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Original Article

Analysis of stool SCFA levels in weaning infants, the timing of weaning, and dietary intake

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Abstract

Background Short-chain fatty acids (SCFA) are metabolites of polysaccharides that cannot be digested by microbiota. Differences in SCFA profiles depend on infant diet and intestinal microbiota. The timing of weaning period from breast milk or formula to solid foods affects the transitional microbiome of the gut. Indonesian Pediatric Society recommend the weaning period at at 6 months old.

Objective To analyze for differences in stool SCFA levels in weaning infants according to timing of weaning to introductory solid foods and dietary intake.

Methods This cross-sectional study was conducted from February to August 2023 in Semarang Primary Health Care Unit. Subjects were infants aged 4-6 months, who underwent fecal SCFA laboratory analysis consisting of acetate, propionic, butyric, and valeric acids, and total SCFA levels, as well as carbohydrate, protein, lipid, and fiber intake assessment through a 3-day food recall processed with Nutrisurvey software of subjects who transitioned to solids before 6 months vs. at 6 months of age. We compared stool SCFA levels in the two groups using T-test analysis.

Results Of 50 infants, 23 children (46%) started weaning from breast milk or formula to food at 6 months of age, and 27 children (54%) started weaning before 6 months of age. In this study, there were no significant differences in SCFA levels between those who started at 6 months and before 6 months ($P>0.05$). There was a weak positive correlation ($r=+0.281$) between protein intake and propionic acid level ($P=0.0481$) in all subjects.

Conclusion There is no significant difference in SCFA level between starting the weaning period at 6 months and before. However, there is a positive correlation between protein intake and propionic acid, when the analysis was done on all subjects. **[Paediatr Indones. 2025;65:279-85; DOI: https://doi.org/10.14238/pi65.4.2025.279-85**].

Keywords: *short chain fatty acid; SCFA; weaning period*

Short-chain fatty acids (SCFA) are metabolites derived from the breakdown of indigestible polysaccharides by colonic microbiota. SCFA have been widely claimed to mediate various conditions associated with changes in gut microbiota. Recent evidence indicates that SCFA play a crucial role in preventing several diseases, linked to systemic effects through diet and microbiota. The SCFA influence the central nervous system, such as changes in neurotransmitter production, as well as mitochondrial function, immune activation, lipid metabolism, and gene expression.[1] Differences in SCFA profiles in infants are influenced bacterial colonization of the gastrointestinal tract that influenced by numerous factors including diet, environment, antibiotic treatment, mucosal maturation, and age.[2] During the period of introduction of weaning foods which marks the transition from breast milk to solid foods, there is a developmental phase of microbiota that affects its composition and diversity, potentially impacting SCFA production. Therefore, we aimed to evaluate

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for possible associations between stool SCFA levels in weaning infants with the timing of introducing solid foods and their dietary intake.

Methods

This study was conducted from February to August 2023 in Semarang Primary Health Care such as Rowosari, Gayamsari, Lamper Tengah and Tlogosari Wetan. Consecutive sampling was used to include infants aged 4 to 6 months who had recently started consuming weaning food. The timing of introducing solid food was categorized into two groups: at six months (on time) and before six months of age (early). Anthropometric measurements were obtained and plotted on the WHO growth chart was categorized into underweight, normoweight, overweight.[3] A questionnaire was used to obtain basic characteristics such as delivery type categorized into caesarian and vaginal, socioeconomic status according to Central Bureau of Statistics on parental income were categorized into low, middle, upper, and parental education were categorized into elementary, junior high, senior high, diploma/bachelors. Food recall data was obtained by a dietitian. Dietary intake was calculated and recorded by a nutrient application software, Nutrisurvey, with subjects' parents asked to remember and report all weaning food consumed in the 3 days prior. Laboratory macroscopic and microscopic evaluations were performed on subjects' fecal specimens. The SCFA levels were measured using gas chromatography-mass spectrometry (GC-MS) at an independent laboratory (*PRODIA®*).

We excluded infants with any congenital digestive tract abnormalities (biliary atresia, duodenal atresia, congenital megacolon), neurological disorders (cerebral palsy), malnutrition, or with a history of antibiotic drug and/or prebiotic use. Parents were fully informed on the procedures, purpose, and benefit of the study before they provided written informed consent. Dietary intake was calculated and recorded for 24-hour food recall for the 3 days before fecal specimen collection using Nutrisurvey, nutrient calculation software, to estimate carbohydrate, protein, lipid, and fiber intake. The percentages of adequate nutrient intake according to the recommended dietary allowance (RDA) were also obtained. Types of complimentary foods were categorized as homemade (complementary food made from natural ingredients and made at home), fortified (commercial complementary food added with important nutritional content according to the baby's requirement), and combination between homemade and fortified.

