



## ORIGINAL ARTICLE

# Synbiotic in the management of infantile colic: A randomised controlled trial

Hamidreza Kianifar,<sup>1</sup> Hamid Ahanchian,<sup>2</sup> Zubin Grover,<sup>2</sup> Seyedali Jafari,<sup>1</sup> Zeinab Noorbakhsh,<sup>1</sup> Ali Khakshour,<sup>1</sup> Majid Sedaghat<sup>1</sup> and Mohammadali Kiani<sup>1</sup>

<sup>1</sup>Pediatric Gastroenterology, Mashhad University of Medical Sciences, Mashhad, Khorasan, Iran and <sup>2</sup>Queensland Children's Medical Research Institute, Royal Children's Hospital, Brisbane, Queensland, Australia

**Aim:** Infant colic is a frequent problem affecting up to 10–30% of infants in first 3 months of life. Results from previous trials have shown that manipulation of gut microbiota can lead to symptomatic improvements. In a randomised clinical trial, we aimed to determine efficacy of synbiotic in reducing average infant crying time at day 7 and day 30 after starting intervention.

**Methods:** Fifty breastfed infants aged 15–120 days with infantile colic randomly assigned to receive either the synbiotic sachet containing 1 billion CFU of: *Lactobacillus casei*, *L. rhamnosus*, *Streptococcus thermophilus*, *Bifidobacterium breve*, *L. acidophilus*, *B. infantis*, *L. bulgaricus* and fructooligosaccharide (Protexin Healthcare, Somerset, UK), or placebo daily for 30 days. Parents were asked to record details of crying times in a symptoms diary. The primary outcome measure was the treatment success (reduction in the daily crying time >50%) and the secondary outcome measure was symptom resolution (reduction in the daily crying time >90%).

**Results:** The treatment success was significantly higher in synbiotic group (82.6%) compared with placebo (35.7%) at day 7 ( $P < 0.005$ ). At day 30, treatment success was 87% and 46% in synbiotic and placebo group, respectively ( $P < 0.01$ ). Symptom resolution was also higher in synbiotic group (39%) compared with placebo (7%) at day 7 ( $P < 0.03$ ) but not at day 30 (56% vs. 36%,  $P = 0.24$ ). We encountered no complication related to synbiotic use.

**Conclusion:** This synbiotic (a mixture of seven probiotic strains plus FOS) significantly improved colic symptoms in comparison with placebo.

**Key words:** infantile colic; probiotic; synbiotic.

## What is already known on this topic

- 1 Infantile colic is a frequent problem in the first 3 months of life with no definite treatment.
- 2 The genesis of excessive crying is considered multi-factorial.
- 3 Previous studies have found manipulation of gut microbiota by probiotics can lead to symptomatic improvements with reduction of crying time.

## What this paper adds

- 1 The effect of synbiotics on infantile colic is an unexplored area of research. This randomised controlled study identifies that a mixture of seven probiotic strains plus fructooligosaccharide significantly improves colic symptoms in comparison with placebo.
- 2 Prebiotic is considered to promote the function and viability of probiotics via its fermentation.
- 3 Prebiotic can also influence immune system showing anti-inflammatory effects.

Infantile colic (IC) is a frequent problem affecting up to 10–30% of apparently healthy infants in first 3 months of life with significant burden on both parent and health-care system. The genesis of excessive, inconsolable crying during this period is considered multifactorial with various underlying psychosocial, neuro-developmental and other physiological factors contributing to this poorly understood and a rather frustrating problem. IC is defined as paroxysms of crying lasting more than 3 hours a day, occurring more than 3 days in any week for 3 weeks in a

healthy baby aged 2 weeks to 3 months.<sup>1,2</sup> Although considered benign but IC associated with greater maternal mood disorder, family functioning, child abuse and long-term behavioral problems in these infants.<sup>1,3</sup>

The proposed mechanisms of excessive crying includes painful intestinal contractions secondary to excessive gas or swallowed air. This theory is supported by significantly higher levels of breath H<sub>2</sub> excretion in infants with colic compared with those without colic; however, no benefits of lactase supplements were observed in trials.<sup>1</sup> It is hypothesised that IC can be as a result of dysbiosis due to disruption or immaturity of immune and epithelial barrier function leading to state of colonic inflammation and motor dysfunction.<sup>4–9</sup> Some studies have demonstrated abnormal levels of fecal calprotectin, a marker of colonic inflammation in infants with colic and perturbed intestinal microbiota with excessive colonisation of

**Correspondence:** Dr Hamid Ahanchian, Queensland Children's Medical Research Institute, Royal Children's Hospital, Brisbane, Qld. 4029, Australia. Fax: +61733655533; email: h.ahanchian@uq.edu.au

Conflict of interest: None declared.

