Magne 2008[79]	Brunser 1989[67]	Bongers 2007[66]	Panigrahi 2008[82]	Augustina 2007[60]	Isolauri 2000[73]	Rautava 2009[83]	Decsi 2005[70]
Baldeon 2008[65]	Mah 2007[80]	Thibault 2004[91]	Euler 2005[71]	Karvonen 1999[96]	Alliet 2007[62]	Knol 2005[75]		Rinne 2005[85]
Chandra 2002[68]	Rinne 2006[86]		Kim 2007[74]	Karvonen 2001[97]	Bakker-Zierikzee 2005[63]	Nopchinda 2002[81]		Velaphi 2008[94]
Kuitunen 2009[76]	Saavedra 2004[88]		Rigo 2001[84]		Bakker-Zierikzee 2006[64]	Rivero 2004[87]		
Kukkonen 2007[77]	Sepp 1993[90]		Savino 2003[89]		Correa 2005[69]	Urao 1999[92]		
Kukkonen 2008[78]	Vendt 2006[95]				Hol 2008[72]	Van der Aa 2010[93]		

Table 2 Summary of 10 included probiotic studies

Probiotic studies	Location	Inclusion criteria	Treatment used in study groups, n =	Treatment duration	Reported outcomes
Brunser 2006[38]	Santiago - Chile	37 – 42 weeks gestation 3000 – 4200 g birth weight	1) Probiotic: *L Johnsonii La1* 10^8 cfu/ g powder n=25 2) Prebiotic: FOS 2g n= 32/L 3) Breastfeeding n= 26 4) In Placebo group: Conventional infant formula no probiotic or prebiotic n= 33	13 weeks	Average formula intake (ml/kg) Fecal excretion of bifidobacteria, Lctobacillus, Enterobacteria (Log10(CFU)/g stool)
Chouraqui 2004[40]	France	Infants < 8 months	1) Probiotic: *B. lactis Strain Bb12* 1.5 X10^6 cfu/g powder, n=46 2) In Placebo group: Conventional infant formula no probiotic or prebiotic, n=44	148 days	Diarrhea, stools/day, Spitting , regurgitation
Gibson 2009[44]	Adelaide -Australia	> 37 weeks gestation, birth weight 2500 - 4500 g,<10 days old	1) Probiotic group: *Bifibacterium lactis.* 3.85 X 10^8 cfu/g 100kcal, n= 72 2) Placebo group: Conventional infant formula no probiotic, n=70	7 months	Growth: Weight, length, head circumference Stool characteristics (data not shown) Stools, colic, spitting up, vomiting and restlessness Mean daily volume of formula intake GI infections, Respiratory infections
Haschke-Becher 2008[45]	Santiago - Chile	36 - 44 weeks gestation, birth weight > 2500 g at 16 weeks of age	1) Probiotic group*: Lactobacillus Johnsonii* 1X10^8 cfu/g powder yielding 0.8 to 1.1 X10^8 cfu/ 200 mls formula, n= 17 2) Placebo group: conventional infant formula no probiotic, n= 18 3) Reference group: Human milk, n==23	4 weeks	Growth: Weight, length, Formula intake
Langhendries 1995[46]	Belgium, St Joseph-Montegnee-Rocourt	Healthy Full term infants	1) Probiotic group: *Bifibacterium Bifidum* 10^6 cfu/g powder, n= 20 2) Placebo group: conventional infant formula no probiotic, n= 20 3) Reference group: Human milk, n= 14	2 months	Bifidobacteria, Bacteriodes, Enterobacteria Log10 (CFU) / g of faeces
Petschow 2005[48]	Iowa, USA	Healthy full term infants, weight >2500g, appropriate for gestational age (0-3 months of age)	1) Probiotic group*: Lactobacillus GG* 1X10^6 cfu/g powder ielding 10^8 cfu/day, n=15 2) Probiotic group: *Lactobacillus GG* 1X10^7 cfu/g powder yielding 10^9 cfu/day, n= 14	7 day baseline, 14 days treatment period, 14 days follow up	Stool frequency, stool consistency

Table 2 Summary of 10 included probiotic studies *(Continued)*

			3) Probiotic group:: *Lactobacillus GG* 1X10^{8} cfu/g powder yielding 10^{10} cfu/day, n= 15 4) Placebo group: Conventional infant formula no probiotic, n= 15		
Urban 2008[50]	Johanesburg South Africa	37 - 42 weeks gestation, 2500 - 4200 g birth weight, born to HIV+ mothers but infants tested HIV-	1) Probiotic group Acidified formula and *Bifidobacterium lactis* n= 29 (cfu/g powder is not specified) 2) No probiotic group: Acidified formula no probiotic, n= 28 3) Placebo group: Conventional infant formula, (whey adapted formula), n= 28	4 months (119 days)	Growth: Males: Weight gain, length and head circumference Females: Weight gain, length and head circumference
Weizman 2005[51]	Beer - Sheva Israel	> 38 weeks gestation, 4-10 months old	1) Probiotic group: *Bifidobacterium Lactis (BB-12)* 1X10^{7} cfu/g powder , n= 73 2) Probiotic group: *Lactobacillus reuteri* 1X10^{7} cfu/g powder, n= 68 3) Placebo group: Conventional infant formula no probiotic, n= 60	12 weeks	Episodes of diarrhea, Volume of feed / day Episodes of respiratory illness, antibiotic use, clinic visits
Weizman 2006[52]	Beer - Sheva Israel	> 38 weeks gestation, < 4 months (3 - 65 days of age)	1) Probiotic group:: *Bifidobacterium Lactis (BB-12)* 1X10^{7} cfu/g powder yielding 2.2 X10^{8} cfu/180 mls reconstituted formula, n= 20 2) Probiotic group: *Lactobacillus reuteri* 1X10^{7} cfu/g powder yielding 2.2 X10^{8} cfu/180 mls reconstituted formula , n= 20 3) Placebo group: Conventional infant formula no probiotic, n=19	4 weeks	Growth: Weight, length, head circumference (final percentiles) Stooling effort score, stooling consistency score Daily crying score and daily crying episodes Formula volume (mls/kg)
Ziegler 2003[55]	Iowa USA	≥ 37 weeks gestation, Birth weight 2500g - 4500g (6 - 10 days of age)	1) No probiotic group: Reduced Protein formula no probiotic or prebiotic n=40 2) Probiotic group: Reduced protein formula, *Bifidobacterium lactis* 3.6 X10^{7} cfu/g powder yielding 4.8 X10^{9} cfu/L reconstituted formula , n= 40 3) Placebo group: Conventional infant formula, no probiotic, n=42	112 days	Growth: Males: Weight, length, Females, weight, length Stool consistency Crying, colic (data not shown) Hospitalization, diarrhea, diarrhea (No. of episodes)

Table 3 Summary of 12 included prebiotic studies

Prebiotic studies	Location	Inclusion criteria	Treatment used in study groups, n =	Treatment duration	Reported outcomes
Bettler 2006[37]	USA	<14 days postnatal age, birth weight and current weight between 10 - 90 percentiles for age,	1) Prebiotic group: FOS 1.5 g/L n=72 2) Prebiotic group: FOS 3.0 g/L n= 74 3) Placebo group: Conventional infant formula no prebiotic, n=66	12 weeks	Growth: Weight, length, Head circumference
Bruzzese 2009[39]	Milan, Napoli, Verona Italy	37 to 42 weeks gestation, > 2500g birth weight, 4 to 6 months old	1) Prebiotic group: GOS, FOS (ratio 9:1) 0.4 g/100 ml n= 96 2) Placebo group: conventional formula with no prebiotic, N= 105	12 months	Growth, Weight, length. Stool consistency Infections: diarrhea episodes / child 12 months, episodes of acute diarrhea, episodes of URTI, antibiotic use
Costalos 2008[41]	Greece	Birth weight between 10th and 90th percentiles, no breastfeeding after age of 14 days	1) Prebiotic group: 90% G0S 10% LcFOS 0.4 g/100 ml n=70 2) Placebo group: Conventional formula no prebiotic n=-70	6 weeks	Growth: Weight gain, length and head circumference gain Stool frequency, consistency. GI Microflora: Bifidobacteria, E coli
Fanaro 2005[42]	Ferrara, Italy	Healthy full term infants, without antibiotic treatment	1) Prebiotic group: Acidic Oligosaccharides 0.2 g/dl, Maltodextrin 0.2 g/dl n= 16 2) Prebiotic group: Acidic Oligosaccharides 0.2 g/dl, Neutral GOS FOS 0.6 g/dl n= 15 3) Placebo group: Maltodextrin at 8g/dl n=15	6 weeks	Growth: Weight and length gain. Stool consistency, Crying, regurgitation and vomiting episodes, GI Microflora
Fanaro 2008[43]	Ferrara, Turin Italy, Las Palmas, Seville Spain	Appropriate for gestational age, birth weight > 1500g, 4 to 6 months old	1) Prebiotic group: GOS 5 g/L n= 56 2) Placebo group: Maltodextrin at 5g/L n=59	18 weeks	Growth: Weight, length, Stool frequency, consistency GI microflora: Bifidobacteria, Lactobacilli, Bacteriodes, Clostridia, Enterobacteriacae
Moro 2002[35] (Moro 2003, considered as one study)	Milan Italy	39 to 40 weeks gestational age	1) Prebiotic group: GOS, FOS 0.4 g/dl n=30 2) Prebiotic group: GOS FOS 0.8 g/dl n= 27 3) Placebo group: Maltodextrin at 0.8g/dl n=33 4) Reference group: Breast milk n=15	28 days	Growth: Weight and length gain Stool frequency, consistency Crying, regurgitation and vomiting, Feeding volume GI microflora: Bifidobaceria, Lactobacilli
Moro 2005[47]	Italy	Healthy full term infants, appropriate for gestational age	1) Prebiotic group: GOS 0.8g/dl, n= 16 2) Placebo group: Maltodextrin at 0. 8g/dl n=16	28 days	Growth: Weight, length gain Feeding volume, GI microflora
Moro 2006[37] (Arslanoglu 2007, Arslanoglu 2008 considered as one study)	Milan Italy	37 - 42 weeks gestational age	1) Prebiotic group: ScGOS Lc FOS at 8g/L, n= 104 2) Placebo group: Maltodextrin at 8g/L, n=102	6 months	Growth: Weight gain, length gain, head circumference Stool frequency, consistency Crying, regurgitation and vomiting GI microflora: Bifidobacteria, Lactobacilli, Infectious episodes: Overall infections, URTI, Otis Media, GI infections, UTI, antibiotic use

Table 3 Summary of 12 included prebiotic studies *(Continued)*

Prebiotic studies	Location	Inclusion criteria	Treatment used in study groups, n =	Treatment duration	Reported outcomes
Schmelzle 2003[49]	Griefswald Germany	37 to 42 weeks gestational age, birth weight between 10 to 90 percentiles, exclusive formula feeding by age 14 days old.	1) Prebiotic group: 90% GOS, 10% FOS 0.8/100ml n=76 2) Placebo group: Conventional infant formula, no prebiotic, n=78	12 weeks	Growth: Males - Weight gain, length gain, head circumference, Females - Growth: Weight gain, length gain, head circumference Volume of feed (formula) GI microflora: Bifidobacteria
Xiao-Ming 2004[53]	Nanjing China	Healthy full term infants	1) Prebiotic group: Galactooligosaccharide 0.24 g/ dl n=69 2) Prebiotic formula with Human milk n= 124 3) Placebo group: Conventional infant formula, no prebiotic, n=52 4) Reference group : Human milk n= 26	6 months	GI Microflora: Bifidobacteria, Lactobacilli, E coli
Xiao-Ming 2008[54]	Nanjing China	> 38 weeks gestation, Birth weight > 3kg.	1) Prebiotic group 1: Galactooligosaccharide 0.24 g/ 100 ml n=37 2) Prebiotic group 2: Prebiotic formula with Human milk n= 58 3) Placebo group: Conventional infant formula, no prebiotic, n=45 4) Reference group : Human milk n= 24	3 months	Growth: Weight gain, length gain Stool consistency Crying, regurgitation and vomiting scores, Volume of feed GI Microflora: Bifidobacteria, Lactobacilli, E coli
Ziegler 2007[56]	USA	> 37 weeks gestation, Birth weight 2500g, solely formula fed	1) Prebiotic group 1: Polydextrose, Galactooligosaccharide n=58 2) Prebiotic group 2: Polydextrose, Galactooligosaccharide, Lactulose n= 48 3) Placebo group: Conventional infant formula, no prebiotic, n=58	120 days	Growth: Weight gain, length gain, head circumference Stool frequency, consistency Intolerance to formula: Vomiting, diarrhea, excessive spitting, colic

Table 4 Summary of 3 included synbiotic studies

Probiotic studies	Location	Inclusion criteria	Treatment used in study groups, n =	Treatment duration	Reported Outcomes
Chouraqui 2008[24]	France (Marseille)	37 – 42 weeks, gestation, ≤ 14 days singletons, 2500 – 4500g birth weight	1) Probiotic group: *Bifibacterium Longum* BL999 1.29 X10^8 cfu/100 ml formula, L.Rhamnosus 6.45 X10^8 cfu/100 ml formula, n=60	4 months, observation: 16 – 52 weeks	Growth: Length, Head circumference Stool frequency, consistency, Incidence of diarrhea during treatment period Frequency of infections
			2) Synbiotic group 1: *Bifibacterium.Longum* BL999 1.29 X10^8 cfu/100 ml, L Rhamnosus 6.45 X108 cfu/100 ml, 90% GOS, 10% ScFOS 0.4 g/100 ml n=54		
			3) Synbiotic group 2: *Bifibacterium Longum* BL999 2.58 X10^8 cfu/100 ml, LParacasei 2.58 X10^8 cfu/100 ml, 90% GOS, 10% ScFOS 0.4 g/100 ml, n=60		
			4) Placebo group: Conventional infant formula no probiotic or prebiotic, n=53		
Puccio 2007[25]	Palermo Italy	Healthy Full term infants with gestational age 39 weeks	1) Synbiotic group: *Bifibacterium Longum* BL 999 2 X 10^7 Cfu/g powder, GOS 90% FOS 10% at 4g/L, n=42, n=67	112 days	Growth: Weight, length, head circumference
			2) Conventional infant formula no synbiotic, n=55		Stool frequency (evacuations/day)
					Crying, restlessness, colic, spitting and vomiting
					Volume of feed tolerated
					Frequency of respiratory tract infections
Vlieger 2009[21]	Niewegein, Netherlands	Healthy Full term infants with gestational age > 37 weeks, < 7 days, formula fed	1) Synbiotic group: : *Bifibacterium animalis ssp Lactis* 1 X 10^7 Cfu/g powder, *Lactobacillus. paracasei* 1 X 107 Cfu/g powder, GOS 0.24 g/100 ml, n=67	6 months	Growth: Weight, length, head circumference
			2) Placebo group: Prebiotic infant formula GOS 0.24 g/100 ml, n=59		

Table 5 Summary of 3 on-going studies

On-going studies	Location	Inclusion criteria	Treatment used in study groups, n =	Outcomes, Estimated date of completion.
Cabana 2010[57]	USA	>37 weeks gestation, birth weight >2500 g and < 4500 g, 14±3 days of age on enrollment, singleton birth, non- breastfed, not received solid foods.	1) Study group 1: Test starter infant formula	Primary: Weight gain (g/day) at 14 to 112 days of life (4 months)
			2) Study group 2: Test starter infant formula with synbiotics	Secondary: Tolerance, morbidity, protein status, metabolic markers, December 2011
			3) Control /placebo group: Standard formula	
Zegerman 2009[58]	Israel	37th and 42 week gestation, birth weight > 2500 g, recruitment age: 0 -28 days, non-breastfed	1) Study group 1: Dietary Supplement: probiotic microorganism and/or prebiotic	Primary: weight, length, head circumference
			2) Dietary Supplement: probiotic microorganism and/or prebiotic	Secondary: Microbiology, August 2012
			2) Dietary Supplement: probiotic microorganism and/or prebiotic	
Ye 2010[59]	Singapore	> 37 weeks to < 42 weeks gestation, singleton birth. Age at enrolment < 14 days old	1) Study group 1: Standard infant formula with prebiotics	Primary: Mean Weight gain
			2) Study group: Infant formula with synbiotics	Secondary: Digestive tolerance, December 2011

Random sequence generation

Fifteen trials described clearly the methods used for random sequence generation [21,24,32,37-41,43,44,46, 49-52]. Random sequence generation was done through computer randomization [21,37,38,43,44,50-52], random number tables [39,46] or block randomization [32,40,41]. The method used for random sequence generation was not clearly described in 10 studies [25,35,42,45, 47,48,53-56].

Allocation concealment

In seven trials, treatment allocation was adequately concealed [32,38,42,44,46,49,50]. Allocation concealment was adequate due to central allocation using a computer [38], use of sealed envelopes [43,44,49], pre – coded or colour coded containers [32,50] and use of independent staff outside of study [46]. In the rest of the 18 studies, allocation concealment was not clearly demonstrated or described [21,24,25,35,37,39-42,45,47,48,51-56].

Blinding

Adequate blinding of study participants, care providers and assessors was done in 9 trials. Blinding was ensured by using pre-coded or colour coded formula tins [21,24,25,32,38,43,44,46,50]. In the other 16 trials, there was not enough information given on the blinding method to make a judgement [35,37,39-42,45,47-49,51-56].

Incomplete outcome data

Reported outcome data was satisfactory for 19 studies. In 3 studies, there was no missing outcome data [38,40,54]. In 16 studies, missing outcome data was balanced across the intervention groups with similar reasons reported [21,24,25,32,37,39,41-45,49-52,55]. In 4 studies there was insufficient information given to permit a judgement [35,46,47,53]. In 2 studies there were no reasons given for missing data [48,56].

Selective reporting

In 7 studies, the pre-specified outcomes in the methods section were reported in the results section [21,25,32,45,49,54,56]. In 18 studies the pre-specified outcomes were not reported [24,35-44,46,48,50-55].

Other potential sources of bias

Nineteen studies appeared to be free from other potential sources of bias [21,24,25,32,38-46,49-52,54,56]. There was insufficient information given to permit a judgment in 6 studies [35,37,47,48,53,55].

Effects of interventions

Synbiotics versus controls

Three studies (N = 475) investigated the effect of synbiotic administration versus no synbiotic or placebo (control group) [21,24,25].

Primary outcomes

Growth parameters

(i) Weight gain

Only one study [24] reported weight gain in terms of grams per day (g/day). In this study, two types of synbiotics (Type 1 and Type 2) were evaluated and results for boys and girls were reported separately. The results of the two synbiotics were combined using the

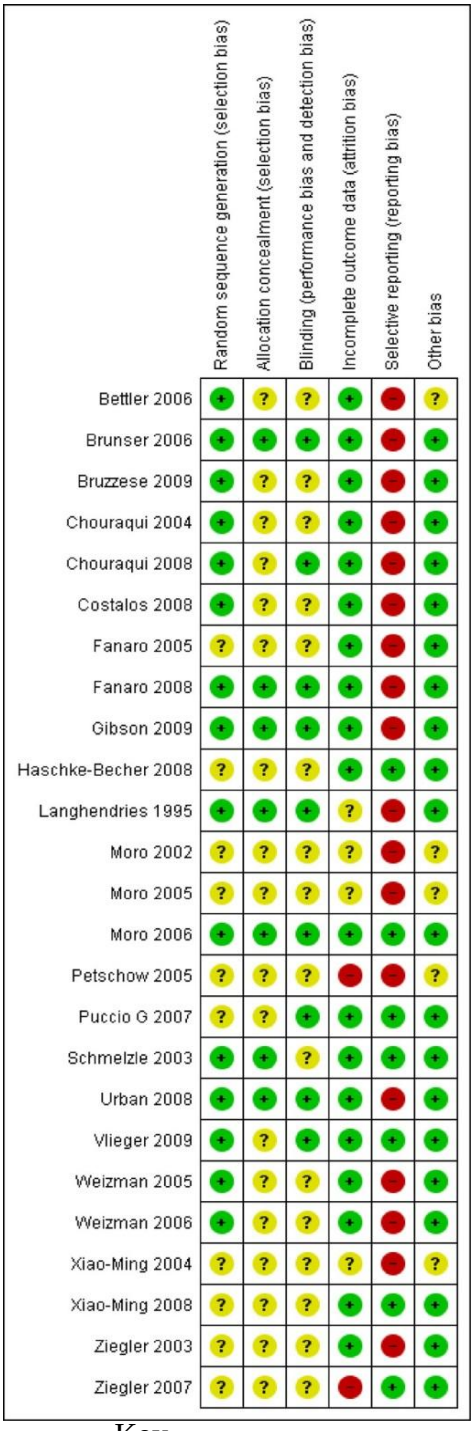

Figure 3 Methodological qualities of included studies.

combined mean and pooled standard deviation. The calculated treatment effects showed that synbiotics failed to significantly increase weight gain for boys (MD 0.90, 95% CI: -1.95 to 3.75, n = 81) and girls (MD 0.90, 95% CI: -1.81 to 3.61, n = 86) compared to the controls. One study [21] reported weight gain in terms of some score scale. A calculated treatment effect showed that synbiotics failed to significantly increase weight gain compared to controls (MD −0.07, 95% CI: -0.43 to 0.29, n = 79). Since the score scale can take negative values, the values of mean and standard deviation in this analysis do not necessarily imply that the data is skewed. One study [25] reported weight gain (g/day) in terms of mean difference (MD) and 90% CI. These values were used in calculating the corresponding standard error (SE). The MD and SE were used in calculating the treatment effect (via the generic-inverse variance method in RevMan). Synbiotics again failed to significantly increase weight gain compared to controls (MD −1.09, 95% CI: -3.54 to 1.36, n= 97).

(ii) Length gain

Two studies [24,25] reported length gain in terms of millimetres per month (mm/month) for boys and girls separately. Results from these two studies were pooled in a meta-analysis but for Chouraqui 2008 [24] results for the two types of synbiotics were combined before meta-analysis. Results from the meta-analysis showed that synbiotics failed to significantly increase length gain compared to controls for both boys (MD 0.75, 95% CI: -0.66 to 2.17, n = 126) and girls (MD 0.75, 95% CI: -0.63 to 2.13, n = 138) [Figure 4]. There was no significant heterogeneity detected between the two studies for boys (Chi^2=0.50, df=1, p=0.48, I^2=0%) and girls (Chi^2=0.53, df=1, p=0.47, I^2=0%).
One study [21] reported length gain in terms of some score scale. A calculated treatment effect showed that synbiotics failed to significantly increase length gain compared to controls (MD 0.01, 95% CI: -0.43 to 0.45, n = 79). Since the score scale can take negative values, the values of mean and standard deviation in this analysis do not necessarily imply that the data is skewed.

(iii) Head circumference gain

Two studies [24,25] reported head circumference gain in terms of mm/month for boys and girls separately. Results from these two studies were pooled in a meta-analysis but for Chouraqui 2008 [24] results for the two types of synbiotics

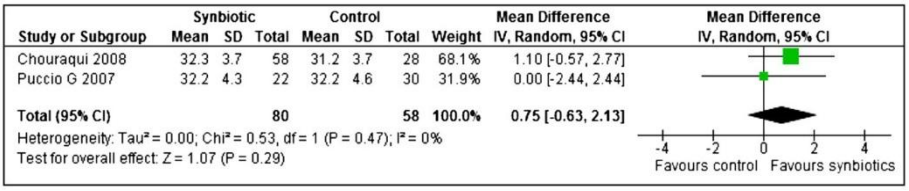

Figure 4 Synbiotics versus controls, Outcome: Length gain (mm/month) for girls.

were combined before meta-analysis. Results from the meta-analysis showed that synbiotics failed to significantly increase head circumference gain compared to controls for both boys. (MD −0.06, 95% CI: -0.96 to 0.85, n = 126) and girls (MD −0.05, 95% CI: -0.94 to 0.85, n = 138). There was no significant heterogeneity detected between the two studies for both boys (Chi^2=0.64, df=1, p=0.43, I^2=0%) and girls (Chi^2=0.67, df=1, p=0.41, I^2=0%).

One study [21] reported head circumference gain in terms of some score scale. A calculated treatment effect showed that synbiotics failed to significantly increase head circumference gain compared to controls (MD 0.01, 95% CI: -0.38 to 0.36, n = 79). Since the score scale can take negative values, the values of mean and standard deviation in this analysis do not necessarily imply that the data is skewed.

Secondary outcomes

Tolerance to formula

(i) Stool frequency

Two studies [21,25] reported stool frequency (evacuations per day) and their results were pooled in a meta-analysis. Synbiotics significantly increased stool frequency compared to the controls (MD 0.28, 95% CI: 0.08 to 0.48, n = 176) and therewas no significant heterogeneity detected between the two trials (Chi^2=0.93, df=1, p=0.33, I^2=0%) [Figure 5].

One study [25] reported stool frequency (evacuations per day) but values for standard deviations were not given and as a result, no treatment effect could be calculated.

(ii) Stool consistency

One study [21] evaluated stool consistency using a consistency score (1=hard to 4=watery and loose) and a calculated treatment effect showed no significant difference between the synbiotic and control treated groups (MD 0.13, 95% CI: -0.15 to 0.41, n = 79).

One study [24] study reported that liquid stools occurred significantly more frequently in the synbiotic group compared to the control group (OR 3.17, 95% CI: 1.59 to 3.60, n = 66).

Puccio 2007 [25] reported that data on stool consistency showed no statistically significant differences between the two study groups (data not shown in study report).

(iii) Incidence of colic, spitting up / regurgitation, vomiting, crying

Data on frequency of crying, restlessness, colic, spitting and vomiting reported by Puccio 2007 [25] showed no statistically significant differences between the two study groups (data not shown in study report).

Results from Vlieger 2009 [21] showed no significant differences in the frequency of vomiting (RR 0.46, 95% CI: 0.12 to 1.72, n = 79) and colic (RR 2.50, 95% CI: 0.46 to 13.73, n = 79) between the two study groups. The same study showed no difference in crying (hours per day) between the two study groups (MD −0.10, 95% CI: -0.46 to 0.26, n = 79).

(iv) Average formula intake

One study [25] reported the mean daily intake of formula in a graph where no values could be retrieved.

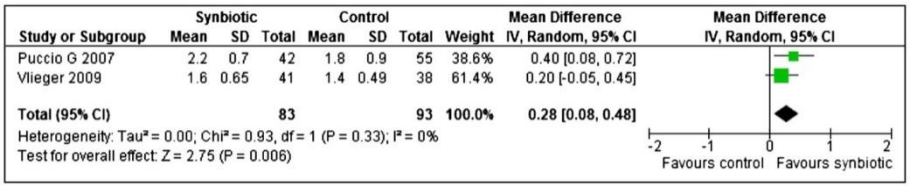

Figure 5 Synbiotics versus controls, outcome: Stool frequency (evacuations per day).

One study [25] reported the mean daily intake of formula in a graph where no values could be retrieved.

Infections

(i) Infections
Puccio 2007 [25] reported data on frequency of respiratory tract infections but there were no significant differences between the synbiotic and control treated groups (RR 0.71, 95% CI: 0.31 to 1.59, n = 97).
Vlieger 2009 [21] reported the mean (SD) of upper respiratory tract infections and gastrointestinal infections (times per month) but no treatment effect could be calculated because the data was skewed (mean < SD).

(ii) Antibiotic intake
Vlieger 2009 [21] reported the mean (SD) of the use of antibiotics (times per month) but no treatment effect could be calculated because the data was skewed (mean < SD).

Probiotics versus controls

Ten studies (N = 933) investigated the effect of probiotic administration versus no probiotic (Control group) [38,40,44-46,48,50-52,55].

Primary outcomes: growth parameters

(i) Weight gain
Four studies [24,44,50,55] reported weight gain (g/day) for boys and girls separately. The results from these four studies were pooled in meta-analyses separately for boys and girls. Probiotics failed to significantly increase weight gain compared to the controls for boys (MD 1.64, 95% CI: -0.36 to 3.64 n = 158), no statistically significant heterogeneity was detected between the studies for boys (Chi^2=3.43, df=3, p=0.33, I^2=13%). However, statistically significant heterogeneity was observed for girls (Chi^2=9.90, df=3, p=0.02, I^2=70%). An investigation of heterogeneity using subgroup analysis with respect to the type of formula (normal/ acidified/ reduced protein) yielded the following results. Two studies [24,44] showed that normal formula with probiotics failed to significantly increase weight gain compared to the controls for girls (MD 1.33, 95% CI: -0.76 to 3.41, n = 113) with no significant heterogeneity between the two studies (Chi^2=0.08, df=1, p=0.78, I^2=0%). Urban 2008 [50] showed that acidified formula with probiotics significantly increased weight gain in probiotic group compared to controls for girls (MD 5.30, 95% CI: 0.46 to 10.14, n = 28). Ziegler 2003 [55] showed that reduced protein formula with probiotics significantly reduced weight gain compared to controls for girls (MD −4.80, 95% CI: -9.18 to −0.42, n = 29) (Figure 6).

(ii) Length gain
Four studies [24,44,50,55] reported length gain for boys and girls separately. Two studies reported in terms of mm/month and two studies reported in terms of mm/day. The latter two studies results were converted to mm/month by multiplying both the mean and SD by 28, assuming a 4 week/ 28-day month. Results from these four

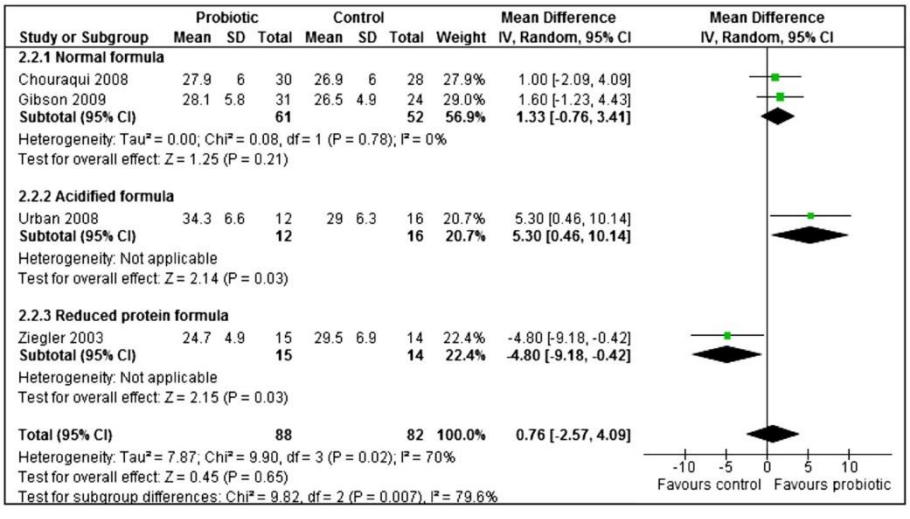

Figure 6 Probiotics versus controls, outcome: Weight gain (g/ day) for girls.

studies were pooled in meta-analyses separately for boys and girls. Probiotics failed to significantly increase length gain compared to the controls for both boys (MD −0.37, 95% CI: -1.64 to 0.90, n = 158) and girls (MD 0.32, 95% CI: -0.81 to 1.45, n =165). No statistically significant heterogeneity was detected between the studies for both boys (Chi^2=3.49, df=3, p=0.32, I^2=14%) and girls (Chi^2=2.94, df=3, p=0.40, I^2=0%).

(iii) Head circumference gain

Three studies [24,44,50] reported length gain for boys and girls separately. Two studies reported in terms of mm/month and one study reported in terms of mm/day. The latter study's results were converted to mm/month by multiplying both the mean and SD by 28 (assuming a 4 week/ 28-day month). Probiotics failed to significantly increase head circumference gain compared to the controls for both boys (MD 0.76, 95% CI: -1.02 to 2.54, n = 125) and girls (MD 0.27, 95% CI: -0.70 to 1.23, n = 139). No statistically significant heterogeneity was detected between the studies for both boys (Chi^2=3.87, df=2, p=0.14, I^2=48%) and girls (Chi^2=1.12, df=2, p=0.57, I^2=0%).

Secondary outcomes

Tolerance to formula

(i) Stool frequency

Two studies [40,48] reported stool frequency (evacuations per day) and meta-analysis of results from these studies showed that probiotics failed to significantly increase stool frequency compared to controls (MD 0.01, 95% CI: -0.44 to 0.46, n = 120). There was no significant heterogeneity between the studies (Chi^2=0.19, df=1, p=0.66, I^2=0%). Since Petschow 2005 [48] evaluated different probiotic dosages, the highest dosage was chosen for the analysis.

