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Full Length Research Paper

Effect of arrowroot (*Maranta arundinacea* L.) diet on the selected bacterial population and chemical properties of caecal digesta of Sprague Dawley rats

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The objective of this research was to study the effect of arrowroot containing diet on the bacterial population and chemical properties of rat digesta. Eighteen male rats of the same age with a body weight of 200-250 g were used in the study. Rats were divided into three groups and fed with standard AIN93 diet (control) and arrowroot diets as source of fiber (ARF) 0.75 g/day and source of starch (ARC) 9.31 g/day for 14 days. Dietary fiber and oligosaccharide content of the arrowroot powder were analyzed. The result showed that arrowroot powder contained 14.86 % of dietary fiber, 396.9 ppm of raffinose, 270.8 ppm of lactulose and low amount of stachyose (<56 ppm) . *In vivo* study indicated that diet containing arrowroot powder increased population of lactobacilli significantly ($p<0.05$), while the viable counts of bifidobacteria, *Eschericia coli* and *Clostridium perfringens* were not significantly ($p>0.05$) different. The digesta of rats fed with arrowroot diet had a lower pH, higher water content, higher butyrate compared to those of control diet suggested that this diet had a better health effects. It can be concluded that supplementation of arrowroot powder in the diet improved bacterial and chemical properties of digesta.

Keywords: Arrowroot powder, colonic bacteria, oligosaccharide, dietary fiber and SCFA.

INTRODUCTION

Since the last decade the use of non-digestible carbohydrates in functional foods has increased significantly. Certain non-digestible carbohydrates are potential substrates for fermentation in colon and can be considered as prebiotics. Prebiotics have been defined as food substances not digested by gastrointestinal enzymes and beneficially affecting the host by a selective stimulation of growth and/or activity of a limited number of colonic bacteria (Roberfroid, 2001). Many efforts have been made to increase the number and/or the activity of the beneficial bacterial groups in the colon and to decrease those considered harmful for the host. In reference to the different intestinal bacterial groups, it is well known that bifidobacteria and lactobacilli can be considered as beneficial bacteria, while some others, like pathogenic *E. coli* and *C. perfringens*, are unfavorable for humans and animals (Tuohy et al., 2001; Fukuda et al., 2002). Most prebiotics, including fructans, lactulose, galacto-oligosaccharides, fructo-oligosaccharides (oligofructose and inulin), soybean oligosaccharides (raffinose, stachyose), have been shown to have bifidogenic properties (Cummings et al., 2001; Bouhnik et al., 2004).

In Indonesia tubers represent major source of carbohydrate but their consumption has decreased dramatically. In parallel, diabetes, obesity and cardiovascular diseases, have emerged as major health problems. It has been suggested that the decrease in fiber intake contributes to the development of these health disorders. Arrowroot (*Maranta arundinacea* L.) is a local tuber commonly used as ingredient for traditional foods. Arrowroot tuber contains plenty of starch and other compounds especially high amount of dietary fiber (Marsono et al., 2005). Arrowroot has a large number of culinary uses. It is used in cookies, cakes, pudding, porridge, fruit pie fillings and glazes as a thickening agent. The arrowroot starch has high digestibility (Kay,

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Table 1. Composition of standard AIN93 and arrowroots diets

Composition (g)	Standard diet (CON)*	Arrowroot fiber diet (ARF)	Arrowroot compound diet (ARC)
Arrowroot Powder	-	365.73	620.7
Corn starch	620.7	241.93	-
Casein	140	2.82	140
Corn oil	40	1.134	40
Sukrosa	100	10.935	100
Mineral mix	35	19.311	35
Cellulose (CMC)	50	-	50
L-cystein	1.8	1.8	1.8
Vitamin mix	10	10	10
Cholin bitartrat	2.5	2.5	2.5

*) Reeves et al. (1993)

1973). Arrowroot is bland, making it suitable for neutral diets, especially for people who are feeling nauseous. Some people believe that arrowroots help to soothe upset stomachs, which is the reason why many health food stores display arrowroot cookies.

