Synbiotic (\textit{L. Plantarum} Dad-13 and Fructo-Oligosaccharide) Powder on Gut Microbiota (\textit{L. Plantarum}, \textit{Bifidobacterium} and \textit{Enterobacteriaceae}) on Stunting Children In Yogyakarta, Indonesia

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Abstract

Synbiotics have a positive effect on the composition of the gut microbiota. They will increase the production of short-chain fatty acid that has modulating effect on gastrointestinal epithelial cell integrity, appetite regulation, and immune function. The aim of this study is to determine the effect of synbiotics (\textit{L. plantarum} Dad-13 and fructo-oligosaccharide) on gut microbiota composition (\textit{L. plantarum}, \textit{Bifidobacterium} and \textit{Enterobacteriaceae}) in stunting children under five in Yogyakarta, Indonesia. The research methods used double blind randomized controlled trials with parallel design. The sample consisted of 39 stunting children under five which was divided into 19 subjects as a synbiotic group given synbiotic (\textit{L. plantarum} Dad-13 1x10\(^{10}\) CFU and fructo-oligosaccharide 700 mg) powder and 20 subjects as a placebo group given skim milk. The intervention was carried out for 90 days. The result showed that, statistically, there were
significant differences in synbiotic group on gut microbiota (increased in *L. plantarum* and *Bifidobacterium*, while decreased in *Enterobacteriaceae*). Protein and carbohydrate were significantly increasing (p=0.000; p=0.001) in synbiotic group compared to placebo group. Body weight and height were significantly different (p=0.000) in both groups. Body weight and height of children on synbiotic group was increasing 1.02 and 1.6 times higher than placebo group. Neither morbidity nor weight loss was recorded throughout consumption period. Synbiotic powder has significantly positive effect on gut microbiota that can induce nutrient intake, height and weight gain of stunting children.

Introduction

Stunting is a form of chronic undernutrition which is height-for-age z-score below -2 standard deviations according to WHO Child Growth Standards median.¹ That occurs during critical periods of growth and development in early life.² One of the main causes of stunting is a lack of nutrients intake. Children with poor nutrient intake have a higher risk of becoming stunted.³ Nowadays, the concept of stunting has changed rapidly and has no longer been related to lack of nutrient intake, but it is related to the presence of gut microbiota like *Lactobacillus* and *Bifidobacterium* which can play a positive role in enhance nutrient absorption.⁴ Based on previous research, it shows that there is an infectious disease that causes inflammation of the small intestine, namely Pediatric Environmental Enteropathy (PEE). PEE causes dysbiosis/imbalance in gut microbiota which is thought to be the main cause of stunting in a group of children.⁵ A recent study in Bangladesh proved that infection with enteric bacteria, particularly Shigella and *E. coli* is associated with PEE in early in life.⁶ In stunted children, inflammatory genus such as *Enterobacteriaceae* is found which is also found in many inflammatory bowel disease patients. Whereas, healthy children had more probiotic species such as *Lactobacillus* and *Bifidobacterium*.⁷

Prevention of stunting is usually focused on improving nutrient, especially micronutrient intake.⁸ Based on previous study, it was reported that micronutrient intervention cannot prevent stunting.⁹,¹⁰,¹¹,¹² It is unfortunate that the prevalence of stunting cannot be reduced significantly using this method, especially in Indonesia.¹² The 2018 Indonesian Basic Health Survey reported that around 30.8% of children under five years are stunted because of the lack of nutrition and infectious disease. Whereas in Yogyakarta, the percentage of stunting children under five years was 21.4% (15.1% stunted; 6.3% severely stunted), higher than the percentage of underweight that around 13% also due to the reasons of lack of nutrition and infectious diseases.¹³

