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Synbiotic Fermented Milk with Double Fortification (Fe-Zn) as a Strategy to Address Stunting: A Randomized Controlled Trial among Children under Five in Yogyakarta, Indonesia

Siti Helmyati [1,2,*], Karina Muthia Shanti [3], Fahmi Tiara Sari [3], Martha Puspita Sari [3], Dominikus Raditya Atmaka [4], Rio Aditya Pratama [5], Maria Wigati [2], Setyo Utami Wisnusanti [1,2], Fatma Zuhrotun Nisa' [1,2] and Endang Sutriswati Rahayu [6]

1 Department of Nutrition and Health, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia; setyo.utami.w@ugm.ac.id (S.U.W.); fatma.znisa@gmail.com (F.Z.N.)
2 Center for Health and Human Nutrition, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia; maria.wigati@mail.ugm.ac.id
3 Department of Biostatistics, Epidemiology and Population Health, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia; karinamuthia.s@gmail.com (K.M.S.); fahmi.tiara.sari@gmail.com (F.T.S.); martha.puspita6@gmail.com (M.P.S.)
4 Faculty of Public Health, Airlangga University, Surabaya 60115, Indonesia; dominikus.raditya@fkm.unair.ac.id
5 Study Program of Public Health Science, Specialization of Health Informatics System, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia; rioadityapratama@outlook.com
6 Faculty of Agricultural Technology, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia; endangsrahayu@ugm.ac.id
* Correspondence: siti.helmyati@gmail.com; Tel.: +62-274-547775

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Abstract: Stunting is one of the public health problems that has yet to be solved in Indonesia. This study developed synbiotic fermented milk with iron and zinc fortification that was then tested in a clinical setting. The product was made from skimmed milk and fructooligosaccharides (FOS) and fermented with *Lactobacillus plantarum*. A sample of 94 stunted children under five years old were randomly assigned to intervention or control groups. The intervention group received double-fortified synbiotic milk, while the control group drank non-fortified milk. After three months, the number of normal children in both groups, according to weight- or height-for-age z-score category, was found to be increasing. However, the difference between the two groups was not significant ($p > 0.05$). The study suggests that fermented milk may have a good effect on child growth. Further research is needed to deepen the potency of synbiotic fermented milk for stunted children.

Keywords: children; double fortification; fermented milk; iron and zinc; stunting; synbiotic

1. Introduction

Developing countries are known for their complex public health problems, including stunting. Globally, it is estimated that 171 million children under five are stunted. Although this number lowered to 149 million children in 2019, stunting remains one of the serious global health challenges that need to be solved. This problem is also happening in Indonesia as one of the developing countries [1–3]. The prevalence of stunting among Indonesian children under five reached 30.8% in 2018. This situation is highly varied in each of the 34 provinces—for example, in East Nusa Tenggara, the stunting prevalence has reached more than 40% [4]. This alarming number emphasizes the need for innovative approaches to combat stunting [5,6].

Stunting is marked by diminished nutritional status and quality of life. Prendergast and Humphrey [7] mentioned that stunting in children is associated with morbidity and mortality, low physical and economic capacity, and an increased risk of metabolic disease in adulthood. This is supported by a study in Indonesia that showed stunting was associated with disease incidence, family income, and parental education [8]. It is estimated that stunting in developing countries costs 13.5% of Gross Domestic Product (GDP) per capita. A one-point increase of child stunting led to a 0.4% reduction of GDP per capita [9].

Stunting is closely related to inadequate nutritional intake. According to Blaney et al. [10], more than 50% of Indonesian children have low energy, likely due to poor iron, zinc, and calcium intake per AKG (*Angka Kecukupan Gizi* or Indonesian Nutrition Adequacy Rate). These are essential minerals that play key roles in human growth [11–13]. The combination of iron and zinc can increase height by 1.1 cm above the standard growth of children [14]. This is in accordance with a meta-analysis by Liu et al. [15] which provided evidence of the potency of zinc supplementation to prevent stunting. Zinc supplementation given after birth can increase height by 0.23 cm and when given to children above 2 years old can increase their height by 1.37 cm. On the other hand, iron supplementation is known to prevent iron deficiency anemia. Larson et al. [16] suggested a strong association between iron supplementation and increased hemoglobin levels and mental development among children. Research on the benefits of zinc and iron combination intake on children's growth is scarce. It is strategic to conduct a clinical trial on the effect of iron and zinc consumption on stunted children in the form of food fortification.

