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Original Article

Impact of *Bifidobacterium lactis* supplementation on fecal microbiota in infants delivered vaginally compared to Caesarean section

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Abstract

Background It has been reported that infants born by Caesarean section have altered gut microbiota, with lower numbers of bifidobacteria and Bacteroides, compared to that of infants who were delivered vaginally. Probiotic supplementation has been reported to have beneficial effects on the immune response, generally in relation to allergies.

Objective To assess the effect of *Bifidobacterium lactis* (*B. lactis*) supplementation on the presence of *B. lactis* and bifidobacteria counts in stool of infants during the first 2 months of life.

Methods We conducted an observational study of 122 healthy, breast-fed infants delivered vaginally or by Caesarean section. Infants assigned to the test group received breast milk and formula supplemented with the *B. lactis* probiotics. Infants in the control group received breast milk and formula without probiotics. The presence of *B. lactis* and stool bifidobacteria counts were determined at 1 month and 2 months of age. Growth, morbidity, serum immune markers, and stool immunoglobulin (Ig) A were also assessed.

Results *B. lactis* was more frequently detected in the stool of infants who received breast milk and probiotic-supplemented formula than in stool of infants who received breast milk and non-supplemented formula, both at 1 month and 2 months of age (OR 1,263; 95%CI 11 to 151,030; P=0.003). Of infants who received probiotic-supplemented formula, *B. lactis* was detected in 80% of those delivered by Caesarean section and in 38% of those delivered vaginally, at the 1-month mark. In infants delivered by Caesarean section, the mean stool bifidobacteria level at 1 month was significantly higher in the probiotic-supplemented group compared to that of the non-supplemented group (P=0.021).

Conclusion Early bifidobacteria supplementation of infants, particularly those delivered by Caesarean section, is associated with higher levels of stool bifidobacteria. Anthropometric data suggests beneficial effects of bifidobacteria supplementation on infant growth, though most are not statistically significant. **[Paediatr Indones. 2013;53:89-98.]**

Keywords: *Bifidobacterium lactis, gut microbiota, Caesarean section, probiotic*

In the early hours and days of life, major changes occur in the composition of intestinal microbiota. The human gut is sterile at birth, but is rapidly colonized by bacteria originating from the mother and the environment.[1,2] One of the first major determinants of gut microbiota is the mode of delivery: infants born vaginally are first colonized by the fecal and vaginal bacteria of the mother, whereas infants born by Caesarean section are initially exposed to bacteria originating from the hospital environment.[2]

A study on 1,032 infants reported that the most important determinants of gut microbiotic

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composition at 1 month of age were the mode of delivery, type of feeding, gestational age at birth, birthing environment and antibiotic use.[3] Compared to vaginally-born infants, infants delivered by Caesarean section had lower numbers of bifidobacteria and *Bacteroides*, but were found to be more often colonized with *Clostridium difficile (C. difficile)*.[4] Formula-fed infants were more often colonized with *Escherichia coli, C. difficile*, *Bacteroides* and lactobacilli than infants who were breastfed. Hospitalization and prematurity were reportedly associated with higher prevalence and counts of *C. difficile*; and antibiotic use was associated with lower numbers of bifidobacteria and *Bacteroides*.[3]

In the mammalian large intestine, there are more than 10 microorganisms/g of intestinal content. It has been well recognized that gut microbiota have an effect on host physiology. In addition, increasing evidence suggests that intestinal microbiota play an essential role in postnatal development of the immune system.[5] Several studies have investigated the effects of Caesarean section delivery on the development of allergic disease during the first years of life. A meta-analysis showed a 20% increase in the risk of asthma in children who had been delivered by Caesarean section.[6] Also, a systematic review showed evidence that the risk of developing IgE-mediated sensitization to food allergens was increased among children delivered by Caesarean section.[7] Furthermore, Caesarean delivery was indicated to be an additional risk factor for wheezing and allergic sensitization to food allergens in infants up to 2 years of age.[8]

