Research Article Open Access

Effect of probiotics on stool characteristic of bottle-fed infants

Yoo Mi Lee, Young Sun Cho, Su Jin Jeong*

Department of Pediatrics, Bundang CHA Medical Center, CHA University, Seongnam, South Korea

Corresponding author: Su Jin Jeong. 351 Yatap-dong, Bundang-gu, Seongnam-si, Gyeonggi-do 463-712, South Korea

Submission date: November 5th, 2018**, Acceptance Date:** March 28th, 2019, **Publication Date:** March 31st, 2019

Citation: Lee Y.M. Cho Y.S., Jeong S.J. Effect of probiotics on stool characteristic of bottle-fed infants. *Functional Foods in Health and Disease* 2019; 9(3):157-165. https://doi.org/10.31989/ffhd.v9i3.579

ABSTRACT

Background: Approximately 70% of all newborns in South Korea are fed a commercial formula as their sole source of nutrition or as a supplement to breast milk. It is common for many formula-fed infants to be switched from one formula to another because of perceived abnormalities in stooling patterns (too much/too little, too hard/too loose). Also, probiotics may have been recommended by a clinician or practitioner when there is thought to be a problem with the babies stool based on many reports.

Aims: The primary objective of this study was to determine whether probiotic (nLp-nF1) exposure during the first 6 months of life is safe. The secondary objective was to assess if nF1 exposure correlated with changes in formula-fed infants' stool characteristics.

Result: We recruited 49 healthy formula-fed infants aged 0-6 months from Bundang Cha Medical Center. Infants were given a 14-day supply of probiotics (nf1: Lactobacillus plantarum 2.5 X 1010/g/pack, 2 pack/day). The overall rate of stool change after probiotics is 83% (41/49). Among them, 75.6% (31/41) participants answered fairly about the change of stool characteristics. The significant change of stool frequency, stool color and flatulence after probiotics intervention was reported. The number of infants who defecated once daily was increased from 53% (26 of 49) to 77.5% (38 of 49) (P=0.075). Mustard yellow colored feces increased from 22 to 40 (p=0.018). Only 9 out of 20 infants with flatulence (>3 times) showed persistence of symptoms after probiotics (p<0.001). After taking probiotics, Bristol Stool Scale 4-5 stool was reported from 23 to 37(p=0.018).

Conclusion: By taking probiotics, the color of the stool changed, and the incidence of flatulence production decreased. Although the results are not statistically significant, the quality and quantity of stool improved according to the Bristol Stool Scale.

Keywords: Probiotics, Bristol Stool Scale, stool

BACKGROUND

Infants are born with a sterile gut. The establishment of the gut microbial population is a continuous and complex process that starts at delivery and proceeds through successive stages under the influence of several factors such as mode of delivery, hygiene measures, feeding habits, and drug therapies [1-3]. These initial differences in early colonization have been implicated in the health differences observed between vaginally and cesarean-delivered infants [4]. After birth, development of infants' gut microbiota is largely influenced by the type of feeding, with breast-fed infants exhibiting bifidobacteria- and lactobacilli-predominant microbiota. Formula-fed infants, on the other hand, have a more diversified microbiota that includes Clostridium perfringens, streptococci, and staphylococci [5-7].

In South Korea, approximately 70% of all newborns are fed a commercial formula as their sole source of nutrition or as a supplement to breast milk [8]. It is common for many formula-fed infants to be switched from one formula to another either by their parents or physicians. Although the reasons for such frequent formula switching are sometimes elusive, most of the changes occur because of perceived abnormalities in stooling patterns (too much/too little, too hard/too loose). Also, probiotics were recommended by a clinician or practitioner when the baby was thought to have a problem with stool based on many reports. [9-10].

In recent years, growing evidence suggests that supplementation with probiotics can modulate intestinal bacterial patterns by aiding the colonization of beneficial bacteria. The implementation of milk formula with prebiotics, probiotics, and lactoferrin has been demonstrated to change newborns' microflora composition toward breast-feeding pattern and stimulate immune response. Several clinical trials have been conducted to examine the effectiveness of probiotics in the treatment of infantile gastrointestinal disorders [11-13].

