Dietary Intervention with Local Arrowroot (*Maranta arundinaceae* L.) Cookies Improves Probiotic Bacteria in Toddler

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ABSTRACT

Prebiotic potential of arrowroot (*Maranta arundinaceae* L.) and starch *in vitro* and *in vivo* have been investigated, but its was limited in experimental animals. Therefore, this study aims to determine the effect of of arrowroot cookies consumption on probiotic bacteria in toddler. Analysis were performed on the components of cookies that have the potential as a prebiotic that is rafinose and FOS, as well as the levels of resistant starch and dietary fiber. Intervention with 5-chip cookies (up to 30g/day) every day was conducted on healthy toddler aged 2-5 years, with the exclusion criteria included use of antibiotics, as well as intake of prebiotic and probiotic. Intervention for 10 days using a pretest-posttest design. Analyses were performed on the profile probiotic bacteria of feces before and after the intervention.The results showed that the arrowroot cookies contain prebiotic components such as resistant starch, soluble and insoluble dietary fiber, as well as raffinose and FOS. Dietary intervention with 10-20g/day arrowroot cookies could improve Bifidobacteria, Lactic Acid Bacteria, *E.coli* and unaerobic bacteria population, but the growth of *E. coli* was inhibited in 20-30g/day arrowroot cookies consumption.

Key words: arrowroot cookies, probiotic bacteria, toddler

INTRODUCTION

Recent years supplementation food products and beverages with a prebiotic component have developed, and proven to improve the beneficial intestinal bacteria. The addition of inulin and oligofruktosa, as prebiotic in infant formula milk and supplementary food for children is recommended by the Food and Agriculture Organization (FAO) because it has been shown to reduce the risk of diarrhea, acute respiratory infections (ARI), fever and antibiotic use (Arslanoglu *et al*., 2007; Kukkonen *et al*., 2008).

Utilization of biological resources in Indonesia, especially the"marginalized" local tubers to improve public health is necessary to work conciderably. Tubers of arrowroot (*Marantha arundinaceae* L) contains a prebiotic components, including soluble fiber 2.37% (db), insoluble fiber 12.49% (db), as well as raffinose, lactulose and stakiosa. Raffinose present in the greatest amount of 396.88 ppm, 270.84 ppm as lactulose, and stakiosa numbered very few, ie less than 56.68 ppm (Kumalasari, 2009). Raffinose and stakiosa can be used by Bifidobacteria and Lactobacilli by breaking it down into monosacharides (Hou *et al.* , 2000). In vitro assay of fresh arrowroot tuber extract could improve the growth of *Lactobacillus, B.longum* and G3, and able to suppress the growth of *S.typhimurium, B.cereus* and *E.coli* (Kusnandar *et al*., 2007). In vivo assay, the dietary intervension with arrowroot starch in mice could to improve the population of *Lactobacilli* in mice digestive tract, although the number of Bifidobacteria colonies have not shown a significant increase (Kumalasari, 2009). However, the research has been conducted on the prebiotic potention of arrowroot but its was limited in experimental animals. Therefore it is necessary to study about the effects of arrowroot cookies consumption to improve the growth of probiotic bacteria and its amount to be recommended for toddler.

MATERIALS AND METHODS

Materials and Analytical Techniques

The cookies were maked from arrowroot flour with 100 mesh flour size. The flour was producted by Indonesian Institute of Sciences (LIPI), Gunung Kidul, Yogyakarta. The cookies were maked using Surawan method (2011). Prebiotic components analysis on the cookies require chemicals such as KCl-HCl buffer pH 1.5, 0.1 M tris-maleate buffer, 0.1 M KOH: 0.4 M acetate buffer pH 4.75, 2 M HCl; pepsin (Merck No. 7190.200 FIT), pancreatin (Sigma A-3176); amiloglucosidase (Sigma A-9913) and glucose GOD FS (Diagnostic System) for the determination of resistant starch (RS) by enzymatic method (Goni *et al*., 1996).

The materials for the determination of dietary fiber (soluble and insoluble) by gravimetric-enzymatik method (Asp, 1983) were α-amylase enzyme necessary thermamyl (Novo Laboratories Inc.), pepsin (Merck No. 7190, 200FIT), cellite 545 (Merck), pancreatin (Sigma A-3176), 90% acetone, and ethanol 95%. Standards required in the determination raffinosa and fosfooligosakharida by HPLC, respectively raffinose (Merck, Germany), and raftilose (Raffinerie Tirlemontoise, Belgium). Analysis of fecal bacteria require media and chemicals were included Man Rogosa Sharpe Agar for the determination of the number of Lactic Acid Bacteria (LAB) using Va'zquez method (2005), Columbia Agar, glucose, cysteine hidrocloride, propionic acid, and 1N NaOH as a pH regulator in determining the number of Bifidobacteria, using Beerens method (1991), Nutrient Agar and nitrogen gas for the determination of total unaerobic bacteriausing Benatti method (2002). Media Tryptone Bile X-Glucoronide agar for the determination of the number of *Escherichia coli* in feces was using methods of the Health Protection Agency (HPA) in MSOP31 (Anonymous, 2005).