Accepted for publication 6 April 2014.

clostridium difficile, coliforms, klebsiella and *Lactobacillus brevis*<sup>10</sup> although fecal calprotectin concentration in infants with IC was normal in another study<sup>11</sup> These findings and results from previous studies have been successful in demonstrating that manipulation of gut microbiota can lead to symptomatic improvements with reduction of crying time.<sup>12-14</sup> In a recent systematic review, Sung *et al.* concluded that although *L. reuteri* may be effective in reduction of crying in exclusively breastfed infants with colic, there is insufficient evidence exist to support general use of probiotics in all infants with colic.<sup>15</sup>

The synergistic combination of probiotics and prebiotics are called synbiotic. In a double blind, placebo controlled randomised trial we aimed to determine efficacy of Synbiotic Protexin Restore (Protexin Healthcare, Somerset, UK), a mixture of seven probiotic strains plus fructooligosaccharide (FOS) in reducing average infant crying time. FOS is considered to promote the function and viability of probiotics via its fermentation. It can also influence immune system showing anti-inflammatory effects.<sup>16</sup> Primary outcome was treatment success defined as achieving at least 50% reduction in average daily crying time; secondary outcomes were weight gain and symptom resolution defined as average 90% reduction in the daily crying. Our hypothesis was that compared with placebo (control) group, the intervention group on synbiotic will have lower crying time.

## Methods

The trial was conducted at Sarvar teaching hospital with the recruitment sample population drawn from community-based pediatrician referrals from April 2010 to May 2012. Ethics approval was granted by Ethic committee of Mashhad University of Medical Sciences. After confirming those suitable for the study by a paediatric gastroenterologist, parents' information sheets were provided, and verbal consent was obtained. The inclusion criteria were: healthy breastfed infants aged 2 weeks to 4 months with infant colic defined as per Wessel's criteria based on care giver's symptom records diary. Those with failure to thrive (Weight deceleration crossing two major percentile lines of growth charts or weight gain less than 500 g per month), congenital abnormalities or syndromes, urinary tract infection, antibiotic or probiotic consumption during 2 weeks before study, any organic cause for abdominal pain and incomplete diary records were excluded.

Computer-generated randomisation was undertaken by university statistician not directly involved with the trial analysis. Following randomisation infants either received a synbiotic sachet containing 1 billion CFU of: *L. casei*, *L. rhamnosus*, *Streptococcus thermophilus*, *Bifidobacterium breve*, *L. acidophilus*, *B. infantis*, *L. bulgaricus* and FOS (Protexin Healthcare, Somerset, UK). The control group received placebo that was matched for size, volume, shape and manufactured by the same company. Both synbiotics and placebo were dispensed by hospital pharmacist. Intervention was concealed from study investigators, pharmacist and parents to minimise treatment bias.

## Study protocol

During first visit following demographic, information was collected by a blinded nurse including sex, gestational age, type of

delivery, type of feeds, that is bottle or breast, birthweight, age at enrolment, maternal depression, perinatal antibiotics, family history of allergies, parent-child interaction, previous exposure to Proton Pump inhibitors, smoking, and type and stool consistency and frequency. Before enrolment in the study, a routine education session focusing on feeding and settling techniques was undertaken by the clinical nurse to make routine care similar in all patients. Parents were advised to give mix synbiotic or placebo sachet with breast milk daily for period of 30 days. Parents were asked to record details of daily crying times, stool consistency and frequency and any side effects every day for 30 days in a symptoms diary and present for the evaluation on day 7 and day 30.

According to Savino study, we estimated a sample size of 20 in each group using  $\alpha = 99\%$  and  $\beta = 80\%$  and possible 20% follow-up loss.

## Statistics

The statistical analysis was performed using the SPSS 11.5 program (SPSS Inc., Chicago, IL, USA). Values were reported as mean  $\pm$  SD for normally distributed variables.

Baseline demographics and clinical characteristics were compared among groups using 'tstudent test', 'chi-square test' and/or 'Fisher exact test' as appropriate. According to 'Kolmogorov-Smirnov' test, the normal distribution in case and control groups was checked.

