Efficacy of *Bifidobacterium animalis* subsp. *lactis*, BB-12® on infant colic – a randomised, double-blinded, placebo-controlled study

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Abstract

To evaluate the administration of *Bifidobacterium animalis* subsp. *lactis*, BB-12® (BB-12) on infant colic in breastfed infants, a double-blind, placebo-controlled randomised study was conducted in Chengdu, China from April 2016 to October 2017 with 192 full-term infants less than 3 months of age and meeting the ROME III criteria for infant colic. After a 1-week run-in the infants were randomly assigned to receive daily BB-12 (1×10^9 cfu/day) or placebo for 3 weeks. Crying/fussing time were recorded using a 24 h structured diary. The primary endpoint was the proportion of infants achieving a reduction in crying and fussing time of $\geq 50\%$ from baseline. Parent's/caregiver's health related quality of life was measured using a modified PedsQL™ 2.0 Family Impact Module and immunological biomarkers were evaluated from faecal samples at baseline and after the 21-day intervention. The percentage of infants achieving a reduction in the daily crying/fussing time $\geq 50\%$ after the 21-day intervention was significantly higher in the infants supplemented with BB-12 ($P < 0.001$). The mean number of crying episodes was significantly reduced in the BB-12 group compared to the placebo group (10.0 ± 3.0 to 5.0 ± 1.87 vs 10.5 ± 2.6 to 7.5 ± 2.8 , respectively) ($P < 0.001$) and the mean daily sleep duration was markedly increased from baseline to end of intervention in the BB-12 group compared to the infants in the placebo group (60.7 ± 104.0 vs 31.9 ± 102.7 min/day, respectively) ($P < 0.001$). The faecal levels of human beta defensin 2, cathelicidin, slgA, calprotectin and butyrate were statistically higher in the BB-12 group compared to the placebo group after the 21-day intervention. At the end of the intervention the parent's/caregiver's physical, emotional and social functioning scores were significantly higher for the BB-12 group compared to the placebo group (all $P < 0.05$). Supplementation of BB-12 is effective in reducing crying and fussing in infants diagnosed with infant colic.

Keywords: *Bifidobacterium*, probiotics, excessive fussing and crying

1. Introduction

Infant colic is a common condition that affects between 17 and 25% of all infants in the first months of life, depending on the region and definitions employed (Ong *et al.*, 2019). Although infant colic is considered a self-limiting and benign disorder with prevalence peaking at about 6 weeks, it may be a major source of anxiety and distress that can negatively impact quality of life, and is a common cause of paediatric consultation (Wolke *et al.*, 2017; Zeevenhooven *et al.*, 2018).

The pathogenesis of infant colic remains elusive and evidence suggests multiple independent causes. Growing evidence has linked the immature gut microbiota to infant colic (Gensollen *et al.*, 2016; Lu and Ni, 2015), and low levels of lactobacilli (Savino *et al.*, 2004) and bifidobacteria have been observed in infants diagnosed with colic (DeWeerth *et al.*, 2013). These alterations suggest that gut dysbiosis could be a possible cause of abnormal gut motility and increased gas production, which has also been a proposed hypothesis with respect to the aetiology of infant colic (Gupta, 2002).

The interest of probiotics as a potential modulator of the intestinal microbiota in infants with colic has increased in recent years. Probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host (Hill *et al.*, 2014), and are widely used in food products, including products for infants.

Recently, a randomised controlled study conducted in Italy reported that supplementation with *Bifidobacterium animalis* subsp. *lactis*, BB-12[®] was effective in the management of infant colic (Nocerino *et al.*, 2020). As evidence suggests that the development of the infant gut microbiota is dependent on region, it is questionable whether results from western countries can be extrapolated to regions with distinct differences in diet and climate (Adlerberth and Wold, 2009; Grzeskowiak *et al.*, 2012).

To explore the potential efficacy of *B. animalis* subsp. *lactis* BB-12 in the management of infant colic in breastfed Chinese infants, this randomised, double-blind, placebo-controlled study was performed.

