

Potency of tubers and rhizomes of Java local foods as prebiotics and their effects toward bacterial diversity in mice cecum

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Abstract. Waskita Y, Pangastuti A, Listyawati S, Sari SLA. 2024. Potency of tubers and rhizomes of Java local foods as prebiotics and their effects toward bacterial diversity in mice cecum. *Biodiversitas* 25: 1544-1553. Tubers and rhizomes (TR) as Java local foods were less attention by people. Local foods contain IP (indigestible polysaccharides) content as prebiotic. The study aimed to analysis the prebiotic potency of TR extracts based on IP content and their effects toward increasing probiotic bacterial number, the TR extracts toward short-chain fatty acid (SCFA) profiles in probiotic media, and the TR extracts toward SCFA profiles and bacterial diversity in mice cecum. Tubers used were purple yam (*Dioscorea alata* var. *purpurea*), air potato (*D. bulbifera*), porang (*Amorphophallus muelleri*), Asiatic yam (*D. esculenta*), Asiatic bitter yam (*D. hispida*), white spot giant arum (*A. paeoniifolius*), yam bean (*Pachyrizus erosus*), and Hausa potato (*Coleus rotundifolius*). Rhizomes used were taro (*Colocasia esculenta*), arrowroot (*Maranta arundinacea*), and canna lilly (*Canna indica*). Work steps were extraction of TR, DNS assay, total sugar assay after acidic and enzymatic digestion, stimulation on increasing probiotic bacterial number (*Lactobacillus plantarum* and *Bifidobacterium bifidum*), SCFA production of probiotics, mice treatment, SCFA extraction in cecum, and determination of cecum bacterial communities. The highest IP contents were arrowroot, purple yam, air potato, porang, Asiatic yam, and taro extracts. These extracts except taro have higher IP content than inulin. The highest increasing probiotic bacterial number and SCFA production were shown by arrowroot extract rather than purple yam, air potato, and inulin. Bacteria communities had a high diversity value that showed micro-ecosystem balance in the cecum. Arrowroot was the same with inulin in increasing probiotics SCFA producers (*Lachnospiraceae* NK4A136, [*Ruminococcus*], *Lactobacillus*, *Bacteroides*, and *Bifidobacterium*) and decreasing pathogenic bacteria (*Lachnoclostridium*, *Helicobacter*, and *Lawsonia*) in mice cecum compared with control feed. *Mucispirillum* as a pathobiont-commensal had the same abundance in three feed treatments. Bacteria fermentation in the cecum produced the highest SCFA concentration in the 5% arrowroot feed treatment. Arrowroot had better prebiotic potency for the future.

Keywords: Arrowroot rhizome, cecum bacteria communities, increase of probiotic bacterial number, indigestible polysaccharide, short-chain fatty acid

Abbreviations: DNS: Dinitrosalicylic Acid, OD: Optical Density, SCFA: Short Chain Fatty Acid, TR: Tubers and Rhizomes, IP: Indigestible Polysaccharide, FID: Flame Ionization Detection

INTRODUCTION

Local foods were optional food sources to replace staple rice food in famine season (Purnomo et al. 2012) that were less consumed than rice and contained carbohydrates (Riptanti et al. 2015). The absence of seed conditions on a commercial scale, low productivity values, and a relatively long harvest period also reduce the cultivation of tubers and rhizomes (TR) as local foods (Diantina and Hutami 2014). These TRs were known as minor foods that were less considered and used by people (Hapsari 2014). Some examples of TR in Java were yam, white spot giant arum, Asiatic yam, canna lily, and arrowroot. They were cultivated on a small scale in Central Java (Rustini et al. 2015). Tubers are a form of plant structure modification containing food reserves like rhizomes. Tubers have a smooth texture and look slippery, whereas rhizomes grow horizontally, branched, and have segments completed by the remaining form of leaves (Tjitrosoepomo 2020). Food

ingredients in the form of TR contain oligosaccharides (Daud et al. 2009). Fructooligosaccharide content as prebiotic fiber was found in TR, previously used for a long time as a nutrition source; it was famous for its resistance to pests and was easy to plant in critical land. The most consumed tubers were cassava, potato, taro, and sweet potato. Some produced tubers in industry scale because of their high prebiotic demand were yacon (*Smallanthus sonchifolius*), burdock (*Arctium*), and Jerusalem artichoke (*Helianthus tuberosus*) (Nabeshima et al. 2020). Prebiotics are indigestible polysaccharides (IP) and oligosaccharides by human and stimulate the specific beneficial gut bacteria (Sebastián et al. 2019) because prebiotics were fermented anaerobically by gut bacteria to produce SCFA such as acetate, propionate, and butyrate (Scott et al. 2013; de Besten et al. 2013). The SCFA as probiotic metabolism products can maintain digestive acidity to benefit commensal microbes to live with optimal growth (Gao et

al. 2022) and prevent the appearance of pathogenic bacteria (Ranjbar et al. 2021).

Inulin is a fiber compound that has a prebiotic role (Tiefenbacher 2017). Most commercial inulin is obtained from *Cichorium intybus* (Asteraceae) (Mudgil and Barak 2019) and is native to Europe (Voss et al. 2021). Asiatic yam (*D. esculenta*), as java local food, was known to have inulin content and higher prebiotic activity score than inulin from *C. intybus* tuber (Winarti et al. 2013). Some different ingredients that had a prebiotic role and could positively influence positively the growth of *Lactobacillus* and *Bifidobacterium* as gut microbiota. Stojanov et al. (2021) reported that wood extract of silver fir (*Abies alba*) stimulate *Lactobacillus* growth and has a prebiotic role. Nicolucci et al. (2017) noted that consuming oligofructose-enriched inulin can increase *Bifidobacterium* spp abundance in children's feces. Vandeputte et al. (2017) reported that inulin-type fructans supplementation could increase *Anaerostipes* and *Bifidobacterium* and decrease *Bilophila* abundances in human feces. *Bifidobacterium* will degrade inulin to produce SCFA in the colon and benefit *Anaerostipes*. The SCFA production will reduce pH in the colon, so *Bilophila* cannot maintain its competitiveness.

