Original Article

# Improved growth of toddlers fed a milk containing synbiotics

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*Bifidobacterium longum* (BL999), *Lactobacillus rhamonosus* (LPR), prebiotics (inulin and fructo-oligosaccharides), and long-chain polyunsaturated fatty acids (LCPUFA) are believed to have health benefits. In a randomized, double-blind, controlled trial we compared growth and development of toddlers fed milk containing synbiotics (BL999, LPR, and prebiotics) and LCPUFA or a control milk. Three hundred and ninety three healthy, 12 month-old toddlers were fed approximately 400 mL/day for 12 months. Anthropometric measurements were taken at 12, 14, and 16 months. Toddlers' response to measles and hepatitis A vaccine was measured at 16 months, and Bayley scale for motor, cognitive, and behavioral functions made at 24 months. The primary outcome was weight gain between 12 and 16 months. Secondary outcomes were gain in length, head circumference, and body mass index, gastrointestinal tolerance (stool characteristics), stool bacterial counts, safety, anti-vaccine IgG, and neurodevelopment. Weight gain was greater in the synbiotics group (mean±SD, 7.57±4.13 g/day) compared with the control group (6.64±4.08 g/day). The difference of 0.93 g/day (with a 95% confidence interval of 0.12 to 1.75) is significant ($p$=0.025). The gain in the synbiotics group resulted in a change in z-score weight-for-age closer to WHO Child Growth Standard. There was a significant increase in lactobacilli and enterococci counts between 12 months and 16 months in the synbiotic group. We conclude that in healthy toddlers milk containing synbiotics and LCPUFA provides better growth and promotes favorable gut colonization, as shown by higher *Lactobacillus* counts.

**Key Words: lactobacillus, bifidobacterium, fructo-oligosaccharide, long chain polyunsaturated fatty acids, growth**

## INTRODUCTION

Bifidobacteria and lactobacilli form the predominant bacterial species of the gastro-intestinal (GI) tract in breast-fed infants, and have been associated with decreased morbidity, especially due to infections. Studies have suggested that both bifidobacteria and lactobacilli may be involved in reducing diarrhea and GI infections.[1-3] This has formed the basis for their selection for use as probiotics in milks for infants and toddlers.

Probiotics protect against GI infections by stimulating the immune system, creating an environment in the GI tract that is unsuitable for growth of pathogens (low pH), and competes for binding sites on the epithelia.[4,5] Some probiotics (mostly combinations of *Lactobacillus* and *Bifidobacterium* species) have also been shown to play an important role in curbing allergy development in high-risk infants.[6-8]

Prebiotics selectively stimulate the growth of certain beneficial bacteria, such as bifidobacteria, in the colon, and may improve the viability of probiotics. Synbiotics, the combination of probiotics and prebiotics, have thus been proposed to have synergistic health effects.[9]

Long chain polyunsaturated fatty acids (LCPUFA) are believed to play a role in the development of brain and visual acuity of infants, based mainly on high concentrations found in the retina and brain of breast-fed infants. There have also been suggestions that LCPUFA may be involved in the modulation of the immune system.[10] However, considering that these lipids are integral components of cell membranes, their addition to milks may contribute to improved health in toddlers.

Although probiotics, prebiotics, and LCPUFA have beneficial health effects in certain groups of infants (e.g., those predisposed to allergy or preterm infants), specific health effects in healthy term infants and toddlers are difficult to demonstrate. Part of the reason for this may be the difficulty in measuring subtle effects on different physiological and immunological parameters that may nevertheless contribute to improved health. Furthermore, the

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different functional components may have synergistic effects. The best measure of the effects of these functional components on overall improvements in health and well-being is growth. The aim of the current study was to evaluate the effects of milk containing synbiotics and LCPUFA on growth of healthy 12-month old toddlers who were fed this milk for 4 months. We compared the effect of a milk containing *Bifidobacterium longum* BL999, *Lactobacillus rhamnosus* LPR, inulin and fructo-oligosaccharide (prebiotics), and LCPUFA with that of a control milk lacking these components, with the hypothesis that the health benefits of the synbiotic plus LCPUFA milk would be demonstrated by better growth in toddlers fed milk containing these components. We also explored possible effects of this milk on immune response to vaccination and on neurodevelopment.