2. Materials and methods

Study design and participants

This study was a randomised, double-blind, placebo-controlled parallel-group study in infants diagnosed with colic according to ROME III criteria, conducted in accordance with the principles of the Declaration of Helsinki and generally scientifically acceptable standards. All procedures involving human subjects were approved by the ethical committee of Chengdu New Century Women's

and Children's Hospital in Sichuan province, China P.R. The legal guardians were informed about the objectives, nature, significance, risks and implications of the study before inclusion and particular about the possibility of withdrawing from the study at any time without losing any benefits their infant was entitled to. Furthermore, how personal and health-related data including stool samples would be collected and used during the study. The infants' parents or caregivers were given time to discuss any questions and decide regarding participation in the study, and written informed consent was obtained from the infants' parents or caregivers before enrolment. The study is registered on the ISRCTN database (International Standard Registry Clinical Number; www.isrctn.com; ISRCTN 13340073).

The study comprised of a screening visit (Visit 1), a 1-week run-in period for assessment of eligibility including confirmation of infant colic, a randomisation visit (Visit 2), a 21-day intervention period, and an end-of-study visit (Visit 3). At Visit 1, a brief physical examination was performed to exclude any other medical reason for the infant's inconsolable crying and fussing. The parents or caregivers were given a 24-h structured diary and instructed on how to use it and fill it in. A faecal sampling kit was provided, and the parents or caregivers were instructed on how to collect a faecal sample from the infant's diaper and how to store the sample.

Each day during the 1-week run-in period, the parents or caregivers filled out the 24-h diary, recording how many minutes the infant had been crying/fussing during the night (00:00-06:00), morning (06:00-12:00), afternoon (12:00-18:00) and evening (18:00-00:00). All episodes of defecation, including the stool form, were recorded (using a modified version of the Amsterdam Stool Chart). Type of feeding and total hours of sleep were also recorded. At the end of the week, the parents or caregivers completed the PedsQL[™] 2.0 Family Impact Module. Infant's sleeping habits were also recorded daily in the diary. Faecal sampling was performed the day before Visit 2.

At Visit 2, the study personnel reviewed the diary to confirm the presence of infant colic and determined if the infant was eligible with respect to inclusion and exclusion criteria. If infant colic was confirmed, the infant was randomised and study product dispensed. As crying on average peaks at 6 weeks of age and then tends to resolve spontaneously, eligible infants were stratified according to age at enrolment (under or above 6 weeks of age) and randomised in the two strata with 1:1 allocation to intervention groups at Visit 2.

During the entire 3-week intervention period, the infants received six drops of the study product every day, preferably at the same time each day. Each day, the parents or caregivers filled in the same 24-h structured diary as used during the run-in period. Parents and caregivers

were instructed to collect faecal samples on Day 20 of the intervention. A 21-day intervention period was chosen as this period of times corresponds to the use of one bottle of the oil drops, and to minimise the risk of dropouts. At Visit 3, the end of-study visit, the parents or caregivers returned the diary, the used oil drop bottle, and the faecal sample. A brief physical examination was performed.

Eligible subjects were full-term infants, less than 12 weeks of age at enrolment, exclusively breastfed at inclusion, birth weight $\geq 2,500$ g, with voluntary written informed consent provided by parents or caregivers. Infants were randomised if they were diagnosed with infant colic according to ROME III, defined as crying or fussing episodes lasting more than 3 h per day and occurring at least 3 days per week as assessed by data collected during the 7-day run-in period and confirmed at Visit 2 (Hyman *et al.*, 2006).

Exclusion criteria included infants fed infant formula or infants fed a combination of breastmilk and infant formula at the time of inclusion, failure to thrive (weight gain < 100 g/week averaged from birth to the last recorded weight), acute or chronic illness, gastrointestinal disorders, and use of antibiotics and/ or probiotics four weeks prior to enrolment.