(ii) Stool consistency

One study [48] reported stool consistency score (1–5: 1=hard, 2=formed, 3=soft, 4=loose, 5=watery). A calculated treatment effect showed that there was no difference in consistency score between the probiotic and control groups (MD 0.00, 95% CI: -0.33 to 0.33, n = 30).

Chouraqui 2008 [24] reported that liquid stools occurred significantly more frequently in the probiotic group compared to the control group (OR 2.79, 95% CI: 1.48 to 5.29, n = 64).

Ziegler 2003 [55] reported stool consistency in terms of mean (SD) separately for hard, formed, soft and liquid stools but no treatment effect was calculated because the data was skewed (mean < SD). Weizman 2006 [52] reported results for stool consistency score but again the data was skewed (mean < SD).

(iii) Episodes of diarrhoea

Ziegler 2003 and Weizman 2005 [52,55] reported episodes of diarrhoea in terms of mean (SD) but no meta-analysis was done because their results show that the data was skewed (mean < SD).

Chouraqui 2004 and Chouraqui 2008 [24,40] reported the frequency of diarrhoea but meta-analysis of their results showed no benefit from probiotic treatment compared to controls (RR 0.80, 95% CI: 0.46 to 1.38, n = 209). No statistically significant heterogeneity was detected between the studies (Chi^2=0.61, df=1, p=0.44, I^2=0%).

(iv) Incidence of colic, spitting up / regurgitation, vomiting, crying

Chouraqui 2004 [40] reported the number of infants having spitting or regurgitation and there was no difference observed between the probiotic and control groups (RR 0.80, 95% CI: 0.26 to 2.42, n = 90). Weizman 2006 [52] reported the daily crying episodes and there were significantly less crying episodes in favour of the control group (MD 0.60, 95% CI: 0.20 to 1.00, n = 59). The results from the two probiotic groups were combined before meta-analysis. Gibson 2009 [44] reported that stools, colic, spitting up, vomiting, restlessness occurred at similar frequencies in the two groups (data not shown in report). Ziegler 2003 [55] reported that that there was no significant formula effects on crying and colic (data not shown in report).

(v) Average formula intake

One study [38] reported the average formula intake (ml/kg body-weight /day) and the calculated treatment effect showed no differences between the probiotic and control groups (MD 5.00, 95% CI: -12.60 to 22.60, n = 58). Two studies [44,51] reported the average formula intake (ml/day) and meta-analysis showed that infants in the probiotic group had a significantly higher formula intake compared to the controls (MD 46.74, 95% CI: 23.93 to 69.54, n = 292). No statistically significant heterogeneity was detected between the studies (Chi^2=0.45, df=1, p=0.50, I^2=0%).

Infections

Infections

One study [44] reported the number of infants having respiratory infections and the calculated treatment effect showed no differences between the probiotic and control groups (RR 0.93, 95% CI:0.74 to 1.17, n = 142). One study [51] reported

episodes of respiratory illness in terms of mean (95% CI). The mean (95% CI) were used in calculating the SDs. However, no treatment effect was calculated because the data was skewed (mean < SD). One study [44] reported the number of infants having gastrointestinal infections and the calculated treatment effect showed no differences between the probiotic and control groups (RR 0.70, 95% CI: 0.45 to 1.11, n = 142).

(i) Antibiotic intake
One study [51] reported prescription of antibiotics in terms of mean (95% CI). The mean (95% CI) were used in calculating the SDs. However, no treatment effect was calculated because the data was skewed (mean < SD).

Hospitalization
Only one study [55] reported hospitalization but no treatment effect was calculated because the data was skewed (mean < SD)

Changes in gastrointestinal microflora

(i) Bifidobacteria
Two studies [38,46] reported results for bifidobacteria measured as log10 (CFU) per gram stool. A meta-analysis showed that the control group had significantly increased counts of bifidobacteria compared to probiotic group. (MD −1.27, 95% CI: -2.03 to −0.51, n = 57). No statistically significant heterogeneity was detected between the studies (Chi^2=0.71, df=1, p=0.40, I^2=0%) [Figure 7].

(ii) Lactobacillus
Only one study [38] reported results for lactobacillus, measured as log10 (cfu) per gram stool and the calculated treatment effect showed that probiotics failed to increase the counts of Lactobacillus compared to the controls (MD 0.22, 95% CI: -0.72 to 1.16, n = 41).

Pathogens

(iii) Enterobacteria
Two studies [38,46] reported results for enterobacteria measured as log10 (cfu) per gram stool and meta-analysis showed that probiotics significantly reduced counts of Enterobacteria compared to the controls (MD −0.61, 95% CI: -1.20 to −0.03, n = 51). No statistically significant heterogeneity was detected between the studies (Chi^2=0.62, df=1, p=0.43, I^2=0%).

(iv) Bacteriodes
Two studies [38,46] reported results for bacteriodes measured as log10 (cfu) per gram stool and meta-analysis showed that probiotics failed to significantly reduce counts of Bacteriodes compared to the controls (MD −0.11, 95% CI: -1.01 to 0.78, n = 51). No statistically significant heterogeneity was detected between the studies (Chi^2=0.95, df=1, p=0.33, I^2=0%).

Prebiotics versus controls
Twelve studies (N = 1563) investigated the effect of pre- biotic administration versus placebo or no prebiotic in for- mula (Control group) [32,35,37,39,41-43,47,49,53,54,56].

Primary outcomes: growth parameters

(i) Weight gain
Eight studies [32,35,41,42,47,49,54,56] reported weight gain (g/day) and meta-analysis of their results showed that prebiotics significantly increased weight gain compared to the controls (MD 0.97, 95% CI: 0.24 to 1.70, n = 861). No statistically significant heterogeneity was detected between the studies (Chi^2=4.67, df=7, p=0.70, I^2=0%). Three studies [35,42,56] evaluated different types of prebiotics (acidic oligosaccharides with maltodextrin or neutral GOS FOS, GOS FOS, GOS with polydextrose alone or with lactulose). The results for the prebiotics in each of these studies were combined before meta-analysis using combined mean and pooled standard deviation (Figure 8).

(ii) Length gain
Seven studies [32,35,41,42,47,49,54] reported length gain either as cm/week or in units that were converted to cm/week. Meta-analysis of their results showed that prebiotics failed to significantly increase length gain compared to the controls (MD 0.01, 95% CI: -0.01 to 0.04, n = 697). No statistically significant heterogeneity was detected

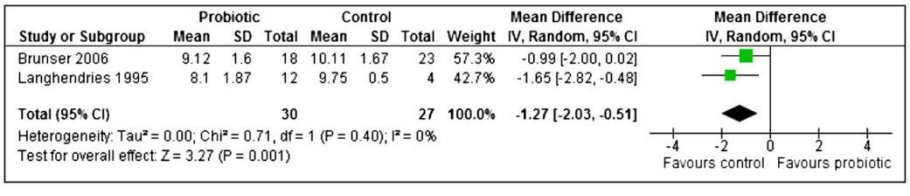

Figure 7 **Probiotics versus controls, outcome: Bifidobacteria -log10(CFU) per gram of stool.**

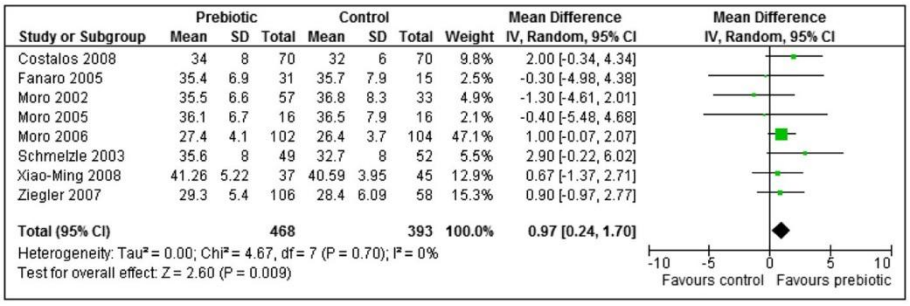

Figure 8 **Prebiotics versus controls, outcome: weight gain (g/day).**

between the studies (Chi2=5.05, df=6, p=0.54, I^2=0%). Two studies [35,42] each evaluated different types of prebiotics (Acidic oligosaccharides 0.2 g/dl with maltodextrin, acidic oligosaccharides 0.2 g/dl with neutral GOS FOS 0.6 g/dl; GOS, FOS 0.4 g/dl and GOS FOS 0.8 g/ dl). The results for the prebiotics in each of these studies were combined before meta-analysis using combined mean and pooled standard deviation.

(iii) Head circumference gain

Three studies [32,41,49] reported head circumference gain either as cm/week or in units that were converted to cm/week. Meta-analysis of their results showed that prebiotics failed to significantly increase head circumference gain compared to the controls (MD −0.01, 95% CI: -0.02 to 0.00, n = 438). No statistically significant heterogeneity was detected between the studies (Chi^2=2.18, df=2, p=0.34, I^2=8%).

Results from Ziegler 2007 [56] were not used because they reported head circumference gain only at 30 days and not at the end of treatment period which was 120 days. (All other studies reported results for end of treatment period).

Secondary outcomes

Tolerance to formula

(i) Stool frequency

Four studies [32,35,43,56] reported stool frequency (evacuations per day) and meta-analysis of their results showed that prebiotics significantly increased stool frequency compared to the controls (MD 0.18, 95% CI: 0.06 to 0.30, n = 539). No statistically significant heterogeneity was detected between the studies (Chi^2=2.97, df=3, p=0.40, I^2=0%). Two studies [35,56] each evaluated different types of prebiotics (GOS FOS; GOS with polydextrose alone or with lactulose). The results for the prebiotics in each of these studies were combined before meta- analysis using combined mean and pooled standard deviation.

Costalos 2008 [41] reported the median (range) of stool frequency (Table 6).

(ii) Stool consistency

Results from the two studies [32,42] using a 5-point scale (1=watery, 2=soft, 3=seedy, 4=formed, 5=hard) were pooled in a meta-analysis but due to significant heterogeneity detected between the two studies, their results are reported separately. Stools from the prebiotic group were significantly softer compared to controls for both Fanaro 2005 [42] (MD −1.20, 95% CI: -1.61 to −0.79, n = 46) and Moro 2006 [32] (MD −0.78, 95% CI: -1.00 to −0.56, n = 206). Fanaro 2005 [42] evaluated two types of prebiotics (acidic oligosaccharides with maltodextrin or neutral GOS FOS), the results for the prebiotics were combined before meta-analysis using combined mean and pooled standard deviation.

Fanaro 2008 [43] used an opposite 5 point scale (1=hard, 2=formed, 3=seedy, 4=soft, 5=watery) and reported the mean (SD) of area under the curve.

Table 6 Stool characteristics

Costalos 2008[41]: Median (range) stool characteristics			
	Prebiotics (n=70)	**Controls (n=70)**	
Stool frequency	1.9 (1.2-2.1)	1.6 (1.1-1.9)	
Stool consistency	3 (2−3.5)	3.1 (2.5-3.5)	
Moro 2002[35]: Median (IQR) Stool consistency score			
	Prebiotic1 (n=30)	**Prebiotic2 (n=27)**	**Control (n=33)**
Stool consistency score	3 (1.5)	2.5 (0.75)	4 (1.5)

A calculated treatment effect showed that stools from the prebiotic group were significantly softer compared to controls (MD 0.53, 95% CI: 0.31 to 0.75, n = 88).

Results from two studies [54,56] used a 4-point scale (1=watery, 2=soft, 3=seedy, 4=formed) were pooled in a meta-analysis but due to significant heterogeneity detected between the two studies, their results are reported separately. Stools from the prebiotic group were significantly softer compared to controls for both Xiao-Ming 2008 [54] (MD −0.65, 95% CI: -0.87 to −0.43, n = 82) and Ziegler 2007 [56] (MD −0.25, 95% CI: -0.44 to −0.06, n = 157). Ziegler 2007 [56] evaluated two types of prebiotics (GOS with polydextrose alone or with lactulose). The results for the prebiotics were combined before meta-analysis using combined mean and pooled standard deviation. Costalos 2008 [41] reported the median (range) of stool consistency score (Table 6).

Moro 2002 [35] reported the median (IQR) of stool consistency score (Table 6).

(iii) Diarrhoea

Two studies [39,56] reported the number of infants having diarrhoea and a meta-analysis showed that prebiotics failed to significantly decrease the incidence of diarrhoea compared to the controls (RR 0.62, 95% CI: 0.19 to 1.99, n = 237). No statistically significant heterogeneity was detected between the studies (Chi^2=1.65, df=1, p=0.20,I^2=39%). Since Ziegler 2007 [56] evaluated two types of prebiotics (GOS with polydextrose alone or with lactulose), the number of events and totals for the prebiotics were summed before meta-analysis.

(iv) Incidence of colic, spitting up / regurgitation, vomiting, crying

Moro 2006 [32] reported no vomiting and very few infants crying but the number of infants experiencing regurgitation were significantly reduced in the prebiotic group compared to the control group (RR 0.11, 95% CI: 0.02 to 0.49, n = 206).

According to Xiao-Ming 2008 [54], there was no difference in crying score (MD 0.01, 95% CI: -0.00 to 0.02, n = 82), regurgitation score (MD −0.01, 95% CI: -0.27 to 0.25, n = 82), and vomiting score (MD −0.03, 95% CI: -0.21 to 0.15, n = 82) between the prebiotic and control groups. All scores were 3 point scores. Crying score: 1= practically not crying, 2 = crying in connection to feeding, 3 = crying independently from meals. Regurgitation score: 1 = no regurgitation, 2 = 1–2 regurgitations, 3 = > 2 regurgitations per day. Vomiting score: 1= no vomiting, 2 = 1 episode of vomiting, 3 = >1 episode of vomiting.

Ziegler 2007 [56] reported that none of the infants had colic; the numbers having excessive spitting were too few; vomiting was similar between the two groups (RR 1.12, 95% CI: 0.43 to 2.89, n = 32). The prebiotic results were summed for the two types before calculation of treatment effect.

Both Moro 2002 and Fanaro 2005 [35,42] reported no difference in the incidence of crying, regurgitation and vomiting episodes (data values not shown in study reports).

(v) Average formula intake

Five studies [35,38,47,49,54] reported formula intake (ml/kg body-weight/ day) and meta-analysis of their results showed statistically significant heterogeneity between the studies (Chi^2=10.80, df=4, p=0.03, I^2=63%,). Sensitivity analysis by removing the one study [49] showing significantly less formula intake for the prebiotics (MD −21.00, 95% CI: -31.86 to −10.14, n = 101) yielded no significant heterogeneity between the four remaining studies (Chi^2=1.79, df=3, p=0.62, I^2=0%) but no significant difference between the two groups (MD 0.31, 95% CI: -8.40 to 9.02, n = 269). The prebiotic results for the two types of prebiotics (GOS, FOS 0.4 g/dl, GOS FOS 0.8 g/dl) in Moro 2002 [35] were combined before meta-analysis using combined mean and pooled standard deviation.

Infections

(i) Infections

According to Moro 2006 [32], prebiotics significantly reduced overall infections compared to the controls (RR 0.45, 95% CI: 0.29 to 0.69, n = 204). The number of infants having gastrointestinal infections, urinary tract infections (UTI) and otitis media were very few [32].

Two studies [32,39] reported the number of infants with upper respiratory tract infections (URTI) and their results were pooled in a meta-analysis. However, due to significant heterogeneity detected between the two studies (Chi^2=7.69, df=1, p=0.006, I^2=87%), their results are reported separately. Although Moro 2006 [32] showed that the prebiotic group significantly reduced the number of infants with URTI compared to the controls (RR 0.48, 95% CI: 0.27 to 0.84, n = 206), there was no difference between the two groups according to Bruzzese 2009 [39] (RR 1.07, 95% CI: 0.86 to 1.33, n = 203).

(ii) Antibiotic intake

According to Moro 2006 [32], prebiotics failed to significantly reduce antibiotic intake compared

to the controls (RR 0.51, 95% CI: 0.26 to 1.00, n = 206).

Changes in gastrointestinal microflora

(i) Bifidobacteria

Five studies [38,42,47,53,54] (n = 280) reported Bifidobacteria (log10 CFU per gram stool) and their results were pooled in a meta-analysis. However, statistically significant heterogeneity was detected between the studies (Chi^2=60.23, df=4, p < 0.00001, I^2=93%). Heterogeneity persisted after conducting subgroup analysis with respect to duration of supplementation or dosage of treatment. The results for each study are therefore reported separately. Four studies showed that prebiotics significantly increased bifidobacteria: Fanaro 2005 [42] (MD 0.30, 95% CI: 0.13 to 0.47, n = 46); Moro 2005 [47] (MD 2.70, 95% CI: 0.37 to 5.03, n = 32); Xiao-Ming 2004 [53] (MD 1.90, 95% CI: 1.51 to 2.29, n = 121); Xiao-Ming 2008 [54] (MD 0.85, 95% CI: 0.16 to 1.54, n = 38). The prebiotic results for the two types of prebiotics (acidic oligosaccharides with maltodextrin or neutral GOS FOS) in Fanaro 2005 [42] were combined before meta-analysis using combined mean and pooled SD. However, Brunser 2006 [38] showed no significant difference in the number of bifidobacteria between the two groups (MD −0.39, 95% CI: -1.49 to 0.71, n = 43) [Figure 9].

Four studies reported their results in median; therefore no conclusions could be made. Costalos 2008 [41] reported the median (range) of Bifidobacteria (log10 CFU per gram stool) as a percentage of total bacteria (Table 7). Three studies [32,35,43] reported the median (IQR) of Bifidobacteria (log10 CFU per gram stool) (Table 8).

(ii) Lactobacillus

Three studies [38,53,54] reported Lactobacillus (log10 CFU per gram stool) and meta-analysis of their results showed statistically significant heterogeneity between the studies (Chi^2=26.44, df=2, p < 0.00001, I^2=92%). Sensitivity analysis was done by removing the one study [38] that showed no difference between the two groups (MD −0.30, 95% CI: -1.08 to 0.48, n = 43). This yielded no significant heterogeneity (Chi^2=0.33, df=1, p =0.57, I^2=0%) between the remaining two studies. Meta-analysis showed that prebiotics significantly increased lactobacillus counts compared to the controls (MD 1.96, 95% CI: 1.58 to 2.34, n = 159).

Three studies reported their results in median; therefore no conclusions could be made. Fanaro 2008, Moro 2002 and Moro 2006 [32,35,43] reported the median (IQR) of Lactobacillus (log10 CFU per gram stool) (Table 8).

Pathogens

(iii) Enterobacteria

According to Brunser 2006 [38], there was no difference in the number of Enterobacteria counts between the prebiotic and control groups (MD −0.48, 95% CI: -1.88 to 0.22, n = 43). Fanaro 2008 [43] reported the median (IQR) of Enterobacteria (log10 CFU per gram stool) (Table 8).

(iv) Bacteriodes

According to Brunser 2006 [38], there was no difference in the number of Bacteriodes between the prebiotic and control groups (MD −0.35, 95% CI: -1.40 to 0.70, n = 43). Fanaro 2008 [43] reported the median (IQR) of Bacteriodes (log10 CFU per gram stool) (Table 8).

(v) E. coli

Two studies [53,54] reported E. coli (log10 CFU per gram stool) and their results were pooled in a meta-analysis. However, statistically significant heterogeneity was detected between the studies (Chi^2=5.96, df=1, p=0.01, I^2=83%). The results are therefore reported separately. Xiao-Ming 2004 [53] showed that prebiotics significantly reduced E. coli counts compared to the controls (MD −0.90, 95% CI: -1.29 to −0.51, n = 121) while Xiao-Ming 2008 [54] showed no significant difference between the two groups (MD 0.67, 95% CI: -0.53 to 1.87, n = 38).

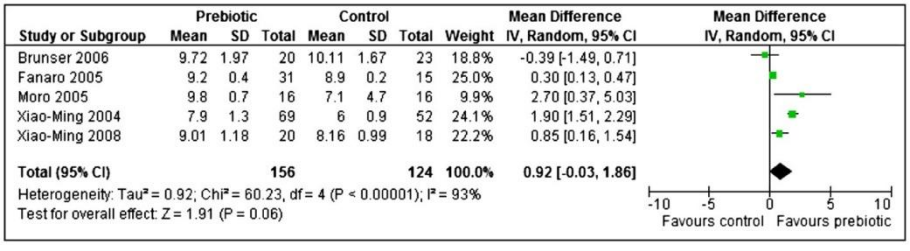

Figure 9 Prebiotics versus controls, outcome: Bifidobacteria -log10(CFU) per gram stool.

Table 7 Summary of findings table: Synbiotic studies

Effects of infant formula containing Synbiotics on clinical outcomes in full term infants

Patient or population: Full term infants, Settings: Multi-centre trials, Intervention: Infant formula with synbiotics, Comparison: Conventional infant formula

Outcomes	Illustrative comparative risks* (95% CI)		Measure of effect (95% CI)	No of Participants (studies)	Quality of the evidence (GRADE)
	Assumed risk	Corresponding risk			
	Conventional formula	Infant formula with synbiotics			
Weight gain (g/day) for boys Follow-up: mean 4 months	The mean (SD) weight gain (g/day) in control group was 30.9 (6.1)	Mean (SD) weight gain in synbiotic group was 31.8 (5.9)	MD (95% CI): 0.90 (-1.95 to 3.75)	81 (1 study)	⊕⊕⊖⊖ **low**[1,2]
Weight gain (g/day) for girls Follow-up: mean 4 months	The mean (SD) weight gain (g/day) in control group was 26.9 (6)	Mean (SD) weight gain in synbiotic group was 27.8 (6)	MD (95% CI): 0.90 (-1.81 to 3.61)	86 (1 study)	⊕⊕⊖⊖ **low**[3,4]
Length gain (mm/mo) for boys Follow-up: mean 4 months	The mean (SD) length gain (mm/month for boys in control group ranged from 32.6 (3.6) to 35.1 (4.4)	The mean length gain (mm/mo) for boys in the intervention groups was **0.75 higher** (0.66 lower to 2.17 higher)	MD (95% CI): 0.75 (-0.66 to 2.17)	120 (2 studies)	⊕⊕⊖⊖ **low**[5,6,7]
Length gain (mm/mo) for girls Follow-up: mean 4 months	The mean length gain (mm/month) for girls in the control groups ranged from 31.2 (3.7) to 32.2 (4.6)	The mean length gain (mm/mo) for girls in the intervention groups was **0.75 higher** (0.63 lower to 2.13 higher)	MD (95% CI): 0.75 (-0.63 to 2.13)	138 (2 studies)	⊕⊕⊖⊖ **low**[8,9,10]
Head circumference gain (mm/mo) for boys Follow-up: 4 to 6 months	The mean head circumference gain (mm/month) for boys in the control groups ranged from 17.4 (2.9) to 18.4 (2.3)	The mean head circumference gain (mm/mo) for boys in the intervention groups was **0.06 lower** (0.96 lower to 0.85 higher)	MD (95% CI): -0.06 (-0.96 to -0.85)	126 (2 studies)	⊕⊕⊖⊖ **low**[11,12]
Head circumference gain (mm/mo) for girls Follow-up: 4 to 6 months	The mean head circumference gain (mm/month) for girls in the control groups ranged from 15.5 (3) to 16.7 (2.4)	The mean head circumference gain (mm/mo) for girls in the intervention groups was **0.05 lower** (0.94 lower to 0.85 higher)	MD (95% CI): -0.05 (-0.94 to 0.85)	138 (2 studies)	⊕⊕⊖⊖ **low**[13,14]
Stool frequency (evacuations per day) Follow-up: 4 to 6 months	The mean (SD) stool frequency (evacuations per day) in the control group ranged from 1.4 (0.49) to 1.8 (0.9)	The mean stool frequency (evacuations per day) in the intervention groups was **0.28 higher** (0.08 to 0.48 higher)	MD (95% CI): 0.28 (0.08 to 0.48)	176 (2 studies)	⊕⊕⊖⊖ **low**[15,16]

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **measure of effect** of the intervention (and its 95% CI).CI: Confidence interval, MD: Mean Difference.

GRADE Working Group grades of evidence. **High quality**: Further research is very unlikely to change our confidence in the estimate of effect. **Moderate quality**: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate. **Low quality**: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate. **Very low quality**: We are very uncertain about the estimate.

[1] Small sample size n=81, 95% CI includes no effect.
[2] Possible publication bias.
[3] Small sample size n=86, 95% CI includes no effect.
[4] Possible publication bias.
[5] Allocation concealment not described in 2 studies.
[6] Small sample size n=126,
[7] Possible Publication bias
[8] Allocation concealment not described in 2 studies,
[9] Small sample size n=138,
[10] Possible Publication Bias
[11] Small sample size n=126,
[12] Possible publication bias,
[13] Small sample size n=138, [14] Possible publication bias,
[15] Small sample size n=176, [16] Possible publication bias.

Table 8 Gastrointestinal microflora

	Costalos 2008[41]: Median (range) as % of total bacteria		
	Prebiotics (n=70)	**Controls (n=70)**	
% Bifidobacteria	39.69 (0–143.3)	14.87 (0–101)	
% E.coli	1.95 (0–69.32)	4.06 (0–59.31)	
	Fanaro 2008[43]: Median (IQR) microflora -log10(CFU) per gram stool		
	Prebiotics (n=56)	**Controls (n=59)**	
Bifidobacteria	9.86 (8.99-10.18)	9.38 (8.35-9.90)	
Lactobacilli	4.62 (2–6.5)	4 (2–5.05)	
Bacteriodes	7.95 (6.64-9.6)	8.16 (6.3-9.04)	
Clostridia	4.3 (3–5.28)	4.29 (2.48-5.43)	
Enterobacteria	8.65 (8.12-9.13)	8.53 (7.96-9.01)	
E. coli	8.50 (7.9-8.99)	8.33 (7.59-8.83)	
	Moro 2002[35]: Median (IQR)		
	Prebiotic1 (n=30)	**Prebiotic2 (n=27)**	**Control (n=33)**
Bifidobacteria	9.3 (1.6)	9.7 (0.8)	7.2 (4.9)
Lactobacilli	5.9 (1.5)	5.6 (2.1)	3.4 (1.8)
	Moro 2006[32]: Median (IQR) log10(CFU) per gram stool		
	Prebiotics (n=50)	**Controls (n=44)**	
Bifidobacteria	10.28 (0.7)	8.65 (1.2)	
Lactobacilli	5.99 (3.6)	5.9 (2)	

Two studies reported their results in median; therefore no conclusions could be made. Costalos 2008 [41] reported the median (range) of E. coli (log10 CFU per gram stool) as a percentage of total bacteria (Table 8). Fanaro 2008 [43] reported the median (IQR) of E. coli and clostridia (log10 CFU per gram stool) (Table 8).

Discussion

The objectives of this systematic review were to deter- mine the effects of infant formula containing probiotics, prebiotics or both (synbiotics) on clinical outcomes in full term infants and to explore if synbiotics are superior over probiotics or prebiotics. Studies that used breast milk or mixed feeds (breast and infant formula or other types of milk) were excluded. All included RCTs evalu- ated either synbiotics, probiotics or prebiotics use in full term infants. The studies varied in enrolment criteria, sample size, intervention and treatment duration.

Summary of main findings

Synbiotics

Addition of synbiotics to infant formula did not have any significant effect on growth (weight gain, length and head circumference). Synbiotics significantly increased stool frequency. However, two studies [21,25] reported no differences in stool consistency, while one study [24] reported an increase in liquid stools in synbiotic group. There were no significant differences between study groups on the incidence and frequency of colic, spitting up / regurgitation, crying, restlessness or vomiting. The effect of synbiotics on the volume of formula tolerated was not reported. Effect of synbiotics on frequency of infections was under reported. In one study [25], there were no differences in the frequency of infections between study groups, while in another study [21], the treatment effect could not be calculated or any conclusions made on the frequency of infections or antibiotic intake. Effects of synbiotics on hospitalization, GI microflora and immune response were not reported in any study therefore these parameters could not be evaluated.

Interpreting the effects of synbiotic supplementation of infant formula on clinical outcomes was difficult due to the limited number of studies. The synbiotic studies had short treatment duration (4 to 6 months) and treatment varied in all 3 studies. There was not enough evidence to state that synbiotics in infant formula have a significant effect on growth or lower the incidence of colic, spitting up / regurgitation, crying, restlessness. There is limited evidence that synbiotics do increase stool frequency and effects on stool consistency were inconclusive. There is not enough evidence to state that synbiotics reduce the risk of infections or decrease use of antibiotics. There is no data on the effects of synbiotics on GI microflora. The available data is very limited to draw reliable conclusions on the effects of synbiotics on clinical outcomes in formula fed infants.

Table 9 Summary of findings table: probiotic studies

Effects of infant formula containing Probiotics on clinical outcomes in full term infants					
Patient or population: Full term infants, Settings: Multi-centre trials (hospitals), Intervention: Infant formula with probiotics, Comparison: Conventional infant formula					
Outcomes	**Illustrative comparative risks* (95% CI)**		**Measure of effect (95% CI)**	**No of Participants (studies)**	**Quality of the evidence (GRADE)**
	Assumed risk	**Corresponding risk**			
	Conventional formula	**Infant formula with probiotics**			
Weight gain (g/day) for boys Follow-up: 4 to 7 months	The mean (SD) weight gain (g/day) for boys in the control group ranged from 30.9 (6.1) to 32.8 (4.1)	The mean weight gain (g/day) for boys in the intervention groups was **1.64 higher** (0.36 lower to 3.64 higher)	MD (95% CI): 1.64 (-0.36 to 3.64)	158 (4 studies)	⊕⊕⊖⊖ **low**[1,2]
Weight gain (g/day) for girls Follow-up: 4 to 7 months	The mean (SD) weight gain (g/day) for girls in the control group ranged from 26.5 (4.9) to 29 (6.3)	The mean weight gain (g/day) for girls in the intervention groups was **0.76 higher** (2.57 lower to 4.09 higher)	MD (95% CI): 0.76 (-2.57 to 4.09)	170 (4 studies)	⊕⊕⊖⊖ **low**[3,4,5]
Length gain (mm/month) for boys Follow-up: 4 to 7 months	The mean (SD) length gain (mm/month) for boys in the control group ranged from 31.36 (4.48) to 37.3 (4.9)	The mean length gain (mm/month) for boys in the intervention groups was **0.37 lower** (1.64 lower to 0.9 higher)	MD (95% CI): -0.37 (-1.64 to 0.90)	158 (4 studies)	⊕⊕⊖⊖ **low**[6,7]
Length gain (mm/month) for girls Follow-up: 4 to 7 months	The mean (SD) length gain (mm/month) for girls in the control group ranged from 28 (3.64) to 32 (4.6)	The mean length gain (mm/month) for girls in the intervention groups was **0.32 higher** (0.81 lower to 1.45 higher)	MD (95% CI): 0.32 (-0.81 to 1.45)	165 (4 studies)	⊕⊕⊖⊖ **low**[8,9]
Head circumference gain (mm/month) for boys Follow-up: 4 to 7 months	The mean (SD) head circumference gain (mm/month) for boys in the control group ranged from 17.5 (3.4) to 35.28 (7)	The mean head circumference gain (mm/month) for boys in the intervention groups was **0.76 higher** (1.02 lower to 2.54 higher)	MD (95% CI): 0.76 (-1.02 to 2.54)	125 (3 studies)	⊕⊕⊖⊖ **low**[10,11]
Head circumference gain (mm/month) for girls Follow-up: 4 to 7 months	The mean (SD) head circumference gain (mm/month) for girls in the control group ranged from16 (3) to 36.68 (15.4)	The mean head circumference gain (mm/month) for girls in the intervention groups was **0.27 higher** (0.7 lower to 1.23 higher)	MD (95% CI):0.27 (-0.70 to 1.23)	139 (3 studies)	⊕⊕⊖⊖ **low**[12,13]

Table 9 Summary of findings table: probiotic studies *(Continued)*

Bifidobacteria -log10(CFU) per gram of stool	The mean (SD) bifidobacteria -log10(cfu) per gram of stool in the control group ranged 9.75 (0.5) to 10.11 (1.67)	The mean bifidobacteria -log10(cfu) per gram of stool in the intervention groups was **1.27 lower** (2.03 to 0.51 lower)	MD (95% CI): -1.27 (-2.03 to -0.51)	57 (2 studies)	⊕⊕⊖⊖ **low**[14, 15]

*The basis for the assumed risk (e.g. the median control group risk across studies) is provided in footnotes. The corresponding risk (and its 95% confidence interval) is based on the assumed risk in the comparison group and the measure of effect of the intervention (and its 95% CI). CI: Confidence interval, CFU: colony forming units, MD: Mean Difference, RR: Risk ratio.
GRADE Working Group grades of evidence. High quality: Further research is very unlikely to change our confidence in the estimate of effect. Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate. Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate. Very low quality: We are very uncertain about the estimate.
[1] Small sample size n=158, 95% CI includes no effect.
[2] Possible publication bias.
[3] Unexplained heterogeneity).
[4] Small sample size n=170.
[5] Possible publication bias.
[6] Small sample size n=158, 95% CI includes no effect.
[7] Possible publication bias.
[8] Small sample size n=165, 95% CI includes no effect.
[9] Possible publication bias.
[10] Small sample size n=125, 95% CI includes no effect.
[11] Possible publication bias. [12]
Small sample size n=139. [13]
Possible publication bias. [14]
Small sample size n=57. [15]
Possible publication bias.