Synbiotics are a combination of probiotics and prebiotics that work together to provide beneficial effects such as nourish the gut microbiota and increase nutrient absorption.¹⁴ *L. plantarum* Dad-13 is an indigenous probiotic from Indonesia which is well developed for its utilization, the highest antimicrobial compared to other indigenous probiotics such as Mut 7, Mut 13, T3, SNP 2 and against such pathogenic bacteria as *A. hydrophilla dky-5, S. dysentriae dky-4, S. typhi dky-3, E. coli* OK, *E. coli* ST.¹⁵ Prebiotics are not only used to increase *Bifidobacterium* but are also shown to have an effect on improving physiological health. An example of physiological effects due to prebiotic consumption is that it can help the absorption of nutrients such as calcium magnesium, trace elements and protein.¹⁶,¹⁷ Fructooligosaccharide (FOS) is a prebiotic that naturally presents in carbohydrates that cannot be digested by humans gut. This FOS also supports the growth of *Bifidobacterium*.¹⁸ The role of synbiotics in the balance of gut microbiota, which is mechanically able to increase the number of beneficial bacteria such as *Bifidobacterium* and *Lactobacillus*.¹⁸,¹⁹ Increased levels of anaerobic bacteria induce production of short-chain fatty acids (SCFA) such as butyrate, propionate and acetate acid.²⁰ SCFA is produced from the microbiota gut fermentation process in the large intestine.²¹ This fermentation will induce changes in metabolic
environment on gastrointestinal lumen. These effects can lower pH in colon environment which can prevent the growth of pathogenic bacteria or pH sensitive bacteria such as Enterobacteriaceae and Clostridia, thereby nourishing the gut and increasing nutrient absorption.\textsuperscript{22,23,24} Healthy intestinal is very important because the intestine contributes to overall health, such as ensuring optimal digestion and absorption of micro and macro nutrients, thereby increasing body weight and height in stunted children.

Therefore, synbiotics are considered as an effective way to treat stunting in early life. Supplementation with synbiotics powder can increase the richness of gut microbiota. The gut microbiota plays an important role in the absorption of nutrients and accelerates the improvement of nutritional status to support growth and development on stunting children. Thus, this study aimed to determine the effect of Lactobacillus plantarum Dad-13 and FOS on gut microbiota (L. plantarum, Bifidobacterium and Enterobacteriaceae) on stunting children.

Materials and Methods

Subject and Study Design

This study was a randomized, double-blind, placebo-controlled trial; 39 subjects were divided into an intervention (synbiotic group) and a control (placebo group) with 19 subjects in synbiotic group and 20 subjects in placebo group. This study used simple randomization, performed with computer-generated random numbers. It was conducted between January to May 2020 at Therapeutic Feeding Centre in Yogyakarta, Indonesia. The sample size was calculated using hypothesis testing for differences in 2 proportion between 2 independence groups. The minimal number of subjects required, with 95% confidence interval, power of 80% and 10% drop out into account. The inclusion criteria of the subject covered having height for age < -2SD, ranged between 12 - 59 months old, and did not take any probiotics in minimum month before the study took place or wash-in period. Figure 1 is a flow diagram showing the subject’s progression during this study period.

Study Procedures

Total period of this study was 90 days. During the study period, the synbiotic group was given 1 g of synbiotic powder, while the placebo group was given 1 g of skim milk without synbiotic each day. Each participant’s representative was asked to fill compliance form to ensure the product was consumed daily. The study product was a symbiotic powder containing L. plantarum Dad 13 1x10\textsuperscript{10} CFU and FOS 700 mg. L. plantarum Dad-13, indigenous

![Flow diagram of the progress through the phases of a parallel randomized trial of two groups (enrolment, intervention allocation, follow-up, and data analysis)](image-url)
probiotic strain was deposited in ampoules at the Food and Nutrition Culture Collection (FNCC), Center for Food and Nutrition Studies, Universitas Gadjah Mada. The FOS was obtained from PT. Beneo GmbH.

**Fecal Sample Collection**
The fecal sample was collected before and after consumption period or day 0 and day 90. Respondents were given a fecal kit box as a place to store fecal sample a day before fecal collection. The fecal kit box consists of fecal bottle, trail paper, rubber gloves, masks and ice gel. Ice gel contained in the stool kit box was previously frozen before being used. The ice gel serves to keep the fecal samples' temperature cold. After the fecal collected, each sample was put into a sterile tube and mixed with 2 ml RNA-Later (Sigma-Aldrich; R0901; Saint Louis, MO, USA) than it was kept at freeze temperature (-180°C until -30°C) immediately before it was used.