Nanometric Lactobacillus plantarum (strain name: nF1) (nLp-nF1) is a biogenics consisting of dead (nonviable) L. plantarum cells pretreated with heat and a nanodispersion process. The number of studies claiming probiotic health effects of Lactobacillus plantarum is escalating. [14-15].

Therefore, the primary objective of this study was to determine whether probiotic (nLp-nF1) exposure during the first 6 months of life is safe. The secondary objective was to assess if nF1 exposure correlated with changes in formula fed infant stool characteristics.

MATERIALS AND METHODS

Enrollment

Infants less than 6 months old (before introducing solid food) who were fed exclusively with cow-milk based formula for at least recent 4 weeks were recruited. Inclusion criteria were being a full-term baby (between 37 weeks and 42 weeks gestation), birth weighing between 2500 g and 4500 g, and normal weight gain. Exclusion criteria were Preterm (before gestational age of 36 weeks), low birth weight, admission history, antibiotics administration within a month, illness (acute gastroenteritis, other GI tract disorder, congenital disorder). All participants were asked to complete a questionnaire about demographic and clinical characteristics of their babies.

Ethical considerations

We obtained written informed consent from the parents or legal guardians of all children enrolled in this study. This study was approved by the Institutional Ethics Committee of CHA Bundang Medical Center, CHA University, Korea (approval No. BD 2017-02-031).

Questionnaire and preparation

After obtaining informed written parental consent, study infants were seen, weighed, and the data for current type of formula, consumption amount, number of daily bowel movements (BM), stool consistency, stool color, stool frequency, gas-out frequency were recorded. The eligible infants received a 14-day supply of probiotics (nLp-nF1 Lactobacillus plantarum 2.5 X 1010/g /pack, 2pack/day). Briefly, nLp-nF1 was manufactured by incubating Lactobacillus plantarum for 20 h under pH control, followed by incubation at high temperature (40°C), high salinity (1.0% (w/w)), and low pH (pH 5.0) for 4 h. Then, the sample was sterilized (80°C, 10 min) and nano-dispersed by high-pressure homogenization [16]. Clinical data were collected at the start of intervention and after 14 day.

Statistical analysis

All statistical analyses were performed using SPSS software (version 24.0; SPSS Inc., Chicago, IL). We examined whether all elements were normally distributed Through Kolmogorov-Smirnov and Shapiro-Wilk test. However, variables did not follow normal distribution. Thus, variables were compared using Wilcoxon's signed-rank test (Non-parametric statistics). Differences with a p-value of < 0.05 were considered statistically significant.

RESULTS

The characteristics of the study infants are shown in Table 1. A total of 55 infants were enrolled and 49 completed the study. Six infants dropped out from the study because of non-milk related problems including antibiotic exposure and failure of parent to adequately complete the diary. Oral probiotics were well tolerated without side effects or adverse reactions.

Table 1. Baseline characteristics of participants.

Gestational age (wk, mean ± SD)	38.6 ± 0.36
Age at entry (d, mean ± SD)	142.1 ± 8.9
Males/females (n (%))	27 (55.1) / 22 (44.9)
Type of delivery, vaginal/cesarean (n (%))	30 (61.2) / 19(38.8)
Birth weight (g, mean ± SD)	3377.2 ± 391.5
Entry weight (g, mean ± SD)	6633.9 ± 669.3

The overall rate of stool change after probiotics is 83% (41/49). Among them, 75.6% (31/41) participants answered fairly about the change of stool characteristics.

The change of stool frequency is shown in figure 1. Before intervention, the rate of infants who defecated once daily was 53% (26 of 49). After probiotics intervention, the rate increased up to 77.5% (38 of 49). The number of children who defecated less than once a day decreased from seven to two. However, there was no statistically significant difference

(P=0.075).

