



eISSN: 2281-7824

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Healthc Low-resour S 2024 [Online ahead of print]

*To cite this Article:*

Rivan Virlando Suryadinata, Heru Wijono, Firstylo Valiant Putra Sanwersko, et al. **The comparison of carbohydrates, fibers, and immunoglobulin-A levels in feces against stunting children in Tuban Regency.** *Healthc Low-resour S* doi: 10.4081/hls.2024.13042

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**Original Article**

**The comparison of carbohydrates, fibers, and immunoglobulin-A levels in feces against stunting children in Tuban Regency**

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**Running title:** The comparison of carbohydrate, fiber, and immunoglobulin-A levels

**Acknowledgement:** The authors sincerely thank the Faculty of Medicine, Universitas Surabaya staff for their valuable assistance during this research.

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**Key words:** carbohydrates; feces; fibers; immunoglobulin-A; stunting

**Contributions:** All authors contributed substantially to the manuscript's development, encompassing the conception, design, data analysis, writing, and revision.

**Conflict of interest:** The authors declare no potential conflict of interest.

**Funding:** This study was financially supported by Faculty of Medicine, Universitas Surabaya.

**Clinical trials:** Not applicable.

**Conference presentation:** Part of this article was presented at the 4th International Nursing and Health Sciences Symposium, from 27<sup>th</sup>-28<sup>th</sup> of October 2023, Universitas Brawijaya, Malang, East Java Indonesia.

**Significance for health:** Stunting events tend to have a negative impact on children development. Various risks of health and intellectual disorders can also cause a decline in the quality of human resources in a country. Decreased digestive tract function is one of the factors contributing to immune system disorders in stunted children, which can trigger the emergence of diseases and decreased absorption and metabolism from the GI Tract. This study seeks to analyze and compare the levels of carbohydrates, fibre, and immunoglobulin-A in the faeces of stunted children, aiming to provide valuable insights into potential dietary and immune factors contributing to stunting.

## **Abstract**

Stunting in children under five years old is a major health problem in Indonesia. A compromised immune system in stunted children increases the risk of infection which has an impact on morbidity and mortality. Moreover, food intake is one of the causes of decreased immunity, where carbohydrate, fiber, and immunoglobulin-A levels in the intestine are related to the immunity of children under five years old. The assessment of feces content can provide an overview of carbohydrates, fiber, and immunoglobulin-A levels in the intestine. Therefore, this study aimed to determine the comparison of carbohydrate, fiber, and immunoglobulin-A levels in feces for stunted children.

An observational method with a case-control design was used, and it was conducted in Tuban Regency from May to July 2023. Forty stool samples were obtained from 20 stunted children and 20 non-stunted children who were tested for carbohydrates, fiber, and immunoglobulin-A. Subsequently, the data were analyzed with a comparison test to determine the differences between the two groups.

The results showed that carbohydrate and immunoglobulin-A levels were higher, while fiber levels in feces were lower in stunted children ( $p \geq 0.05$ ).

It was concluded that there were differences in levels of carbohydrates, fiber, and immunoglobulin-A in the intestines of stunted children.

## **Introduction**

The problem of malnutrition in children under five years old is a global health concern, with developing countries recording over 50% of death cases. Malnutrition in this age group can be prevented through treatment and intervention strategies.<sup>1</sup> Even though there has been a reduction in the prevalence of malnutrition, the decline has not been significant. In low-income communities, the risk of mortality before the age of 20 is doubled, similar to the risk of stunting children due to chronic malnutrition.<sup>2</sup> Stunting is a chronic nutritional problem attributed to inadequate nutrient intake over a long period, resulting in impaired growth in children. Globally, the prevalence of stunting among children under five is 21.3%.<sup>3</sup> with Africa recording a prevalence of 30.9% in 2015. More than 155 million children under five suffer from stunting globally, with over 1 million deaths and a third experiencing Disability Adjusted Life Years (DALYS).<sup>4</sup> Asian regions recorded 83.6 million cases, with the highest proportion originating from South Asia (58.7%), and Indonesia (27.67%) in 2019.<sup>5</sup>