The FAO and WHO referred to probiotics as "live microorganisms which, when consumed in an adequate amount, confer a health effect on the host".[9] Allergy prevention by way of probiotic administration to change infantile gut microbiota was first reported in 2001 by Kalliomaki *et al.*,[10] who also showed that supplementation with *Lactobacillus rhamnosus* GG resulted in a reduction in atopic eczema persisting to age 7 years.[5] In a randomized trial of 1,223 families at high risk for allergies, pregnant mothers received probiotic mixtures consisting of two lactobacilli, *Bifidobacterium breve*, and a propionibacterium. From birth to 6 months, their infants received the same probiotics as well as a galacto-oligosaccharide prebiotic. In the interim analysis at 2 years, 29% less atopic disease was observed in supplemented infants. Although the probiotic supplementation did not extend an allergy-preventative effect to 5 years of age in the overall study group, it did confer protection to IgE-associated allergic disease in Caesarean-delivered children.[11]

The primary objective of this study was to evaluate the impact of probiotic *B. lactis* supplementation on gut microbiota of babies born in two developing countries. More specifically, the effect of *B. lactis* supplementation in vaginally-delivered and Caesarean-delivered infants was assessed by *B. lactis* presence and measuring bifidobacteria levels in the stool at 1 month and 2 months of age. We also investigated the effect of *B. lactis* supplementation on growth, morbidity, and immune markers observed in these infants.

Methods

An open, non-randomized, controlled, parallel-group trial was carried out in two centers in Indonesia and Thailand from November 2009 to September 2010. Healthy infants delivered by Caesarean section or vaginally were recruited from either Padjadjaran University Medical School, Bandung, Indonesia, or Khon Kaen University Medical School, Thailand. Inclusion criteria were maximum age of 2 weeks at enrollment, birth weight ≥ 2500 g and ≤ 4500 g, gestational age ≥ 37 weeks, willing to consume a minimum of 150 ml of test product daily, likely to be compliant, and having informed consent from a legal representative. Approval was obtained from the Ethics Committees of Padjadjaran University Medical School, Bandung, Indonesia and Khon Kaen University, Khon Kaen, Thailand. Infants were predominantly breast fed for 2 weeks, followed by formula supplementation/mixed feeding of at least 150 ml/day. We excluded infants with congenital illness, significant pre-natal and/or post-natal disease, immunocompromised mothers, parents with a history of allergies, who received antibiotics at the time of enrollment, could not be expected to comply with the feeding regimen, or already participated in another clinical trial.

Subjects were assigned to either the test group, who received breast milk and *NAN 1*® formula (*Nestlé Nutrition, Indonesia*) supplemented with probiotics (*Bifidobacterium lactis* BSMZ 10140), or

the control group, who received breast milk and *NAN 1*® formula without probiotics. Both *NAN 1*® products were specifically formulated for the nutritional support of infants aged 0-6 months and were obtained as powdered form in 400 g tins. Infant formula was administered orally, in accordance with the manufacturer's instructions.

A total of 168 subjects were recruited for the study, consisting of 87 subjects allocated to the test group and 81 subjects allocated to the control group. Within each of these groups, approximately half of the infants were born by Caesarean section and half by vaginal delivery. A required minimum sample size of 140 subjects (70 per group) was determined based on the ability to detect a mean difference of 15% in levels of fecal bifidobacteria between the two study groups at a significance level of 5%. The intent-to treat population (ITT) consisted of all infants who received the study formulations and provided any pertinent data. Given the non-randomized nature of the study, subjects were analyzed according to the product received. The per-protocol population (PP) consisted of infants in the ITT population who adhered to all protocol requirements without any major protocol violations or deviations from the statistical analysis plan. Major protocol violations included: hospitalization and/or serious adverse reactions during the study period, introduction of prohibited treatment/medication during the study period, and non-compliance and/or being off study formulations during the study period.

The primary outcomes of the study were presence of *B. lactis* and bifidobacteria counts in the stool of supplemented infants born by Caesarean section or vaginal delivery, compared to non-supplemented infants, at the ages of 1 month and 2 months. Secondary outcomes were growth, morbidity, and levels of select immune markers, at 1 month and/or 2 months of age.