## Results

Of the 50 colicky infants enrolled, 26 were assigned randomly to be treated with synbiotic and 24 with placebo. Four patients in placebo group were excluded from the analysis, for the following reasons: lost to follow-up ( $n = 2$ ) and discontinued intervention ( $n = 2$ ) and one patient in synbiotic group due to lost to follow up.

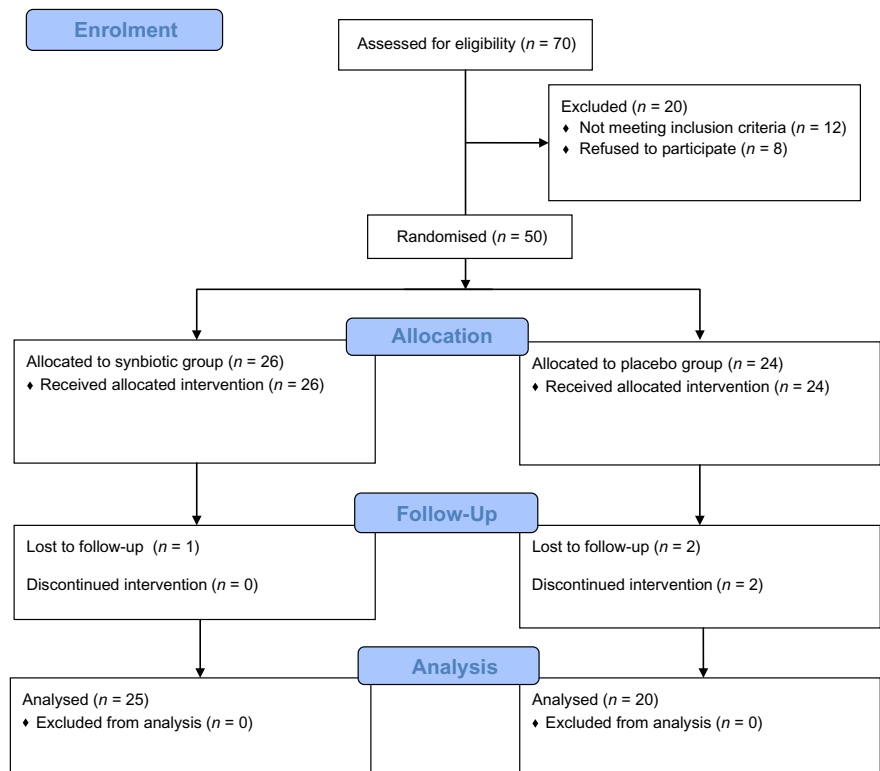
Forty-five infants completed the study, 25 were treated with synbiotic and 20 were treated with placebo (Fig. 1). No adverse effect related to the trial was seen.

Baseline characteristics are shown in Table 1. According to this table, there are no significant difference between groups in sex, birth order, delivery methods, maternal drug history, history of maternal depression, passive smoking, history of allergy, frequency of daily colic bouts and previous siblings with colic and gastrointestinal disease history ( $P > 0.05$ ). The mean age was 40.92 days in synbiotic group and 43.73 days in placebo group. Baseline daily crying time recorded before nurse education session was also same in both groups.

Table 2 shows that the primary outcome measure (treatment success) was significantly higher in synbiotic group (82.6%) compared with placebo group (35.7%) at day 7 ( $P < 0.005$ ). At day 30, treatment success was 87% and 46% in synbiotic and placebo group, respectively ( $P < 0.01$ ).

The secondary outcome measure (symptom resolution) was also higher in synbiotic group (39%) compared with placebo group (7%) at day 7 ( $P < 0.03$ ) but not at day 30 (56% vs. 36%,  $P = 0.24$ ).

Table 2 also shows that although weights were increased in both groups, it was not statistically significant ( $P = 0.057$ ).



**Fig. 1** Flow diagram of the patients' progression throughout the study.

**Table 1** Baseline characteristics of patients

		Placebo group (n = 20)	Synbiotic group (n = 25)	P-value
Sex(male/female), n	—	7/13	15/10	0.102
Birth order (1st child), n		10	10	0.296
Vaginal/Caesarean delivery, n		9/11	10/15	0.68
Familial history of allergy, n	Yes	15	20	0.137
Frequency of colic/day	—	4.13 ± 3.3	5.33 ± 3.9	0.338
Gestational age		1.3 ± 37.7	1.3 ± 37.5	0.710
Birthweight, g		468.3 ± 3070.6	392.8 ± 3170.8	0.476
Mean age(first visit), d		43.73 ± 10.82	40.92 ± 21.13	0.582

—, inconclusive results.