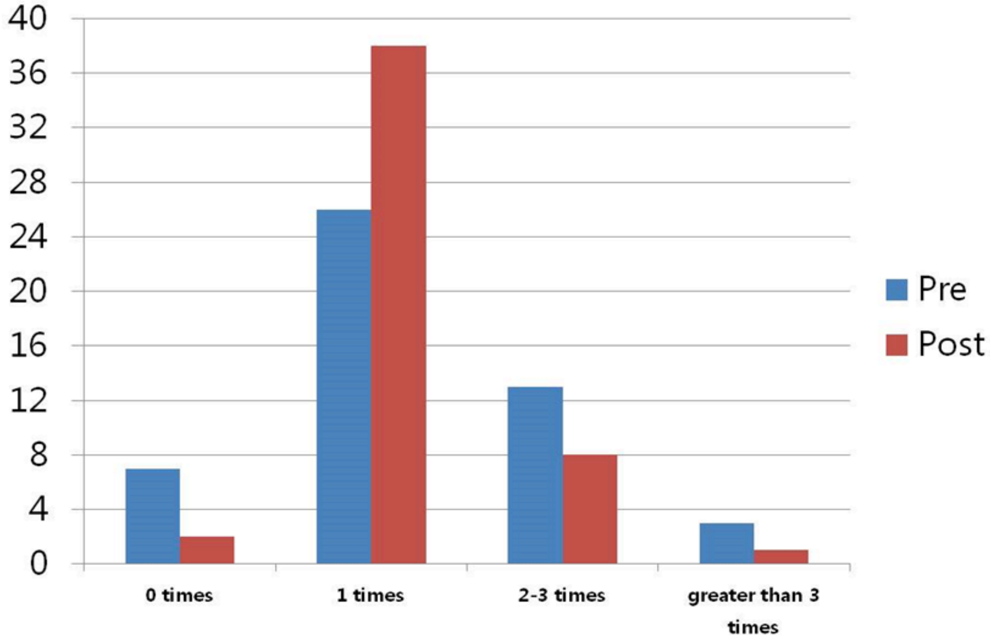

Figure 1. The change of stool frequency. Change of Stool color, frequency flatulence, and stool consistency (Bristol stool scale point) are shown in Figure 2, 3 and 4.

In figure 2, green stool color was reported in 11 infants before intervention. After 14 days of oral trial of probiotics, only 3 infants showed green stool color. The number of children exhibited mustard yellow-colored feces increased from 22 to 40. We compared stool color before and after probiotic use by Wilcoxon's signed-rank test, and there was a statistically significant difference (p=0.018).

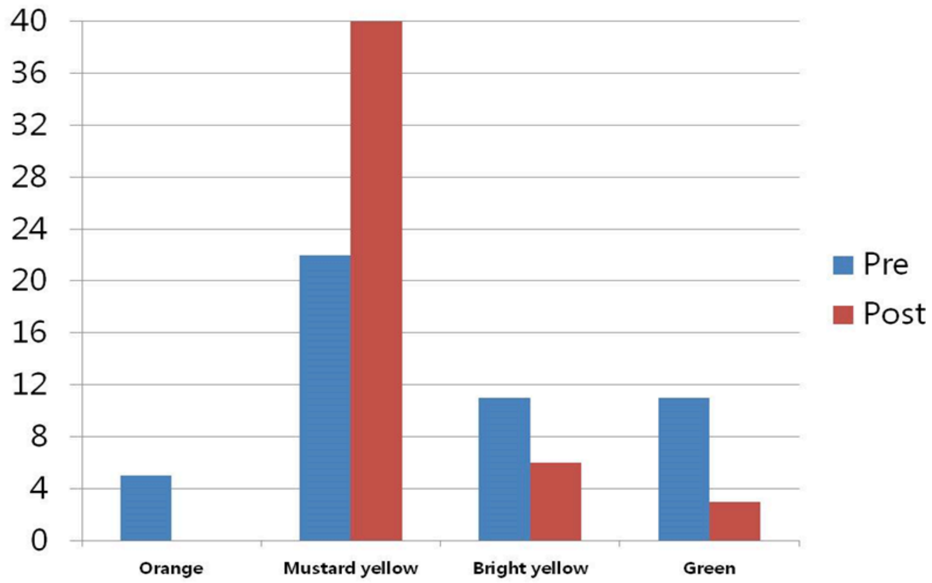

Figure 2. The change of Stool color.

In figure 3, the change in the rate of flatulence was statically significant difference (p<0.001). Before infants use probiotics, 20 infants exhibited flatulence greater than 3 times a day (20 of 49, 40.8%). After the 14-day trial of probiotics, 21 infants exhibited flatulence once (21 of 49, 41%).