The presence of *B. lactis* in stool samples was determined by real time PCR using bifidobacteria specific primers. For analysis purposes, stool samples with undetected *B. lactis* were considered as 'absent'. Presence or absence of *B. lactis* in the stools of test and control groups was compared using chi square test. A logistic regression analysis was performed to assess the effect of probiotic supplementation, allowing for the mode of birth. Fecal bifidobacteria (cells/g) were measured by quantitative real-time PCR using bifidobacteria-specific primers, as described previously.[11] Stool samples were collected at 1-month and 2-month time points. For analysis purposes, bifidobacteria counts below the detection limit were set to the lower value of the detection limit (10^6 cells/g). Counts were $\log_{11}$ transformed and the resulting data assessed for normality. The non-parametric Mann-Whitney test was used to compare the test and control groups. Given the exploratory nature of the hypotheses studied, no correction of significance level was employed. Subsequently, all P values presented were unadjusted.

The effect of *B. lactis* supplementation on growth was measured by progression of weight, recumbent length/height, and head circumference at the time of enrollment, at 1 month and at 2 months of age. Infants were weighed without clothing on electronic scales. In each of the study centers, the same scale was used for all infants at all visits. Throughout the study, the electronic weighing scales were calibrated in accordance with the manufacturer's recommendations. If duplicate measurements did not agree within 10 g, the infant was weighed again. The individual measurements and the final mean of one set of measurements were recorded. Recumbent length/height was measured to the nearest 1 mm using a standardized length board. At least two people were present to maintain proper body alignment and full body extension with feet flexed. If the infant was able to stand with its full body extended, the height was taken. Head circumference was measured to the nearest 1 mm using a standard non-elastic, plastic-coated measuring tape. The measurement was taken approximately 2.5 cm above the eyebrows, directly over the largest circumference of the skull. If duplicate measurements were not within 2 mm, a second set of two measurements was obtained. The individual head circumference measurements and the final mean of one set of measurements were recorded.

Morbidity, which was defined as upper respiratory infections, diarrhea, use of antibiotics, or the presence of fever, was assessed at each visit from enrollment to 2 months. These illnesses were recorded by parents in a diary, reviewed by investigators following each scheduled visit, and recorded as adverse events. Medication was noted by the parents in the diary and consolidated by the investigators in the Concomitant

Medication section of the case report form. The number of times the infants were seen at any health care facility in the period between scheduled visits was also documented. At each visit the mother/caregiver was asked about interim health problems. Diarrhea was defined as having three or more unformed stools in a 24 hour period; a diarrhea episode was defined as being separated from another diarrhea episode by at least three diarrhea-free days.[14] Fever was defined as a baby's axillary temperature above 38°C, reaching 38.5°C at least once during a 24 hour period.

Analyses of the immune markers interleukin (IL)-4, IL-12, IL-23 and transforming growth factor (TGF)-β in blood specimens taken from 49 infants delivered by Cesarean section enrolled in the Indonesian center were performed at the 1 month time point using ELISA kits as per the manufacturer's instructions. Stool Immunoglobulin (Ig)-A was also measured in a subsample of 79 Indonesian infants at 1 month. Further analyses were performed at 2 months in a subsample of 61 infants from both countries. Stool IgA was measured using an IMMUNO-TECH Human IgA ELISA Kit (*ZeptoMetrix*®) as per manufacturer's instructions.

An adverse event was defined as any untoward occurrence in a subject who had been administered the supplemented or non-supplemented formula product and included illnesses, signs or symptoms occurring or worsening, abnormal laboratory findings during the course of the study, and/or when a subject's mother/caregiver contacted the investigator or their private physician and the subject was examined or given medical direction. An adverse event may or may not have resulted in withdrawal of the subject from the study. Any adverse event occurring during the study was reported and documented whether or not it was considered to be non-serious, serious, or related/unrelated to the treatment. The following information was recorded: description of the adverse event (including duration, frequency, intensity, and seriousness), action taken, outcome, sequelae, and relationship to the formula.