## Discussion

In the present study, a mixture of seven probiotic strains and FOS in comparison to placebo was associated with a higher treatment success, symptoms resolution and a lower duration of crying after 7 and 30 day of intervention. These results are impressive as 82.6% of infants in synbiotic group had treatment success 7 days after intervention compared with 35.7% in placebo group. No adverse events were recorded during the study.

In 2010, Savino *et al.*, in a randomised trial of 46 breastfed colicky infants compared probiotic *L. reuteri DSM 17938* with placebo. Infants in the *L. reuteri*-treated-group showed significantly reduced crying compared with the placebo group on day 7, 14 and 21. The authors suggested that gut microbiota changes

induced by probiotic may be involved in clinical improvement.<sup>17</sup> Our findings are consistent with the findings of this study in treatment success or crying time, although they only used a single strain of probiotic compared with our study using seven strains of probiotics. In 2013, Szajewska *et al.*, concluded that exclusively or predominantly breastfed infants with IC benefit from *L. reuteri* DSM 17938 compared with placebo.<sup>3</sup> Saavedra *et al.*, showed that a formula containing two strains of probiotic including *B. lactis* and *S. thermophilus* was safe and reduced reporting of colic or irritability.<sup>12</sup> Some studies showed that using a probiotic mixture might be more effective but as shown in other studies efficacy of the probiotic mixtures may be reduced by inhibitory effects between different probiotic strains.<sup>18</sup>

**Table 2** Primary and secondary outcome measures

Primary outcome	Synbiotic group (n = 25)	Placebo group (n = 20)	NNT	P-value
Treatment success (reduction in the daily average crying time >50%)	—			
Day 7	82%	35%	2.1	<0.005
Day 30	87%	46%	2.5	<0.01
Secondary outcomes	—			
Symptom resolution (reduction in the daily average crying time >90%)				
Day 7	39%	7%	3	<0.03
Day 30	56%	36%	5.5	0.24
Duration of colic (minutes/day)	—			
Before intervention	193.04 ± 26	185.0 ± 24	—	0.635
Day 7	70.00 ± 10	97.5 ± 12	—	<0.001
Day 30	28.80 ± 9.7	63.46 ± 10	—	<0.001
Weight (gram)	—			
Before intervention	4300.2 ± 1098	4640.66 ± 914	—	0.320
Day 30	5269.8 ± 1736	5060.0 ± 743	—	0.057

—, inconclusive results; NNT, number needed to treat.

Prebiotics have been useful in management of IC in some studies but some trials failed to show their effectiveness.<sup>19</sup> Moro *et al.*, in a prospective controlled trial showed that a formula containing a mixture of galacto and FOS can reduce crying time. They commented that prebiotics can influence immune system not only via the intestinal microbiota but also by direct interaction with immune cells.<sup>20</sup> Partty *et al.* showed that early administration of prebiotics, probiotics or placebo during the first 2 months of life to infants born preterm provided relief to their crying and fussing. They suggested that delayed colonisation by *B. infantis* is linked to the risk of irritability in preterm infants.<sup>21</sup> As *B. infantis* is one of the seven probiotics used in our study, our results can support their suggestion.

The effect of synbiotics on IC is an unexplored area of research. The prebiotic in the synbiotic improves the survival of probiotics and stimulates the host endogenous bacteria. Mugambi *et al.*, in a review of three studies using infant formula containing synbiotic with total 457 participants concluded that synbiotics had no impact on incidence and frequency of colic, crying and restlessness.<sup>22</sup> The therapeutic effects of synbiotics depend on the type of strains, number of strains, dosage, duration of intervention, study population and environmental background.<sup>23–25</sup> The probiotic strains used in these studied were *B. longum* at dose of  $1.29 \times 10^8$  CFU or  $2 \times 10^7$  CFU and *B. animalis*  $1 \times 10^7$  CFU plus combination of galacto and FOS as prebiotics. Compared with this study, we used a of a mixture of higher dose ( $1 \times 10^9$ ) plus FOS alone.

