

Effect of arrowroot (*Marantha arundinacea*) cookies intervention on fecal secretory immunoglobulin A and physical properties of children under five years

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ABSTRACT

The purpose of this research was to study the effect of arrowroot cookies on levels of fecal secretory immunoglobulin A (sIgA) and fecal physical properties of children under five years. Total of 17 healthy children aged 2 to 5 years at a Day Care Facility in Yogyakarta received the following treatments: arrowroot cookies and wheat cookies (as control). Intervention of cookies as much as 30 g per child per day for 10 days, and the consumption of cookies was recorded per day. The proportion of arrowroot or wheat flour in the cookies was 41.80%. The average of fecal sIgA (3055 µg/ml) and pH (7.05) of intervened children with arrowroot cookies showed no significantly different from the control. However, the fecal moisture content of children treated with arrowroot cookies was higher (79.23%) compared to the control (73.75%) ($P < 0.05$). Therefore, the arrowroot cookies could provide a mucosal immune response similar to wheat cookies, and improve the physical properties of faeces become softer.

Keywords: Arrowroot Cookies, sIgA, Moisture, pH, Children under Five Years.

INTRODUCTION

Malnutrition is one of the most important underlying causes of child mortality in developing countries, particularly during the first 5 years of life (Rodríguez et al., 2011). The prevalence of malnutrition in Indonesia is still relatively high despite a declining trend from 2007 to 2010. The proportion of malnourished children under five in Indonesia decreased from 18.4% in 2007 to 17.9% in 2010. While the prevalence of malnutrition decreased from 5.4% in 2007 to 4.9% in 2010. The prevalence of stunted children decreased from 18.0% in 2007 to 17.1% in 2010. The prevalence of very thin children under five years slightly declined from 13.6% in 2007 to 13.3% in 2010 (RISKESDAS, 2010).

The inadequate density of complementary foods may cause undernutrition in young children. The plant-based diet contains little animal protein and low amounts of zinc,

which places the infants at high risk of suffering from macro- and micronutrient deficiencies (Ninuk, et al., 1997; Lind et al., 2003). Cookies are suitable to be given as a snack for under five years. One type of food that is good to address malnutrition by giving cookies as a supplement of energy and nutrient needs (Shah, 2011). Cookies, also known as biscuit are ideal for nutrient availability, palatability, compactness and convenience, because of having low moisture content, comparatively free from microbial spoilage and long shelf life of the product (Sharif et al., 2011)

The increased demand for functional foods in recent years is closely related to the growing concern of society with health and quality of life. Moreover, consumers are more informed and aware about the foods that can benefit health. Several industrial products containing added prebiotics can be found in the consumer market, for example in dairy products, dairy desserts, milk, yogurt, and biscuits (de Sousa et al., 2011). Arrowroot plant (*Marantha arundinacea*) is one of the indigenous tuber in Indonesia which has been known for its prebiotic properties, because the arrowroot contained dietary fiber,

raffinose, lactulose, stachyose (Harmayani et al., 2011), and fructooligosaccharides (Fitri, 2011). According to Gibson (2004), those oligosaccharides have prebiotic effect. Arrowroot starch and flour characterized by smooth and easily digestible making it suitable used as baby food and the patients (Anonymous, 2009).

The largest organ of the human body is the gastrointestinal tract (GIT). The large surface area of the GIT is perfect for absorption of essential nutrients. It is, however, also a perfect location for invasion by foreign bacteria and viruses. As infectious agents pass through the intestine and colon they are met by immunoglobulins such as sIgA. Secretory IgA is the primary immunoglobulin that is excreted from mucosal cells, where it binds to antigens (Mantis and Forbes, 2010). It was estimated that the human body produces approximately 66 mg of IgA per kg of bodyweight per day (Shimada et al., 1999). Secretory IgA is also secreted in large quantities in breast milk in order to protect the infant from infection while his/her own immune system is developing (Amarri et al., 2006; Maruyama et al., 2009). While, protein calorie malnutrition (PCM) is a major cause of secondary immune deficiency in the world (Rodríguez et al., 2011).

A prebiotic is a nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health (Gibson and Roberfroid, 1995). The previous study showed that percentages of *Clostridium* spp. and *E. coli* tended to be lower in the infants that received the galactooligosaccharides (GOS)/fructooligosaccharides (FOS) formula, and increased percentages of bifidobacteria may have been involved in the stimulation of the production of sIgA (Scholtens et al., 2008). The major fermentation products of prebiotic metabolism in large bowel are short-chain fatty acids (SCFAs), which had different effects on colon morphology and function such as supply of energy to the intestinal mucosa, lowering of the pH, and stimulation of sodium and water absorption (Scheppach, 1994).