Food fortification is a cost-effective effort to tackle the problem of micronutrient deficiencies [17,18]. Cost-effectiveness is generally defined as the affordable cost to achieve a certain outcome. In this article, it means the cost to avert one case of stunting among children under five. It can also be calculated as the cost per disability-adjusted life-year (DALY) saved [18]. Cost-effectiveness is related to other types of interventions. An analysis in 48 countries showed that food fortification would cost between USD 1 and USD 134 per DALY saved [19].

We planned to develop functional foods by fortifying fermented milk with iron and zinc. Fermented milk was chosen as the vehicle to increase its nutritional value. A meta-analysis by Matsuyama et al. [20] revealed that fortified milk could lower the risk of anemia but could not increase height. On the other hand, several studies about fermented milk development showed its positive effects on health [21–24]. A clinical trial with 494 children in Indonesia demonstrated beneficial effects in that the consumption of probiotics modestly increased weight and height [25]. Onubi et al. [26] suggested that probiotic consumption is beneficial to improving child growth in developing countries. The fermentation process in milk is also essential since it will be fortified with iron and zinc. A higher intake of iron is associated with various side effects including diarrhea, increasing pathogens, and other inflammatory diseases in the gut [27,28]. Lin et al. [29] mentioned that consumption of probiotic and prebiotic could ameliorate those side effects. The presence of pre- and probiotics in diets, called synbiotic foods, which are good to the gut microbiota balance, can reduce the likelihood of micronutrient utilization by pathogenic bacteria in the colon and increase nutrients absorption [30].

Fermented milk with double fortification has potential as a functional food to promote children's growth. Bearing the complex causes of stunting among children under five, the innovation of food development is essential to support stunting management programs in the country. We carried out this research and aimed to determine the effect of synbiotic fermented milk with double fortification on the height and nutritional status of stunted children in Indonesia. We expected this research to give a novel evidence-based intervention for Indonesia and the other low- and middle-income countries which face the same problem, stunting.

2. Materials and Methods

2.1. Subjects and Design of the Study

The study was conducted between May 2017 and October 2018 among under-5-year-olds in Seyegan District, Yogyakarta Province, Indonesia. Informed consent was obtained from the parents or guardians of the children. The Medical and Health Ethics Committee of the Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada gave ethical permission with the reference no. KE/FK/0640/EC/2017. The study is also registered in ClinicalTrial.gov no NCT03495401.

The study used a double-blind, randomized, controlled trial design. There were three main parts: screening, intervention, and a follow-up phase. Screening was conducted with inclusion and exclusion criteria (n = 212). The inclusion criteria were children aged between 2 and 5 years old and categorized as stunted, according to the height-for-age z-score (HAZ < −2 standard deviation). Exclusion criteria were one or more congenital abnormalities, chronic disease, presence of edema or weight-for-height z-score (WHZ) below −3SD, and allergy to milk, iron, or zinc supplements. There were 7 children who refused to be measured, 101 who did not meet the inclusion criteria, and 10 who refused to participate. The subjects were then randomly divided into the intervention (n = 47) and control groups (n = 47). In the intervention phase, the researchers gave synbiotic fermented milk with double fortification (Fe-Zn) to the intervention group and the same milk without fortification to the control group for three months. In the middle of the study, nine subjects from the intervention group and four from the control group dropped out. Anthropometric measurements were conducted twice, namely before and after intervention. Dietary intake was assessed eight times throughout the intervention phase. The number of subjects who remained through the entire study were 38 children from the intervention group and 43 from the control group. The baseline characteristics can be seen in Table 1.

Table 1. Baseline sociodemographic characteristics.