## MATERIALS AND METHODS

### *Population*

The study population was healthy 12-month-old toddlers. To be included in the study, toddlers had to: have weight z-score between -2 and +2 and height z-score between -2 and +2 according to WHO growth standards; be vaccinated against measles at 9 months; be able to drink 400 mL of the study milks daily; and have had their parents give consent to participate in the study. Toddlers were excluded from the study if they: were receiving breast milk at the time of enrollment; had documented allergies, including to cow's milk protein or lactose intolerance; had received vaccination against hepatitis A; had hemoglobin concentrations <10 g/dL; had major deformities or illness including cardiovascular, GI, renal, neurological, or metabolic; were receiving treatment with lactulose-containing preparation during the 2 weeks preceding enrolment; were receiving concomitant medication other than non-steroid anti-inflammatory drugs during the 4 weeks preceding enrollment; were expected not to be able to comply with the study requirements; or were currently participating or had participated in another clinical trial during the last 3 months.

### *Study design*

This was a randomized, controlled, double-blind, parallel-group study performed in the division of Gastroenterology in the Department of Child Health at the Faculty of Medicine, University of Indonesia, Jakarta. The study was conducted according to the principles and rules laid out by the Declaration of Helsinki and its subsequent amendments; and was approved by the ethics committee of Faculty of Medicine, University of Indonesia. The study adhered to good clinical practices.

Toddlers were screened when they were younger than 12 months old and eligible toddlers whose parents/ guardians consented were enrolled and randomly assigned to one of the two milk groups. At baseline, milks and diaries were distributed and milk reconstitution and use of diaries was demonstrated. At this visit, toddlers underwent clinical examination, had anthropometric and hematological measurements (including anti-measles and hepatitis A IgG) taken, assessed for motor, cognitive, and behavioral functions, and had stool bacterial, pH, and stool blood (hematest) measurements taken.

Following baseline measurements, toddlers started taking their assigned study milks (200 mL twice daily) in addition to their normal diet. Visits took place every two months thereafter until they were 24 months old. Parents/guardians recorded daily stool pattern and volume of milk intake during the 3 days prior to each visit. On the 14-month visit, toddlers had clinical examinations, anthropometric measurements, stool pH and hematest taken, and they received their first hepatitis A and measles booster vaccinations. The investigator reviewed diaries and recorded any adverse events (AE) and intake of concomitant medication. At the 16-month visit, similar assessments were made but additionally, hematological measurements (including assays for anti-measles and hepatitis A IgG) were taken, and microbiological determination of stools were performed in a subset (n=53) of toddlers. At the following visits, the investigator only reviewed the diaries and made assessments of safety (AEs) and intake of concomitant medications. At the last (24 months) visit, motor, cognitive, and behavioral developments assessments were also conducted.

### *Milks*

Both the synbiotics and control milks were cow's milk-based and contained protein, carbohydrate, fat, vitamins, and minerals in amounts sufficient for normal growth of toddlers aged 12-24 months as supplementary food. In addition, the synbiotics milk also contained the probiotics *Bifidobacterium longum* BL999 (ATCC: BAA 999) and *Lactobacillus rhamonosus,* LPR (CGMCC 1.3724), the prebiotics inulin (30%) and fructo-oligosaccharide (70%), and the LCPUFA, arachidonic acid (AA) and docosahexaenoic acid (DHA) (Table1). One hundred milliliters of the reconstituted milk contained 14.4 g of this combination.

The study milks were coded by the sponsor/ manufacturer and the study investigator, staff, and toddlers' parents/guardians were blinded to milk identity.

### *Outcome measures*

Primary outcome was weight gain per day between 12 months and 16 months. Secondary outcomes were change in length, head circumference, and body mass index (BMI); stool characteristics (frequency, consistency, color,

**Table 1.** Nutritional composition per 100 g of study formulas

| | Experimental | Control |
|---|---|---|
| Energy (kcal/kJ) | 449/1880 | 506/2120 |
| Fat, total (g) | 18.0 | 28.2 |
| Linoleic acid (g) | 4.3 | 0.0 |
| Linolenic acid (mg) | 0.48 | 0.00 |
| ARA (mg) | 24 | 0 |
| DHA (mg) | 23 | 0 |
| Protein (g) | 18.4 | 25.7 |
| Carbohydrates, total (g) | 54.0 | 37.4 |
| Prebiotics, total (g) | 3.4 | 0.0 |
| Inulin (g) | 1.02 | 0.00 |
| Fructo-oligosaccharide (g) | 2.38 | 0.00 |
| BL999 (CFU/g) | $1\times10^7$ | 0 |
| LPR (CFU/g) | $2\times10^7$ | 0 |

CFU, colony forming unit

and smell), pH, microbiota composition, and presence of blood in stool; immune response to measles and hepatitis A vaccination; hematology assessments; and the toddlers' motor, cognitive and behavioral development.