**Fecal Microbiota Populations with Quantitative PCR (qPCR) DNA Extractions Protocol for Fecal Sample**
DNA extraction was extracted from stool samples by using the bead-beating method. Immediately, RNA-later was diluted 10 fold (w/v) and washed with 1 ml of Phosphate-buffered saline (PBS), feces samples were mixed with 300 µL of Tris-Sodium dodecyl sulfate (SDS) solution and 500 µL of TE saturated phenol breaking the cell bead beater (Fast Prep - 24TM, MP Biomedials, USA) at a speed of 4000 rpm for 60 seconds. The supernatant obtained was added with 400 µL of phenol/chloroform/isoamyl alcohol (25:24:1; v/v) (Sigma-Aldrich; P2069; Saint Louis, MO, USA) than bead beater (Fast Prep -24TM, MP Biomedials, USA) for 30 seconds, followed by centrifugation at 13,000 rpm for 5 minutes at 4°C. After centrifugation, 250 µL of the supernatant was mixed with 25 µL of 3 M sodium acetate (pH 5.2) (Sigma-Aldrich; 567422; Saint Louis, MO, USA) and incubated for 30 minutes on ice. Three hundred microliters of isopropanol were added and centrifuged at 13,000 rpm for 5 minutes at 4°C. The DNA pellets were washed with 500 µL of 70% cold ethanol and shaken by hand and centrifuged at 13,000 rpm for 5 minutes at 4°C. Removing the supernatant was by decantation than drying the pellets in the tube at room temperature for approximately one night. The last step was adding 20 µl Tris-EDTA (TE) buffer pH 8 and dissolving the pellets and then, keeping them at 180°C to 300°C.

**Quantitative Real-Time PCR**
The microbiota analysis stage used the quantitative real time PCR method including DNA dilution from the results of DNA isolation, making PCR master mix, reading, making standard curves, and calculating the results of the total number of bacteria. DNA dilution was carried out to equalize the concentration of DNA included in the PCR master mix mixture. The concentration of bacterial DNA was made to 20 ng/µL for *L. plantarum*, *Bifidobacterium* and *Enterobacteriaceae*. The mastermix PCR was made every time you run PCR, consisted of a mixture of Eva Green (5 µL), forward primer (0.5 µL x 1000nM), reverse primer (0.5 µL x 1000nM), sample DNA (1 µL) and nuclease free water (3 µL). The real-time PCR tool used the Bio-Rad CFX-96. The results of multiplication and reading of bacterial DNA quantification were known using the analysis software Bio-Rad CFX Manager Software 3.0. The results of the analysis using the software showed the total *L. plantarum*, *Bifidobacterium* and *Enterobacteriaceae* bacteria in the sample. Primers used had a DNA base sequence as shown in table 1.

### Table 1: Primers

<table>
<thead>
<tr>
<th>Target</th>
<th>Primer</th>
<th>Sequence (5’ → 3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lactobacillus Plantarum</em>²⁸</td>
<td>sg-Lpla-F</td>
<td>CTC TGG TAT TGA TTG GTT CTT GCA T</td>
</tr>
<tr>
<td></td>
<td>sg-Lpla-R</td>
<td>GTT CGC CAC TCA CTC AAA TGT AAA</td>
</tr>
<tr>
<td><em>Bifidobacterium</em>²⁹</td>
<td>g-Bifid-F</td>
<td>CTC CGT GAA ACG GGT GG</td>
</tr>
<tr>
<td></td>
<td>g-Bifid-R</td>
<td>GGT GTT CTT CCC GAT ATC TAC A</td>
</tr>
<tr>
<td><em>Enterobacteriaceae</em>³⁰</td>
<td>En-Isu-3F</td>
<td>TGC CGT AAC TTC GGG AGA AGG CA</td>
</tr>
<tr>
<td></td>
<td>En-Isu-3R</td>
<td>TCA AGG ACC AGT GTT CAG TGT C</td>
</tr>
</tbody>
</table>
Nutrient Intake
Nutrient intake was measured using semi quantitative food frequency questioner (SQFFQ) method. SQFFQ is a method to describe the individual nutrients intake habits at a certain as in use in time. This method is the same as the method of frequency of food both in format but modified by adding question about quantity or size of food. SQFFQ was carried out before and after consumption period by interviewed mother of the children at Therapeutic Feeding Centre. Nutrient intake was analyzed using Nutri Survey 2007 software based on subject’s SQFFQ Nutri Survey is English translation of a professional German nutrition software (EBISpro).

Anthropometric Measurement
Measurement of weight was taken to the nearest of 0.1 kg, using a standardized 20 kg infant digital scale while measurement of height was taken to nearest of 0.1 cm, using measuring board for child under 2 years and microtoise for child over 2 years. Body weight and height were measured every month during consumption period.

Data Analysis
Statistical analysis was performed in R program (v.4.0.3). A comparison between the group was analyzed using t-test. The data have a normal distribution based on Kolmogorov Smirnov analysis. Therefore, a comparison between the groups was analyzed using t-test and chi-square analysis. All analysis was performed on an intention to-treat basis where p-value< 0.05 was considered statistically significant.