Moreover, there has been no research discussing the effect of TR as java local foods and prebiotic sources toward in vitro SCFA profile fermented by *L. plantarum* FNCC 0020 (aerobically) and *B. bifidum* BRL 130 (anaerobically), in vitro and in vivo SCFA profiles, bacterial communities and gut health in vivo using a metagenomic approach. The study examined the prebiotics sources that have been used to determine the microbe composition structure of fructooligosaccharide, galactooligosaccharide, and fibersol-2 metagenomically for increasing biotransformation and bioavailability of ginsenosides (Zhang et al. 2021). Also, studying the purified fraction Fuzhuan brick tea for growing SCFA producer bacteria (Chen et al. 2022), brown alga *Himanthalia elongata* for obtaining prebiotic effect and SCFA analysis of in vitro human distal colon (Lopez-Santamarina et al. 2022), and mixture of xylooligosaccharide from birchwood xylan for producing xylooligosaccharides such as xylobiose, xylotriose and xyloetraose (Nieto-Domínguez et al. 2017). The study aimed to analysis the prebiotic potency of TR extracts based on IP content and their effects toward increasing of probiotic bacterial number, the TR extracts as carbon source of prebiotic candidate toward SCFA profiles in probiotic media, and the TR extracts as prebiotic feeds toward SCFA profiles and bacterial diversity in mice cecum. Eventually, it is hoped there will be new food product commodities with prebiotics as an added value from TR as beneficial for health especially in regulating probiotic bacteria communities in the gut.

MATERIALS AND METHODS

Materials

Tubers used were purple yam (*Dioscorea alata* var. *purpurea*), air potato (*D. bulbifera*), porang

(*Amorphophallus muelleri*), Asiatic yam (*D. esculenta*), taro (*Colocasia esculenta*), Asiatic bitter yam (*D. hispida*), white spot giant arum (*A. paeoniifolius*), yam bean (*Pachyrizus erosus*), and Hausa potato (*Coleus rotundifolius*). Rhizomes used were arrowroot (*Maranta arundinacea*) and canna lily (*Canna indica*). Both were obtained from traders located at Karanganyar, Sukoharjo, and Sragen Districts, Central Java. Other materials were 80% ethanol (Onemed, Indonesia), aqua distilled, HCl buffer pH 2 (Merck, Germany), sodium hydroxide (Emsure, Germany), phosphate buffer pH 7 (Merck, Germany), dinitrosalicylic acid (Sigma-Aldrich, USA), glucose (Sigma-Aldrich, USA), phenol (Smart Lab, Indonesia), sodium sulfite (Emsure, Germany), potassium tartrate tetrahydrate (Sigma-Aldrich, USA), sulfuric acid (Merck, Germany), inulin food grade from local supplier, MRS media (Himedia, India), bacteriological agar (Oxoid, UK), multifeed BR 1-7 RT for mice, drinking water for mice (Le Minerale, Indonesia), primer 341F and 806R for V3-V4 region *16S rRNA*, PCR mix KOD-Multi & Epi™ (Toyobo, Japan), 1% TBE (Tris Borat EDTA) agarose gel, and DNBSEQ-G400 (MGI Tech, China).

Test organism

Male mice (*Mus musculus*) BALB/c strain age 2-3 months, weight 20-25 g were obtained from Commanditaire Vennootschap (CV) Dunia Kaca, Indonesia. The use of mice as animal models was approved by the Animal Ethics Committee Sebelas Maret University, Indonesia (date: 25 May, 2023; number: 778/UN.27.2.0.1/PT.02/2023). *Lactobacillus plantarum* FNCC 0020 as an aerobic probiotic model and *Bifidobacterium bifidum* BRL 130 as an anaerobic probiotic model were obtained from Food and Nutrition Culture Collection, Gadjah Mada University, Indonesia.

Tubers and rhizomes extraction

All TR were extracted to get polysaccharide extracts by polar solvent 80% ethanol based on Wichienchot et al. (Wichienchot et al. 2011) with modification. First, TRs were chopped, dried, and ground in a blender. Extraction was conducted for 3 days and repeated twice. Filtrate was evaporated using a rotary evaporator (Bibby RE200, Spain) and vacufuge concentrator (Eppendorf, Germany), and the extraction yield was collected.

DNS assay

The standard glucose curve of the dinitrosalicylic acid (DNS) assay was measured to obtain its regression equation at 540 nm using a microplate reader (LabGeni, China). All extracts were measured for their reducing sugar content by DNS assay from their absorbance and standard glucose curve of DNS assay.

Resistance to acidic and enzymatic digestion

All extracts were tested for acidic and enzymatic digestion by HCl buffer pH 2 for 4 hours and amylase for 6 hours, respectively, in an incubator at 37°C (Thermo Scientific, USA) (Wichienchot et al. 2011; Korakli et al. 2002). Next, the standard glucose of the phenol-sulfuric

acid assay was measured using a microplate reader to obtain its regression equation at 490 nm. All extracts were measured in their carbohydrate total after digestion by phenol-sulfuric acid assay from their absorbance and standard glucose curve of phenol-sulfuric acid assay. All extracts were selected based on the top three highest IP content by reducing total carbohydrates with reducing sugar content.