Toddlers were weighed naked on electronic scales that had been calibrated according to the manufacturer's instructions, and weights to the nearest 10 g were recorded. All toddlers in the study were weighed on the same scale. Recumbent length and head circumference were measured to the nearest 1 mm.

Stool counts and characteristics were recorded for 3 days prior to each visit, and frequency calculated as mean stool counts per day. Stool consistency was recorded using the Bristol Stool chart and classified as Type 1-Type 7.[11] Stool color was recorded as yellow, brown, green, or black, and smell as normal or unusual. Fecal occult blood was assessed using Hematest (ACON Laboratories, California, USA).

For measurements of stool microbiota composition, approximately 5 g stools were collected in sterile tubes and stored at ≤20°C. Quantification of bacteria was performed using fluorescence *in-situ* hybridization (FISH) by a commercial partner (Biovisible Analytical Center, The Netherlands). Specific primers used were Erec482 for Clostridia/Eubacteria, Bac303 for Bacteroides/Prevotella, Wbif164 for Bifidobacteria, Eco1531 for Enterobacteria, and Lab158 for Lactobacilli/Enterococci.[12,13]

For hematology, red blood cell (RBC), hematocrit, mean corpuscular volume (MCV), hemoglobin, white blood cell (WBC), and eosinophile measurements were preformed using standard clinical laboratory procedures. Antibody titers were determined by enzyme linked immunosorbent assay (ELISA).

Toddlers' development functions were assessed using the Bayley Scale of Infant and Toddler Development (Bayley-III) by a growth-developmental and community pediatrician. Composite scores were recorded and scaled to a metric with a mean of 100 and standard deviation (SD) of 15, and a range of 40 to160.

### *Safety*

At each visit, the investigator assessed safety based on the occurrence of AEs, the diary records, interviews with parents, and hematology results. The investigator assessed AEs for seriousness, severity, and relatedness to the milks. AEs were coded using the World Health Organization Adverse Reaction Terminology (WHOART) medical dictionary.

### *Statistical analyses*

Sample size determination was based on the primary endpoint of detecting a difference in mean weight gain per day between the two study groups. A clinically significant weight gain was defined as 3.3 g/day and a SD of 11, which was based on a gain of 20% of the mean weight gain in the control group of toddlers of similar age from a previous study in Indonesia. Using a two-sample t-test with a power of 80% and alpha of 5%, 175 toddlers per group would be required to show a significant difference in weight gain. Assuming a 10% drop-out rate, 194 toddlers per group would need to be enrolled.

Randomization was performed by dynamic allocation (Nestlé Trial Balance program) and stratified by sex. All randomized toddlers were included in the intent-to-treat (ITT) population. The per protocol population (PP) consisted of all toddlers who completed the study without any major protocol violations and had weight measurements at 16 months. Primary outcome was analyzed in both the ITT and PP populations and secondary outcomes in the PP population. Summary data are presented as mean±SD or median and interquartile or minimum and maximum ranges.

Weight gain between groups was compared using a linear mixed model correcting for sex. Other anthropometric measures and changes from baseline measurements were summarized. WHO growth standards were used to calculate weight-for-age, height-for-age, BMI-for-age, and head-circumference-for-age z-scores. Comparisons of the change from baseline at the different visits were compared between groups using ANCOVA with sex, age at baseline, and outcome variables at baseline as covariates.

Comparison of stool frequency was performed using ANCOVA using sex as a covariate. For stool consistency, color, and smell, counts in each category (seven categories for consistency, four for color and two for smell) were recorded and comparisons between the two groups were made using Poisson regression, using sex as a covariate and the number of total stools collected as the overdispersion parameter.

