Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.14.6564

World J Gastroenterol 2008 November 14; 14(42): 6564-6568
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Low level of galacto-oligosaccharide in infant formula stimulates growth of intestinal *Bifidobacteria* and *Lactobacilli*

Xiao-Ming Ben, Juan Li, Zong-Tai Feng, Sheng-Yun Shi, Ya-Dong Lu, Rui Chen, Xiao-Yu Zhou

Xiao-Ming Ben, Zong-Tai Feng, Ya-Dong Lu, Rui Chen, Xiao-Yu Zhou, Department of Neonatology, Nanjing Children's Hospital of Nanjing Medical University, Nanjing 210008, Jiangsu Province, China
Juan Li, Department of Pediatric Immunology, Nanjing Children's Hospital of Nanjing Medical University, Nanjing 210008, Jiangsu Province, China
Sheng-Yun Shi, Pediatric Research Institute, Nanjing Children's Hospital of Nanjing Medical University, Nanjing 210008, Jiangsu Province, China
Author contributions: Ben XM and Li J contributed equally to this work; Ben XM designed the research; Li J, Feng ZT, Lu YD, Chen R, Zhou XY performed the research; Shi SY contributed to the new reagents/analytic tools; Ben XM and Li J analyzed the data, and wrote the paper.
Correspondence to: Dr. Xiao-Ming Ben, Department of Neonatology, Nanjing Children's Hospital of Nanjing Medical University, 72 Guangzhou Road, Nanjing 210008, Jiangsu Province, China. benxm@163.com
Telephone: +86-25-51554501 Fax: +86-25-83304239
Received: July 11, 2007 Revised: October 13, 2008
Accepted: October 20, 2008
Published online: November 14, 2008

Abstract

AIM: To investigate the effect of a new infant formula supplemented with a low level (0.24 g/100 mL) of galacto-oligosaccharide (GOS) on intestinal micro-flora (*Bifidobacteria*, *Lactobacilli* and *E. coli*) and fermentation characteristics in term infants, compared with human milk and a standard infant formula without GOS.

METHODS: Term infants (n = 371) were approached in this study in three hospitals of China. All infants started breast-feeding. Those who changed to formula-feeding within 4 wk after birth were randomly assigned to one of the two formula groups. Growth and stool characteristics, and side effects that occurred in recruited infants were recorded in a 3-mo follow-up period. Fecal samples were collected from a subpopulation of recruited infants for analysis of intestinal bacteria (culture technique), acetic acid (gas chromatography) and pH (indicator strip).

RESULTS: After 3 mo, the intestinal *Bifidobacteria*, *Lactobacilli*, acetic acid and stool frequency were significantly increased, and fecal pH was decreased in infants fed with the GOS-formula or human milk, compared with those fed with the formula without GOS. No significant differences were observed between the GOS formula and human milk groups. Supplementation with GOS did not influence the incidence of crying, regurgitation and vomiting.

CONCLUSION: A low level of GOS (0.24 g/100 mL) in infant formula can improve stool frequency, decrease fecal pH, and stimulate intestinal *Bifidobacteria* and *Lactobacilli* as in those fed with human milk.

Key words: Human milk; Prebiotic; Probiotic; Safety; Chinese

Peer reviewer: Lynne V McFarland, Puget Sound VA, HSR&D, 1100 Olive Street, Suite #1400, Seattle, Washington WA 98101, United States

Ben XM, Li J, Feng ZT, Shi SY, Lu YD, Chen R, Zhou XY. Low level of galacto-oligosaccharide in infant formula stimulates growth of intestinal *Bifidobacteria* and *Lactobacilli*. *World J Gastroenterol* 2008; 14(42): 6564-6568 Available from: URL: http://www.wjgnet.com/1007-9327/14/6564.asp DOI: http://dx.doi.org/10.3748/wjg.14.6564

INTRODUCTION

Breastfeeding is the primary choice for newborns. However, for some reason, many infants are formula fed. Breast milk is superior over artificial formula in many aspects, including its effect on the development of intestinal microflora. Breast-fed infants have a higher level of intestinal *Bifidobacteria* and *Lactobacilli*[1,2], both of which are known to be potentially beneficial to the health of their hosts[3,4]. It is likely that oligosaccharides in human milk are more beneficial to intestinal flora[5-7].