Table 10 Summary of findings table: prebiotic studies

Effects of infant formula containing Prebiotics on clinical outcomes in full term infants

Patient or population: Full term infants, Settings: Multi-centre trials, Intervention: Infant formula with prebiotics, Comparison: Conventional formula

Outcomes	Illustrative comparative risks* (95% CI)		Measure of effect (95% CI)	No of Participants (studies)	Quality of the evidence (GRADE)
	Assumed risk	Corresponding risk			
	Conventional formula	Infant formula with prebiotics			
Weight gain (g/day)s Follow-up: 1 to 6 month	The mean (SD) weight gain (g/day) in the control group ranged from 26.4 (3.7) to 40.59 (3.95)	The mean weight gain (g/day) in the intervention groups was **0.97 higher** (0.24 to 1.7 higher)	MD (95% CI): 0.97 (0.24 to 1.70)	861 (8 studies)	⊕⊕⊖⊖ **low**[1,2,3]
Length gain (cm/week) Follow-up: 1 to 6 months	The mean (SD) length gain (cm/week) in the control group ranged from 0.74 (0.1) to 0.96 (0.11)	The mean length gain (cm/week) in the intervention groups was **0.01 higher** (0.01 lower to 0.04 higher)	MD (95% CI): 0.01(-0.01 to 0.04)	697 (7 studies)	⊕⊕⊖⊖ **low**[4,5,6]
Head circumference gain (cm/ week) Follow-up: 1.5 to 6 months	The mean (SD) head circumference gain (cm/ week) in the control group ranged from 0.34 (0.05) to 0.63 (0.1)	The mean head circumference gain (cm/ week) in the intervention groups was **0.01 lower** (0.02 lower to 0 higher)	MD (95% CI): -0.01 (-0.02 to 0.00)	438 (3 studies)	⊕⊕⊖⊖ **low**[7,8]
Stool frequency (evacuations per day) Follow-up: 1 to 6 months	The mean (SD) stool frequency (evacuations per day) in the control group ranged from1.5 (0.6) to 2.4 (1.64)	The mean stool frequency (evacuations per day) in the intervention groups was **0.18 higher** (0.06 to 0.3 higher)	MD (95% CI): 0.18 (0.06 to 0.30)	579 (4 studies)	⊕⊕⊖⊖ **low**[9,10]
Diarrhea Follow-up: 4 to 12 months	**Study population**		**RR 0.62** (0.19 to 1.99)	237 (2 studies)	⊕⊕⊖⊖ **low**[11,12]
	23 per 100	**14 per 100** (4 to 46)			
	Moderate				
	19 per 100	**12 per 100** (4 to 38)			
URTI Follow-up: 6 to 12 months	**Study population**		**RR 0.74** (0.32 to 1.73)	409 (2 studies)	⊕⊕⊖⊖ **low**[13, 14, 15]
	45 per 100	**33 per 100** (14 to 77)			
	Moderate				
	44 per 100	**33 per 100** (14 to 76)			
Bifidobacteria -log10(CFU) per gram stool Follow-up: 1 to 6 months	The mean(SD) bifidobacteria -log10(cfu) per gram stool in the control group ranged from 6(0.9) to 10.11 (1.67)	The mean bifidobacteria -log10(cfu) per gram stool in the intervention groups was **0.92 higher** (0.02 lower to 1.86 higher)	MD (95% CI): 0.92 (-0.03 to 1.86)	280 (5 studies)	⊕⊕⊖⊖ **low**[16, 17, 18]

Table 10 Summary of findings table: prebiotic studies *(Continued)*

Lactobacilli -log10(CFU) per gram stool Follow-up: 3 to 6 months	The mean (SD) lactobacilli -log10 (cfu) per gram stool in the control group ranged from 3.95 (1.57) to 4.27 (2.02)	The mean lactobacilli -log10(cfu) per gram stool in the intervention groups was **1.12 higher** (0.44 lower to 2.67 higher)	MD (95% CI): 1.12 (-0.44 to 2.67)	202 (3 studies)	⊕⊕⊖⊖ **low**[19,20,21]

*The basis for the assumed risk (e.g. the median control group risk across studies) is provided in footnotes. The corresponding risk (and its 95% confidence interval) is based on the assumed risk in the comparison group and the measure of effect of the intervention (and its 95% CI). CI: Confidence interval, CFU: Colony Forming Units, MD: Mean Difference, RR: Risk ratio.
GRADE Working Group grades of evidence: High quality: Further research is very unlikely to change our confidence in the estimate of effect. Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate. Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate. Very low quality: We are very uncertain about the estimate.

[1] Allocation concealment not clearly described in 6 studies.
[2] Blinding not clearly demonstrated or described in 7 studies.
[3] Possible publication bias.
[4] Allocation concealment not clearly demonstrated in 5 studies.
[5] Blinding not clearly demonstrated in 6 studies.
[6] Possible publication bias.
[7] Blinding not clearly described in 2 studies.
[8] Possible publication bias.
[9] Incomplete outcome data (with no reasons given for missing data) was present in 1 study.
[10] Possible publication bias.
[11] Small sample size n=237, 95% CI includes no effect.
[12] Possible publication bias.
[13] Unexplained heterogeneity.
[14] 95% CI includes no effect.
[15] Possible publication bias.
[16] Unexplained heterogeneity.
[17] Small sample size n=280.
[18] Possible publication bias.
[19] Unexplained heterogeneity.
[20] Small sample size n=202.
[21] Possible publication bias.

Probiotics

A limited number of studies analyzed the effects of probiotic supplementation on growth by gender. These studies had small sample sizes and the follow-up periods were short. Addition of probiotics to infant formula did not have any significant effect on growth (weight gain, length gain or head circumference) in boys or girls. No study reported any weight loss. Probiotic infant formula was well tolerated. The limited available data shows that probiotics did not have any significant effect on stool frequency or consistency. Probiotic supplementation was not associated with fewer episodes of diarrhoea, a lower incidence of colic, spitting up / regurgitation, restlessness, vomiting. In one study [52] there were fewer crying episodes in the control group than probiotic group. Probiotic effects on infections, antibiotic use and length of hospitalization were inconclusive. Probiotic supplementation did result in a significantly higher formula intake compared to controls.

Effects of probiotic supplementation on intestinal microflora were conflicting. Probiotics failed to increase counts of bifidobacteria and lactobacillus. Probiotics significantly reduced counts of enterobacteria but failed to reduce counts of bacteriodes. None of the studies reported on immune response (CRP, IL-6), therefore the impact of probiotics on these parameters could not be evaluated. All 10 probiotic studies used various strains of bifidobacteria and lactobacillus with different doses. Treatment duration also varied from 14 days to 7 months. This confirms the ESPGHAN Committee on nutrition statement that there is a lack of published evidence on clinical benefits from long term use of probiotic containing infant formula [95]. Well designed long term follow – up RCTs using similar treatment regimens (same probiotics strains, dose and treatment duration) are needed to establish the effects of probiotics on healthy formula fed infants.

Prebiotics

Prebiotic addition to infant formula did have a significant effect on weight gain but had no significant effect on length and head circumference. None of the studies reported any weight loss. Prebiotic supplementation increased stool frequency but failed to improve stool consistency or decrease incidence of diarrhoea. Prebiotic supplementation did not reduce the incidence of spitting up / regurgitation, vomiting or crying (no study reported colic) or increased volume of formula tolerated. Prebiotic supplementation failed to significantly reduce upper respiratory infections. However, one study [32] did report a significant reduction in overall infections and antibiotic intake. Prebiotics supplementation failed to increase counts of bifidobacteria, lactobacillus or decrease the levels of pathogens (enterobacteria, bacteriodes, E – coli). None of the studies reported on hospitalization (days in hospital) and immune response (CRP, IL-6), therefore the impact of prebiotics on these parameters could not be evaluated.

Majority of the studies had a short treatment duration ranging from 28 days to 12 months. The prebiotic doses ranged from 0.15 g to 0.8 g/100 ml which did not exceed the level recommended by the European Committee on food in order to minimize intolerance and maximize the bifidogenic effect of the prebiotic.

Quality of the evidence and potential biases in the review process

We used guidelines from GRADE working group and GRADEpro 3.6 software to assess the quality of evidence in this review (Table 7, 9, 10). Overall the quality of evidence for primary outcomes is low, meaning that further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate. The quality of the evidence was compromised by: Imprecision (majority of studies had a small sample size ranging from 97 to 227 in the synbiotic studies, 54 to 201 in probiotic studies, 32 to 271 in the prebiotic studies); limitations in study design and execution (inadequate information was published to assess methodological quality of the study. Information was missing on sequence generation, allocation concealment, blinding, incomplete outcome data, selective reporting, free of other bias domains; Inconsistency of results); unexplained heterogeneity; use of different study preparations (types of synbiotic, probiotic, prebiotics) and different doses regimens were used and publication bias.

At the conclusion of the review process and preparation of the manuscript (for this review), one on-going study [96] was recruiting, one study [97] was not yet recruiting, one study [98] was still on-going, no longer recruiting. Therefore data from these studies could not be included in this review. The reviewers used thorough comprehensive search strategies adopted for the available databases. All attempts were made to minimize publication bias. All steps of this review were conducted independently by the reviewers. Only randomised controlled studies were included in this review.

Breast feeding statement

By conducting this review on exclusively formula fed infants, the authors do not seek to diminish the importance of breastfeeding and promote formula feeding. The reviewers acknowledge the importance of breastfeeding for infants. They support exclusive breastfeeding for 6 months, thereafter safe complementary feeding from 6 months of age with continued breastfeeding up to 2 years and beyond as per the global recommendations for

optimal infant feeding of WHO and United Nations Children's Fund (UNICEF). This is because breastfeeding is the ideal feeding method for infants [99].

Conclusion

There is not enough evidence to state that supplementation of term infant formula with synbiotics, probiotics or prebiotics does result in improved growth and clinical outcomes in full term infants. There is no data available to establish if synbiotics are superior to probiotics or prebiotics. Therefore this review does not support the routine supplementation of term infant formula with synbiotics, probiotics or prebiotics.

Implications for practice

Probiotics: The limited evidence shows synbiotic or probiotic supplementation of infant formula did not have any adverse effects, significant impact on growth or clin- ical outcomes. All studies used different probiotic strains, the effects of one type of probiotic cannot be extrapolated to other types of probiotic bacteria. Pre- biotic supplementation of infant formula also did not result in any adverse effects on infants. There are some clinical benefits such as improved weight gain and stool frequency.

Implications for research

For clear recommendations to be made, well designed large RCTs with long term follow - up are required on exclusively formula fed term infants to investigate the effect of the same synbiotic combinations on clinical outcomes; the effect of the same probiotics (with similar doses and treatment duration) on clinical outcomes because available studies used different probiotic doses and treatment durations; the effect of the same prebiotics (with similar doses and treatment duration) on clinical outcomes because available studies used similar prebiotics with different doses and treatment duration; the effects of synbiotics, probiotics or prebiotics on clinical outcomes that have not been adequately addressed in previous studies; if synbiotics are superior to probiotics or prebiotics. Future RCTs should have treatment arms that include both synbiotics, probiotic and prebiotics.

Abbreviations

Cfu: Colony Forming Units; CI: Confidence Interval; cm: Centimetres; ESPGHAN: European society for paediatric, gastroenterology, hepatology and nutrition; FOS: Fructooligosaccharide; g/day: Grams per day; GI: Gastrointestinal; GOS: Galactooligosaccharide; GRAS: Generally regarded as safe; IQR: Inter quartile range; IL-6: Interleukin – 6; MD: Mean difference; mm: millimetres; RCTs: Randomized controlled trials; RR: Risk ratio; SD: Standard deviation; UNICEF: United nations children's fund; UTI: Urinary tract infections; URTI: Upper respiratory tract infections; WHO: World Health Organisation.

Competing interests

All reviewers declared no competing interests.

Authors' contributions

The reviewers contributed the following: MM: Developed review protocol (unpublished), selected RCTs, conducted data extraction, assessment of risk of bias in included studies, developed, edited and critically reviewed the manuscript. ML: Selected RCTs, conducted data extraction, assessment of risk of bias in included studies, critically reviewed the manuscript. AM: Conducted the statistical analysis, interpretation of results and critically reviewed the manuscript. TY: Assisted in designing the review and critically reviewed the manuscript. RB: Assisted in designing the review and critically reviewed the manuscript. All authors' read and approved the final manuscript.

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Chapter 4: Association between funding source, methodological quality and research outcomes in randomized controlled trials of synbiotics, probiotics and prebiotics added to infant formula: A systematic review

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RESEARCH ARTICLE Open Access

Association between funding source, methodological quality and research outcomes in randomized controlled trials of synbiotics, probiotics and prebiotics added to infant formula: A Systematic Review

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Abstract

Background: There is little or no information available on the impact of funding by the food industry on trial outcomes and methodological quality of synbiotics, probiotics and prebiotics research in infants. The objective of this study was to compare the methodological quality, outcomes of food industry sponsored trials versus non industry sponsored trials, with regards to supplementation of synbiotics, probiotics and prebiotics in infant formula.

Methods: A comprehensive search was conducted to identify published and unpublished randomized clinical trials (RCTs). Cochrane methodology was used to assess the risk of bias of included RCTs in the following domains: 1) sequence generation; 2) allocation concealment; 3) blinding; 4) incomplete outcome data; 5) selective outcome reporting; and 6) other bias. Clinical outcomes and authors' conclusions were reported in frequencies and percentages. The association between source of funding, risk of bias, clinical outcomes and conclusions were assessed using Pearson's Chi-square test and the Fisher's exact test. A p-value < 0.05 was statistically significant.

Results: Sixty seven completed and 3 on-going RCTs were included. Forty (59.7%) were funded by food industry, 11 (16.4%) by non-industry entities and 16 (23.9%) did not specify source of funding. Several risk of bias domains, especially sequence generation, allocation concealment and blinding, were not adequately reported. There was no significant association between the source of funding and sequence generation, allocation concealment, blinding and selective reporting, majority of reported clinical outcomes or authors' conclusions. On the other hand, source of funding was significantly associated with the domains of incomplete outcome data, free of other bias domains as well as reported antibiotic use and conclusions on weight gain.

Conclusion: In RCTs on infants fed infant formula containing probiotics, prebiotics or synbiotics, the source of funding did not influence the majority of outcomes in favour of the sponsors' products. More non-industry funded research is needed to further assess the impact of funding on methodological quality, reported clinical outcomes and authors' conclusions.

Keywords: Synbiotics, Probiotics, Prebiotics, Funding source, Methodological quality

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Background

There are numerous studies that explore the relationship between industrial sponsorship of biomedical research and published outcomes [1]. Several reviews have documented how trials funded by industry are more likely to report results in favour of the sponsor's products [2-5]. These reviews focused on trials sponsored by the pharmaceutical industry. Few reviews have explored the impact of funding by the food industry on outcomes of research trials [6,7]. A review by Nkansah et al. also found that majority of trials investigating the effects of calcium supplementation in healthy children were industry funded and all supported calcium supplementation, in favour of the sponsor [8]. Similarly, a review by Lesser et al. found that scientific nutrition related articles (intervention trials, observational studies and scientific reviews) on common consumed beverages (soft drinks, juice, milk) funded by the food industry, were more likely to be favourable to the sponsor than articles that did not have industry funding [6].

Reporting only positive outcomes in a research trial significantly reduces a sponsors' financial risk. Pressure to show a food product causes favourable outcomes in a specific population, may result in biases in trial design (methodology) and reporting of outcomes in industry sponsored research. This type of bias in nutrition research could adversely affect public health. Results from nutrition research also influence policy formulation, professional dietary guidelines, design of public health interventions and regulation of food product health claims. In addition, findings from nutrition research often receive publicity from the media, which influences consumer behaviour [6].

More studies are needed to explore the relationship between the food industry and nutrition research [7]. There is little or no information available on the impact of funding by the food industry on trial outcomes and methodological quality of synbiotics, probiotics and prebiotics research in infants. There are no systematic reviews that have explored if sources of funding affects outcomes and methodological quality of randomized controlled trials (RCTs) conducted on infants given probiotics, prebiotics or synbiotics supplemented infant formula.

Probiotics are defined as "live microorganisms" which when administered in adequate amounts may confer a health benefit to the host [9]. The main probiotic organisms that are currently used worldwide belong to the genera Lactobacillus and Bifidobacteria and are found in the gastrointestinal microflora [9]. The probiotics preparations of interest for this review are those added to infant formulas. Prebiotics are non- digestible food ingredients that may benefit the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon and improving the host's health [10-12]. The most widely studied prebiotics are galactooligosaccharides (GOS), inulin and fructooligosaccharide (FOS) [13,14]. GOS, FOS and inulin are added to different foods as fat and sugar replacements to improve texture or for their functional benefits [10,15,16]. When probiotics and prebiotics are administered simultaneously, the combination is termed Synbiotics.

The aim of this review was to explore whether financial sponsorship by the food industry affects outcomes and methodological quality of trials on synbiotics, probiotics or prebiotics used in infants. Methodological quality may be compromised when insufficient information is provided regarding sequence generation, allocation concealment, blinding, bias introduced from other sources and incomplete outcome reporting.

Objective

The objective of this systematic review was to compare the methodological quality and outcomes of food industry sponsored trials versus non industry sponsored trials with regards to supplementation of synbiotics, probiotics and prebiotics in infant formula.

Hypothesis

The source of funding in research trials using probiotics, prebiotics or synbiotics supplemented formula in infants is associated with outcomes in favour of the sponsor's products and authors' conclusions.

Methods

Criteria for considering studies for this review

Types of studies

All randomized controlled trials (RCTs) conducted from 1980 to 2012 (irrespective of language) on synbiotics, probiotics, or prebiotics added to infant formula were included. Study participants were healthy full term infants (>37 weeks gestation or > 2.5 kg birth weight, 0–12 months old), preterm infants (born < 37 weeks gestation), low birth weight (<2.5 kg at birth) and extreme low birth weight infants (<1000 g at birth). Infants were fed either infant formula (preterm or full term formula), mixed feeds (breast milk with infant formula) with added synbiotics, probiotics or prebiotics or conventional infant formula with or without placebo. RCTs were excluded if they included infants with cardiac defects, pulmonary diseases, gastrointestinal diseases, major congenital abnormalities or chromosomal abnormalities. Commentaries, editorials, letters to the editor and studies that were not RCTs were excluded.

Types of outcome

The outcomes included: 1) Source of funding, 2) Methodological quality (Risk of bias), 3) Clinical outcomes in RCTs, Conclusions (Overall study conclusions and conclusions on reported clinical outcomes) and 5) Association between

source of funding and methodological quality, clinical outcomes and author's conclusions.

Search methods for identification of studies

A literature search regardless of language was conducted on electronic databases including The Cochrane CENTRAL Register for Controlled Trials (2012), EMBASE (1980+), Scopus (1980 to 2012), EBSCO host (1960 to 2012), PUBMED / MEDLINE (1966 to 2012), OVID (1950 to 2012), SPORTDiscus (1960 to 2012), Web of Science (1970 to 2012), Science Direct (1950 to 2012), CINAHL (1980 to 2012), Science citation index (1970 to 2012), Latin American Caribbean Health Sciences literature (LILACS) (1965 to 2012), NLMGateway (1950–1966). RCTs published in non-English language journals were translated by independent translators who were familiar with the subject matter.

The search strategy used to search PUBMED for studies on full term infants is: (synbiotic* and probiotic* OR prebiotic*) AND (FOS or fructooligosaccharide or inulin or GOS or galactooligosaccharide) AND (infant formula* OR infant feeding OR formula OR formula milk) AND (infant* or baby or babies) NOT (preterm or premature or low birth weight babies or allergy or eczema) AND (randomized controlled trial* OR controlled clinical trial* OR random allocation*) Limits: Humans. This search strategy was modified to search other electronic databases and for studies on preterm infants.

A hand search was conducted on abstracts of major conference proceedings such as the Pediatric Academic Society meetings from 1990 (www.pas-meetings.org), cross checked references cited in RCTs and in recent reviews (published from 2003 to 2012) for additional RCTs not identified by electronic searches and speciality journals which were not included in any database such as Pediatrika and Chinese Journal of Microecology. To identify on-going and unpublished trials, experts in the field, manufacturers of infant formula containing probiotics and prebiotics were contacted. Web sites of companies that have conducted or were conducting RCTs on probiotics and prebiotics were searched.

Examples: Pfizer (www.pfizerpro.com/clinicaltrials), Chris Hansen Laboratory (www.chr-hansen.com/research_development/documentation.html). A search was conducted on prospective trial registries such as World Health Organization (WHO) International Clinical Trials Registry Platform Search Portal (www.who.int/trialsearch), Clinical Trials.gov register (www.clinicaltrials.gov), Current Controlled Trials *meta*Register of Controlled Trials [*mRCT*] (www.controlled-trials.com/mrct) and www.clinicaltrialresults.org.

Selection of studies

One reviewer (MM) independently reviewed all abstracts, citations and identified potentially eligible RCTs. The full reports of eligible RCTs were retrieved by one reviewer (MM) and the pre-specified selection criteria applied independently by two reviewers (MM, ML) using a study eligibility form designed for this review. If more than one publication of a study existed, all reports of the study were grouped together under one name. Any disagreements between the reviewers were resolved through discussion. Unresolved disagreements were resolved by a third party (RB).

Data extraction and management

Two reviewers (MM, ML) independently extracted data using a pretested data extraction form that was designed for this review. The reviewers (MM, ML) cross checked data and resolved any differences through discussion. Unresolved disagreements were resolved by a third party (RB). One reviewer (MM) entered the data in SPSS version 19 and the other reviewer (AM) conducted quality control checks. The data obtained from each RCT included:

A) Source of funding or support of RCTs

The source of funding or support of the RCTs was defined and categorized as:

1) Industry included:
   - For – profit company, donation of study product by a for – profit company which manufactured the study product,
   - Not – for profit company that promoted the consumption of synbiotics, probiotics or prebiotics,
   - Mixed sources (for-profit company and other source).
2) Non – industry included:
   - Government: National, regional (provincial, county) government body with NO industry association.
   - Foundation / Philanthropies: examples include Rockefeller foundation, Bill and Melinda Gates foundation.
   - Institution: University, Research centres, teaching and academic hospitals.
   - Other source of funding.
3) None: No source of funding was disclosed in study report.

B) Assessment of methodological quality of evidence (Risk of bias)

Two reviewers (MM, ML) independently assessed the risk of bias of included RCTs as described in the Cochrane Handbook for Systematic Reviews for Interventions according to the following 6 components: 1) sequence generation; 2) allocation concealment; 3)

Table 1 Included studies and on-going studies

Included studies						On-going studies	
Author publication year	**Full term/Preterm infant**	**Sponsor**	**Author publication year**	**Full term/Preterm infant**	**Sponsor**	**Author, Year study commenced**	**Full term/Preterm infant**
Allen 2010 [18]	Full Term	Knowledge exploitation fund, collaborative industrial research, others	Soh 2009 [19]	Full Term	National Medical Research Council, Singapore	Jacobs 2007 [20]	Pre-Term
Alliet 2007 [21] Scholtens 2008 [22]	Full Term	Numico	Urban 2008 [23]	Full Term	Nestle	Patole 2009 [24]	Pre-Term
Ashley 2012 [25]	Full Term	Mead Johnson	Velaphi 2008 [26]	Full Term	Nestle	Underwood 2009 [27]	Pre-Term
Bakker-Zierikzee 2005 [28] Bakker-Zierikzee 2006 [29]	Full Term	None/Not clear	Vendt 2006 [30]	Full Term	Valio Ltd		
Bettler 2006 [31]	Full Term	Wyeth Nutrition	Vlieger 2009 [32]	Full Term	Friesland		
Brunser 2006 [33]	Full Term	None/Not clear	Weizman 2005 [34]	Full Term	Materna Laboratories		
Bruzzese 2009 [35]	Full Term	Numico	Weizman 2006 [36]	Full Term	Marterna Laboratories		
Chouraqui 2004 [37]	Full Term	Nestle	Xiao-Ming 2004 [38]	Full Term	Friesland		
Chouraqui 2008 [39]	Full Term	Nestle	Xiao-Ming 2008 [40]	Full Term	None / Not clear		
Copper 2010 [41]	Full Term	Nestle	Ziegler 2007 [42]	Full Term	Mead Johnson		
Costalos 2008 [43]	Full Term	Numico	Bin-Nun 2005 [44]	Pre-Term	Mr and Mrs Stephen Hammerman, Mirsky Research fund		
Decsi 2005 [45]	Full Term	Numil Ltd	Boehm 2002 [46] Boehm 2003 [47] Knol 2005 [48]	Pre-Term	Numico		
Fanaro 2005 [49]	Full Term	None / Not clear	Chrzanowska-Liszewska 2012 [50]	Pre-Term	None/Not clear		
Fanaro 2008 [51]	Full Term	Humana GmbH	Costalos 2003 [52]	Pre-Term	None/Not clear		
Gibson 2009 [53]	Full Term	Nestle	Dani 2002 [54]	Pre-Term	None/Not clear		
Gil-Campos 2012 [55]	Full Term	Puleva	Indrio 2008 [56]	Pre-Term	Bio Gaia		
Hascoet 2011 [57]	Full Term	Nestle	Indrio 2009 [58]	Pre-Term	Numico		
Holscher 2012a [59]	Full Term	Nestle	Kapiki 2007 [60]	Pre-Term	None/Not clear		
Holscher 2012b [61]	Full Term	Nestle	Kitajima 1992 [62]	Pre-Term	None/Not clear		
Kim 2010 [63]	Full Term	Ministry of Health, Welfare and family affairs. Republic of Korea	Lin H-C 2008 [64]	Pre-Term	National Science Council of Taiwan		
Knol 2005 [65]	Full Term	Numico	Mihatsch 2006 [66]	Pre-Term	Milupa GmbH		
Magne 2008 [67]	Full Term	Numico	Mihatsch 2010 [68]	Pre-Term	Nestle		
Mah 2007 [69]	Full Term	National Medical Research Council Singapore	Millar 1993 [70] Stansbridge 1993 [71]	Pre-Term	Wessex Regional Health Authority and childrens Research fund		
Maldonado 2010 [72]	Full Term	Puleva	Modi 2010 [73]	Pre-Term	Danone		

Table 1 Included studies and on-going studies *(Continued)*

Moro 2002 [74] Moro 2003 [75]	Full Term	None/Not clear	Mohan 2006 [76]	Pre-Term	None/Not clear
Moro 2005 [77]	Full Term	None/Not clear	Reuman1986 [78]	Pre-Term	None/Not clear
Moro 2006 [79] Arslanoglu 2007 [80] Arslanoglu 2008 [81] Van Hoffen 2009 [82] Schouten 2011 [83]	Full Term	Numico	Riskin 2009 [84]	Pre-Term	None/Not clear
Piemontese 2011 [85]	Full Term	Danone	Rouge 2009 [86]	Pre-Term	French Ministry of Health
Puccio 2007 [87]	Full Term	Nestle	Sari 2011 [88]	Pre-Term	None/Not clear
Rautava 2006 [89] Rautava 2009 [90]	Full Term	Microbes and Man Research program, Academy of Finland, others	Stratiki 2007 [91]	Pre-Term	Nestle
Rinne 2005 [92]	Full Term	Academy of Finland, Turku University Central Hospital Research Funds	Westerbeek 2010 [93] Westerbeek 2011a [94] Westerbeek 2011b [95]	Pre-Term	Danone
Saavedra 2004 [96]	Full Term	Nestle	Yong 2009 [97]	Pre-Term	None/Not clear
Scalabrin 2009 [98]	Full Term	Mead Johnson			
Scalabrin 2012 [99]	Full Term	Mead Johnson			
Schmelzle 2003 [100]	Full Term	Numico			

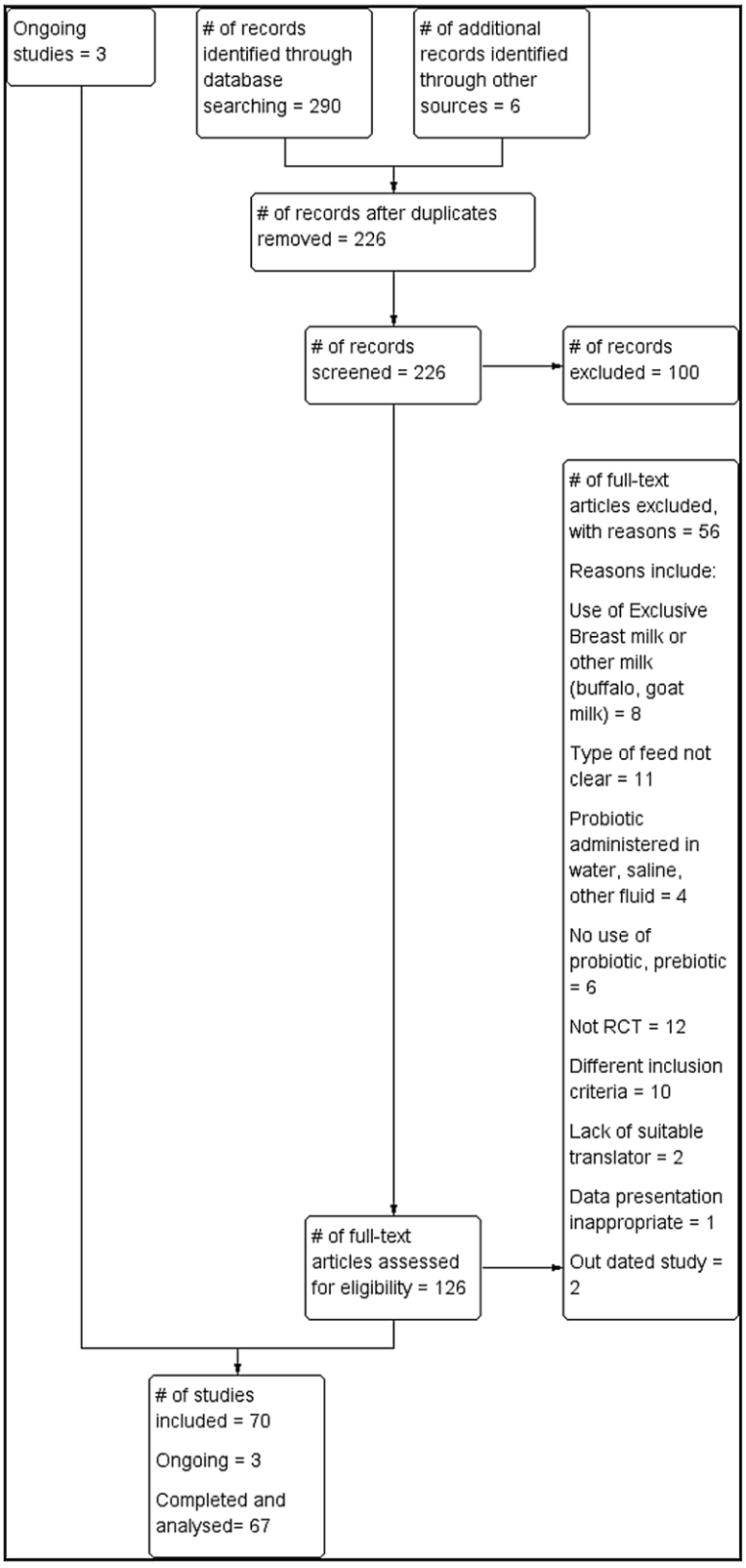

Figure 1 **Process of study selection.**

Table 2 Table of 56 Excluded studies with reasons for exclusion

Use of Exclusive breast milk or Other milk feeds (buffalo, goat milk)	Type of feed not clear/specified	Probiotic administered in water, saline or other fluid that is not infant formula	No use of probiotic, prebiotic	Not RCT, (Cross over, Follow up, Observational study)	Different inclusion criteria	Lack of suitable/ knowledgeable translator	Data presentation inappropriate	Out dated (published before 1980)
Agarwal 2003 [101]	Al Hosni 2012 [102]	FengJuan 2008 [103]	Morisset 2011 [104]	Huet 2006 [105]	Agustina 2007 [106]	Akiyama1994a [107] (Japanese)	Grzéskowak 2012 [108]	Andrews 1969 [109]
Baldeon 2008 [110]	Campeotto 2011 [111]	Kuitunen 2009 [112]	Patole 2005 [113]	Bongers 2007 [114]	Correa 2005 [115]	Akiyama1994b [116] (Japanese)	Robinson 1952 [117]	
Braga 2011 [118]	Cukrowska 2002 [119]	Kukkonen 2007 [120]	Rochat 2007 [121]	Chou I-C 2009 [122]	Hol 2008 [123]			
Chandra 2002 [124]	*Karvonen 1999 [125]	Kukkonen 2008 [126]	Taipale 2011 [127]	Euler 2005 [128]	Isolauri 2000 [129]			
Lin H-C 2005 [130]	*Karvonen 2001 [131]		Taylor 2009 [132]	Hoyos 1999 [133]	Nopchinda 2002 [134]			
Manzoni 2006 [135]	*Karvonen 2002 [136]		Thibault 2004 [137]	Kim 2007 [138]	Rivero 2004 [139]			
Rinne 2006 [140]	Li 2004 [141]			Lee 2007 [142]	Urao 1999 [143]			
Samanta 2009 [144]	Panigrahi 2008 [145]			Lidesteri 2003 [146]	Van der Aa 2010 [147]			
Rojas 2012 [148]				Marini 2003 [149]	Waliogora-Dupriet 2007 [150]			
Taylor 2007 [151]				Rigo 2001 [152]	Wang 2007 [153]			
	Underwood 2009 [154]			Savino 2003 [155]				
				Sepp 1993 [156]				

Key: * Unpublished trials.

blinding; 4) incomplete outcome data; 5) selective outcome reporting; and 6) other sources of bias [17]. Each domain was assessed as having either a low risk of bias, high risk of bias or unclear to permit judgment. Any disagreements regarding risk of bias were resolved through discussion between MM, ML and RB. The association between risk of bias (domains) and type of funding (industry, non – industry, none declared) was explored.