Ethical Consideration
Parents or legal guardians were fully informed about the aim of the study, and they signed informed consent obtained from at least one parent or legal guardians because the subjects were minors. Protocol was approved by the Ethical Committee, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia. (Approval date: 25 November 2019; Ref. number: KE/FK/1388/EC/2019) The study was conducted in accordance with Declaration of Helsinki.

Results
There was no significant difference on synbiotic and placebo group (p>0.05), so it could be concluded that the subjects had the same characteristics at the baseline. Characteristics of research subjects including age, sex, weight, and height of children under five is shown in Table 2.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Synbiotic Group (n=19)</th>
<th>Placebo Group (n=20)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>12</td>
<td>7</td>
<td>0.905a</td>
</tr>
<tr>
<td>Male</td>
<td>13</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, month</td>
<td>26±8.34</td>
<td>29±5.78</td>
<td>0.194b</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>8.5±0.94</td>
<td>9.0±0.82</td>
<td>0.103b</td>
</tr>
<tr>
<td>Height, cm</td>
<td>78.96±5.4</td>
<td>80.9±4.55</td>
<td>0.241b</td>
</tr>
</tbody>
</table>

aChi-Square test, bIndependent t-test, significant if p-value<0.05, Values are expressed as mean± SD.

Effect Consumption of Synbiotics Powder on *L. plantarum*, *Bifidobacterium* and *Enterobacteriaceae*

The difference in population of *L. plantarum*, *Bifidobacterium* and *Enterobacteriaceae* was analyzed after consuming synbiotics powder for 90 days. There was a significant increase in the population of *L. plantarum* and *Bifidobacterium* and a significant decrease in the population of *Enterobacteriaceae* in the synbiotic group and not in the placebo group (Table 3).

Energy, carbohydrate, protein intake in placebo group did not significant change after intervention (p>0.05) whereas, carbohydrate and protein intake in synbiotic group significantly changed after 90 days intervention (p<0.05) (Table 4).
The mean of body weight gain in synbiotic group was 1.44±0.65 kg, from 8.53±0.92 to 9.97±1.34 kg while in placebo group was 0.78±0.62 kg, from 9.02±0.82 kg to 9.8±0.89 kg. Figure 2 shows the slope at synbiotic group was higher than placebo group, which was 0.083 and 0.081, consecutively. It showed that within 90 days of synbiotic consumption might be increase 0.083 kg of bodyweight every week and 0.081 kg in placebo group every week. Coefficient of determination ranges from 0 to 1 on both groups, it can be said that the effect of consuming synbiotic powder on body weight is large. This means that the model used is good to explain the effect of these variables. There was a significant difference in bodyweight after 90 days intervention on both groups (p-value<0.05) (table 5).

The mean of height in synbiotic group was 3.89±0.80 cm, from 78.95±5.56 to 80.92±4.66 cm while in placebo group was 0.85±0.74 cm, from 80.92±4.66 cm to 83.43±4.52 cm. Figure 3 shows the slope at symbiotic group was 0.3375, greater than placebo group 0.2107. It means that every week of symbiotic consumption could increase 0.3375 cm in synbiotic group, while the height in placebo group increased 0.2107 cm every week. Coefficient of determination ranges from 0 to 1 on both groups, it can be said that the effect of consuming symbiotic powder on body weight is large. This means that the model used is good to explain the effect of these variables. There was a significant difference in height after 90 days intervention on both groups (p-value<0.05) (table 5).
Fig. 2: Linear regression of weight in synbiotic and placebo group, $y_1$ represents synbiotic group and $y_2$ represents placebo group.

Fig. 3: Linear regression of height in synbiotic and placebo group, $y_1$ represents synbiotic group and $y_2$ represents placebo group.