Randomisation and blinding

The randomisation list was generated by a person not directly involved in the study using the Excel RAND function (Microsoft, Redmond, WA, USA). Randomisation to the two groups was performed in a 1:1 ratio in blocks of eight and stratified by age at the time of study enrolment (< 6 weeks versus ≥ 6 weeks) due to the prevalence of colic peaking at around six weeks of age and naturally resolving. Study products were labelled according to the randomisation lists and only identifiable by the randomisation number. Allocation was performed by the investigator in consecutive order by assigning each eligible infant the first available randomisation number for the relevant stratum. Parents/ caretakers, investigators, and clinical staff involved in the study were blinded until the final database was locked. Only the appointed staff at Chr. Hansen A/S (Hoersholm, Denmark) had access to the randomisation list to perform labelling of the study products.

Study conduct and study product

The infants were recruited from the outpatient departments of eight hospitals in the Qingyang, High-tech, Qing Baijing, Jinniu, Dayi, and Longquanyi districts of Chengdu city and Huili County in Xichang city, Sichuan province, western China between April 2016 and October 2017.

The active study product, *B. animalis* subsp. *lactis* BB-12 (BB-12[®] is a registered trademark of Chr. Hansen),

was provided in bottles in a suspension of sunflower oil containing 10^9 cfu BB-12 per 6 drops. Placebo products were an identical sunflower oil suspension but without any probiotics. The active and placebo products had a similar appearance, taste and smell and were provided in identical bottles with identical labelling except for the subject specific randomisation number. Both active and placebo were administered orally, as six drops once-daily for 21 days. Study products were produced by Chr. Hansen A/S. For the entire study period, infants and the mother were not allowed to consume any probiotics or fermented dairy products.

Endpoints

The primary endpoint was treatment success, defined as the proportion of infants achieving a reduction in crying and fussing time of $\geq 50\%$ from baseline. Crying and fussing time were recorded using the 24-h structured diary, where parents/caregivers were asked to record the number of minutes the infants spent crying inconsolably (minutes) and the number of crying episodes that occurred throughout the day.

Secondary endpoints were reduction in daily inconsolable crying and fussing time (min/day) from baseline until end of intervention and stool consistency and frequency. The parents/caregivers recorded stool consistency and frequency daily by using the Amsterdam Stool Form Scale in the 24-h structured diary (Bekkeali *et al.*, 2009).

Parent's/caregiver's Health Related Quality of Life (HRQoL), infant's sleep duration and faecal levels of secretory immunoglobulin A (sIgA), calprotectin, human beta-defensin 2 (HBD-2), cathelicidin (LL-37) and butyrate were exploratory endpoints. Parent's/caretaker's HRQoL was assessed weekly using the PedsQL Family Impact Module (Varni *et al.*, 2004) in the 24-h structured diary. The Parent HRQoL Summary Score (20 items) is computed as the sum of the items divided by the number of items answered in the Physical, Emotional, Social, and Cognitive Functioning scales. In the current study, we used a modified version to only include the physical, emotional, and social scales, with the aim of exploring the usefulness of the scales in relation to infant colic.

Faecal samples were randomly collected before and after the intervention to measure level of faecal sIgA, faecal calprotectin, HBD-2, LL-37 and butyrate. Levels of HBD-2, LL-37 and butyrate were analysed post-hoc. Parents or caregivers received a faecal sample kit consisting of a spoon and faecal sampling tube at Visit 1 and Visit 2 and were instructed to collect the faecal sample 24 h before the next visit, to keep it in a freezer between -18 and -20 °C, and to bring the stool sample to the next visit. When delivered, faecal samples were stored at -80 °C until analysis.

All adverse events, defined as any untoward medical occurrence in an infant during the intervention period, were recorded.

