

Effect of Bifidobacterium Bb12 with or without *Streptococcus thermophilus* Supplemented Formula on Nutritional Status

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Abstract

Acute diarrhea is a common cause of infant morbidity and mortality. Probiotic supplemented infant formula is one of the effective methods for prevention of rotavirus diarrhea. Other benefits of the probiotics supplemented formula were evaluated by monitoring the growth of the children. A double-blind, placebo-controlled trial was done in 148 children aged 6-36 months. They were divided into 3 groups: the Bb12 group, 51 children received infant formula with Bifidobacteria Bb12 supplement; the Bb12+ST group, 54 children received infant formula with Bb12 and *Streptococcus thermophilus* supplement; and the control group, 43 children received infant formula without supplement. The mean weight Z-score according to WHO reference standard of the Bb12 group was -1.8 ± 0.12 , the Bb12+ST group was -1.4 ± 0.11 and the control group was -1.8 ± 0.13 at entry. The mean weight Z-score of children after 6 month showed that the children in the Bb12+ST group had the highest increase in weight which was increased from -1.4 ± 0.11 to -0.9 ± 0.12 compared to the Z-score of the Bb12 group which had increased from -1.8 ± 0.12 to -1.2 ± 0.13 and in the control group from -1.8 ± 0.13 to -1.7 ± 0.25 . In terms of the mean height Z-score, the Bb12 group was -2.7 ± 0.14 to -1.7 ± 0.16 which was higher than the Bb12+ST group (-2.2 ± 0.13 to -1.7 ± 0.13) but was not different from the control group. However, the mean weight/height Z-score of the Bb12+ST group had approached the reference standard (Bb12 group -0.1 ± 0.11 to -0.1 ± 0.13 , Bb12+ST group -0.1 ± 0.10 to 0.3 ± 0.17 , control group -0.4 ± 0.12 to -0.1 ± 0.16). Data showed that children who received the probiotics supplement formula had better growth during the 6 month period.

Key word : Bifidobacterium Bb12, *Streptococcus thermophilus*, Probiotic

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The term probiotic refers to live microorganisms that survive through the passage of the gastrointestinal tract and have beneficial effects on the host (1,2). The interest in probiotics has resulted in a growing number of publications which have reported biological effects of various bacterial strains(3). Probiotics can protect the host against pathogens through two mechanisms: the barrier effect or colonization resistance and modulation of the host defense mechanisms.

The anaerobic bacteria of the genus *Bifidobacterium* constitute the predominant colonic flora of breast-fed infants(4) and are thought to exert some of the protective effect against diarrhea(5). Other non-pathogenic bacteria may also improve gastrointestinal function in infants, for example, lactic-acid-producing bacteria such as *Streptococcus thermophilus* (ST) replicate within the human gastrointestinal tract and generate lactase activity(6). This enzymatic activity facilitates the digestion of lactose in formula and milk and consequently decreases the symptoms of malabsorption, which accompany acute infectious diarrhea.

Formula and fermented milk products containing *Bifidobacterium bifidum*, ST and other bacteria have been used for their potential benefit on intestinal digestion and function for many years(7,8). The formula supplemented with *Bifidobacterium bifidum* Bb12 (Bb12) and ST may enhance the absorption of the nutrients and may promote growth of children. The present study presents the nutritional status in infants and children receiving formula supplemented with either Bb12 alone or together with ST during a 6-month period.

Objective

The purpose of this study was to assess the nutritional status focusing on the growth of children, by comparing the Z-score of weight, height and the

change of weight and height after consuming *Bifidobacterium* Bb12 milk formula with or without *Streptococcus thermophilus*.

SUBJECTS AND METHOD

A randomized, double-blinded and placebo controlled trial was conducted to evaluate the efficacy of formula supplemented with either Bb12 alone or together with ST compared to infant formula without probiotics supplement as the control group. The project was approved by the Ethics Committee, Faculty of Medicine, Ramathibodi Hospital, Mahidol University. The consent was signed by the Director of the Babies Home.

One hundred and forty-eight children, who were not suffering from chronic diarrhea at the Babies Home in Bangkok, were enrolled in the study. Their ages ranged from 6 to 36 months. They were randomized into 3 groups based on the feeding milk formula contained in 3 different cans coloured blue, yellow and red. The milk formula were coded by the manufacturer to ensure that they were blinded to both house personnel and researchers. The 3 study milk formulas were kept in an air conditioned room until used. The infants and children received 3-6 servings of formula (at least 400-600 ml per day depending on their age) over a period of 6 months. The formula was prepared with cool boiled water in the formula room. All the infants and children received the same house food according to age. Medical treatment was provided by pediatricians. Measurements of weight and height were performed monthly during the study.