Subjects' parents were asked to report about breastmilk, formula, or combination intake. Normality testing was performed to determine the distribution of numerical data. Data are presented as mean (standard deviation) for normal distribution, while numerical data with a non-normal distribution are presented as median (range). Categorical data are presented as frequency distribution. Hypothesis testing was conducted using independent T-test or Mann-Whitney test, if the data distribution was non-normal. Bivariate analysis of types of dietary intake composition and SCFA levels were performed with Pearson's correlation for normal data distribution and Spearman's rho for non-normal data distribution. We used *SPSS version 24* for *Windows* software. This study was approved by the Ethics Committee of the Faculty of Medicine of Universitas Diponegoro.

Results

A total of 53 infants participated in the study from February to August 2023; 3 infants were excluded due to lack of parental consent. Subjects were grouped by age at weaning: at 6 months (46%) and before 6 months (54%) of age. Of these, 30 subjects (60%) were still receiving breast milk and 15 subjects (30%) were given a combination of breast milk and formula. The majority of parents had completed high school education and 56% had middle socioeconomic status. Baseline characteristics of subjects were not significantly different between groups. Seventeen (34 %) subjectsreceived homemade complementary feeding, and 24 (48%) subjects were given a combination of homemade and fortified complementary foods (**Table 1**). This table showed the characteristic in infant weaning period at 6-month-old and before with the average anthropometry and the overall mean age of infants with introducing weaning food was 5.92 (SD 0.34) months, The majority were male (54%) and had normal nutritional status (90%).

Table 1. Baseline characteristics according to timing of weaning

Subject characteristics	At 6 months of age (n=23)	Before 6 months of age (n=27)
Mean age (SD), months	6 (0)	5.85 (0.45)
Mean WHZ (SD)	-0.01 (1.1)	-0.25 (1.3)
Mean WAZ (SD)	-0.53 (1.09)	-0.46 (0.95)
Mean HAZ (SD)	-0.71 (1.11)	-0.32 (1.3)
Gender, n		
Male	12	15
Female	11	12
Nutritional status,n		
Underweight	1	1
Normoweight	21	24
Overweight	1	2
Delivery type, n		
Caesarian section	10	13
Vaginal	13	14
Family socioconomic status, n		
Low	8	7
Middle	10	18
Upper	5	2
Parental education		
Elementary	3	3
Junior high	1	1
Senior high	10	12
Diploma/Bachelor's	9	11
Type of milk consumed, n		
Breast milk	12	18
Formula	3	2
Combination	8	7
Type of solid food consumed, n		
Homemade	8	9
Fortified	5	4
Combination	10	14
Dietary intake		
Mean carbohydrate (SD), g	88.88 (11.10)	83.06 (16.74)
Mean protein (SD), g	14.65 (3.09)	14.48 (4.03)
Mean lipid (SD), g	35.69 (4.96)	33.65 (6.56)
Mean fiber, g	1.23 (0.91)	1.45 (1.53)
Fecal SCFA level		
Mean acetate (SD), %	77.7 (10.72)	75.81 (11.04)
Mean propionate (SD), %	14.39 (7.86)	14.07 (6.87)
Mean butyrate (SD), %	4.61 (3.10)	5.91 (4.75)
Mean valerate (SD), %	1.204 (1.36)	1.55 (1.49)
Mean total SCFA (SD), mg/mL	12.13 (6.37)	12.89 (5.45)

There were no significant differences between the timing of weaning groups and acetate levels (P=0.546) or total SCFA (P=0.652). There were also no significant differences in propionate levels (P=0.922), butyrate levels (P=0.576), valerate levels (P=0.483) between groups (**Table 2**).

The results showed a significant, weak, positive correlation (r=0.281) between all subject protein intake and propionate level (P=0.048). However, no correlations were found between carbohydrate, lipid, and fiber intake with all fecal SCFA level (**Table 3**).

Table 2 . Analysis of fecal SCFA levels between timing of weaning period groups

Timing of weaning	Acetate, %		Propionate, %		Butyrate, %		Valerate, %		Total SCFA, mg/mL	
	Mean (SD)	P value	Mean (SD)	P value	Mean (SD)	P value	Mean (SD)	P value	Mean (SD)	P value
At 6 months	77.70 (10.73)	0.546	14.39 (7.86)	0.922	4.61 (3.10)	0.576	1.20 (1.37)	0.483	12.13 (6.37)	0.652
Before 6 months	75.81 (11.05)		14.07 (6.87)		5.91 (4.75)		1.56 (1.49)		12.89 (5.45)	

