Effects of a nine-strain bacterial synbiotic compared to simethicone in colicky babies – an open-label randomised study

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Abstract

The aim of the study was to determine effects of administration of simethicone and a multi-strain synbiotic on the crying behaviour of colicky babies. The study design consisted of an open-label, two parallel treatment group study involving 87 infants aged 3-6 weeks with infantile colic (defined as crying episodes lasting 3 or more hours per day and occurring at least 3 days per week within 3 weeks prior to enrolment) randomly, unequally [1:1.5] assigned to receive simethicone (n=33) or a multi-strain synbiotic (n=54) orally for 4 weeks. The multi-strain synbiotic contained *Lactobacillus acidophilus* LA-14, *Lactocaseibacillus casei* R0215, *Lactocaseibacillus paracasei* Lp-115, *Lactocaseibacillus rhamnosus* GG, *Ligilactobacillus salivarius* Ls-33, *Bifidobacterium lactis* Bl-04, *Bifidobacterium bifidum* R0071, *Bifidobacterium longum* R0175 and fructooligosaccharides). Primary outcome measures were the responder rates (effect ≥50% reduction from baseline) of the measures ‘crying days last 3 weeks’, ‘average evening crying duration last 3 weeks’ and ‘reduction of average number of crying phases per day last three weeks’ at the end of treatment. The study is registered at [ClinicalTrials.gov](https://clinicaltrials.gov) under NCT 04487834. Significantly higher responder rates (effect ≥50% reduction from baseline) of the multi-strain synbiotic compared to simethicone were found for the measures ‘crying days last 3 weeks’ (72% vs 18%, $P<0.0001$) and ‘average evening crying duration last 3 weeks’ (85% vs 39%, $P=0.0001$). No significant difference was found for the measure ‘reduction of average number of crying phases per day last three weeks’ (50% vs 42%, $P=0.4852$). No adverse effects were reported for the two treatment groups. Based on these results, the multi-strain synbiotic can be considered as an interesting therapeutic possibility for the treatment of infantile colic, worthwhile to be investigated further in non-clinical and clinical studies.

Keywords: infantile colic, extensive crying, gut microbiota, probiotic

1. Introduction

Infantile colic or extensive crying is a major burden for newborns, their parents and healthcare providers (Landgren and Hallström, 2011). In most of the cases infantile colic will disappear after the first three to five months of life (Sung, 2018). Nevertheless, due to its stressful nature for parents, infant colic is among the leading causes to consult a health care professional during early infancy (Lucassen, 2010). The parental burden associated with infantile colic finally results in enormous pressure on paediatricians to prescribe

at least some kind of remedy. Diagnosis of infantile colic can be based on the Wessel’s criteria, defined by crying and restlessness for more than 3 h a day for more than three days per week for more than three weeks (Wessel *et al.*, 1954). Depending on diagnostic details, occurrence rates between 3 to 40% of all infants have been found (Helseth and Begnum, 2002; Lucassen *et al.*, 2001).

The aetiology of infant colic remains unclear (Sarasu *et al.*, 2018), with a variety of potential causes (gastrointestinal, hormonal, neurodevelopmental, and psychosocial)

discussed. This has resulted in a broad range of therapeutic approaches aiming to address the problem (Lucassen, 2010; Mai *et al.*, 2018).

Simethicone (Anonymous, nd), a mixture of dimethicone and SiO₂, is a rather old product that, it is claimed, acts as a topical barrier for protecting the gut mucosa against irritants. It is not absorbed and is virtually non-toxic. While its use in diagnostic procedures is well-established, the therapeutic effects in a number of gastroenterological indications are contradictory (Meier and Steuerwald, 2007). In infantile colic, a number of smaller simethicone trials (Danielsson and Hwang, 1985; Metcalf *et al.*, 1994; Sethi and Sethi, 1988) have been published, but evidence did not reach the threshold of significance (Biagioli *et al.*, 2016). Despite the absence of evidence for beneficial effects, simethicone is widely used for the treatment of infantile colic in some countries.