Statistic analysis

Z-scores of weight and height were calculated by using the WHO reference standard(9). Comparisons among the groups were analyzed by one-way ANOVA. Statistically significant difference was considered at p-value of less than 0.05.

Table 1. Baseline characteristics of the studied children.

Parameter	Bb12	Bb12+ST	Control
Total number	51	54	43
Male : female	28 : 23	31 : 23	32 : 11
Mean age \pm SD (day)	487 \pm 189	526 \pm 193	684 \pm 210
Mean weight Z-score \pm SD	-1.8 \pm 0.12 ^a	-1.4 \pm 0.11	-1.5 \pm 0.13 ^a
Mean height Z-score \pm SD	-2.7 \pm 0.14 ^b	-2.2 \pm 0.13	-2.3 \pm 0.19

a = significant difference : control, Bb12 < Bb12+ST with p < 0.05

b = significant difference : Bb12 < Bb12+ST with p < 0.05

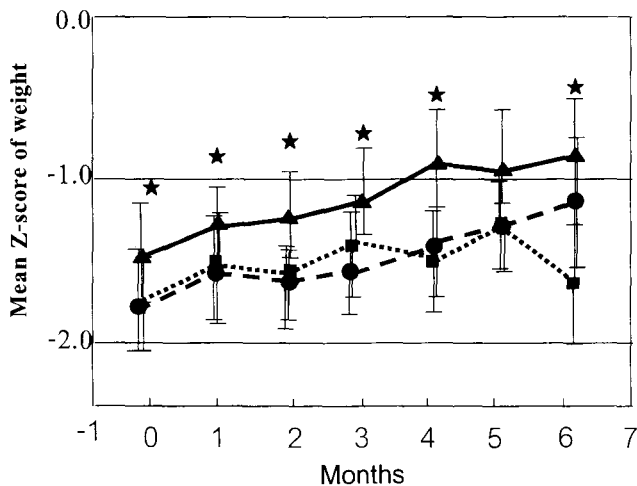


Fig. 1. Mean Z-score of weight during 6 months of treatment.

---■--- Control, ---●--- Bb12, ---▲--- Bb12+ST
★ Significant difference between groups with $p < 0.05$

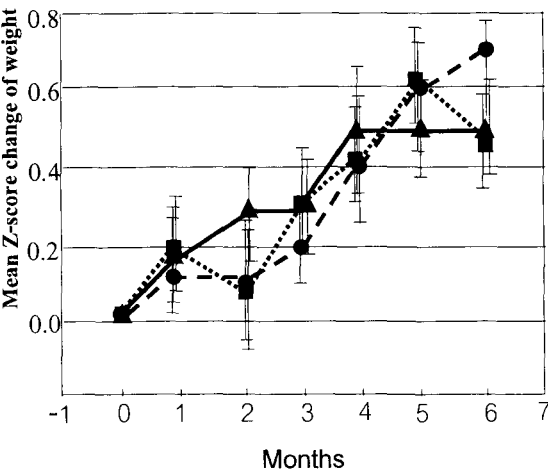


Fig. 2. Mean Z-score change of weight during 6 months of treatment.

---■--- Control, ---●--- Bb12, ---▲--- Bb12+ST
No significant difference between group.

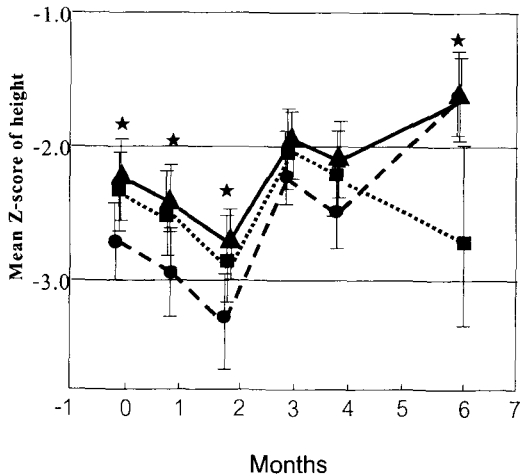


Fig. 3. Mean Z-score of height during 6 months of treatment.