Analyses were performed by Newpharing Bio-medical Science Co, Ltd, Chengdu, China P.R. Commercial human enzyme-linked immunosorbent assay (ELISA) kits were used to measure HBD-2 (Shanghai of Shanghai Ding Biological Technology Co, Ltd., Shanghai, China P.R.), LL-37 (Nanjing Peptide Biological Technology Co, Ltd., Nanjing, China P.R.), sIgA (Shanghai of Shanghai Ding Biological Technology Co, Ltd.) and calprotectin (Zhengzhou Cell Antibody and Antigen Biotechnology Co Ltd., Zhengzhou, China P.R.) from the supernatants of faecal homogenates, as per manufacturer's instructions. Faecal butyrate levels were assessed using gas chromatography and expressed as mM (Xi'an Tianmao Chemical Co, Ltd., Xi'an, China P.R.).

Statistical analysis and sample size

The sample size was based on the primary endpoint and the ability to achieve a significant difference of 20% in treatment success between active and placebo. With a significance level of 0.0500, a difference of 20 percentage points between the probiotic and placebo groups, a 2-sided approach, power of 80% and a potential dropout rate of 40%, it was planned that a total of 224 infants would be included, i.e. 112 in each treatment group. During the study phase, the observed drop-out rate was below the anticipated 40%, therefore, recruitment was stopped for ethical reasons once 192 infants had been recruited with written informed consent.

All analyses of efficacy data were performed on the intention-to-treat (ITT) dataset, defined as infants who were randomised and consumed at least one dose of study product and who had available efficacy data. For sensitivity analysis, analyses were also performed on the per protocol set (PPS), consisting of infants who were randomised, had consumed at least one dose of study product, who had available efficacy data and who had no major protocol deviations. All safety data were reported on the safety analysis set (SAS), defined as infants who were randomised and consumed at least one dose of study product regardless of any protocol deviations. Definition of major protocol deviations and selection of infants accordantly were performed prior to database lock and unblinding of the study. SAS version 9.2 for Windows (SAS Institute Inc., Cary, NC, USA) was used for all analyses.

The main analysis for the primary endpoint, treatment success in infants, was based on the proportion of responders versus non-responders in each group using the χ^2 test with 95% confidence intervals. A responder was defined as an infant achieving a reduction in daily average crying time $\geq 50\%$ from baseline to day 21. All statistical tests

were two-sided and performed at the 5% level. A *P*-value of <0.05 was considered statistically significant. Wilcoxon sign-rank test was used for skewed distribution data.

3. Results

Infant characteristics

A total of 192 infants were enrolled and randomised into the study and included in the ITT analysis. 96 were randomised to the BB-12 group and 96 to the placebo group. No infants were lost to follow up, and all infants completed Visit 3. Sixteen infants were excluded from the PP analysis due to major protocol deviations, the most common reasons were 'using another probiotic' ($n=5$) and 'difficulty in completing the diary' ($n=8$). All allocated infants who received at least one dose of study product were included in the ITT. 176 infants were included in the PP dataset (89 in the BB-12 group and 87 in the placebo group). No adverse events related to study product intake were reported during the study. Figure 1 is a flowchart illustrating participant involvement.

Based on the characteristics measured, the two study groups were similar at baseline (Table 1). Compliance was calculated for the 21-day intervention period based on the weights of the returned bottles and the daily dose taken (determined using the 24-h diary recordings). No difference in compliance was observed between study groups, with a compliance of 97.6% in the BB-12 group and 94.8% in the placebo group.

Efficacy results

The primary endpoint, treatment success, was defined as the percentage of infants achieving a reduction in the daily crying/fussing time $\geq 50\%$ after the 21-day intervention. ITT analysis of the primary efficacy endpoint showed a statistically significant difference in treatment success between the study groups in favour of BB-12 compared to the placebo group (61.45 vs 21.87%, 59/96 vs 21/96) ($P<0.001$) (Figure 2).

Secondary efficacy outcome

A statistically significant reduction in crying time after the 21-day intervention was observed in the BB-12 group compared to placebo ($P<0.0001$) (Table 2).

The mean number of daily crying episodes after the 21-day intervention was significantly reduced in the BB-12 group compared to the placebo group (10.0 ± 3.0 to 5.0 ± 1.9 vs 10.5 ± 2.6 to 7.5 ± 2.8 , respectively) ($P<0.001$). The mean daily sleep duration was markedly increased from baseline to end of intervention in the BB-12 group compared to the infants in the placebo group (60.7 ± 104.0 minutes

Figure 1. Disposition of the subjects.