Results

A total of 168 subjects were recruited for the study, with 87 (51.8%) allocated to the test group and 81 (48.2%) to the control group. Twenty-three subjects dropped out during the study, with similar proportions dropping out from the test (13/23) and control (10/23) groups. The most common reasons for dropping out were: a delay in the passing of stool creating difficulties in obtaining specimens as per criteria (11/23), or parents deciding to exclusively breastfeed (9/23). There were fewer dropout subjects with parents educated above university level (3/23) than subjects with parents educated below university level (20/23). A total of 145 subjects completed the study: 74 (51%) in the test group and 71 (49%) in the control group. These subjects were defined as the intent-to-treat (ITT) population. Of the 145 ITT subjects, 23 failed to adhere to all protocol requirements without any major violations or deviations. Subsequently, a total of 122 subjects completed the study as per-protocol (PP), with 64 (52%) in the test group and 58 (47%) in the control group. Within the PP population test group, 29 infants were delivered by Caesarean section and 35 were delivered vaginally. Within the control group, 26 were delivered by Caesarean section and 32 were born vaginally.

Of the 145 subjects in the ITT population, 85 were male (58%) and 60 female (41%); 62.1% had siblings; 46.9% received antibiotics during delivery; and 68.3% had a father who smoked. At birth, the mean infant weight was 3.13 (SD 0.36) kg; the mean length was 48.65 (SD 1.90) cm; and the mean head circumference was 33.76 (SD 1.47) cm. These demographic and baseline physical characteristics were similar between the test and control groups. The mean age at enrollment was 2.1 (SD 4.0) days in the test group and 1.3 (SD 2.7) days in the control group; the mean age at completion was 8.3 (SD 0.6) weeks in both groups. The median AGPAR score (at 1 minute) of 8 was similar for both groups. The mean maternal age was 29.3 years (SD 5.5) in the test group and 28.1 (SD 5.8) years in the control group, while the mean paternal age was 33.7 (SD 7.2) years in the test group and 33.2 (SD 6.9) years in the control group.

B. lactis was detected in stool samples at a significantly higher frequency in the test group (*B. lactis* supplemented) than in the control group ($P < 0.001$), at both the 1 month and 2 month time points (**Table 1**).

When both the treatment type and mode of birth were considered, we found that the frequency of *B. lactis* detection was significantly higher in supplemented

infants born by either Caesarean section or vaginal delivery compared to non-supplemented infants, both at the 1 month and 2 month time points (**Table 2**).

Logistic regression analysis revealed a strong positive effect of *B. lactis* supplementation on the detection of *B. lactis* in the stool. An odds ratio of 1,263 (P= 0.003; 95%CI 11 to 151,030) indicated that infants given the *B. lactis* probiotic were 1,263 times more likely to have stool positive for *B. lactis* when compared to infants given formula without the probiotic.

The geometric mean of bifidobacteria (cells/g) measured in the stool did not significantly differ between the test and control groups at either the 1 month or the 2 month time points (**Table 3**).

When the treatment type and mode of birth were considered, we found that infants born by Caesarean section who received the *B. lactis* supplement had a significantly higher mean bifidobacteria level at 1 month than those born by Caesarean section who received the non-supplemented control (P= 0.021). However, at the end of the second month there was no longer a significant difference in bifidobacteria levels between these two groups. The stool bifidobacteria levels of infants born vaginally who were supplemented with *B. lactis* probiotic were not significantly different at either time point to levels measured in vaginally-born non-supplemented infants (**Table 4**).

Although not statistically significant, a trend for higher mean body weight and head circumference was observed in infants from the test group compared to the control group, at 1 and 2 months of age. The mean gain in head circumference from the first to the second month was 4.63 (SD 2.83) cm in the test group compared with 4.42 (SD 2.72) cm in the control group. Spearman's coefficient correlation test revealed a positive correlation between body length and bifidobacteria level (P=0.045) in the test group

Table 1. Detection of *B. lactis* in stools by treatment group

B. lactis detection	Test group	Control group	P value*	RR (95%CI)
At 1 month**				
Positive, n (%)	29 (59)	1 (2)	<0.001	25.45 (3.62-179.05)
Negative, n (%)	20 (41)	42 (98)		
At 2 months**				
Positive, n (%)	22 (34)	0	<0.001	
Negative, n (%)	42 (66)	58 (100)		

* Chi square test
** Only subjects in Indonesia had stool samples collected at 1 month; all subjects in the PP population had stool samples collected at 2 months

