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Probiotics, Prebiotics and Synbiotics for Infant Feeding – A Review

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Abstract

Breast milk is considered as the most ideal food for infants. In absence of breast milk, various infant formulae may not be an appropriate substitute as they have not yet been able to meet the critical nutritional and physiological demands of infants. Diversity in microbiota of formula-fed infants in comparison to breast-fed infants has been reported and microbiota could be manipulated by dietary interventions such as probiotics, prebiotics and synbiotics. Research revealed that both probiotics and prebiotics could be safely incorporated into infant food; however safety assessment of the product through *in vitro* and studies on animal and human must be carried to evaluate their immediate and long-term effect on the composition and development of the intestinal microflora especially during the first months of life. In the present review, an attempt has been made to highlight the significance of a healthy intestinal microbiota and the health benefits of probiotics and prebiotics for their inclusion in infant formulae. More exhaustive research for safety and efficacy of infant formula supplementation with probiotics and prebiotics must be evaluated prior to its commercialization. Emergence of a standard methodology for assessing the intestinal flora and its mandatory adoption for conducting related studies is required to arrive upon a conclusive result.

Keywords: Probiotics; Prebiotics; Synbiotics; Infant nutrition

Introduction

Human breast milk is always seen as the preferred choice for infant nutrition [1]. Human milk has been promulgated to be entirely sufficient and must be initiated within first hour after birth [2]. Owing to its inherent therapeutic and nutritional features [3,4]. United Nations Children's Fund [5], American College of Obstetricians and Gynecologists [6], American Academy of Family Physicians [7], World Health Organization [8], American Academy of Pediatrics [9] and various health organizations have recommend exclusive breastfeeding for the first 6 months of life. Prolonged exclusive breast feeding may require iron supplementation starting from 6 months [10,11]. An early supplementation at < 6 months is suggested in case of preterm and low birth weight infants and infants with hematologic disorders or infants who had inadequate iron stores at birth [12,13]. It was pointed out that even human milk does not completely satisfy the nutritional requirement of infants and required to be supplemented with minerals and vitamins [11,14,15]. Finally, the World Health Organization and United Nations Children's Fund (UNICEF) recommend exclusive breastfeeding for all newborns with no supplementation [16]. Higher protein and mineral contents in cow and buffalo milk exerts renal osmolar load due to under developed kidney function of infants and therefore are not suitable alternative for infant feeding [17]. Further, technological innovations made in the commercial infant milk powder have not yet been able to meet the critical nutritional and physiological demands of infants [18]. It was noted that modification of infant formulae are constantly being carried with the characterization of components of human milk and identification of nutritional needs of infants, resulting in formulation of special humanized milk with specific functions [19].

Diversity in microbiota of formula-fed infants in comparison to breast-fed infants due to presence of oligosaccharide in human milk [20] have led to suggest that intestinal microflora of formula-fed infants could be manipulated through dietary interventions such as probiotics, prebiotics and synbiotics. Compared to probiotics, which introduces exogenous bacteria into the colonic microflora, a prebiotic aim at stimulating the growth of one or a limited number of potentially health promoting indigenous bacteria, thus modulating the composition of the natural microbial ecosystem [21], modifying immune responses in the GALT (Gut Associated Lymphoid Tissue), enhanced cytokine production and other immune function [22]. Probiotic dairy products containing human isolates of lactobacilli and bifidobacteria as well as prebiotic food formulae, capable of exhibiting health benefits are recently available in the market [23]. In the present review, an attempt has been made to highlight suitability of probiotics, prebiotics and synbiotics for their inclusion in infant formulae.

Significance of colonic microbiota

Recent studies on intestinal microbes have demonstrated that the microbial ecosystem within the human gastrointestinal tract is exceedingly complex [24]. Development of a normal intestinal flora is a gradual and complex process and resulted from a complex interplay of nutritional, immunological and environmental factors [25] and can be classified broadly into 5 phases of microbial succession (Table 1). Various factors influencing the development of the intestinal microflora are mode of delivery, environment during birth, hygiene measures, environmental contaminants, faecal, vaginal and skin flora of mother, developmental stage of the gastrointestinal tract and type of feed used [27].

