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Effect of Fiber Drink Consumption Containing Glucomannan and Isomaltooligosaccharide on Digesta Profile and Gut Microbiota

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Abstract

Dietary fibers have long been recognized for their numerous health benefits, including supporting gut health, aiding in weight management, and reducing the risk of cardiovascular disease. Glucomannan, derived from konjac and porang tubers, has gained attention for its diverse health-promoting properties. However, its application in beverages is limited due to its high viscosity. Combining glucomannan with isomaltooligosaccharide (IMO) is suggested to synergistically enhance functionality, particularly improving gastrointestinal health. This study investigates the functional effects of porang glucomannan and isomaltooligosaccharide-containing fiber drinks (GIFD) on body weight, feed intake, digesta profiles (digesta weight, moisture content, pH) and gut microbiota using *in vivo* method. Twenty-four male Sprague Dawley rats were divided into 4 groups, including control, inulin, and 2 doses of glucomannan and isomaltooligosaccharide fiber drink (GIFD). Rats were fed with AIN-93M. Adaptation was carried out for 5 days followed by 28 days of intervention. Results indicate that GIFD consumption led to reduced feed intake, likely due to increased satiety effects and glucomannan's gel-forming ability, resulting in lower body weight gain compared to other groups. While GIFD did not significantly alter digesta weight and moisture contents, it significantly decreased pH values. Analysis of short-chain fatty acids (SCFAs) showed a shift in propionic acid levels, suggesting a beneficial effect on weight control. Moreover, GIFD treatment reduced abundance percentages of enteric bacteria like *E. coli* and *Clostridium*, possibly due to increased SCFA production. However, GIFD consumption did not significantly alter sIgA concentration, although there was a trend towards increased levels in GIFD-2, suggesting potential immunomodulatory effects. Overall, it was indicated that porang glucomannan and IMO-containing fiber drinks has the potential to be used as beverages to support body weight regulation and to help maintaining digestive tract health.

Keywords: Digesta profile, GIFD, Gut microbiota, Porang Glucomannan, sIgA

Introduction

Over decades, the consumption of dietary fibers has long been associated with various health benefits, such as supporting a healthy gut microbiome, enhancing metabolic health, body weight management, and even associated with a reduced risk for the development of cardiovascular disease and mortality [1]. Glucomannan, a type of fiber derived from the roots of the konjac plant, has gained attention for its remarkable health-promoting properties [2]. Porang tubers, similar to the konjac tuber, also contains glucomannan as its high water-soluble polysaccharides. Glucomannan health benefit ranging from increasing satiation and satiety to immune enhancement and improving gastrointestinal well-being [3,4]. Because of glucomannan's remarkable qualities and functional effects, glucomannan are further developed as a raw material for functional food products [5,6]. It has been added to a number of processed food items, including glucomannan konjac flour, pancake mixes, and pudding mixes aimed at health-conscious customers. However, the creation of these items frequently calls for additional processing or isn't convenient enough, which emphasizes the need for alternative options for convenient consumption. To meet this need, instant high-fiber drinks is potential due to its convenience to delivers the health benefits of glucomannan. In addition, the global market of dietary fiber reached US$ 9.1 Billion in 2023 and expected to grow by 6.5 % during 2024 - 2032 [7].

There are several factors affecting the quality of fiber drink including fermentability, bulking ability, binding ability, viscosity and gel formation, water-holding capacity and solubility [8,9]. Glucomannan exhibits high viscosity due to its exceptional water-binding capacity, making its application as beverages primarily limited [10]. While enzymatic methods are capable of reducing its viscosity, they are sometimes quite expensive and may alter functional attributes of glucomannan. Hence, adding alternative fiber sources becomes essential to maintain its functionality. IMO is another water-soluble dietary fiber renowned for its health benefits, which is known to lower blood cholesterol levels and reduce constipation [11]. IMO can also increase the weight and water content of the digesta and lower the pH of rat digesta [12]. In addition, as a form of dietary fiber, IMO could induce the afferent signal that cause appetite suppression [13].

Combining IMO with glucomannan is anticipated to synergistically enhance the functionality of these beverages, particularly in promoting gastrointestinal health. However, products incorporating both types of prebiotics remain relatively scarce, and their effects on gastrointestinal health, including digesta profiles, microbiota diversity, and immune response, remain largely unexplored. It is expected that IMO and glucomannan together will improve these drinks functionality in a synergistic way, particularly when it comes to supporting gastrointestinal health. Products containing both kinds of prebiotics are still somewhat uncommon, and little is known about how they affect immune response, digesta profiles, and microbial diversity in the gastrointestinal tract. In addition, most *in vivo* studies have focused on evaluating the pure forms of these prebiotics, however there is a scarce information on *in vivo* effects of these prebiotics in product form. Therefore, the current study was aimed to investigate the impact of fiber drink consumption containing glucomannan and IMO on digesta profiles and gut microbiota. The current study paves the way for future developments in the field of prebiotic research.