Research Subjects

The research was conducted with the permission ofthe Medical Research Ethics Committee and Health, Faculty of Medicine, Gadjah Mada UniversityNo.KE/FK/559/EC. Recruitment of the participants performed at the Sardjito Hospital and Tungga Dewi day cares. Recruitment results on the both of daycares were gained 20 toddler aged 2 to 5 years, which has returned the informed consent and has been declared healthy by a pediatrician. Exclusion criteria included use of antibiotics, as well as intake of prebiotic and probiotic. Of the 20 infants was only17 participants can follow this study up to the end.

The Experimental Design

The research wasconductedbased on thepre test-post test design. Prior to the intervention diet, first performed taking feces specimens for analyzing the bacterial profiles. Feeding 30g/day cookies was done every day for 10 days. The calculation of cookies consumsion was calculated each day. Further collecting feces specimens has done to determine the bacterial profile of feces on the post-intervention diet.

Statistical analysis

The data obtained were grouped based on the amount of cookies consumption. Unbalance univariate analysis of variance and pair wise comparison with LSD at the significance 0.05 (SPSS 17) was performed to compare between the data pre and post-intervention arrow root cookies in the same individual, based on the amount of cookies consumption.

Result and Discussion

Analysis of prebiotic components in arrowroot cookies among others resistant starch (RS), soluble dietary fiber (SDF), insoluble dietary fiber (IDF), also raffinose and FOS was showed in Table 1. Table1 showed that the arrowroot cookies contain resistant starch was 36.67% (% wb), and the content of FOS was higher than rafinosa.

In this study, each child was given cookies as much as 30g/day and grouped based on the numberof cookies consumed, namely1-10g/day, 10-20g/day and 20-30g/day. Fecal bacterial profile in arrowroot cookies intervention based on the amount of consumption can be seen in Figure 1 - 3. In Figure 1 shows that the intervention is less than arrowroot cookies 10g/day affects only the number of Bifidobacteria. This means arrowroot cookies consumption of less than10g/day is still relatively small so do not affect the balance of fecal bacteria.

Table 1. Arrowroot cookies prebiotic component

Parameter	Arrowroot Cookies (% wb)
Resistant starch (RS)	36.67
Soluble dietary fiber (SDF)	2.48
Insoluble dietary fiber(IDF)	10.83
Total dietary fiber	13.31
Rafinosa	0.0059
Fructooligosachcaride (FOS)	0.49

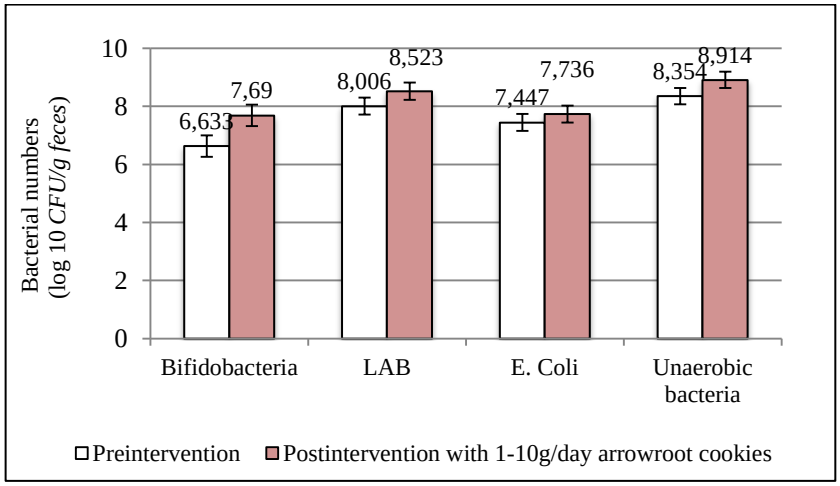

Figure 1. Fecal bacterial profile pre and post-intervention with 1-10g/day arrowroot cookies