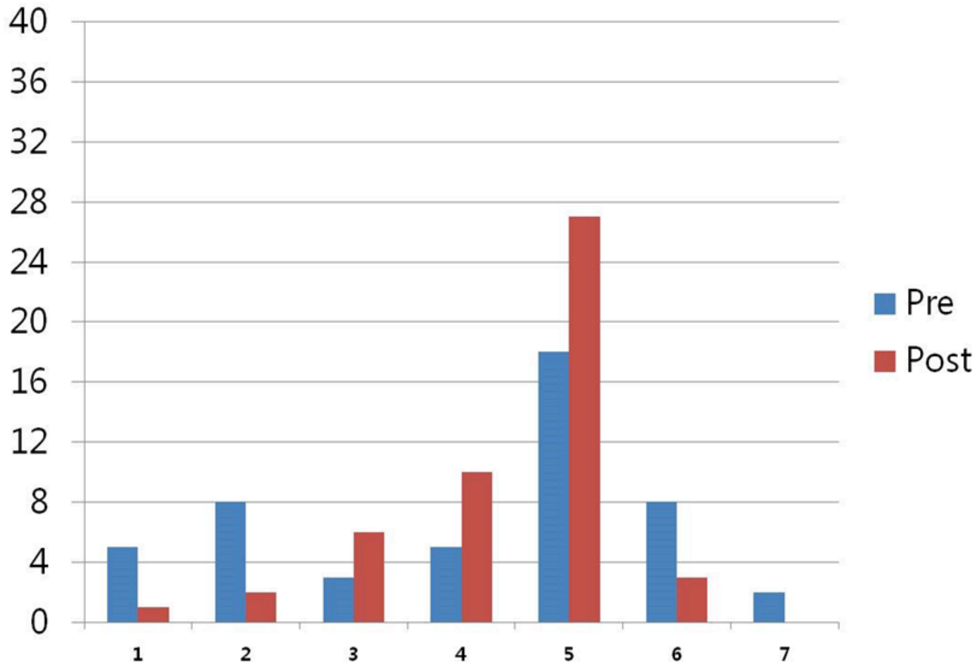

Figure 3. The change of Gas out times.

In Figure 4, Several infants had hard, pebble-sized stool (BSS 1, 2) before probiotics use, some of them showed BSS 4-5 stool after intervention. However, there was no statistically significant difference (P=0.061).

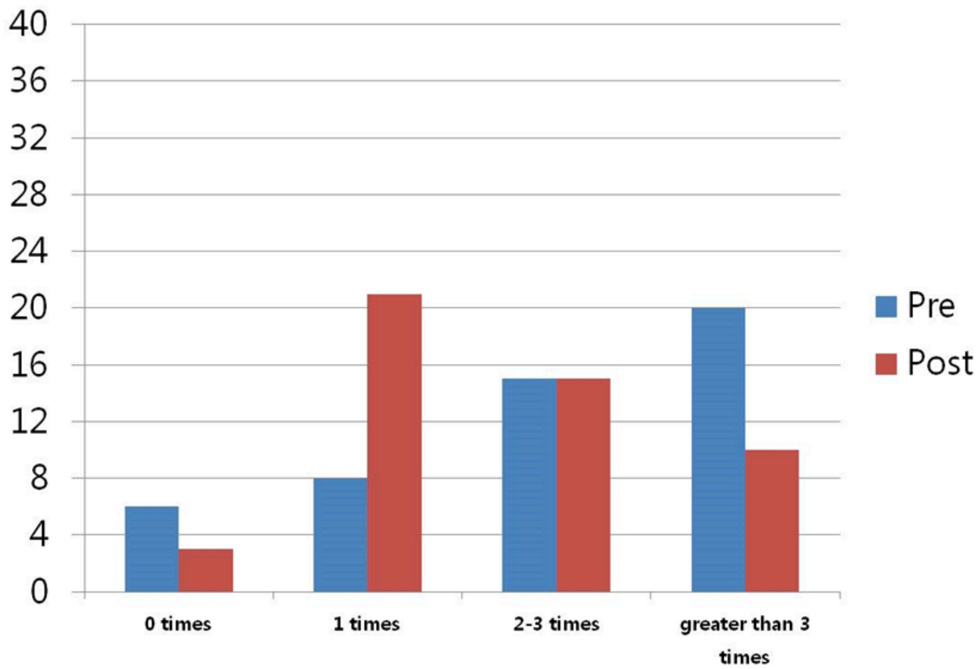

Figure 4. The change of Bristol stool scale.