The objective of this study was to investigate the effect of arrowroot cookies on the level of fecal sIgA and fecal physical properties of children under five years at a Day Care Facility in Yogyakarta - Indonesia.

MATERIALS AND METHODS

Materials

The materials ingredients of cookies were arrowroot flour (100 mesh) produced by LIPI-Gunungkidul Yogyakarta, soft wheat flour (8-10% protein), margarine, fine sugar, milk powder, egg yolk, and essence from local market in Yogyakarta Indonesia. Human sIgA ELISA Kit used for fecal sIgA analysis.

Preparation of cookies

The arrowroot cookies in this research were still substituted with 25% wheat flour for produce not too hard cookies. Margarine and sugar were mix with high speed for 5 minutes, and then egg yolk was added and mix for 5 minutes. Milk powder, arrowroot or wheat flour and essence were added and homogenized. The mix that formed was moulded and heated in oven at 140°C for 10 minutes. Cookies formula showed on Table 1.

Cookies intervention in under five years children

This research were conducted following the recommendation of the Ethical Committee of Medicine and Health, Universitas Gadjah Mada (No. KE/FK/559/EC). Some of under five years healthy children (2-5 years) at Day Care Facility in Yogyakarta-Indonesia, were use in this research. Exclusive criteria including antibiotic, prebiotic and probiotic consumption. Before cookies intervention, fecal sample of children was sampled for sIgA, moisture and pH analysis.

The arrowroot cookies was giving at the first intervention for 10 days, and then wash out for 14 days. The second intervention, was done after wash out, and then giving wheat cookies for 10 days. Giving of cookies as much as 30 g/day, and cookies consumption was recorded. After cookies intervention, fecal of under five years children were sampled for sIgA, moisture and pH analysis.

Analysis of fecal sIgA

Fecal sIgA was assayed according to instruction in Human sIgA ELISA Kit (Immundiagnostik AG, Stubenwald-Alee 8a, Bensheim).

Sample preparation: Fecal sample (100 mg) are suspended in 5 ml of extraction buffer, and then centrifugation of the suspension (dilution factor 1: 50). After centrifugation, the supernatant was diluted 1: 250 in wash buffer. For analysis, 100 µl of dilution (1: 250) was pipetted in each well. Final dilution factor is 50 x 250 = 12.500.

Assay procedure. Microtiter plate was washed 5x with 250 µl ELISA wash buffer. Standards (STD), controls (CTRL) and samples (faeces) were added in each well. Microtiter plate was incubated for 1 hour, shaking on horizontal mixer at room temperature. The wells were aspirated and washed 5x with 250 µl ELISA wash buffer. Conjugate (CONJ) was added in each well, and incubated for 1 hour, shaking on a horizontal mixer at room temperature. The conten of the plate was decanted and washed the wells 5x with 250 µl wash buffer. Substrate TMB was added 100 µl per well, and incubated for 10-20 minutes at room temperature. Stop solution was added 50 µl per well and mixed shortly. The absorbtion was determined with an ELISA reader at 450 nm.

Table 1. Cookies formulation

Ingredients	%
Flour (Arrowroot or wheat)	41.80
Sugar	16.50
Margarine	27.50
Milk powder	10.45
Egg yolk	3.74

Analysis of fecal moisture

Fecal moisture contents was analyzed according to AOAC (Anonymous,1995). Sample weighed 1-2 g in bottle that was known their weight. Bottle and sample were dried in oven at 105°C for 3-5 hours. Sample was cooled in desicator and then weighed. Sample was dried in oven for 1 hour, cooled in desicator and weighed. This procedure was repeated until their sample weight reach constant (weight differences less than 0.02 mg). Decreasing of weight showed the sample moisture content.

Moisture content = (Evaporated water / Sample weight) x 100% = [(A+B) – C] / A x 100%

A = initial sample weight

B = initial bottle weight

C = (weight of sample + bottle) after constant weight

Measurement of fecal pH

Measuring of fecal pH according to Tenorio et al. (2010) method with slight modification. Fecal was suspended in aqubidest (1:10) w/v, and then homogenized and measured the pH by pH-meter.

Statistical analysis

The data from cookies consumption, sIgA, moisture content and pH of fecal in children under five years that intervened with arrowroot and wheat cookies were analyzed by Compare Means Independent- Samples T Test. Whereas the data of sIgA, moisture and pH of fecal in children under five years before and after intervention with arrowroot or wheat cookies were analyzed by Compare Means Paired-Samples T Test using SPSS.