Characteristics	Intervention Group n = 38	Control n = 43	*p*
Age (months) (n, %)			
24–35 months	18 (47)	20 (47)	
36–47 months	11 (29)	10 (23)	0.345 [a]
48–59 months	9 (24)	13 (30)	
Birth weight (gram) (n, %)			
<2500	10 (26)	4 (9)	0.347 [a]
≥2500	28 (74)	39 (91)	
Birth length (cm) (n, %)			
Unknown	0 (0)	1 (2)	
<48	14 (37)	11 (26)	0.700 [b]
≥48	24 (63)	31 (72)	
Sex (n, %)			
Male	21 (55)	22 (51)	0.712 [a]
Female	17 (45)	21 (49)	
Number of sibling (n, %)			
0–1	12 (31)	9 (21)	0.291 [a]
2–3	25 (66)	31 (72)	
≤4	1 (3)	3 (7)	
Mothers' educational level (n, %)			
Did not attend school	0 (0)	0 (0)	
Elementary school	3 (8)	2 (5)	
Junior high school	11 (29)	9 (20)	0.723 [a]
Senior high school	22 (58)	30 (70)	
Bachelor	2 (5)	2 (5)	

Table 1. *Cont.*

Characteristics	Intervention Group n = 38	Control n = 43	*p*
Fathers' educational level (n, %)			
Did not attend school	1 (3)	0 (0)	0.498 [b]
Elementary school	5 (13)	4 (9)	
Junior high school	10 (26)	10 (23)	
Senior high school	19 (50)	28 (65)	
Bachelor	3 (8)	1 (3)	
Household income (IDR) (n, %)			
<1,000,000	4 (10)	5 (12)	0.347 [b]
1,000,000–<1,500,000	17 (45)	13 (30)	
1,500,000–<2,000,000	6 (16)	14 (33)	
2,000,000–<2,500,000	3 (8)	7 (16)	
2,500,000–<3,000,000	2 (5)	0 (0)	
≤3,000,000	6 (16)	4 (9)	
Episode of illness (past 3 months) (n, %)			
0	8 (21)	9 (21)	0.853 [a]
1	15 (39)	16 (37)	
2	12 (32)	12 (28)	
3	3 (8)	6 (14)	
Illness duration (days) (n, %)			
0	8 (21)	9 (21)	0.482 [a]
1–5	11 (29)	16 (37)	
6–10	8 (21)	10 (23)	
11–15	6 (16)	4 (9)	
16–20	2 (5)	1 (2)	
<20	3 (8)	3 (7)	
Height-for-age z-score categories (HAZ) (n, %)			
Stunted	38 (100)	43 (100)	N/A
Normal	0 (0)	0 (0)	
Weight-for-age z-score categories (WAZ) (n, %)			
Underweight	19 (50)	27 (63)	0.246 [a]
Normal	19 (50)	16 (37)	

N/A, not available; [a] chi-squared test; [b] Fisher's exact test; significant if $p < 0.05$; intervention group, subjects given synbiotic fermented milk fortified with iron and zinc; control, subjects given synbiotic fermented milk without fortification.

2.2. Production of Synbiotic Fermented Milk with Double Fortification (Fe-Zn)

The researchers collaborated with CV Violla Foods to produce synbiotic fermented milk with and without fortification. Ingredients consisted of skim milk, sugar, probiotic *Lactobacillus plantarum* (*L. plantarum*), prebiotic fructooligosaccharides (FOS), iron fortificant (ferrous sulfate, Merck, Branchburg, NJ, USA), and zinc fortificant (zinc acetate, Merck). The skim milk (Lactona, Yogyakarta, Indonesia) and sugar (Gulaku, Lampung, Indonesia) were procured from the local public market in Yogyakarta, the probiotic was obtained from the Food and Nutrition Culture Collection at Universitas Gadjah Mada, and the prebiotic was from Beneo Orafti, Indonesia. Skim milk is commonly used as an ingredient for making fermentation products. Before we carried out this research, we conducted trials by comparing skim milk, whole milk, and ultra-high-temperature milk as the main ingredient. We concluded that using skim milk will have better consistency and sensory characteristics that the other two. In line with our trials, several studies mentioned the advantages of using skim milk including lower price and a positive relation with probiotic viability and fermented milk flavor [31–33].