Comparisons of bacterial counts were performed by comparing the 16-month values or the change in bacterial counts (CFU/g of feces or %) from baseline to 16 months in each group using ANCOVA and sex and baseline values as covariates. Antibody titers were log transformed and compared between groups using ANCOVA with sex baseline values as covariates.

A clinically relevant difference in toddler development at 24 months measured by Bayley Scale for Infant and Toddler Development (BSID III) was considered to be a value of 5 (SD=12.5) based on a previous report.[14] At a power of 80% and alpha of 5%, 100 toddlers would have to be assessed to observe a significant difference. Composite Bayley scores of the two groups were compared using ANCOVA with sex, age, and baseline scores as covariates. At 24 months, each toddler was classified as increase, no change, or decrease by computing the change in the seven categories between baseline and 24 months, and the study groups were compared using the Cochran-Mantel-Haenszel test.

Statistical analyses were performed using SAS 8.2 (SAS Institute, Cary, NC, USA).

## RESULTS

### *Study population*

Three hundred and ninety-three healthy 12-month-old toddlers were enrolled and randomized into the synbiotics (n=199) and the control milk (n=194) groups. Similar proportions of toddlers dropped out from each group before the 16-month follow-up (Figure 1). There were no significant differences in the reasons for drop-out between the two groups. Demographics and baseline characteristics were similar between groups (Table 2).

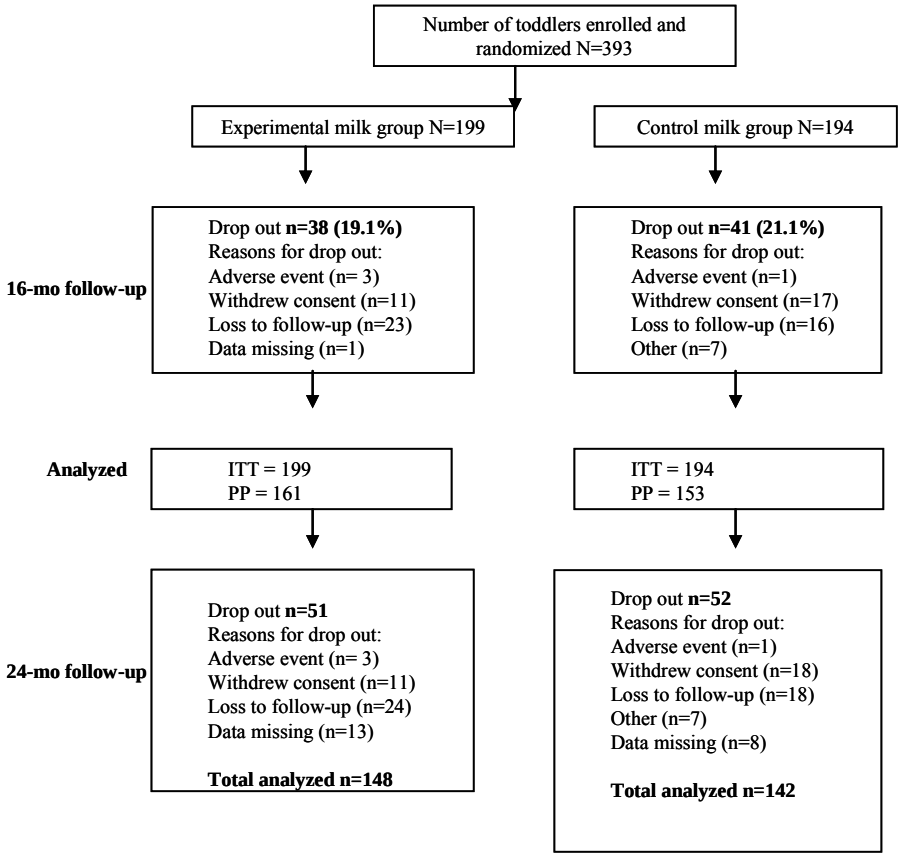


**Figure 1.** Flow of toddlers' participation in the study.

**Table 2.** Demographics n (%) and mean (SD) baseline characteristics of toddlers in the study (ITT)

| | Synbiotic Milk Group n=199 | Control Milk Group n=194 |
|---|---|---|
| Girl | 98 (49) | 92 (47) |
| Boy | 101 (51) | 102 (53) |
| Age (days) | 377 (6.42) | 377 (6.81) |
| Weight (g) | 8757 (1035) | 8810 (1175) |
| Length (cm) | 73.8 (3.05) | 73.5 (3.13) |
| BMI (kg/m$^2$) | 16.1 (1.55) | 16.3 (1.59) |
| Head circumference (cm) | 45.7 (1.33) | 45.6 (1.51) |
| z-score for weight | -0.66 (1.01) | -0.64 (1.08) |
| Breast fed before 12 months (days) | 107 (54) | 91 (47) |