DISCUSSION

Colonization of the intestinal microbiome during infancy represents a critical time for shaping infant's immunity [2]. Consequently, there has been an increase in consumer interest in probiotic products during the neonatal period as a non-invasive attempt to optimize infant microbiota [17]. However, there is little evidence to show that probiotics colonize the neonatal gut of healthy, term infants. Recently probiotics were produced as variable formula including foods, infant formula, dietary supplements, and pharmaceutical products. As a result, probiotics encompass a range of regulatory infrastructures with varying requirements for demonstrating safety and efficacy [18,19].

Currently, the results of observational studies and randomized controlled trials for probiotic products in infants are promising [20,21]. For example, systematic reviews concluded that probiotics may be beneficial in preventing eczema in infants and infantile colic [21,22]. The typical probiotic strains manufactured include *Bifidobacterium* and lactic acid bacteria such as *Lactobacillus* species. While these bacteria are present in low abundance in the human intestine, their use dominates the probiotic industry because they are relatively easy to cultivate given their historical use in the dairy industry allowing for large scale production. However, there is a lack of evidence that these probiotics influence the gut microbiome when given exogenously and their effect on health outcomes in infants is largely unknown.

During our study, oral administration of probiotics (5 X 10^{10}/g /day *Lactobacillus plantarum*) in infants less than 6 months of age was well-tolerated. Most infants' stool characteristics were changed after 14 days of intervention. Other reports also support the effects of L. plantarum in benefiting digestive tract health [15,16].

Bowel movements are one measure of infants' health and nutrition status. Formula-fed babies generally produce fewer bowel movements than breast-fed babies. Neonates typically pass several bowel movements in a day, which continues for several weeks. After 1 or 2 months, infant's bowel movements will likely decline to just one a day, but other babies may pass stool less frequently. As long as stool has the right texture, the frequency is usually not a problem. Additionally, if the baby with infrequent stools have pebble-size stools, when the legs are pulled up to her stomach, and the infant grunts, turns red in the face, and cries or makes a fuss while trying to produce bowel movement, it may be the sign of constipation [23]. Various studies have investigated the potential mechanisms of action by which probiotics might play a role in the treatment of constipation in children [24,25]. One proposed mechanism is that probiotics beneficially change the gastrointestinal microbiota [26]. Another is that some probiotics have a role in which they inhibit contraction of the colonic epithelial cell cytoskeleton, which opens the tight junctions and induces direct or indirect effects of nitric oxide in the gastrointestinal tract [27]. A third proposed mechanism is that some probiotics increase the amounts of lactate and short-chain fatty acids as well as decrease the luminal pH, thus enhancing gut peristalsis [28]. The outcomes of this study demonstrate that probiotics result in increased stool frequency (Figure 1) and improved Bristol stool scale (Figure 4).

Formula-fed babies have pasty, peanut butter-like stool on the brown color spectrum: tan-brown, yellow-brown, or green-brown [29]. Passing of green stool is not something to be worried about as long as the infant is healthy, happy, and gaining weight at a normal pace. However, the color of stool is the main cause of frequent infant formula switching. This study showed the change of stool color after probiotics intervention. The results are statistically significant ($p=0.018$). One more interesting thing in our study was that the change of flatulence patterns was statically significant ($p<0.001$). Before the use of probiotics, 20 infants had flatulence greater than 3 times (20 of 49, 40.8%). After a 14-day trial of probiotics, 21 infants had flatulence 1 time (21 of 49, 41%) (Figure 3). We did not investigate infantile colic for the baby who participated in the study. However, intestinal gas production is thought to be one of the causes of abdominal discomfort in infants suffering from colic in many articles [30]. In addition, there are many reports that probiotics are effective in the treatment of infantile colic. So, the relationship between intake of probiotics and gas production can be inferred [11-13].

Although many studies have investigated the influence of probiotics and prebiotics on stool outcomes in the hope of achieving a stool profile closer to that of the breast-fed infant, breast-feeding is the natural way to achieve optimal gut flora.

We think that the study is limited because of the time constraints when administering the questionnaire.