At birth, the infant's gastrointestinal tract (GIT) is sterile and is subsequently colonized within hours by microorganisms from various sources such as environment and maternal vagina and faeces [28]. It has been mentioned that factors like mode of delivery influences the colonization of intestine during neonatal period [29] and bifidobacteria

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capable of producing conjugated linoleic acid (CLA) develop in infants shortly after birth, resulting considerable genomic diversity in neonatal bifidobacteria population [30]. Extent of colonization of gut with Bifidobacterium and Lactobacillus was faster (10 days vs. 1 month) for vaginally delivered infants than those delivered caesarean, which remained lower (36 vs. 76%) even after 6 months. Cesarean delivered infants were significantly less often colonized with *Bacteroides fragilis* group than vaginally delivered infants [31]. Studies based upon PCR technique revealed less diverse microbiota containing no bifidobacteria species in cesarean delivered infants but predominance of *Bifidobacterium longum* and *Bifidobacterium catenulatum* in vaginally delivered infants [32].

Intestinal environment of neonates shows a positive oxidation/reduction potential at birth and the gastrointestinal tract is colonized first by facultative aerobes and gradual uptake of oxygen by these bacteria changes creates a more-reduced one, permitting the subsequent growth of strict anaerobes [33]. Initially infant's gut flora constituted of facultative anaerobes such as enterobacteria, *Streptococcus* and *Staphylococcus*. After the first week, *Bifidobacterium* and *Lactobacillus* species appear and the former constitute as the predominant bacterial species [34-36] and remain an important component of the gut microbiota in infancy [37]. Constituents of intestinal flora vary within the GIT. Colonic microflora was reported to be consisted of facultative anaerobes like *enterobacter, streptococci, staphylococci, lactobacilli, Propionibacterium* and bacilli in the upper portion of the colon and strictly anaerobes like Bacteroides, *Bifidobacterium,* eubacteria, peptococci, *Fusobacterium* and *Clostridium* on lower portion of colon [38]. Ratio of anaerobic: facultative organisms remains at 1000:1 at the centre of lumen and dropped to 10:1 at the epithelial surface within the intestinal crypts [39] and bacteroides and *Bifidobacterium* accounts for 30 and 25% of the total anaerobic constituents, respectively [40]. Bacterial density increased from a level of 10^3 cfu/ml in the stomach to 10^4-10^6 cfu/ml in the small intestine or more than 10^{12} cfu/ml in the colon [38]. Density and nature of bacteria in the human GIT is shown in Table 2.

Formula and breast-fed infants have very different early intestinal bacterial flora ` Predominant flora in breast-fed infants comprises of Lactobacilli and Bifidobacteria, whereas in a formula-fed infant, it consisted of more *bacteroides*, *Clostridium* and *Enterobacteriaceae*

Phases	Stage of life	Dominant flora
Phase 1 Birth		*Escherichia coli*, *Clostridium* sp.,
	(0-24h)	*Streptococcus* sp., *Bacteroides*
Phase 2 Infancy		*Lactobacillus* sp., *Lactobacillus* sp.,
	(after 24h)	bifidobacteria, Few clostridia, Few *Bacteroides*
Phase 3	Weaned	
Phase 4	Adults	*Bacteroides*, *Eubacterium*, *Peptostreptococcus*, *Streptococcus*, *Clostridium*, *Bifidobacterium*, *Veillonella*, *Escherichia coli*, *Fusobacterium*
Phase 5	Old age	*Clostridium perfringens*, *Lactobacillus*, *Streptococcus*, *Enterobacteriaceae*

Table 1: Different phases of development of human intestinal flora [26].

Site	Density/ml	Type of flora
Stomach and proximal ileum	10^3-10^4	Predominant Gram(+)ve
Terminal ileum	10^6-10^7	Predominant Gram(-)ve
Colon	10^{11}-10^{12}	Predominant Gram(-)ve

Table 2: Density and nature of bacteria in the human GIT [41].