Food hypersensitivity is a possible mechanism of colic as shown in recent studies; IC has a higher frequency in children with a family history of atopy.<sup>17</sup> As gut is a central player in ontogenic development of immune tolerance, modulation of immune responses and inflammation by synbiotic may play a role in reduction of symptoms.<sup>26,27</sup>

The rate of weight gain was higher in the probiotic group, but this increase was not statistically significant ( $P = 0.837$ ), which

is same as other trials.<sup>1,28</sup> Gut microbiota may play a role in weight gain by different mechanisms including improvement of mineral bioavailability, vitamin synthesis, regulation of gastrointestinal motility and regulation of energy extraction from the diet.<sup>29–31</sup>

Roos *et al.*, in an RCT compared the global microbial composition in fecal samples from colicky infants given *L. reuteri* DSM 17938 or placebo. They concluded that the increase of Bacteroidetes in responder infants indicated that a decrease in colicky symptoms was linked to the changes of the microbiota.<sup>32</sup> A limitation of the study was that we did not evaluate the stool samples before and after intervention. Other weaknesses of our study were small sample size, non-validated outcome measure, no measure of synbiotic/placebo compliance and just relying on parents' reports in the diaries.

Strength of this study is its novelty in using synbiotic for treatment of infantile colic and its design as double blind, placebo-controlled randomised trial.

## Conclusion

A synbiotic containing a mixture of seven probiotic strains plus FOS may reduce infantile colic symptoms. For clear recommendations, well-designed large RCTs with longer follow-up, larger sample size, well-defined outcome measures and different mixtures are needed to be able to determine the ideal effective product for treatment or even prevention of infant colic.

## References

- 1 Savino F. Focus on infantile colic. *Acta Paediatr.* 2007; **96**: 1259–64.
- 2 Kheir AE. Infantile colic, facts and fiction Abdelmoneim E. *Ital. J. Pediatr.* 2012; **38**: 34.
- 3 Szajewska H, Gyrzczuk E, Horvath A. *Lactobacillus reuteri* DSM 17938 for the management of infantile colic in breastfed infants: a