Stunting is an indicator for determining the well-being of children, reflecting environmental and social inequalities. Various risk factors contribute to stunting, including parental (physical condition and nutritional status of the mother during pregnancy, parental education level, and socio-economic status), child (genetic, anthropometric, infection, food intake during infancy, gender, and age), environment (hygiene, sanitation, drinking water sources, culture, and beliefs) among others.<sup>6</sup> Stunting process often begins during pregnancy, influenced by the mother's dietary history during gestation.<sup>7</sup> The impacts of disease on stunted children include inflammation, disruption of the leptin hormone, and increased glucocorticoids. These factors can trigger neurological development disorders, neurogenesis, and apoptosis, affecting brain areas related to cognition and memory in children.<sup>8</sup> Others may include digestive tract disorders in the form of intestinal inflammation. These lead to hampered nutrients absorption, further worsening the condition of stunted children. Intestinal disorders can also compromise the immune system, facilitating microbial invasion, diseases, and systemic inflammation.<sup>9</sup>

Intestinal inflammation can impact children nutritional status through impaired absorption of carbohydrates and proteins. It also disrupts the intestinal mucosa and microbial metabolic activity, which is essential for preventing the entry of pathogens.<sup>10</sup> Microbiota in the food tract plays a significant role in children health, such as increasing metabolism and immunology. This condition is influenced

by various factors, including food intake, particularly fiber. Fiber, a type of polymeric carbohydrate found in food, cannot be absorbed and undergoes fermentation, thereby affecting the bacterial community and microbial metabolic activity.<sup>11</sup> Immunoglobulin A (Ig A), secreted by the mucous membrane lining the gastrointestinal tract plays a role in protecting or providing immunity in the digestive tract. Adequate IgA production enhances gut-associated lymphoid tissue (GALT) and functions as a mechanical barrier in the digestive tract.<sup>12</sup>

Stunting in children can lead to various vulnerabilities in the body, increasing the risk of mortality. Genetic, racial, and ethnic differences, as well as the provision of food, also tend to influence the risk of stunting.<sup>13</sup> Different interventions, including nutritional support and education, are required for each stunted child in various regions. Therefore, assessing the levels of carbohydrates, fiber, and immunoglobulin-A in feces of stunted children in Tuban Regency is necessary to determine differences between the groups.

## **Materials and Methods**

This study used an observational with a case-control method and was conducted in Tuban Regency from May to July 2023. Research approval was issued by Tuban Regency One-Stop Investment and Integrated Services Service (No.070/298/1.S/414.111.3/2023). Furthermore, a non-random purposive sampling method was used. Respondents were 20 stunting and 20 non-stunting children residing in Tuban Regency, East Java, who met the inclusion criteria (aged 2-5 years, free from autoimmune or congenital diseases related to GI Tract, and not suffering from diseases affecting digestion during the study period such as gastroenteritis, hepatobiliary, parasites, or worms) and exclusion criteria (fecal samples showing abnormal characteristics such as changes in color, texture, and odor).

Children who agreed to participate were accompanied by village cadres to receive further information about the study and to sign informed consent. Height/body length measurements were taken before fecal collection, and mothers were educated on the proper collection procedure, including separation from urine by urinating first. Fecal samples were collected in plastic wrap and transferred to clean, dry, and tightly closed pots using a stick or spoon. Each pot was labeled, stored in an ice box at

temperatures of 4-8°C, and transported to the nutrition laboratory of the Faculty of Public Health, Airlangga University for analysis. Carbohydrate levels were examined using the Luff School test, fiber levels through gravimetric test, and Immunoglobulin-A levels using enzyme-linked immunosorbent assay (ELISA) method.

The obtained data were analyzed using a comparison test. Normality test was first carried out to assess data distribution, respectively followed by homogeneity, and Independent T-test for comparison. Mann-Whitney test can be carried out when data are not normally distributed or heterogeneous.