Probiotic growth stimulation and SCFA production

Lactobacillus plantarum was cultured aerobically and *B. bifidum* was cultured anaerobically on MRS broth media to obtain sufficient cell density at their optimal temperature of 37°C (Matejčková et al. 2016; Shah 2011) for 24 hours. Anaerobic culture was obtained in an anaerobic jar (Merck, Germany). Incubation was obtained at an incubator at 37°C (Thermo Scientific, USA). Liquid cultures of both probiotics were made serial dilution and then measured their absorbance in Optical Density (OD) 600 using a microplate reader. Serial dilution of both probiotics was cultured in MRS agar media to count the number of probiotics on MRS agar media at 37°C for 24 hours. The number of probiotics and their absorbance were measured to obtain a regression equation of each bacteria cell number.

Lactobacillus plantarum and *B. bifidum* were cultured separately in MRS broth media with the modification that are top three highest IP extracts, i.e., arrowroot, purple yam, and air potato. Each in 2%, 2% inulin, and 2% glucose were cultured separately at 37°C for 24 hours. The bacterial cell numbers were counted on 0 and 24 hours of culturing using a regression equation and their absorbance in OD 600. The increase in bacteria number was counted based on Wang et al. (2015) and the highest of increased bacterial number was selected for in vivo treatment in mice. *Lactobacillus plantarum* and *B. bifidum* liquid cultures were centrifuged separately at 5,000 rpm 4°C for 3 minutes. Supernatants were purified and sterilized in a 0.22 µm nylon syringe filter (AllPure™, USA) to obtain the profile of SCFA contents using gas chromatography (Shimadzu GC-2010 Plus, Japan). The measured SCFA profiles were acetic, propionic, and butyric acid. Gas chromatography was equipped with flame ionization detection (FID) and BP21 capillary column (25.0 m × 0.53 mm × 0.50 µm). The injector and detector temperature were 240.0°C and 250.0°C respectively with helium as a gas carrier. The best extract was selected for the next assay based on probiotic growth stimulation and SCFA production.

Treatment in mice

Mice were divided into 3 feed treatments: 5% inulin, 5% arrowroot extracts, and control separately for 4 weeks. Control feed treatment does not contain prebiotics and every feed treatment requires 5 mice. Powder feed increased calorie and food intake (Yan et al. 2011). Drinking water was provided ad libitum. Mice were measured for their weight and naso-anal length to obtain the Lee index of obesity in mice (Hariri and Thibault 2010).

SCFA extraction from cecal

The cecum of mice was separated from the cecal and each mice cecal was extracted to obtain SCFA content based on Liu et al. (2021) and Kamil et al. (2021) with modification. Cecal in 0.150 g was extracted by sterile aqua distilled 1 mL. The extracts were centrifuged at 5,000 rpm 4°C for 8 minutes. Supernatants of extracts were purified and sterilized in a 0.22 µm nylon syringe filter to obtain the profile of SCFA contents using gas chromatography using gas chromatography. The measured SCFA profiles were acetic, propionic, and butyric acid. Gas chromatography was equipped with a FID detector and RT-CW20M F & F capillary column (30.0 m × 0.25 mm × 0.25 µm). The injector and detector temperature were 220.0°C with helium as a gas carrier.

Molecular work

Five mice cecum were pooled together based on each feed treatments separately. DNA extraction of mice cecum was conducted using ZymoBIOMICS™ DNA Miniprep Kit (Zymo Research, USA). Extracted DNA was checked for their concentration and purity using a biophotometer (Eppendorf, Germany), nanodrop (Thermo Fisher Scientific, USA), and qubit (Thermo Fisher Scientific, USA). The gDNA samples were amplified with V3-V4 16S rRNA-specific primers (341F & 806R). Library preparation was performed using the final amplicons. The final library was sequenced on DNBSEQ-G400 (MGI Tech, China) to generate paired-end raw reads.

Statistical analysis

The IP contents, increase of probiotic bacterial number, and SCFA contents were analyzed using SPSS software version 22 (USA) to obtain a descriptive homogeneity test and then continued to the Tukey post-hoc test.

Molecular data analysis

Adapter and PCR primer sequences from the paired-end-reads were removed using Cutadapt (Martin 2011). DADA2 was used to correct sequencing errors and remove low-quality sequences and chimera errors (Callahan et al. 2016). The resulting ASVs data was used for taxonomic classification against SILVA (silva_nr99_v138.1). Downstream analysis and visualizations were performed using packages in RStudio (R version 4.2.3).

RESULTS AND DISCUSSION

All yields of tuber and rhizome extracts were classified as poor yield because their percentages were >0.1% to <1% based on Furniss et al. (1989), which declared poor yield extracts in <40%. The poor yield extracts were caused by high moisture content in TR in the 60-90% (Chandrasekara et al. 2016). The IP extract of TR had variable concentration with significant differences from a to c (p <0.05) (Figure 1). The top three highest extracts were selected based on IP calculation, i.e., arrowroot, purple yam, and air potato. These three extracts had IP content higher than inulin significantly. Thus, the two extracts,

porang and Asiatic yam had IP contents higher than inulin although their three contents were classified similarly. The taro extract had IP content similar to inulin but in lower content. The tuber and rhizome extracts with negative contents did not have IP contents because their total sugar after acidic and enzymatic hydrolysis contents were lower than their reducing sugar contents.

The highest increase in cell number of *L. plantarum* were glucose, inulin, arrowroot, purple yam, and air potato extracts, respectively, and 2% of every carbon source was mixed in MRS broth media separately (Figure 2). The increase of cell number *L. plantarum* in glucose was highest because *L. plantarum* can digest it more easily than complex compounds like inulin and polysaccharide extracts from arrowroot, purple yam, and air potato. The increase of cell number *L. plantarum* in arrowroot extract had a higher increase than purple yam and air potato. The cell number increase in arrowroot extract was still lower than inulin and higher than purple yam. Therefore arrowroot extract was selected for in vivo mice treatment.