The amount of oligosaccharides in mature human milk is in range of 12-15 g/L. Most of oligosaccharides are fermented in the large intestine[8,9]. Galacto-oligosaccharide (GOS) contained in oligosaccharides, is a short-chain galactose with a terminal glucose molecule[10], which is produced from lactose and commercially available. Studies have shown that GOS can selectively stimulate development of *Bifidobacteria*[11]. However, only a few studies are available on oligosaccharides in infant

formula[12,13], and there is no study using only GOS in infant formula.

Recently, Moro *et al*[13] showed that the number of *Bifidobacteria* and *Lactobacilli* increases significantly compared with a control formula with maltodextrin instead of the oligosaccharides in term infants fed with formula at the dose of 0.4 g and 0.8 g of oligosaccharide per 100 mL (90% GOS and 10% low-molecular weight fructo-oligosaccharides). There was no difference between the two supplemented groups. These values have been adopted by the Scientific Committee on Food (SCF: April, 2003) of the European Commission and probably will be implemented in European regulations. In preterm infants, the same mixture of oligosaccharide (1.0 g/100 mL) could stimulate growth of *Bifidobacteria* and result in stool characteristics as seen in human milk-fed infants[12]. Recently, however, a rat study[14] showed that the intestinal cell wall is irritated and the risk of bacterial translocation increases (following orally *Salmonella enteritidis* infection) when fructooligosaccharides are provided at a high (3% and 6% of dry matter), but not an unrealistic amount (maximum level adopted by the SCF is about 6% of dry matter). Therefore, positive effects of oligosaccharides on intestinal bacteria may not always justify the levels tested.

Our hypothesis is that a prebiotic effect of the human milk-like GOS is already present at a much lower level than that currently adopted by the SCF. This study was to investigate the effect of 0.24 g GOS per 100 mL on intestinal microflora colonization and fermentation in formula-fed term infants compared with breast-fed and control formula-fed counterparts, and to detect the lowest and safe effective level of GOS.

MATERIALS AND METHODS

Subjects

Three hundred and seventy-one healthy term infants, appropriate for gestational age, were approached in the study by Nanjing Children's Hospital of Nanjing Medical University (n = 181), Nanjing Maternal Hospital of Nanjing Medical University (n = 90) and Affiliated Hospital of South East University (n = 100). Finally, 164 infants were recruited for the 3-mo follow-up (Table 1). Those excluded from the study were due to refusal of their parents and failure in taking the fresh fecal samples. The Ethics Committees of the three hospitals approved the study and all the parents gave their informed consent before enrolment in the study.

Diets

When not breast-fed, the infants were randomly assigned to test formula group and control formula group (Table 1). The test formula (Frisolac Advanced, Friesland Nutrition, Netherlands) group was supplemented with GOS (0.24 g/100 mL), while the control formula (Frisolac H, Friesland Nutrition, Netherlands) group was not supplemented with GOS. The other infants were either breastfed or received a combination of breast milk and test formula.

Growth and stool characteristics of, and side effects in the infants were recorded during the 3-mo follow-up. The body weight of all infants was measured using a scale with an accuracy of ± 5 g. The crown-heel length and head circumference were measured using a special board for newborn infants with an accuracy of ± 1 mm. Stool consistency (score 1-4: 1 = watery, 2 = loose/mushy, 3 = soft formed, 4 = hard formed) and frequency were recorded based on an interview with the mother. Crying (score 1-3: 1 = practically not crying, 2 = crying in connection with feeding, 3 = crying independently from the meals), regurgitation (score 1-3: 1 = no regurgitation, 2 = 1-2 regurgitations, 3 = > 2 regurgitations per day), and vomiting (score 1-3: 1 = no vomiting, 2 = 1 episode of vomiting, 3 = > 1 episode of vomiting per day) were also recorded based on an interview with the mother.