Materials and methods

Materials

Chocolate flavored fiber drink containing glucomannan and IMO (GIFD) is produced at PT. Lautan Natural Krimerindo. Chocolate flavored GIFD is made with FiberCremeTM, sugar, milk powder, glucomannan, salt, cocoa powder, and chocolate flavoring. FiberCremeTM contains isomalto-oligosaccharide, coconut oil, sodium caseinate,

phosphate stabilizer, vegetable emulsifier and anti-caking agent. Chemicals were provided by Sigma Aldrich (Burlington, Massachusetts, United States) and Merck (Darmstadt, Germany).

Experimental animals

This research was approved by the Medical & Health Research Ethics Committee of Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada. The ethical clearance document has been issued under the number KE/FK/1307/EC/2022. In this study, 24 male Sprague Dawley rats aged 8 weeks weighing 150 - 200 g were obtained from Center for Food and Nutrition Studies (CFNS) UGM (Yogyakarta, ID). The flow chart regarding experimental animals in this study is shown in **Figure 1**. Rats were divided into 4 treatment groups, namely control (KON) was given 3.6 mL of aquadest; the Inulin (INL) group was given 0.072 g inulin/3.6 mL; the glucomannan and IMO fiber drink (GIFD) group dose 1 was given 0.54 g/3.6 mL; the glucomannan and IMO fiber drink (GIFD) group dose 2 was given 1.08 g/3.6 mL. Rats were housed individually in cages under controlled conditions (temperature: 23 ± 3 °C, humidity: 50 ± 10 %, and a 12/12-h light-dark cycle. The acclimatization was done for 5 days followed by intervention period for 28 days. Treatment samples were given by oral gavage. All animals were allowed free access to water and standard AIN 93M diet during the study [14]. The standard AIN-93 M contained 620.7 g/kg diet of corn starch; 140 g/kg diet of casein 85 % protein, 100 g/kg diet of sucrose, 40 g/kg diet of soybean oil, 50 g/kg diet of fiber from agar, 35 g/kg diet of mineral mix, 10 g/kg diet of vitamin mix, 1.8 g/kg per diet of L-cysteine, and 2.5 g/kg diet of choline bitartrate with 3.326 kcal energy total.

During the intervention period, feed intake was calculated every day and its body weight was weighed every 7 days. Rats were sacrificed followed by the collection of cecal digesta and duodenal intestinal fluid. The cecal digesta was analyzed for weight, water content, pH, microbiota abundance, and SCFA concentration. Duodenal intestinal fluid was analyzed for sIgA concentration.

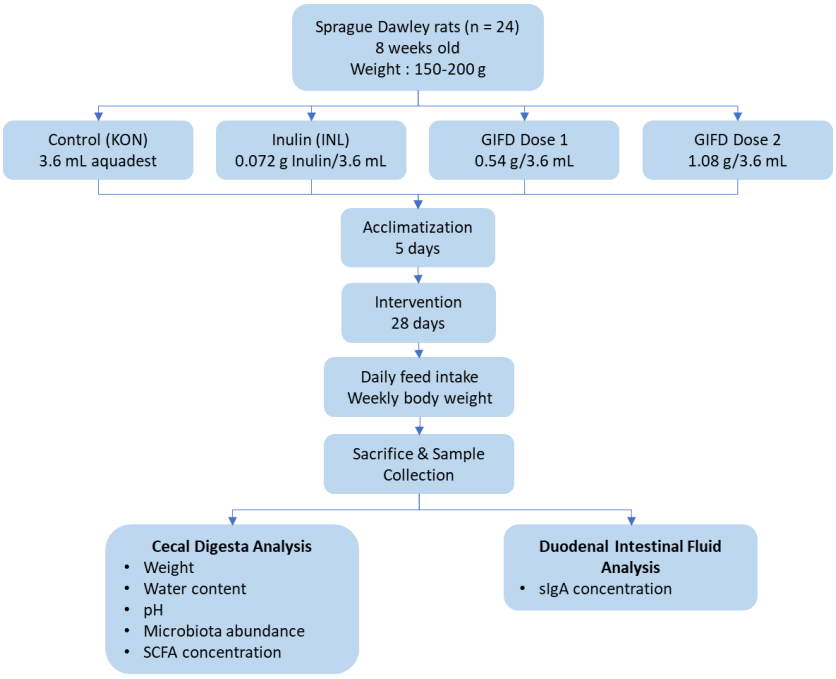

Figure 1 Flow chart experimental animals.