There is growing evidence that the gut microbiotas of colicky infants significantly differ from those of non-colicky babies (Savino *et al.*, 2004, 2005, 2009). In the gut microbiota of colicky babies a lower level of commensal bacteria like *Lactobacilli* and *Bifidobacteria* and higher numbers of *Proteobacteria* have been found. Among these *Proteobacteria* were *Escherichia* and *Klebsiella* bacteria (Savino *et al.*, 2011), which are well known for their gas-producing properties, as well as the potential production of inflammatory lipopolysaccharides (LPS). In addition, it has been described that the gut microbiota of colicky infants exhibits a slower bacterial colonisation, a reduced microbiota diversity and a lower microbiota stability (De Weerth *et al.*, 2013). These findings have triggered a number of studies investigating the effect of supplementation of the gut microbiota of colicky babies with products containing probiotic bacteria without a prebiotic component (probiotics), or with (synbiotics).

Although infantile colic is in most cases a self-limiting condition, paediatricians are writing a prescription for most of the affected infants. Unpublished results from surveys, performed by our research team among German and Polish paediatricians, revealed that in these two countries simethicone and pro-/synbiotics were the most frequently prescribed products for infantile colic. The objective of the present study was to compare the effects of simethicone with those of a food supplement multi-strain synbiotic on the crying behaviour in colicky babies. This multi-strain synbiotic preparation, which is frequently used by German and Polish paediatricians, contains one *Lactobacillus* (*Lactobacillus acidophilus*), three lactaseibacilli (*Lactobacillus casei*, *Lactobacillus paracasei*, *Lactobacillus rhamnosus* GG), one *Lactiplantibacillus* (*Lactiplantibacillus plantarum*), one *Ligilactobacillus* (*Ligilactobacillus salivarius*), three bifidobacteria (*Bifidobacterium lactis*, *Bifidobacterium*

bifidum, *Bifidobacterium longum*) and the prebiotic fructooligosaccharides (FOS). In a study, recently published by us (Piątek *et al.*, 2020), it was found that this multi-strain synbiotic antagonises the *in vitro* growth of the pathogenic bacteria *Escherichia coli* EPEC, *Shigella sonnei*, *Salmonella typhimurium*, *Klebsiella pneumoniae* and *Clostridioides difficile*.

During the preparation phase of this study, we learned that pediatricians willing to participate were not ready to treat colicky babies with a placebo, as this would not be acceptable for the majority of parents of the colicky babies. In addition, placebo preparations were neither available for us for simethicone nor for the multi-strain synbiotic. Consequently, our study had to be performed as an open trial, comparing the two products without a placebo control group. Effects of simethicone in infantile colic have been shown not to differ significantly from treatment with a placebo (Danielsson and Hwang, 1985; Metcalf *et al.*, 1994; Sethi and Sethi, 1988), therefore the simethicone treatment group in our study has to be considered as the control group against which the effect of the multi-strain synbiotic has to be evaluated.

2. Materials and methods

This was an open-label, unequally randomised [1:1.5], two treatment group study conducted in Poland during April and July 2020. The trial was registered at [ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study?term=NCT04487834) (NCT 04487834). Parents of all infants gave their informed consent for participation before the infants were included in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of The President Stanisław Wojciechowski State University of Applied Sciences in Kalisz (Project identification code 1/2020). Assuming a significance level of 5%, a power of 80%, a responder rate of 50% in the simethicone group and of 80% in the multi-strain synbiotic group and a dropout rate of 0%, a required sample size of 36 patients was calculated by using the Sample Size Calculator of Sealed Envelope Ltd. (London, UK). Recruitment for the study was carried out in hospitals in Poznań, Poland. Infants (aged 3–6 weeks) of parents who agreed were assessed for eligibility to participate in the study. After organic causes of crying were carefully excluded, parents, supported by paediatricians, midwives or nutritionists, filled out a questionnaire to obtain data concerning type of delivery, birth weight, gestational age, type of feeding and details of the crying behaviour of the babies. Babies who fulfilled the Wessel criteria (Wessel *et al.*, 1954) of extensive evening crying for at least 3 h per day, during at least three days per week, during the last three weeks, were included in the study. Exclusion criteria were prior treatment with probiotics, synbiotics or antibiotics. The study population comprised breastfed and formula-fed, as well as mixed-fed new-borns.