Fecal samples were collected from a subpopulation (Tables 2 and 3) for analysis of intestinal bacteria (n = 82), short chain fatty acids (SCFAs) (n = 96) and pH (n = 112). For analysis of *Bifidobacteria* and *Lactobacilli*[1,12,13], one gram of fresh feces was homogenized and diluted in 10 mL of a pre-reduced brain and heart infusion broth in an anaerobic glove box within 1 h of collection. Ten μL of each dilution was spread on the surfaces of Rogosa SL agar (Difco, Detroit, USA) dishes and incubated anaerobically at 37℃. Colony forming units (cfu) of *Lactobacilli* were marked after 2 d, whereas cfu of *Bifidobacteria* were counted after 4 d. For detection of *E. coli*, 1 g of fresh feces was homogenized and diluted in 10 mL of a pre-reduced brain and heart infusion broth in a clean airflow bench. Ten μL of each dilution was spread on the surfaces of MacConkey agar (Difco, Detroit, USA) dishes and incubated aerobically at 37℃ for 1 d. Cfu were expressed as per gram of feces.

Concentration of SCFA acetic acid was determined by gas chromatography as previously described[15]. Briefly, weighed fecal samples were diluted at approximately 1:10 in a 0.1 mol/L sodium phosphate broth (pH 6.5), and suspensions were used to determine the concentration of acetate using a Hewlett-Packard 5890A Series Ⅱ gas chromatograph (Agilent, Wilmington, DE) and a glass column (180 cm × 4 mm i.d.) packed with 10% SP-1200/1% H_3PO_4 on 80/100 mesh Chromosorb WAW (Supelco, Bellefonte, PA). Nitrogen was the carrier gas with a flow rate of 75 mL/min. Temperature of the oven, detector and injector was 125℃, 175℃ and 180℃, respectively. SCFA concentrations in the blank tube were used to correct non-substrate SCFA production. pH of the fresh stool sample was measured with a piece of multicolor indicator paper with an accuracy of 0.2 U (Spezialindicatorpapier Merck Eurolab GmbH, Darmstadt, Germany).

Statistical analysis

All data were given as mean ± SD. An overall group effect on a measured variable was evaluated by ANOVA with F value. When significant, this was followed by t test for single factor group comparisons. $P < 0.05$ was considered statistically significant. SPSS 12 (SPSS Institute Inc., Chicago, USA) was used in analysis of data.

Table 1 Clinical data of the infants enrolled in the study

	GOS formula	GOS formula & human milk	Human milk	Control formula	*F*/*P* value
Babies recruited in 3 mo follow-up	*n* = 37	*n* = 58	*n* = 24	*n* = 45	
Male/Female	20/17	30/28	13/11	24/21	
Gestational age (wk)	38.7 ± 0.6	39.1 ± 0.8	39.4 ± 0.7	38.8 ± 0.8	1.40/0.24
Weight at birth (kg)	3.30 ± 0.42	3.20 ± 0.43	3.34 ± 0.43	3.35 ± 0.45	1.28/0.28
Length at birth (cm)	49.50 ± 0.96	49.47 ± 1.15	49.61 ± 1.23	49.57 ± 1.03	0.13/0.94
Head circumference at birth (cm)	34.15 ± 0.55	33.98 ± 0.72	34.07 ± 0.81	34.25 ± 0.77	1.35/0.26
Feeding volume (mL/kg-BW/d)	162 ± 27			157 ± 34	
Weight gain during study period (g/d)	41.26 ± 5.22	43.35 ± 4.87	40.97± 5.06	40.59 ± 3.95	1.54/0.21
Length gain during study period (cm/wk)	0.95 ± 0.11	1.01 ± 0.11	0.93 ± 0.10	0.96 ± 0.11	1.94/0.13

Data are expressed as mean ± SD. GOS formula: Galactooligosaccharides in an amount of 0.24 g/dL. Control formula does not contain added GOS.

Table 2 Levels of intestinal bacteria at the end of a 3-mo feeding period as measured in fresh feces

	GOS formula (*n* = 20)	GOS formula & human milk (*n* = 29)	Human milk (*n* = 15)	Control formula (*n* = 18)	*F*/*P* value
Bifidobacteria	9.01 ± 1.18	8.97 ± 0.85	9.25 ± 0.93	8.16 ± 0.99	4.08/0.01
Lactobacilli	5.91 ± 1.61	5.99 ± 2.12	5.45 ± 2.16	4.27 ± 2.02	3.17/0.03
E. coli	6.35 ± 1.59	5.90 ± 1.84	5.74 ± 1.68	5.68 ± 2.11	0.52/0.67

Data are presented as mean ± SD Log_{10} cfu/g wet faeces. Control formula does not contain added GOS.