The steps in the process to make synbiotic fermented milk were as follows: (1) dissolve sugar, skim milk, and add FOS and fortificants into water; (2) sterilize; (3) inoculate *L. plantarum*; and (4) incubate. CV Violla Foods had the authority to encode the milk, and the code was not known to the research team. The code was revealed only after the research ended. All types of milk were packed in 100 mL bottles and had the same color, flavor, and taste so that participants could not differentiate the products. Before the study began, we made sure that all subjects had received deworming medication to minimize the potential effects of worm infection. The participants consumed synbiotic fermented milk every day for three months. Considering the study subjects are children, we did not give rigid dietary rules but advised the parents to not change the dietary habits of their children during the study. Children were permitted to consume the milk at any meal each day. Each bottle of synbiotic fermented milk with double fortification contained 79.93 kcal energy, 2.26 g protein, 1.95 g fat, 13.67 g carbohydrate, 1.27 g crude fiber, 90 mg calcium, 2.26 mg iron, 1.22 mg zinc, and 3.23×10^8 CFU/mL *L. plantarum*. Synbiotic fermented milk without fortification contained 85.75 kcal energy, 2.05 g protein, 1.51 g fat, 16.44 g carbohydrate, 0.74 g crude fiber, 88 mg calcium, 0.73 mg iron, 0.18 mg zinc, and 3.19×10^8 CFU/mL *L. plantarum*.

2.3. Socio-Demographic Characteristics Assessment

Socio-demographic characteristics were collected from the parents or guardians of the subjects using questionnaires before the intervention began. The variables assessed were birth weight, birth length, sex, number of siblings, parents' educational levels, household income, illness frequency in the last three months, and illness duration. The variability of these factors between the two groups was assessed.

2.4. Anthropometry Measurements

Body weight and height were measured before and after the intervention phase using a standardized procedure [34]. The weights of the children were assessed using a digital weight scale (GEA Medical, Jakarta, Indonesia) with an accuracy of 0.1 kg; the height was measured using a microtoise with an accuracy of 0.1 cm. We assessed HAZ and WAZ to categorize the nutritional status of the subjects.

2.5. Dietary Intake Assessment

Dietary intake information was collected using 24-h food recall from the parents or guardians of the children. Trained enumerators conducted eight recalls in non-consecutive time with a range of 1–2 weeks for each assessment. The enumerators used a food model book to help with data collection. NutriSurvey software was used to analyze dietary data consumed by the subjects as energy, carbohydrates, protein, fat, iron, and zinc.

2.6. Statistical Analysis

Data were analyzed using STATA 13. Baseline characteristics were written as categorical data, then assessed using a chi-squared test or Fisher's exact test. A chi-square test was used to compare categorical data between the intervention and control groups; if the chi-square assumption could not be fulfilled (there is an expected value < 5 in the cell), then we used Fisher's Exact test [35]. Normality of the data was determined using the Shapiro–Wilk Test. Normally distributed data are presented as a mean $\pm$ standard deviation (SD) while abnormally distributed data are presented as a median (interquartile range). For normal data, the mean difference between the intervention and control groups was measured using an independent *t*-test. The mean difference between pre- and post-intervention was assessed using the paired *t*-test. For data that were not normally distributed, the Mann–Whitney U test was used instead of the independent *t*-test, and the Friedman test instead of the paired *t*-test. The differences in the nutritional status categories between the intervention and control groups were evaluated using the chi-squared test or Fisher's exact test. Multiple linear regression was also used to assess the influence of several nutrients on subjects' nutritional status according to HAZ.

3. Results

Ninety-four (n = 94) children under five years of age were divided into two groups: intervention and control. Throughout the research, four subjects dropped out from the control group and nine dropped out from the intervention group. Subject characteristics were evenly distributed between the two groups. In the current study, the age of the subjects ranged from 24 to 59 months with the number of boys and girls nearly equal. More than 50% of the subjects had birth lengths of 48 cm and birth weights of more than 2500 g. The complete results can be seen in Table 1.

After drinking synbiotic fermented milk for three months, the heights and weights of the subjects remained nearly the same. Table 2 shows that there was no difference in nutritional status between the intervention and control groups ($p > 0.05$), according to their HAZ and WAZ data.

Table 2. Height-for-age z-score (HAZ) and Weight-for-age z-score (WAZ) category after intervention.