Table 1. Demographic and baseline characteristics of the infants.¹

Characteristics	BB-12	Placebo	t/ χ^2 values	P-value
No. of infants	96	96	–	–
Female [n (%)]	52 (54.2)	46 (47.9)	0.750	0.386
Caesarean delivery [n (%)]	49 (51.0)	54 (56.3)	0.524	0.493
Gestational age [weeks (mean $\pm$ SD)]	39.3 $\pm$ 5.1	39.7 $\pm$ 6.2	0.488	0.626
Age when recruited [weeks (mean $\pm$ SD)]	4.5 $\pm$ 0.8	4.3 $\pm$ 0.9	1.627	0.106
Birth weight [kg (mean $\pm$ SD)]	3.52 $\pm$ 0.74	3.66 $\pm$ 0.81	0.106	0.213
Maternal intake of probiotic during pregnancy [n (%)]	21 (21.9)	18 (18.8)	0.290	0.590
No. of infants with allergic history [n (%)]	8 (8.3)	11 (11.5)	0.526	0.468
No. of infants exposed to passive smoking [n (%)]	3 (3.1)	2 (2.1)	0.205	0.650
Living environment (n)	–	–	0.021	0.884
Rural	42	41	–	–
Town	54	55	–	–
Levels of parents' education (n)	–	–	3.813	0.149
High school	22	23		
Junior College	38	49		
University	36	24		

¹ SD = standard deviation.

Figure 2. Primary endpoint treatment success.

per day vs 31.9 ± 102.7 min/day, respectively) ($P < 0.001$). No significant change was observed for mean daily stool frequency from baseline to end of intervention in the BB-12 group (5.0 ± 2.5 to 4.7 ± 2.3), but in the placebo group a statistically significant decrease was observed (4.8 ± 2.5 to 3.7 ± 2.8).

The faecal levels of slgA, butyrate, calprotectin, HBD-2, and LL-37 for the two study groups at baseline and after the 21-day intervention are presented in Figure 3. The faecal levels of HBD-2, LL-37, slgA, calprotectin and butyrate were statistically higher in the BB-12 group compared to the placebo group after the 21-day intervention.

At the end of the intervention, the physical functioning, emotional functioning, and social functioning scores were significantly higher for the BB-12 group compared to the placebo group (all $P < 0.05$) (Table 3).

Safety and healthy growth

The study product was well tolerated, and no adverse events were reported. Normal growth, i.e. weight, length and head circumference, increased within the normal range from visit to visit, with no difference between the two study groups. No use of antibiotics was reported.

4. Discussion

This study suggests that supplementation with *B. animalis* subsp. *lactis* BB-12 is effective for the treatment of infant colic. The study showed that the BB-12 strain, administered in a daily dose of 1×10^9 cfu for 21 days, was associated with treatment success (defined as the proportion of infants achieving a reduction in crying and fussing time of $\geq 50\%$ from baseline), reduced crying duration and improved sleep duration. These variables have all been considered clinically relevant in previous studies and meta-analyses (Dryl and Szajewska, 2018; Sung *et al.*, 2014, 2018).

Table 2. Mean daily crying time (min/day) in study population.¹

	BB-12 (n=96)	Placebo (n=96)	P-value ²
Baseline (mean $\pm$ SD)	139.5 $\pm$ 35.2	137.0 $\pm$ 33.8	0.5863
End of 1 st week of intervention (mean $\pm$ SD)	122.4 $\pm$ 33.7	139.1 $\pm$ 36.7	0.001
End of 2 weeks of intervention (mean $\pm$ SD)	102.3 $\pm$ 28.1	131.6 $\pm$ 31.5	<0.0001
End of intervention (mean $\pm$ SD)	60.8 $\pm$ 23.4	95.8 $\pm$ 26.0	<0.0001

¹ SD = standard deviation.
² t-test for comparison between groups.

