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Profiling of the intestinal microbiota of stunted children in Semarang, Indonesia

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Abstract. *Susanti R, Zaenafreee I, Christijanti W, Mustikaningtyas D, Yuniastuti A. 2024. Profiling of the intestinal microbiota of stunted children in Semarang, Indonesia. Biodiversitas 25: 1337-1343.* Stunting is a top global health priority. Gut microbiota profiles correlate with growth biomarkers, including stunting. This study aims to profile the intestinal microbiota of stunted children in Semarang, Indonesia. This research is an observational analytical study with a cross-sectional design, conducted on children aged 3-3.5 years with stunting and normal nutritional status in Bandarharjo Village, North Semarang District, Semarang City, Central Java Province, Indonesia. Fecal samples were collected, kept in a cooler box, and taken to the laboratory to be analyzed for pH, worm eggs, Alpha-1-Antitrypsin (AAT) levels, and microbiota profile. Microbial DNA was extracted from stool samples. The diversity and abundance of the gut microbiota were determined using the 16S rRNA V3-V4 gene marker region. The diversity and abundance of the intestinal microbiota of stunted children are smaller than those of non-stunted children. The ratio of Firmicutes/Bacteroidetes in intestinal stunting is 96.45, while in healthy, it is 26.05. The intestinal microbes of healthy children were dominated by *Lactobacillus rhamnosus* (30.92%) and *Bifidobacterium breve* (21.49%). The intestinal microbiota of stunted children is dominated by the bacterial species *Blautia obeum* (23.5%) and *Faecalibacterium prausnitzii* (19.13%). The concentration of AAT in the feces of stunted children is higher (75.49±7.68) than that of healthy children (26.73±4.48). The pH value of the feces of stunted children was higher (6.84±0.31) than that of healthy children (6.29±0.43). In conclusion, the composition of the microbiota indicates a condition of dysbiosis in intestinally stunted children. The intestines of stunted children may experience inflammation based on data on higher pH values, higher AAT levels, and deficiencies in bacterial species that benefit the host.

Keywords: Alpha-1-Antitrypsin, dysbiosis, Firmicutes/Bacteroidetes ratio, intestinal microbiota, stunted children

INTRODUCTION

Stunting is defined as being too short for the child's age; that is, the height-for-age Z-score is more than 2 standard deviations below the median of the WHO Child Growth Reference Standard (WHO 2009). Due to growth disorders, the child's height does not correspond to his age. UNICEF/ WHO/World Bank Group data in 2021 revealed that more than a fifth of children under five, or around 149.2 million worldwide, experience stunted, and 45.4 million are wasted. Compared to the previous year, the number of children experiencing stunting has decreased in all regions except Africa (WHO 2021). This stunting is largely irreversible after the child's second birthday. Stunting is a major global health priority (de Onis and Branca 2016). The prevalence of stunting in Indonesia decreases yearly, namely 27.7% in 2019, 24.4% in 2021, and 21.6% in 2022. WHO standards regarding the prevalence of stunting must be less than 20%. The Indonesian government targets a reduction in stunting of 3.8% yearly to reach 17.8% in 2023 and 14% in 2024 (Indonesian Ministry of Health 2023). Even though the percentage has decreased, the number of stunted children under five is still 4.6 million in 2019. With this number, the burden of stunting in Indonesia is still above the child stunting rate in the Southeast Asia region (24.7%) (Central Bureau of Statistics-Ministry of Health Indonesia 2019).

The stunting prevention action recommended by the WHO is to increase understanding, identification, measurement, and early diagnosis of stunting (WHO 2018). Most stunting is caused by poor nutritional intake, malnutrition, or chronic infections (Black et al. 2013). Persistent malnutrition in childhood will change the composition of the gut microbiota (dysbiosis) (Hoffman et al. 2017). Dysbiosis of the gut microbiota is associated with decreased plasma levels of essential amino acids (Kumar et al. 2018). Gut microbiota influences body weight regulation in obesity (Guida and Venema 2015) and anorexia nervosa (Canfora et al. 2017). Metabolites produced by the gut microbiota have been shown to play a role in body weight regulation, particularly Short-Chain Fatty Acids (SCFA) (Canfora et al. 2017, 2019). Dysbiosis is associated with the pathophysiology of stunting (Vonaesch et al. 2018a). Gut microbiota profiles correlate with growth biomarkers, including stunting (Gehrig et al. 2019). Microbiota profiles are related to age and diet and are individual and regionally specific, with different compositions in Asia, Europe, the US, and Africa (Kolde et al. 2018).