Table 3. Analysis of dietary intake with SCFA levels in weaning infants

Variables	Acetate		Propionate		Butyrate		Valerate		Total SCFA	
	r	P value	r	P value	r	P value	r	P value	r	P value
Carbohydrate	-0.005	0.970	0.165	0.251	-0.264	0.064	0.114	0.430	0.040	0.780
Protein	-0.067	0.645	0.281*	0.048*	-0.144	0.317	0.060	0.681	-0.119	0.412
Lipid	0.008	0.954	0.117	0.420	-0.090	0.535	0.253	0.076	-0.136	0.348
Fiber	0.163	0.668	0.010	0.947	-0.269	0.059	0.118	0.414	0.087	0.546

Discussion

In this study, SCFA levels were measured using GC-MS. Of the 50 subjects, 23 (46%) started weaning food on time at 6 months of age, and 27 (54%) started early before 6 months of age. There were no significant differences in acetate, butyrate, propionate, valerate, or total SCFA levels between the timing of weaning groups (at 6 months and before 6 months of age). Our finding were similar to a previous studcy which showed that all infants acetate is higher than other SCFAs in all group, that showed acetate and propionate is positively correlated with Bifidobacterium.[4] Study from Canadian healthy infant that showed that SCFAs levels on 4 month infants with exclusive breastfeeding was lower than not breastfed in concentration of total SCFAs, acetate, butyrate, propionate, and valerate. However, infants with exclusively breastfed had four times higher levels of acetate compared to not breastfed. This was because of breastfed influences the composition of fecal microbial metabolites in infancy (OR 4.50, 95%CI 1.58 to 12.82).[5] Gut microbiota during infancy were influenced by environment, mode of delivery, and exposure to breast milk which in our study, middle socioeconomic status 28 (56%), breastmilk 45 (90%) and caesarian section 23 (46%).[6,7] Human milk provides optimal nutrition for infants in the first 6 months of life with contains carbohydrates identified as human milk oligosccharides (HMOs) which are preferred substrate to produce SCFA.[3] In our study, 24 % infants had exclusively breastfeeding (breastmilk only until 6-month-old), so carbohydrate intake in infants weaning period as HMO at 6 months of age higher than before 6 months of age. A study found that gut microbiota composition also differed by breastfeeding exclusivity and duration, Bifidobacteriaceae family to be enriched with breastfeeding and had been shown to metabolize HMO to produce acetate.[8] Exclusive breastfeeding has been associated with a number of beneficial health outcomes in early childhood.[5,8]

In contrast, a study analyzed stool specimens from children between 2 group who started early complementary feeding (before 3-month-old) and later (more than 3-month-old). They were examined microbiota and SCFAs in stool when at ages 3 and 12 months, and found infants introduced to complementary foods early had significantly higher diversity and higher abundance microbiota at ages both 3 and 12 months and early complementary feeding was not associated with butyric acid, propionic, acetate at ages 3 months old but higher fecal butyric acid, propionic, and acetate at ages 12 months old.[1] In our study, there were no significant difference between early introduction group (before 6 months old) and on time (at 6 months old) which stool sample was collected at 4 to 6 months old who had recently started weaning food and we didn't conduct the follow up SCFA level at 1 year of life. On a previous study, extend the finding by showing impact of introduction of solid food before age of 3 months on the gut microbiome could be persist beyond the initial microbial transition, at least 1 year of age. Measurement of gut microbiome diversity at 3 months

in early introduction group (before 3 months old) was correlated with fecal butyrate and total SCFAs concentration at 12 months. The SCFAs composition at 12 months of age on early complementary feeding had significantly associated with higher butyric acid and higher total SCFAs that showed changes in the gut microbiome may lead changes in SCFA concentrations in the later age. Early introduction of complementary foods was associated with decreased Bifidobacteria and increased Bilophila wadsworthia and *Lachnospiraceae roseburia*, that produced higher butyrate production at ages of 12 months than 3 months, leading to changes in gut microbiota up to 1 year.[1] Higher diversity at 3 months of age is associated with higher risk for overweight later in life.[9] In our study, on early weaning food group (before 6 months), there were no significant difference in all SCFAs levels but microbiota diversity at the time of examination and SCFA evaluation at 1 year of age were not assessed in our study.