- randomized, double-blind, placebo-controlled trial. *J. Pediatr.* 2013; **162**: 257–62.
- 4 Vandenplas Y, Veereman-Wauters G, De Greef E *et al.* Probiotics and prebiotics in prevention and treatment of diseases in infants and children. *J. Pediatr. (Rio J)* 2011; **87**: 292–300.
  - 5 Grajek W, Olejnik A, Sip A. Probiotics, prebiotics and antioxidants as functional foods. *Acta. Biochim. Pol.* 2005; **52**: 665–71.
  - 6 Kunze WA, Mao YK, Wang B *et al.* *Lactobacillus reuteri* enhances excitability of colonic AH neurons by inhibiting calcium-dependent potassium channel opening. *J. Cell. Mol. Med.* 2009; **13** (8B): 2261–70.
  - 7 Wang B, Mao YK, Diorio C *et al.* *Lactobacillus reuteri* ingestion and IK(Ca) channel blockade have similar effects on rat colon motility and myenteric neurones. *Neurogastroenterol. Motil.* 2010; **22**: 98–107, e33.
  - 8 Ma X, Mao YK, Wang B, Huizinga JD, Bienenstock J, Kunze W. *Lactobacillus reuteri* ingestion prevents hyperexcitability of colonic DRG neurons induced by noxious stimuli. *Am. J. Physiol. Gastrointest. Liver Physiol.* 2009; **296**: G868–75.
  - 9 Eberl G, Lochner M. The development of intestinal lymphoid tissues at the interface of self and microbiota. *Mucosal. Immunol.* 2009; **2**: 478–85.
  - 10 Rhoads JM, Fatheree NY, Noroni J *et al.* Altered fecal microflora and increased fecal calprotectin in infants with colic. *J. Pediatrics.* 2009; **155**: 823–8.
  - 11 Olafsdottir E, Aksnes L, Fluge G, Berstad A. Faecal calprotectin levels in infants with infantile colic, healthy infants, children with inflammatory bowel disease, children with recurrent abdominal pain and healthy children. *Acta. Paediatr.* 2002; **91**: 45–50.
  - 12 Saavedra JM, Abi-Hanna A, Moore N, Yolken RH. Long-term consumption of infant formulas containing live probiotic bacteria: tolerance and safety. *Am. J. Clin. Nutr.* 2004; **79**: 261–7.
  - 13 Savino F, Bailo E, Oggero R *et al.* Bacterial counts of intestinal *Lactobacillus* species in infants with colic. *Pediatr. Allergy Immunol.* 2005; **16**: 72–5.
  - 14 Savino F, Cresi F, Pautasso S *et al.* Intestinal microflora in breastfed colicky and non-colicky infants. *Acta. Paediatr.* 2004; **93**: 825–9.
  - 15 Sung V, Collett S, de Gooyer T, Hiscock H, Tang M, Wake M. Probiotics to prevent or treat excessive infant crying: systematic review and meta-analysis. *JAMA Pediatr.* 2013; **167**: 1150–7.
  - 16 Vieira AT, Teixeira MM, Martins FS. The role of probiotics and prebiotics in inducing gut immunity. *Frontiers Immunol.* 2013; **4**: 445. eCollection 2013. Review.
  - 17 Savino F, Cordisco L, Tarsaco V *et al.* *Lactobacillus reuteri* DSM 17938 in infantile colic: a randomized, double-blind, placebo-controlled trial. *Pediatrics* 2010; **126**: e526–33.
  - 18 Chapman CM, Gibson GR, Rowland I. In vitro evaluation of single- and multi-strain probiotics: inter-species inhibition between probiotic strains, and inhibition of pathogens. *Anaerobe* 2012; **18**: 405–13.
  - 19 Xiao-Ming B, Li J, Feng Z-T *et al.* Low level of galactooligosaccharide in infant formula stimulated growth of intestinal bifidobacteria and lactobacilli. *World J. Gastroenterol.* 2008; **14**: 6564–8.
  - 20 Moro G, Arslanoglu S, Stahl B, Jelinek J, Wahn U, Boehm G. A mixture of prebiotic oligosaccharides reduces the incidence of atopic dermatitis during the first six months of age. *Arch. Dis. Child.* 2006; **91**: 814–19.
  - 21 Partty A, Luoto R, Kalliomaki M, Salminen S, Isolauri E. Effects of early prebiotic and probiotic supplementation on development of gut microbiota and fussing and crying in preterm infants: a randomized, double-blind, placebo-controlled trial. *J. Pediatr.* 2013; **163**: 1272–7.
  - 22 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.
  - 23 Ahanchian H, Jones CM, Chen Y, Sly PD. Respiratory viral infections in children with asthma: do they matter and can we prevent them? *BMC Pediatr.* 2012; **12**: 147.
  - 24 Kianifar HR, Farid R, Ahanchian H, Jabbari F, Moghiman T, Sistani A. Probiotics in the treatment of acute diarrhea in young children. *Iran J. Med. Sci.* 2009; **34**: 204–7.
  - 25 Heydarian F, Kianifar HR, Ahanchian H, Khakshure A, Seyedi J, Moshirian D. A comparison between traditional yogurt and probiotic yogurt in non inflammatory acute gastroenteritis. *Saudi Med. J.* 2010; **31**: 280–3.
  - 26 Pfeufferle PI, Prescott SL, Kopp M. Microbial influence on tolerance and opportunities for intervention with prebiotics/probiotics and bacterial lysates. *J. Allergy Clin. Immunol.* 2013; **131**: 1453–63.
  - 27 Farid R, Ahanchian H, Jabbari F, Moghiman T. Effect of a new synbiotic mixture on atopic dermatitis in children: a randomized-controlled trial. *Iran J. Pediatr.* 2011; **21**: 225–30.
  - 28 Surono IS, Koestomo FP, Novitasari N, Zakaria FR, Yulianasari, Koesnandar. Novel probiotic *Enterococcus faecium* IS-27526 supplementation increased total salivary sIgA level and bodyweight of pre-school children: a pilot study. *Anaerobe* 2011; **17**: 496–500.
  - 29 LeBlanc JG, Laino JE, del Valle MJ *et al.* B-group vitamin production by lactic acid bacteria-current knowledge and potential applications. *J. Appl. Microbiol.* 2011; **111**: 1297–309.
  - 30 Turnbaugh PJ, Ridaura VK, Faith JJ, Rey FE, Knight R, Gordon JI. The effect of diet on the human gut microbiome: a metagenomic analysis in humanized gnotobiotic mice. *Sci. Transl. Med.* 2009; **1**: 6ra14.
  - 31 Ly NP, Litonjua A, Gold DR, Celedón JC. Gut microbiota probiotics, and vitamin D: interrelated exposures influencing allergy, asthma, and obesity? *J. Allergy Clin. Immunol.* 2011; **127**: 1087–94.
  - 32 Roos S, Dicksved J, Tarasco V *et al.* 454 Pyrosequencing analysis on faecal samples from a randomized DBPC trial of colicky infants treated with *Lactobacillus reuteri* DSM 17938. *PLoS ONE* 2013; **8**: e56710.