Table 2. Detection of *B. lactis* in stools by treatment group and mode of birth

B. lactis detection	Test group	Control group	P value*	RR (95%CI)
At 1 month**				
Caesarean section, n				
Positive	20	1	<0.001	18.40 (2.68-126.38)
Negative	5	22		
Natural delivery, n				
Positive	9	0	0.002	
Negative	15	20		
At 2 months**				
Caesarean section, n				
Positive	12	0	0.000	
Negative	17	26		
Natural delivery, n				
Positive	10	0	0.001	
Negative	25	32		

* Chi square test
** Only subjects in Indonesia had stool samples collected at 1 month; all subjects in the PP population had stool samples collected at 2 months

at the 1 month time point (**Table 5**).

No serious morbidity was encountered by infants in either the test or control groups during this study (Table 6).

None of the immune markers IL-4, IL-12, IL-23, or TGF-β analyzed in blood samples taken from Indonesian subjects at the 1 month time point (n=49) were significantly different between the control and

Table 3. Level of bifidobacteria in stools by treatment group

Bifidobacteria (cells/g of stool)	Test group	Control group	P value*
At 1 month*	**n = 48**	**n = 42**	
Geometric mean	$1.5x10^9$	$0.6x10^9$	0.158
Median	$2.4x10^9$	$1.8x10^9$	
Minimum	$6.0x10^6$	$6.0x10^6$	
Maximum	$7.8x10^{10}$	$2.2x10^{10}$	
At 2 months **	**n = 64**	**n = 58**	
Geometric mean	$2.5x10^9$	$2.7x10^9$	0.146
Median	$4.6x10^9$	$6.4x10^9$	
Minimum	$4.8x10^6$	$6.0x10^6$	
Maximum	$4.0x10^{10}$	$7.2x10^{10}$	

* Chi square test
** Only subjects in Indonesia had stool samples collected at 1 month; all subjects in the PP population had stool samples collected at 2 months

Table 4. Level of bifidobacteria in stools by treatment group and mode of birth

Bifidobacteria (cells/g of stool)	Test group	Control group	P value*
At 1 month**	**n = 48**	**n = 42**	
Caesarean section	n = 24	n = 22	
Geometric mean	$8.0x10^9$	$3.0x10^9$	0.021
Median	$5.0x10^9$	$3.0x10^9$	
Minimum	$7.1x10^6$	$6.0x10^6$	
Maximum	$3.0x10^{10}$	$1.0x10^{10}$	
Vaginal delivery	n = 24	n = 20	
Geometric mean	$6.0x10^9$	$5.0x10^9$	0.887
Median	$8.0x10^8$	$9.0x10^8$	
Minimum	$6.0x10^6$	$6.0x10^6$	
Maximum	$8.0x10^{10}$	$2.0x10^{10}$	
At 2 months **	**n = 64**	**n = 58**	
Caesarean section	n = 29	n = 26	
Geometric mean	$7.0x10^9$	$1.0x10^{10}$	0.296
Median	$4.0x10^9$	$8.0x10^9$	
Minimum	$9.0x10^7$	$6.0x10^6$	
Maximum	$4.0x10^{10}$	$7.0x10^{10}$	
Vaginal delivery	n = 35	n = 32	
Geometric mean	$6.0x10^9$	$1.0x10^{10}$	0.328
Median	$6.0x10^9$	$5.0x10^9$	
Minimum	$4.8x10^6$	$6.0x10^6$	
Maximum	$2.0x10^{10}$	$6.0x10^{10}$	

* Chi square test
** Only subjects in Indonesia had stool samples collected at 1 month; all subjects in the PP population had stool samples collected at 2 months

Table 5. Correlation between bifidobacteria, anthropometry and *B. lactis* supplementation

Variables		Test group		Control group	
		r_s*	P value	r_s	P value
At 1 month**		**n = 48**		**n = 42**	
	Body weight	0.226	0.123	-0.126	0.426
	Body length	0.290	0.045	-0.134	0.397
	Head circumference	0.045	0.761	-0.035	0.827
At 2 months**		**n = 64**		**n = 58**	
	Body weight	-0.022	0.861	-0.014	0.918
	Body length	0.228	0.070	0.119	0.375
	Head circumference	-0.008	0.952	-0.059	0.660

*Spearman's rank coefficient correlation
** Only subjects in Indonesia had stool samples collected at 1 month; all subjects in the PP population had stool samples collected at 2 months

Table 6. Morbidity observed during the course of the study.