## **Result and Discussion**

Characteristics of respondents included gender, history of exclusive breastfeeding, history of diarrhea frequency each year, and type of additional food intake. Respondents were 20 stunting and 20 non-stunting children residing in Tuban Regency, East Java. Based on Table 1, the majority were female, namely stunting (60%) and non-stunting (65%) children. Furthermore, there were 85% exclusive breastfeeding non-stunting children. Both groups demonstrated a similar history of diarrhea frequency of once per year (40%), with porridge being the most common food intake.

Stunted children were affected by various factors such as socio-economics, environmental conditions, and behavior. Gender differences in the risk of stunting varied significantly between regions, with several studies showing no effect due to wrong parenting patterns.<sup>14</sup> A mother's behavior in complying with exclusive breastfeeding had been proven to be associated with an increased risk of stunting. Examples of poor food intake for children include micronutrient content, low food diversity, and wrong feeding patterns in terms of timing, consistency, and quantity. Infant nutrition is crucial for the health and development of children.<sup>15</sup> Optimal breastfeeding and provision of healthy and appropriate complementary foods to babies until 2 years can increase immunity factors.<sup>16</sup> Diarrhea is known to be a complication of stunting and environmental conditions, affecting approximately 13.5%. Sampling from a single population tends to have similarities in terms of environmental conditions and sanitation hygiene.<sup>17</sup> A significant relationship was found between children who received complementary foods and the risk of stunting. However, the additional food in question was not solely

based on the quantity or type of food provided, but also on diversity, balance of macronutrients and micronutrients, as well as intake of vitamins and minerals. The most important period of a children growth is up to the age of 60 months when any slowdown can cause disruption.<sup>18</sup>

The results of fecal examination are presented in Table 2. The average carbohydrate content was  $\pm$  SD-value of  $11.39 \pm 1.18$  mg in feces of stunted children and  $6.45 \pm 1.17$  mg for non-stunted. The average fiber content was  $\pm$  SD-value of  $5.28 \pm 0.78\%$  for stunted and  $2.98 \pm 0.73\%$  for non-stunted. The average immunoglobulin-A level was  $\pm$  SD-value of  $31.47 \pm 4.08$  ng/mL for stunted and  $12.94 \pm 1.38$  ng/mL for non-stunted.

The absorption of carbohydrate macronutrients is influenced by starch, lactose, and sucrose consumed. Foods containing cellulose cannot be digested by the small intestine, while monosaccharides are absorbed more rapidly by the body. Digestion of carbohydrates initiates in the mouth through the action of amylase enzyme, and continues further in the microvillus membrane. However, monosaccharides can be directly absorbed, and unabsorbed carbohydrates, such as cellulose, are fermented by bacteria in the large intestine and used for energy conversion. Excessive carbohydrate fermentation in malabsorption disorders can lead to bloating.<sup>19</sup> Absorption disorders in stunted children are related to adaptation mechanisms to chronic calorie, protein, and environmental deficiencies, as well as the extent of malnutrition.<sup>20</sup> Malnutrition contributes to a decrease in the secretion of pancreatic enzymes (lipase, trypsin, chymotrypsin, and amylase), atrophy of the villi in the intestine, changes in blood flow, and increased intestinal permeability. This can result to a decrease in digestive enzymes and absorption function in the large intestine.<sup>21</sup> Changes in the microbiota of the digestive tract, thinning of the mucosal layer and intestinal wall, atrophy of the microvilli (brush border), and changes in mucosal cells lead to increased intestinal mucosal permeability, more than threefold, in stunted children.<sup>22</sup> Consequently, this affects carbohydrate absorption, resulting in higher carbohydrate levels in feces of stunted children compared to non-stunted.

Based on Table 3, data on carbohydrate, fiber, and immunoglobulin-A levels showed a normal distribution ( $p > 0.05$ ). In the homogeneity test, the homogeneous variables were carbohydrates and fiber ( $p > 0.05$ ), while Immunoglobulin-A was heterogeneous ( $p < 0.05$ ). Independent t-test was conducted to compare carbohydrate and fiber variables, which showed significant differences between the two



groups ( $p > 0.05$ ). Furthermore, immunoglobulin variable was assessed using Mann Whitney test, showing significant differences ( $p > 0.05$ ).