Additional research, from clinical observation to microbiologic analysis, is needed to confirm the beneficial effects of probiotics. In reality, the safety and efficacy of probiotics depends upon the amount and dosage, the characteristics of the consumer, and the context in which they are used. A potential future direction is to assess the role of other probiotic species and to identify the ideal strain for specific clinical states because specific probiotic strains have specific properties and targets in the human intestinal microbiota, exerting different health effects.

CONCLUSION

By taking probiotics, the color of the stool changed, and the incidence of flatulence production decreased. Although the results are not statistically significant, the quality and quantity of stool improved according to the Bristol Stool Scale.

Conflicting Interests: All authors and BIOGENICS KOREA declared no potential conflicts of interest in terms of the research, authorship and/or publication of this article.

Authors' Contributions: YM Lee, YS Cho and SJ Jeong participated in the design of the study and contributed to data analysis. YS Cho collected the data. YM Lee drafted the manuscript. SJ Jeong supervised the investigation. All authors reviewed and approved the final version of the article.

Acknowledgements and Funding: We are grateful to BIOGENICS KOREA for the donation of the probiotics assays. We also thank the Department of Pediatrics, Bundang CHA Medical Center for help with testing our study participants.

REFERENCES

1. Adlerberth I, Wold A: Establishment of the gut microbiota in Western infants. Acta paediatrica 2009, 98(2):229-38.
2. Martin R, Nauta A, Ben Amor K, Knippels L, Knol J, Garssen J: Early life: gut microbiota and immune development in infancy. Beneficial microbes 2010, 1(4):367-82.
3. Bäckhed F, Roswall J, Peng Y, Feng Q, Jia H, Kovatcheva-Datchary P, et al.: Dynamics and stabilization of the human gut microbiome during the first year of life.

Cell host & microbe 2015, 17(5):690-703.

4. Huurre A, Kalliomäki M, Rautava S, Rinne M, Salminen S, Isolauri E: Mode of delivery–effects on gut microbiota and humoral immunity. Neonatology 2008, 93(4):236-40.
5. Balmer S, Wharton B: Diet and faecal flora in the newborn: breast milk and infant formula. Archives of Disease in Childhood 1989, 64(12):1672-7.
6. Fallani M, Young D, Scott J, Norin E, Amarri S, Adam R, et al.: Intestinal microbiota of 6-week-old infants across Europe: geographic influence beyond delivery mode, breast-feeding, and antibiotics. Journal of pediatric gastroenterology and nutrition 2010, 51(1):77-84.
7. Guaraldi F, Salvatori G: Effect of breast and formula feeding on gut microbiota shaping in newborns. Frontiers in cellular and infection microbiology 2012, 2:94.
8. Choi EY, Park BS, Park SJ: Integrative review of domestic research papers on breast-feeding intervention. Journal of the Korea Academia-Industrial Cooperation Society 2016, 17(1):441-52.
9. Coccorullo P, Strisciuglio C, Martinelli M, Miele E, Greco L, Staiano A: Lactobacillus reuteri (DSM 17938) in infants with functional chronic constipation: a double-blind, randomized, placebo-controlled study. Journal of Pediatrics 2010, 157:598–602.
10. McFarland LV: Meta-analysis of probiotics for the prevention of antibiotic associated diarrhea and the treatment of Clostridium difficile disease. American Journal of Gastroenterology 2006, 101:812–22
11. de Weerth C, Fuentes S, Puylaert P, de Vos WM: Intestinal microbiota of infants with colic: development and specific signatures. Pediatrics 201, peds: 2012-1449.
12. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, et al.: Expert consensus document: The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nature Reviews Gastroenterology and Hepatology 2014, 11(8):506.
13. Oncel MY, Sari FN, Arayici S, Guzoglu N, Erdeve O, Uras N, et al.: Lactobacillus reuteri for the prevention of necrotising enterocolitis in very low birthweight infants: a randomised controlled trial. Archives of Disease in Childhood-Fetal and Neonatal Edition 2014, 99(2): F110-F5.
14. Choi DW, Jung SY, Kang JS, Nam YD, Lim SI, Kim KT, et al.: Immune-Enhancing Effect of Nanometric Lactobacillus plantarum nF1 (nLp-nF1) in a Mouse Model of Cyclophosphamide-Induced Immunosuppression. Journal of Microbiology and Biotechnology 2018, 28(2):218–226.
15. Hamza AS, Farida B, Frédérique G, Ismail F, Giuseppe S, Djamel D: Lactobacillus plantarum and Its Probiotic and Food Potentialities. Probiotics and Antimicrobial Proteins 2017, 9(2): 111–122.
16. Lee HA, Kim H, Lee KW, Park KY: Dead Lactobacillus plantarum stimulates and skews immune responses toward T helper 1 and 17 polarizations in RAW 264.7 cells and mouse splenocytes. Journal of Microbiology and Biotechnology 2016, 26: 469-476.
17. de LeBlanc AdM, LeBlanc JG: Effect of probiotic administration on the intestinal microbiota, current knowledge and potential applications. World Journal of