The microbiota in the intestine is a consortium of commensal and non-commensal bacteria closely related to food digestion, absorption, and intestinal function. Likewise, metabolites produced by the gut microbiota play a role in body growth, especially during growth. The number of Enterobacteriaceae from the phylum Proteobacteria, often associated with human pathogenicity in stunting, increases with digestive disorders of absorption and local intestinal inflammation (Vonaesch et al. 2018b). The Firmicutes/ Bacteroidetes ratio is related to homeostasis, so changes in this ratio indicate the presence of intestinal inflammation (Mbuya and Humphrey 2016; Ezeh et al. 2021). Low Bacteroidetes can result in poor energy extraction from dietary fiber (Arini et al. 2020). *Ruminococcus* (Phylum Firmicutes) increases carbohydrate intake, degrades starch into simple sugars, and ferments substrates into energy (Nasrin et al. 2023). The genus *Succinivibrio* (Phylum Proteobacteria) is a marker of intestinal dysbiosis because it can produce proinflammatory succinate and increase intestinal permeability (Wasihun et al. 2018; Setyani and Anwar 2022). Long-term dietary habits determine intestinal microbiota composition. *Prevotella* and *Bacteroides* are enterotypes of the gut microbiota that diet influences. The high abundance of *Prevotella* is associated with a plant-based diet. In contrast, the high abundance of *Bacteroides* is associated with a rich animal protein diet (Indonesian Ministry of Health 2020).

This study aims to profile the intestinal microbiota of stunted children in Semarang, Indonesia. Based on 2022 data, the number of stunted children in the Regency City of Semarang is 1,465. Of this number, the largest number was recorded in the North Semarang District, namely 236 children, and 58 of them were in Bandarharjo Village.

MATERIALS AND METHODS

This research is an observational analytical study with a cross-sectional design. A cross-sectional study was conducted on children aged 3-3.5 years with stunting (n = 10) and normal nutritional status (n = 10) in Bandarharjo Village, North Semarang District, Semarang City, Central Java Province, Indonesia. The Universitas Negeri Semarang Health Research Ethics Committee approved the protocol (No.260/KEPK/EC/2023). Written informed consent was obtained from the parents or guardians of the children in the presence of a third person.

Fecal samples were collected on-site from stunted children and healthy children, kept in a cooler box, and taken to the laboratory to be analyzed for pH, worm eggs, AAT levels, and microbiota profile.

Analysis of pH and worm eggs

Fecal pH measurements are carried out using a pH meter (OHAUS pH Meter ST2200-F). *Ascaris lumbricoides*, *Trichuris trichiura*, and *Ancylostoma duodenale* worm eggs were examined qualitatively using the flotation method. One gram of feces plus 8 mL of distilled water was put into a tube and centrifuged at 3,000 rpm for 5 minutes. The upper liquid was taken with a pipette, put into another tube, then added 8 mL of distilled water and centrifuged again. The centrifugation process is carried out until a clear liquid is obtained. The final liquid obtained is added with NaCl (concentration 43%) until the tube is full. The surface of the tube was covered with deck glass and left for 30 minutes. The deck glass is removed, and the part containing the liquid is covered with a glass object dripped with 1% eosin. Next, the preparations were observed with a microscope with a magnification of 10x40 in a 100 field of view (Collender et al. 2015).