[43]. Predominance of *Bacteroides fragilis* within 7 days of age in approximately two-thirds of formula-fed infants compared with only 22% of breastfed infants was noticed [44]. *B. catenulatum* was the most prevalent group in infants after 5 days and *Bifidobacterium longum* after 3 weeks of age [45]. Other bifidobacteria species detected in breast-fed infants are *Bifidobacterium bifidum* and *Bifidobacterium breve* and *B. longum* [46,47]. Due to variance in the intestinal flora formula-fed infants are more prone to gastrointestinal infections than breast-fed infants [48], however, recent studies revealed no significant differences between these groups [49].

Factors explaining discrepancies between studies comparing the microflora of breast and formula-fed infant are buffering capacity of formula feeds, changes in formulation, obstetric practices and environment and bacterial identification and methodology [27]. Most of studies on human intestinal microbiota have been based upon the assumption that faecal flora is reflecting the prevalent intestinal microbiota. The conventional culture based methods being time consuming and failed to grow more than 20-50% of intestinal bacteria led to their replacement by a more sensitive, specific and rapid molecular 16S DNA detection system based on the polymerase chain reaction [50-52]. Conventional Polymerase chain reaction (PCR) method is a faster and more reliable than culturing techniques, but post-PCR steps required for evaluation of the amplification product are laborious and further, these end-point results are not very precise and are only semi-quantitative [53]. A modified PCR technique called real-time PCR technique was developed to combat with the drawbacks of the conventional PCR technique which allow the continuous quantification of DNA as the reaction is proceeding [54]. Real-time PCR analysis of bacterial DNA isolated from faecal specimens has become increasingly used for the quantification of indigenous intestinal microbiota. It has been reported that neonate's microflora obtained by means of culture-independent analysis are in agreement with those produced by selective media [55]. A standard methodology for assessing the intestinal flora should be formulated and its adoption must be made mandatory for conducting related studies to arrive upon a conclusive result.

Preterm infants are particularly susceptible to abnormal colonization. Diversity in gut flora of preterm infants and term infants [56,57] and delayed bifidobacteria colonization [56] coupled with higher prevalence of *Clostridium difficile* [58] in preterm infants may be attributable to the use of parenteral nutrition and antibiotic therapy for extended periods [56]. Various factors such as the immature intestinal function, frequent use of broad-spectrum antibiotics, delay in initiating enteral feeding, infection control procedures and sterilization of milk limit the exposure of preterm infants to normal commensal microorganisms [59] and are therefore prone to systemic infections due to increased intestinal permeability to potentially pathogens [60]. Stool specimens of extremely low birth-weight (<1000 g) infants were predominated with *Enterococcus faecalis, Escherichia coli, Staphylococcus epidermidis, Enterbacter cloacae, Klebsiella pneumoniae*, *Staphylococcus haemolyticus*, however *Lactobacillus* and bifidobacteria spp could be identified in only one stool specimens [61].

Substantial compositional change in the intestinal microbiota occurs during weaning [62] and the intestinal microflora shifts to a more adult like pattern with fewer *E. coli*, *Clostridium* sp. [63], bifidobacteria [64] and more bacteroides, Gram (-) ve cocci [63], fungi and enterobacter and the flora remain s relatively stable after 2 years [64]. Intestinal microbiota contains a complex and diverse society of both pathogenic and non-pathogenic organisms [65]. Establishment

of pathogens impairs the integrity of intestinal microbiota [66] and alterations in the gut composition induced manifestation of atopic disease [67]. A healthy intestinal microbiota is considered to be important for priming of infant's mucosal and systemic immunity [68] and could be modulated by dietary interventions.

Postulated health benefits of probiotics

Probiotics are live bacteria which transit GIT and benefit the health of consumers [69] and must be ingested in sufficient quantities to deliver the relevant dose of live bacteria to the gut [70]. Recently, probiotic research has received significant attention and has been exploited of as potential therapeutics for the developing world, for the treatment of chronic and acute enteric infections. Postulated health benefits of probiotics are enumerated below.