Several types of dietary fiber especially non digestible oligosaccharides (fructooligosacharide, lactulose, rafinose, stachyose, inulin) capable of enhancing beneficial bacterial growth such as bifidobacteria and lactobacilli in the gastrointestinal tract (Hou et al., 2000; Bielecka et al., 2002; Wang et al., 2003; Mikkelsen and Jensen, 2004). Lactobacilli and bifidobacteria have different fermentation capability on prebiotic carbohydrate. Both of them need specific substrate in fermentation (Van den Broek and Voragen, 2008). Colonic bacteria composition dominated by beneficial bacteria population will reduce Gram negative pathogenic bacteria such as bacteriodes, coliforms and clostridia. Insoluble dietary fiber (IDF) are generally more resistant to colonic fermentation than soluble dietary fiber (SDF) and play a role in volume caecal increase (Wong et al., 2006). According to Grasten et al. (2002), dietary fibers are not digested in the small intestine and soluble dietary fiber fermented faster and produce higher amount of short chain fatty acid (SCFA) than that of IDF. Butyrate potentially reduces colorectal cancer risk by inhibiting histone deacetylase (HDAC) activity which affects the differentiation and apoptosis proliferation of precancer or tumor cell (Waldecker et al., 2008). Combination of butyrate with propionate and acetate more effectively prevent colorectal cancer compared to butyrate alone (Curi et al., 1997).

Information on the effect of oligosaccharide components of local tubers which have potential function as prebiotic is still limited. The objective of the study was to examine the non-digestible oligosaccharides content of arrowroot powder and the effect of diet supplemented with arrowroot powder on the population of bifidobacteria, lactobacilli, *E. coli,* and *C. perfringens* chemical characteristics and weight of digesta in rats.

MATERIALS AND METHODS

Materials

Arrowroot powder (water content 8.18%) was obtained from Sukoharjo, Central Java, Indonesia. Pancreatin and pepsin (Merck KGaA, Darmstadt, Germany), α amylase Termamyl, and all HPLC standard i.e. raffinose, stachyose and lactulose (Sigma, St. Louis, Missouri, USA).

Animals and diets

Eighteen male Sprague Dawley rats of the same age with a body weight of 200-250 g were supplied by the breeding center in Jakarta, Indonesia. All rats were fed with a standard maintenance. Animals were housed in individual metabolic cages in a room throughout the 14-day experimental period. Animals received water and diet *ad libitum*. The study was approved by Department of Food Science and Technology Gadjah Mada University. The protocol of the commission of ethics in animal experimentation of Gadjah Mada University was observed. Rats divided into three groups of 6 rats and were fed with standard diet (CON); arrowroot fiber diet (ARF) 0.75 g/day in which source of fiber was obtained from arrowroot powder; and arrowroot compound diet (ARC) 9.31 g/day in which corn starch was replaced with arrowroot powder (Table 1). The basal diet composition referred the AIN93 diet (Reeves et al., 1993). There were two arrowroot diets used in the study. The difference

among them was the addition of arrowroot as source of fiber or as source of starch.
Food intake, body weight and fecal weight were monitored every 3 days. After the experimental period, the animals were anaesthetized by ether and sacrificed. The abdominal cavity was open to expose the gastrointestinal tract. The digesta of caecum were removed immediately and were put into sterile container for further analysis. The caecal content (digesta) were taken and analyzed for weight, water content, pH, SCFA and bacterial number.

Bacterial enumeration

Caecal digesta samples from 18 rats were collected in sterile tubes. All bacterial enumerations were done immediately after sampling. Fresh caecal digesta were collected directly from each rat, weighed and immediately homogenized and serially diluted with 1% peptone water. From each of the dilutions, 1 ml was pour plated in triplicate onto selective media: TBX Agar for *E. coli*, OPSP Agar (Oxoid, Unipath Ltd, Basingstoke, UK) for *C. Perfringens*, rogosa agar, supplemented with 1.32 ml glacial acetic acid, for *Lactobacillus* spp.; Columbia agar containing 5 g glucose, 0.5 g cysteine HCl, bromo cresol purple and 0.5 ml propionic acid per litre, pH 5.0, for *Bifidobacterium* spp.; nutrient agar, for total aerobes. All agars were purchased from Oxoid and prepared according to the supplier's instructions. TBX agar and rogosa agar plates were incubated aerobically at 37 ^{0}C for 24 h. Colombia agar and OPSP agar media were incubated anaerobically at 37^{0}C for 48–72 h (Roy, 2001). Representative colonies of each selective medium were identified by color and colonies morphology. After identification, colonies were averaged from triplicate plates.

Evaluation of caecal digesta pH

Caecal digesta pH was determined directly in the digesta sample. Digesta were diluted 1:10 with distilled water and pH values of the samples were measured using pH meter.