Method of analysis

Analysis weight and moisture content of the digesta

The rat digesta that had been taken was then collected and weighed using an analytical balance (Mettler Toledo, Greifensee, Switzerland). Analysis of the digesta moisture content was carried out by taking 0.2 g of rat digesta and then drying it in the oven for 24 h at 105 °C [15].

Analysis of the pH and SCFA digesta

The pH value of the digesta cecum was determined by dissolving the cecal digesta in a phosphate-buffered saline solution (pH 7.4) with a ratio of 1:5, then measured with a pH meter (Mettler Toledo, Greifensee, Switzerland). SCFA concentration analysis was performed using a modified method from Abreu *et al*. [16]. The digesta cecum was centrifuged at 13,000 rpm for 25 min (T: 28 °C), followed by the addition of 25 % metaphosphoric acid to the collected supernatant with a ratio of 5:2. Homogeneous samples were injected into a gas chromatography column (Shimadzu GC-2010, Kyoto, Japan) with T:240 °C, carrier gas He, pressure 18.9 kPa, total flow 102.1 mL/min, column flow 3.2 mL/min, linear speed 28.4 cm/s, and spit ratio 30.

Gut microbiota analysis

Analysis of the abundance of all 4 bacteria was carried out using the q-PCR method. Digest samples from each rat were collected and DNA isolation and purification was carried out using a DNA kit (FavorPrep TM Stool DNA Isolation Mini Kit) according to the protocol. The total DNA obtained was then calculated using a spectrophotometer (GeneQuant 1300) at wavelength n = 260/280 nm. The total DNA obtained was then diluted to a concentration of 25 ng/µL. Before testing using q-PCR, annealing temperature optimization was carried out using conventional PCR (Bio-Rad T100 Thermal Cycler brand produced in California, USA). The PCR results were then visualized using an electrophoresis device (Bio-Rad PowerPac Basic brand produced in California, USA and Hercuvan MiniGEL Electrophoresis produced in Cambridge, United Kingdom). DNA samples were run using a q-PCR tool (brand Bio-Rad CFX96 Real-Time System produced in California, USA) by making a q-PCR mix

of 10 µL containing q-PCR mix 2× Fast ExcelTaq TM q-PCR mix 5 µL, Nuclease Free Water 2.5 µL, forward primer 0.25 µL, reverse primer 0.25 µL, and DNA sample with a concentration of 25 ng/µL as much as 2 µL. The stages of the q-PCR cycle are tailored to each specific primer.

Table 1 Primer for q-PCR.

Bacterial target	Primer (5`-3`)	Primer	Reference
Biffidobacterium spp.	*Forward*: ATCTTCGGACCBGAYGAGAC *Reverse*: CGATVACGTGVACGAAGGAC	xfp_F xfp_R	[17]
E. coli	*Forward*: CATTGACGTTACCCGCAGAAGAACG *Reverse*: CTCTACGAGACTCAAGCTTGC	1457F 1652R	[18]
Clostridium	*Forward:* GCCAAAGGATTTATTCGCTATGA *Reverse:* ACCCGCGGCGCATTAGCTTGT		[19]
Universal	*Forward:* CCTACGGGGNGGCWGCAG *Reverse:* GACTACHVGGGTATCTAATCC	341F 805R	[20]

The calculation of bacteria is carried out by calculating the percent abundance of the total bacteria with a calculation formula: One divided by the result of calculating the Cq value of the target gene divided by the Cq value of the Gene Reference and multiplied by 100 % [21].

Analysis sIgA concentration of intestinal fluid

sIgA (secretory immunoglobulin A) analysis was performed using the Rat Secretory Immunoglobulin A (sIgA) ELISA Kit KTE100261 (Abbkine, Wuhan, China) according to the manufacturer's instructions. Samples of the small intestine of the duodenum (10 cm) were flowed with 1 mL of PBS (phosphate buffer saline) to obtain intestinal fluid (temperature 5 °C and pH 7.4) then centrifuged (3000 rpm, 10 min at 4 °C) then the supernatant was taken. The supernatant was stored at – 20 °C until ready to be analyzed. Optical Density readings were carried out with an ELISA reader (ZENIX ZN-320, Shenzen, China) at a wavelength of 450 nm. The concentration of sIgA is expressed as µg/mL which is the multiplication between the concentration read from the standard curve and the dilution factor [22].