The increase in cell number of *B. bifidum* was counted as *L. plantarum* counting (Figure 2). The highest increase

in cell number were glucose, arrowroot, air potato, purple yam, and inulin, respectively, and every 2% carbon source was mixed in MRS broth media separately. Like glucose as a carbon source in *L. plantarum*, glucose also had the highest increase in cell number because *B. bifidum* can digest it more easily than complex compounds like inulin and polysaccharide extracts from arrowroot, purple yam, and air potato. These three extracts had an increase in cell number *B. bifidum* higher than inulin. Arrowroot extract had an increase in cell number *B. bifidum*, so higher than other extracts that it was selected for in vivo mice treatment.

The increase of cell number in arrowroot extract was highest among the other two extracts, both in *L. plantarum* and *B. bifidum*, because the IP content in arrowroot extract was higher than other tuber and rhizome extracts. The IP content of prebiotics gives an effect toward the increase of probiotic bacterial numbers or proliferative effects as was noted by Wang et al. (2015) as one of the probiotic proliferative mechanisms. The higher the IP contents in MRS broth media, the higher its probiotic proliferative effects.

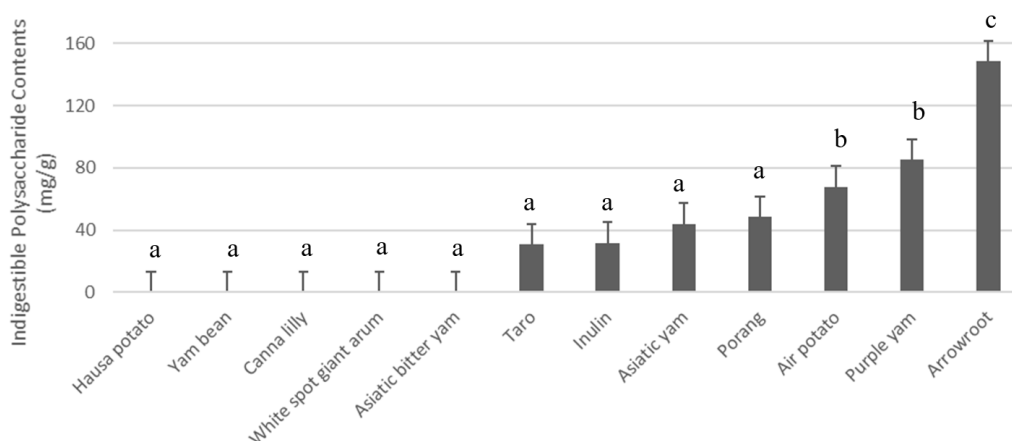


Figure 1. IP content in tuber and rhizome extracts from a to c, a significant difference at $p < 0.05$

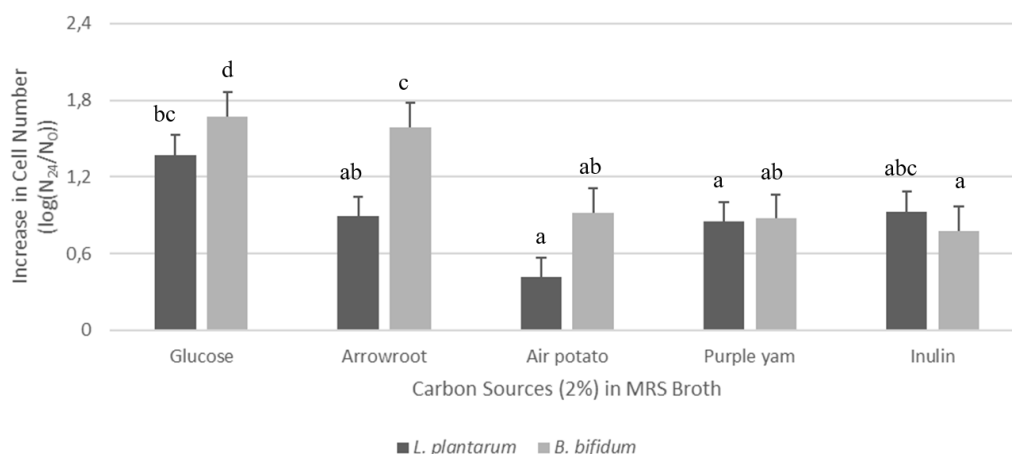


Figure 2. Increase of probiotics bacterial numbers in 37°C incubation at 24 hours from a to d, a significant difference at $p < 0.05$

The acetic, propionic, and butyric acid contents in aerobic fermentation *L. plantarum* FNCC 0020 that were given 2% carbon sources such as: glucose, inulin, purple yam, air potato, and arrowroot extracts separately on MRS broth media (Table 1). All butyric acid and propionic acid contents on MRS broth media had similar amounts although there were no detected amounts in purple yam extract and inulin. The acetic acid content in all carbon sources in MRS broth media had varied significantly; the highest amount of acetic acid in *L. plantarum* fermentation used arrowroot extract as a carbon source. Arrowroot extract was more excellent than air potato, inulin as a prebiotic model, purple yam extract, and glucose as a carbon source from monosaccharide in acetic acid, propionic acid, and butyric acid contents.

The acetic, propionic, and butyric acid contents on anaerobic fermentation *B. bifidum* BRL 130 were given 2% carbon sources such as: glucose, purple yam, inulin, air potato, and arrowroot extract separately on MRS broth media (Table 1). All acetic acid contents on MRS broth media had variable and significant amounts, except in purple yam extract and glucose with similar contents. Acetic and propionic acid contents in arrowroot extract had higher amounts than other carbon sources, although its butyric acid content was lower than the glucose carbon source.