- Improves digestion
- Increases natural resistance to infectious intestinal diseases [71]
- Decrease in fecal enzyme and mutagenicity
- Exhibit hypocholesterolemic effect [72]
- Enhance immune system
- Synthesis and enhancement in bioavailability of nutrients
- Reduce incidence of lactose-intolerance
- Reduce risk of cancer
- Prevent allergy [73]
- Down-regulate hypersensitivity reactions
- gut mucosal dysfunction [74]
- Protection of mucosa from colonization by pathogens
- Increase anti-inflammatory cytokines [25]
- Enhancing intestinal microbial balance
- Restore intestinal permeability [75]

Probiotic foods for infants

Recently, an aggravated inclination towards health foods have led to the introduction of probiotic organisms in cultured milk products intended for infant feeding. Probiotics exert therapeutic effects by positively influencing normal microbe-microbe and host–microbe interactions [76] and may augment the protection against infections by commensal flora through competitive interactions, direct antagonism of pathogens, and/or production of antimicrobial factors [77] or by altering the intestinal lumen pH by producing potentially microbicidal short-chain volatile fatty acids, which may inhibit the proliferation of pathogenic microorganisms [61,78,79]. Probiotic combinations are required to counteract the complex microbiota deviations at different sites of the intestinal tract and combinations of specific probiotics strains may exhibit enhance health effects on the host. Bacterial adherence to the intestinal epithelium or invasion of pathogenic bacteria into the mucosa is preceding steps for both colonization and infection in the gastrointestinal tract [80]. Reviewed literature indicated that a number of cultured milk products containing probiotics have already been developed [81,82] and most of them could be successfully employed for weaned as well as lactose-intolerant infants [83]. Cultured milk products prepared employing specially selected strains of lactobacilli and bifidobacteria was noted to play an important role in nutrition of children aged < 8 years [84].

Ability of bifidobacteria to withstand the conditions prevailing in the GIT and transiently colonizing the intestine [85], reducing the *E. coli* population and protecting against an increase in bacteroides [86] have led to their introduction in cultured milk products for infant feeding. Cultured milk products based on bifidobacteria were reported to be suitable for the treatment of convalescent children, lactose-intolerant children, maintenance of intestinal flora, prevention of food allergy [87], recovery of antibiotic associated diarrhoea [88] and sustaining healthy intestinal flora during and after weaning [85]. Health beneficial effects of bifidobacteria based fermented milk products is influenced by the strains of bifidobacteria employed. Children feeding trials revealed higher efficacy of *B. breve* than erythromycin for eradication of *Camylobacter jejuni* from stools [89] and *Bifidobacterium lactis* for reducing the incidence of diarrhoea [90]. A controlled, double-blind, randomized trial, revealed that amongst various combinations of additives in formula a specific mixture containing *B. longum* BL999 and *Lactobacillus rhamnosus* LPR exhibited a prolonged effect in reducing the incidence of diarrhea in healthy full-term infants [91]. Another double-blind, placebo-controlled, randomized clinical study reported that *B. lactis* Bb12 supplementation induced a significantly higher faecal bifidobacteria population ($\log_{10}$ 8.18±0.54 vs. $\log_{10}$ 4.82± 0.51) and lower viable counts of *Enterobacteriaceae* ($\log_{10}$ 7.80±0.34 vs. $\log_{10}$ 9.03±0.35) and *Clostridium* spp. ($\log_{10}$ 4.89±0.30 vs. $\log_{10}$ 5.99±0.32) than the infants in the placebo group [92]. Specific probiotic bacteria have been reported to stabilize the gut microbial environment and the intestine's permeability barrier thus enhancing systemic and mucosal IgA responses [93] and supplementation of infant milk formula with *Bifidobacterium animalis* BB-12 or *Lactobacillus* GG was noted to modify the allergic inflammation in infants with atopic eczema [94]. Clinical trials have shown significant reduction in incidence of allergy during the first 6 months of life, resulting from the use of extensively hydrolyzed infant formula containing *L. rhamnosus* GG or *B. lactis* Bb-12, however, the exact mode of action are not yet known [95].

Probiotics may benefit the immature gut by preventing the overgrowth of pathogens, such as members of the *Enterobacteriaceae* family and coagulase-negative staphylococci, which are common pathogens in NICU infections [96]. Lower incidence of death or necrotizing enterocolitis (9 of 180 vs. 24 of 187) was noted in very low birth weight (<1500 g) infants receiving Infloran containing *Lactobacillus acidophilus* and *Bifidobacterium infantis* with breast milk in comparison to those receiving breast milk alone [97]. Prophylactic probiotic supplementation is recommended for premature neonates with a weight range of 1000-1500g [98] and systematic reviews of randomized, controlled trials indicated lower mortality and necrotizing enterocolitis in preterm very low birth weight neonates [99].