### *Milk intake and growth*

Toddlers in both groups had a daily milk intake of ≥400 mL/day during the study. There was no significant difference in the mean daily volume of milk consumed in the two groups between 12 months and 24 months: mean (±SD) intakes were 426±25.9 ml and 426±31.2 ml in the synbiotics and control groups, respectively. At 16 months, toddlers in the synbiotics group weighed more than those in the control group (mean±SD of 9711±1142 g vs. 9643 ±1218 g). The mean weight gain between 12 months and 16 months in both the ITT and PP population was significantly higher in the synbiotics group compared with the control group ($p$=0.025, Table 3). Furthermore, comparison with the WHO standards also showed that the change in z-score from baseline to 16 months was significantly higher in the synbiotics compared with the control groups (Table 3). The change in weight-for-age z-score was closer to WHO Child Growth Standard in the synbiotic group (Figure 2).

At 16 months, there were no significant differences in length (mean ±SD, 77.8±3.0 cm and 77.9±3.4 cm in the synbiotics and control groups, respectively) and head circumference (mean ±SD, 46.3±1.3 cm and 46.4±1.4 cm for the synbiotics and control groups, respectively) between the two groups. Similarly, there were no significant differences in length gain and head circumference between the two groups (data not shown). BMI (in kg/m$^2$)

**Table 3.** Growth of toddlers between 12 months and 16 months

| | Synbiotic Milk Group (n = 199) | | Control Milk Group (n = 194) | | Difference between groups | | |
|---|---|---|---|---|---|---|---|
| | LS-mean (SD) | 95% CI | LS-mean (SD) | 95% CI | LS-mean (SD) | 95% CI | $p$-value |
| Weight gain (g/day), ITT | 7.57 (4.13) | 7.00-8.14 | 6.64 (4.08) | 6.06-7.21 | 0.93 (0.41) | 0.12-1.75 | 0.025* |
| Weight gain (g/day), PP | 7.67 (3.83) | 7.08-8.26 | 6.71 (3.83) | 6.12-7.31 | 0.96 (0.42) | 0.12-1.79 | 0.025* |
| Change in weight-z-score, ITT | 0.11 (0.40) | 0.05-0.17 | 0.02 (0.40) | -0.04-0.08 | 0.09 (0.04) | 0.01-0.18 | 0.036* |

SD, standard deviation; ITT, intent to treat; PP, per protocol; CI, confidence interval; LS-mean, least-square means, are within-group means appropriately adjusted for all covariates in the model; *$p < 0.05$.

was slightly higher in the synbiotics group compared with the control group at the 16 months visit (16.0 vs 15.9). Compared with 12 months, there was a slight decrease in BMI in both groups at the 16-month visit (-0.02 vs -0.09, in the synbiotics and control groups, respectively).

***Stool characteristics***

There was no significant difference in stool frequency or any stool characteristics between the two groups at 16 months (data not shown). Both groups had more soft stools (Type 4 and Type 5) than any other type, and the majority of stools were yellow (70% and 74% in the synbiotics and control groups, respectively). In both groups more than 90% were classified as smelling normal.

Similarly, at 16 months there was no difference in stool pH between the two groups and it did not change as compared to the 12 months measurement (data not shown). Stool hematest was negative (no occult fecal blood) in >90% of toddlers in both groups and there was no difference between groups

***Microbiota composition (Table 4)***

Microbiological data were collected from 23 toddlers in the synbiotics and 30 toddlers in the control groups. Baseline total and specific bacterial counts were similar between the two groups. Toddlers fed the synbiotics milk had an increase in lactobacilli/enterococci counts as well as an increase in the proportion of lactobacilli/enterococci between 12 months and 16 months whereas in toddlers fed the control milk there was a decrease. There was no significant difference in these bacterial counts between the two groups, but the change in counts between 12 months and 16 months was significantly different between the two groups ($p$=0.023). Bifidobacteria counts did not change significantly between the two visits in either group, though there was a slight decrease in bifidobacteria counts between 12 months and 16 months in the synbiotics group.