Arrowroot extract as a carbon source on MRS broth media for aerobic and anaerobic probiotic fermentation obtained higher acetic and propionic acid than other extracts even inulin and glucose. However, the butyric acid content in arrowroot extract was still lower than glucose in anaerobic fermentation. Therefore the arrowroot rhizome was selected for in vivo mice treatment.

The substance amount comparison of acetic, propionic, and butyric acid in all carbon sources MRS broth media both in aerobic fermentation by *L. plantarum* and anaerobic fermentation by *B. bifidum* were higher than molar ratio 3:1:1 respectively as was noted by Cuervo-Zanatta et al. (2019) and Cummings et al. (1987). Especially in the content of acetic acid production was higher significantly compared to other SFCA contents exhibited there was a potential effect in physiology that was a mucous layer

thickness of digestive tracts. Hademann et al. (2009) reported that a high acetic acid content will increase the mucous layer thickness of digestive tracts in rats, while a high butyric acid content will decrease it. This regulation of both SCFAs can modulate mucous layer thickness in digestive tracts. Based on Hansson (2012), the function of the mucous layer in digestive tracts was cleaner and separator in digestive epithelial cells from pathogenic bacteria that caused inflammation and infection. Therefore, all prebiotic sources can increase the mucous layer thickness in digestive tracts for health maintenance with a more optimal prebiotic source by arrowroot. Propionic acid was detected in arrowroot, air potato extracts, and glucose, except in purple yam extract and inulin. According to Xia et al. (2017), propionic acid plays a major role in maintaining digestive health and immune homeostasis. Besides that, propionic acid concentration can increase barrier function in colonic epithelial cells in vitro so it has potential in disease therapy for necrotizing enterocolitis in premature newborns by nutritional regulation.

All mice were categorized into no obesity (Table 3) because their values were less than 310 and all Lee index values had no significant differences ($p > 0.05$). An ideal body weight condition is required to maintain normal mice respond consistency toward cecum bacterial diversity until observed bacteria are an expression of normal-weight mice.

The community of cecum bacteria in control, 5% inulin, and 5% arrowroot feeds were detected successfully based on the rarefaction curve (Figure 3) because their curve shapes were flat. The frequently and rarely found cecum bacteria were collected. Thus, the collected species numbers of bacteria were the same as those in mice cecum.

The observed cecum bacteria in three feed treatments varied around 300 species. The cecum bacteria diversities were classified into high categories because the Shannon-Wiener index values were more than three, and the Simpson indices value were close to one (Table 4). The micro-ecosystem condition in mice cecum could be considered balanced and very stable because of high bacterial diversity. These show that cecum in three feed treatments had good digestive health (Yin et al. 2019; Li et al. 2022).

Table 1. SCFA profiles of probiotic fermentation in 37°C incubation at 24 hours from a to j, a significant difference at $p < 0.05$

Probiotics	<i>L. plantarum</i>	<i>B. bifidum</i>	<i>L. plantarum</i>	<i>B. bifidum</i>	<i>L. plantarum</i>	<i>B. bifidum</i>
SCFA	Acetic acid (mmol)		Propionic acid (mmol)		Butyric acid (mmol)	
Arrowroot	61.14 ± 0.87j	57.99 ± 0.30i	0.94 ± 0.17ab	1.25 ± 0.02b	0.08 ± 0.06a	0.14 ± 0.02ab
Inulin	40.59 ± 0.35e	50.45 ± 0.08h	0a	0a	0a	0a
Air potato	41.82 ± 0.16f	46.63 ± 0.09g	0.25 ± 0.01ab	0.30 ± 0.04ab	0.07 ± 0.04a	0.03 ± 0.01a
Glucose	31.10 ± 0.30c	38.51 ± 0.66d	0.22 ± 0.04ab	0.20 ± 0.05ab	0.12 ± 0.01a	0.36 ± 0.35ab
Purple yam	37.48 ± 0.57d	38.32 ± 0.44d	0a	0a	0a	0a

Note: The mice feed consumption in all treatments had similar weights (Table 2). No significant differences ($p > 0.05$) existed in all feed consumption weights. Mice were kept for 4 weeks

Table 2. Mice feed consumption ($p > 0.05$)

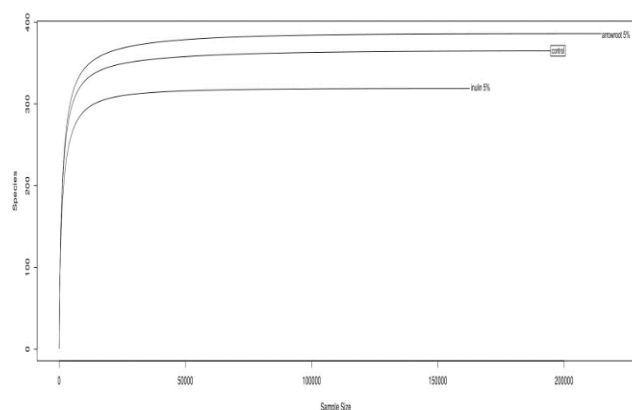
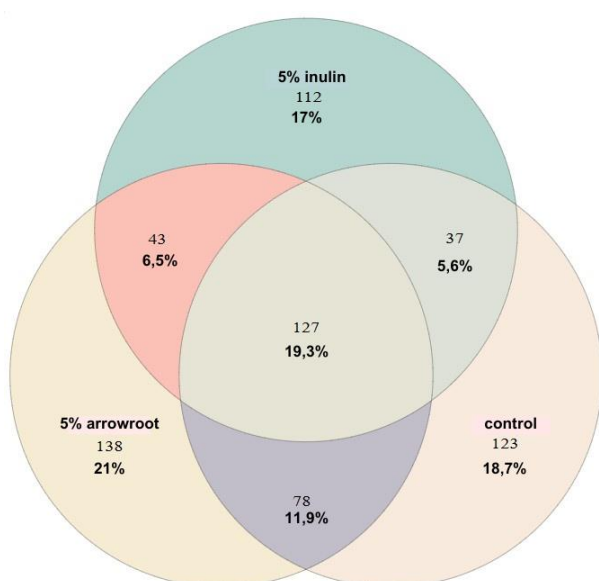
Feed treatments	Feed consumption weight
Control	2.91 ± 0.10a
5% Inulin	2.93 ± 0.09a
5% Arrowroot	2.96 ± 0.12a