Enzyme-Linked Immunosorbent Assay (ELISA) for Alpha-1-Antitrypsin (AAT) analysis

Alpha-1-Antitrypsin (AAT) levels were analyzed using sandwich ELISA (Sakamoto et al. 2018). First, a standard AAT solution was prepared with graded concentrations (200 ng/mL, 100 ng/mL, 50 ng/mL, 25 ng/mL, 12.5 ng/mL, 6.25 ng/mL, 3.13 ng/mL, and 0 ng/mL). Next, 100 µL of fecal samples and 100 µL of standard solution were added to each well. Next, the well plate was closed and incubated at 37°C for 2 hours. After incubation, each well was washed thrice using 1X wash buffer (350 µL/well). A working biotin conjugate antibody was added to each well in 100 µL and incubated at 37°C for 1 hour. After incubation, each well was washed thrice using 1X wash buffer (350 µL/well). Working streptavidin-horseradish peroxidase (HRP) was added to each well in 100 µL and incubated at 37°C for 0.5 hours. After incubation, each well was washed thrice using 1X wash buffer (350 µL/well). A total of 90 µL of tetramethylbenzidine (TMB) substrate was added to each well and then incubated at 37°C for 15-20 minutes in the dark. A total of 50 µL stop solution was added to each well. Next, the plate is inserted into a Biobase microplate ELISA reader (BK-EL10C; Biobase Biodusty (Shandong), Co., Ltd.) and read at 450nm wavelength. The absorbance measurement results were made into a standard curve using Microsoft Excel curve fitting software.

DNA isolation and Next Generation Sequencing (NGS)

Therefore, the microbial DNA was extracted from stool samples using the QIAamp DNA Stool Mini Kit (Qiagen, San Diego, California, US) following the manufacturer's protocol. The extracted DNA was stored in a -20°C freezer before being used for further analysis.

Bacterial DNA from each stool sample was extracted separately. Then, DNA isolated from 10 fecal samples of stunted children was collected and combined thoroughly. Likewise, DNA isolated from 10 healthy children's fecal samples was also collected and combined thoroughly. Next, the 16S rRNA V3-V4 gene marker region was used to find out the variety and abundance of the gut microbiota (Yarza et al. 2014). The amplification process used the Illumina HiSeq 2500 platform for 20 cycles using primer-forward 338F (5′-GGACTACHVGGGTWTCTAAT-3′) and primer-reverse 806R (5′-GGACTACHVGG GTWTCTAAT-3′), which binds to the barcode, which is a sequence of eight bases specific to each sample. The amplification program with PCR (Polymerase Chain Reaction) was 94°C denaturation for 3 minutes, followed by 27 cycles (denaturation at 95°C for 30 seconds, annealing at 55°C for

30 seconds, extension at 72°C for 45 seconds), final extension at 72°C for 10 minutes. The PCR reactions were conducted with a total volume of 20 mL containing 0.5 µL (5 U/µL) Easy Taq DNA polymerase, 2 µL 10× Easy Taq buffer, 2 µL 0.25 mmol/L dNTPs, 0.2 µmol/L primers, 10 ng template DNA and up to 20 µL ddH_2O (Holm et al. 2019). Next, metagenomic analysis was used to generate sequencing amplification.

Data analysis

Metagenomic analysis of microbes in feces was conducted by the Quantitative Insights Into Microbial Ecology software package (QIIME2) Ver. 2019.4 (Caporaso et al. 2010). Paired files are demultiplexed using the demux plugin. Quality control on each sample was done using the DADA2 plugin (Callahan et al. 2019). The diversity index value was measured using 4 diversity indices: Shannon, Simpson, InvSimpson, and Observed OTUs. Microbial taxonomy was compiled based on the Greengenes 13_8 99% OTU database (McDonald et al. 2012). Heatmaps were prepared using the heatmap plugin (Hunter 2007), and taxa bar plots were prepared using Microsoft Excel 2010.

Data on pH, eggs, worms, and AAT levels in fecal samples were analyzed descriptively by correlating the diversity and abundance of microbes in each fecal sample of stunted children and healthy children.

RESULTS AND DISCUSSION

The microbiota OTUs analyzed in this study were 286,345. The number of OTUs in the intestinal healthy was higher (166,827) than in the intestinal stunted (119,518). Diversity analysis of the intestinal microbiota showed that the Shannon and Simpson indexes were higher in healthy than stunted children. The index of species observed in healthy intestines was higher (585) than in stunted intestines (459) (Table 1); 113 species were observed in both stunted and healthy intestines (Figure 1). The differences in microbial diversity and abundance between the intestines of stunted and healthy children indicate a condition of dysbiosis in stunted children.