	Nutritional Status	Intervention Group	Control	*p*
Height-for-age z-score (HAZ)				
Post-intervention	Stunted	29 (76)	39 (91)	0.078 [a]
	Normal	9 (24)	4 (9)	
Weight-for-age z-score (WAZ)				
Post-intervention	Underweight	15 (39)	26 (60)	0.059 [a]
	Normal	23 (61)	17 (40)	

Data presented in frequency n (%); [a] chi-squared Test; * significant at $p < 0.05$; intervention group, given synbiotic fermented milk fortified with iron and zinc; control, given synbiotic fermented milk without fortification.

In the preliminary phase of the intervention group, all 38 subjects were categorized as stunted. When the subjects were measured again three months after intervention, nine were categorized as normal according to height-for-age ($p < 0.05$). Six months after the intervention, 11 subjects had dropped out. Of these children, four were categorized as stunted in the past two measurements, and two as severely stunted. Two more were severely stunted before the intervention but improved to stunted after the intervention. Three subjects who were stunted before the intervention were re-categorized as normal afterwards. Although four subjects in the control group were also categorized as normal after the intervention, it was not considered statistically significant.

Table 3 shows there was a significant difference of energy, carbohydrates, protein, fat, and iron intake between the intervention and control groups. The intake was higher in the control group than in the intervention group.

Table 3. Comparison of dietary intake between intervention and control group.

Dietary Intake	Intervention Group	Control	*p*
Energy [2] (kcal)	1005.37 ± 197.86	1118.08 ± 189.48	0.011 [b]
Carbohydrate [2] (g)	130.03 ± 32.34	143.95 ± 26.82	0.037 [b]
Protein [2] (g)	32.32 ± 6.62	36.59 ± 8.76	0.016 [b]
Fat [2] (g)	41.85 ± 8.60	47.09 ± 10.59	0.017 [b]
Iron [1] (mg)	5.10 (2.4)	5.23 (2.07)	0.011 [a]
Zinc [1] (mg)	3.43 (1.42)	3.61 (1.56)	0.116 [a]

[1] Data presented in median (IQR); [2] data presented in mean ± standard deviation (SD); [a] Mann–Whitney U test; [b] independent *t*-test; significant if $p < 0.05$.

4. Discussion

The research subjects were stunted young children aged 2–5 years. After the subjects were randomized, there were some who dropped out during the study. The drop-out rate in the intervention group was 19.1%, while in the control group, it was 8.5%. According to Dumville et al. [36], the stunting level of the dropouts did not bias the results of the study. Statistical test results also showed no significant difference between subjects in the control group and the intervention group.

Synbiotic fermented milk in this study was fortified with as much as 2.26 mg (28% AKG) of iron and 1.22 mg (30% AKG) of zinc per 100 mL serving per day. In this study, the amount of fortification complied with the WHO Recommended Dietary Allowance (RDA), which mentions requirements for dairy products and their preparations in the range of 15–30% [18]. A study by Sazawal et al. [37] and El Menchawy et al. [38] also used 30% RDA for the amount of fortification added to milk, which proved to have a positive impact on growth. Antagonistic interactions between iron and zinc in research can be minimized with a ratio of iron to zinc of 1.8:1. Another study noted that iron and zinc fortification in milk formula, with a ratio of 1.3:1, had no effect on reducing zinc absorption [39]. Iron and zinc content of 1:1, however, can cause interactions that reduce the concentration of iron in the plasma, while a $\geq$ 2:1 ratio inhibits the absorption of zinc in the intestine [40].

Three months after being given the product, the bodyweight of subjects in the intervention group increased by 0.7 kg, while height increased by 2.58 cm. Weight and height gains in the control group were 0.6 kg and 2.5 cm. Based on the WHO Child Growth Standards, the increase in body weight and height of children aged 2–5 years over the course of three months ranged from 0.5 to 0.6 kg and 1.5 to 2.5 cm, respectively [41]. This shows that the results in both groups equaled or exceeded normal growth. Growth velocity of body weight and height in stunted children exceeded the speed of growth of children with normal height after the phase of growth restriction [42]. Every 1 cm in height was associated with a lower growth rate of 0.03 cm, and each one-unit increase in HAZ was associated with a decrease in growth speed of 0.08 standard deviation (SD) per year [43].