Table 3. Lee index in mice ($p > 0.05$)

Feed treatments	Lee index	Annotation
Control	280.02 ± 11.32a	No obesity
5% Inulin	283.86 ± 5.73a	No obesity
5% Arrowroot	283.33 ± 8.58a	No obesity

Table 4. Cecum bacteria numbers and their diversity indices

Feed treatments	Observed species	Shannon-Wiener Index	Simpson index
Arrowroot 5%	386	4.20	0.96
Control	365	4.15	0.95
5% Inulin	319	4.28	0.97

**Figure 3.** Rarefaction curve of cecum bacteria**Figure 4.** Venn diagram of cecum bacteria in feed treatments

Mice with 5% arrowroot feed treatment had higher bacterial numbers than other treatments (Figure 4). The second highest cecum bacterial number was the intersection between 5% inulin-5% arrowroot-control feed treatments. The third highest cecum bacterial number was the control feed treatment, while 5% inulin feed treatment was in the fourth position.

The three feed treatments' most abundant bacterial phyla were Firmicutes, Deferribacterota, Campylobacterota, Bacteroidota, and Desulfobacterota. In comparison, bacterial phyla with lower abundance were Actinobacterota, Proteobacterota, Spirochaetota,

Verrucomicrobiota, and Patescibacteria, respectively (Figure 5). Firmicutes were abundant in three feed treatments, especially in 5% inulin and 5% arrowroot, because of polysaccharide hydrolysis, as Firmicutes' role produces butyric and other SCFA. Firmicutes and Bacteroidota were more abundant than Proteobacteria, Verrucomicrobiota, and Actinobacteria, which show a healthy mice digestive. Actinobacteria and Proteobacteria were observed in low abundance but could produce butyric acid (Fusco et al. 2023). Bacteroidota could transform toxic compounds and produce butyric acid with anticancer activity to maintain a healthy digestive (Gryaznova et al. 2022). Proteobacteria were the first colonizing bacteria and role in the next preparation colonization by other bacteria for healthy digestive so Proteobacteria's abundance will be low in healthy hosts (Shin et al. 2015). Verrucomicrobiota also increased healthy digestive and glucose homeostasis and induced immunity regulation of the digestive microbiome. Actinobacteria also increase digestive health (Gryaznova et al. 2022).

Some studies showed that obese individuals had a higher Firmicutes/Bacteroidota ratio than normal individuals (Magne et al. 2020; Stojanov et al. 2020), while inflamed individuals had a lower Firmicutes/Bacteroidota ratio. Firmicutes had nutrition and metabolism roles through SCFA synthesis and regulated hunger and fullness. Bacteroidota had an immunomodulation role and increased immune reaction through cytokine synthesis. A balanced ratio between both phyla will maintain digestive health and prevent dysbiosis in the form of obesity and digestive inflammation. Administration of *Lactobacillus* and *Bifidobacterium* as probiotics, and even *Saccharomyces boulardii* as yeast, could also prevent dysbiosis by reducing weight and suppressing the immune system (Stojanov et al. 2020). However, the Firmicutes/Bacteroidota ratio cannot be used as a definitive mark for obesity because all mice are normal based on Lee index (Table 3), and there was no comparison between cecum bacteria communities in normal and obese mice in this study. Magne et al. (2020) noted that it was difficult to associate between Firmicutes/Bacteroidota ratio and individual health status, especially in obesity biomarkers, because of the complexity affecting digestive microbiota composition and resulting interpretation. The microbiome studies should focus on identifying taxonomic markers to classify individuals appropriate to microbiome rather than investigating taxonomic markers of obesity in the form of Firmicutes/Bacteroidota ratio (Magne et al. 2020). Besides that, in the cecum bacteria communities in three feed treatments, there were still *Lactobacillus* and *Bifidobacterium* (Figure 6) that both prevented dysbiosis, especially in obesity (Stojanov et al. 2020).

Deferribacterota, Campylobacterota, and Desulfobacterota were pathogenic bacteria phyla contributing in host systemic inflammation activation (Shi et al. 2021). Campylobacterota were associated with ulcerative colitis development. Desulfobacterota had a butyric degradation role through beta-oxidation in the catabolic metabolism pathway, caused inflammatory damage, and exacerbated energy metabolism disorder.

Some Spirochaeta was pathogenic, but its abundance decreased when digestive disease occurred (Gryaznova et al. 2022). These pathogenic phyla had lower abundance, so their role was defeated by Firmicutes and Bacteroidota communities. Prebiotic feed treatment in 5% inulin and 5% arrowroot could decrease Campylobacteria abundance, although Deferribacterota was similar in three feed treatments. Besides that, SCFA concentration in cecum (Figure 7) on 5% arrowroot feed treatment was higher than 5% inulin. This did not correlate with Firmicutes abundance that was similar in both treatments; even Bacteroidota, Actinobacteria, and Proteobacteria abundances in 5% inulin were higher than 5% arrowroot.