At the phylum level, all DNA sequences of the intestinal microbiota identified six phyla. Six phyla were identified in the healthy intestine and four in the stunted intestines. The microbial phyla that dominated the healthy intestine were Firmicutes (77.08%), Actinobacteria (17.73%), Bacteroidetes (2.96%), and Verrucomicrobiota (2.12%). Meanwhile, the microbiota in the intestines of stunted children is dominated by Firmicutes (86.76%) and Actinobacteria (12.28%) (Table 2). Figure 2 shows the proportional contribution of dominant bacteria in the intestines of healthy and stunted children. The data in Table 2 also shows dysbiosis in the intestines of stunted children, characterized by the composition changes in their microbiota phyla. In individuals who receive adequate nutritional intake, the gut microbiota is mostly dominated by the Firmicutes and Bacteroidetes phyla and, to a lesser extent, Actinobacteria and Proteobacteria (Yadav et al. 2018). The abundance and diversity of the gut microbiome are strongly influenced by feed composition and nutrient intake and modulate the host's immune quality in the gut (Schofield and Palm 2018). There is a symbiotic relationship between the gut microbiota and the host. The commensal microbiota contributes to increased resistance to infection, differentiation of the host immune system, and the synthesis of certain nutrients, such as short-chain fatty acids (Ramakrishna 2013).

Firmicutes have a high ability to ferment and metabolize carbohydrates and lipids (Bourdon et al. 2019). Firmicutes bacteria produce carbohydrate and dietary fiber-degrading enzymes (Pham et al. 2019). The Firmicutes/Bacteroidetes (F/B) ratio in the intestinal microbiota is also a marker of dysbiosis, an imbalanced diet, and inflammation. The F/B ratio shows the level of fiber digested in the intestine. Fiber consumption can increase the population of Bacteroidetes, thereby reducing the F/B ratio (Magne et al. 2020). The F/B ratio in stunted children in this study was very high at 96.45. Meanwhile, for health, it was 26.05. The high F/B ratio was also reported in stunted children in Aceh (Rinanda et al. 2023), West Java (Surono et al. 2021), West Sumatra (Masrul et al. 2020) and Mexico (Méndez-Salazar et al. 2018). A high F/B ratio indicates a higher abundance of Firmicutes than Bacteroidetes, correlating with high sugar and low fiber consumption (Méndez-Salazar et al. 2018).

Table 1. Diversity and abundance of data

Index	Healthy	Stunted
OTUs (Operational taxonomic units)	166,827	119,518
Observed-species	585	459
Simpson	0.93	0.95
Shannon	3.76	3.73
InvSimpson	13.30	22.20

Table 2. Relative abundance of stunted and healthy intestinal microbiota (phylum taxa)

Phylum	Healthy	Stunted
Firmicutes	77.08%	86.76%
Actinobacteria	17.73%	12.28%
Bacteroidetes	2.96%	0.90%
Verrucomicrobiota	2.12%	0
Proteobacteria	0.10%	0.06%
Desulfobacterota	0.003%	0

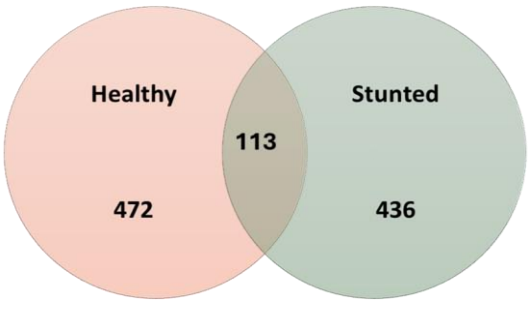

Figure 1. Venn diagram showing the presence of shared species (in the middle) between the habitat of stunted and healthy intestines

At the species level, 52,836 OTUs were recorded in the gut microbiota of healthy children, which were grouped into 65 species. Moreover, 26 species of bacteria are only found in the intestines of healthy children, and 23 species are only found in the intestines of stunted children. The intestinal microbes of healthy children were dominated by the species *Lactobacillus rhamnosus* (30.92%), *Bifidobacterium breve* (21.49%), *Fusicatenibacter saccharivorans* (9.32%), *Bifidobacterium longum* (8.4%), *Akkermansia muciniphila* (6.69%), *Sarcina ventriculi* (4.67%), and *Bifidobacterium bifidum* (3.12%) (Figure 3). Bacterial species that dominate the intestines of healthy children act as probiotics, living microorganisms that provide health benefits to their hosts. Specific probiotic strains were reported from the *Lactobacillus*, *Bifidobacterium*, *Saccharomyces*, *Streptococcus*, *Pediococcus*, *Enterococcus*, *Leuconostoc*, and *Bacillus*. This study found probiotic species from the genera *Lactobacillus* and *Bifidobacterium*.