Therapeutic significance of yoghurt ingestion for children with persistent diarrhoea is dependent upon its severity and co-existing systemic infection [100] and its efficacy augmented with the conjugated application of *Lactobacillus casei* [101] and *B. bifidum* [102]. It has been reported that *Streptococcus thermophilus*, when grown in association with *B.bifidum* enhanced the efficacy to reduce rotavirus shedding [103] and in combination with *B. lactis* reduced the episodes of colonic irritability and resulted in a lower prevalence of antibiotic use [104]. Feeding of dietetic yoghurt supplemented with *B.bifidum* and *Propionibacterium freudenreichii* subsp. *shermanii* containing 10^8 cells/g, to infants induced a diminution in faecal viable bacteria and coliforms accompanied by an increment in counts of lactobacilli and bifidobacteria after 7 days [105].

Ingestion of acidophilus milk for a short duration induced recovery of only 40-70% of children with dysentery, however long-term feeding

resulted in complete recovery [106]. Associative application of propionic acid bacteria with *L. acidophilus*, *B.bifidum* and *Leuconostoc citrovorum* are recommended for obtaining dietary supplements with intensified viability of these organisms in the human GIT [107]. Inclusion of *B. bifidum* in *kefir* [108] or *P. freudenreichii* subsp. *shermanii* in acidophilus milk [109] proved to be more efficacious than ordinary *kefir* or acidophilus milk in eliminating intestinal infection and preventing GIT disorders in children and infants.

Postulated health benefits of prebiotics

"A prebiotic is a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microbiota, that confers benefits upon host wellbeing and health" [110]. A more recent definition stated that a prebiotic is a non-digestible food ingredient that benefits the host by selectively stimulating the favorable growth and/or activity of one or more indigenous probiotic bacteria [111,112]. Postulated health benefits of prebiotics are enumerated below.

- enhanced resistance to invading pathogens
- improved bowel function
- Anti-colon cancer properties
- Lipid lowering action
- improved calcium bioavailability [21]
- Osteoporosis management
- Alleviation of menopausal symptoms [113]
- Beneficial for cardiovascular disease associated with dyslipidemia and insulin resistance, obesity and possibly type 2 diabetes [72]
- Improvement of vitamin supply
- Effect on defacation and flatulence [114]

Prebiotic foods for infants

Diversity in the intestinal microbiota of bottle-fed and breast-fed infants [115], could be attributed to the prebiotic effect of oligosaccharide (OS) presenting human milk [20]. Human milk OS acts as prebiotic soluble fiber and plays a significant role in post-natal development of the intestinal flora [116] and it has been suggested that early intestinal bifidobacteria composition of infant formulae could be achieved with the supplementation of infant formulae with Galacto-oligosaccharide (GOS) + Fructo-oligosaccharide (FOS) up to the age of 6 months [117].

Concentration and composition of OS in human milk differ considerably among individuals and during the course of lactation [118] and contains more than 130 different oligosaccharides at a concentration of 15–23 g/L in colostrum and 8–12 g/L in transitional and mature milk [119,120]. Prebiotics are introduced in infant formulae with the objective of obtaining a bifidogenic effect identical to breast-fed infants [121] and inclusion of OS may beneficially modify the composition of the gut microbiota of formula-fed infants [122] and in immunomodulation [119] by acting as a receptor for bacteria and viral adhesion molecules [123] and stimulate the bifidus flora in the colon [124]. Prebiotics change the composition of fecal bacteria either by increasing beneficial bacteria such as lactobacilli and bifidobacteria, which modulate the activity of the immune system or by decreasing organisms such as clostridia and protein-degrading *Bacteroides*, which can produce tumor-promoters from metabolism of proteins that escaped digestion in the upper gut [125].