C) Assessment of clinical outcomes

The primary and secondary outcomes from each study report were evaluated and categorized as:

1. Positive: synbiotic, probiotic or prebiotic supplementation had a statistically significant effect, $p < 0.05$.

Examples of ***positive outcomes*** included: adequate growth (weight gain, length gain, head circumference), tolerance (no feeding problems), microflora (increase in colony forming units of bifidobacteria, lactobacillus, decrease in pathogens), decreased infections (decrease in frequency, incidence of infections).

2) Negative: synbiotic, probiotic or prebiotic supplementation had a statistically significant effect *in an adverse event / negative outcome such as weight loss, diarrhoea*, $p < 0.05$
3) Neutral: synbiotic, probiotic or prebiotic supplementation **did not have** a statistically significant effect, $p > 0.05$, no significant differences between study groups. Clinical outcomes included: growth parameters, gastrointestinal parameters (tolerance to feed, stool characteristics, microflora); immune response, infections and mortality.

D) Overall study conclusions and conclusions on reported outcomes

The authors' overall study conclusion and conclusions on reported clinical outcomes were evaluated and categorized as:

1. Positive: The author's conclusion preferred the sponsor's products over control/placebo. Interpretation of data **supported** the sponsor's products over control.
2. Negative: The sponsors' products were not preferred over control / placebo. Interpretation of data did **NOT** support the sponsors' products.
3. Neutral: The author's conclusion was neutral to the sponsor's products.
4. No clear conclusion was offered by author.

In this review, the "conclusions on reported outcomes" referred to the authors' conclusions on individual reported RCTs outcomes. Examples include conclusions on weight gain, length gain, vomiting, necrotizing enterocolitis, sepsis.

Table 3 Source of funding and study participants

Sponsor	Study participants		Total
	Full term	Preterm Infant	
	n	n	n (%)
Industry	33	7	40 (59.7)
None / Not Clear	6	10	16 (23.9)
Non Industry	6	5	11 (16.4)
Total	45	22	67 (100.0)

Statistical Analysis

All the outcomes in this review were dichotomous and are described in frequencies and percentages. The association between source of funding (industry/non-industry/ none) and methodological quality (low/unclear/high risk of bias), clinical outcomes and author's conclusions were assessed using both the Pearson's Chi-square test and the Fisher's exact test. A p-value of less than 0.05 was considered statistically significant. SPSS version 19 statistical software was used. A statistician (AM) was consulted throughout the review process.

Ethics

The Human Research Ethics Committee at Stellenbosch University, South Africa reviewed the protocol for this review, ruled that all data to be collected for this review was from the public domain and was therefore exempt from ethical approval.

Results

Results of the search and description of studies

Electronic search of available databases yielded 290 citations. After reading titles and abstracts, duplicate reports were removed, 226 articles were screened and 100 articles were excluded. A hand search yielded 6 more articles. Potentially relevant full text reports were retrieved, reviewed for eligibility and a further 56 RCTs were excluded. Studies that had multiple publications were considered as one trial. Sixty seven RCTs and three on-going RCTs were

Table 4 Methodological quality (Risk of bias)

Quality of studies N = 67	N (%)		
	Low risk	High risk	Unclear
Sequence generation	42 (62.7)		25 (37.3)
Allocation concealment	32 (47.8)		35 (52.2)
Blinding	31 (46.3)		36 (53.7)
Incomplete Outcome data	52 (77.6)	1 (1.5)	14 (20.9)
Selective reporting	57 (85.1)	7 (10.4)	3 (4.5)
Other bias	53 (79.1)		14 (20.9)

Table 5 Reported outcomes and conclusions

Variable		N (%)		
Reported Outcomes	**N=**	**Positive***	**Neutral***	**Negative***
Weight gain	56	4 (7.1)	52 (92.9)	
Length gain	40	3 (7.5)	37 (92.5)	
Head circumference	31	4 (12.9)	27 (87.1)	
Colic	13	1 (7.7)	12 (92.3)	
Spitting up/Regurgitation	26	2 (7.7)	23 (88.5)	1 (3.8)
Vomiting	31	1.5 (3.2	30 (96.8)	
Crying/Fussiness	22	3 (13.6)	18 (81.8)	1 (4.5)
Gastric Residuals, Abdominal distension	5	1 (20)	4 (80)	
Volume of formula consumed	31	3 (9.7)	27 (87.1)	1 (3.2)
Time to full enteral feeds	9	2 (22.2)	7 (77.8)	
Stool frequency	37	10 (27)	27 (73)	
Stool consistency	37	18 (48.6)	19 (51.4)	
Stool pH	13	11 (84.6)	2 (15.4)	
Short chain fatty acids	9	3 (33.3)	6 (66.7)	
Flatulence/Gas	16		16 (100)	
Diarrhoea, Diarrhoea episodes	19	3(15.8)	15 (78.9)	1(5.3)
Constipation	3	1 (33.3)	2 (66.7)	
Microflora - Bifidobacteria	31	23 (74.2)	8 (25.8)	
Microflora - Lactobacillus	19	8 (42.1)	11 (57.9)	
Microflora - Pathogens	25	5 (20)	19 (76)	1 (4)
Immune response CRP, IL6, Cytokines	0			
Immunoglobulins (IgA,IgG, Ig-Flc, IgE)	10	6 (60)	4 (40)	
Allergy	3	1 (33.3)	2 (66.7)	
Eczema, Dermatitis, Rash, Skin Alterations	7	2 (28.6)	4 (57.1)	1 (14.3)
Infections - Acute Otitis Media	3		3 (100)	
Respiratory Infections	9	3 (33.3)	6 (66.7)	
Gastrointestinal infections	6	1 (16.7)	5 (83.3)	
Total infections, other unspecified infections	8	1 (12.5)	7 (87.5)	
Urinary tract infections	2		2 (100)	

Variable		N (%)			
Overall study conclusion	**N=**	**No conclusion**	**Positive**	**Neutral**	**Negative**
	67	**4 (6)**	**49 (73.1)**	**7 (10.4)**	**7 (10.4)**
Conclusion on reported outcomes					
Weight gain	56	40 (71.4)	15 (26.8)		1 (1.8)
Length gain	40	26 (65)	14 (35)		
Head circumference	31	17 (54.8)	14 (45.2)		
Colic	13	11 (84.6)	2 (15.4)		
Spitting up/Regurgitation	26	23 (88.5)	3 (11.5)		
Vomiting	32	24 (75)	8 (25)		
Crying/Fussiness	20	12 (60)	8 (40)		
Gastric Residuals, Abdominal distension	6	3 (50)	3 (50)		
Volume of formula consumed	30	26 (86.7)	3 (10)		1(3.3)
Time to full enteral feeds	8	5 (62.5)	2 (25)		1 (12.5)
Stool frequency	38	27 (71.1)	11 (28.9)		
Stool consistency	39	23 (59)	16 (41.0)		
Stool pH	12	7 (58.3)	5 (41.7)		
Short chain fatty acids	9	5 (55.6)	4 (44.4)		
Flatulence/Gas	15	11 (73.3)	4 (26.7)		
Diarrhoea, Diarrhoea episodes	18	12 (66.7)	5 (27.8)		1 (5.6)
Constipation	4	3 (75)	1 (25)		
Microflora - Bifidobacteria	30	10 (33.3)	17 (56.7)	2 (6.7)	1 (3.3)
Microflora - Lactobacillus	19	9 (47.4)	8 (42.1)	1 (5.3)	1 (5.3)
Microflora - Pathogens	25	12 (48)	11 (44)		2 (8)
Immune response CRP, IL6, Cytokines	1	1 (100)			
Immunoglobulins (IgA,IgG, Ig-Flc, IgE)	10	4 (40)	6 (60)		
Allergy	3	2 (66.7)	1 (33.3)		
Eczema, Dermatitis, Rash, Skin Alterations	7	5 (71.4)	1 (14.3)		1 (14.3)
Infections - Acute Otitis Media	3	1 (33.3	2 (66.7)		
Respiratory Infections	8	5 (62.5)	3 (37.5)		
Gastrointestinal infections	4	1 (25)	3 (75)		
Total infections, other unspecified infections	10	6 (60)	2 (20)		2 (20)
Urinary tract infections	2	1 (50)	1 (50)		

Table 5 Reported outcomes and conclusions *(Continued)*

Necrotizing Enterocolitis	11	2 (18.2)	9 (81.8)	Necrotizing Enterocolitis	12	7 (58.3)	3 (25)	2 (16.7)
Sepsis	10		10 (100)	Sepsis	10	9 (90)	1 (10)	
Fever, Febrile Episodes	4	2 (50)	2 (50)	Fever, Febrile Episodes	2	2 (100)		
Antibiotic use	19	4 (21.1)	15 (78.9)	Antibiotic use	16	13 (81.3)	3 (18.8)	
Hospitalization	12		12 (100)	Hospitalization	10	10 (100)		
Biochemical measures	9		9 (100)	Biochemical measures	6	5 (83.3)	1 (16.7)	
Adverse events	18	2 (11.1)	16 (88.9)	Adverse events	17	13 (76.5)	4 (23.5)	
Death / Mortality	7	1 (14.3	6 (85.7)	Death/Mortality	8	7 (87.5)	1 (12.5)	
Intestinal permeability	3	1 (33.3)	2 (66.7)	Intestinal permeability	3	1 (33.3)	2 (66.7)	
Duration of TPN	5		5 (100)	Duration of TPN	5	4 (80)	1 (20)	

*__Positive__: synbiotic, probiotic or prebiotic supplementation **had a** statistically significant effect, $p < 0.05$.
*__Neutral__: synbiotic, probiotic or prebiotic supplementation **did not have** a statistically significant effect, $p > 0.05$.
*__Negative__: synbiotic, probiotic or prebiotic supplementation **had a** statistically significant *increase **in an adverse event / negative outcome**,* $p < 0.05$.

Table 6 Association between Sponsor and methodological quality (risk of bias)

Methodological quality	Source of funding	Yes (Low risk)	No (High risk)	Unclear	Chi-square p value	Fisher's exact p value
N = 67 studies		n (%)[$$]	n (%)[$$]	n (%)[$$]		
Sequence generation	Industry	26 (38.8)		14 (20.9)	0.435	0.465
	None/Not clear	8 (11.9)		8 (11.9)		
	Non industry	8 (11.9)		3 (4.5)		
Allocation concealment	Industry	21 (31.3)		19 (28.4)	0.315	0.338
	None/Not clear	5 (7.5)		11 (16.4)		
	Non lindustry	6 (9.0)		5 (7.5)		
Blinding	Industry	18 (26.9)		22 (32.8)	0.395	0.457
	None/Not clear	6 (9.0)		10 (14.9)		
	Non industry	7 (10.4)		4 (6.0)		
Incomplete outcome data	Industry	36 (53.7)	1 (1.5)	3 (4.5)	0.023*	0.005*
	None/Not clear	9 (13.4)		7 (10.4)		
	Non industry	7 (10.4)		4 (6.0)		
Selective reporting	Industry	36 (53.7)	2 (3.0)	2 (3.0)	0.224	0.188
	None/Not clear	11 (16.4)	4 (6.0)	1 (1.5)		
	Non industry	10 (14.9)	1 (1.5)	0		
Free of other bias	Industry	35 (52.2)		5 (7.5)	0.033*	0.038*
	None/Not clear	9 (13.4)		7 (10.4)		
	Non industry	8 (13.4)	1 (1.5)	2 (3.0)		

*Significant $p < 0.05$.
[$$]Overall percentage.

included in this review. (Table 1) The selection process is shown in Figure 1. Table 2 gives a list of 56 RCTs which were excluded for: use of exclusive breast or non-formula milk (8 RCTs), type of feed not clear (11 RCTs), probiotic administered in saline, water or other fluid (4 RCTs), no use of probiotic or prebiotic (6 RCTs), not RCT (12 studies), different inclusion criteria (10 studies), lack of suitable translator (2 RCTs), data presentation inappropriate (1 RCT) and out of date [published before1980] (2 RCTs). Three excluded RCTs were unpublished trials.

Characteristics of included studies

Table 1 lists included and on-going trials. Sixty seven RCTs were included, 45 (67.2%) on full term infants, 22 (32.8%) on preterm infants. All included RCTs were published trials. All trials were conducted on healthy full

Table 7 Association between Sponsor and clinical outcomes: Growth

Growth	Source of funding	Assessment of outcome		Chi-square p value	Fisher's exact p value
		Positive*	Neutral*		
		n (%)[$$]	n (%)[$$]		
Weight gain N = 56	Industry	2 (3.6)	35 (62.5)	0.309	0.266
	None/Not clear	2 (3.6)	10 (17.9)		
	Non industry	0	7 (12.5)		
Length gain N = 40	Industry	3 (7.5)	29 (72.5)	0.667	1.00
	None/Not clear		6 (15)		
	Non industry		2 (5)		
Head Circumference N = 31	Industry	4 (12.9)	23 (74.2)	0.712	1.00
	None /Not clear		3 (9.7)		
	Non industry		1 (3.2)		

[$$]Overall percentage.
*__Positive__: synbiotic, probiotic or prebiotic supplementation **had a** statistically significant effect, $p < 0.05$. **There were significant differences** between study groups **(in favour of experimental group)**.
*__Neutral__: synbiotic, probiotic or prebiotic supplementation **did not have** a statistically significant effect, $p > 0.05$, **No significant differences** between study groups.

term or preterm infants and used standard (full term or preterm) infant formula (Table 3).

Funding

Out of 67 trials, 40 (59.7%) were funded by food industry, 11 (16.4%) were funded by non-industry entities, and 16 (23.9%) did not specify their source of funding,
10 RCTs on preterm infants, 6 RCTs on full infants (Table 3).

Methodological quality (Risk of bias)

In this review, several domains were not adequately reported, particularly, the domains of sequence generation, allocation concealment and blinding. Out of 67 RCTs,
25 (37.3%) failed to report sequence generation, 35 (52.2%) failed to report allocation concealment and 36 (53.7%) did not report blinding. Majority of the RCTs were assessed as having a low risk of bias in the domains of incomplete outcome data 52 (77.6%), selective reporting 57(85.1%) and other bias 53 (79.1%) (Table 4).

Outcomes and study conclusions

In most RCTs, majority of outcomes were assessed as neutral, (intervention did not have a statistically significant effect, $p > 0.05$). A total of 49 (73.1%) of RCTs had a positive overall study conclusion in favour of the sponsors' products, while 7 (10.4%) had negative, 7 (10.4%) had neutral conclusions and 4 (6%) had no clear conclusion. The included RCTs either did not provide any conclusion on their reported clinical outcomes or, they provided a positive conclusion for their reported outcome in-favour of the sponsors' products. Few RCTS had either negative or neutral conclusions on their reported clinical outcomes (Table 5).

Association between source of funding (sponsor) and methodological quality of studies

There was no significant association between the source of funding and the domains of sequence generation (Chi – square $p = 0.435$, Fisher exact $p = 0.465$), allocation concealment (Chi – square $p = 0.315$, Fisher exact $p = 0.338$), blinding (Chi – square $p = 0.395$, Fisher exact $p = 0.457$)

Table 8 Association between Sponsor and clinical outcomes: Tolerance symptoms

Tolerance	Source of funding	Positive*	Negative*	Neutral*	Chi-square	Fisher's exact
		n (%)[$$]	n (%)[$$]	n (%)[$$]	p value	p value
Colic N = 13	Industry	1 (7.7)		11 (84.6)	0.764	1.00
	None/Not clear					
	Non industry			1 (7.7)		
Spitting up/Regurgitation N = 26	Industry	2 (7.7)	1 (3.8)	17 (65.4)	0.907	1.00
	None/Not clear			4 (15.4)		
	Non industry			2 (7.7)		
Vomiting N = 31	Industry	1 (3.2)		23 (74.2)	0.860	1.00
	None/Not clear			5 (16.1)		
	Non industry			2 (6.5)		
Crying fussiness N =22	Industry	3 (13.6)	1 (4.5)	14 (63.6)	0.581	1.00
	None/Not clear			4 (18.2)		
	Non industry			0		
Gastric residuals, Abdominal distension N = 5	Industry			1 (20)	0.659	1.00
	None/Not clear			1 (20)		
	Non industry	1 (6.7)		2 (40)		
Volume of formula consumed/daily intake N = 31	Industry	3 (9.7)	1 (3.2)	18 (58.1)	0.758	1.00
	None/Not clear			4 (12.9)		
	Non industry			5 (16.1)		
Days to full enteral feeding N = 9	Industry			4 (44.4)	0.325	0.444
	None/Not clear	1 (11.1)		1 (11.1)		
	Non industry	1 (11.1)		2 (22.2)		

[$$]Overall percentage.
*__Positive__: synbiotic, probiotic or prebiotic supplementation **had a** statistically significant effect, $p < 0.05$. **There were significant differences** between study groups (in favour of experimental group).
*__Neutral__: synbiotic, probiotic or prebiotic supplementation **did not have a** statistically significant effect, $p > 0.05$, **No significant differences** between study groups.
*__Negative__: synbiotic, probiotic or prebiotic supplementation **had a** statistically significant ***increase in an adverse event / negative outcome,*** $p < 0.05$.

Table 9 Association between sponsor and clinical outcomes: stool characteristics

Stool characteristics	Source of funding	Positive* n (%)[$$]	Negative* n (%)[$$]	Neutral* n (%)[$$]	Chi-square p value	Fisher's exact p value
Stool Frequency N = 37	Industry	7 (18.9)		22 (59.5)	0.501	0.540
	None/Not clear	3 (8.1)		4 (10.8)		
	Non industry			1 (2.7)		
Stool Consistency n =37	Industry	14 (37.8)		15 (40.5)	0.562	1.00
	None/Not clear	4 (10.8)		3 (8.1)		
	Non industry			1 (2.7)		
Stool pH N =13	Industry	7 (53.8)		2 (15.4)	0.305	1.00
	None/Not clear	4 (30.8)				
	Non industry					
Stool Short Chain Fatty Acids N = 9	Industry	2 (22.2)		4 (44.4)	0.687	1.00
	None / Not clear	1 (11.1)		1 (11.1)		
	Non industry			1 (11.1)		
Flatulence / Gas N = 16	Industry			15 (93.8)	Not valid	
	None/Not clear			1 (6.3)		
	Non industry			0		
Diarrhoea, Diarrhoea episodes N = 19	Industry	3 (15.8)	1 (5.3)	10 (52.6)	0.771	1.00
	None/Not clear			2 (10.5)		
	Non industry			3 (15.8)		
Constipation N = 3	Industry	1 (33.3)		1 (33.3)	0.386	1.00
	None/Not clear			1 (33.3)		
	Non industry			0		

[$$]Overall percentage.
*Positive: synbiotic, probiotic or prebiotic supplementation had a statistically significant effect, $p < 0.05$. There were significant differences between study groups (in favour of experimental group).
*Neutral: synbiotic, probiotic or prebiotic supplementation did not have a statistically significant effect, $p > 0.05$, No significant differences between study groups.
*Negative: synbiotic, probiotic or prebiotic supplementation had a statistically significant increase *in an adverse event / negative outcome,* $p < 0.05$.

Table 10 Association between sponsor and clinical outcomes: Microflora

Microflora	Source of funding	Positive 4* n (%)[$$]	Negative 5* n (%)[$$]	Neutral 6* n (%)[$$]	Chi-square p value	Fisher's exact p value
Bifidobacteria N = 31	Industry	12 (38.7)		6 (19.4)	0.416	0.583
	None/Not clear	8 (25.8)		2 (6.5)		
	Non industry	3 (9.7)				
Lactobacillus N = 19	Industry	2 (10.5)		6 (31.6)	0.155	0.176
	None/Not clear	4 (21.1)		5 (26.3)		
	Non industry	2 (10.5)		0		
Pathogens N = 25	Industry	2 (8.0)		11 (44.0)	0.532	0.612
	None/Not clear	3 (12.0)	1 (4.0)	6 (24.0)		
	Non industry			2 (8.0)		

[$$] Overall percentage.
*__Positive__: synbiotic, probiotic or prebiotic supplementation **had a** statistically significant effect, $p < 0.05$. **There were significant differences** between study groups (in favour of experimental group).
*__Neutral__: synbiotic, probiotic or prebiotic supplementation **did not have** a statistically significant effect, $p > 0.05$, **No significant differences** between study groups.
*__Negative__: synbiotic, probiotic or prebiotic supplementation **had a** statistically significant ***increase in an adverse event / negative outcome,*** $p < 0.05$.

Table 11 Association between sponsor and clinical outcomes: Necrotizing enterocolitis, sepsis and antibiotic use

	Source of funding	Positive* n (%)[$$]	Neutral* n (%)[$$]	Chi-square p value	Fisher's exact p value
Necrotising enterocolitis N = 11	Industry		4 (36.4)	0.118	0.273
	None/Not clear		3 (27.3)		
	Non industry	2 (18.2)	2 (18.2)		
Sepsis N = 10	Industry		2 (20)	Not Valid	
	None/Not clear		3 (30)		
	Non industry		5 (50)		
Antibiotic use N = 19	Industry	4 (21.1)	4 (21.1)	0.031[#]	0.039[#]
	None/Not clear		5 (26.3)		
	Non industry		6 (31.6)		

[$$] Overall percentage.
*__Positive__: synbiotic, probiotic or prebiotic supplementation **had a** statistically significant effect, $p < 0.05$. **There were significant differences** between study groups (in favour of experimental group).
*__Neutral__: synbiotic, probiotic or prebiotic supplementation **did not have** a statistically significant effect, $p > 0.05$, **No significant differences** between study groups.
[#] Significant $p < 0.05$.

and selective reporting (Chi – square p = 0.224, Fisher exact p = 0.188) (Table 6).

There was a significant association between funding and the domains of incomplete outcome data (Chi – square p = 0.023, Fisher exact p = 0.005) and free of other bias (Chi – square p = 0.033, Fisher exact p = 0.038) (Table 6). The association between source of funding and incomplete outcome data was such that industry-funded trials had significantly less missing data than non-industry funded trials. The association between source of funding and free of other bias (such as outcomes bias) was such that a significantly higher percentage of industry-funded trials were free of other bias compared to non-industry-funded trials.

Association between source of funding (sponsor) and clinical outcomes

There was no significant association between source of funding and reporting of clinical outcomes: Growth parameters, stool characteristics, microflora, infections (Tables 7, 8, 9, 10 and 11), immune parameters, adverse events and mortality (data not shown). There was a significant association between the source of funding and reporting of antibiotic use in formula fed infants (Chi-square p = 0.031, Fisher exact p = 0.039) such that industry funded trials were more likely to decrease the use of antibiotics than non-industry funded trials (Table 11).

Association between source of funding (sponsor) and overall study conclusion

There was no significant association between sources of funding and overall study conclusion (Chi-square p = 0.505, Fisher exact p = 0.373). Majority of RCTs, 49 (73.1%), had a positive study conclusion; 32 (47.8%) of these RCTs, were industry sponsored, 7 (10.4%) non- industry and 10 (14.9%) which did not declare their source of funding (Table 12). A sensitivity analysis was conducted with respect to combining industry sponsored studies with those that had not declared their source of funding. There was no change in the results. There was no significant association between source of funding and overall study conclusion (Chi-square p = 0.483, Fisher exact p = 0.425).

Association between source of funding (sponsor) and conclusion on reported clinical outcomes

There was a significant association between source of funding and conclusion on weight gain (Chi-square p = 0.037, Fisher exact p = 0.024) such that industry-funded

Table 12 Association between sponsor and OVERALL study conclusion

Source of funding		Positive n (%)[$$]	Negative n (%)[$$]	Neutral n (%)[$$]	No clear conclusion n (%)[$$]	Total n (%)[$$]	Chi-square p value	Fisher's exact p value
Overall conclusion N = 67	Industry	32 (47.8)	2 (3.0)	3 (4.5)	3 (4.5)	40 (59.7%)	0.505	0.373
	None/Not clear	10 (14.9)	3 (4.5)	2 (3.0)	1 (1.5)	16 (23.9%)		
	Non industry	7 (10.4)	2 (3.0)	2 (3.0)	0	11 (16.4%)		
	Total	49 (73.1%)	7 (10.4%)	7 (10.4%)	4 (6.0%)	67 (100)		

[$$] Overall percentage.

Table 13 Association between sponsor and conclusion on reported outcome: Growth parameters

Authors conclusion on:	Source of funding	No conclusion on reported outcome n (%)[$$]	Positive n (%)[$$]	Negative n (%)[$$]	Chi-square p value	Fisher's exact p value
Weight gain N = 56	Industry	23 (41.1%)	14 (25.0%)		0.037[#]	0.024[#]
	None/Not clear	10 (17.9%)	1 (1.8%)	1 (1.8%)		
	Non industry	7 (12.5%)				
Length gain N = 40	Industry	18 (45%)	14 (35%)		0.068	0.051
	None/Not clear	6 (15%)				
	Non industry	2 (5)				
Head circumference N = 31	Industry	13 (41.9)	14 (45.2)		0.151	0.232
	None/Not clear	3 (9.7)				
	Non industry	1 (3.2)				

[#]Significant $p < 0.05$, [$$] Overall percentage.

trials were more likely to report positive conclusions on weight gain than non-industry-funded trials (Table 13). There was no significant association between source of funding and conclusion on other reported clinical out- comes (Tables 14, 15, 16 and 17).

Discussion

This review revealed that majority of RCTs (from 1980 to 2012) on infants fed formula supplemented with pro-biotics, prebiotics or synbiotics are funded by the food industry. This is consistent with the trend that

Table 14 Association between Sponsor and conclusion on reported outcome: Tolerance symptoms

Tolerance	Source of Funding	No conclusion on reported outcome n (%)[$$]	Positive n (%)[$$]	Negative n (%)[$$]	Chi-Square p value	Fisher's Exact p value
Colic N = 13	Industry	10 (76.9)	2 (15.4)		0.657	1.00
	None / Not Clear					
	Non Industry	1 (7.7)				
Spitting up / Regurgitation N = 26	Industry	19 (73.1)	1 (3.8)		0.032	0.062
	None / Not Clear	2 (7.7)	2 (7.7)			
	Non Industry	2 (7.7)				
Vomiting N = 32	Industry	19 (59.4)	5 (15.6)			
	None / Not Clear	3 (9.4)	3 (9.4)			
	Non Industry	2 (6.3)				
Crying Fussiness N =20	Industry	10 (50)	6 (30)		0.648	1.00
	None / Not Clear	2 (10)	2 (10)			
	Non Industry	0				
Gastric Residuals, Abdominal distension N = 6	Industry		1 (16.7)		0.513	1.00
	None / Not Clear	1 (16.7)	1 (16.7)			
	Non Industry	2 (33.3)	1 (16.7)			
Volume of formula consumed / daily intake N = 30	Industry	19 (63.3)	2 (6.7)	1 (3.3)	0.867	0.733
	None / Not Clear	3 (10.0)				
	Non Industry	4 (13.3)	1 (3.3)			
Days to full enteral feeding N = 8	Industry	2 (25)		1 (12.5)	0.547	1.00
	None / Not Clear	1 (12.5)	1 (12.5)			
	Non Industry	2 ()25	1 (12.5)			

[$$]Overall percentage

Table 15 Association between sponsor and conclusion on reported outcome: Stool characteristics

Stool characteristics	Source of funding	No conclusion on reported outcome Positive n (%)[$$]	n (%)[$$]	Negative n (%)[$$]	Pearson's chi Square	Fisher's exact p value
Stool frequency N = 38	Industry	21 (55.3)	9 (23.7)		0.809	1.00
	None / Not clear	5 (13.2)	2 (5.3)			
	Non industry	1 (2.6)				
	Total	27 (71.1)	11 (28.9)			
Stool consistency n =39	Industry	18 (46.2)	13 (33.3)		0.699	1.00
	None / Not clear	4 (10.3)	3 (7.7)			
	Non industry	1 (2.6)				
	Total	23 (59)	16 (41)			
Stool pH N =12	Industry	5 (41.7)	3 (25)		0.679	1.00
	None / Not clear	2 (16.7)	2 (16.7)			
	Non industry					
	Total	7 (58.3)	5 (41.7)			
Stool short chain fatty acids N = 9	Industry	3 (33.3)	3 (33.3)		0.638	1.00
	None / Not clear	1 (11.1)	1 (11.1)			
	Non industry	1 (11.1)				
	Total	5 (55.6)	4 (44.4)			
Flatulence/Gas N = 15	Industry	10 (66.7)	4 (26.7)		0.533	1.00
	None / Not clear					
	Non industry	1 (6.7)				
	Total	11 (73.3)	4 (26.7)			
Diarrhoea, Diarrhoea episodes N = 18	Industry	7 (38.9)	5 (27.8)	1 (5.6)	0.484	0.557
	None / Not clear	2 (11.1)				
	Non industry	3 (16.7)				
	Total	12 (66.7)	5 (27.8)	1 (5.6)		
Constipation N = 4	Industry	2 (50)	1 (25)		0.505	1.00
	None / Not clear					
	Non industry	1(25)				
	Total	3 (75)	1 (25)	1 (25)		

[$$] Overall percentage.

biomedical research is increasingly being funded by in- dustry [1,2] There was a trend that more RCTs on pre- term infants failed to report their source of funding. The reason(s) for this trend needs to be explored further.

Cochrane guidelines were used to assess the risk of bias of included RCTs. The reporting of several domains was however suboptimal particularly sequence generation, allocation concealment and blinding domains. Considering completed data, there was no significant association between funding source and methodological quality of RCTs in the domains of sequence generation, allocation concealment, blinding and selective reporting. There was a significant association between funding and methodological quality of RCTs in the domains of incomplete outcome data and free of other bias. Industry funded trials had significantly less missing data than non-industry funded trials. A higher percentage of industry funded trials were free of other bias compared to non-industry funded trials. More industry sponsored trials had low risk of bias in 5 out of 6 domains, even though our results did not show a statistical significant association between funding and methodological quality in most domains. Our results confirm findings from previous reviews on infants given enteral feeds with probiotics, prebiotics and synbiotics [157-159].