The most abundant genera in three feed treatments were *Lachnospiraceae* NK4A136, *Mucispirillum*, *Helicobacter*, [*Ruminococcus*] *torques*, and *Bacteroides*, respectively (Figure 6). The lower abundance genera were *Lachnoclostridium*, *Lactobacillus*, *Lawsonia*, and *Bifidobacterium*. *Lachnospiraceae* NK4A136 has not been further classified based on the NCBI database. Its temporary scientific name is *Lachnospiraceae bacterium* NK4A136. Thus, for [*Ruminococcus*] *torques*, it was temporary scientific name that would be replaced with *Mediterraneibacter torques* (Schoch et al. 2020). Both bacteria, *Lactobacillus*, *Bacteroides*, and *Bifidobacterium*, were SCFA producers (Fusco et al. 2023; Akhtar et al. 2022; LeBlanc et al. 2017).

Lachnoclostridium was a member of the Lachnospiraceae Family; some members were SCFA producers, but others were diarrhea-causing bacteria (Vacca et al. 2020; Du et al. 2023). *Mucispirillum* and *Helicobacter* were involved in gut inflammation (Gryaznova et al. 2022); even *Helicobacter* could exacerbate gut diseases until colitis and colonic carcinoma appeared (Erdman et al. 2003). *Mucispirillum* was considered a pathobiont that had a role as commensal

bacteria or could cause disease in the Rodentia gut (Loy et al. 2017). Pathobionts were opportunistic microbes that appeared because of microbiome imbalance in the healthy gut and complex interaction between genetics, environment, microbe, and host factors (Chandra et al. 2021). *Lawsonia* could infect wild mice and rats onto appear gut diseases like: ileitis, typhus, colitis, or intestinal hemorrhage (Whary et al. 2015). Wild mice and rats were susceptible infected because they were *Lawsonia* reservoirs for infecting other animals (Collins et al. 2011).

The 5% inulin and 5% arrowroot feed treatments in mice could decrease *Helicobacter* compared to the control feed treatment; 5% inulin was more excellent than 5% arrowroot. *Mucispirillum* abundance was similar in three feed treatments involving both genera in the gut inflammation process (Gryaznova et al. 2022); it also had a commensal role even though it was useful for gut health. Herp et al. (2019) reported that *Mucispirillum schaedleri* antagonized toward *Salmonella enterica* serotype Typhimurium that caused colitis so its infection and virulence factor can be prevented. Competition between *M. schaedleri* and *S. enterica* in anaerobic respiration substrate protects mice successfully from colitis threatening. The 5% arrowroot and 5% inulin also could decrease *Lachnoclostridium* that caused diarrhea (Vacca et al. 2020; Du et al. 2023) rather than control feed that 5% arrowroot was better than 5% inulin feed. *Lawsonia* abundance in 5% arrowroot feed treatment was a special note because it was higher and not detected in 5% inulin and control feed treatments. Collins et al. (2011) noted that *Lawsonia* did not cause dangerous effects for laboratory mice and rats because both Rodents were little affected by colonic lesions from *Lawsonia*. Therefore negative effects of *Lawsonia* abundance in 5% arrowroot feed treatment for mice gut can be ignored.