Table 3. Impact of intervention on PedsQL™ Family Impact subscales.¹

	BB-12 (n=96)	Placebo (n=96)	P-values
Physical functioning score			
Baseline (mean $\pm$ SD)	43.1 $\pm$ 11.4	40.8 $\pm$ 13.3	0.814
After intervention (mean $\pm$ SD)	56.8 $\pm$ 15.2	49.6 $\pm$ 12.5	0.011
Emotional functioning score			
Baseline (mean $\pm$ SD)	50.6 $\pm$ 10.6	51.6 $\pm$ 11.4	0.515
After intervention (mean $\pm$ SD)	66.7 $\pm$ 15.1	59.5 $\pm$ 13.1	0.005
Social functioning score			
Baseline (mean $\pm$ SD)	42.7 $\pm$ 13.1	44.5 $\pm$ 11.4	0.770
After intervention (mean $\pm$ SD)	57.8 $\pm$ 14.7	51.2 $\pm$ 16.2	0.024

¹ SD = standard deviation.

The results of this study are in line with data from other studies that have investigated the BB-12 strain in infants with colic (Nocerino *et al.*, 2020; Xinias *et al.*, 2017). In particular, results from a recent randomised, double-blind, placebo-controlled study with a similar study design in terms of dose tested and primary endpoint, also found that the BB-12 strain was associated with treatment success (same definition as the current study), reduced crying time and a beneficial effect on sleep duration (Nocerino *et al.*, 2020). Additionally, the faecal analysis results on the immunity biomarkers HBD-2, LL-37, secretory IgA and butyrate from our study are in line with the results reported in the comparable study (Nocerino *et al.*, 2020). We found these immunity biomarkers increased in both study groups, but to a greater extent in the BB-12 group suggesting that the BB-12 strain may have an immunomodulatory action in the infant gut which could play a beneficial role in the context of infant colic. Butyrate, which is a gut microbiota metabolite synthesised from non-absorbed carbohydrate by

Figure 3. Immunological faecal biomarkers at baseline and after the 21-day intervention. (A) Stool immunoglobulin A; (B) stool butyrate; (C) stool calprotectin; (D) stool human beta defensin 2; (E) stool human cathelicidin.

colonic microbiota, has, among other effects, been shown to play a role in intestinal motility, visceral perception and to exert an anti-inflammatory action, all effects relevant for infant colic (Canani *et al.*, 2011). Increases in butyrate were seen in both treatment groups in our study, but again to a higher extent in the BB-12 group.

In contrast to the Nocerino *et al.* (2020) study, we found that the levels of calprotectin increased to a higher level in the BB-12 group compared to the placebo group. In the current study, we observed a relatively high level of calprotectin in both intervention groups. Newer research has suggested that calprotectin is important for the postnatal development of the intestinal microbiota and immune system in infants (Willers *et al.*, 2020). Faecal calprotectin levels in apparently healthy infants aged 1 to 18 months have been reported with inconsistent results, thus with the common denominator that the values are substantial higher, with a high interindividual variation, than in adults and exhibit a downward trend with increasing age (Kapel *et al.*, 2010; Li *et al.*, 2015; Willers *et al.*, 2020). The role of calprotectin in infant colic needs further investigation.

B. animalis subsp. *lactis* BB-12 has shown in several studies to increase the total amount of bifidobacteria in the infant gut (Mohan *et al.*, 2006; Nocerino *et al.*, 2020) and the amount of bifidobacteria has been associated with decreased duration of colicky crying (Nocerino *et al.*, 2020; Pärtty *et al.*, 2012). Moreover, infant colic has been associated with dysbiosis due to an increase in the abundance of proteobacteria and decrease in the abundance of bifidobacteria (DeWeerth *et al.*, 2013; Pärtty *et al.*, 2012; Savino *et al.*, 2009). These data support the hypothesis that infant colic may be caused by alterations to the infant gut microbiota which may lead to abnormal gut motility and gas production (Gupta, 2002). Furthermore, it underlines that the gut microbiota is an important target intervention against infant colic.