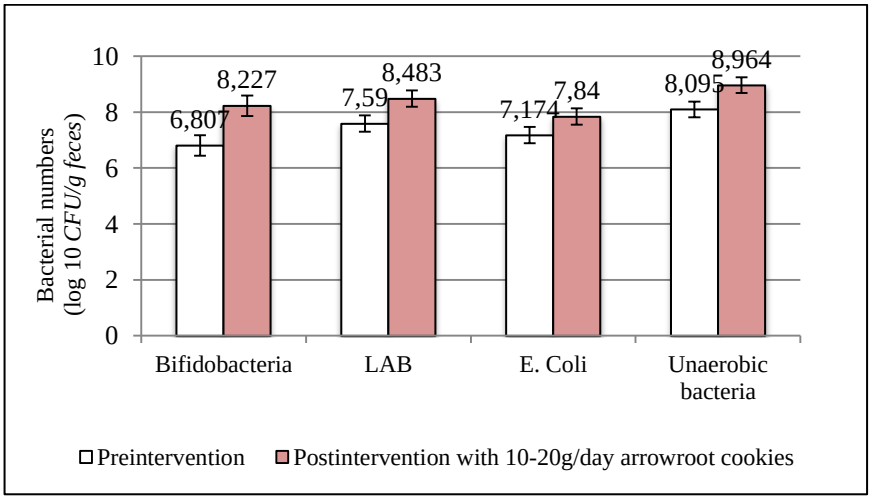

Figure 2. Profile of fecal bacteria in the pre and post-intervention with 10-20 g/day arrowroot cookies

In Figure 2, intervention with 10-20 g/day arrowroot cookies could significantly increase the number of Bifidobacteria, LAB, *E. coli*, and anaerobic bacteria, compared to the amount before the intervention. Increasing of Bifido bacteria and LAB was caused by its complex carbohydrate content, especially FOS and DF in arrowroot cookies. According to Roberfroid (1998), 82% of FOS intake in the human digestive tract could be fermented by Bifido bacteria. Increasing the number of Bifido bacteria and LAB also caused the total anaerobic bacteria population increase significantly, because of both are classified of anaerobic bacteria (Holt *et al.*, 1994). The number of *E.coli* population was also increased by intervension with 10-20g/day arrowroot cookies significantly, but less then Bifido

bacteria and LAB population. Increasing the number of *E.coli* may be correlated with increased nutrients in the feces, either the degradation of carbohydrates and protein products which could not been absorbed in the small intestine. According to Schneeman and Gallaher (2001), a source offiber in the diet will decrease the activity of digestive enzymes and intestinal absorption. Decrease indigestibility is caused by inhibition of enzyme and substrate interaction, because the substrate is trapped in the matrix of dietary fiber.

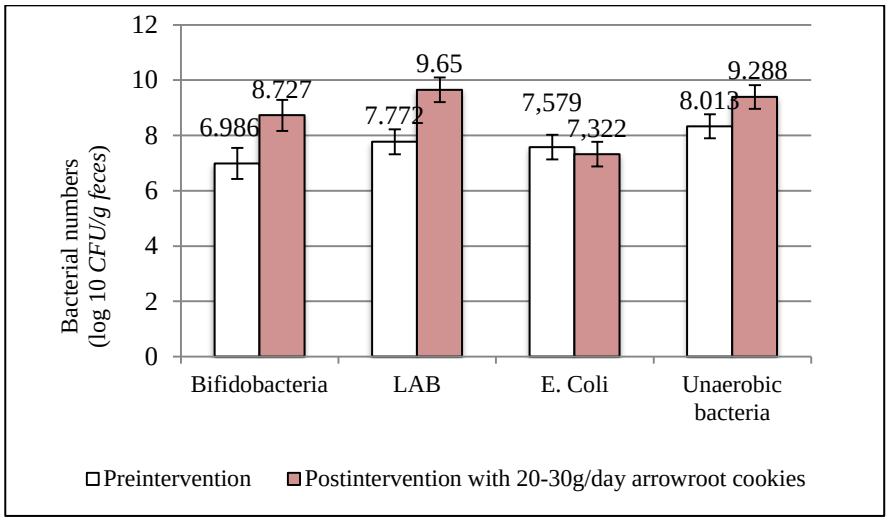

Figure 3. Fecal bacterial profile in the pre and post-intervention with 20-30 g / day arrowroot cookies

Figure 3. showed that the intervention with 20-30g/day arrowroot cookies could improve the number of Bifidobacteria and LAB populations significantly, but the number of *E. coli* showed no significant improvement. This fenomena may be caused by *E. coli* growth was inhibited, so its number was not different before and after arrowroot cookies intervention. According to Woods and Gorbach (2001), the growth of Bifido bacteria could inhibit the growth of pathogenic bacteria, such as *E. coli*.

CONCLUSION

The Prebiotic components of Arrowroot Cookies such as resistant starch, soluble and insoluble dietary fiber, as well as raffinose and FOS value were 36,67; 2.48; 10,83; 0.0059; and 0.49% (% wb), respectively. Dietary intervention with 10-20g/day arrowroot cookies could improve Bifido bacteria, LAB, *E.coli* and unaerobic bacteria population, but the growth of *E. coli* was inhibited in 20-30g/day arrowroot cookies consumption.

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