The *Bifidobacterium* genus is an anaerobic, non-motile, and Gram-positive bacteria. In the intestines of healthy children in this study, there were 3 probiotic species from the genus *Bifidobacterium* with a total relative abundance of 33.01%. Meanwhile, 1 species from the genus *Bifidobacterium* was found in the intestines of stunted children with a total relative abundance of 3.91% (Table 3). *Bifidobacterium breve* is widely used as a pediatric supplement because this species can stimulate immunity and has antimicrobial activity against human pathogens (Cionci et al. 2018). *Bifidobacterium longum* is a probiotic that benefits the host by protecting the intestinal epithelial barrier and tissue structure, producing various active metabolites, inhibiting inflammation by regulating the immune system's balance, and increasing acetate production. *Bifidobacterium longum* influences interactions between the digestive, endocrine, cardiovascular, immune, and nervous systems to maintain the body's health (Yao et al. 2021). *Bifidobacterium bifidum* could interact with the host and other gut microbiota members, activate host immunity, adhere to the intestinal mucosa, and metabolize host glycans, such as mucin (Turroni et al. 2019).

Moreover, *L. rhamnosus* is resistant to acid and bile to survive in the digestive tract. This bacterial species also has a great adhesion capacity to the intestinal epithelial layer, inhibiting several pathogens' growth and attachment. Because of this ability, *L. rhamnosus* is widely used in the food and health supplement industry (Mathipa-Mdakane and Thanthsa 2022). The presence of *A. muciniphila* in the intestine is negatively correlated with Inflammatory Bowel Disease (IBD), obesity, and diabetes; this bacterium acts as an immunomodulatory probiotic for chronic inflammatory diseases and autoimmune (Rodrigues et al. 2022).

The intestinal microbiota of stunted children differs from the microbiota of healthy children. In the intestinal microbiota of stunted children, 43,979 OTUs were recorded, grouped into 63 bacterial species. The intestinal microbiota of stunted children is dominated by the bacterial species *Blautia obeum* (23.5%), *Faecalibacterium prausnitzii* (19.13%), *Collinsella aerofaciens* (7.57%), *Culturomica massiliensis* (6.36%), and *Clostridium tertium* (5.45%) (Figure 3). *Faecalibacterium prausnitzii* is one of the main butyric acid-producing bacteria in the intestine. This bacterial species is also reported to produce metabolites that function as an epithelial barrier that prevents the severity of inflammation (Lopez-Siles et al. 2017). The proportion of *B. obeum* in the intestinal microbiome of stunted children was higher (23.5%), while in the intestinal microbiome of healthy children, it was only 0.47%. Likewise, the *Dorea formicigenerans* and *Dorea longicatena* species in the intestinal microbiome of stunted children were 4.48% and 3.56%, respectively. Meanwhile, the intestinal microbiome of healthy children was recorded at 0.79% (*D. formicigenerans*) and 0.82% (*D. longicatena*) (Table 3). High levels of *Blautia* and *Dorea* species indicate high-fat consumption and are significantly associated with inflammation and disruption of the digestive tract barrier (Vacca et al. 2020).

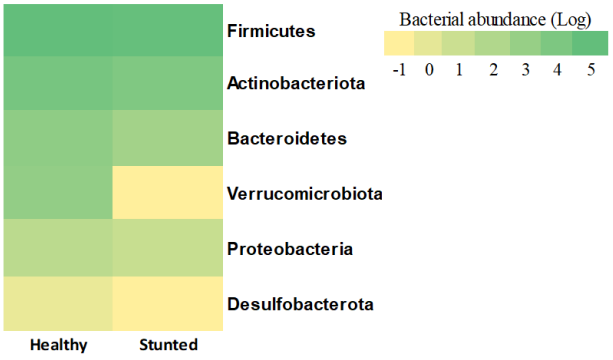

Figure 2. Phylum heatmap distribution of intestinal bacteria density and abundance