Nutrition intake, health status, and biological systems interact with each other [44]. The mechanisms underlying this interaction occur locally as well as systemically. The phase of stunted growth causes a decrease in the speed of cell proliferation and molecular changes. Increased levels of pro-inflammatory cytokines in the body can inhibit growth-promoting protein [45]. After this phase is completed, there is an increase in the speed of proliferation at the growth plate, or non-skeletal organs, beyond the normal speed according to age, because the organ feels a growth that is not in accordance with age [46].

Comparisons between weight, height, HAZ, and WAZ of the intervention and control groups did not show a significant difference. Various studies show inconsistent results related to the effect of micronutrient fortification on growth and growth indicators. This was not in accordance with the research of [47], which showed a significant difference in all growth indicators—body weight, height, WAZ, WHZ, and HAZ—in the larger group of children aged 1–4 years, given milk fortified with iron, zinc, and several other micronutrients for one year.

The provision of micronutrients showed mixed results on the growth of children. A meta-analysis by Ramakrishnan et al. [48] noted that iron and zinc supplements affected young children's height and weight gains, though the effect size was minor. Lind et al. [49] said that in his group, giving iron and zinc supplements had no effect on the HAZ after 12 months of intervention. Fahmida et al. [14] found a significant increase in HAZ and height compared to those given placebos, as well as stunted children given zinc after just 10 mg iron and 10 mg zinc supplements for four months. Iron and zinc produce varying effects on children's growth, especially those aged < 24 months who are not iron-deficient but, on the contrary, experience iron repletion. Iron repletion is negatively related to growth because iron in this condition can be a pro-oxidant that increases the number of pathogenic microorganisms [50].

Sufficient energy intake (≥80% RDA) was found to affect the HAZ. Energy plays a role in the synthesis of new tissue to form normal body composition, including adipose tissue, lean tissue, and skeletal tissue. The existence of a positive energy balance in the body can increase weight and height [51]. Micronutrients alone cannot support growth; the adequacy of macronutrients in the body also plays an important role. The lack of significant differences in growth between the intervention and control groups could be caused by the synbiotic milk given to both. Synbiotic milk contains energy of around 80 kcal/serving/day, and protein of around 2 g/serving/day that can support growth.

The addition of synbiotics to milk influences growth. A study by Sazawal et al. [47] showed a significant difference in the speed of weight gain that was greater in children who were given the synbiotic milk *Bifidobacterium lactis* HN019 and oligosaccharides compared to children who were given normal milk. Agustina et al. [25] stated that children who were given milk supplemented with *Lactobacillus reuteri* DSM 17938 for six months experienced significantly faster growth in body weight and height, and a significantly higher WAZ than those without probiotic supplementation. Another strain, *Lactobacillus plantarum* FNCC 260 from Indonesia, also has microbial activity against pathogens that is beneficial for gut health [52].

This study had several limitations. First, the duration was only three months. Although it is appropriate to measure height changes due to an intervention, it is difficult to conclude whether the effects of the intervention will continue. Secondly, the study group only consisted of the intervention and control groups. Both groups were given synbiotic fermented milk; the only difference was the presence of fortified iron and zinc. This design left the researchers without an exact conclusion regarding whether the synbiotic milk has a truly beneficial effect on growth, since the two groups' results were almost identical.

This study introduces an innovation to address stunting using a food-based approach. This research serves as the beginning of further studies in Indonesia, where many local resources can be utilized to address the country's nutritional problems. As a contribution to society, the development of the product in this study has been following the Innovator Innovation Indonesia Expo (*Inovator Inovasi Indonesia Expo*) in 2018 and is in the process of commercialization with the name of "Forty Milk".

5. Conclusions

There were no significant differences in height between the intervention and the control groups after they were given synbiotic milk, with or without double fortification (Fe-Zn). Nutritional status improvement according to HAZ and WAZ tended to be higher in the intervention group than in the control. However, statistical analysis revealed no significant difference observed, which suggests that the consumption of synbiotic milk alone had a good effect on the nutritional status of the children. Further study to include a third group who are not given fermented milk is needed for better comparison.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data available on request due to privacy issues.

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