Babies diagnosed to have infantile colic were randomly assigned to two treatment-groups. A two-treatment unequal allocation [1:1.5] randomisation scheme was generated by using the Simple Randomiser of Sealed Envelope Ltd. Product was allocated to patient by paediatrician using the randomisation scheme based on entry into the study. One group was treated for four weeks with simethicone (Espumisan®, 100 mg/ml, Berlin-Chemie/Menarini Polska Sp. z o.o., Warsaw, Poland). Simethicone was administered 3-6 times per day with each treatment comprising 6 drops of the 100 mg/ml emulsion. The second group was treated with one stick pack of a multi-strain synbiotic (Multilac® Baby, Vivatrex GmbH, Aachen, Germany) per day. Each stick pack of Multilac Baby contains a total of 10^9 cfu, with equal cfu amounts of the following probiotic bacteria: *L. acidophilus* LA-14, *L. casei* R0215; *L. paracasei* Lpc-3; *L. plantarum* Lp-115; *L. rhamnosus* GG, *L. salivarius* Ls-33, *B. lactis* Bl-04, *B. bifidum* R0071, *B. longum* R0175 and 1.43 g of the prebiotic FOS. Parents were provided with a diary (24 h Parental Daily Report) to protocol crying behaviour, drug administration and side effects.

After the end of the treatment period changes in crying behaviour were assessed with the same set of questions used for the enrolment into the study. In addition, it was investigated if antibiotics were administered during the treatment period. Numerical measures (days of crying during the last three weeks, duration of evening crying, average number of crying phases per day) were analysed by calculation of the average and the standard deviation. In addition, the rate of treatment success was determined, which was defined as a reduction of a given measure (days of crying during the last three weeks, average duration of evening crying during last three weeks, average number of crying phases per day during last three weeks) by equal or more than 50%, compared to the value at the time of enrolment (Sung, 2018). For non-numerical measures (questions answered either by yes or no), the percentages of 'yes' (or 'no') answers were calculated.

The statistical analyses were conducted with GraphPad Prism software version 8.2 (GraphPad Software, San Diego, CA, USA) or MedCalc Statistical Software version 19.2.1 (MedCalc Software Ltd., Ostend, Belgium). The Student t-test was used to compare mean values of continuous variables approximating a normal distribution. For non-normally distributed variables, the Mann-Whitney U test was used. The X2 test or Fisher exact test was used, as appropriate, to compare percentages. The MedCalc Statistical Software version 19.2.1 was used to calculate the relative risk (RR) and number needed to treat (NNT), all with a 95% confidence interval (CI). The difference between the treatment groups was considered significant when the *P*-value was less than 0.05, when the 95% CI for RR did not include 1.0, or when the 95% CI for mean difference did not include 1.0, or when the 95% CI for mean difference

did not include 0. One-way ANOVA followed by Dunnett's multiple comparisons test as well as Cochran-Armitage Chi-squared test for trend were performed using GraphPad Prism software. All statistical tests were two-tailed and performed at the 5% level of significance. All analyses were conducted on an intention-to-treat basis, including all patients in the groups to which they were allocated.

3. Results

Figure 1 shows the flow of patients through the study. During the recruitment period of the study a total of 915 babies were born. Parents of 211 babies agreed to have their infants assessed for eligibility to participate in the study. Of these, 87 (41.2%) fulfilled the Wessel's criteria used for enrolment. One baby was fulfilling the Wessel's criteria of enrolment but was excluded because of a previous antibiotic treatment. 123 new-borns were excluded for not fulfilling the Wessel's criteria. Of the babies diagnosed for infantile colic, 33 were treated with simethicone and 54 with the multi-strain synbiotic. No patient was lost or had to be excluded during the remaining phase of the trial. Table 1 shows the baseline characteristics of both treatment groups. Statistical analyses revealed that there were no significant differences between the two treatment groups.