There was no significant association between funding source and clinical outcomes or majority of authors' conclusions. There was a significant association between funding and conclusion on weight gain. Regardless of the reported clinical outcomes, nearly all RCTs in this review reported neutral results. That is supplementation with probiotics, prebiotics or synbiotics did not have a significant effect or there were no significant differences between study groups of infants given supplemented formula or placebo. Our findings confirm the results of two

Table 16 Association between sponsor and conclusion on reported outcome: Microflora

Microflora	Source of funding	No conclusion on reported outcome	Positive n	Negative	Neutral	Chi-square	Fisher's exact
		n (%)[$$]	(%)[$$]	n (%)[$$]	n (%)[$$]	p value	p value
Bifidobacteria N = 30	Industry	7 (23.3)	11 (36.7)			0.249	0.195
	None/Not clear	2 (6.7)	5 (16.7)	1 (3.3)	1 (3.3)		
	Non industry	1 (3.3)	1 (3.3)		1 (3.3)		
Lactobacillus N = 19	Industry	5 (26.3)	4 (21.1)			0.084	0.294
	None/Not clear	3 (15.8)	4 (21.1)		1 (5.3)		
	Non industry	1 (5.3)		1 (5.3)			
Pathogens N = 25	Industry	7 (28)	6 (24)			0.152	0.269
	None/Not clear	4 (16)	5 (20)	1 (4)			
	Non industry	1 (4)		1 (4)			

[$$]Overall percentage.

systematic reviews which found that supplementation with probiotics, prebiotics or synbiotics did not offer any distinct advantage over placebo [158,159]. However, results of this review did not agree with two nutrition related reviews or reviews on pharmaceutical industry supported RCTs, which reported that industry sponsored RCTs had results and conclusions in favour of the sponsor [2-4,6,8,160-162]. Despite reporting neutral outcomes, authors from industry sponsored RCTs had a tendency to advocate for the consumption of the sponsors' products. Similar findings were reported by Nestle, who reported that research investigators "who received company grants tended to publish results, give advice and prescribe in favour of the sponsor." This applied to research that was supported by pharmaceutical and food industries [163].

Effects of sponsorship on overall study conclusion have been equally documented in biomedical literature. Reviews by Lessor and Nkansah reported positive conclusions in favour of the sponsor [6,8]. Although no statistically significant association between funding and authors conclusion was found in this review, more than 70% of RCTs reported positive conclusions, 47.8% of these were industry sponsored. Often, these positive conclusions in the RCTs were not supported by the reported data as demonstrated by the neutral clinical outcomes. Our findings are consistent with those of previous reviews, which found that, results from RCTs may be accurate, but authors may distort the meaning of the results, present conclusions that are more favourable, and that were not supported by the data presented [2,5,163]. Even meta – analyses were not spared from this trend [2,5,163]. Despite overwhelming positive overall study conclusions, majority of RCTs did not have any conclusion on their reported clinical outcomes. The RCTs that reported any conclusion on their clinical outcomes, majority were positive in favour of the sponsors' products.

Limitations

This review did not document the role of the sponsor in study design, data collection, and analysis. Few RCTs

Table 17 Association between sponsor and conclusion on reported outcome: Necrotising Enterocolitis Sepsis and antibiotic use

	Source of funding	No conclusion on reported outcome	Positive	Negative	Pearson's chi Square	Fisher's exact
		n (%)[$$]	n (%)[$$]	n (%)[$$]		p value
NEC N = 12	Industry	3 (25)		1 (8.3)	0.511	0.782
	None/Not clear	2 (16.7)	1 (8.3)	1 (8.3)		
	Non industry	2 (16.7)	2 (16.7)	0		
		7 (58.3)	3 (25)	2 (16.7)		
Sepsis N = 10	Industry	2 (20)			0.274	0.500
	None/Not clear	2 (20)		1 (10)		
	Non industry	5 (50)				
Antibiotic use N = 16	Industry	4 (25)	3 (18.8)		0.093	0.141
	None/Not clear	4 (25)				
	Non industry	5 (31.3)				

[$$] Overall percentage.

reported this. More detailed documentation and disclosure in RCT reports would help evaluate if there was an association between funding and reported outcomes or conclusions. Many RCTs had missing data especially on the domains of sequence generation, allocation concealment and blinding. Attempts were made to contact authors for missing information but none responded. The sample size (number of RCTs) was small and skewed towards industry.

Conclusion

This study assessed the impact of funding by the food industry on trial outcomes and methodological quality of synbiotics, probiotics and prebiotics research in in- fants. There was no significant association between source of funding and methodological quality of study in the domains of sequence generation, allocation concealment and blinding. Industry funded trials had less missing data and were free of other bias than non-industry funded trials.

There was no significant association between funding and majority of reported clinical outcomes or authors' conclusions. However, there was a significant association between funding source and reported antibiotic use and conclusion on weight gain. Majority of RCTs were industry funded, more non-industry funded research is needed to further assess the impact of funding on methodological quality, reported clinical outcomes and authors' conclusions.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
The reviewers contributed the following: MM: Developed review protocol (unpublished), selected RCTs, conducted data extraction, assessment of risk of bias in included RCTs, developed, edited and critically reviewed the manuscript. ML: Selected RCTs, conducted data extraction, assessment of risk of bias in included RCTs, critically reviewed the manuscript. AM: Conducted the statistical analysis, interpretation of results and critically reviewed the manuscript. TY: Contributed to designing the review methodology and critically reviewed the manuscript. RB: Contributed to designing the review, acted as third party arbitrator and critically reviewed the manuscript. All authors read and approved the final manuscript.

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Chapter 5: Application of evidence on probiotics, prebiotics and synbiotics by food industry: A descriptive study

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Application of evidence on probiotics, prebiotics and synbiotics by food industry: A descriptive study.

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Abstract

Background: This study assessed how the food industry applies the knowledge and evidence gained from synbiotics, probiotics or prebiotics research in infants, on the general paediatric population. This study also explored: what happens after the clinical trials using infant formula are completed, data is published or remains unpublished; the effectiveness and type of medium the formula manufacturers use to educate consumers on probiotic, prebiotic or synbiotic infant formula.

Methods: This was a descriptive study (a survey) that used a structured questionnaire. All listed companies that manufacture and / or market food products with added probiotics, prebiotics or synbiotics for infants were identified and invited to participate. People responsible for research and development were invited to participate in the survey. A letter of invitation was sent to selected participants and if they expressed willingness to take part in the study, a questionnaire with a written consent form was sent. Descriptive statistics and associations between categorical variables were to be tested using a Chi-square test, a $p<0.05$ was statistically significant.

Results: A total of 25 major infant formulas, baby food manufacturers were identified, invited to participate in the survey. No company was willing to participate in the survey for different reasons: failure to take any action 5 (20%), decision to participate indefinitely delayed 2 (8%), sensitivity of requested information 3 (12%), company does not conduct clinical trials 1 (4%), company declined without further information 4 (16%), erroneous contact information 6 (24%), refusal by receptionists to forward telephone calls to appropriate staff 3 (12%), language barrier 3 (12%), company no longer agrees to market research 1(4%).

Conclusion: Due to a poor response rate in this study, no conclusion could be drawn on how the food industry applies evidence gained through probiotics, prebiotics or synbiotics research on infants for the benefit of the general paediatric population. More information and greater transparency is needed from the infant formula manufacturers on how they apply the evidence gained from the research on probiotics, prebiotics and synbiotics on infants.

Key words: Food industry, infant formula, evidence, probiotics, prebiotics, synbiotics.

Background

Scientific evidence from numerous studies in the last 25 years confirms that breastfeeding is the optimal way to feed infants, since breast milk contains all the essential nutrients to meet babies' needs, as well as antibodies that fight off infection. [1, 2, 3, 4] The World Health Organisation (WHO) estimates, that if women breastfed their infants, up to 1.5 million infant deaths or 13% of deaths in children under 5 years old could be prevented annually. [5] Despite the well documented benefits of breastfeeding, more women are choosing formula feeding, either exclusively or giving mixed feeds (both formula and partial breastfeeding). Globally, this has resulted in sales of infant formula skyrocketing creating stiff competition among infant formula companies to manufacture new and innovative products. [5]

One objective of the infant food industry is to provide infants with nutrition support, that accommodates the development and function of rapidly developing organ systems. [6] A factor driving research and innovation in the infant food industry is the need to understand the composition and functional characteristics of breast milk. Therefore, scientists continuously conduct research to identify how infant formula can be adapted to more closely resemble the composition and function of human milk. This has resulted in different components being added to infant formula such as docosahexaenoic acid (DHA), arachidonic acid, synbiotics, probiotics or prebiotics. [6 - 9]

Probiotics, prebiotics and synbiotics

Probiotics are defined as "live microorganisms" which when administered in adequate amounts may confer a health benefit to the host. [9, 10, 11] The main probiotics that are used worldwide are Lactobacillus and Bifidobacteria which are found in the gastrointestinal (GI) microflora. [11, 12] Formula companies have

been adding probiotics to infant formula. Probiotics are also consumed in the form of fermented foods, dairy products such as yogurt and cheese and can be added to other foods such as cereals, biscuits, soy milk, sausages and numerous other foods. [11 - 16]

Prebiotics are non- digestible food ingredients that may benefit the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon and improving the host's health. [9, 10, 17] A prebiotic is also an ingredient that is selectively fermented by indigenous bacteria (especially lactobacilli, bifidobacteria) resulting in changes in the composition and / or activity of the GI microbiota resulting in optimum colon function which improves the host's health. [18] The most widely studied prebiotics are inulin and fructooligosaccharide (FOS). FOS and inulin are added to different foods as fat and sugar replacements to improve texture or for their functional benefits. [9, 10] The latter is why formula companies now add prebiotics to infant formula. Adding prebiotics to formula stimulates the growth of only beneficial bacteria in the gastrointestinal tract to levels found in breastfed infants. [17, 18, 19]

When probiotics and prebiotics are administered simultaneously, the combination is termed Synbiotics. [9, 10, 19] A new trend in the infant food industry is the addition of synbiotics to infant formula.

How strong is the evidence for adding probiotics, prebiotics and synbiotics to infant formula?

There is evidence that a healthy GI microflora in infants is necessary to achieve optimal health and growth. [20] For infants who are not breastfed, there is a rational to adapt infant formulas to promote an intestinal microbiota resembling that of breastfed infants, which has a greater concentration of bifidobacteria, fewer potentially pathogenic bacteria than formula fed infants. Strategies to achieve this goal include the addition of probiotics, prebiotics or synbiotics to infant formula for full term and preterm infants to improve growth, development and decrease infections. [11] Adding these ingredients to infant formula changes the intestinal microbiota of infants. [19, 21, 22]

Guidelines from the Oxford Centre for Evidence- based Medicine state that a systematic review of randomized clinical trials offers the highest level of evidence for information on the effectiveness of an intervention. This is followed by RCT(s). [23, 24, 25] Health benefits conferred by probiotic bacteria are strain specific. [12, 20] Systematic reviews on full term infants given probiotics show certain strains of probiotics improve stool consistency and frequency (*Lactobacillus GG*) [26], other strains increase average formula intake (*L. reuteri, .B. lactis*) [22], and support normal growth (*B. lactis, B. longum* BL999, *L. rhamnosus LPR, Lactobacillus* GG, *L. reuteri* ATCC 55730). [26] For preterm infants, administration of probiotics results in reduced risk of Necrotising Enterocolitis (from combinations of *Lactobacillus bifidus, streptococcus thermophillus,* and *bifidobactrium infantis*) and mortality (*L acidophilus* and *B infantis*). [27]

Two systematic reviews on formula fed infants show that addition of synbiotics, probiotics or prebiotics to infant formula does not always have a significant effect on clinical outcomes in infants. [22, 28] In full term infants, addition of synbiotics to infant formula did not have any significant effect on growth, frequency of colic, regurgitation or vomiting. Addition of probiotics to infant formula did not have any significant effect on growth, episodes of diarrhoea, stool frequency or consistency. Addition of prebiotics to infant formula had no significant effect on length gain, head circumference, stool consistency or incidence of diarrhoea, regurgitation, counts of bifidobacteria or lactobacillus. [22] For preterm infants, probiotics did not have any significant effect on growth, risk reduction of NEC, sepsis and death. [28]

Probiotics have been granted GRAS (generally regarded as safe) status by the Food and Drug Administration (FDA) due to the long history of safe use, and the bacteria used in the probiotic preparations are identical to those found in the GI tract. [29] In healthy people, probiotics rarely cause disease. The risk of developing bacteraemia from ingested lactobacilli is less than 1 per 1 million users; risk of developing fungaemia (from Saccharomyces Boulardii) is less than 1 per 5.6 million users. [30, 31, 32] Systemic infections such as endocarditis, fungaemia are extremely rare. Predisposing factors include immunosuppression, prior hospitalization, severe underlying co-morbidities, previous antibiotic therapy, invasive procedures that involve the gastrointestinal tract and other organs. [33, 34, 35] Risk factors for probiotic associated sepsis are prematurity in infants, presences of a central venous catheter, impairment of the intestinal epithelial barrier and concurrent administration of broad spectrum antibiotics to which the probiotic is resistant. [36]

Prebiotics have a good safety record at levels found in existing food components. Flatulence or abdominal bloating are reported at doses greater than 20g / day. Abdominal cramps or diarrhoea are reported at doses greater than 50 g / day. [19, 29] Adding prebiotics to formula stimulates the growth of only beneficial bacteria in the gastrointestinal tract to levels found in breastfed infants. [12, 37, 38] As these beneficial bacteria increase, they exclude pathogens; the gut mucosal barrier improves preventing infections with enteric pathogens or trans-located gut bacteria. [10, 18, 39] Other benefits reported from consuming prebiotics are improved intestinal architecture from increased villi height, thicker mucus layer, deeper crypts and increased globlet cells which improves intestinal permeability [19, 29, 40]

Communication of best evidence to the consumer

Communicating effectively with the consumer is challenging. Communication of health and nutrition messages comes from many sources. The consumer is inundated with information from the media, government, non-profit groups, advocacy organizations, food and beverage industry. [41] Furthermore, the environment for communicating health and nutrition information has changed in recent years due to an increase of television channels, internet usage and new media such as social networking sites, podcasts and webinars. [42]

Health information is not always disseminated by experts. Fewer media outlets have medical and health reporters in their staff to cover complex topics. As a result, new scientific information maybe reported by people who do not have the necessary background to understand the content of complex journal articles or presentations. Often due to time pressure, these people depend on single sources of information such as press releases or wire services. This increases the chances of inaccurate information being disseminated to the consumers. Ultimately, the consumers are overwhelmed and confused. [41] In addition, more than 50 percent of literate adults are actually NOT health literate. They are unable to obtain, interpret or understand basic health information and make sound decisions. [43] The health information messages have to be tailored to the target audience, using appropriate communication channels and techniques. [44]

To communicate with the consumers, the food industry uses multiple channels to promote and sell their products with a goal of achieving profitable growth. The food industry uses subtle messages of better nutrition as part of their promotional activities. [41] In the context of probiotics, prebiotics containing food products, the consumer may not understand the meaning or importance of scientific terms such as probiotics, *Lactobacillus*, fructooligosaccharide or inulin. Thus, there is a great need for clear information in a language that the consumer can understand.

Rationale for research

To our knowledge, there are no studies that have assessed how the food industry applies the knowledge and evidence gained from research on probiotics, prebiotics or synbiotics on the general paediatric population. This study attempted to explore what happens after research trials using infant formula have been conducted and the data is published or remains unpublished. Based on the new scientific evidence, do the companies routinely develop and market a new probiotic, prebiotic or synbiotic containing infant formula, or improve on one that is already sold on the market?

Probiotic infant formulas have been sold in Europe and Asia in the last 15 years but are not used widely in North America. [45] A physical check of several retail outlets in the Western Cape, South Africa, yielded few brands (sometimes only two) of probiotic containing infant formula. Yet several companies (in collaboration with academic institutions) have conducted research projects using probiotics and prebiotics on infants in Southern Africa. [46, 47] There is little or no information on the differences between the study formula and the retailed product. It is not clear how the manufacturers of probiotic, prebiotic or synbiotic containing infant formula educate the consumers on their products. This study set out to answer product specific questions on genera of probiotics used, product viability at end of shelf life, differences between study and retailed product. As well as, explore the effectiveness and type of medium the infant formula manufacturers use to educate the consumers on probiotic, prebiotic or synbiotic infant formula.

Safety issues are also an area of concern. The two probiotic infant formula brands available in the Western Cape, South Africa retail outlets state that using water with temperatures above 40 ^{0}C (degrees centigrade) will compromise the natural cultures. This contradicts the WHO "Guidelines for safe preparation, storage and handling of powdered infant formula" which recommends that water with a minimum temperature of 70 ^{0}C should be used to minimize the risk of potentially deadly infections caused by *Enterobacter Sakazakii*, bacteria that has been found in infant formula. [48] In addition there is a lack of published evidence on clinical benefits from long term use of probiotic containing infant formula. [26, 49] This study tried to explore how the infant formula companies address the contradiction to WHO guidelines on formula preparation and safety issues of long term usage of probiotic infant formula.

Research question

How does the food industry apply the evidence gained through probiotics, prebiotics and synbiotics research on infants for the benefit of the general paediatric population?

Research Aim

To investigate how the infant food industry applies evidence gained through probiotics, prebiotics and synbiotics research on infants.

Objectives:

The objectives of this study were to determine the following:

Application of evidence:

1. If new research evidence resulted in new infant formula products been developed,
2. If there were any differences in study and retailed infant formula,
3. The frequency of conducting research using probiotics, prebiotics or synbiotics containing infant formula

Publication of results:

4. If the infant formula companies had intentionally NOT published study results that were viewed as negative or having no clinical benefit to infants?
5. If study results perceived to be negative, were these withheld and was new research conducted to confirm the results?

Medium for consumer education:

6. The type and effectiveness of medium used to educate the consumer,
7. The presence of bias in promoting formula feeding more than breastfeeding.

Compliance to WHO guidelines:

8. How formula companies complied with WHO guidelines on formula preparation with a focus on high water temperature and its effects on probiotics, synbiotics containing infant formula?

Safety of long term use of probiotic or synbiotic containing infant formula,

9. How companies addressed safety, since there is a lack of published evidence on the clinical benefits of long term consumption of probiotic containing formula (longer than 1 year).

Product viability,

10. If the probiotic, synbiotic containing infant formula remain viable throughout storage or were there substantial changes in the number of colony forming units at the end of shelf life?

How companies keep abreast of the latest research on probiotics, prebiotics and synbiotics in infant formula and weaning foods?

11. If the formula companies had staff designated to keep track of research or was it on " *ad hoc*" basis?

Methodology

Study design

This was a descriptive study (a survey) employing the use of a structured questionnaire developed by the researcher.

Company selection

Companies that manufacture and / or market food products with added probiotics, prebiotics or both (synbiotics) for infants and children were identified through several databases such as EBSCOhost, Business Source Premier and DATAMONITOR[360]. In addition, company websites were visited to acquire the contact information of individual companies. The person / people responsible for research and development were invited to participate in the survey. Study participants included clinical research managers and individual researchers in the infant food companies. Worldwide, the numbers of infant food companies (especially infant formula manufactures) are few. Therefore all listed companies were invited to participate in the study. The number of study participants per company was one or two.

Data collection and processing

A letter of invitation was sent to selected participants, inviting them to take part in the study. The letter of invitation explained all aspects of the study, and if they expressed willingness to take part in the study, a questionnaire with a written consent form was sent via post, email or fax. If the questionnaire was posted, a stamped envelope was included for returning the completed questionnaire to the researcher. A maximum of four reminders were given to the participants to complete the questionnaire. The participants were free to withdraw from the study at any time without any consequence.

Due to the expected small sample size, maintaining anonymity of study participants with the corresponding company name was difficult. Therefore, data processing was done according to product and company name.

However, during report writing, all identifying details (name of study participant, product and company name) were excluded. Only the researcher and statistician had access to the data.

Questionnaire description

A questionnaire was designed for this study based on relevant published information. The questionnaire focused on product specific questions, research based questions, education of consumers and safety issues. It was validated for content by sending it to experts in the field of probiotics, prebiotics and infant nutrition, who were able to judge if the questionnaire met the objectives of the study. These experts did not partake in the study nor were they associated with the infant food industry.

Data analysis

Researchers planned to enter the collected data into SPSS (Statistical Program for Social Sciences) for analysis. The data was to be analysed using descriptive statistics and associations between categorical variables, be tested using a Chi-square test. A $p<0.05$ was considered statistically significant. A statistician was consulted at every step of the study process.

Ethics approval

Ethics approval to conduct this study was given by the Human Research Ethics Committee at the University of Stellenbosch, Faculty of Medicine and Health Sciences, reference number N11/07/203.

Results

A total of 25 major infant formula and baby food manufacturers were identified from around the world and invited to participate in the survey. (Table 1) A total of 5 (20%) companies initially agreed to participate but took no action by not signing the informed consent form and completing the questionnaire. The decision to participate in the study was delayed indefinitely for 2 (8%) companies since their head of department was too busy to make a final decision. Sensitivity that the requested information would give the competition an

advantage was cited by 3 (12%) companies for not participating in the study, while 1 (4%) company stated they manufacture baby food and distribute it for retail without conducting any clinical trials. A total of 4 (16%) companies declined to participate without giving any further information. Erroneous contact information given on company websites hindered any contacted being made with 6 (24%) companies. Company representatives from 3 (12%) companies refused to forward telephone calls from the researchers to the appropriate department and staff. Three (12%) companies cited language barrier (Mandarin, German, Dutch) as a reason for not participating in the study, despite offers to professionally translate the study documents into a language of their choice. One (4%) company stated that it was overwhelmed with people making requests for market research, as a result it had restructured and "market research was no-longer a priority." (Table 2) In the end no company was willing to participate in the survey.

Table 1. List of 25 baby food companies and infant formula manufacturers invited to participate in survey.

Company Name	**Company Name**
Abbott Laboratories / Abbot Nutrition	Milupa
Aspen Phamarcare	Morinaga Milk industry Co. Ltd
Beech-nut nutrition corporation	Nestle (South Africa and Switzerland)
Danone baby and medical nutrition BV	Organix brands
Earth's Best (Hain Celestian Group)	Pfizer Inc (SA) and Pfizer Head office
FrieslandCampina (Netherlands)	Raptakos Brett & Co. Ltd.
Gerber products company	SMA Nutrition (Ireland and UK)
Hangzhou Beingmate Group Co Ltd.	Synutra International
HiPP GmbH & Co Vertrieb KG	Tiger brands
JH J Heinz	Wakodo Co. Ltd
Kewpie	Wockhardt Limited
Mead Johnson	Hero AG
Meiji Dairies	

Table 2. Reasons for not participating in survey.

Reason(s) for not participating in survey	N = 25 **Number of companies n (%)**
No Action taken by company after agreeing to participate in survey	5 (20%)
Head of department too busy to make decision	2 (8%)
Requested information too sensitive - may give competition an advantage	3 (12%)
Company does not conduct clinical trials, just manufacture infant food, distribute it for retail	1 (4%)
No reason given for declining to participate in survey	4 (16%)
Researchers unable to make contact with company through use of internet (emails, "contact us" features in company websites), telephone, fax or post office.	6 (24%)
Company receptionist / contact person refuses to forward call / put researchers in touch with appropriate person to answer questions	3 (12%)
Quote: "Too many people conducting market research on company, company has other priorities than answering market research questions."	1 (4%)
Language barrier – "prefer questionnaire in local dialect" such as Mandarin, Dutch, German.	3 (12%)
Note: Several companies gave more than one reason for not participating in survey	

Discussion

To our knowledge, this was the first study to explore how the food industry applies evidence gained through research on probiotics, prebiotics and synbiotics on infants for the benefit of the general population. As a direct result of the poor response rate in this survey, several key questions remain unanswered. These are discussed below.

Application of evidence

Despite more than 30 years of research on probiotics, prebiotics or synbiotics on infants and children, any differences between studied and retailed infant formula such as the strains of probiotic bacteria used could not be established. It remains unknown if new evidence from clinical trials led to the improvement of existing formula, development of new infant formula or weaning foods containing probiotics or synbiotics.

Publication of results (Publication bias)

Publication bias is defined as "the tendency for investigators, journal editors and reviewers to submit or accept a manuscript for publication based on the directions or strength of the study findings. [50] Publication bias can have far reaching consequences on the public. For example, if an intervention that is not effective is falsely considered effective and administered to patients, an effective treatment that is available is withheld. Not publishing results from research where the intervention is discovered to be harmful; may indirectly harm study participants taking part in future research. This is because other investigators will (unknowingly) repeat the same research, testing the harmful intervention, causing suffering on a different group of people. [50] This study was not able to establish if companies engaged in research had intentionally NOT published study results that were viewed as negative or not having any clinical benefits to infants and children. Whether companies conducted new research to confirm results that may have been perceived as negative could not be established.

Medium for consumer education

The type and effectiveness of medium used to educate the consumer on probiotics, prebiotics or synbiotics containing formula or baby foods could not be established. The numerous techniques used by the formula and baby food industry to increase awareness of their products are beyond the scope of this study and are described elsewhere. Only one education and promotion technique is illustrated below.

Internet

The internet is an important source of health information for parents. [51, 52, 53] Company websites offer advice on infant feeding, child rearing and health care issues. Some websites have useful product information, most websites use information on breastfeeding to jump to the second best option; formula feeding. [3]

Most websites of formula manufacturers have product specific content concerning infant formula brands. Websites present images of branded packs linked with information about specific infant formula. These website links are accessible to the public, health and medical professionals. Research has shown consumers (mothers) get confused with formula advertising. [51] In situations where infant formula and follow-on formula share brand identities, consumers recall advertising and messages for follow-on formula and think it also applies to infant formula. As a result, information and promotional messages designed around follow-on formula are transferred to infant formula products. This type of confusion has far reaching implications. [51]

Navigating the websites of the 25 companies invited to participate in this study, in addition to the product specific content in the websites, only eight companies had brief descriptions of probiotics or prebiotics, five companies had health claims on probiotics and one company had a health claim on prebiotics. There was no mention of the strains of probiotics or type of prebiotics in their products. In addition, the information on probiotics and prebiotics was difficult to obtain from the websites and could be inaccessible to consumers without advanced computer skills, tertiary education or sufficient knowledge on what to look for.

In South Africa (SA), formula companies are able to market probiotics, prebiotics or synbiotics supplemented formula without providing evidence for health claims. Furthermore, South Africa's

"Regulations Relating to Foodstuffs for Infants and Young Children, Government Gazette number 35941"prohibit health or nutritional claims on the formula labels. The government gazette strictly prohibits the distribution of any information or education material on nutrition or feeding of infants and young children that promotes produsts such as infant formula or follow-on formula. However, for formula for infants with specific medical conditions, the government gazette does make provision for information leaflets (in 5 official languages) to be inserted inside the label. (54)

The SA government gazette could be amended to allow for distribution of educational material on feeding of infants and young children. For example, the SA Government gazette would be changed to allow the formula companies to provide information leaflets inside the labels of all formula types including probiotics, prebiotics or synbiotics supplemented formula. For all health claims, supporting evidence would be included in the information leaflets (in 5 or more official languages). These information leaflets would be strictly regulated to offer only relevant information and not promote any products, brands or formula feeding.

Compliance to WHO guidelines

The position of formula companies on how they comply with WHO guidelines on water temperature during formula preparation could not be established. WHO recommends diluting the powdered formula in water at a temperature of at least 70^0 C to inactivate *cronobacter spp* (*Enterobacter sakazakii*). [48] South Africa's "Regulations Relating to Foodstuffs for Infants and Young Children, Government Gazette number 35941" state that labels for any infant formula, follow-up formula must "provide instructions for appropriate use according to the latest FAO / WHO guidelines." The gazette requires the labels to state that infant formula is not always sterile and may contain harmful microorganisms, emphasizing appropriate preparation. [54] Yet the labels of infant formula found in retail stores of Western Cape, South Africa do not recommend to use water above 40^0C.

The European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) committee on nutrition and French Food Safety Agency (AFSSA) disagree with WHO guidelines and state that heating water to temperatures greater than 70^0C is not necessary and maybe harmful to the nutritional quality of formula. Using hot water (greater than 70^0C) may lead to formation of curds, risk of severe burns and the

loss of 10 to 25% of some nutrients: Thiamine, Vitamins B1, B6, B12, Folic acid, and Vitamin C. [55, 56] The effect of water temperature on *Cronobacter spp* (*Enterobacter sakazakii*) is striking. At 37 to 39^{0}C, there is optimal growth, at 5.5 to 8^{0}C there is minimal growth. At room temperature, *Cronobacter spp* has the potential for rapid growth. [55, 56] It is worth noting the rate of contamination with *Cronobacter spp* has decreased over the years from 14% in 1980s to 2.4% in mid 2000s. [55, 56, 57]

A study by Sani et al found preparation instructions on formula labels were "insufficient, ambiguous or difficult to follow." The study concluded that formula labels could be improved to cater for special consumer groups such as the less educated and the preparation instructions must be consistent with WHO guidelines. [58]

Safety of long term use of probiotics or synbiotics containing formula

The way companies address the question on safety of long term consumption of probiotics, synbiotics of infant formula could not be established. Safety of long term use is an important issue since majority of consumers of probiotics, synbiotics containing formula and baby foods use these products for more than a year. According to ESPGHAN committee on nutrition, there is a lack of published evidence on the clinical benefits and safety from long term consumption of probiotic containing formula. [26, 49] How the formula and baby food companies educate the consumer on this issue is yet to be determined or observed.

Product viability

Whether bacteria in retailed probiotics or synbiotics containing infant formula remain viable throughout shelf life was not established in this study. There are few reports on the stability of probiotics in powdered formula for infants and toddlers. [59] Several studies have conducted long term stability tests on bifidobacteria in powdered formula and results show the viability of live bacteria (such as bifidobacteria) decreased with length of time in storage and with increase in temperature. [59, 60] Consumers usually store powdered formula at room temperature. However, the formula may be exposed to high temperatures during transportation, during hot seasons or, in countries with hot weather conditions. If there is a large reduction in

viable cell counts of probiotic bacteria, the consumer does not benefit from the expected probiotic effects due to the insufficient number of viable cells in the infant formula. [59] The change in stability at various storage temperatures should be made clear by formula manufacturers.

How companies keep abreast of the latest research on probiotics, prebiotics and synbiotics in infant formula and weaning foods.

How companies keep abreast of the latest research on probiotics, prebiotics and synbiotics in infants could not be established. This study tried to find out if there are any formal mechanisms in place to ensure that employees or researchers keep abreast of the latest research. That is, are the employees or researchers offered regular training programs, workshops, symposiums, refresher courses, lectures, conferences or other methods for them to stay abreast of the latest research on probiotics, prebiotics and synbiotics in infant formula? The formula and baby food industry needs to be more open on this issue.

Limitations

Sampling frame

Only online electronic databases were used to identify the companies around the world that manufacture infant food products with probiotics, prebiotics or synbiotics. Small regional companies that were not listed in the electronic databases were missed and subsequently not invited to participate in the study. Different methods could have been used to identify small regional companies. For example: contacting regional chambers of commerce (such as European Union Chamber of Commerce, American Chamber of Commerce, All-China Federation of Industry and Commerce or International Chamber of Commerce Netherlands) would provide the contact details of small companies that manufacture infant formula. Another method would to browse unknown small business databases such as Business Monitor international. Use of business listings such as local directories (example white or yellow pages, phone books) would have been unfeasible.

Selection bias (under-coverage bias)

Efforts were concentrated on inviting people responsible for research and development such as clinical research managers and individual researchers. Other staff such as product managers could have been invited to participate in the study.

Survey participation rates,

Survey participation rates were nil. Many company staffs were cautious after the initial contact and invitation to participate in the study. After continued dialogue, they were unwilling to participate in the survey. During telephone conversations with the some company employees, the researchers were perceived to be in collaboration with the competition.