RESULTS

Cookies consumption

The average of arrowroot and wheat cookies consump-

tion of children under five years showed not significantly different. The range of arrowroot cookies consumption of children under five years was 4.20-30.00 g (average 12.04 g) per day, while the wheat cookies consumption was 6.64-30 g (average 15.79 g) per day. The cookies consumption of under five years children during this research, showed on Table 2.

Fecal sIgA

The results showed that fecal sIgA level in under five years was not significantly different between before and after intervention with arrowroot cookies, and also before and after intervention with wheat cookies (Figure 1). The average of fecal sIgA before and after arrowroot cookies intervention were 2683.61 and 2816.66 µg/ml, respectively. Whereas the fecal sIgA level before and after wheat cookies intervention were 3153.95 and 3772.15 µg/ml, respectively. The kinds of cookies intervention did not affect on fecal sIgA of children under five years (Table 3).

Fecal moisture

The moisture contents of fecal in under five years was significantly different (P<0.05) between before and after intervention with arrowroot cookies, while before and after intervention of wheat cookies showed not significantly different (Figure 2). The average of fecal moisture contents before and after arrowroot cookies intervention were 73.65 and 78.97%, respectively. Whereas the moisture contents before and after wheat cookies intervention were 75.26 and 73.76%, respectively. Fecal moisture content of children under five years that intervened with arrowroot cookies was higher (P<0.05) than wheat cookies (Table 3).

Fecal pH

The fecal pH of children under five years was not significantly different between before and after intervention with arrowroot cookies, and also before and after intervention with wheat cookies (Figure 3). The

Table 2. The consumption of arrowroot and wheat cookies in under five years children

Children code	Arrowroot cookies (g/day)	Wheat cookies (g/day)
1	8.40	13.24
2	4.20	11.73
3	6.90	16.00
4	6.20	14.29
5	3.00	11.40
6	9.95	6.64
7	22.80	18.55
8	15.00	21.0
9	12.13	14.50
10	13.62	16.6
11	13.09	13.50
12	13.40	8.44
13	16.50	7.59
14	30.00	30.00
15	15.14	19.09
16	4.85	30.00
17	9.43	-
Average	12.04 ^a	15.79 ^a

Superscript with the same letter indicates not significantly different



Figure 1. Fecal sIgA levels of children under five years before and after intervention with arrowroot and wheat cookies. Note: superscript with the same letter indicates not significantly different .

Table 3. Levels of fecal sIgA, moisture contents and pH of children under five years after intervention with arrowroot and wheat cookies.

Intervention	sIgA (µg/ml)	Moisture (%)	pH
Arrowroot cookies	3055.38 ^a	79.23 ^a	7.05 ^a
Wheat cookies	4078.50 ^a	73.35 ^b	6.98 ^a

Superscript with different letter in the same column and different row indicates significantly different (P<0.05)

average of fecal pH before and after arrowroot cookies intervention were 7.09 and 7.04, respectively. Whereas the fecal pH before and after wheat cookies intervention

were 7.13 and 6.98, respectively. The kinds of cookies intervention did not affect on fecal pH of children under five years (Table 3).

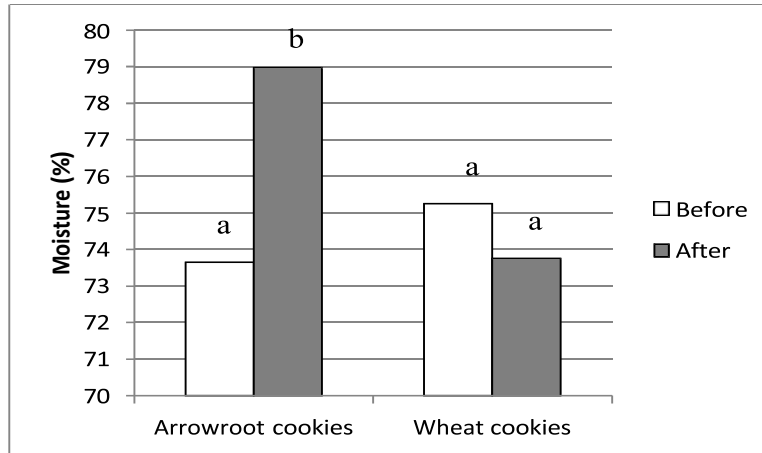


Figure 2. Fecal moisture contents of children under five years before and after intervention with arrowroot and wheat cookies. Note: superscript with different letter indicates significantly different ($P < 0.05$)



Figure 3. Fecal pH of children under five years before and after intervention with arrowroot and wheat cookies. Note: superscript with the same letter indicates not significantly different .