Results for the three main measures of the study: (1) number of days of crying during the last three weeks, (2) average duration of evening crying during the last three weeks and (3) average number of crying phases per day during the last three weeks are shown in Figure 2. As is visible in Figure 2A and 2B, simethicone and the multi-strain synbiotic treatment caused a significant reduction in both the number of days of crying during the last three weeks and the average duration of evening crying during the last three weeks. Comparing the effects of the multi-strain synbiotic with that of simethicone revealed that the multi-strain synbiotic had significantly ($P \leq 0.0001$) superior effects on these two main measures. The average number of crying phases during the last three weeks was significantly reduced by the multi-strain synbiotic, but not by simethicone (Figure 2C).

Treatment success rates for the reduction of days of crying during the last three weeks and for the reduction of average evening crying duration last three weeks were significantly higher in the multi-strain synbiotic group than in the simethicone group (Table 2). The treatment success rate for the reduction of average number of crying phases during the last three weeks showed no significant difference between simethicone and the multi-strain synbiotic treatment groups.

In addition to the main measures, a number of other crying behaviour observations were assessed by the study (Table 3). In order to verify if the effects of the multi-strain synbiotic were significantly different from those of simethicone, a

Figure 1. Patient enrolment and study progress.

Table 1. Baseline characteristics of simethicone and multi-strain synbiotic treatment groups.¹

	Simethicone (n=33)	Multi-strain synbiotic (n=54)	P-value ²
Gender (F/M)	18/15	29/25	0.9394 ^a
Type of delivery (normal/caesarean)	24/9	39/15	0.9594 ^a
Birthweight (g)	3,480±412	3,500±404	0.8246 ^b
Feeding (breast/formula/mixed)	22/11/0	27/17/10	0.0943 ^a
Days of crying last 3 weeks (days)	18.2±1.7	18.3±2.1	0.8246 ^b
Duration of evening crying (h/day)	2.7±0.5	2.8±0.7	0.4760 ^b

¹ Data are expressed as average ± standard deviation or numbers.

² Statistical tests indicated by superscript letter (a) Chi-squared test, and (b) Student t-test.

Cochran-Armitage Chi-squared test for trend was used. The linear trend of data ordered by treatment efficiency was tested. Data were ordered in three groups: before treatment (n=87) < simethicone (n=33) < multi-strain synbiotic (n=54). In conclusion, statistically significant, greater effectiveness of the multi-strain synbiotic was confirmed for: (1) reducing the number of babies who cannot be calmed during crying, (2) reducing the number of babies with problems falling asleep, (3) reducing the number of babies with bloated belly, and (4) reducing the number of babies with retracted legs. Neither simethicone, nor the multi-strain probiotic, had an effect on the number of babies with a red face. No adverse effects were reported for the simethicone or the multi-strain synbiotic treatment group.

4. Discussion and conclusions

Infantile colic is a self-limiting condition and most cases are improving over time without any treatment (Lucassen, 2010). Nevertheless, for most colicky patients paediatricians in Germany and Poland are prescribing simethicone or a pro-/synbiotic (according to the unpublished results from surveys performed by our research team among German and Polish paediatricians). Despite its wide use, there is little evidence that simethicone has significant effects in infantile colic compared to a placebo treatment (Biagioli *et al.*, 2016; Lucassen, 2010; Meier and Steuerwald, 2007). The role of probiotics in the treatment of infantile colic has been reviewed by a number of authors (Anabrees *et al.*, 2013; Dryl *et al.*, 2018; Ong *et al.*, 2019; Schreck

Figure 2. Effects of simethicone or multi-strain synbiotic treatment in colicky babies on (A) days of crying during three weeks, (B) average duration of daily evening crying during three weeks and (C) average number of crying phases per day during three weeks. Results are mean $\pm$ standard deviation. Data were analysed by one-way ANOVA followed by Dunnett's multiple comparisons test: ** $P \leq 0.0001$.

Table 2. Effect of simethicone and the multi-strain synbiotic on main measures of crying behaviour.¹

Outcome	Simethicone (n=33)	Multi-strain synbiotic (n=54)	RR (95% CI)	NNT (95% CI)	P-value ²
Number of patients with treatment success: reduction of days of crying $\geq 50\%$ (% responder)	6 (18%)	39 (72%)	0.2838	1.427	<0.0001
Number of patients with treatment success: reduction of avg. duration of evening crying $\geq 50\%$ (% responder)	13 (39%)	46 (85%)	0.2444	2.184	0.0001
Number of patients with treatment success: reduction of avg. number of crying phases per day $\geq 50\%$ (% responder)	14 (42%)	27 (50%)	0.8684	13.200	0.4852

¹ NNT = number needed to treat is the estimated number of patients who need to be treated with multi-strain synbiotic rather than with simethicone for one additional patient to benefit (Altman, 1998); RR = risk ratio; CI = confidence interval.