Most of the experiments with prebiotics is confined with GOS and FOS and generally a combination of 90 % GOS and 10 % FOS is employed as it exhibits a synergistic effect towards growth promotion of bifidobacteria and lactobacilli in the intestine [126]. Infants ingesting a standard formulae containing FOS+ GOS (0.8g / 100g) induced an increase in faecal bifidobacteria population from 54.8 ± 9.8 to 73.4 ± 4.0 % [68] with a diversity of bifidobacteria species similar to breast-fed infants [127] and stool characteristics identical to those recorded for pre-term infants with human milk [128]. Other infant feeding trials reported an increase in bifidobacteria population by 43-57 % [129] or 59.6 % [130] and the population reached to a level of 10.0 ± 2.05 log cfu/g [131] or 9 x 10^{12} cfu/g faeces [115]. An elevation in the bifidobacteria population [10] folds) and faecal lactate (4 folds) accompanied by lowering of faecal pH were observed with the inclusion of 0.7 % FOS in infant formulae [132]. Extent of bifidogenesis by prebiotics is dose-dependent, higher being noted at higher dosages (0.8g/ 100ml) than at lower dosages (0.4g/ 100ml) during infant feeding trials [133] and reviewed literature suggested a dosage of 4g/ day to be optimum [134]. Stool analysis of bottle-fed preterm infants receiving an infant formula containing 0.4 g/dL FOS revealed higher population of bifidobacteria and bacteroides but a lower *E. coli* and enterococci in comparison to those receiving infant formula which contained maltodextrin [135]. Supplementation of an infant formula with 0.4g/100 ml GOS+FOS induced significantly lower faecal clostridia and higher faecal bifidobacteria population as determined by fluorescent in situ hybridization in contrast to infants fed with infant formula without prebiotic [136]. Recent studies revealed that introduction of prebiotic mixture in infant formulas might extend clinical benefits by decreasing the risk of developing atopic dermatitis in high risk infants and a reduced incidence of intestinal as well as upper airway infections in the first year of life [137].

Synbiotic foods for infants

Synbiotics, a combination of probiotics and prebiotics have also been employed to enhance the health benefits of infant formulae. Published literature indicated that continued probiotic administration resulted in an initial colonization with probiotics but only few viable probiotics could be recovery in the stools at 10-12 days. Further, permanent colonization of bifidobacteria in low birth weight infants were noted due to introduction of a preterm formula enriched with prebiotics and remained stable at variance from foreign administered probiotics [138-140].

Clinical reports have identified that a combination of milk derived OS and *B. lactis* can confer protection to children suffering from diarrhoea and severe illness and reduced the need of antibiotics therapy [141]. Bifidus factor in infant formulae containing *B. bifidum*, *B. infantis* and *B. longum* could be enhanced with the introduction of 0.5 % lactulose (another prebiotic) or FOS [142], maximum growth stimulation of bifidobacteria being recorded in infant formulae based on milk [143]. A randomized double blind study indicated higher abatement in bifidobacteria population due to inclusion of FOS/ GOS in infant formulae containing *B. animalis* (59.20 %) than those noted for formulae containing only probiotic culture (52.70 %) or a standard formula (51.80 %) after 16 weeks of infant feeding [144]. Recently, a double-blind, placebo-controlled multi-centre trial, revealed that synbiotic mixture containing *Bifidobacterium breve* M-16V and a galacto-/fructooligosaccharide mixture induced significantly higher population of bifidobacteria (54.7 vs. 30.1%) accompanied by lower population of *Clostridium lituseburense*/*Clostridium histolyticum* (0.5

vs. 1.8%) and *Eubacterium* rectale/*Clostridium coccoides* (7.5 vs. 38.1) but failed to demonstrate beneficial effect on atopic dermatitis severity in infants [145]. Thus synbiotics may be more advantageous than probiotics or prebiotics for promoting infant's health but long-term safety must be evaluated.

Safety of probiotics and prebiotics infant feeding

Worldwide regulations related to probiotics are incoherent and therefore efficacy of probiotic, prebiotic and synbiotic products available in the market varied considerably in terms of food safety. In Europe, dietary supplements intended for use by infants and young children have specific compositional legal requirements [146] but in United States, pre-market review and Food and Drug Administration approval are generally not required for dietary supplements but are prerequisites for as biological products, marketed specifically for the treatment or prevention of a disease. Pre-market review for probiotics marketed for specific health benefits by the Therapeutic Goods Administration in Australia and by the Health Ministry in Japan are required [147].