SCFA measurement (Zoran et al., 1997)

Digesta was centrifuged 3500 rpm for 20 minutes and the supernatant was collected and analyzed by gas chromatography (Shimadzu GC-8A Kyoto, Japan) for SCFA content using a capillary column (180 cm x 4 mm). All analyses were carried out in triplicate.

Soluble and insoluble dietary fiber of arrowroot

Dietary fiber was analyzed according to the method of Asp et al., (1983). Starch was gelatinized and hydrolyzed using α amylase at 85 ^{0}C for 15 minutes. Protein was hydrolyzed with pepsin at pH 1.5 for 1 hour and incubated again with pancreatin at pH 7 for 1 hour at 40 ^{0}C. The hydrolyzed product was filtered. Non soluble dietary fiber was obtained from the residue of the filtrate collected in crucible glass containing 0.5 g cellite. Soluble dietary fiber was obtained from filtrate precipitated with ethanol.

Characterization of non digestible oligosaccharide of arrowroot flour

Non-digestible oligosaccharide (NDO) types and content of arrowroot powder were analyzed using method of Black and Bagley (1976). Arrowroot powder was extracted in ethanol-water solution (80: 20). Samples was analyzed using HPLC (KNAUER HPLC RID, Shimadzu, Kyoto, Japan) equipped with detector RI, dimensions (300 × 7.8mm), eluent H_2O, flow 0.7 ml/min and temperature 85^{o}C. Oligosaccharide standards used in this research were raffinose, stachyose and lactulose (Sigma, St. Louis, Missouri, USA).

Statistical analysis

All analyses were carried out in triplicate. Data concerning bacterial population, water content, weight, SCFA and pH of caecal digesta were subjected to analysis of variance using SPSS 6.0 program. Differences among treatments were further analyzed by a Duncan's multiple range tests. Differences were considered significant when P-values were less than 0.05.

RESULTS

Dietary fiber and oligosaccharides content of arrowroot

The result showed that arrowroot powder contained 14.86 % of total dietary fiber consisted of 2.37 % (db) soluble dietary fiber 12.49 % (db) insoluble dietary fiber. Chromatogram of oligosaccharide in arrowroot powder was shown at Figure 1. Arrowroot powder contained 396.9 ppm of raffinose, 270.8 ppm of lactulose and low amount of stachyose (<56 ppm). In Figure 1, several other peaks were detected but these compounds were