---■--- Control, ---●--- Bb12, ---▲--- Bb12+ST
★ Significant difference between groups with $p < 0.05$

RESULTS

A total of 148 children were enrolled at the baseline. The code of formula was opened after completing the study. The blue can contained formula supplemented with live lyophilized Bb12 approximately 3×10^7 colony forming unit (cfu) per gram, the yellow can contained formula supplemented with live lyophilized Bb12 and ST approximately 3×10^7 cfu per gram and the red can contained infant formula without probiotic supplementation. The Bb12 group (51 children) received infant formula with Bifidobacteria Bb12 supplement; the Bb12+ST group (54 children) received infant formula with Bb12 and *Streptococcus thermophilus* supplement; and the

control group (43 children) received infant formula without supplement. The characteristics of the children are shown in Table 1. However, complete data of the studied children in the 3 groups gradually decreased and finally, a total of 84 children were enrolled at 6 months comprising 36 children in the Bb12 group, 23 children in the Bb12+ST group and 25 children in the control group.

For the weight, all 3 groups had low mean weight values compared to the WHO reference standard, most of the Z-score mean values were between -2 and -1. At the beginning, a significant difference of mean weight Z-score existed between the Bb12 and the control group and the Bb12+ST group. The

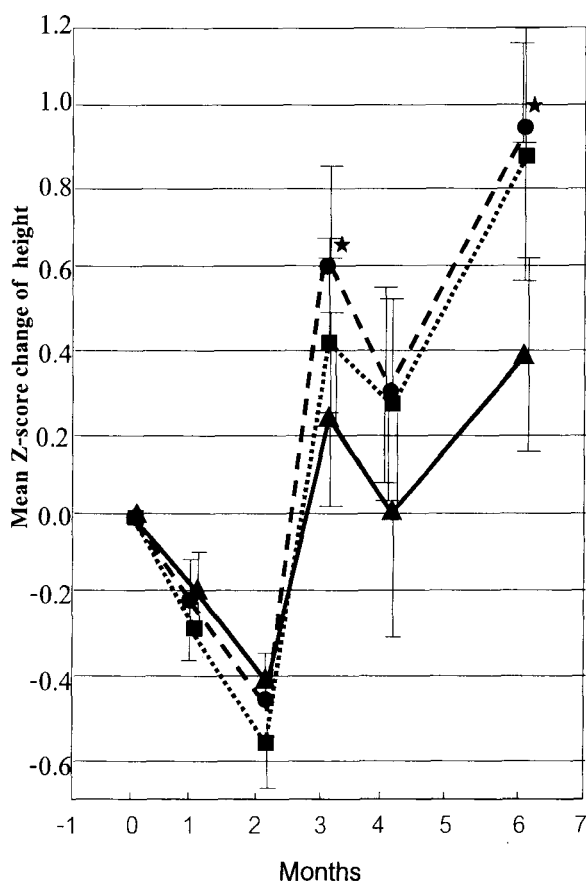


Fig. 4. Mean Z-score change of height during 6 months of treatment.

---■--- Control, ---●--- Bb12, ---▲--- Bb12+ST
 * Significant difference between groups with $p < 0.05$

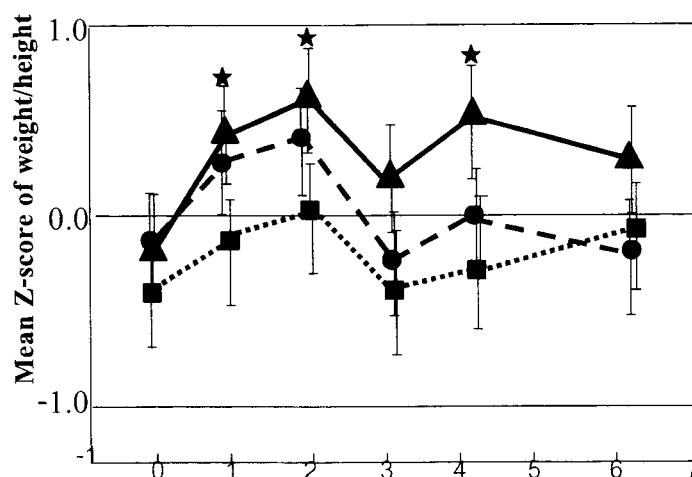


Fig. 5. Mean Z-score of weight/height during 6 months of treatment.