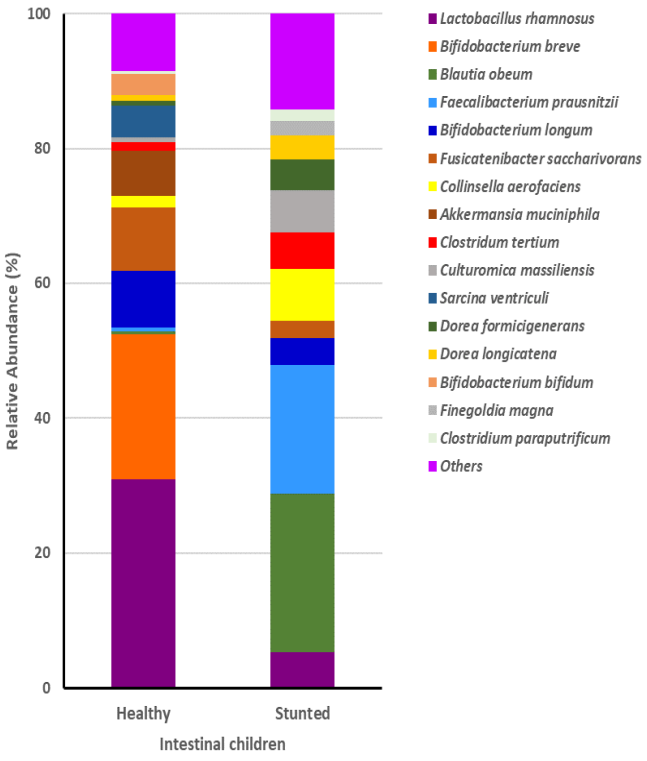

Figure 3. Spatial distribution of the top 16 species in feces pooled-sample content of stunted and healthy children

Table 3. Relative abundance of species of genera *Bifidobacterium, Lactobacillus*, *Clostridium*, *Ruminococcus*, *Bacteroides, Blautia*, and *Dorea* in intestinal of healthy and stunted children

Species	Healthy (%)	Stunted (%)
Bifidobacterium		
Bifidobacterium breve	21.49	0
Bifidobacterium longum	8.40	3.91
Bifidobacterium bifidum	3.12	0
Total	33.01	3.91
Lactobacillus		
Lactobacillus rhamnosus	30.92	5.29
Clostridium		
Clostridium tertium	1.41	5.45
Clostridium paraputrificum	0.41	1.76
Clostridium butyricum	0.16	0.05
Clostridium durum	0.01	0.03
Clostridium mayombei	0	1.51
Clostridium hathewayi	0.04	0.06
Clostridium ramosum	0	0.05
Total	2.03	8.91
Ruminococcus		
Ruminococcus bicirculans	0	0,02
Ruminococcus bromii	1.12	0.65
Ruminococcus callidus	0.02	0.07
Total	1.14	0.74
Bacteroides		
Bacteroides coprocola	0	0.15
Bacteroides cutis	0.02	0
Bacteroides eggerthii	0.02	0
Bacteroides faecis	0.79	0.17
Bacteroides finegoldii	0.40	0
Bacteroides fragilis	0.12	0.04
Bacteroides plebeius	0.02	0
Bacteroides uniformis	0.33	0.23
Bacteroides vulgatus	0.10	0.56
Total	1.80	1.16
Blautia		
Blautia obeum	0.47	23.50
Blautia glucerasea	0	0.07
Blautia intestinalis	0.14	0.29
Total	0.60	23.86
Dorea		
Dorea formicigenerans	0.79	4.48
Dorea longicatena	0.82	3.56
Total	1.62	8.04

Table 4. The results of pH, worm eggs, and AAT analysis of feces of stunted and healthy children

Parameter	Healthy	Stunted
pH	6.29±0.43	6.84±0.31
Alpha-1-Antitrypsin (AAT) (ng/mL)	26.73±4.48	75.49±7.68
Ascaris lumbricoides worm eggs	Negative	Negative
Trichuris trichiura worm eggs	Negative	Negative
Ancylostoma duodenale worm eggs	Negative	Negative

In the intestines of healthy children in this study, there were 2 species from the genus *Ruminococcus* with a total relative abundance of 1.14%. Meanwhile, in the intestines of stunted children, three species from the genus *Ruminococcus* were found with a total relative abundance of 0.74% (Table 3). The genus *Ruminococcus* increases carbohydrate intake, degrades starch into simple sugars, and ferments substrates into energy (Nasrin et al. 2023). In the intestines of healthy children in this study, there were eight species from the genus *Bacteroides* with a total relative abundance of 1.8%. Meanwhile, five species from the genus *Bacteroides* were found in the intestines of stunted children, with a total relative abundance of 1.16% (Table 3). The high abundance of *Bacteroides* is associated with a diet rich in animal protein (Indonesian Ministry of Health 2020).