Both milk groups saw an increase in clostridia/ eubacteria count as well as an increase (non significant) in the proportion of these bacteria from 12 months to 16 months but there was no significant difference between groups.

***Immune response***

Toddlers in both groups had an increase in antibody titers to both the measles booster and hepatitis A primary vaccination (data not shown). There was no difference in mean antibody titers to measles vaccine between the two groups at 16 months (ANCOVA, $p$=0.715). At baseline more than a third of toddlers in both groups already had anti-hepatitis A antibodies. There was no difference in anti-hepatitis A antibody titers at 16 months between the two groups both among toddlers who were positive at baseline and among toddlers who were negative at baseline (data not shown).

***Neurodevelopment***

There were no significant differences in any of the mental and motor developmental measures between the two groups. The change in cognitive and adaptive behavior scores between 12 and 16 months was higher but not significantly different in the synbiotics group compared with the control group. (Table 5)

***Safety***

Serious AEs were reported in two toddlers in the syn-

**Table 4.** Comparison of bacterial counts, mean $\log_{10}$ CFU/g stool (SD) and proportion (%) of total bacterial counts

| | Synbiotic Milk Group (n = 23) | | Control Milk Group (n = 30) | | $p$-value |
|---|---|---|---|---|---|
| | 12 mo | 16 mo | 12 mo | 16 mo | |
| Total Counts | 10.2 (0.35) | 10.2 (0.31) | 10.2 (0.38) | 10.3 (0.27) | 0.457 |
| Bifidobacteria counts | 9.74 (0.96) | 9.32 (1.49) | 9.53 (1.17) | 9.60 (1.10) | 0.369 |
| % total (SD) | 50.2 (26.6) | 47.1 (32.1) | 44.1 (31.7) | 42.4 (30.6) | 0.707 |
| Lactobacillus/Enterococcus | 7.16 (0.94) | 7.54 (0.93) | 7.49 (0.83) | 7.13 (0.82) | 0.023* |
| % total (SD) | 0.24 (0.34) | 0.77 (1.42) | 0.62 (1.34) | 0.23 (0.33) | 0.037* |
| Clostridia/Eubacteria | 8.63 (1.08) | 9.26 (0.64) | 9.16 (0.85) | 9.29 (0.70) | 0.901 |
| % total (SD) | 11.1 (14.63) | 21.1 (17.4) | 14.72 (19.3) | 22.1 (21.8) | 0.838 |
| Enterobacteria | 6.90 (0.89) | 6.89 (0.83) | 7.25 (0.83) | 7.20 (0.89) | 0.295 |
| % total (SD) | 0.20 (0.36) | 0.31 (0.59) | 0.37 (0.52) | 0.55 (1.28) | 0.342 |
| Bacteroides/Prevotella† | 6.01 (0.06) | 6.02 (0.11) | 6.01 (0.04) | 6.04 (0.14) | 0.569 |
| % total (SD) | 0.01 (0.01) | 0.01 (0.01) | 0.01 (0.01) | 0.01 (0.01) | 0.853 |

CFU, colony forming unit; SD, standard deviation. *The $p$-values stand for the significance of the difference between the two groups of the change in bacterial counts between 12 and 16 months. †For Bacteroides/Prevotella counts were below detection limit in >95% of toddlers in both groups at both visits.

**Table 5.** Neurodevelopment measures according to change in Bayley scores between 12 and 24 months, mean (SD)

| | Synbiotic Milk Group (n = 76) | Control Milk Group (n = 77) | Difference between groups ($p$-value) |
|---|---|---|---|
| Cognitive | 5.6 (22.5) | 1.9 (20.4) | 0.726 |
| Language | 15.7 (16.5) | 16.2 (16.6) | 0.631 |
| Motor | 18.0 (23.5) | 20.6 (24.2) | 0.454 |
| Social emotional | 37.8 (31.8) | 37.0 (25.6) | 0.607 |
| Adaptive behavior | 24.2 (20.1) | 18.4 (20.8) | 0.218 |

SD, standard deviation. The comparison between the two groups was performed using an analysis of covariance with sex, age at 12 months and 12 months composite scores as covariates and the 24 months composite scores as outcome variables.