---■--- Control, --●-- Bb12, —▲— Bb12+ST
 ★ Significant difference between groups with $p < 0.05$

mean Z-score \pm SD of weight in the Bb12, Bb12+ST and control group were -1.8 ± 0.12 , -1.4 ± 0.11 and -1.8 ± 0.13 , respectively (Fig. 1). The mean weight Z-score of the control and Bb12 groups were parallel and significantly lower than that of the Bb12+ST group from 0-4 months. At 5 months, the significant difference disappeared but the Bb12+ST group still remained the highest. At the end of the trial of 6 months, the Bb12 group was not significantly different from the Bb12+ST group but the mean of the control group was the lowest (Fig. 1). While the means of Z-score change (mean of individual difference with the initial Z-score), showed no significant differences between the 3 groups but the children in the Bb12+ST group had the highest increment in weight which had increased from -1.4 ± 0.11 to -0.9 ± 0.12 compared to the Z-score of the Bb12 group which had increased from -1.8 ± 0.12 to -1.2 ± 0.13 and in the control group from -1.8 ± 0.13 to -1.7 ± 0.25 (Fig. 2).

For the height, all 3 groups had low mean height values compared to the WHO reference standard, most of the Z-score mean values were between -2 and -3. At the beginning a significant difference of mean height Z-score already existed between the Bb12 and Bb12+ST groups and the control group.

The mean value of height Z-score of the Bb12 group was the lowest (Fig. 3). This difference remained the same after 1 and 2 months of the trial. No difference appeared at 3 and 4 months. At 6 months, the mean height Z-score of the Bb12 group had caught up with the mean height Z-score of the Bb12+ST group while the mean value in the control group remained the lowest. A beneficial effect of Bb12+ST and Bb12 supplemented formula compared to the infant formula in the control group could be demonstrated by the changing of the mean values of the height Z-score above zero as shown in Fig. 4. From this figure, there was no difference between the Bb12 and control groups.

The mean weight/height of the Z-score was closer to 0 (Fig. 5) compared to those of the weight and height. The weight/height ratios of the studied children reflected the catch up growth to the reference standard. Although there was no significant difference between the 3 groups at the beginning, the Bb12+ST group had a significantly higher increment of weight/height ratio than that of the Bb12 and control groups at 1 month. These results suggested that children receiving Bb12+ST supplemented formula had better catch up in weight while children receiving

Bb12 supplemented formula, whose height was the lowest at baseline, had gained and caught up with the Bb12+ST group and more so over the control group.

DISCUSSION

This study was to determine the beneficial effect of milk formula supplemented with either *Bifidobacterium* alone or together with *Streptococcus thermophilus* on the growth of the children. The addition of live bifidobacteria to infant formula and follow-up formula is an interesting alternative approach to induce bifidogenic gut flora in non-breast-fed infants and toddlers. Saavedra *et al* reported that administration of 2 probiotics of Bb12 and ST reduced the incidence of diarrhea in 55 hospitalized infants (7). The lactic-acid-producing ST is also added to the formula because it replicates within the human gastrointestinal tract and generates lactase activity.

This enzymatic activity facilitates the digestion of lactose in milk formula. Thus, children receiving bifidobacteria supplemented milk-based formula have less rotavirus diarrhea⁽¹⁰⁾ and have better lactose digestion so they could absorb more nutrients for catch up growth. However, evaluation of the safety and efficacy of formulas with the selected strain is a necessity for registration in industrialized and developing countries and proof of scientific claims.

SUMMARY

The authors compared weight and height (length) of malnourished infants and toddlers receiving Bb12 supplemented formula with or without *Streptococcus thermophilus*. Both formula resulted in better rapid catch up growth than those receiving follow-up formula without probiotics supplementation.