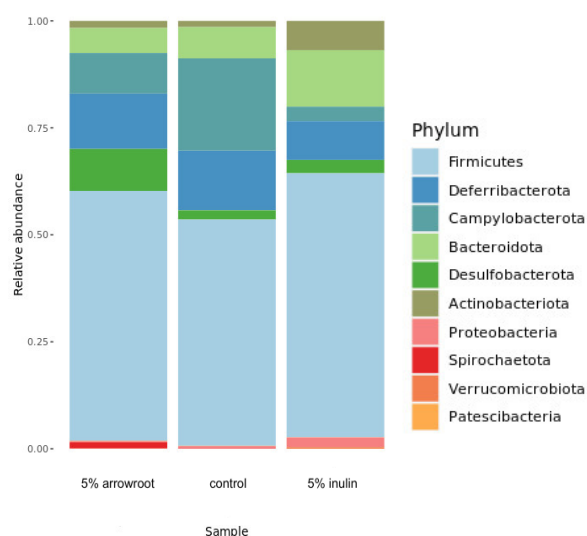


Figure 5. Relative abundance of cecum bacteria community in phylum taxa

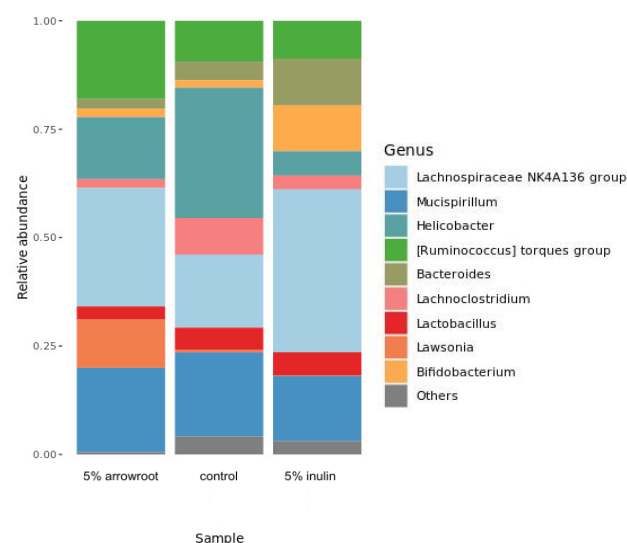


Figure 6. Relative abundance of cecum bacteria community in genera taxa

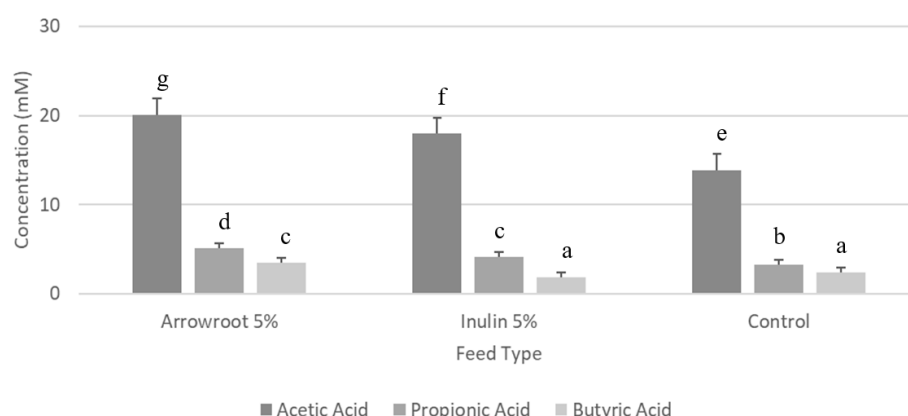


Figure 7. SCFA profiles in mice cecum from a to g, a significant difference at $p < 0.05$

The 5% inulin feed treatment was more excellent than the 5% arrowroot feed treatment in increasing *Lachnospiraceae* NK4A136, *Bacteroides*, *Lactobacillus*, and *Bifidobacterium* abundances, but the 5% arrowroot feed treatment was better than the 5% inulin feed in increasing [*Ruminococcus*] abundance. *Lachnospiraceae* NK4A136 abundance was lower in the control feed treatment than in both treatments because it doesn't contain prebiotics. However, control feed treatment could increase *Bacteroides* abundance rather than the 5% arrowroot feed treatment. Besides that, [*Ruminococcus*] and *Lactobacillus* were similar in control and 5% inulin feed treatments. These two things were an anomaly because control feed treatment seems more excellent than 5% inulin feed treatment if not compared with bacterial abundances and SCFA profiles in cecum.

Short-chain fatty acid profile in mice cecum showed variable concentrations between acetic, propionic, and butyric acid with significant differences from a to g (Figure 7). Concentration comparison of acetic, propionic, and butyric acid in all feed treatments were higher than the molar with a ratio of 3:1:1 (Cuervo-Zanatta et al. 2019; Cummings et al. 1987) as SCFA profiles of *L. plantarum* and *B. bifidum* fermentations. Generally, acetic acid is higher than propionic and butyric acid in all feed treatments. It was suitable with Hademann et al. (2009), who reported that higher acetic acid concentration will increase mucous layer thickness in digestive tracts so that it can clean and separate digestive epithelial cells from inflammation and infection from pathogenic bacteria (Hansson 2012). Acetic acid concentration was highest in mice fed with 5% arrowroot, 5% inulin, and control, significantly. Thus, arrowroot is more excellent than inulin and the control for propionic and butyric acid concentration. This was suitable with the previous assay that by in vitro increase in probiotics bacterial numbers and SCFA productions by probiotics that arrowroot more excellent than inulin as a prebiotic model. This emphasizes that arrowroot rhizome has a strong potential as a good prebiotic source.

In three feed treatments, short-chain fatty acid profiles in mice cecum were fermentation products of gut

microbiota in mice cecum. Acetic acid concentration was generally higher than propionic and butyric acid. Bacteroidota produced acetic and propionic acid, whereas Firmicutes produced butyric acid. Acetic acid was absorbed and spread in adipose tissues, muscles, liver, and brain. Acetic acid stimulates lipid synthesis in the liver related to dyslipidemia; in the brain, acetic acid stimulates insulin secretion by the pancreas and ghrelin by gastric mucosa. Propionic acid stimulates glucagon-like-peptide-1 (GLP-1) and peptide YY (PYY) by L-enteroendocrine cells for inhibiting appetite. Acetic acid has a gluconeogenesis role and decreases fatty acid enzyme and cholesterol expression in the liver. Butyric acid increases insulin-sensitivity and anti-inflammation activity and regulates energy metabolisms, and leptin gene expression (Magne et al. 2020).

This study noted that Firmicutes/Bacteroidota ratio was higher (Figure 5); besides that, Firmicutes had a butyric acid producer role, whereas Bacteroidota, as acetic and propionic acid producers, should have butyric acid concentration higher than acetic acid and propionic acid. Otherwise, SCFA profiles in the cecum noted that the concentration of acetic acid was higher than propionic and butyric acid (Figure 7). According to Magne et al. (2020), acetic acid was strongly suspected in obesity risk contributing. Acetic acid was the intermediate product after IP degradation by *Bifidobacterium* (Actinobacteria), *Lachnospiraceae* (Firmicutes), and [*Ruminococcus*] (Firmicutes) that will be processed into butyric acid by Firmicutes (Baxter et al. 2019). There is no specific limit on when all acetic acid will be processed into butyric acid or vice versa. Propionic acid production was through a different pathway from acetic and butyric acid, i.e., succinate pathway by Bacteroidota, acrylate pathway by Firmicutes, and propanediol by Enterobacteria and Firmicutes (Nogal et al. 2021). Enterobacteria was not observed in three feed treatments, so Firmicutes only carried out the propanediol pathway. The main role of Firmicutes in SCFA-producing made the direction of the metabolism pathway interesting because this phylum had a SCFA-producing role, especially in high acetic acid concentration rather than propionic and butyric acid.

Another assumption is colonocytes used most butyric acid because 70% of their main energy was supplied by butyric acid. In comparison, propionic and acetic acid were energy sources for peripheral organs (Cuervo-Zanatta et al. 2021). Hence, their concentration was higher than butyric acid along with distance and target organs.

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