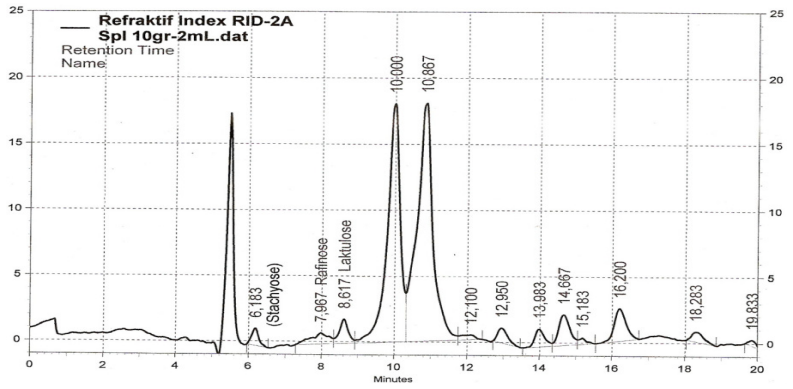

Figure 1. Chromatographic profiles of oligosaccharide in Arrowroot flour

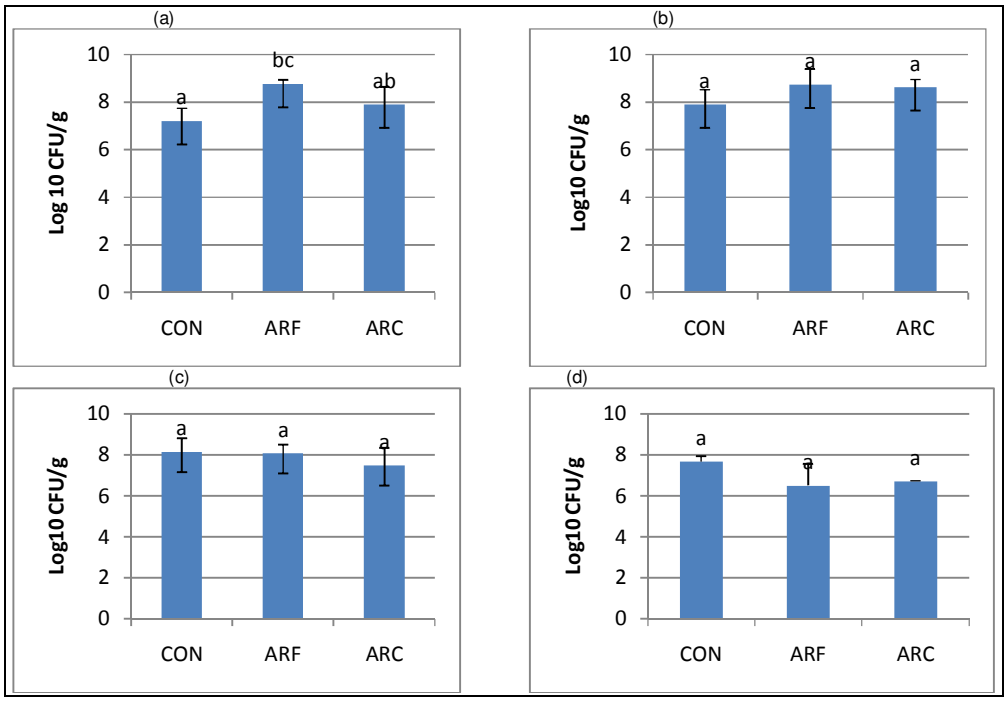

Figure 2. Total viable counts of Lactobacilli (a), Bifidobacteria (b), *E. coli* (c); and *C. perfringens* (d) in rat digesta.
Note: Bars represent mean ± SD. Superscript with different letter indicates significantly different ($p<0.05$

not identified yet.

Bacterial population of digesta.

The rat digesta consisted of a relatively higher viable counts of lactobacilli (8.8 $\log_{10}$ CFU/g) in Arrowroots (ARF and ARC) groups as compared to control group (CON) (Figure 2).

The viable counts of Lactobacilli in ARF (8.8 $\log_{10}$ CFU/g) was significantly ($p<0.05$) higher, compared to other groups. The viable counts of bifidobacteria in groups of arrowroot powder diets were not significantly ($p>0.05$) different compared to control. As shown in Figure 2(c), caecal concentrations of *E. coli*, was not significantly ($p>0.05$) different in all groups. The viable counts of *C. perfringens* in digesta in rats fed of arrowroot was not significantly ($p>0.05$) different.

Weight and water content of caecal digesta

The changes of physical and chemical properties of the caecal digesta as a result of feed supplementation were also observed. The weight of caecal digesta with arrowroot diet treatments were not significantly ($p>0.05$)

Table 2. Weight (g) and water content (%) of caecal digesta

Groups	Weight	water content
CON	1.48[a]	56.06[a]
ARF	1.83[a]	73.83[b]
ARC	1.90[a]	86.30[c]

Note: superscript with different letter in same column indicates significantly different ($p < 0.05$)

Table 3. Molar proportion (%) of SCFA in the caecal digesta of the rats

No	Groups	Acetate	Propionate	Butyrate
1	CON	66.33	25.51	8.16
2	ARF	55.36	28.57	16.07
3	ARC	59.59	23.97	16.44

Table 4. pH values of rat digesta with arrowroots diet (ARF and ARC) and standard diet (CON)

Groups	pH
CON	7.42[a]
ARF	6.63[b]
ARC	6.63[b]

Note: superscript with different letter in same column indicates significantly different ($p<0.05$)

different compared to the control (Table 2).

However, water content of caecal digesta with arrowroot diet treatments were significantly ($p<0.05$) higher (73.83% in ARF and 86.30 in ARC) compared to the control group (56.06%).

Molar proportion of SCFA

Molar proportion of SCFA in the caecal digesta of the rats was shown in Table 3. Caecum molar proportion of arrowroot diets groups tends to have high amount of butyrate.

pH value of the caecal digesta of the rats

The pH values of the caecal digesta of the rats were shown in Table 4. Rats treated with arrowroots diets had lower pH values (6.63) than that of the control (7.42). The results well correlated with high amount of SCFA in rats fed with arrowroots diets.