² Fisher's exact test was used to calculate the P-values.

Table 3. Effect of simethicone and the multi-strain synbiotic on other crying behaviour observations.¹

Observation	Before treatment (n=87)	Simethicone (n=33)	Multi-strain synbiotic (n=54)	Chi-squared (trend)	P-value ²
Patients who cannot be calmed during crying	50.6% (44)	24.2% (8)	13.0% (7)	22.062	<0.0001
Patients with problems falling asleep	100.0% (87)	57.6% (19)	42.6% (23)	60.499	<0.0001
Patients with bloated belly	62.1% (54)	48.5% (16)	33.3% (18)	11.071	0.0009
Patients with retracted legs	90.8% (79)	69.7% (23)	61.1% (33)	17.150	<0.0001
Patients with red face	89.7% (78)	87.9% (29)	90.7% (49)	0.0275	0.8683

¹ Results are percentage of total (number of babies).

² Cochran-Armitage Chi-squared test for trend was used to calculate the trends as well the P-values.

Bird *et al.*, 2017; Tintore *et al.*, 2017). Probably the most extensively investigated probiotic in infantile colic is *Limosilactobacillus reuteri* DSM 17938. A number of trials have been published, however, with conflicting results. Four double-blind randomised studies of breastfed infants with colic demonstrated *L. reuteri* DSM 17938 to be effective (Chau *et al.*, 2015; Roos *et al.*, 2013; Savino *et al.*, 2010; Szajewska *et al.*, 2013). In contrast, one double-blind randomised study of both breastfed and formula-fed infants with colic concluded it was ineffective even for those who were breastfed (Sung *et al.*, 2014). A recently published individual participant data meta-analysis (IPDMA), pooling raw data from four individual studies, found that *L. reuteri* DSM 17938 had a positive effect in breastfed infants, however had no effect in formula-fed infants (Sung *et al.*, 2018). In a single-blind randomised trial in predominantly or exclusively breastfed colicky babies, *L. reuteri* was found to reduce daily crying time, improve parent satisfaction and reduce maternal depression (Mi *et al.*, 2015). In a non-blinded randomised controlled trial with breastfed babies with infantile colic, *L. reuteri* exhibited colic symptom relief superior to simethicone (Ashraf *et al.*, 2015). *L. reuteri* has been demonstrated to decrease faecal calprotectin, which is a clinical marker of intestinal inflammation, and resulted in improvements of blood inflammatory markers (Savino *et al.*, 2018).

A mono-strain probiotic containing only the probiotic strain *L. rhamnosus* GG is well known by paediatricians for its effects against antibiotic-associated diarrhoea (Guo and Goldenberg, 2019). When evaluated for efficacy in infantile colic, no significant treatment effects compared to placebo were found (Fatheree *et al.*, 2016; Pärtty *et al.*, 2015). In infants treated in tandem with behavioural support and cow's milk elimination diet, *L. rhamnosus* GG did not provide additional treatment effect for diary-verified colic crying, although parental report of crying suggested the probiotic intervention was effective.

Another mono-strain probiotic, *Bifidobacterium animalis* subsp. *lactis* BB-12s has been found to have positive effects in exclusively breastfed colicky infants (Nocerino *et al.*, 2020).