Historical data suggested that lactobacilli and bifidobacteria can be safely employed for human use [148,149] and these organisms are considered as GRAS (Generally Regarded as Safe) and traditionally been exploited during the manufacture of cultured milk products [150,151]. Probiotic foods intended for human consumption generally employ various lactic acid bacteria such as *Lactobacillus*, *Bifidobacterium* and *Streptococcus*, which are the inhabitants of the intestine [152,153] and probiotic therapy appeared as safer than pharmaceutical agents [154]. Human trials have confirmed that lactobacilli such as *L. rhamnosus*, *Lactobacillus reuteri*, *Lactobacillus delbrueckii* subsp. *lactis* and *L. casei* subsp. *alactus* are safe [155] and *L. rhamnosus*, *L. acidophilus* and *B. lactis* did not degrade gastric mucin [156,157], which acts as mucosal barrier and any disturbance to the mucin layer will imbalance the host's mucosal defence function [158]. It was concluded that the probiotic products available in the market are safe but zero risk does not exist as probiotics may have also some side effects, therefore further epidemiological and clinical studies are emergent for consumer safety [159]. Probiotic bacteria should be characterized during preclinical and clinical evaluations as each strain has specific properties that cannot be extrapolated from other and must be obtained from acceptable sources with a proven safety record and efficacy to guarantee their future clinical applications [93]. Probiotics besides having an excellent overall safety record, their suitability for premature infants or in infants with immune deficiency, must be evaluated because of limited data regarding the mechanisms of probiotic action, appropriate administrative regimens, and probiotic interactions. Further efficacy of probiotic is strain-specific and prophylactic effects of one probiotic strain should not be generalized for strains [160]. Safety assessment of the product through *in vitro* and studies on animal and human has already been recommended [161]. Following approaches are suggested for assessing the safety of a probiotic strain [159].

- Evaluating the intrinsic properties of the strain
- Evaluating the pharmacokinetics of the strain
- Investigate the interactions between the strain and the host.

Infant formulae and follow-on formulae must not contain any substance in a concentration that may cause health hazards to the infants and young children. Based upon various research reports European Commission has approved inclusion of 90% GOS and 10% FOS at a maximum level of 8 g/L in infant formulae and follow-on formulae [162,163]. Later, Scientific Committee on Food had confirmed the statements in the SCF Report on Essential Requirements of Infant Formulae and Follow-on Formulae [164]. FDA has approved FOS as a GRAS ingredient, but no pre-market notifications have been submitted for infant formula containing oligo-saccharides [165]. Incorporation of prebiotics in infant formulas has been reported in Japan and Europe but intervention studies must be carried to evaluate their immediate and long-term effect on the composition and development of the intestinal microflora, and on the resulting bacterial-bacterial and bacterial-host interactions are not known especially during the first months of life [166]. Long-term benefit of prebiotics must be evaluated by confirmatory well-designed clinical research studies [167] and safety and efficacy of infant formula supplemented with prebiotics must be evaluated through at least two randomized control studies prior to its commercialization [168]. The Committee on Nutrition of the European Society for Paediatric Gastroenterology, Hepatology, and Nutrition reported that presently, there is insufficient data to recommend the routine use of probiotic-and/or prebiotic-supplemented formulae [169] and stressed upon the need to define the mechanisms, optimal doses and intake durations, as well as provide more information about the long-term safety of probiotics and/or prebiotics [169-171].

Conclusion

Colonization and composition of the intestinal microbiota after birth play a pivot role towards conferring immunity to infant to the new environment. Composition of the intestinal microbiota can be modulated either by administration of probiotics, the living health-promoting bacteria that survive the gastrointestinal tract or by introduction of prebiotics, the non-digestible dietary ingredients that selectively stimulate health promoting colonic bacteria. Routine application of probiotic- and/or prebiotic-supplemented formulae is not recommended and certain parameters such as mechanisms, optimal doses and intake durations, as well as long-term safety of these products require extensive clinical trials. A standard methodology for assessing the intestinal flora should be formulated and its adoption must be made mandatory for conducting related studies to arrive upon a conclusive result.

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