The microbiota composition suggests dysbiosis in intestinally stunted children, particularly the reduced degree of variety and abundance of microbiota, the F/B ratio, and an imbalance in microbiota phylum and species composition. These results are in line with the AAT and pH data. The concentration of AAT in the feces of stunted children is higher (75.49±7.68) than that of healthy children (26.73±4.48) (Table 4). This indicates that stunted children's intestinal walls are more susceptible than healthy children's. AAT is an indicator protein for diagnosing diseases of the small and large intestines; as a neutrophil elastase inhibitor, it is released during the inflammatory process. AAT acts as a proteolytic enzyme in areas of inflammation. High levels of AAT in feces indicate an intestinal inflammatory process characterized by high intestinal wall permeability (Kulanov et al. 2022). Dysbiosis of gut microbiota can trigger Environmental Enteric Dysfunction (EED). Inflammation and increased permeability of the intestinal wall indicate the presence of EED, a state of subclinical intestinal inflammation. EED is one of the pathogenesis of stunting, because EED can reduce the delivery, absorption and utilization of nutrients resulting in linear growth faltering (Budge et al. 2019).

Indications of intestinal inflammation in stunted children are most likely not due to worm infections because the results of the analysis of the worm eggs of *Ascaris lumbricoides*, *Trichuris trichiura* and *Ancylostoma duodenale* showed negative results (Table 4). *Ascaris lumbricoides* (roundworm), *T. trichiura* (whipworm) and *A. duodenale* (hookworm) are the most dominant species among the group of Soil-Transmitted Helminths (STH) (WHO 2023). The results of the stunting research in Semarang are not in line with previous research in Central Lombok Regency, NTB Province, which reported a significant relationship between intestinal worm infection and stunting (Benvenuto et al. 2022).

The results of the study showed that the pH value of the feces of stunted children was higher (6.84±0.31) than that of healthy children (6.29±0.43) (Table 4). Increased fecal pH was significantly associated with stunted growth. Stool pH is an indicator of intestinal microbiota status. Stool pH is an indirect determining factor in the occurrence of stunting during the growth period of children. An increased fecal pH indicates a reduction in these two species in the

intestine, namely *Bifidobacteria* and *Clostridia* (Hossain et al. 2019). This study's high fecal pH value in stunted children aligns with intestinal microbiota data (Table 4). In this study, *Bifidobacteria* was found in the intestines of stunted children, *Bifidobacterium longum* at 3.91%, but *Clostridia* was found at 8.91%. In the intestines of healthy children, *Bifidobacteria* was found at 33.01% and *Clostridia* at 2.03% (Table 3); commensal *Bifidobacteria* and *Clostridia* contribute to fecal acidity. The composition of the intestinal microbiota, enriched with *Bifidobacteria* and *Clostridia* species, indicates optimal child growth (Hossain et al. 2019).

Gut microbiota dysbiosis can stimulate immune responses and inflammation in the intestine, so it is indirectly related to stunting. The condition of gut microbiota dysbiosis can be caused by individual-specific factors, such as genetics, health status, diet, and environmental factors. The main factors that negatively impact gut microbiota dysbiosis are environmental factors, such as unhealthy diet and medications (Hrncir 2022). In this research only focuses on highlighting the intestinal condition of stunted children in one of the areas with the highest number of stunting in Semarang. Further research is needed regarding the analysis of factors causing gut microbiota dysbiosis in stunted children in this study. Although the sample size in this study is limited, the results of this study can be used as a reference to follow up with relevant research and as a consideration in determining policies to reduce stunting rates. Based on research results, stunted children who experience dysbiosis need to be given gut microbiota-based therapy. Gut microbiota can be used for therapeutic purposes, namely FMT (Fecal Microbiota Transplantation) and probiotic treatment (Hrncir 2022).

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