Table 5: The difference of weight and height

<table>
<thead>
<tr>
<th>Group</th>
<th>Before</th>
<th>After</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Synbiotic group</td>
<td>8.53±0.97</td>
<td>9.96±1.41</td>
<td>0.000*</td>
</tr>
<tr>
<td>Placebo group</td>
<td>9.02±0.84</td>
<td>9.80±0.91</td>
<td>0.000*</td>
</tr>
<tr>
<td>Height (cm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Synbiotic group</td>
<td>78.95±5.56</td>
<td>83.08±5.56</td>
<td>0.000*</td>
</tr>
<tr>
<td>Placebo group</td>
<td>80.92±4.66</td>
<td>83.43±4.51</td>
<td>0.000*</td>
</tr>
</tbody>
</table>

Data are presented as mean±SD *Independent sample t-test, a significantly different (p<0.05)

Table 6: Changes in Body Weight and Height

<table>
<thead>
<tr>
<th></th>
<th>Synbiotic group (Mean±SD)</th>
<th>Placebo group (Mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Day 30</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>8.5±0.94</td>
<td>9.0±1.01</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>78.9±5.48</td>
<td>80.5±5.86</td>
</tr>
</tbody>
</table>
The consumption of synbiotics for 90 days was able to increase body weight and height in stunted children. Changes in body weight and height before and after intervention shows growth rate has improved (Table 6).

Discussion
Based on real-time PCR analysis shown after 90 days intervention, the population of L. plantarum and Bifidobacterium increase significantly and a significant decrease in the Enterobacteriaceae population. The increase of Bifidobacterium population could be resulted from increase in L. plantarum. In addition, the benefits of FOS, among others are to increase Bifidobacteria, Lactobacillus and to reduce Enterobacteriaceae and Clostridium perfringen. The increase in Bifidobacterium in the colon can have a positive impact on health. Bifidobacterium found in the colon helps maintaining health and is a bacterium that is much more important for life. The decrease or loss of Bifidobacterium in the human colon indicates that we are not healthy. In line with previous study, giving a synbiotic containing L. casei shirota (3 x 10^{10} CFU) and galacto-oligosaccharide (GOS) (2.5 g) for every 80 ml for 2 weeks can significantly increase Bifidobacterium. The increase in Bifidobacterium in the colon can have an impact positive for health. Bifidobacterium found in the colon helps in nourishing health and is a bacterium that is much more important to life. Decrease or loss of Bifidobacterium in the human colon is indicative that we are not well. Low number of Bifidobacterium presents in malnutrition and inflammatory bowel syndrome patients. Overall there is a trend towards low levels of Bifidobacterium with various disease, so it plays a role in health. Lactobacillus and Bifidobacterium (which can decrease inflammation, strengthen gut barrier function, inhibit pathogens, and mediate other beneficial effects under certain conditions), are deficient in stool on undernourished children.

Conversely, the decreasing population of Enterobacteriaceae can cause inflammation or pathogens bacteria that can cause PEE. The previous study revealed that overall microbiota of stunted children were enriched in inflammogenic bacteria belonging to the Proteobacteria phylum, whereas those of children who were not stunted were enriched in probiotic species such as B. longum. The previous studies stating that 30 out of 30 healthy adult subjects experienced an increase in the population of L. plantarum after consuming the probiotic L. plantarum Dad-13 for 20 days. Previous research also stated that 20 out of 20 healthy adolescent subjects experienced an increase in the amount of L. plantarum (mean increase of 6.14 log 10 CFU/g of feces) after consuming the probiotic L. plantarum Dad-13 for 2 months. This shows that L. plantarum Dad-13 is able to live in the human gastrointestinal tract and has a beneficial impact on health. In line with previous research which states that L. plantarum Dad-13 is a strain that can survive in the digestive system, is resistant to bile salt and gastric juice, and is found in human feces who consume it. Subjects who experienced a decrease were higher than the increase in the population of the Enterobacteriaceae after drinking L. plantarum Dad-13 probiotics for 2 months, and it occurred in healthy adolescents in Yogyakarta, namely 11 out of 20 subjects that experienced a decrease in E. coli and 15 and 20 subjects that experienced a decrease in Coliform non E. coli. The consumption of L. plantarum Dad-13 for 20 days can reduce the Enterobacteriaceae population in 19 of 30 subjects (the average decrease is 0.71 log10 CFU/g of feces). This shows that it can reduce Enterobacteriaceae in the feces of some subjects. Fecal microbial communities from both undernourished cohorts included increased proportions of pathogenic taxa within Proteobacteria, including Enterobacteriaceae, Escherichia, Klebsiella, and Shigella, as confirmed elsewhere. It should be noted that a similar pattern (increased proportions of Proteobacteria with decreased microbial diversity) is found in inflammatory bowel disease. On the other hand, genera containing potentially beneficial organisms are depleted in the undernourished gut.