Variables	Test group n = 74	Control group n = 71	P value*	RR (95%CI
At 1 month, n (%)				
diarrhea +	0	0	-	
diarrhea -	74 (100)	71 (100)		
acute respiratory infections +	1 (1)	3 (4)	0.360	0.32
acute respiratory infections -	73 (99)	68 (96)		(0.03-3.00)
At 2 months, n (%)				
diarrhea +	0	0	1.0	
diarrhea -	74 (100)	71 (100)		
acute respiratory infections +	2 (3)	1 (1)	1.0	1.92
acute respiratory infections -	72 (97)	70 (99)		(0.18-20.70)

*Fisher's exact test

test groups. Immunoglobulin A levels measured in the stool of a subsample of Indonesian infants at 1 month and a subsample of infants from both countries at 2 months were also not significantly different between the test and control groups (**Table 7**), although there was a trend for higher mean values in *B. lactis*-supplemented Caesarean-delivered infants compared to that of non-supplemented Caesarean-delivered infants. No adverse effects observed in either the test or control groups.

Discussion

To assess the effects of *B. lactis* probiotic-supplemented formula, we conducted an open, non-randomized, placebo-controlled, 2-treatment, parallel-group trial of healthy babies born by Caesarean section or vaginal delivery. A total of 145 breastfed infants born in Indonesia and Thailand were assigned to either the test group, who received NAN 1 formula supplemented with *B. lactis* probiotics, or the control group, who received non-supplemented NAN 1 formula. The primary endpoint of the study was the presence of *B. lactis* in the stool and bifidobacteria count, measured at 2 months of age. Measurements at 1 month of age were also performed in infants from the Indonesian study population.

Stool *B. lactis* detection and bifidobacteria levels were consistent with previously published study.[15] *B. lactis* detection in the stool was significantly more frequent in 1-month-old infants who received a combination of breast milk and probiotic-supplemented formula compared to those who received breast milk and non-supplemented formula ($P < 0.001$). *B. lactis* was detected in 59% of the test group and in 2% of

Table 7. Stool Ig A levels in infant subsamples from the PP population.

Stool Ig A (µg/mL)	Test group *B. lactis* suppl.*	Control group Non-suppl.	P value**
At 1 month #	**n = 42**	**n = 37**	
Caesarean section	n = 25	n = 24	
Geometric mean	4.3	4.2	0.327
Median	4.5	4.3	
Min	3.5	3.0	
Max	4.6	4.6	
SD	0.3	0.3	
Vaginal delivery	n = 17	n = 13	
Geometric mean	4.2	4.3	0.408
Median	4.2	4.3	
Min	3.6	3.9	
Max	4.6	4.6	
SD	0.3	0.2	
At 2 months ##	**n = 31**	**n = 30**	
Caesarean section	n = 10	n = 9	
Geometric mean	2.3	2.1	0.447
Median	2.3	2.1	
Min	1.7	1.2	
Max	2.8	2.8	
SD	0.3	0.5	
Vaginal delivery	n = 21	n = 21	
Geometric mean	2.5	2.4	0.734
Median	2.4	2.5	
Min	2.0	1.8	
Max	2.9	2.8	
SD	0.2	0.3	

* supplemented

** Mann-Whitney test

\# A subsample of the PP population from Indonesia had stool IgA measured at 1 month

\## A subsample of the PP population from Indonesia and Thailand had stool IgA measured at 2 months

the control group at 1 month of age (**Table 1**). *B. lactis* detection rates in infants who received the probiotic-supplemented formula were 80% and 38%, for Caesarean section and vaginal delivery, respectively, after 1 month (**Table 2).** These differences were still observed after 2 months. *B. lactis* detection in the test group was 34% compared to 0% in the control group (**Table 1**), while *B. lactis* detection in infants who received probiotics was 41% and 29% for Caesarean section and vaginal delivery, respectively (**Table 2**).