Table 3 Fecal concentration of acetic acid and pH values at the end of a 3-mo feeding period

	GOS formula	GOS formula & human milk	Human milk	Control formula	*F*/*P* value
Acetic acid (*n*)	25.93 ± 6.84 (21)	25.09 ± 5.49 (34)	23.76 ± 5.65 (17)	19.42 ± 5.35 (24)	6.03/< 0.01
pH (*n*)	5.22 ± 0.25 (25)	5.27 ± 0.25 (41)	5.32 ± 0.24 (19)	5.56 ± 0.51 (27)	5.57/< 0.01

Data of acetic acid are presented as mean ± SD mmol/g wet faeces. Control formula does not contain added GOS.

Table 4 Scores of stool characteristics and intensity of digestive symptoms in the infants enrolled in the study (mean ± SD)

	GOS formula	GOS formula & human milk	Human milk	[1]Control formula	*F*/*P* value
Babies recruited in 3 mo follow-up	*n* = 37	*n* = 58	*n* = 24	*n* = 45	
Stool consistency	2.46 ± 0.62	2.55 ± 0.66	2.37 ± 0.83	3.11 ± 0.34	3.27/0.02
Crying	1.06 ± 0.03	1.04 ± 0.02	1.08 ± 0.05	1.05 ± 0.03	1.29/0.27
Regurgitation	1.34 ± 0.55	1.28 ± 0.63	1.41 ± 0.58	1.35 ± 0.67	1.18/0.34
Vomiting	1.22 ± 0.43	1.18 ± 0.34	1.14 ± 0.46	1.25 ± 0.38	1.24/0.30

[1]Control formula does not contain added GOS.

RESULTS

At the end of a 3-mo feeding period, the number of intestinal *Bifidobacteria* and *Lactobacilli* was significantly increased both in GOS-supplemented formula-fed infants and in breast-fed infants, compared with those fed with the control formula. No difference was seen between the GOS formula-feeding and breast feeding groups. The number of cfu in *E. coli* did not differ between the 3 groups (Table 2).

Intestinal acetic acid production and stool frequency were significantly increased in infants fed with GOS formula or with breast milk, compared with those fed with standard formula. Fecal pH was significantly higher in those fed with the control formula. No difference in stool frequency, fecal pH and intestinal acetic acid production was found between GOS formula-fed and breast-fed infants, (Tables 3 and 4).

The GOS formula did not influence the incidence of side effects (crying, regurgitation, vomiting) (Table 4). Weight gain and body height increase were similar among the groups (Table 1).

DISCUSSION

This study showed that a 3-mo feeding period of a relatively low amount of GOS (0.24 g/dL) in infant formula stimulated the growth of *Bifidobacteria* and *Lactobacilli* as seen in breast-fed infants but not the growth of potentially harmful *E. coli*. Stool frequency, fecal pH and amount of produced acetic acid were also comparable in the breast-fed infants, indicating hat a low GOS formula has the same prebiotic effect as a high GOS formula, but a minimized risk of intestinal irritation.

Breast-fed infants have an intestinal microflora dominated by *Bifidobacteria* and *Lactobacilli*[1,2] and are

quite different from those fed with a standard infant formula[16]. Both *Bifidobacteria* and *Lactobacilli* are beneficial to infants. As a result, manufacturers of formula try to mimic the gastrointestinal flora in breast-fed infants by adding probiotics and/or prebiotics such as GOS. Although the addition of probiotics is able to manipulate the infants' microflora towards the breast-fed infants' microflora[17,18], this concept may be regarded as unphysiological since breast milk itself does not contain bacteria. Prebiotics are, therefore, the first choice.

Prebiotics serve as food for *Bifidobacteria* and *Lactobacilli* and increase their number by their competitive edge over the pathogenic bacteria. During the fermentation of prebiotics, organic acids (lactic acid and SCFA) are produced that can inhibit the growth (increase colonization resistance) of acid-sensitive pathogens like salmonella[19]. SCFA, also the preferred source of energy for colonic epithelial cells, may stimulate colonocyte proliferation, and are suggested to enhance small intestinal glucose uptake[20]. On the other hand, the same organic acids may induce injury to the intestinal mucosa and impair its barrier function, as indicated by increased cytotoxicity of fecal water and fecal mucin excretion[14]. The fermentation rate might play an important role in this detrimental effect. Since slow fermentation as seen in case of resistant starch does not increase fecal mucin and luminal cytotoxicity, providing a lower amount of fast fermentable prebiotics to the intestine may also prohibit irritation[21].