Conclusion

Due to a total lack of response from the formula companies, no conclusion could be drawn on how the food industry applies evidence gained through probiotics, prebiotics or synbiotics research on infants and children for the benefit of the general paediatric population. More information with greater transparency is needed from the infant formula and baby food companies on how they apply the evidence gained from the extensive research conducted using probiotics, prebiotics and synbiotics on infants and children. Legislation (in each country that the formula companies operate in) and recommendations from international bodies such as WHO, ESPGHAN, must be introduced to compel the industry to be transparent on how they apply the evidence gained from research.

List of abbreviations

AFSSA: French Food Safety Agency

^{0}C: Degrees centigrade

DHA: Docosahexaenoic acid

ESPGHAN: European Society of Pediatric Gastroenterology, Hepatology and Nutrition

FOS: Fructooligosaccharide

GOS: Galactooligosaccharide

SPSS: Statistical Program for Social Sciences

WHO: World Health Organisation

Competing interests

All authors declared no competing interests.

Authors' contributions

The authors contributed the following:

MM: Developed study protocol (unpublished), contacted companies for information, processed data, developed, edited and critically reviewed the manuscript.

TY: Assisted in designing the study and critically reviewed the manuscript.

RB: Assisted in designing the study, gave further information to companies when contacted and critically reviewed the manuscript.

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Tables and captions

Table 1. List of 25 baby food companies and infant formula manufacturers invited to participate in survey.

Table 2. Reasons for not participating in survey.

Chapter 6: Conclusion and recommendations

6.0 Conclusion and recommendations

6.1 Summary of findings

6.1.1 Probiotics, prebiotics infant formula use in preterm or low birth weight infants: a systematic review

Objective of review was: To assess if addition of probiotics or prebiotics to preterm infant formula led to improved growth and clinical outcomes in preterm or low birth weight infants.

Hypothesis for this review was: Consumption of probiotics, prebiotics by preterm infants leads to improved clinical outcomes.

Probiotics:
The four included probiotic studies had short treatment duration of 30 days, with small sample sizes ranging from 20 to 87 study participants, a total of 212 participants. All studies used different live probiotics at various doses. There was information missing on the methodological quality (risk of bias) domains including sequence generation, allocation concealment, blinding, incomplete outcome data, selective reporting and other biases. For preterm infants, addition of probiotics to formula had no significant effect on weight, linear and head growth, amount of feed volume (ml/day) and frequency of vomiting. Probiotic supplementation failed to significantly reduce the risk of complications, such as NEC, sepsis and death. Preterm infant formula with probiotics was well tolerated as no gastric aspirates, abdominal distension or diarrhoea were reported. Probiotic effects on stool characteristics were under-reported. Outcomes, such as number of days on parenteral nutrition and other infections were not reported. Effects on intestinal permeability could not be evaluated because two different laboratory tests were reported and their results could not be pooled. Outcomes, such as age at full enteral feeds and intestinal micro flora (pathogens) could not be evaluated as medians (inter quartile ranges) were reported. There was no data on low birth weight infants, therefore no conclusions could be made on this population.

Prebiotics:
The four included prebiotic studies also had short treatment duration, ranging from 15 to 28 days, with small sample sizes ranging from 20 to 56, a total of 126 study participants only. The doses of the prebiotics used (GOS, FOS) varied from 0.4 g/dl to 1g/dl. Addition of GOS /FOS or FOS alone to preterm infant formula did not have any significant effect on weight gain or head growth. Addition of GOS / FOS to preterm infant formula did not have any effect on linear growth but addition of FOS alone did have a significant effect on linear growth. There was no significant effect on the age at which infants reached full enteral feeds, or volume of feed tolerated. There were no reports of vomiting, gastric aspirates, abdominal distension or

diarrhoea reported, showing that prebiotic preterm formula was well tolerated. Prebiotic supplementation did result in a higher stooling frequency; effects on stool consistency were inconclusive. There was an effect on intestinal micro flora as evidenced by significant increases of bifidobacteria counts, however effects on lactobacillus levels were not clear as actual figures were unavailable. The sum of studied pathogens and some selected pathogens (E- coli, enterococci) were significantly fewer in the prebiotic group. No prebiotic study reported any data on low birth weight infants, therefore no evaluations could be made on this group. Prebiotic effects on NEC, sepsis, other infections, mortality (death), parenteral nutrition, or changes in intestinal permeability were not reported; therefore these outcomes could not be evaluated.

6.1.2 Synbiotics, probiotics or prebiotics in infant formula for full term infants: a systematic review

Objectives of the review were:
1. To determine the effects of infant formula containing synbiotics, probiotics, or prebiotics on clinical outcomes in full term infants

2. To explore if synbiotics are superior over probiotics or prebiotics.

Hypothesis for this review was: Consumption of probiotics, prebiotics or both (synbiotics) by full term infants leads to improved clinical outcomes

Synbiotics:
The three synbiotic studies had: a short treatment duration, ranging from 4 to 6 months; a small sample size, ranging from 122 to 227 study participants; a total of 475; and treatment (combinations of probiotics and prebiotics) also varied among the studies. Synbiotic addition to infant formula did not have any significant effect on growth (weight gain, length and head circumference), or on the incidence and frequency of colic, spitting up / regurgitation, crying, restlessness, or vomiting. There was increased stool frequency but effects on stool consistency were inconclusive. Synbiotic effects on frequency of infections and antibiotic intake were also inconclusive. Several outcomes were not reported, including volume of formula tolerated, hospitalization, GI microflora and immune response.

Probiotics:
The 10 included probiotic studies had: a short treatment duration, ranging from 14 days to 7 months; small sample sizes, ranging from 54 to 142 study participants; and a total of 933 participants. All 10 probiotic studies used various strains with different doses of bifidobacteria and lactobacillus. A limited number of studies analysed the effects of probiotic supplementation on growth by gender. Probiotics did not have any significant effect on growth (weight gain, length gain or head circumference) in boys or girls. No study reported any weight loss. Probiotics did not have any significant effect on stool frequency or consistency,

episodes of diarrhoea, incidence of colic, spitting up / regurgitation, restlessness, or vomiting. Effects on infections, antibiotic use and length of hospitalization were inconclusive. There was a significant effect on volume of formula intake, in the probiotic group. Effects on intestinal microflora were conflicting as there was no increase either in bifidobacteria or lactobacillus counts.. Probiotics significantly reduced enterobacteria counts but failed to reduce bacteriode counts. Probiotic effects on immune response (CRP, IL-6) were not reported.

Prebiotics:
The 12 included prebiotic studies had a short treatment duration, ranging from 28 days to 12 months; small sample sizes, ranging from 32 to 206 study participants, with a total of 1563 participants. The prebiotic doses ranged from 0.15 g to 0.8 g/100 ml. Addition of prebiotics to infant formula did have a significant effect on weight gain but had no effect on length gain, or head circumference. None of the studies reported any weight loss. There was an increase in stool frequency but no improvement in stool consistency. Prebiotic supplementation did not reduce incidences of diarrhoea, spitting up / regurgitation, vomiting / crying, or increasing volume of formula tolerated. There was no significant reduction in upper respiratory infections, however there was a significant reduction in overall infections and antibiotic intake. Prebiotics supplementation did not increase counts of bifidobacteria, lactobacillus, or decrease pathogen levels (enterobacteria, bacteriodes, E – coli). Outcomes not reported in any study included colic, hospitalization (days in hospital) and immune response (CRP, IL-6).

6.1.3 Associations among funding source, methodological quality and research outcomes in randomized controlled trials of synbiotics, probiotics and prebiotics added to infant formula: A Systematic Review

Objective of review was: To compare the methodological quality and outcomes of industry sponsored trials versus non- industry sponsored trials, with regard to synbiotics, probiotics and prebiotics supplementation in infant formula.

Hypotheses for this review were: The source of funding in research trials using probiotics, prebiotics or synbiotics supplemented formula in infants is associated with outcomes in favour of the sponsor's products and authors' conclusions. Methodological qualities of non-industry sponsored trials are equivalen to industry sponsored trials.

Sixty seven published RCTs were included: 45 (67.2 %) on full term infants; 22 (32.8%) on preterm infants. Of these, 40 (59.7%) were funded by food industry; 11 (16.4%) were funded by non-industry; and 16 (23.9%) did not specify funding source.

Methodological quality: For risk of bias several domains were inadequately reported; particularly, the domains of sequence generation (37.3%), allocation concealment (52.2%) and blinding (53.7%). Most RCTs had a low risk of bias in the domains of incomplete outcome data, selective reporting and other biases. There was no significant association among funding source and the domains of sequence generation, allocation concealment, blinding and selective reporting. There was a significant association among funding and the domains of incomplete outcome data and there were no other sources of bias such as RCT being stopped early, baseline imbalances of characteristics of study participants or inappropriate administration of intervention (that is deviating from the pre-specified methods).

Outcomes: In most RCTs, outcomes were assessed as neutral, that is, supplementation with synbiotics, probiotics or prebiotics did not have a significant effect. There was no significant association between source of funding and reporting of clinical outcomes, except antibiotic use in infants.

RCT conclusions: A total of 73.1% of RCTs had a positive overall study conclusion in favour of sponsor products. There was no significant association between sources of funding and overall study conclusion; and conclusions on reported clinical outcomes, except for weight gain.

6.1.4 Application of evidence on probiotics, prebiotics and synbiotics by food industry: A descriptive study

The research aim was to investigate how the infant food industry applies the evidence gained through synbiotics, probiotics and prebiotics research on infants.

The objectives were to determine the following: application of evidence on probiotics, prebiotics on infants by the food industry; publication of results; type and effectiveness of medium used for consumer education; compliance to WHO guidelines, regarding water temperature for formula preparation; safety issues, regarding long term use of probiotic or synbiotic infant formula; product viability following long term storage; and how companies keep abreast of the latest research.

Hypothesis for this study was: Evidence gathered through research trials that use probiotics, prebiotics and synbiotics in infants is implemented by the food industry for the benefit of the general paediatric population.

A total of 25 major infant formula and baby food manufacturers were identified and invited to participate in the survey. No company was willing to participate in the survey. As a result, none of the study objectives could be met. The hypothesis could not be tested.

The reasons for companies declining to participate in the survey were: No action by company (refusal to sign informed consent form or complete questionnaire); indecision by department heads, sensitivity of requested information; company does not conduct clinical trials; company declined with no further information; erroneous contact information on company websites; telephone calls not forwarded to key staff; language barriers; and market research no longer a company priority.

6.2 Limitations

Phase 1: Systematic reviews on preterm and full term infants; Phase 2: Systematic review on source of funding, methodological quality and research outcomes

6.2.1 Language bias

Accessing non - English language RCTs indexed in foreign language databases (such as Chinese Biomedical database, Chinese Medical Current Content database) was difficult, as the researcher is primarily English speaking. Similarly, accessing foreign language RCTs (such as RCTs in Spanish, Chinese, Dutch languages) not indexed in English language databases, such as PubMed and LILACS was also difficult. A number of RCTs, thus may have been unintentionally missed and not included in the systematic reviews. For the identified foreign language RCTs, (especially Japanese language RCTs), the lack of a knowledgeable translator further resulted in some RCTs being excluded, or poorly translated, using computer software; this may have compromised the study report content.

6.2.2 Selection bias

All efforts were made to locate unpublished RCTs from experts in the field, researchers, research institutions and companies conducting research using synbiotics, probiotics and prebiotics on infants and children. Only one company agreed to avail three unpublished RCTs. There were as a result, few unpublished RCTs in the systematic reviews. The meta analyses were based mainly on published RCTs. As a result, the effectiveness of an intervention may have been over estimated. Accessing more unpublished RCTs, may change the results and conclusions of the systematic reviews.

6.2.3 Inconclusive findings and failure to establish an association

In the RCTs included in the systematic reviews, many outcomes were not reported or data presentation was inappropriate for a meta-analysis. As a result, there were many inconclusive findings and the systematic reviews could not establish an association or effect of the intervention (probiotics, prebiotics or synbiotics). Examples include (in the review on preterm infants) no reporting on lactobacillus counts, stool consistency or intestinal permeability. In the review on full term infants, there was no reporting on effects on immune response or GI microflora.

6.2.4 Loss of power

In the phase 2 systematic review (on source of funding, methodological quality and research outcomes), majority of clinical outcomes were categorized as neutral; majority of the conclusions categorized as "no clear conclusion." This resulted in loss of information and the review losing power to detect significant associations between source of funding and clinical outcomes or study conclusions.

6.2.5 Type 1 and type 2 errors

Type 1 error occurs if a difference is concluded when no difference actually exists. There is a possibility that a type 1 error could have occurred in the preterm and full term systematic reviews. When multiple tests are done (as in our systematic reviews), the probability of type 1 errors (significance level) increases. One way of compensating for multiple tests is to divide the significance level by the number of tests. However, no adjustments for multiple tests were done in all our reviews.

Type 2 error occurs if a difference is not detected when a difference actually exists. In the systematic reviews, a type 2 error could not be avoided due to the following reasons. In the reviews on preterm and full term infants, for majority of outcomes analysed, the number and sample sizes of RCTs were too low to provide sufficient power to detect a difference even when the difference could actually have existed. In chapter 2, there is a distinct possibility that a true effect of probiotics on preventing NEC was missed. There were few RCTs reporting NEC and each had low power. The risk ratio was 0.42 ($p=0.09$) and if there were more RCTs, there could have been a significant effect

In the phase 2 review (on source of funding, methodological quality and research outcomes) a type 2 error could not be avoided as many outcomes and study conclusions were inconclusive. That is, they were categorized as "neutral outcome and no clear conclusion."

Phase 2: Descriptive study

6.2.6 Sampling Frame

Identification of manufacturers of infant food products with probiotics, prebiotics or synbiotics was done using online electronic databases only. Regional companies not listed in the electronic databases were missed and as a result, not invited to participate in the study. Different methods could have been used to identify small regional companies to participate in the survey.

6.2.7 Selection bias (under-coverage bias)

Efforts were concentrated on inviting people responsible for research and development, such as clinical research managers and individual researchers to participate in the survey. Other staff, such as product managers could have been invited to participate in the study.

6.3 Study conclusions

Phase 1

6.3.1 Probiotics, prebiotics in infant formula use in preterm or low birth weight infants: A systematic review

The evidence on the effects of probiotics or prebiotics on formula-fed, preterm infants was based on RCTs: of short duration, few in number, having small sample sizes; using different strains and doses of probiotics; or using similar prebiotics with different doses. For most clinical outcomes (except weight gain), only 2 to 3 RCTs reported or assessed any given outcome. The null hypothesis is accepted for majority of clinical outcomes. Therefore in formula fed preterm infants, there is not enough evidence at this time to state that supplementation of preterm infant formula with probiotics or prebiotics does result in improved growth and majority of clinical outcomes.

The null hypothesis is rejected for the outcomes of linear growth, bifidobacteria counts and stool frequency. There was enough evidence to support that supplementation of preterm infant formula with prebiotics FOS alone significantly increased linear growth and bifidobacteria counts. There was also enough evidence indicating that supplementation with a combination of GOS/ FOS or FOS alone significantly increased stool frequency.

6.3.2 Synbiotics, probiotics or prebiotics in infant formula for full term infants: A systematic review

The evidence on the effects of infant formula containing synbiotics on full term infants was based on only three studies, using different probiotic strains and doses; similar prebiotics with different doses; and different treatment duration. Similarly, the effects of probiotics or prebiotics on full term infants was based on RCTs with different probiotic strains, doses and duration; similar prebiotics with different doses; and different duration. Therefore in formula fed full term infants, there is not enough evidence at this time to state that supplementation of term infant formula with synbiotics, probiotics or prebiotics does result in improved growth and majority of clinical outcomes in full term infants. There was no data available to establish if synbiotics are superior to probiotics or prebiotics.For this study, the null hypothesis is accepted for majority of clinical outcomes except weight gain. Supplementation of term infant formula prebiotics significantly increased weight gain.

6.3.3 Association between source of funding, methodological quality and research outcomes:

The null hypothesis is **accepted** for methodological quality (in the domains of sequence generation, allocation concealment and blinding), majority of study outcomes and authors' conclusions (except for the outcome of antibiotic use and conclusion on weight gain). Therefore in RCTs on infants fed infant formula supplemented with synbiotics, probiotics or prebiotics, there was no significant association between source of funding and methodological quality of study in certain domains (sequence generation, allocation concealment and blinding). There was no significant association among funding source and most reported clinical outcomes and conclusions (overall study conclusion, conclusions on reported outcomes). Source of funding is not associated with methodological quality (in the domains of sequence generation, allocation concealment and blinding), majority of outcomes and authors' conclusions in favour of sponsor products.

The null hypothesis is **rejected** for methodological quality (in the domains of incomplete outcome data, and free of other bias), antibiotic use and conclusion on weight gain.
Therefore there was a significant association between source of funding and methodological quality (in the domains of incomplete outcome data, and free of other bias), outcome of antibiotic use, and conclusions on weight gain

6.3.4 Application of evidence on probiotics, prebiotics and synbiotics by food industry: A Descriptive study

The food industry was unwilling to participate in the descriptive study.The hypothesis, as a result was not tested; and the study objectives were not met. Therefore no conclusion could be drawn on how the food industry applies evidence gained through synbiotics, probiotics or prebiotics research on infants for the benefit of the general paediatric population. It is necessary for the food industry to be transparent on how it implements the evidence gained from probiotic, prebiotic or synbiotic research on infants for the benefit of the general paediatric population. Legislation (in each country that the formula companies operate in) and recommendations from international bodies such as WHO, ESPGHAN, must be introduced to compel the industry to be transparent with how they apply the evidence gained from research.

6.4 Overall thesis conclusion

The included studies had several limitations (small sample size, use of different strains of probiotics, different prebiotics and short-duration); a few studies could be combined in a meta-analysis or even in meta-analysis, there was not enough power to demonstrate an effect. Therefore at this time, there is not enough evidence to: state that consumption of probiotics, prebiotics or synbiotics leads to improved growth, or clinical outcomes in formula fed infants; or support the routine supplementation of infant formula with probiotics, prebiotics or synbiotics.

In RCTs using infant formula with probiotic, prebiotics or synbiotics in preterm, low birth weight and full term infants, the source of funding is not associated with outcomes and authors' conclusions in favour of sponsor products. It is necessary for the food industry to be transparent on how it applies the evidence gained from probiotic, prebiotic or synbiotic research on infants for the benefit of the general paediatric population.

6.5 Summary of contributions

New knowledge generated by this research project includes the following:

Phase 1

At this time, there is not enough evidence to state that:
1. Probiotics or prebiotics supplemented infant formula has a distinct advantage, or adverse effects compared to conventional infant formula, in strictly formula fed preterm infants.
2. Synbiotics, probiotics or prebiotics supplemented infant formula has a distinct advantage, or adverse effects compared to conventional infant formula, in strictly formula fed full term infants.
3. Synbiotics supplemented infant formula is superior to either probiotics or prebiotics containing infant formula.

Phase 2

1. In RCTs using synbiotics, probiotics or prebiotics supplemented infant formulas, the funding source has no impact over methodological RCT quality, study outcomes, or conclusions.

2. The food industry is unwilling to reveal how they implement the evidence gained from synbiotics, probiotics or prebiotics research in infants, for the benefit of the general paediatric population.

6.6 Implications for practice using synbiotics, probiotics or prebiotics supplemented infant formula

The limited evidence shows that synbiotics or probiotics supplemented infant formula did not have any adverse effects; significant impact on growth; or clinical outcomes in infants. All studies used different probiotic strains, the effects of one type of probiotic cannot be extrapolated to other types of probiotic bacteria. In addition, the limited evidence shows prebiotic supplemented infant formula did not result in any adverse effects on infants. There are some clinical benefits, such as improved weight gain and stool frequency. The limited evidence does not support the routine supplementation of infant formula with synbiotics, probiotics or prebiotics. However, the researcher acknowledges that in South Africa and many parts of the world, synbiotics, probiotics and prebiotics supplemented infant formulas are sold directly to the public in retail outlets.

6.7 Recommendations for further research

6.7.1 Research on preterm and low birth weight infants:

The evidence on probiotics and prebiotics use in preterm and low birth weight infants is based on RCTs having short treatment duration, small sample sizes and different interventions. Therefore, well designed, long term, large RCTs on exclusively formula fed preterm and low birth weight infants are required to:

1. investigate the effects of probiotics and prebiotics supplementation in preventing NEC, sepsis, death / mortality; changes in intestinal micro flora; and intestinal permeability
2. explore the effectiveness of different doses of the same probiotic on clinical outcomes, because available studies used different probiotic doses
3. explore the effectiveness of different doses of the same prebiotic on clinical outcomes, because available studies used similar prebiotics with different doses and treatment duration.

6.7.2 Research on full term infants

The evidence on synbiotics, probiotics and prebiotics use in full term infants is based on RCTs also having short treatment duration, small sample sizes and different interventions. Therefore, well designed, large RCTs with long term follow-up are required on exclusively formula fed term infants to investigate the following:

1. effects of the same synbiotic combinations on clinical outcomes
2. effects of the same probiotics (with similar doses and treatment duration) on clinical outcomes, because available studies used different probiotic doses and treatment durations
3. effects of the same prebiotics (with similar doses and treatment duration) on clinical outcomes, because available studies used similar prebiotics with different doses and treatment duration
4. effects of synbiotics, probiotics or prebiotics on clinical outcomes not adequately addressed in previous studies
5. synbiotics superiority to probiotics or prebiotics: RCTs should have treatment arms to include both synbiotics, probiotic and prebiotics.

6.7.3 Research exploring the associations among funding sources, methodological quality and research outcomes in RCTs, using synbiotics, probiotics or prebiotics supplemented formula in infants .

Most RCTs using supplemented formula in infants were industry funded. More non-industry funded research is needed to further assess funding impact on methodological quality (risk of bias); reported clinical outcomes; and conclusions (overall study conclusion, conclusions on reported outcomes) in RCTs, using synbiotics, probiotics or prebiotics supplemented formula in infants.

6.7.4 Research on the implementation of evidence on synbiotics, probiotics and prebiotics by food industry:

Greater transparency is needed from the infant formula and baby food companies on how they apply the evidence gained from the extensive research they have conducted (from 1980 to 2012), using probiotics, prebiotics and synbiotics in infants.

Appendix 1: Ethics approval

UNIVERSITEIT·STELLENBOSCH·UNIVERSITY
jou kennisvennoot · your knowledge partner

08 February 2011

MAILED

Mrs M Mugambi
Department of Human Nutrition
3rd Floor
Clinical Building

Dear Mrs Mugambi

Probiotics, Prebiotics and Synbiotics in Neonates: A critical appraisal of the evidence and evaluation of its application by the food industry.

ETHICS REFERENCE NO: N11/02/035

RE : ETHICAL REVIEW NOT REQUIRED

According to the protocol all data to be collected for the systematic review will be from the public domain. In light of this, the cluster head for Research Ethics has considered this proposal to be exempt from ethical review.

This letter confirms that this project is now registered and you can proceed with the work

Yours faithfully

MS CARLI SAGER
RESEARCH DEVELOPMENT AND SUPPORT
Tel: +27 21 938 9140 / E-mail: carlis@sun.ac.za
Fax: +27 21 931 3352

08 February 2011 14:48

Fakulteit Gesondheidswetenskappe · Faculty of Health Sciences

Verbind tot Optimale Gesondheid · Committed to Optimal Health
Afdeling Navorsingsontwikkeling en -steun · Division of Research Development and Support
Posbus/PO Box 19063 · Tygerberg 7505 · Suid-Afrika/South Africa
Tel.: +27 21 938 9075 · Faksi/Fax +27 21 931 3352

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jou kennisvennoot · your knowledge partner

05 August 2011 **MAILED**

Mrs M Mugambi
Department of Human Nutrition
3rd Floor
Clinical Building

Dear Mrs Mugambi

An evaluation of the application of evidence on probiotics, prebiotics and synbiotics by the food industry.

ETHICS REFERENCE NO: N11/07/203

RE : APPROVAL WITH STIPULATIONS

It is a pleasure to inform you that the Health Research Ethics Committee has approved the above-mentioned project with STIPULATIONS at a meeting on 3 August 2011, including the ethical aspects involved, for a period of one year from this date.

1. Please remove the employees name from the questionnaire.

This project is therefore now registered and you can proceed with the work. Please quote the above-mentioned project number in ALL future correspondence.

Please note a template of the progress report is obtainable on www.sun.ac.za/rds/ and should be submitted to the Committee before the year has expired. The Committee will then consider the continuation of the project for a further year (if necessary). Annually a number of projects may be selected randomly and subjected to an external audit.

Translations of the consent document in the languages applicable to the study participants should be submitted.

Federal Wide Assurance Number: 00001372
Institutional Review Board (IRB) Number: IRB0005239

The Health Research Ethics Committee complies with the SA National Health Act No.61 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 Part 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes 2004 (Department of Health).

Please note that for research at a primary or secondary healthcare facility permission must still be obtained from the relevant authorities (Western Cape Department of Health and/or City Health) to conduct the research as stated in the protocol. Contact persons are Ms Claudette Abrahams at Western Cape Department of Health (healthres@pgwc.gov.za Tel: +27 21 483 9907) and Dr Hélène Visser at City Health (Helene.Visser@capetown.gov.za Tel: +27 21 400 3981). Research that will be conducted at any tertiary academic institution requires approval from the relevant hospital manager. Ethics approval is required BEFORE approval can be obtained from these health authorities.

Approval Date: 3 August 2011 Expiry Date: 3 August 2012

Fakulteit Gesondheidswetenskappe · Faculty of Health Sciences

Verbind tot Optimale Gesondheid · Committed to Optimal Health
Afdeling Navorsingsontwikkeling en -steun · Division of Research Development and Support
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Appendix 2: Study eligibility form (Preterm infants)

Probiotics, prebiotics infant formula use in preterm or low birth weight infants: A systematic review

STUDY ELIGIBILITY FORM

Review title:	**Probiotic, prebiotics infant formula use in preterm or low birth weight infants: A systematic review**
Study ID (Author last name, initials)	
Refworks ID number	
Date of review for eligibility (DD-MM-YYYY)	
Journal title	
Title of study/article	
Year/volume/issue/page	
Extractor (Last name, initials)	

Type of study	**Put a check (√) mark in appropriate box.**		
Is this study a Randomized controlled study?	YES	UNCLEAR	NO
	↓	↓	↓
	Go to next question		**Exclude**
Trial intervention			
Preterm infant formula containing probiotic(s)	YES	UNCLEAR	NO
Preterm infant formula containing prebiotic(s)	YES	UNCLEAR	NO
Conventional preterm formula / placebo	↓	↓	↓
	Go to next question		**Exclude**
Study Participants			
Premature infants <37 weeks gestation	YES	UNCLEAR	NO
Low birth weight infants ≤ 2.5 kg at birth	YES	UNCLEAR	NO
	↓	↓	↓
	Go to next question		**Exclude**
Study Outcomes (≥1 outcomes below)			
Short term growth parameters (Wt, Ht, Hd Circum)	YES	UNCLEAR	NO
Adverse events (# days on parenteral, full enteral nutrition, maximal enteral feed, vomiting, GI aspirates, abdomen distension, stool characteristics- consistency, frequency)	YES	UNCLEAR	NO
Complications (NEC, Sepsis, other infection, death)	YES	UNCLEAR	NO
Intestinal permeability	YES	UNCLEAR	NO
GI Microflora (Bifidobacteria, Lactobacillus, pathogen cfu)	YES	UNCLEAR	NO
	↓	↓	↓
		Clarify missing information	
Other reasons for excluding study	**NO**	↔	**Yes**
	↓	↓	↓
Final decision	**Include**	**Unclear**	**Exclude**
		↓	
		For discussion	

Comments

Appendix 3: Data extraction form (Preterm infants)

Probiotics, prebiotics infant formula use in preterm or low birth weight infants: A systematic review

Data Extraction Form

Review Ttitle: Probiotic, prebiotic formula versus conventional preterm formula for feeding preterm or low birth weight infants.

Section 1

Study ID (Author last name, initials) _____ Extractor ID (Last name, Initials) _____

Refworks ID _____ Date of reviewing eligibility (dd/mm/yyyy) _____

Source ID (e.g PMID: 19707025 or database name) _____

Author(s) [Last name, initials]_____

Title of study: _____

_____

Journal name, yyyy/vol/iss/page: _____

_____

Language of publication _____ Translation Needed? YES____ NO_____

Date of trial (dd/mm/yyyy) _____ Duration of trial _____

Ethics approval: NO___ Unclear____YES ____ If yes Who granted ethics approval_____

Informed consent: NO____ YES _____if yes, consent was: Oral ____ Written _____ Not clear _____

Location of study (country) _____ Sponsor _____

Section 2

Eligibility Criteria

Does this study meet the following eligibility criteria?

Type of study: Randomized Controlled trial	YES	NO	Not Clear
Study participants: Preterm infants ≤ 37 weeks gestation Low Birth weight infants ≤ 2.5 kg at birth	YES	NO	Not Clear
Intervention: Preterm infant formula containing probiotic(s) Preterm infant formula containing probiotic(s) Conventional preterm formula or placebo	YES	NO	Not Clear
Outcomes: Does this study have any of these outcomes? Growth parameters, adverse events, complications (NEC,infections), Changes in GI permeability, GI microflora	YES	NO	Not Clear

Include or exclude this study?

Include this study?	Yes	NO
Exclude this study ?	Yes	NO
Reasons fro excluding		

Methodological design

Sequence generation / Randomization method			
	YES	NO	Unclear
Allocation concealment			
Adequate: • Central allocation (including telephone, web based, pharmacy randomization) • Onsite computer systems which can be assessed after entering the characteristics of an enrolled participant. • Precoded or sequentially numbered containers identical in appearnce. • Sequentially numbered, opaque, sealed envelopes.	YES	NO	
Inadequate: • Date of birth, case record number • Open random allocation schedule (list of random numbers) • Unsealed, non opaque envelopes • Alternation or rotation	YES	NO	
Unclear • Sealed envelopes but not sequentially numbered, opaque • Randomization stated but no details given • Insufficient informationto permit judgement of YES or NO	YES	NO	
Not reported	YES	NO	

Blinding	YES	NO	Unclear
Who was blinded? (Participant, care giver, asssessors)			

Loss to Follow –up (attrition Bias)	Adequate	Unclear	Inadequate	Not used

Intervention

N= _____	Arm 1 _____ n= ___	Arm 2 _____ n=____	Arm 3 _____ n=____	**Comments**
Name / Strain of probiotic(s)				
DOSE: Probiotics only Number of Viable cells (cfu) / _____.				
Name of prebiotic(s)				
DOSE: Prebiotics only g/__ml				
Name of Placebo				

Intervention continued

N=	Arm 1 n= ____ _____	Arm 2 n=_____ _____	Arm 3 n=_____ _____	Comments
Volume of feed given (mls/24 hours)				
Frequency of administration per 24 hours				
Treatment Duration				
Time points				
Specify time points used for measurements. (e.g Day 1,2,3 or week 1,2,3 or Month 1,2,3.				
Withdrawl / losses				
Reasons for withdraw/ losses				
1				
2				
3				
4				
6				
5				
6				
7				

Outcomes

Description of Outcomes

Primary Outcomes
Secondary Outcomes

Description of Outcomes continued

Adverse events due to treatment. Definition of Adverse event.

Section 3

Results

	N	%	Unclear	
Number recruited		Number randomized		
	Arm 1 n= ____ _____	**Arm 2 n=_____ _____**	**Arm 3 n=_____ _____**	***Comments***
Total Number				
Male				
Female				
Gestation Age Unit of measurement:				

1. Primary Results

N=					
Growth Parameters	**Arm 1 n= ____ _____**	**Arm 2 n=_____ _____**	**Arm 3 n=_____ _____**	P value	Statistical test used / Comments
Weight gain Unit of measurment ___					
Linear growth Unit of measurment ___					
Head growth Unit of measurment ___					

Secondary Results

2. Adverse events

Feed intolerance	**Arm 1 n=___ _____**	**Arm 2 n=___ _____**	**Arm 3 n=____ _____**	P value	95% CI	Statistical test used/ Comments
Gastric aspirate (mls/day)						

Adverse events continued

N=	Arm 1 n= ___ _____	Arm 2 n=____ _____	Arm 3 n=____ _____	P value	95% CI	Statistical test used / Comments
Colour of aspirate (Green, milky, clear)						
Abdominal distention						
Number of days on Parenteral nutrition						
Number of days to full enteral nutrition						
Stool characteristics (Hard, firm, loose, watery)						
Code for Hard, firm,lose, watery stool						
Stooling Frequency / evacuations						
Other adverse events						

3. Complications

N=	Arm 1 n= ___ _____	Arm 2 n=____ _____	Arm 3 n=____ _____	P value	95% CI	Statistical test used / Comments
Necrotizing Enterocolitis (NEC)						
Sepsis						
Mortality / Death						

Complications continued.