Bifidobacteria levels in stool samples were not significantly different between the test and control groups, at either the 1 month or the 2 month time points (**Table 3**). Similarly, the level of bifidobacteria in the stools of infants who received probiotics was not different between Caesarean-born infants and those born by vaginal delivery (**Table 4**). A significant difference was, however, observed between probiotic-supplemented and non-supplemented infants born by Caesarean section. At 1 month of age, stool bifidobactera levels were significantly higher in Caesarean-born infants who received probiotics (P=0.021). This difference was no longer observed at the 2 month time point. These results suggest that the mode of delivery impacts the composition of intestinal microbiota soon after birth. Intestinal microbiota after Cesarean delivery is characterized by an absence of bifidobacteria species.[16] Vaginally-delivered neonates, even if they showed individual microbial profiles, were characterized predominantly by groups such as *B. longum* and *B. catenulatum*.[17] This effect may partly explain the lack of difference in bifidobacteria levels observed between the test and control vaginally-delivered populations, particularly at the 1 month time point. Another possible contributing factor is the previously documented bifidogenic effect of breast milk.[15] All infants in this study were on a mixed-feeding regimen. As such, the breastfeeding component likely affected bifidobacteria levels.

The microbiota colonization of infants born vaginally is generally dominated by bifidobacteria and lactobacilli strains, while *C. difficile* and *E. coli* are found in small amounts. Although there are many beneficial bacterial species in the intestine, such as *L. acidophyllus*, *L. casei*, *L. salivarius*, *B. longum*, and *B. bifidum*, the bifidobacteria group has been reported as the predominant microbiota and is known to have a role in maintaining the gut ecology.[17] Previous research has compared bifidobacteria levels in term infants who were fed formula milk containing bifidobacteria, infants who were fed formula milk without bifidobacteria, and exclusively breastfed infants. Stool bifidobacteria levels of infants fed supplemented formula were found to be similar to those of exclusively breastfed infants, but significantly higher than the levels measured in infants who were fed non-supplemented formula milk.[15]

A study reported that administration of *B. lactis* Bb12 to premature infants increased the number of bifidobacteria and reduced the number of enterobacteria and clostridia, but did not reduce the colonization of organisms which were resistant to antibiotics.[17] A recent review also suggested that *B. lactis* supplementation has the potential to increase the total number of bifidobacteria in feces and to reduce enterobacteria and clostridia.[18] A significantly reduced intestinal permeability in preterm infants receiving Bifidus BL (*B. lactis*) supplementation has also been reported.[19]

The baseline anthropometry data of the test and control groups was similar. At the first and second month, the mean weight and mean head circumference were consistently higher in the probiotic-supplemented group. A statistically significant positive correlation between body length and bifidobacteria level was observed in the test group at the 1 month time point. Overall, anthropometry results suggested that formula supplemented with *B. lactis* probiotic may assist infant growth, though these results were generally not statistically significant.

The immune markers measured were not significantly different between the test and control groups. It is noteworthy to mention that all immune parameters were measured in infants who were breastfed for at least the first two weeks of life. This certainly impacted the IgA outcomes and likely also affected the cytokine profiles.[20] In this context, supplementation of breastfeeding with formula (with or without *B. lactis* probiotics) did not add benefits beyond breastfeeding alone. Although it is possible that a benefit of *B. lactis* supplementation may be seen in infants who encounter an early cessation of breast feeding, the duration of this study may have been too brief to see such an effect. A recently published study investigated the effect of *B. lactis* supplementation on intestinal antibody responses in 6-week-old infants.[20] Infants were fed either a whey-based starter formula or the same formula supplemented with a *B. lactis* Bb12 probioticS, for a period of 6 weeks. Interestingly, infants born by Caesarean section who received the probiotic had enhanced immune responses to poliovirus and rotavirus vaccines, as measured by increases in fecal anti-rotavirus- and anti-poliovirus-specific IgA, when compared to Caesarean-born infants who received non-supplemented formula.

In conclusion, results of this study suggest that infants, in particular those born by Caesarean section, may benefit from formula supplementation with *B. lactis* probiotics. Although probiotic supplementation do not appear to affect the levels of immune markers measured, it may be interesting in future to investigate possible correlations between immune parameters and *B. lactis* presence.

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