A cohort study evaluated gut microbiota composition in the feces of 5-year-old children who were introduced to solid food before 4 months of age and after 4 months of age. Breastfeeding duration and nutritional status up to 5 years of age were also assessed. Children with early complementary feeding (before 4 months of age) with breastfed duration for 4 months was associated with higher BMI at 5 years of age than who breastfed duration less than 4 months. Children with early introduction of solid food (before 4 months old) had a higher abundance in *Ruminococcaceae Ruminococcus bromii*, Lachnospiraceae (genus and sp. unknown), Prevotellaceae and lower abundance of *Bifidobacterium animalis* than introduction solid food after 4 months of age. This matter showed that infant microbiota changes from pre-weaning (6 week of age) to post weaning (4 weeks after introduction of solids food). Children who not breastfeeding at 4 months and received solids earlier had lower abundance of Bifidobacterium animalis which most prevalent genera in infant microbiota.[10,11]

In our study, a weak positive correlation was found between protein intake and propionate levels ($r=0.281$; $P=0.0481$) in our total sample population. However, no correlation was observed between carbohydrate, fat, or fiber intake and SCFA levels (acetic acid, propionate, butyrate, valerate, total SCFA). Other studies reported that high-protein diet groups had increased Firmicutes and decreased Proteobacteria (genus Bifidobacterium), along with increased Ruminococcus and Roseburia, leading to higher levels of butyrate and acetate.[12,13] Higher concentrations of proteolytic metabolites in formula-fed infants may also be due to reduced carbohydrate availability in the absence of HMOs and hence to greater derivation of energy from protein metabolism so when fermentable carbohydrates were not present in fecal cultures from both breast and formula-fed infants, metabolites indicative of protein fermentation were mainly produced. Differences in microbiota composition were noted with the introduction of solid foods based on breastfeeding duration.[13]

Studies had shown that infants who were exclusively breastfed had lower microbial diversity, with a predominance in Proteobacteria and Actinobacteria (bifidobacteria), whereas formula-fed increased abundance of Clostridia and Bacteroides species. Gut microbiota dysbiosis and metabolite profiles changes presented a critical window of opportunity influencing of future metabolic and immune health.[5,14-16] A previous study evaluated the impact of meat and dairy based complementary feeding within formula-fed infants on gut microbiota in stool specimens with intervention meat or dairy as primary source of protein from complmentary food with total protein intake of 3 g/kg/day. A significant change of butyric acid from 5 to 12 months was found in the meat group, this showed that meat and dairy group had different bacterial profiles, Ruminococcus was enriched in the meat group.[12] In our study, protein intake was measured in all subject on breastfed or formula-fed and had positive correlated with propionate acid in all subjects. In our study, there were 9 infants (18%) who was given formula milk and weaning food before 6-month-old had higher butyrate and valerate acid. This production of butyrate could be explained by the presence of some amount of intestinal microbiota and food diet.[4,12] Proteins and amino acids which are not digested fermented by microbiota until they reach the large intestine about ten percent or more dependent on protein quality that may result in differing availabilities of dietary proteins to gut microbiota.[17] Children who still were breastfed at least 4 months of age and started weaning on time (at 6 month of age) had significantly increased Roseburia in the stool. Early introduction of complementary feeding

may lead to changes in other microbes to replace the decreased Roseburia microbiota in children starting introduction to solid food and experiencing reduced breast milk. Roseburia species are abundant in breast milk and are producers of butyrate.[10] Loniewska et al.[18] showed an increase in butyrate in children with higher body weight who received formula.

Studies on the impact of breastfed and formula-fed on SCFA in the first year of life mentioned that children who breastfeed is associated with change in propionate and butyrate concentration in the first 9 months of life, that showed higher levels in stool in children with infant formula than breastfed in butyrate and propionate at difference stage of life.[4,19] In our study acetate and propionate were higher at 6-month- old weaning period but butirat, valerate, and total SCFA higher in early (before 6 months) weaning period. This showed that breastfeeding was associated with higher Bifidobacteria abundance and lower Firmicutes, while formula feeding was associated with higher Bacteroides, Escherichia, Enterobacteriaceae, and Clostridium.[5] Acetate was most abundant in infants receiving breast milk, followed by propionate and butyrate. This indicates that breast milk contains HMOs and was associated with lower microbiota diversity, with Proteobacteria and Acinobacteria (Bifidobacteria) predominating.[5,10,20]

A limitation of the study was the recall bias in the food recall method. To minimize this, the researchers provided education before conducting the recall. For future research, it may be beneficial to control the types and amounts of dietary content, with the help of researchers and nutritionists. Also, stool SCFA levels in our study were not compared to serum SCFA levels. Additionally, our study did not account for the quantity and diversity of gut microbiota in children receiving weaning food. Objective measurements of hygiene practices and assessment of parental knowledge about weaning food were not conducted.

In conclusion, our study showed that composition of dietary intake had correlation with SCFA levels in infants on the weaning period. Timing on weaning period in our study didn't have significant difference SCFAs levels that shaped by early-life events as infancy period present a rapid period of gut microbial development. Our study can be considered as the leading study to know about overview SCFA levels in healthy infants on weaning period, hopefully, for further research can be conducted on children of various ages and conditions.

Conflict of interest

None declared.

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