Ten Bruggencate *et al*[14], in a rat study, showed that 6% of FOS on dry matter increases the number of *Bifidobacteria*, but 3% of FOS significantly (100-fold) increases the number of Enterobacteria, indicating that the selectivity of fibers can be questioned. The increased levels of Enterobacteria in combination with an impaired barrier function may increase the risk of bacterial translocation[14]. Moro *et al*[13] reported that the number of *Bifidobacteria* and *Lactobacilli* increases significantly in full term infants following oligosaccharide supplementation of 0.4 g/dL and 0.8 g/dL (3% and 6% on dry matter respectively). Although the authors reported no significant increase in the number of infants with positive culture of Enterobacteria, this statement does not say anything about the levels of these gram negative bacteria in those with a positive culture. Boehm *et al*[22] showed that 1.0 g of a mixture of GOS and FOS per 100 mL preterm formula has a bifidogenic effect but no significant effect on the number of *Lactobacilli* and Enterobacteria.

In the present study, the effect of only 0.24 g/dL of GOS on intestinal microflora and fermentation was observed in term infants. The results show that even such a low amount of GOS could stimulates the growth of *Bifidobacteria* and *Lactobacilli* as in breast-fed counterparts, decrease fecal pH, and increase the production of intestinal SCFA. The frequency of stools was shorter and the stools became softer, as seen in breast milk-fed infants. These changes in stool characteristics could not be explained by the increased osmolarity (about 1 mOsmol/L) of the formula because of the addition of GOS, and are, therefore, probably related to the changes in bacterial flora and fermentation. Studies in adults showed that a greater amount of dietary oligosaccharides may lead to adverse effects, flatulence in particular[23]. In the present study, no adverse side effects were reported.

This study certainly has its limitations. The number of infants involved in bacterial and SCFA analysis as very small mainly due to the refusal of parents and the failure in taking fresh fecal samples. However, despite such limitations, the difference between GOS-fortified and non-fortified groups was significant. Furthermore, files of infants are not complete because of the poor communication and traffic facility for the follow-up. Therefore, this study was a pilot study with promising results that need to be confirmed in a larger and more appropriate study.

In summary, supplementation of GOS stimulates the growth of *Bifidobacteria* and *Lactobacilli*. Both bacteria are beneficial to infants. However, an increase in Enterobacteria cannot be excluded, although it may be dose-dependent. A small amount of GOS can stimulate the growth of *Bifidobacteria* and *Lactobacilli*, but not the growth of Enterobacteria in breast-fed infants.

COMMENTS

Background

Breast milk is superior over artificial formula in terms of newborn nutrition. Breast-fed infants have a higher level of intestinal *Bifidobacteria* and *Lactobacilli*, both of which are potentially beneficial to the health of their hosts. Oligosaccharides in human milk are more beneficial to intestinal flora. The amount of oligosaccharides in mature human milk ranges 12-15 g/L. Galacto-oligosaccharide (GOS) is a short chain galactose with a terminal glucose molecule. Studies have shown that GOS can selectively stimulate the development of *Bifidobacteria*. However, a large amount GOS (3%-6% of dry matter) supplementation to the artificial formula showed irritation of the intestinal cell wall and increased risk of bacterial translocation. This study investigated the effects of artificial formula supplemented with 0.24 g GOS per 100 mL (1.8% of dry matter) on intestinal microflora colonization and fermentation in infants, and detected the lowest and safe effective level of GOS.