Gastroenterology: WJG 2014, 20(44):16518.
18. Martinez RCR, Bedani R, Saad SMI: Scientific evidence for health effects attributed to the consumption of probiotics and prebiotics: an update for current perspectives and future challenges. British Journal of Nutrition 2015, 114(12):1993-2015.
19. Clauson ER, Crawford P: What you must know before you recommend a probiotic. Journal of Family Practice 2015, 64(3):151-6.
20. Indrio F, Di Mauro A, Riezzo G, Civardi E, Intini C, Corvaglia L, et al.: Prophylactic use of a probiotic in the prevention of colic, regurgitation, and functional constipation: a randomized clinical trial. JAMA pediatrics 2014, 168(3):228-33.
21. Pärtty A, Lehtonen L, Kalliomäki M, Salminen S, Isolauri E: Probiotic Lactobacillus rhamnosus GG therapy and microbiological programming in infantile colic: a randomized, controlled trial. Pediatric research 2015, 78(4):470.
22. Van der Aa L, Heymans H, Van Aalderen W, Sillevis Smitt J, Knol J, Ben Amor K, et al.: Effect of a new synbiotic mixture on atopic dermatitis in infants: a randomized-controlled trial. Clinical & Experimental Allergy 2010, 40(5):795-804.
23. Vandenplas Y, Benninga M, Broekaert I, Falconer J, Gottrand F, Guarino A, et al.: Functional gastro-intestinal disorder algorithms focus on early recognition, parental reassurance and nutritional strategies. Acta Paediatrica 2016, 105(3):244-52.
24. Tabbers M, DiLorenzo C, Berger M, Faure C, Langendam M, Nurko S, et al.: Evaluation and treatment of functional constipation in infants and children: evidence-based recommendations from ESPGHAN and NASPGHAN. Journal of pediatric gastroenterology and nutrition 2014, 58(2):258-74.
25. Coccorullo P, Strisciuglio C, Martinelli M, Miele E, Greco L, Staiano A: Lactobacillus reuteri (DSM 17938) in infants with functional chronic constipation: a double-blind, randomized, placebo-controlled study. The Journal of pediatrics 2010, 157(4):598-602.
26. Grehan MJ, Borody TJ, Leis SM, Campbell J, Mitchell H, Wettstein A: Durable alteration of the colonic microbiota by the administration of donor fecal flora. Journal of clinical gastroenterology 2010, 44(8):551-61.
27. Ait-Belgnaoui A, Han W, Lamine F, Eutamene H, Fioramonti J, Bueno L, et al.: Lactobacillus farciminis treatment suppresses stress induced visceral hypersensitivity: a possible action through interaction with epithelial cell cytoskeleton contraction. Gut 2006, 55(8):1090-4.
28. Waller PA, Gopal PK, Leyer GJ, Ouwehand AC, Reifer C, Stewart ME, et al.: Dose-response effect of Bifidobacterium lactis HN019 on whole gut transit time and functional gastrointestinal symptoms in adults. Scandinavian journal of gastroenterology 2011, 46(9):1057-64.
29. Bhinde SM: Importance of Stool Examination in Babies. Journal of Indian System of Medicine Vol 2014, 2(3):139.
30. Savino F, Cordisco L, Tarasco V, Locatelli E, Di Gioia D, Oggero R, et al.: Antagonistic effect of Lactobacillus strains against gas-producing coliforms isolated from colicky infants. BMC microbiology 2011, 11(1):157.