DISCUSSION

The results indicated that arrowroot powder contained dietary fiber, raffinose, lactulose and stachyose. According to Gibson (2004), those oligosaccharides have prebiotic effect. Our results indicated a significant ($p<0.05$) increase in lactobacilli during feeding with arrowroot powder. The study also demonstrated selective stimulation of lactobacilli compared to *C. perfringens* and *E. coli*. However the total viable counts of bifidobacteria was not significantly ($p>0.05$) different. This result is in agreement with other previous studies reported by Bielecka (2002) and Matteuzzi et al., (2004). Increased soluble dietary fiber content in arrowroot diet could enhance the viable counts of lactobacilli. The increase of lactobacilli population however did not followed by the decreased of *C. perfringens* and *E. coli*. Oligosaccharide compound in arrowroot powder were identified as raffinose, lactulose and stachyose, while others have not been identified. According to (Mateuzzi et al., 2005) raffinose can increase the population of lactobacilli and bifidobacteria and decreased both *C. perfringens* and *E.*

coli. Lactulose content in arrowroot powder might have role in decreasing Gram negative bacteria such as *C. perfringens* and *E. coli* (Mizota, 1996). However, the amount of nondigestible oligosaccharides was too low in arrowroot powder, so that it might not effective in decreasing the viable counts of *C. perfringens* and *E. coli*. High viable counts of lactobacilli may produce high amount of acid which can suppress sporulation of *C. perfringens* which may also reduce the toxins released by these bacteria (Wrigley, 2004). Growth of lactic acid bacteria such as bifidobacteria and lactobacilli can lower the pH (6 to 6.5) in the colonic ecosystem environment.

Raffinose and other indigestible polysaccharides are available for microbial fermentation and can modify the colonic microbiota by lowering some Gram negative bacteria such as coliform and increasing potentially health promoting bacteria such as bifidobacteria and lactobacilli. Furthermore its selective effect did not change the viable counts of total bacteria (Bielecka et al., 2002).

Microbiota composition inside the colon is relatively stable, so the increase or decrease in one type of microbial population does not provide a sharp change of total bacteria. Provision of arrowroot containing diets for 14 days had not been able to give a drastic effect because of the stable nature of the colonic ecosystem. However, the results of this study showed change in the composition microbiota towards a better composition.

Caecal microbiota that produced major SCFA such as acetate, propionate and butyrate has potential in reducing colonic pH. High value of SCFA content will lower the pH. In addition to the high SCFA, the low pH in the arrowroot diet treatments may be caused by lactic acid produced by lactobacilli. Molar proportion of butyric acid was higher in diets containing arrowroot powder than the standard diet. This is probably due to the lactic acid bacteria such as lactobacilli that can use the oligosaccharide components that are likely to exist in soluble fiber of arrowroot powder. These bacteria produce lactic acid which can be converted into acetyl CoA which has NADP regeneration into butyrate. Arrowroot starch containing resistant starch is predicted to influence the molar ratio of SCFA. According to Robertson et al., (2001) resistant starch can be modified to butyrate. Butyric acid has been shown to inhibit carcinogenesis at the promotion phase of abnormal cells (Cummings and Bingham, 1987; Schrenk et al., 2008).

Arrowroot powder consumption can improve the physical properties of digesta. Soluble fiber fermented by bacteria will increase the amount of mucus, the viscosity and the osmotic pressure of digesta. Therefore, the digesta became more bulky, soft and increased its water binding capacity. Soluble fiber easily hydrolyzed by bacteria so that the amount of soluble fiber degrading bacteria increased and increased biomass of rat's digesta, thereby increasing the volume and weight of rat digesta (Wong et al., 2006). The weight of caecal digesta in groups supplemented with arrowroot flour slightly enhanced. According to Bielecka (2002), weight of the caecal digesta significantly ($p<0.05$) higher in group fed with fructooligosaccharide and lactulose. In their study the laxative effect was correlated with weight of the caecal digesta and significantly ($p<0.05$) higher in groups fed FOS and lactulose. Digesta that have a lot of mucus is bulky. Therefore, increased stool transit time in colon and easy to remove (Wong et al., 2006). With the reduction colonic transit time, it provides health benefits such as reductions of toxin absorption that may exist in the digesta and the risk of colon cancer and tumors. In conclusion, the research showed that arrowroot powder potentially improved colonic microbiota SCFA and chemical and physical properties of digesta toward healthy composition.

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