Research frontiers

Moro *et al*[13] showed that in term infants fed with formula at the doses of 0.4 and 0.8 g of oligosaccharides per 100 mL (90% GOS and 10% low-molecular weight fructo-oligosaccharides), the number of *Bifidobacteria* and *Lactobacilli* increased significantly compared with a control formula with maltodextrin instead of oligosaccharides. These values have been adopted by the Scientific Committee on Food of the European Commission and probably will be implemented in European regulations. In preterm infants, the same mixture of oligosaccharides (1.0 g/100 mL) stimulated the growth of *Bifidobacteria* and resulted in stool characteristics as seen in human milk-fed infants. However, a recent rat study showed irritation of the intestinal cell wall and increased risk of bacterial translocation (following orally *Salmonella enteritidis* infection) when a large amount of fructooligosaccharide (3% and 6% of dry matter), but not an unrealistic amount (maximum level adopted by the SCF is about 6% of dry matter) was provided. Therefore, positive effects of oligosaccharides on intestinal bacteria may not always justify the levels tested.

Innovations and breakthroughs

This study showed that a 3 mo feeding period of a relatively small amount of GOS (0.24 g/dL) in infant formula could stimulate the growth of *Bifidobacteria* and *Lactobacilli*, but not the growth of potentially harmful *E. coli* in breast-fed infants. Stool frequency, fecal pH and the amount of produced acetic acid were also comparable, indicating that low GOS formula may have the same prebiotic effect as high GOS formula, but a minimized risk of intestinal irritation.

Applications

The present study was designed to investigate the effect of only 0.24 g/dL

of GOS on intestinal microflora and fermentation in term infants. The data show that even such a small amount of GOS could stimulate the growth of *Bifidobacteria* and *Lactobacilli*, decrease fecal pH, and increase the production of intestinal SCFA. Stools came more frequently and became softer in breast milk-fed infants, indicating that a mall amount of GOS (0.24 g/dL) supplementation can stimulate the growth of *Bifidobacteria* and *Lactobacilli*, but not the growth of *Enterobacteria* in breast-fed infants. It is, therefore, safe and effective when used in artificial infant formula.

Terminology

Prebiotics are "selectively fermented ingredients that allow specific changes both in composition and/or activity of gastrointestinal microflora that confers benefits to host well being and health". Probiotics are defined viable microorganisms, a sufficient amount of which can reach the intestine in an active state and thus exerting positive health effects.Synergistic combinations of pro- and prebiotics are called synbiotics. Today, only bifidogenic, non-digestible oligosaccharides (particularly inulin, its hydrolysis product oligofructose, and galactooligosaccharides), fulfill all the criteria for prebiotic classification.

Peer review

This study determined the effect of a lower-than-normal dose of a prebiotics on the gastrointestinal tract of infants. Its results show that a mall amount of GOS (0.24 g/dL) in artificial formula could stimulate the growth of intestinal *Bifidobacteria* and *Lactobacilli* but not *E. coli* in term infants, indicating that it is safe and effective when used in artificial infant formula.

REFERENCES

1 **Fanaro S**, Vigi V, Chierici R, Boehm G. Fecal flora measurements of breastfed infants using an integrated transport and culturing system. *Acta Paediatr* 2003; **92**: 634-635

2 **Harmsen HJ**, Wildeboer-Veloo AC, Raangs GC, Wagendorp AA, Klijn N, Bindels JG, Welling GW. Analysis of intestinal flora development in breast-fed and formula-fed infants by using molecular identification and detection methods. *J Pediatr Gastroenterol Nutr* 2000; **30**: 61-67

3 **Gronlund MM**, Arvilommi H, Kero P, Lehtonen OP, Isolauri E. Importance of intestinal colonisation in the maturation of humoral immunity in early infancy: a prospective follow up study of healthy infants aged 0-6 months. *Arch Dis Child Fetal Neonatal Ed* 2000; **83**: F186-F192

4 **Saavedra J**. Probiotics and infectious diarrhea. *Am J Gastroenterol* 2000; **95**: S16-S18

5 **Newburg DS**. Oligosaccharides in human milk and bacterial colonization. *J Pediatr Gastroenterol Nutr* 2000; **30** Suppl 2: S8-S17

6 **Picciano MF**. Nutrient composition of human milk. *Pediatr Clin North Am* 2001; **48**: 53-67

7 **Miller JB**, McVeagh P. Human milk oligosaccharides: 130 reasons to breast-feed. *Br J Nutr* 1999; **82**: 333-335