DISCUSSION

There was no differences in cookies consumption between arrowroot and wheat cookies, although the fiber of arrowroot cookies was higher than fiber of wheat cookies. The total fiber content of arrowroot cookies was 13.98%, while the total fiber content of wheat cookies only 3.25% (Fitri, 2011). According to Harmayani et al. (2011), arrowroot powder contained 396.9 ppm of raffinose, 270.8 ppm of lactulose and low amount of stachyose (<56 ppm). Arrowroot is expected to play a role as prebiotic that can improve the health of GIT in under five years children.

According to previous study, the fecal sIgA in the middle school students that treated with whole grain

foods (rich of fiber) can cause fecal bulking. A rich fiber fecal sample may have less “dry” mass and more “wet” mass when compared to the same volume of a lower fiber fecal sample. This dilution effect may have skewed the data and caused no effect to be observed when there may have been one if dry mass was used (Etter, 2012).

The level of fecal sIgA before and after cookies intervention in under five years children in this study showed no significantly different. This results also similar to the previous study by Etter (2012) in healthy middle school students, with whole grain foods and refined-grain foods intervention showed the same of fecal sIgA. Because under five years children that treated with cookies in this research was healthy, there were no metabolic disorder including metabolic in sIgA produc-

tion. According to Abrams and Levitt (2011), not healthy children including obese have risk experiences of developing metabolic function disorder and their complications. Obese individuals that treated with whole grain foods showed the lower fecal sIgA levels than healthy individuals (Etter, 2012).

Obesity condition may influence nonspecific and specific immune responses mediated by humoral and cell mediated mechanisms (Martí et al., 2001). Consequently, obesity has also been associated with decreased immune function, thus lower sIgA levels would be expected compared to healthy weight individuals (Etter, 2012). Another obesity in children, PCM also cause decreasing immune function. Several studies have demonstrated that PCM impairs host immune responses, including cell-mediated immunity (Rodríguez et al., 2011). However, secretory IgA levels were not altered in children with mild-moderate PCM. This finding is of considerable practical importance since a majority of the children in poor communities suffer from milder grades of malnutrition. It has generally been held that the increased incidence of infections seen in undernourished children is due to their impaired immunological status and on this basis it has been suggested that improvement in their nutritional status is one way of reducing infective episodes in malnourished children (Reddy et al., 1976).

Fecal sIgA and other immunoglobulins may be used as markers for immune health due to their importance in the defense of our body from foreign pathogens (Etter, 2012). According to the previous study (Scholtens et al., 2008) concentration of fecal sIgA was higher in infant that fed infant formula for 26 weeks with 6 g/L scGOS/lcFOS (in ratio 9:1) compared to the control. Dietary FOS also increases the total IgA levels in tissue extracts from the jejunum, ileum, and colon in infant mice. Moreover, dietary FOS upregulated ileal and colonic pIgR (polymeric immunoglobulin receptor) expression (Nakamura et al., 2004). Another important function of sIgA is the maintenance or regulation of commensal gut flora homeostasis by demonstrating that the absence of normal IgAs leads to a significant shift in anaerobe populations in the small intestine. The compensatory mechanisms in response to the anaerobic expansion involve adaptive immune responses rather than the local antimicrobial defenses (Suzuki et al., 2004). According to Kumalasari et al. (2012), feeding of the arrowroot tuber powder for 14 days significantly enhanced IgG, IgM, and IgA levels in mice serum. SCFA production, particularly butyrate, in the colon may reduce the requirement of epithelial cells for glutamine, thereby sparing it for other cells, such as those of the immune system. Glutamine is an essential energy source for immune lymphocytes (Schley and Field, 2002).

There was no increase fecal sIgA levels in under five years children that consumed of arrowroot and wheat cookies with limited FOS content in this study. According to Debbabi et al. (1998), the immune responses depend

on the amount of antigen administered. The kinds of cookies could not effect on fecal sIgA, because in the same of cookies consumption in this study showed not significantly different in fecal sIgA. Differ from animal experiments, the same feeding standard can be prepared and administered. Thus the effect of intervention will obtained clearly. Duration of intervention of cookies, and bulking effect of fiber also effect on fecal sIgA in under five years children.

Arrowroot cookies containing more fiber than wheat cookies, leads to more water absorption and causing fecal consistency become softer. According to Fitri (2011), fiber in the arrowroot cookies was 13.98%, while fiber in the wheat cookies was only 3.25%. Changes in bowel function such as reduced transit time, more frequent bowel movements, increased fecal bulk or softer stools may be a beneficial physiological effect (EFSA, 2012).