Consumption 90 days of synbiotic powder significantly changes in nutrient intake especially protein and carbohydrate. In summary, synbiotic treatment for 90 days might have promotes protein and carbohydrate intake. The consumption of protein and carbohydrate is more than 70% RDA in Indonesia. Children in developing country typically consume plant-based diets rich in complex plant polysaccharides. In line with previous study, the consumption of gut microbiota in school-
In this study weight and height were significantly increasing after 90 days synbiotic treatment. Consumption of synbiotic (L. plantarum Dad-13 and FOS) group for 90 days may increase the bodyweight and height of children by 1.02 and 1.6 times higher than placebo group. It is line with the previous study stating that supplementation of E. faecium IS27526 at 108 cfu/day and 125 ml low fat milk for 90 days may increase the bodyweight of children by 1.5 times higher than supplementation of 125 ml low fat milk only.\textsuperscript{44} It might be due to the synergy between probiotics and prebiotics stimulate cell proliferation in order to expand the surface of mineral absorption thereby increasing mineral bioavailability so that facilitating better nutrient absorption, mostly of magnesium and calcium.\textsuperscript{45,46,47} Hence, the increasing on weight and height might be related to the role of synbiotic.

The main aim of prebiotics is to stimulate the growth and activity of beneficial bacteria in the gastrointestinal tract, which confers a health benefit on the host. Through mechanisms including antagonism (the production of antimicrobial substances) and competition for epithelial adhesion and nutrients, the intestinal microbiota acts as a barrier for pathogens. Final products of carbohydrate metabolism are mostly SCFAs, namely: acetic acid, butyric acid, and propionic acid, which are subsequently used by the host as a source of energy.\textsuperscript{48} As a result of the fermentation of carbohydrates, Bifidobacterium or Lactobacillus may produce some compounds inhibiting the development of gastrointestinal pathogens, as well as causing a reduction in the intestinal pH.\textsuperscript{49} Moreover, Bifidobacterium demonstrates tolerance to the produced SCFAs and reduced pH. Therefore, due to their favorable effect on the development of beneficial intestinal bacteria, the administration of prebiotics may participate in the inhibition of the development of pathogens. SCFAs are a subset of fatty acids that are produced by the gut microbiota during the fermentation of partially and non-digestible polysaccharides.\textsuperscript{50} Diet composition and intake (e.g., types of fibers and iron) have been reported to influence the microbiota composition and the gut SCFA concentration and to impact gut motility and to strengthen the gut barrier functions. SCFAs are estimated to contribute 6%–10% of total energy requirements, and the contribution is expected to be higher for humans consuming high-fiber diets and for herbivorous species.\textsuperscript{51}

Efficient energy metabolism requires communication between the gut and peripheral organs such as the pancreas, liver, adipose tissue, and brain. Information about nutritional status in the gut is relayed by various signals, including gut derived hormones such as glucagon-like peptide-1 (GLP-1). Transient postprandial increases in GLP-1 have many effects on metabolism, including the stimulation of insulin secretion (incretin effect), inhibition of gastric emptying, and an increased feeling of satiety. Secretion of GLP-1 from enteroendocrine L cells can be stimulated by sugars, amino acids, and long-chain fatty acids. Dietary supplementation with fermentable fibers has been shown to increase GLP-1 levels in rodents and humans and SCFAs can stimulate GLP-1 secretion in vitro. Thus, it has been suggested that the gut microbiota increases GLP-1 levels through the production of SCFAs. The absence of SCFAs-producing microbes in GF colon results in significantly higher plasma GLP-1 levels. This colonic-derived GLP-1 has an important role in slowing small intestinal transit, which may be an adaptive response for promoting nutrient absorption.\textsuperscript{52} It can impact to increase in body weight and height. Synbiotic treatment for 90 days is able nourish gut microbiota than can give...
a positive effect on nutrient intake and anthropometric parameters (weight and height) on stunting children.

**Conclusion**

Consumption synbiotic (*L. plantarum* Dad-13 and fructo-oligosaccharide) powder for 90 days was significant in gut microbiota composition (increasing on population of *L. plantarum* and *Bifidobacterium*; decreasing on population of *Enterobacteriaceae*). In addition, it is also significant in nutrient intake such as protein and carbohydrates. Synbiotic also may increase the bodyweight and height of children by 1.02 and 1.6 times higher than placebo group. Synbiotic powder has significantly positive effect on gut microbiota that can induce nutrient intake, height and weight gain of stunting children.

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**Conflict of Interest**

All authors report that there is no conflict of interest.

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