Other complications continued	Arm 1 n= ___ _____	Arm 2 n=____ _____	Arm 3 n=____ _____	P value	95% CI	Statistical test used / Comments

4. Changes in intestinal permiability

N= L/M ratio	Arm 1 n= ___ _____	Arm 2 n=____ _____	Arm 3 n=____ _____	P value	Statistical test used / Comments

5. Changes in Gastrointestinal Microflora

N=	Day/Week 1			Day / Week 2				
List of bacteria	Arm 1 n= ___ _____	Arm 2 n=___ _____	Arm 3 n=___ _____	Arm 1 n= ___ _____	Arm 2 n=___ _____	Arm 3 n=___ _____	P Value	Statistical test used / Comments
	cfu/g of feces			cfu/g of feces				
1								
2								
3								
4								
Statistical test used								

Changes in Gastrointestinal Microflora continued.

N=	Day/Week 3			Day / Week 4				
List of bacteria	Arm 1 n= ___ _____	Arm 2 n=___ _____	Arm 3 n=___ _____	Arm 1 n= ___ _____	Arm 2 n=___ _____	Arm 3 n=___ _____	P Value	Statistical test used / Comments
	cfu/g of feces			cfu/g of feces				
1								
2								
3								
4								
5								
6								
7								
8								
Statistical test used								

Section 4

Author's contact: Telephone_____ Cell Phone _____ Fax _____

Email _____

Address:_____

_____

Correspondence with Author

Additional information needed from Author

_____

_____

_____

Information received from Author: _____

_____

Section 5

Information from Reference list.

Reference scaned Yes _____ NO _____

Additional studies identified from reference list.

1._____

_____

_____ Sent to Refworks Yes_____NO_____

2._____

_____

_____ Sent to Refworks Yes_____NO_____

3._____

_____

_____ Sent to Refworks Yes_____NO_____

4._____

_____

_____ Sent to Refworks Yes_____NO_____

5._____

_____

_____ Sent to Refworks Yes_____NO_____

6._____

_____

_____ Sent to Refworks Yes_____NO_____

7._____

_____

_____ Sent to Refworks Yes_____NO_____

Appendix 4: Study eligibility form (Full term infants)

Synbiotics, probiotics or prebiotics in infant formula for full term infants: A systematic review

STUDY ELIGIBILITY FORM

Review title:	**Effects of probiotic, prebiotic and, synbiotic containing infant formula on clinical outcomes in term infants**
Study ID (Author last name, initials)	
Refworks ID number	
Date of review for eligibility (DDMMYYYY):	
Title of study/article	
Journal title	
Year/volume/issue/page	
Extractor (Last name, initials)	

Type of study	**Put a check (√) mark in appropriate box.**			
Is this study a Randomized controlled study?	YES	UNCLEAR	NO	
	↓	↓	↓	
	Go to next question		**Exclude**	**Intervention used in study:**
Trial intervention				Circle below
Experimental group: Term infant formula containing either probiotic(s) or prebiotic(s) or synbiotic(s)	YES	UNCLEAR	NO	Probiotic
Control group: Conventional term formula with or without placebo	YES	UNCLEAR	NO	Prebiotic
Note: All formulas are Cow based milk formula, no soy based formula. Breastmilk when used as reference only	YES	UNCLEAR	NO	Synbiotic
	↓	↓	↓	
Study Participants	Go to next question		**Exclude**	
Healthy Full term infants (≥37 weeks gestation or ≥ 2.5 kg birth weight), Age: 0-12 months. Strictly formula fed infants only.	YES	UNCLEAR	NO	
Exclude: If infants have Congenital malformations, chromosomal abnormalities. Breastfed infants (unless they are used as a reference group)	**NO**	UNCLEAR	**YES**	
	↓	↓	↓	
Study Outcomes. Does the study have one or more of the following outcomes:	Go to next question		**Exclude**	
Growth parameters: (weight, Height/length, Head Circum)	YES	UNCLEAR	NO	
Tolerance to feed: stool pattern (frequency, consistency), vomiting, diarrhoea, volume of feed tolerated	YES	UNCLEAR	NO	
Infections: frequency, type, use of meds	YES	UNCLEAR	NO	
Immune respone: CRP, IL-6, other immune system parameters	YES	UNCLEAR	NO	
Stool Microbiology: levels of bifidobacteria, lactobacilli, pathogens	YES	UNCLEAR	NO	
Hospitalization: # days in hospital, ICU (if any)	YES	UNCLEAR	NO	
	↓	**Clarify missing information**	↓	
Other reasons for excluding study	**NO**	↔	**Yes**	
	↓	↓	↓	
Final decision	**Include**	**Unclear**	**Exclude**	
		↓		
		For discussion		

Comments

Appendix 5: Data extraction form (Full term infants)

Synbiotics, probiotics or prebiotics in infant formula for full term infants: A systematic review

Data Extraction Form

Review Ttitle: Protective effects and tolerance of Synbiotic containing infant formula compared to probiotic, prebiotic infant formula.

Section 1

Study ID (1st Author last name, initials)_____ Extractor ID (Last name, Initials) _____

Refworks ID _____ Date of reviewing eligibility (dd/mm/yyyy) _____

Author(s) [Last name, initials]_____

Title of study: _____

_____

_____

Journal name, yyyy/vol/iss/page: _____

Language of publication _____ Translation Needed? YES____ NO_____

Date of trial (dd/mm/yyyy) _____ Duration of trial _____

Ethics approval: NO___ Unclear____YES ____ If yes Who granted ethics approval_____

Informed consent: NO____ YES _____if yes, consent was: Oral ____ Written _____ Not clear _____

Location of study (country) _____ Sponsor _____

Section 2

Eligibility Criteria

Does this study meet the following eligibility criteria?

Type of study: Randomized Controlled trial	YES	NO	Not Clear
Study participants: Full term infants, non hospitalized, strictly formula feed only	YES	NO	Not Clear
Intervention: **Experimental group:** Infant formula containing synbiotics, probiotic(s), or prebiotic(s) **Control group:** conventional infant formula with placebo or without placebo.All formulas will be cow based milk formula (soy based formulas will be excluded	YES	NO	Not Clear
Outcomes: Does this study have any of these outcomes: **Growth parameters:** (weight, Height/length, Head circum, **Tolerance to feed:** stool pattern, vomiting, colic **Infections:** frequency type, use of meds, **Immune respone:** CRP, IL-6, **Stool Microbiology**: levels of Bifidobacteria, lactobacilli, pathogens, **Hospitalization:** # days in hospital, ICU (if any)	YES	NO	Not Clear

Include or exclude this study?

Include this study?	Yes	NO
Exclude this study ?	Yes	NO
Reasons fro excluding		

Methodological design

Item	**Judgment**			**Description**
Adequate Sequence generation?	YES	NO	UNCLEAR	
Allocation concealment?	YES	NO	UNCLEAR	
Blinding of study participants, study personnel, assessors?	YES	NO	UNCLEAR	
Incomplete outcome data addressed?	YES	NO	UNCLEAR	
Free of selective reporting?	YES	NO	UNCLEAR	
Free of othe bias?	YES	NO	UNCLEAR	

Intervention

N= _____	Arm 1 _____ n= ___	Arm 2 _____ n=____	Arm 3 _____ n=____	**Comments**
Name / Strain of probiotic(s), Synbiotic(s)				
DOSE: Probiotics only Number of Viable cells (cfu) / _____.				
Name of prebiotic(s)				
DOSE: Prebiotics only g/__ml				
Name of Placebo				

Intervention continued

N=	Arm 1 n= ____ _____	Arm 2 n=_____ _____	Arm 3 n=_____ _____	Comments
Volume of feed given (mls/24 hours)				
Frequency of administration per 24 hours				
Duration of study/ treatment				
Time points				
Specify time points used for measurements. (e.g Day 1,2,3 or week 1,2,3 or Month 1,2,3.				
Withdrawl / losses				
Reasons for withdraw/ losses				
1				
2				
3				
4				
5				

Outcomes

Description of Outcomes

Primary Outcomes
Secondary Outcomes
Adverse events due to treatment. Definition of Adverse event.

Section 3

Characteristics of study participants

	N	%	Unclear	
Number recruited		Number randomized		
	Arm 1 n= ____ _____	**Arm 2 n=_____ _____**	**Arm 3 n=_____ _____**	**Comments**
Total Number				
Male				
Female				
Gestation Age Unit of measurement:				
Birth weight Unit of measurement				

1. Results: Growth

N=					
Growth Parameters	**Arm 1 n= ____ _____**	**Arm 2 n=_____ _____**	**Arm 3 n=_____ _____**	P value	Statistical test used / Comments
Weight gain Unit of measurment ___					
Linear growth Unit of measurment ___					
Head growth Unit of measurment ___					

2. Tolerance to formula

N=	Arm 1 n= ___ _____	Arm 2 n=____ _____	Arm 3 n=____ _____	P value	95% CI	Statistical test used / Comments
Stool characteristics (Hard, firm, loose, watery)						
Code for Hard, firm,lose, watery stool						
Stooling Frequency / evacuations						
Vomiting						
Spitting up						
Colic						
Crying episodes						
Other forms of feed intolerance						

3. Infections

N=	Arm 1 n= ___ _____	Arm 2 n=____ _____	Arm 3 n=____ _____	P value	95% CI	Statistical test used / Comments
Type of infection						
1						
2						
3						
4						

3. Infections continued

N=	Arm 1 n= ___ _____	Arm 2 n=____ _____	Arm 3 n=____ _____	P value	95% CI	Statistical test used / Comments
Use of Medication (antibiotic)						
Number of days on antibiotic						

4. Immune Response

N=	Arm 1 n= ___ _____	Arm 2 n=____ _____	Arm 3 n=____ _____	P value	Statistical test used / Comments
C- reactive protein (CRP)					
IL-6					
Other					
1					
2					

5. Hospitalization, Adverse events

N=	Arm 1 n= ___ _____	Arm 2 n=____ _____	Arm 3 n=____ _____	P value	Statistical test used / Comments
Number of days in hospital					
General ward					
ICU					
Other adverse events					
1 Mortality / Death					
2					
3					
4					

6. Stool Microbiology: Changes in Gastrointestinal Microflora

N=	**Day/Week 1**			**Day / Week 2**				
List of bacteria	Arm 1 n= ___ _____	Arm 2 n=___ _____	Arm 3 n=___ _____	Arm 1 n= ___ _____	Arm 2 n=___ _____	Arm 3 n=___ _____	P Value	Statistical test used / Comments
	cfu/g of feces			cfu/g of feces				
1								
2								
3								
4								
Statistical test used								

Changes in Gastrointestinal Microflora continued.

N=	**Day/Week 3**			**Day / Week 4**				
List of bacteria	Arm 1 n= ___ _____	Arm 2 n=___ _____	Arm 3 n=___ _____	Arm 1 n= ___ _____	Arm 2 n=___ _____	Arm 3 n=___ _____	P Value	Statistical test used / Comments
	cfu/g of feces			cfu/g of feces				
1								
2								
3								
4								
Statistical test used								

Section 7

Author's contact: Telephone_____ Cell Phone _____ Fax _____

Email _____

Address:_____

_____

Correspondence with Author

Additional information needed from Author

_____

_____

_____

Information received from Author: _____

_____

_____

Section 5

Information from Reference list.

Reference scaned Yes _____ NO _____

Additional studies identified from referencen list.

1._____

_____

_____ Sent to Refworks Yes_____NO_____

2._____

_____

_____ Sent to Refworks Yes_____NO_____

3._____

_____

_____ Sent to Refworks Yes_____NO_____

4._____

_____ Sent to Refworks Yes_____NO_____

Appendix 6: Study eligibility forms (Systematic review on food industry)

Association among funding source, methodological quality and research outcomes in randomized controlled trials of synbiotics, probiotics and prebiotics added to infant formula: A Systematic Review

STUDY ELIGIBILITY FORM - (*PRETERM INFANTS*)

Review title:	**Association between funding source, methodological quality and research outcomes in RCTs of synbiotics, probiotics and prebiotics in infant formulas: A systematic review**
Study ID (Author last name, initials)	
Date of review for eligibility (DD-MM-YYYY)	
Title of study/article	
Journal title	
Year/volume/issue/page	
Extractor (Last name, initials)	

Type of study	**Put a check (√) mark in appropriate box.**			
Is this study a Randomized controlled study?	YES	UNCLEAR	NO	**Intervention used in study:**
	↓	↓	↓	Circle below
	Go to next question		**Exclude**	Probiotic
Trial intervention				Prebiotic
Preterm infant formula containing probiotic(s)	YES	UNCLEAR	NO	Synbiotic
Preterm infant formula containing prebiotic(s) OR Synbiotics	YES	UNCLEAR	NO	
Conventional preterm formula / placebo	↓	↓	↓	
	Go to next question		**Exclude**	
Study Participants				
Premature infants <37 weeks gestation	YES	UNCLEAR	NO	
Low birth weight infants ≤ 2.5 kg at birth	YES	UNCLEAR	NO	
	↓	↓	↓	
	Go to next question		**Exclude**	
Study Outcomes (≥1 outcomes below)				
Short term growth parameters (Wt, Ht, Hd Circum)	YES	UNCLEAR	NO	
Adverse events (# days on parenteral, full enteral nutrition, maximal enteral feed, vomiting, GI aspirates, abdomen distension, stool characteristics- consistency, frequency)	YES	UNCLEAR	NO	
Complications (NEC, Sepsis, other infection, death)	YES	UNCLEAR	NO	
Intestinal permeability	YES	UNCLEAR	NO	
	YES	UNCLEAR	NO	
GI Microflora (Bifidobacteria, Lactobacillus, pathogen cfu)	↓	↓	↓	
		Clarify missing information		
Other reasons for excluding study	**NO**	↓	**Yes**	
	↓	↓	↓	
Final decision	**Include**	**Unclear**	**Exclude**	
		↓		
		For discussion		

Comments

STUDY ELIGIBILITY FORM- *(FULL TERM INFANTS)*

Review title:	**Association between funding source, methodological quality and research outcomes in RCTs of synbiotics, probiotics and prebiotics in infant formula: A systematic Review**
Study ID (Author last name, initials)	
Date of review for eligibility (DDMMYYYY):	
Title of study/article	
Journal title	
Year/volume/issue/page	
Extractor (Last name, initials)	

Type of study	**Put a check (√) mark in appropriate box.**			
Is this study a Randomized controlled study?	YES	UNCLEAR	NO	
	Go to next question		**Exclude**	**Intervention used in study:**
Trial intervention				Circle below
Experimental group: Term infant formula containing either probiotic(s) or prebiotic(s) or synbiotic(s)	YES	UNCLEAR	NO	Probiotic
Control group: Conventional term formula with or without placebo	YES	UNCLEAR	NO	Prebiotic
Note: All formulas are Cow based milk formula, no soy based formula. Breastmilk when used as reference only	YES	UNCLEAR	NO	Synbiotic
Study Participants	Go to next question		**Exclude**	
Healthy Full term infants (≥37 weeks gestation or ≥ 2.5 kg birth weight), Age: 0-12 months. Strictly formula fed infants only.	YES	UNCLEAR	NO	
Exclude: If infants have Congenital malformations, chromosomal abnormalities. Breastfed infants (unless they are used as a reference group)	**NO**	UNCLEAR	**YES**	
Study Outcomes. Does the study have one or more of the following outcomes:	Go to next question		**Exclude**	
Growth parameters: (weight, Height/length, Head Circum)	YES	UNCLEAR	NO	
Tolerance to feed: stool pattern (frequency, consistency), vomiting, diarrhoea, volume of feed tolerated	YES	UNCLEAR	NO	
Infections: frequency, type, use of meds	YES	UNCLEAR	NO	
Immune respone: CRP, IL-6, other immune system parameters	YES	UNCLEAR	NO	
Stool Microbiology: levels of bifidobacteria, lactobacilli, pathogens	YES	UNCLEAR	NO	
Hospitalization: # days in hospital, ICU (if any)	YES	UNCLEAR	NO	
		Clarify missing information		
Other reasons for excluding study	**NO**		**Yes**	
Final decision	**Include**	**Unclear**	**Exclude**	
		For discussion		

Comments

Appendix 7: Data extraction form (Systematic review on food industry)

Association among funding source, methodological quality and research outcomes in randomized controlled trials of synbiotics, probiotics and prebiotics added to infant formula: A Systematic Review

Data Extraction Form

Review Ttitle: Association between funding source, methodological quality and research outcomes in randomized control studies of Synbiotics, probiotics and prebiotics infant formula: A Systematic Review.

A) Study ID (1st Author last name,)_____ Publication date _____

Extractor Last name, Initials _____

Journal name, yyyy/vol/iss/page: _____

_____

Title of study: _____

_____

B) Location of study:1) Country _____2) City _____

3) Hospital(s) _____

C) Source of funding or support

1	**Name of Sponsor(s):**		**For official use**
	Category of sponsor (Tick only one)		
2	**<u>Industry</u>**: ▪ For – profit company, ▪ Donation of study product by a for – profit company which **manufactures** the study product, ▪ Not - for – profit company that promotes the consumption of synbiotics, probiotics or prebiotics. ▪ Mixed funding **(For profit company and other source)** Specify sources of funding:_____ ***(Note:** No assumptions are to be made: the study report must **<u>state</u>** who the sponsor is. If study report lists the name of a formula and puts a company name next to it, there should be no assumption that the company is sponsoring the study)*		**2**
3	**<u>Non-Industry:</u>** Government: National government, Regional (provincial, county) government body with NO industry association.		**3**
	▪ Foundation / Philanthropies: examples include Rockefeller foundation, Bill and Melinda Gates foundation.		
	Institution: University, Research centres, hospitals, teaching and academic hospitals.		
	Other:(Specify the source of funding)_____		
4	**<u>None / Not Clear</u>**. No source of support is disclosed in study report.		**4**

Source of funding for this study was: (CHECK ONE)	**Industry**	**Non- Industry**	**None / Not clear**

<u>General comments:</u>

D) Methodological quality of RCTs

	Item	Judgment (circle one)			For official use
1	Adequate Sequence generation? **For low risk**: there is description of a random component in the sequence generation process such as: ▪ Referring to a random number table; ▪ Using a computer random number generator; ▪ Coin tossing; ▪ Shuffling cards or envelopes; ▪ Throwing dice; ▪ Drawing of lots ▪ Minimization	YES / (Low risk)	NO / (High risk)	Unclear	**1**
2	Allocation concealment? **For low risk**: Investigators enrolling participants could not foresee assignment because one of the following, or an equivalent method, was used to conceal allocation: ▪ Central allocation (including telephone, web-based and pharmacy-controlled randomization); ▪ Sequentially numbered drug containers of identical appearance; ▪ Sequentially numbered, opaque, sealed envelopes.	YES / (Low risk)	NO / (High risk)	Unclear	**2**
3	Blinding of study participants, study personnel, assessors? **Low risk:** Any one of the following: ▪ No blinding or incomplete blinding, but the review authors judge that the outcome is not likely to be influenced by lack of blinding; ▪ Blinding of participants and key study personnel ensured, and unlikely that the blinding could have been broken.	YES / (Low risk)	NO / (High risk)	Unclear	**3**
4	Incomplete outcome data addressed? **For low risk**: Any one of the following: ▪ No missing outcome data; ▪ Reasons for missing outcome data unlikely to be related to true outcome (for survival data, censoring unlikely to be introducing bias); ▪ Missing outcome data balanced in numbers across intervention groups, with similar reasons for missing data across groups; ▪ For dichotomous outcome data, the proportion of missing outcomes compared with observed event risk not enough to have a clinically relevant impact on the intervention effect estimate; ▪ For continuous outcome data, plausible effect size (difference in means or standardized difference in means) among missing outcomes not enough to have a clinically relevant impact on observed effect size; ▪ Missing data have been imputed using appropriate methods.	YES / (Low risk)	NO / (High risk)	Unclear	**4**
5	Free of selective reporting? **For low risk**: Any of the following: ▪ The study protocol is available and all of the study's pre-specified (primary and secondary) outcomes that are of interest in the review have been reported in the pre-specified way;	YES / (Low risk)	NO / (High risk)	Unclear	**5**

	▪ The study protocol is not available but it is clear that the published reports include **all expected outcomes, including those that were pre-specified**				
6	Free of other bias? **For Low risk:** The study appears to be free of other sources of bias. **For High risk:** Had a potential source of bias related to the specific ▪ Study design used; ▪ Has been claimed to have been fraudulent; ▪ Had some other problem.	YES / (Low risk)	NO / (High risk)	Unclear	**6**
Please refer to Cochrane guidelines for further description on each item					

E) Intervention.

	N= _____	Arm **n**= ___	Arm 2 **n**= ___	Arm 3 **n**= ___	Arm 4 **n**= ___	
1	Name(s) intervention (probiotics, prebiotic, synbiotic)					**1**
2	**DOSE:** (cfu) / ____.					**2**
3	Treatment duration					**3**

F) Study outcomes (At END of study / intervention)

	In General Outcomes were: (Circle either YES or NO for the answer that applies)			**For official use**
1	**<u>Positive</u>:** synbiotic, probiotic or prebiotic supplementation **had a** statistically significant effect, $p<0.05$. **There <u>were</u> significant differences** between study groups **(in favour of experimental group)**.			**1**
1a	**Growth Parameters:** Weight gain, Length gain, Head circumference	**YES**	**NO**	**1a**
1b	**Tolerance: Significant differences in frequency / incidence of:** Colic	**YES**	**NO**	**1b1**
	Spitting up / regurgitation	**YES**	**NO**	**1B2**
	Vomiting	**YES**	**NO**	**1B3**
	Crying	**YES**	**NO**	**1B4**
	Volume of infant formula consumed / daily intake of formula	**YES**	**NO**	**1B5**
1c	**Stool characteristics:** Frequency,	**YES**	**NO**	**1c**
1d	Consistency	**YES**	**NO**	**1d**
1e	**Microflora: Significant difference** in colony forming units of Bifidobacteria, lactobacillus	**YES**	**NO**	**1e**
1f	**Significant difference** in colony forming units of pathogens	**YES**	**NO**	**1f**
1g	**Immune response:** CRP, Interleukin 6, other cytokines, Other immunity parameters	**YES**	**NO**	**1g**
1i	**Infections:** Frequency, incidence of different type of infections Specify type of infection(s)_____ _____	**YES**	**NO**	**1i**
1f	**Other parameters. Specify:** 1) _____ 2) _____	**YES**	**NO**	**1f**

	3) _____			
2	**Negative:** synbiotic, probiotic or prebiotic supplementation **had a** statistically significant ***increase*** ***in an adverse event / negative outcome*** ***such as weight loss, diarrhoea, vomiting,*** p<0.05.			
2a	**Specify:** 1) _____ 2) _____ 3) _____	**YES**	**NO**	**2a**
3	**Neutral:** synbiotic, probiotic or prebiotic supplementation **did not have** a statistically significant effect, p>0.05, **No significant differences** between study groups.			**3**
3a	**Growth Parameters:** Weight gain, Length gain, Head circumference	**YES**	**NO**	**3a**
3b	**Tolerance: No significant difference in frequency / incidence of:** Colic	**YES**	**NO**	**3b1**
	Spitting up / regurgitation	**YES**	**NO**	**3B2**
	Vomiting	**YES**	**NO**	**3B3**
	Crying	**YES**	**NO**	**3B4**
	Volume of infant formula consumed / daily intake of formula	**YES**	**NO**	**3B5**
3c	**Stool characteristics:** Frequency,	**YES**	**NO**	**3c**
3d	Consistency	**YES**	**NO**	**3d**
3e	**Microflora: No significant difference** between study groups in colony forming units of bifidobacteria, lactobacillus	**YES**	**NO**	**3e**
3f	**No significant difference** in colony forming units of pathogens	**YES**	**NO**	**3f**
3g	**Immune response:** CRP, Interleukin 6, other cytokines, Other immunity parameters	**YES**	**NO**	**3g**
3i	**Infections:** Frequency, incidence of different type of infections Specify type of infection(s)_____ _____	**YES**	**NO**	**3i**
3f	**Other parameters. Specify:** 1) _____ 2) _____ 3) _____	**YES**	**NO**	**3f**

G) Authors' conclusions

	Auhors conclusions were: (Tick only one)		**For official use**
1	**Positive**: Interpretation of data supports the sponsor's products over control. The sponsor's products were preferred over control / placebo.		**1**
2	**Negative**: Interpretation of data does **NOT** support the sponsors' products. The sponsor's products were **NOT** preferred over control / placebo.		**2**
3	**Neutral**: The authors' conclusion was neutral to the sponsor's product		**3**
4	**No clear conclusion offered by author**		**4**

H) Authors' conclusions on *outcomes*

Primary Outcome(s)	**Author's conclusion** **(Positive, negative, neutral, not clear/not mentioned)**
1	1
2	2
3	3
4	4
5	5
6	6
7	7
8	8
9	9
Secondary Outcome(s)	**Author's conclusion** **(Positive, negative, neutral, not clear/not mentioned)**
1	1
2	2
3	3
4	4
5	5
6	6
7	7
8	8
9	9
10	10
11	11
Comments	

Appendix 8: Sample invitation letter to participate in descriptive study

Application of evidence on probiotics, prebiotics and synbiotics by food industry: A descriptive study

Mr. John Doe
Clinical Project Manager
(Insert company name)
Tel: +01 21 123. 456 789
Fax: +01 21 123 456 789
E-mail: John.doe@gmail.com

Mrs. Mary Mugambi
Researcher & PhD student
Division of Human Nutrition
Faculty of Health Sciences
University of Stellenbosch
P.O box 19063, Tygerberg, 7505. South Africa
Fax +27 21933 2991
Cell Phone: +27 73 992 4774
Email: nkmugambi@hotmail.com

October 17th 2011

Dear Mr. John Doe

Re: Invitation to participate in a research study

You are being invited to take part in a unique research study being conducted at the Division of Human Nutrition at the University of Stellenbosch in Cape Town, South Africa. The aim of the study is to investigate how the infant food industry applies evidence gained through probiotic, prebiotic and synbiotic related research on infants. The study is being conducted since there is little or no information on how the food industry **applies** the knowledge and evidence gained in research on probiotics, prebiotics or synbiotics on the general paediatric population. The study will explore what happens after the research trials using infant formula have been conducted and the data is published or remains unpublished.

This study is unique and the first of its kind since it seeks information directly from people within the infant food industry. The study is targeting companies that market and /or retail food products with added probiotics, prebiotics or both (synbiotics) for infants and children. The person / people responsible for research and development will be invited to participate in the study. Study participants will include clinical research managers and individual researchers in the infant food companies. The study is a descriptive study (a survey) employing the use of a structured questionnaire developed by the researcher.

If you agree to participate in the study the following will be sent directly to you:

1) Informed consent form,
2) Structured questionnaire.

As a study participant, you will be required to do the following:

1) Read, sign the informed consent form,

2) Complete the questionnaire which will take 20 to 25 minutes to fill out,

3) Send back (by fax, email, post) the signed informed consent form and completed questionnaire to the researcher. (contact details are provided above)

Please note: The researcher will sign your signed informed consent form and send it to you, to keep for your own records.

The questionnaire will have 5 sections. Information from the following sections includes:

1. Contact details: Company name, position in company, fax number, email address, mailing address.

2. Product specific questions: Company brands of infant food containing or not containing probiotics, prebiotics, synbiotics and reasons for exclusion. Types of infant food: formula, weaning products. Type and dose of probiotic, prebiotic and synbiotic. Product viability issues.

3. Research based questions: frequency of research, application of evidence from research, scientific proof for claims on labels, differences between study formula and retail formula

4. Education of consumers: Medium used, perceived efficacy of specified medium, translation to sales,

5. Safety issues: complying with WHO guidelines for formula preparation instructions, safety of long term consumption of products.

As a study participant, you will be free to withdraw from the study at any time. The information you provide will be extremely valuable; will be used for publication in a scientific peer reviewed journal and university thesis as part of my PhD studies. All the information you provide will be kept confidential and protected.

To ensure confidentiality of data and anonymity of study participants in relation to the information given, the following will take place:

1) Data processing will be done according to product and company name,

2) Only the researcher and statistician will have access to the data,

3) In the final report:

- All identifying details (product and company name) will be excluded,
- Only a list of participating companies will be included,
- The formula brands will be given generic names such as “brand X, brand Y or brand Z,”

- Study participants will be anonymously acknowledged for contributing to the success of the study. The following clause will be inserted to thank the participants: “We wish to thank all the people who participated in the study. Your insights contributed to the successful completion of this study.”

This study has been approved by the Committee for Human Research at Stellenbosch University and will be conducted according to the ethical guidelines and principles of the international Declaration of Helsinki, South African Guidelines for Good Clinical Practice and the Medical Research Council (MRC) Ethical Guidelines for Research. If you have questions about this study, first discuss them with the researcher (contact details are provided above). You can also get more information from the Committee for Human Research by contacting Dr Lyn Horn (contact details are provided below). After you have consulted the researcher or the Committee for Human Research and if they have not provided you with answers to your satisfaction, you should write to Professor Renee Blaauw of the Division of Human Nutrition at:

Prof Renee Blaauw.
Division of Human Nutrition,
University of Stellenbosch
Faculty of Health Sciences
P.O Box 19063, Tygerberg,
7505 South Africa
Telephone: +27 21 938 9135
Fax: +27 21 933 2991;
E-mail rb@sun.ac.za

Dr Lyn Horn:
Committee for Human Research
University of Stellenbosch
PO Box 19063, Tygerberg, 7505
Cape Town, South Africa
Tel: +27 21 938 9677 or +27 21 938 9207
Fax: +27 21 931 3352
Email: lhorn@sun.ac.za

It is my sincere hope that you agree to participate in this unique study. If you agree to participate in the study, please contact me using either fax, email or postal address as listed above. I will then send you the informed consent form and questionnaire.

Thank you

Sincerely

Mrs. Mary Mugambi
Researcher, PhD student
Division of Human Nutrition
University of Stellenbosch,
Cape town, South Africa

Appendix 9: Informed consent form for descriptive study

Application of evidence on probiotics, prebiotics and synbiotics by food industry: A descriptive study

PARTICIPANT INFORMATION LEAFLET AND CONSENT FORM

TITLE OF THE RESEARCH PROJECT:

An evaluation of the application of evidence on probiotics, prebiotics and synbiotics by the food industry.

REFERENCE NUMBER: N11/07/203

PRINCIPAL INVESTIGATOR: **Mary Mugambi**

ADDRESS: **University of Stellenbosch, Tygerberg campus**
Division of Human Nutrition
P.O Box 19063
Tygerberg, 7505

Contact Number (cell phone): **+27 73 992 4774**
E-mail: **nkmugambi@hotmail.com**

You are being invited to take part in a research project. Please take some time to read the information presented here, which will explain the details of this project. Please ask the principle investigator any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied and clearly understand what this research entails and how you will be involved. Also, your participation is **entirely voluntary** and you are free to decline to participate. If you say no, this will not affect you negatively in any way whatsoever.

This study has been approved by the **Committee for Human Research at Stellenbosch University** and will be conducted according to the ethical guidelines and principles of the international Declaration of Helsinki, South African Guidelines for Good Clinical Practice and the Medical Research Council (MRC) Ethical Guidelines for Research. This

What is this research study all about?

Venue: This study is being conducted at the Division of Human Nutrition, Faculty of Health Sciences, University of Stellenbosch.

Objectives of study:

- To evaluate the application of evidence on probiotics, prebiotics and synbiotics by the food industry for the benefit of the general paediatric population.

Methodology:

- A structured questionnaire will be sent to the companies that manufacture and / or retail infant formula (for premature or full term infants) containing probiotic(s), prebiotic(s) or synbiotic(s).
- People responsible for research and development in these companies will be asked to participate in the survey.

Your responsibilities will be:

- Reading and signing this consent form,
- Completing the provided questionnaire on probiotics, prebiotic and synbiotics. The questionnaire is divided into sections which are: general questions, product specific questions, education of consumers and safety issues,
- The questionnaire will take 15 to 20 minutes to complete,
- Email, fax or mail the completed questionnaire to the address above.