There are a number of published clinical studies which investigated the effects of multi-strain synbiotics in the treatment of infantile colic. A mixture of *B. lactis* BB-12 and *Streptococcus thermophilus* has been reported to reduce the incidence of infant colic (Saavedra *et al.*, 2004). A mixture of two *Bifidobacterium breve* strains (BR03 and B632) was found to have no significant effects in the full population of the study. A sub-group analysis revealed that there was a significant reduction of crying time in the formula-fed group only (Giglione *et al.*, 2016). Treatment of colicky babies with a mixture of *Pediococcus pentosaceus* CECT 8330 and *B. longum* CECT 7894 was

found to increase the overall microbiota diversity and to reduce the relative abundance of *Enterobacteria* (mostly of *Escherichia* and *Shigella*) and *Staphylococcus*, compared to placebo (Tintore and Cune, 2017). In addition, a trend towards greater reduction in crying time in the probiotic group compared to placebo was observed (Santas *et al.*, 2015). A placebo-controlled study (Kianifar *et al.*, 2014) found a significant treatment effect (reduction of average infant crying time) in breastfed colicky babies by a synbiotic preparation containing seven probiotics (*L. acidophilus*, *Lactobacillus bulgaricus*, *L. casei*, *L. rhamnosus*, *B. breve*, *Bifidobacterium infantis*, *S. thermophilus*) and the prebiotic FOS. Another study (Dorreh *et al.*, 2017) investigated the effect of a synbiotic containing three probiotic bacteria (*L. reuteri*, *L. rhamnosus* and *B. infantis*) and the prebiotic FOS. The study included breastfed and formula-fed colicky infants and no significant difference in crying time and the number of crying attacks between the treatment group and the control group was found.

Over recent years, we collected a number of anecdotal reports from paediatricians indicating that the multi-strain synbiotic tested in the present study had positive effects in colicky babies. Our trial is the first investigating the effects of this product in a clinical trial setting. The present study has used, as most other studies evaluating the effects of probiotics in infantile colic, the measures (1) reduction of crying days during the last three weeks, (2) reduction of the average evening crying duration during the last three weeks and (3) reduction of average number of crying phases per day during the last three weeks. Improvements in these measures are clinically and practically relevant as they are potentially translating into stress relief in the affected newborns and their parents.

The improvements observed in the simethicone treatment group of the present study would have most likely been observed as well if the colicky babies had received no treatment. However, as improvements caused by the multi-strain synbiotic were significantly larger than those of simethicone, it can be concluded that the product has treatment effects superior to a placebo treatment in infantile colic.

In addition to the superior effects on the main measures, the multi-strain synbiotic showed positive effects on some other crying behaviour observations. The number of babies who could not be calmed during crying, or who had problems falling asleep, was reduced significantly more by the multi-strain synbiotic than by simethicone. These effects might be very relevant for parents of colicky babies, as not being able to calm a crying baby or getting it to sleep is experienced as utterly frustrating (Landgren and Hallström, 2011). The multi-strain synbiotic also had a significantly better effect in reducing the number of babies with a bloated belly and with retracted legs in comparison to simethicone. These

measures of symptoms are sometimes used as indicators for infantile colic, however, treatment effects on these symptoms might not be perceived as too relevant in daily practice. In contrast, neither simethicone nor the multi-strain synbiotic was found to have a significant effect on the numbers of babies with a red face.

The strength of the present study is that the effects of the multi-strain synbiotic were observed in a study with a relatively small number of participants and without selecting a subgroup of colicky babies (e.g. breast-fed babies only). Whether the treatment effects are different in certain subgroups will remain open for clarification in future larger clinical trials. A limitation of the study is the use of parental diaries to measure infants' crying behaviour. Using paper diaries (Barr *et al.*, 1988) is not without problems (Stone *et al.*, 2003). Nevertheless, it has been shown that paper diary recordings of common infant behaviour can provide reasonably good estimates of durations, while behavioural frequencies may be underestimated (Lam *et al.*, 2010). Another major limitation of the study is that medication was not blinded. Past experience or non-experience with either of the two medications might therefore exhibit a bias on the results. However, this might have influenced results for some patients negatively (e.g. preference of a liquid formulation over a powder formulation), while in others it might have had a positive influence, which, at least to a certain extent, might have neutralised this bias.

While the necessity of treating this self-limiting condition can be questioned, the pressure from parents on paediatricians to prescribe is high. Results of the present study can support paediatricians in their prescribing decisions and during their communication with parents. Results of the present clinical study and of preclinical studies published earlier (Piatek *et al.*, 2020) render the multi-strain synbiotic an interesting candidate for further clinical and non-clinical research in infantile colic.

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