According to Harmayani et al. (2011), the moisture content in rats caecal digesta with arrowroot diet treatments was higher compared to the control group. In other study using animal experiments as well as in human studies, the amount of time needed in order to detect significant effect of prebiotics on gut microbiota was at least two weeks, indicating that a preventive approach could be more relevant than a therapeutic approach (Vaisman et al., 2010).

SCFAs are believed to stimulate sodium and water absorption from the colon, although not all experiments have confirmed this. There is weak positive correlations between faecal water content and the major SCFAs, acetic, propionic and butyric acids (Siigur et al., 1994). Carbohydrates entering the large bowel can alter colonic physiology in two ways: physical presence and fermentation. Undigested mono-, di-, and oligosaccharides induce osmotic diarrhea if consumed to excess. Fecal bulking was an important component of the fiber hypothesis and is a recognized attribute of foods such as cereal brans. Fiber analogs such as plastic "bran" flakes also speed transit and promote laxation, indicating the importance of the roughage effect of fiber (Topping and Clifton, 2001). However, supplementation of prebiotics to the diet of children with acute diarrhea has no significant effect on stool characteristics over the first 10 days of treatment. Data from animals and preterm infants indicate that at least 14 days are necessary in order to develop the desired intestinal microbiota. Thus, prebiotics reacting via the development of the intestinal microbiota can preferentially be used for prevention rather than for treatment (Vaisman et al., 2010).

Arrowroot cookies intervention did not effect on fecal pH of children under five years. Probably the prebiotic intake could not enough for fermentation process in the colon, and leads the SCFA production could not decrease fecal pH. Because the prebiotic FOS content in the arrowroot cookies is 0.52% (Fitri, 2011), and the range of the arrowroot consumption is 12.04-15.79 g/day,

which means that FOS consumption only 0.06 - 0.08 g/day. Consequently, the dose of prebiotics in this study could not enough for induce the bifidogenic effect, because according to Tuohy et al. (2005), FOS consumption less than 5 g/day have no significantly bifidogenic effect.

The composition of the intestinal microbiota can be influenced by application of dietary ingredients that are nondigestible during the passage through the small intestine, reach the colon, and stimulate selectively health promoting colonic bacteria or by combining both principles in a "synbiotic" approach (Boehm and Moro, 2008).

SCFAs are organic acids produced by intestinal microbial fermentation of mainly undigested dietary carbohydrates, specifically resistant starches and dietary fiber, but also in a minor part by dietary and endogenous proteins. SCFAs are 2-carbon to 5-carbon weak acids, including acetate (C2), propionate (C3), butyrate (C4), and valerate (C5). SCFAs are essentially produced in the colon. As a result of increasing concentrations of acidic fermentation products, the luminal pH in the proximal colon is lower. This pH seems to boost the formation of butyrate, as mildly acidic pH values allow butyrate-producing bacteria to compete against Gram-negative carbohydrate-utilizing bacteria, such as *Bacteroides spp.* (Canani et al., 2011).

The average of stool pH did not reflect the total SCFA concentrations, which may be due to the presence of other anionic compounds (succinate, lactate, bicarbonate) in the stool. SCFA production shifts from butyrate to acetate and propionate as colonic pH increases (5.5–6.5). The observed change in pH was not reflected in the percentages of acetate, propionate and butyrate recovered in the stool. The pH change may have been too small to produce a measurable difference in SCFA production. Other studies with resistant starch (RS) have shown a change in SCFA and fecal pH, using doses ranging from 30 to 50 g/day. A larger sample size and more specific fermentation metabolite analysis may be required to see a relationship between pH and SCFA concentration (Stewart et al., 2010). Stool pH of the infants supplemented group (infant formula supplemented with GOS and FOS 9:1 ratio, 8 g/L, for 6 weeks) was significantly lower than that of the control group that received infant formula (pH of 5.7 vs. 6.3), and did not differ from that of a breastfed reference group (Stewart et al., 2010).

CONCLUSION

The study showed that arrowroot cookies had similar effect with wheat cookies on mucosal immune response and fecal pH of healthy children under five years. Although the arrowroot cookies could not increase the fecal sIgA response and decrease of fecal pH of children, but may be considered as bulking effect that improve the

fecal physical properties of children become softer. The amount of prebiotics contents in the arrowroot cookies that consumed is important for significantly bifidogenic effect associated with decreasing of fecal pH. Optimal consumption of prebiotic in the cookies, duration of cookies intervention, individual health condition, and bulking effect of fiber may effect the fecal sIgA response.

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