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ผลการให้นมผงผสมเชื้อไบฟิโดแบคทีเรียม บีบี12 และสเตรปโตค็อกคัส เทอร์โมฟิลลัส ต่อภาวะโภชนาการของเด็ก

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เนื่องจากโรคอุจจาระร่วงยังคงเป็นปัญหาที่ทำให้เกิดความเจ็บป่วยและความตาย การเสริมจุลินทรีย์เชื้อโปรไบโอติกลงในนมผสมสำหรับทารกจึงเป็นวิธีการหนึ่งที่มีผลในการป้องกันการเกิดโรคอุจจาระร่วง หลายการศึกษารายงานว่าโปรไบโอติก ปลอดภัยและยังป้องกันการเกิดโรคอุจจาระร่วงซึ่งมีสาเหตุมาจากเชื้อไวรัสโรต้า ดังนั้นการได้รับนมผงผสมเชื้อไบฟิโดแบคทีเรียม (Bb12) หรือเชื้อ Bb12 ร่วมกับสเตรปโตค็อกคัส เทอร์โมฟิลลัส (ST) อาจจะมีผลดีต่อการเจริญเติบโตของเด็กด้วย ผู้วิจัยจึงทำการศึกษาคู่มือเด็กที่สถานเลี้ยงเด็กอ่อน จำนวน 148 คน อายุระหว่าง 6-36 เดือน แบ่งออกเป็น 3 กลุ่ม โดยวิธีสุ่มตามชนิดของนมผสมสำหรับทารกสูตรต่อเนื่อง 3 ชนิด คือ กลุ่ม Bb12 มีเด็ก 51 คนได้รับนมผงผสมเชื้อไบฟิโดแบคทีเรียม (Bb 12) กลุ่ม Bb12+ST มีเด็ก 54 คนได้รับนมผงผสมเชื้อ Bb12 ร่วมกับ ST และกลุ่มควบคุม มีเด็ก 43 คนได้รับนมผสมสูตรต่อเนื่อง รับประทานเป็นเวลา 6 เดือน ซึ่งน้ำหนักและวัดส่วนสูงทุกเดือน นำ น้ำหนักทั้ง 3 กลุ่ม โดยคำนวณเป็นค่า Z-score นำมาเปรียบเทียบระหว่างกลุ่ม และเทียบกับเกณฑ์มาตรฐานขององค์การอนามัยโลก ปรากฏว่าก่อนเริ่มการศึกษาค่า mean ของ weight Z-score ของกลุ่ม Bb12 เท่ากับ -1.8 ± 0.12 กลุ่ม Bb12+ST เท่ากับ -1.4 ± 0.11 และกลุ่มควบคุมเท่ากับ -1.8 ± 0.13 หลัง 6 เดือนพบว่ากลุ่ม Bb12+ST มีค่าเฉลี่ยเพิ่มขึ้นของน้ำหนักมากที่สุด โดยเพิ่มขึ้นจาก -1.4 ± 0.11 เป็น -0.9 ± 0.12 ส่วนกลุ่ม Bb12 เพิ่มขึ้นจาก -1.8 ± 0.12 เป็น -1.2 ± 0.13 และกลุ่มควบคุมเพิ่มจาก -1.8 ± 0.13 เป็น -1.7 ± 0.25 ค่าเฉลี่ยของส่วนสูงของกลุ่ม Bb12 เพิ่มขึ้นจาก -2.7 ± 0.14 เป็น -1.7 ± 0.16 มากกว่ากลุ่ม Bb12+ST ซึ่งมีค่าเพิ่มขึ้นจาก -2.2 ± 0.13 เป็น -1.7 ± 0.13 แต่กลุ่มควบคุมไม่มีการเปลี่ยนแปลง อย่างไรก็ตามอัตราส่วนของน้ำหนักต่อส่วนสูงของทั้ง 3 กลุ่มใกล้เคียงกับเกณฑ์มาตรฐาน โดยกลุ่ม Bb12 จาก -0.1 ± 0.11 เป็น -0.1 ± 0.13 กลุ่ม Bb12+ST จาก -0.1 ± 0.10 เป็น 0.3 ± 0.17 และกลุ่มควบคุมจาก -0.4 ± 0.12 เป็น -0.1 ± 0.16 จะเห็นว่ากลุ่ม Bb12+ST มีค่าใกล้เคียงกับเกณฑ์มาตรฐานมากที่สุด ผลการศึกษาแสดงให้เห็นว่าการได้รับนมผงผสมเชื้อไบฟิโดแบคทีเรียมชนิดเดียวหรือมีเชื้อสเตรปโตค็อกคัส เทอร์โมฟิลลัสรวมอยู่ด้วยจะมีผลดีต่อการเจริญเติบโตของน้ำหนักและส่วนสูงของเด็ก

คำสำคัญ : ไบฟิโดแบคทีเรียม, สเตรปโตค็อกคัส เทอร์โมฟิลลัส, โปรไบโอติก

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