**Table 6.** Number (%) of toddlers with adverse events (AEs) coded by WHO ART reported during the study

| AE Preferred Term | Synbiotic Milk Group (n = 199) | Control Milk Group (n = 194) | Relative risk | *p*-value |
|---|---|---|---|---|
| Rhinitis | 154 (77.4) | 140 (72.2) | 1.15 | 0.233 |
| Upper respiratory tract infection | 106 (53.3) | 102 (52.6) | 1.01 | 0.891 |
| Diarrhea | 110 (55.3) | 86 (44.3) | 1.25 | 0.030* |
| Fever | 108 (54.3) | 88 (45.4) | 1.20 | 0.077 |
| Coughing | 57 (28.6) | 42 (21.2) | 1.22 | 0.110 |
| Stomatitis | 26 (13.1) | 29 (15.0) | 0.93 | 0.590 |
| Conjunctivitis | 15 (7.5) | 19 (9.8) | 0.87 | 0.426 |
| Vomiting | 16 (8.0) | 16 (8.3) | 0.99 | 0.940 |
| Furunculosis | 8 (4.0) | 10 (5.2) | 0.88 | 0.591 |
| Dermatitis | 10 (5.0) | 6 (3.1) | 1.33 | 0.333 |

biotic group (typhoid fever in one and typhoid fever and dengue encephalopathy in the other) and in four toddlers in the control group (typhoid fever; fever and febrile seizures; fever, diarrhea, and dehydration; fever, icteric and alcoholic stool, and hepatitis). These were not considered to be related to the study milks.

Most toddlers experienced at least one AE during the study. There was no difference in the frequency of AEs between the two groups (94.5% and 94.9% of toddlers in the synbiotics and control groups, respectively, Table 6). The risk of diarrhea was higher in the synbiotics group compared with the control group (relative risk: 1.25, $p$=0.030), but there was no difference in the frequency of other AEs between the two groups (Table 6).

Although there were some differences in some of the hematology values between groups, these were not considered to be safety issues.

## DISCUSSION

In the current study we showed that 12-month old toddlers fed a synbiotics milk containing LCPUFA for 4 months had higher weight gain compared with toddlers fed a control milk lacking these components (difference in mean weight gain of 0.93 g/day, $p$<0.05). Even though the study was powered to detect a difference in 3.3 g/day, we observed a significant difference in weight gain at a lower margin because the SD in the current study was lower than had been anticipated (4 vs. 11), and therefore the study was powered to detect smaller differences. We did not detect significant differences in gains in length or head circumference during this period, which may be due to the difficulty in measuring the small changes that occur during a 4-month period. Both milks were tolerated well and there were no safety issues identified. Diarrhea was reported more often in the synbiotics group compared with the control group. It is important to note however, that this was based on the parents/guardians interpretation of loose stools, and it was not considered to be clinically significant.

Most published studies that have evaluated the effect of probiotics and prebiotics on growth have evaluated equivalence in growth since the goal was to demonstrate safety. Nevertheless, there has been a report of a study that demonstrated higher growth among young infants (≤6 months) fed probiotics,[15] and another report of improved growth among a sub-population of vulnerable infants.[16] In the current study we hypothesized that in healthy toddlers fed synbiotics and LCPUFA-containing milks, specific subtle beneficial changes in physiological and immunological parameters may be difficult to measure, but that these effects would be manifested through improved growth compared with toddlers fed milks lacking these components. Thus, our study was designed to demonstrate superiority in promoting growth. The small but significant differences in weight gain suggest that probiotics, prebiotic, and LCPUFA may have synergistic effects. Additional studies controlling for confounding factors (such as differences in diet) are required to confirm the association of synbiotics and LCPUFA with increased weight gain in healthy toddlers.

Our study also showed that stool lactobacilli and enterobacteria counts increased after the 4 months of milk intake among the synbiotics group but not the control group. Lactobacilli and enteroccocci also made up a slightly higher (and statistically significant) percentage of the total microbiota at 16 months compared with 12 months in the synbiotic group. On the other hand, among toddlers in the control group, there was a decrease in these species during this period, suggesting that the synbiotic milk may promote transient colonization by lactobacilli and enteroccocci.