As several studies have reported distinct differences in the gut microbiota of infants from different parts of the world (Adlerberth and Wold, 2009; Grzeskowiak *et al.*, 2012) one could question if infant colic study results from western countries can be extrapolated to other distinct regions of the world. With the current study, positive results now exist for the treatment of infant colic with the BB-12 strain in two different regions of the world. Both studies show a significant effect on treatment success and crying duration after supplementation with the same probiotic strain.

Traditionally, the definition of infant colic was based on the rule of three; i.e. unexplained episodes of crying for more than three hours per day for three days per week for at least three weeks, often referred to as Wessel's Criteria (Wessel *et al.*, 1954). The Rome III criteria expanded on Wessel's

Criteria for infant colic by further adding age criteria and no evidence of a failure to thrive (Hyman *et al.*, 2006). In 2016, the updated Rome IV redefined and simplified the criteria to recurrent and prolonged periods of infant crying, fussing, or irritability in an infant below 5 months who displays no failure to thrive. However, in a research setting the diagnostic criteria also includes crying or fussing for at least 3 hours per day during 3 or more days over a 7 day period, which is similar to the criteria used in the current study (Benninga *et al.*, 2016). The change in criteria was based on new research, and an aim of developing a more effective clinical diagnostic scheme, including adding aspects other than the amount of crying, as the amount of crying has been found to distress caregivers less than prolonged, hard-to-soothe, and unexplained crying episodes does (Fujiwara *et al.*, 2011).

Parents and caregivers often assume that the cause of prolonged, hard-to-soothe, and unexplained crying episodes originate from gastrointestinal pain. The crying bouts occur without obvious cause and the unexplained nature of infant colic is associated with maternal post-partum depression, parental guilt and frustration, early cessation of breastfeeding and multiple physician visits (Zeevenhooven *et al.*, 2018). These inconsolable crying bouts are one of the main reasons for parents' and caregivers' concerns and may have a major impact on the establishment of a new family and on parents'/caregivers' HRQoL. We used a modified version of the PedsQL Family Impact Module, an instrument designed to assess the impact of chronic disease on parents and families by examining family functioning and parent HRQoL, to explore the impact of infant colic on the social, emotional and physical function of parents and caregivers. No significant difference in the physical, emotional and social function scores from the PedsQL Family Impact Module was observed between the groups at baseline, but at the end of the intervention, parents of infants receiving the BB-12 strain had significant higher HRQoL scores than the placebo group, indicating that they experience a better quality of life despite the presence of infant colic in the family.

To our knowledge, this study is the first to investigate a possible effect of probiotics on the HRQoL of parents with a colicky infant. The strengths of this study are the blinded randomised design and the application of well-defined and acknowledged infant colic diagnostic criteria. The study was well-powered and the estimated sample size, without the added estimated drop-out rate, was met. In order to take into account the observed peak of infant colic at 6 weeks of age and the natural resolution of infant colic, the study population was stratified into two groups (below or above 6 weeks at study inclusion) to ensure equal distribution in both groups. The data reported are from the ITT, which is appropriate as the data are conservative, reflect clinical practice and increase generalisability as non-compliant

and compliant subjects are analysed together, reflecting the real world.

The main limitation of this study is the exclusion of formula fed infants and the lack of microbiota analysis. However, a minor group converted to mixed feeding during the study. Regardless of the effect of BB-12 in infants with mixed feeding, further studies are needed to confirm the beneficial effect in exclusively formula fed infants.

5. Conclusions

In conclusion, the results of this study strongly support a clinically relevant benefit of the probiotic strain *B. animalis* subsp. *lactis* BB-12 on reducing crying and fussing in infants diagnosed with infant colic.

Supplementary material

Supplementary material can be found online at <https://doi.org/10.3920/BM2020.0233>.

Table S1. Raw data biomarkers.

Conflicts of interest

Cathrine Melsaether is employed by Chr. Hansen A/S. None of the other authors have any conflicts of interest.

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