The microbiota in humans comprises bacteria, fungi, archaea, protozoa, and viruses found in the digestive tract. It plays a crucial role in processing dietary fiber to release antioxidant or anti-inflammatory components. The breakdown of fiber by colonic microbiota can prevent various diseases, including digestive (colitis and infections) and metabolic disorders (diabetes, cardiovascular disease, and obesity).<sup>23</sup> Gut microbiota is related to digestion, absorption, and intestinal function. Stunted children experience changes in the composition of gut microbiota, leading to an imbalance or dysbiosis.<sup>24</sup> This dysbiosis can be related to malnutrition and a decrease in essential amino acid levels in plasma. Furthermore, gut microbiota is essential in regulating body weight, particularly Short Chain Fatty Acid (SCFA) production. Changes in gut microbiota are also related to the pathophysiology of stunting and can be detected before growth retardation occurs.<sup>25</sup> Consumption of dietary fiber influences the microbial composition in the human gut and microbiome function, potentially leading to the development of chronic inflammatory diseases.<sup>26</sup> The increase in fecal fiber levels in stunted children is attributed to the absence of microbiota in the digestive tract.

Immunoglobulin (Ig) A contributed to host bacterial homeostasis in the intestine, which showed nutritional deficiency was associated with changes in the interaction between IgA and gut microbiota.<sup>27</sup> Chronic nutritional deficiency tends to alter IgA recognition of the microbiota. Microbes bound to IgA in feces are significantly higher in stunted children. Stunting is associated with an increase in antibodies, leading to intestinal dysbiosis and inflammation.<sup>28</sup> IgA also functions as a mucosal antibody in the intestine. A substantial increase of IgA in stunted and non-stunted children should be considered due to the potential as a pathogen.<sup>29</sup> The chronic inflammatory process directly induces body inflammation and cell damage.<sup>30</sup> Therefore, increased Ig A levels are often observed in stunted children with infections and associated with a high risk of death.

## **Conclusions**

In conclusion, carbohydrate and immunoglobulin-A levels were higher in feces of stunted children due to digestive disorders and inflammatory processes, while fiber levels were lower ( $p \leq 0.05$ ).

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**Table 1. Distribution of Respondents' Characteristics**

Characteristics		Stunting		Non-Stunting	
		Frequency	%	Frequency	%
Gender	Male	8	40	7	35
	Female	12	60	13	65
Exclusive breastfeeding	Yes	5	25	17	85
	No	15	75	3	15
History of frequency of diarrhoea	1x/year	8	40	8	40
	2x/year	10	50	7	35
	3x/year	1	5	4	20
	Every drinking milk	1	5	1	5
Types of additional food intake	Fine Porridge	14	70	15	75
	Packaged Porridge	2	10	0	0
	Family Food	4	20	5	25

**Table 2. Results of Children Feces Examination**

<b>Variable</b>		<b>Average <math>\pm</math> SD</b>	<b>Maximum</b>	<b>Minimum</b>
<b>Carbohydrate (mg)</b>	Stunting	11.39 $\pm$ 1.18	13.02	8.99
	Non-stunting	6.45 $\pm$ 1.17	8.12	4.18
<b>Fiber (%)</b>	Stunting	5.28 $\pm$ 0.78	6.58	3.64
	Non-stunting	2.98 $\pm$ 0.73	4.13	1.49
<b>Immunoglobulin-A (ng/mL)</b>	Stunting	31.47 $\pm$ 4.08	39.90	25.92
	Non-stunting	12.94 $\pm$ 1.38	15.02	11.04

**Table 3. Difference Test Results in Children Feces**

<b>Variable</b>		<b>Normality Test</b>	<b>Homogeneity Test</b>	<b>P-Value</b>
<b>Carbohydrate (mg)</b>	Stunting	0.252	0.798	< 0.001
	Non-stunting	0.359		
<b>Fiber (%)</b>	Stunting	0.525	0.645	< 0.001
	Non-stunting	0.631		
<b>Immunoglobulin-A (ng/mL)</b>	Stunting	0.124	< 0.001	< 0.001
	Non-stunting	0.053		