We did not observe a difference in stool bifidobacterial counts after 4 months of milk intake. In the current study toddlers were 12 months old at the start of the study and their microbiota is mature and less likely to be modulated with the concentrations of bifidobacteria used. Most studies of probiotics and prebiotics in the young are performed with infants, typically younger than 6 months, when the microbiota may be more malleable.

Probiotics are considered to provide health benefits primarily by modulating the immune system. The most documented benefits are associated with the reduction of diarrhea and incidence of allergy, especially with *Lactobacillus* and *Bifidobacterium* species.[3] However, it has also been suggested that these probiotics may improve antibody production in response to vaccines,[17,18] though there is no sufficient evidence for this. In the current study, we did not see an effect on the response to either measles booster nor to primary vaccination against hepatitis A. Since the study was not powered to evaluate these parameters, additional studies would be required to address effect on immune response to vaccination. Additionally, as discussed above, in this age group with a relatively mature microbiota (compared with infants) it may be difficult to see a measurable effect of the dietary probiotics on immune response.

We did not observe a significant effect of the synbiotics plus LCPUFA milk on neurodevelopment. However, neurodevelopmental evaluation had been planned for 100 toddlers from each group, but data from sufficient numbers of toddlers were not available at 24 months (77 in the synbiotics and 76 in the control groups) to make a conclusion regarding the effect of LCPUFA. Thus, this would still need to be evaluated with a larger number of toddlers in future studies. Although the presence of high concentrations of LCPUFA in retina and brain especially in breast-fed infants have been taken as indications of their beneficial effect on neurodevelopment, recent systematic reviews of studies in both term and pre-term infants have shown this effect has not been demonstrated unequivocally.[19,20]

In conclusion, our studies show that 4 months of feeding milk containing synbiotics and LCPUFA lead to higher weight gain in toddlers compared to those fed a control milk lacking these components. However, an effect on neurodevelopmental measures was not observed. There was an increase in colonization with lactobacilli and enteroccocci in the synbiotics milk group but not in the control group.

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**AUTHOR DISCLOSURES**

Agus Firmansyah, MD, PhD, Pramita G Dwipoerwantoro, MD, PhD; Muzal Kadim, MD; Safira Alatas, MD declare that we have no financial interest in and have received no funding for the work presented in this paper and/or APJCN which would create a conflict of interest for us.

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Original Article

# Improved growth of toddlers fed a milk containing synbiotics

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# 含合生元的奶粉有助幼兒成長

雙歧桿菌屬的 *Bifidobacterium longum* (BL999)、乳酸菌屬的 *Lactobacillus rhamonosus* (LPR)、益菌助生質(菊糖和果寡糖)和長鏈不飽和脂酸(LCPUFA)被認為對健康有益。在一項隨機、雙盲控制試驗中，比較有添加合生元(BL999、LPR和益菌助生質)及長鏈不飽和脂酸的奶粉和沒有添加的奶粉，兩者對幼兒成長發育的影響。計 393 位健康的 12 個月大幼兒每天被餵食約 400 毫升配方奶，持續 12 個月。於第 12、14 和 16 個月測量體位。於第 16 個月檢測幼兒對痲疹和 A 型肝炎疫苗的反應，並於第 24 個月以貝氏量表評估運動、認知和行為。首要結果是第 12 個月到第 16 個月間增加的體重。次要結果是身長、頭圍和 BMI 的增加、腸胃道耐受性(糞便特色)、糞便中細菌量、安全性、抗疫苗 IgG 抗體和神經發展情形。與控制組(6.64±4.08 克/天)相比，實驗組體重增加較多(7.57±4.13 克/天)。兩組體重差值 0.93 克/天，有統計上顯著差異($p$=0.025)。實驗組體重增加使得年齡別體重值的 Z 分數更接近世界衛生組織孩童生長標準。在第 12 個月和第 16 個月之間，實驗組的乳酸菌和腸內菌數量顯著增加。總結，添加合生元和長鏈不飽和脂酸的奶粉對幼兒成長有益處，且從乳酸菌屬總量較高，顯示對腸道菌落也有好處。

**關鍵字：乳酸菌、雙歧桿菌、果寡醣、長鏈不飽和脂酸、成長**