8 **Collins MD**, Gibson GR. Probiotics, prebiotics, and synbiotics: approaches for modulating the microbial ecology of the gut. *Am J Clin Nutr* 1999; **69**: 1052S-1057S

9 **Engfer MB**, Stahl B, Finke B, Sawatzki G, Daniel H. Human milk oligosaccharides are resistant to enzymatic hydrolysis in the upper gastrointestinal tract. *Am J Clin Nutr* 2000; **71**: 1589-1596

10 **Kobata A**, Yamashita K, Tachibana Y. Oligosaccharides from human milk. *Methods Enzymol* 1978; **50**: 216-220

11 **Fanaro S**, Boehm G, Garssen J, Knol J, Mosca F, Stahl B, Vigi V. Galacto-oligosaccharides and long-chain fructo-oligosaccharides as prebiotics in infant formulas: a review. *Acta Paediatr Suppl* 2005; **94**: 22-26

12 **Boehm G**, Lidestri M, Casetta P, Jelinek J, Negretti F, Stahl B, Marini A. Supplementation of a bovine milk formula with an oligosaccharide mixture increases counts of faecal bifidobacteria in preterm infants. *Arch Dis Child Fetal Neonatal Ed* 2002; **86**: F178-F181

13 **Moro G**, Minoli I, Mosca M, Fanaro S, Jelinek J, Stahl B, Boehm G. Dosage-related bifidogenic effects of galacto- and fructooligosaccharides in formula-fed term infants. *J Pediatr Gastroenterol Nutr* 2002; **34**: 291-295

14 **Ten Bruggencate SJ**, Bovee-Oudenhoven IM, Lettink-Wissink ML, Van der Meer R. Dietary fructo-oligosaccharides dose-dependently increase translocation of salmonella in rats. *J Nutr* 2003; **133**: 2313-2318

15 **Bouhnik Y**, Flourie B, D'Agay-Abensour L, Pochart P, Gramet G, Durand M, Rambaud JC. Administration of transgalacto-oligosaccharides increases fecal bifidobacteria and modifies colonic fermentation metabolism in healthy humans. *J Nutr* 1997; **127**: 444-448

16 **Rubaltelli FF**, Biadaioli R, Pecile P, Nicoletti P. Intestinal flora in breast- and bottle-fed infants. *J Perinat Med* 1998; **26**: 186-191

17 **Langhendries JP**, Detry J, Van Hees J, Lamboray JM, Darimont J, Mozin MJ, Secretin MC, Senterre J. Effect of a fermented infant formula containing viable bifidobacteria on the fecal flora composition and pH of healthy full-term infants. *J Pediatr Gastroenterol Nutr* 1995; **21**: 177-181

18 **Parracho H**, McCartney AL, Gibson GR. Probiotics and prebiotics in infant nutrition. *Proc Nutr Soc* 2007; **66**: 405-411

19 **Bovee-Oudenhoven IM**, Termont DS, Heidt PJ, Van der Meer R. Increasing the intestinal resistance of rats to the invasive pathogen Salmonella enteritidis: additive effects of dietary lactulose and calcium. *Gut* 1997; **40**: 497-504

20 **Chen CC**, Walker WA. Probiotics and prebiotics: role in clinical disease states. *Adv Pediatr* 2005; **52**: 77-113

21 **Bovee-Oudenhoven IM**, ten Bruggencate SJ, Lettink-Wissink ML, van der Meer R. Dietary fructo-oligosaccharides and lactulose inhibit intestinal colonisation but stimulate translocation of salmonella in rats. *Gut* 2003; **52**: 1572-1578

22 **Boehm G**, Lidestri M, Casetta P, Jelinek J, Negretti F, Stahl B, Marini A. Supplementation of a bovine milk formula with an oligosaccharide mixture increases counts of faecal bifidobacteria in preterm infants. *Arch Dis Child Fetal Neonatal Ed* 2002; **86**: F178-F181

23 **Bouhnik Y**, Vahedi K, Achour L, Attar A, Salfati J, Pochart P, Marteau P, Flourie B, Bornet F, Rambaud JC. Short-chain fructo-oligosaccharide administration dose-dependently increases fecal bifidobacteria in healthy humans. *J Nutr* 1999; **129**: 113-116

S- Editor Li DL **L- Editor** Wang XL **E- Editor** Lin YP