Why have you been invited to participate?

- You have been invited to participate in this study because as clinical research manager / researcher you have one or more unique roles such as:

    - Keeping track of:
        - All research trials being conducted or sponsored by your company
        - Individual trial results
        - Published and unpublished trials conducted by your company
    - Dissemination of information to interested parties
    - Liaise with manufacturing sector on how to use new evidence gained from research on probiotic, prebiotic or synbiotic

Benefit of taking part in this research?

There is little or no information on how the food industry uses evidence gathered from research on probiotics, prebiotics and / or synbiotics for the benefit of the general paediatric population. By participating in this study, the information you provide will help answer the question of "what happens after research trials have been concluded, the results published / unpublished?" The information you provide will also help answer the question of "how does the general paediatric population benefit from probiotic, prebiotic research outside of the clinical setting?" That is, does your company come up with new commercial infant formula, or products containing probiotics, prebiotics or synbiotics for use by the general paediatric population? Or does your company improve on already existing infant formula or products sold on the market?

Are there any risks involved in taking part in this research?

- There are no risks involved.
- All identifying information will be kept confidential and protected. There will be no mention of any names or any information that may identify you. Only company names will be used.
- Preliminary study results will be sent to you for further comment.
- The information collected will be published in a scientific paper and University Thesis.
- You will be anonymously acknowledged for your contribution to the success of this study during publication of the scientific paper and university thesis.

What If you do not agree to take part in the study?

- This study is purely on a voluntary basis. Refusal to participate in the study will not affect you in any way.

Who will have access to the information you provide?

The following people will have access to the information you provide:

- Principle investigator (researcher).
- Biostatistician.

Will you be paid to take part in this study and are there any costs involved?

No, you will not be paid to take part in this study. Your participation is purely on a voluntary basis. There are little or no costs involved. There will be the cost of sending (by email, fax or post) the completed questionnaire to the researcher.

Is there anything else that you should know or do?

- You can contact the Committee for Human Research at:

    Tel: +27 21 938 9677 or +27 21 938 9207
    Fax: +27 21 931 3352

    Postal address:

    Attention: Dr Lyn Horn:
    Committee for Human Research
    University of Stellenbosch
    PO Box 19063, Tygerberg, 7505
    Cape Town, South Africa

Email: lhorn@sun.ac.za

- If you have questions about this trial you should first discuss them with the researcher or the Committee for Human Research (contact details as provided above). After you have consulted the researcher or the Committee for Human Research and if they have not provided you with answers to your satisfaction, you should write to Professor Renee Blaauw of the Division of Human Nutrition at:

  Prof Renee Blaauw.
  Division of Human Nutrition, University of Stellenbosch
  Faculty of Health Sciences
  P.O Box 19063, Tygerberg, 7505 South Africa
  Telephone: +27 21 938 9135
  Fax: +27 21 933 2991;
  E-mail rb@sun.ac.za

- You will receive a copy of this signed consent form for your own records.

A. Declaration by study participant:

By signing below, I *(name)* ……..…….. agree to take part in a research study entitled: An evaluation of the application of evidence on probiotics, prebiotics and synbiotics by the food industry.

I declare that:

- I have read and understood this consent form and that it is written in a language with which I am fluent and comfortable.
- I have had a chance to ask questions and all my questions have been adequately answered.
- I understand that taking part in this study is **voluntary** and I have not been pressurised to take part.
- I may choose to withdraw and will not be penalised or prejudiced in any way.

Declaration to participate in the study:

Signed at (*place*) .....…..…….. On (*date*) ……..…….. 20__ .
DD/MM/YYYY

_____
Name of study particpant (Print letters)

....
Signature of study participant **Signature of witness**

Declaration by the investigator:

I *(name)* ……..…… declare that:

- I explained the information in this document to the study participant
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understand all aspects of the research, as discussed above

- I did/did not use a translator (i*f a translator is used, then the translator must sign the declaration below).*

Signed at (*place*) .....…….. on (*date*) ……...…….. 20__ .
DD/MM/YYYY

_____
Name of investigator (print letters)

…… ……
Signature of investigator **Signature of witness**

Appendix 10: Questionaire for descriptive study

Application of evidence on probiotics, prebiotics and synbiotics by food industry: A descriptive study

An evaluation of the application of evidence on probiotics, prebiotics and synbiotics by the infant food industry

Questionnaire for Study Participants in Infant Food Industry

This questionnaire has been sent to companies that manufacture and / or retail infant foods containing probiotic(s) or prebiotic(s) or synbiotic(s) such as infant formula (for premature or full term infants), weaning foods and beverages.

This study seeks the view point of the infant food industry, making it unique and the first of its kind. The information you provide is extremely valuable, will be kept confidential and protected. Only the Principle investigator (researcher) and statistician will have access to the information you provide. During data processing only initials, pseudonyms and Product names will be used. In the final published report of this study all identifying details will be omitted. You may consult the appropriate professional in your company to obtain the required technical information.

Please complete and send this form to the researcher:
Mary Mugambi,
Division of Human Nutrition, University of Stellenbosch, Tygerberg.
P.O Box 19063 Tygerberg 7505 South Africa
Email: nkmugambi@hotmail.com, Fax: +27 21 933 2991

Participant Contact details

Company Name		
Address		
Email address	Telephone	Fax
Your position in the company		

Product questions

Put an "X" next to the answer(s) that apply to your company

1 My company routinely manufactures infant formula containing:		
a. Probiotics	Yes	If yes, what is the target age group(s): Preterm ___, 0 – 5 months____, 6 – 11 months____, >12 months ___
	No	If no, give reason not having any probiotic in your company formula
	Uncertain	
b. Prebiotics	Yes	If yes, what is the target age group(s): Preterm ___, 0 – 5 months____, 6 – 11 months____, >12 months ____
	No	If no, give reason not having any prebiotic in your company formula
	Uncertain	
c. Synbiotics	Yes	If yes, what is the target age group(s): Preterm ___, 0 – 5 months____, 6 – 11 months____, >12 months ___
	No	If no, give the reason(s) for not having any synbiotic in your company's formula
	Uncertain	

Definitions: **Synbiotic**s are combinations of probiotics and prebiotics in same food product. **Probiotics** are "live microorganisms" which when administered in adequate amounts may confer a health benefit to the host. **Prebiotics** are non- digestible food ingredients that benefit the host by selectively stimulating the growth and/or activity of a limited number of bacteria in the colon, improving the host's health.

Put an "X" next to the answer(s) that apply to your company

2 My company routinely manufactures other infant foods (such as cereals, porridges, other beverages) containing:		
a. Probiotics	Yes	If yes, specify type of infant food / beverage:
	No	If no, give reason for omitting probiotics in infant food – if any
	Uncertain	
b. Prebiotics	Yes	If yes, specify type of infant food / beverage
	No	If no, give reason for omitting prebiotics in infant food – if any
	Uncertain	
c. Synbiotics	Yes	If yes, Specify type of infant food / beverage:
	No	If no, give reason for omitting synbiotics in infant food – in any
	Uncertain	

3. Please provide the following information on your company's <u>Probiotic</u> infant formula or follow-on formula

	Formula 1	**Formula 2**	**Formula 3**	**Formula 4**	**Formula 5**	**Formula 6**
a. Formula name						
b. Target age group (preterm, full term, > 1 year)						
c. Type of probiotic used (Genus, species)						
d. Shelf life period (months)						
e. Dose of probiotic on label: Colony forming units/__						
f. Dose at manufacture: Colony forming units/ __						
g. Dose of probiotic at mid shelf life. Colony forming units/ __						
h. Dose of probiotic at expiration date, Colony forming units/ __						
i. Type of bioavailability tests conducted						
j. Specify the time intervals when bioavailability tests are conducted						
k. Not applicable (company does not manufacture infant formula) (Place an X here)						
For additional products, use / add a separate page						

4. Please provide the following information on your company's Prebiotic infant formula or follow-on formula.

	Formula 1	Formula 2	Formula 3	Formula 4	Formula 5	Formula 6
a. Formula name						
b. Target age group (preterm, full term, > 1 year)						
c. Type of prebiotic used						
d. Shelf life period (months)						
e. Dose of prebiotic on label: __ g / __						
f. Dose of prebiotic at manufacture: __ g / __						
g. Dose of prebiotic at mid shelf life. __ g / __						
h. Dose of prebiotic at expiration date __ g / __						
i. Type of stability tests conducted						
j. Specify the time intervals when stability tests are conducted						
k. Not applicable (company does not manufacture infant formula) (Place an X here)						
For additional products, use / add a separate page						

5. Please provide the following information on your company's Synbiotic infant formula or follow-on formula.

	Formula 1	Formula 2	Formula 3	Formula 4	Formula 5	Formula 6
a. Formula name						
b. Target age group (preterm, full term, > 1 year)						
c. Type of **probiotic** (Genera, strain)						
Shelf life period (months)						
d. Dose of **probiotic** on label: Colony forming units/ ____						
e. Dose of **probiotic** at manufacture: Colony forming						

units/ ____						
f. Dose of probiotic at mid shelf life Colony forming units/ _						
g. Dose of probiotic at expiration date: Colony forming units / __						
h. Type of **prebiotic**						
i. Dose of **prebiotic** on label: Grams / ____						
j. Dose of **prebiotic** at manufacture: Grams / ____ prebiotic						
k. Dose of prebiotic at expiration date: Grams / __						
l. Type of bioavailability tests conducted						
m. Specify the time intervals when bioavailability tests are conducted						
o. Not applicable (company does not manufacture infant formula) (Place an X here)						
For additional products, use / add a separate page						

6. Please provide the following information on your company's Probiotic weaning foods such as cereals, porridges, other beverages.

	Product 1	**Product 2**	**Product 3**	**Product 4**	**Product 5**	**Product 6**
a. Product name						
b. Target age group						
c. Type of probiotic used (Genus, species)						
d. Shelf life period (months)						
e. Dose of probiotic on label: Colony forming units/ __						
f. Dose of probiotic at manufacture: Colony forming units/ __						
g. Dose of probiotic at mid shelf life. Colony forming units/ __						
h. Dose of probiotic at expiration date. Colony forming units/ __						

i. Type of bioavailability tests conducted						
j. Specify the time intervals when bioavailability tests are conducted						
k. Not applicable (company does not manufacture weaning foods) (Place an X here)						
For additional products, use / add a separate page						

7. Please provide the following information on your company's <u>Prebiotic weaning foods</u> such as cereals, porridges, other beverages.

	Product 1	Product 2	Product 3	Product 4	Product 5	Product 6
a. Product name						
b. Target age group						
c. Type of probiotic used (Genus, species)						
d. Shelf life period (months)						
e. Dose of prebiotic on label: __ g / __						
f. Dose of prebiotic at manufacture: __ g / __						
g. Dose of prebiotic at mid shelf life. __ g / __						
h. Dose of prebiotic at expiration date. __ g / __						
i. Type of stability tests conducted						
j. Specify the time intervals when stability tests are conducted						
k. Not applicable (company does not manufacture weaning foods) (Place an X here)						
For additional products, use / add a separate page						

8. Please provide the following information on your company's <u>Synbiotic weaning foods</u> such as cereals, porridges, other beverages

	Product 1	Product 2	Product 3	Product 4	Product 5	Product 6
a. Product name						
b. Target age group						
c. Type of **<u>probiotic</u>** (Genera, strain)						
d. Shelf life period (months)						

e. Dose of **probiotic** on label:Colony forming units/ _						
f. Dose of **probiotic** at manufacture: Colony forming units/ ____						
g. Dose of **probiotic** at mid shelf life Colony forming units/ ____						
h. Dose of **probiotic** at expiration date: Colony forming units / __						
i. Type of **prebiotic**						
j. Dose of **prebiotic** on label: __ g / __						
k. Dose of **prebiotic** at manufacture: __ g / __						
l. Dose of prebiotic at expiration date: __ g / __						
m. Type of bioavailability tests conducted						
n. Specify the time intervals when bioavailability tests are conducted						
o. Not applicable (company does not manufacture weaning foods) (Place an X here)						
For additional products, use / add a separate page						

Put an "X" next to the answer(s) that apply to your company

9. In which parts of the world are your company's probiotics(s) / prebiotic(s) / synbiotic(s) infant formula or follow-on formula sold?

America: North ___, Central ___, South ___

Europe: East ___, Central ___, West ___,

Africa: North ___, East ___, Central ___, West ___, South ___

Australia: North ___, West___, South ___, Queensland ___, New South Wales ___, Victoria ___, Tasmania ___,

New Zealand ___,

Asia: North___, Central ___, South ___, East ___, South East Asia ___, West ___,

Oceania: Melanesia___, Micronesia ___, Polynesia ___,

Other: Please specify _____

_____

Research questions

Put an "X" next to the answer that apply to your company.

10 How does your company officially keep abreast of latest research on the type of probiotic(s) / prebiotic(s) / synbiotic(s) used in your company's infant formula, follow – on formula, weaning foods or beverages?	
Designated person with a specific job description. Specify job title of this designated person.	
Each researcher / technician is responsible for keeping up to date	
Focus group discussions. **Specify with whom.**	
Workshops: invited researchers presenting their work	
Other, please specify	
Uncertain	
Not applicable	

Put an "X" next to the answer that applies to your company while providing the following information

11. In the last 10 years, what proportion of research in your company was conducted in collaboration with independent / external entities such as universities or research institutions?			
	a. For Probiotics in infant formula, follow – on formula	**b. For Prebiotics in infant formula, follow – on formula**	**c. For Synbiotics in infant formula, follow – on formula**
Uncertain			
0% – all research is conducted inside our company			
__ % of research conducted in collaboration with other institutions (**specify)**			
All company research is done independently by external institutions **without any collaboration with our company**.			
Not applicable			

Put an "X" next to the answer that apply to your company while providing the following information

12. In the last 10 years, what proportion of research in your company was conducted in collaboration with independent / external entities such as universities or research institutions?			
	a. For Probiotics in <u>weaning</u> foods	**b. For Prebiotics in <u>weaning</u> foods**	**c. For Synbiotics in <u>weaning</u> foods**
Uncertain			
0% – all research is conducted **inside** our company			
__ % of research conducted in collaboration with other institutions **(specify)**			
All company research is done independently by **external** institutions **<u>without any collaboration with our company</u>**.			
Not applicable			

Questionnaire continued below.

Put an "X" next to the answer(s) that apply to your company while providing the following information.

13. Has your company's infant formula(s) been studied using one or more randomized controlled clinical trials (RCTs)?								
a. Probiotic formula	Yes	**If yes, specify:**	Formula 1	Formula 2	Formula 3	Formula 4	Formula 5	Formula 6
		Formula Name						
		Target group						
		Estimated Number of RCTs						
		Month/Year RCTs conducted						
		Were the RCTs published? Y/N						
	No	Why not? Please give reason						
	Uncertain							
	Not applicable							
b. Prebiotic formula	Yes	**If yes, specify:**	Formula 1	Formula 2	Formula 3	Formula 4	Formula 5	Formula 6
		Formula Name						
		Target group						
		Estimated Number of RCTS						
		Month/Year RCTs conducted						
		Were the RCTs published? Y/N						
	No	Why not? Please give reason						
	Uncertain							
	Not applicable							
c. Synbiotic formula	Yes	**If yes, specify:**	Formula 1	Formula 2	Formula 3	Formula 4	Formula 5	Formula 6
		Formula Name						
		Target group						
		Estimated Number of RCTS						
		Month/Year RCTs conducted						
		Were the RCTs published? Y/N						
	No	Why not? Please give reason						

	Uncertain	
	Not applicable	
For additional products, use / add a separate page		

Put an "X" next to the answer(s) that apply to your company while providing the following information.

14. Does your company sell infant formula or follow – on formula that is **identical** to its study formula after RCTs produced positive results?								
a. Probiotic formula	Yes	**If yes, specify:**	Formula 1	Formula 2	Formula 3	Formula 4	Formula 5	Formula 6
		Formula Name						
		Target group						
		Estimated Number of RCTS						
		Month/Year RCTs conducted						
		Were the RCTs published? Y/N						
	No	Why not? Please give reason						
	Uncertain							
	Not applicable							
b. Prebiotic formula	Yes	**If yes, specify:**	Formula 1	Formula 2	Formula 3	Formula 4	Formula 5	Formula 6
		Formula Name						
		Target group						
		Estimated number of RCTS						
		Month/Year RCTs conducted						
		Were the RCTs published? Y/N						
	No	Why not? Please give reason						
	Uncertain							
	Not applicable							
c. Synbiotic	Yes	**If yes, specify:**	Formula 1	Formula 2	Formula 3	Formula 4	Formula 5	Formula 6
		Formula Name						

formula		**Target group**						
		Estimated number of RCTS						
		Month/Year RCTs conducted						
		Were the RCTs published? Y/N						
	No	Why not? Please give reason						
	Uncertain							
	Not applicable							
For additional products, use / add a separate page								

Questionnaire continued below.

Put an "X" next to ALL the answers that apply to your company while providing the following information

15. How **often** does your company conduct research trials to **re-evaluate infant formula or follow on formula** that is already in the market?			
	a. For Probiotic infant formula or follow – on formula, every:	**b. For Prebiotic infant formula or follow – on formula, every:**	**c. For Synbiotic infant formula or follow – on formula, every:**
Never			
Every ____ Months (Specify)			
Every ____ Years (Specify)			
Other specify			
Only when a new formulation / ingredient is added / changed ___			
Uncertain			
Not Applicable			

16. How often does your company conduct research trials to **re-evaluate weaning foods** (such as infant cereals / porridges) already in the market			
	a. For other Probiotic infant foods / beverages	**b. For other Prebiotic infant foods / beverages:**	**c. For other Synbiotic infant foods/ beverages**
Never			
Every ____ Months (Specify)			
Every ____ Years (Specify)			
Other specify			
Only when a new formulation / ingredient is added / changed ___			
Uncertain			
Not applicable			

17. How often does your company conduct research trials to **re-evaluate infant beverages** already in the market			
	a. For <u>Probiotic</u> infant beverages	**b. For <u>Prebiotic</u> infant beverages**	**c. For <u>Synbiotic</u> infant beverages**
Never			
Every ____ Months (Specify)			
Every ____ Years (Specify)			
Other specify			
Only when a new formulation / ingredient is added / changed ___			
Uncertain			
Not applicable			

Questionnaire continued below.

Put an "X" next to the answer(s) that apply to your company while providing the following information.

18. Based on **scientific evidence** that has emerged **since the year 2000**, has your company **developed and marketed a new infant formula or follow – on formula** containing probiotics(s) / prebiotic(s) / synbiotic(s):

a. Probiotic formula	Yes	**If yes, specify:**	Formula 1	Formula 2	Formula 3	Formula 4	Formula 5	Formula 6
		Formula Name						
		Target group						
		Month/Year of introduction						
	No							
	Uncertain							
	Not applicable							
b. Prebiotic formula	Yes	**If yes, specify:**	Formula 1	Formula 2	Formula 3	Formula 4	Formula 5	Formula 6
		Formula Name						
		Target group						
		Month/Year of introduction						
	No							
	Uncertain							
	Not applicable							
c. Synbiotic formula	Yes	**If yes, specify:**	Formula 1	Formula 2	Formula 3	Formula 4	Formula 5	Formula 6
		Formula Name						
		Target group						
		Month/Year of introduction						
	No							
	Uncertain							
	Not applicable							

For additional products, use / add a separate page

Put an "X" next to the answer(s) that apply to your company while providing the following information.

19. Based on **scientific evidence** that has emerged **since the year 2000**, did your company **improve** on infant formula or follow – on formula containing probiotics(s) / prebiotic(s) / synbiotic(s) that was **already sold** in the market?

a. Probiotic formula	Yes	**If yes, specify:**	Formula 1	Formula 2	Formula 3	Formula 4	Formula 5	Formula 6
		Formula Name						
		Target group						
		Month/Year of improvement						
		Specify type of improvement						
	No							
	Uncertain							
	Not applicable							
b. Prebiotic formula	Yes	**If yes, specify:**	Formula 1	Formula 2	Formula 3	Formula 4	Formula 5	Formula 6
		Formula Name						
		Target group						
		Month/Year of improvement						
		Specify type of improvement						
	No							
	Uncertain							
	Not applicable							
c. Synbiotic formula	Yes	**If yes, specify:**	Formula 1	Formula 2	Formula 3	Formula 4	Formula 5	Formula 6
		Formula Name						
		Target group						
		Month/Year of improvement						
		Specify type of improvement						
	No							
	Uncertain							

	Not applicable	
For additional products, use / add a separate page		

Put an "X" next to ALL the answers that apply to your company while providing the following information

20. Based on **scientific evidence** that has emerged **since the year 2000**, has your company **developed and marketed a new weaning food or beverage** containing probiotics(s) / prebiotic(s) / synbiotic(s):

			Product 1	Product 2	Product 3	Product 4	Product 5	Product 6
a. Probiotic weaning food or beverage	Yes	**Product Name**						
		Target group						
		Month/Year of introduction						
	No							
	Uncertain							
	Not applicable							
b. Prebiotic weaning food or beverage	Yes		Product 1	Product 2	Product 3	Product 4	Product 5	Product 6
		Product Name						
		Target group						
		Month/Year of introduction						
	No							
	Uncertain							
	Not applicable							
c. Synbiotic weaning food or beverage	Yes		Product 1	Product 2	Product 3	Product 4	Product 5	Product 6
		Product Name						
		Target group						
		Month/Year of introduction						
	No							
	Uncertain							
	Not applicable							
For additional products, use / add a separate page								

Put an "X" next to the answer(s) that apply to your company while providing the following information.

21. Based on **scientific evidence** that has emerged **since the year 2000**, did your company **improve** on **weaning** food or beverage that was **already sold** in the market?

			Product 1	Product 2	Product 3	Product 4	Product 5	Product 6
a. Probiotic weaning food or beverage	Yes	**Product Name**						
		Target group						
		Month/Year of improvement						
		Specify type of improvement						
	No							
	Uncertain							
	Not applicable							
b. Prebiotic weaning food or beverage	Yes		Product 1	Product 2	Product 3	Product 4	Product 5	Product 6
		Product Name						
		Target group						
		Month/Year of improvement						
		Specify type of improvement						
	No							
	Uncertain							
	Not applicable							
c. Synbiotic weaning food or beverage	Yes		Product 1	Product 2	Product 3	Product 4	Product 5	Product 6
		Product Name						
		Target group						
		Month/Year of improvement						
		Specify type of improvement						
	No							
	Uncertain							
	Not							

	applicable	
For additional products, use / add a separate page		

Put an "X" next to the answer(s) that apply to your company while providing the following information.

22. Have the health claims on the **infant formula labels been substantiated** through randomized controlled clinical trials for each infant formula that your company markets / sells.

		Formula 1	Formula 2	Formula 3	Formula 4	Formula 5	Formula 6
a. Probiotic formula	Formula name						
	Yes						
	No						
	Uncertain						
	Not applicable						
b. Prebiotic formula		Formula 1	Formula 2	Formula 3	Formula 4	Formula 5	Formula 6
	Formula name						
	Yes						
	No						
	Uncertain						
	Not applicable						
c. Synbiotic formula		Formula 1	Formula 2	Formula 3	Formula 4	Formula 5	Formula 6
	Formula name						
	Yes						
	No						
	Uncertain						
	Not applicable						
For additional products, use / add a separate page							

Put an "X" next to the answer(s) that apply to your company while providing the following information.

23. Have the health claims on the **weaning food labels been substantiated** through randomized controlled clinical trials for each weaning food product that your company markets / sells.

		Product 1	Product 2	Product 3	Product 4	Product 5	Product 6
a. Probiotic weaning food	Product name						
	Yes						
	No						
	Uncertain						
	Not applicable						
		Product 1	Product 2	Product 3	Product 4	Product 5	Product 6
b. Prebiotic weaning food	Product name						
	Yes						
	No						
	Uncertain						
	Not applicable						
		Product 1	Product 2	Product 3	Product 4	Product 5	Product 6
c. Synbiotic weaning food	Product name						
	Yes						
	No						
	Uncertain						
	Not applicable						

For additional products, use / add a separate page

Put an "X" next to the answer(s) that apply to your company while providing the following information.

24. Are there any differences between your company's study **probiotic** formula (used in RCTs) and retailed infant formula or follow-on formula?							
	Study formula	Formula 1	Formula 2	Formula 3	Formula 4	Formula 5	Formula 6
a. No (study and retailed formula are identical)							
b Uncertain							
c. Not applicable							
d. Yes (specify difference below)							
i. Formula name							
ii. Type of probiotic(s)							
iii. Dose cfu / g							
iv. Target group							
v. Other ingredient							
vi. Other difference (liquid / powder formulation)							
Reason(s) for the change (Please specify)							
For additional products, use / add a separate page							

25. Are there any **differences** between your company's **<u>study prebiotic</u>** formula (used in RCTs) and retailed infant formula or follow-on formula?							
	Study formula	Formula 1	Formula 2	Formula 3	Formula 4	Formula 5	Formula 6
a. No (study and retailed formula are identical)							
b Uncertain							
c. Not applicable							
d. Yes (specify difference below)							
i. Formula name							
ii. Type of prebiotic(s)							
iii. Dose __ g/__							
iv. Target group							
v. Other ingredient							
vi. Other difference (liquid / powder formulation)							
Reason(s) for the change (Please specify)							
For additional products, use / add a separate page							

26. Are there any **differences** between your company's **study synbiotic** formula (used in RCTs) and retailed infant formula or follow-on formula?							
	Study formula	Formula 1	Formula 2	Formula 3	Formula 4	Formula 5	Formula 6
a. No (study and retailed formula are identical)							
b Uncertain							
c. Not applicable							
d. Yes (specify difference below)							
i. Formula name							
ii. Type of **probiotic(s)**							
iii. Probiotic dose __ cfu / g							
iv. Type of **prebiotic(s)**							
v. Prebiotic dose __ g/ __							
vi. Target group							
vii. Other ingredient							
viii. Other difference (liquid / powder formulation)							
Reason(s) for the change (Please specify)							
For additional products, use / add a separate page							

Put an "X" next to the answer that applies to your company while providing the following information.

27. Has your company used scientific evidence gained through research on company infant formula to **manufacture other products** (containing probiotics, prebiotics or synbiotics) for infants and children?						
No (no new product developed)						
b Uncertain						
c. Not applicable						
d. Yes (Give details below)	Product 1	Product 2	Product 3	Product 4	Product 5	Product 6
i. Type of product (milk drink, fruit drink, chewable tablets)						
ii. Type of **probiotic(s)**						
iii. Probiotic dose __ cfu / g						
iv.Type of **prebiotic(s)**						
v. Prebiotic dose __ g/ __						
vi. Target group						
For additional products, use / add a separate page						

Put an "X" next to the answer(s) that apply to your company while providing the following information.

28. Has your company intentionally **not published** any RCT results on probiotics / prebiotics / synbiotics use in infants that were viewed as being negative or having no clinical benefit(s) to infants or children?						
Uncertain						
No						
Not applicable						
b. Yes (Please answer questions below)	RCT 1	RCT 2	RCT 3	RCT 4	RCT 5	RCT 6
i. Type of product being tested / studied						
ii. Probiotic / prebiotic name						
iii. Study population						
iv. **Reason(s) for not publishing study results**						
c. Was new research was conducted to confirm negative results? YES / NO / Uncertain						
d. Were the new study results published in peer review journals YES / NO / Uncertain						
i. If Yes, specify: author, journal name, title of article, year, volume and page						
ii. If no, specify the reason(s) for not publishing the new study results						

Education of consumers

Put an "X" next to <u>all</u> the option(s) that apply to your company

29. What type(s) of method does your company use to **<u>educate</u>** the consumer on your probiotic(s) / prebiotic(s) / synbiotic(s) infant formula or follow – on formula?			
Company websites (Provide website address)		Formal workshops	
Brochures / leaflets		Home visits by staff	
Press releases		Mass media (Television, radio, newspaper)	
Focus group discussions		Information booklets in health care facilities	
Free Telephone hotlines for consumers		Uncertain	
Educate health care workers who then educate the consumers		None: our company **does not** educate consumers on this topic.	
Not applicable (company does not manufacture infant formula)			
Other, Please specify			

30. What type(s) of method does your company use to **<u>educate</u>** the consumer on your probiotic(s) / prebiotic(s) / synbiotic(s) **weaning foods** such as cereals, porridges, other beverages?			
Company websites (Provide website address)		Logos, slogans on posters	
Brochures/leaflets		Mass media advertising (Television, radio, newspaper)	
Formal workshops of parents and potential consumers		In-store promotion: tasting	
Free samples distributed by medical professionals		Distribution of trial size samples by company	
Information booklets		Product education to health workers	
Home visits		None	
Discount coupons		Uncertain	
Gift items with logos and slogans		Not applicable (company does not manufacture weaning foods)	
Other please specify			

Questionnaire continued next page

31. Which education method is the most effective based on **consumer usage.** That is which method of education resulted in ***increased use*** of company infant formula, follow-on formula or weaning foods?

Rank your answer from very effective (5) to not effective (1).

	Not effective		**Neutral**		**Very effective**
	1	**2**	**3**	**4**	**5**
Focus group discussions					
Brochures / leaflets					
Press releases					
Home visits by staff					
Free Telephone hotlines for consumers					
Educate health care workers who then educate the consumers					
Formal workshops					
Company website					
Mass media (Television, radio, newspaper)					
Information booklets in health care facilities					
Other, Please specify					
Uncertain					
Not applicable					
None (our company **does not** educate consumers on infant formula, follow- on formula or weaning foods)					

Please provide the following information.

32. How does your company test the effectiveness of the method(s) used to educate consumers?
Uncertain
Not applicable

Put an "X" next to the answer that applies to your company

Since 2005 has your company given:					
33. Any **type of literature** (such as brochures, leaflets) to **potential consumers** of infant formula or follow – on formula in health care institutions such as in hospital maternity or pediatric wards, clinics, doctor's offices?	Uncertain	No	Not applicable	Yes	Please specify
34. Any **type of literature** (such as brochures, leaflets) to **distributors or retailers** of infant formula or follow – on formula to give to potential consumers?	Uncertain	No	Not applicable	Yes	Please specify
35. **Formula samples** to potential consumers in health care institutions such as in hospital maternity or pediatric wards, clinics, doctor's offices to promote this type of formula?	Uncertain	No	Not applicable	Yes	Please specify

Safety issues

World Health Organization's (WHO) "Guidelines for safe preparation, storage and handling of powdered infant formula" recommends that water with a minimum temperature of 70^0 C should be used to minimize the risk of potentially deadly infections caused by Enterobacter sakazakii, a bacteria that has been found in infant formula. (WHO 2007) Preparation instructions on **majority** of probiotic or synbiotic containing formula tin labels state to boil water for 5 minutes, allow to cool down to 37^0 C or to lukewarm temperature (similar to body temperature). Then add powder according to the provided feeding table.
36. How does your company address the above contradiction to WHO guidelines on formula preparation?

Put an "X" next to the answer that applies to your company

37 Has your company **routinely** tested for pathogens in formula prepared as per the instructions on the label?			
Uncertain	**No**	**Not applicable**	**Yes**
If Yes, the main pathogens **routinely** tested for are:			

Put an "X" next to the answer that applies to your company while providing the following information.

A commentary by the ESPGHAN committee on nutrition states that "There is a lack of published evidence on clinical benefits from long term use of probiotic containing infant formula." (ESPGHAN 2004)				
38. In the last 10 years has your company conducted randomized controlled trials using probiotic(s) or synbiotic(s) infant formula in which the **supplementation and test period was greater than 12 months**?	Yes	No	Not applicable	Uncertain
39. Have these results been published in a peer reviewed journal?	Yes	No	Not applicable	Uncertain
If yes, please specify: Author, Journal, Title of article, Year, volume and page				
40. How does your company address concerns on safety of long term use (longer than 12 months) of probiotic or synbiotic containing infant formula or follow-on formula?				
Uncertain	Not applicable			

References:

ESPGHAN 2004

ESPGHAN Committee on Nutrition. Probiotic Bacteria in dietetic products for infants: a commentary by the ESPGHAN Committee on Nutrition. J Pediatr Gastroenterol Nutr 2004; 38:365-374.

WHO 2007

WHO. Safe preparation, storage and handling of powdered infant formula guidelines. 2007. http://www.who.int/foodsafety/publications/micro/pif_guidelines.pdf. Accessed 17 March 2011

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