Data analysis

Statistical analysis using IBM SPSS Statistics version 27.0 (SPSS Inc., Chicago, IL, USA) with one-way analysis of Variance (ANOVA) followed by post

hoc test using Tukey HSD at the 95 % confidence level ($\alpha \leq 0.05$).

Results and discussion

Feed intake and body weight gain of rat

The consumption effect of fiber drinks containing glucomannan and IMOs (GIFD) on the general condition of Sprague Dawley rats can be observed, one of which is through feed consumption and body weight gain. Based on **Figure 2**, the trends of feed intake in all groups remain relatively constant. The average feed intake of rats in **Table 2** shows that the average feed intake of GIFD-2 is lower than the inulin group. This indicates that GIFD are able to reduce feed intake because they contain IMOs and glucomannan which provide a longer satiety effect thereby reducing energy intake because consumption of fermented fiber can increase appetite suppressing hormones such as glucagon like peptide (GLP-1) and proglucagon expression and increase glucose homeostasis [23]. These hormones are known to improve satiety by inhibits gastric emptying which might slow down glucose delivery to the small intestine and its subsequent transport across the intestine into the systemic circulation [24,25]. The addition of glucomannan can increase the viscosity of fiber drinks thus have an impact on reducing feed intake. Rats and humans fed a high-viscosity dietary fiber diet reported consuming less food at the subsequent test diet than when consuming a low-viscosity diet [21,26,27,28]. The feed intake of the Sprague Dawley rats in this study was not substantially different from the control group after 28 days of GIFD consumption (**Figure 2**).

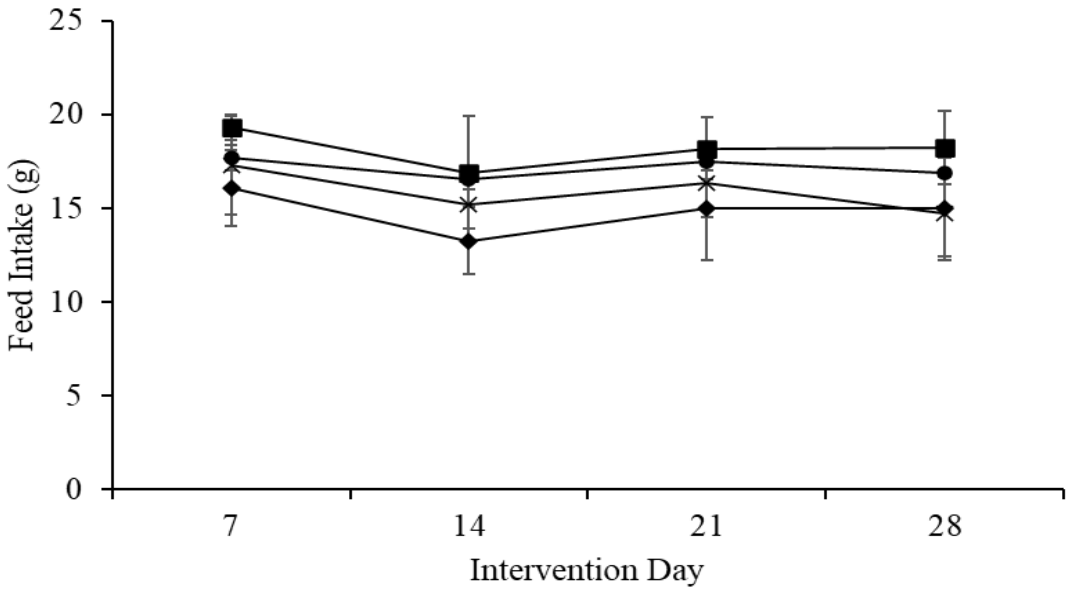

Figure 2 Feed intake of Sprague Dawley rats during 28 days of intervention supplemented by aquadest/control (●), inulin (■), GIFD dose 1 (╳), and GIFD dose 2 (♦).

The rat's body weight increased in all groups (**Figure 3**). On the other hand, **Table 2** shows that the GIFD-1 and GIFD-2 groups gained less body weight than the other groups ($p < 0.05$). However, rats' body weight did not change significantly when the GIFD dosage was increased. The growth rate of the rat's body weight, apart from being influenced by the quality and availability of food, was also influenced by several factors, including strain and environmental factors such as temperature and social situation [29]. This study demonstrated how glucomannan-containing fiber drinks can minimize weight gain in rats. Consistent with the study conducted by Xu *et al.* [30], the present investigation revealed that rats administered konjac glucomannan experienced a reduction in daily weight

gain compared to the control. Thus, the introduction of konjac glucomannan to the diet changed the eating behaviors in this case. The suppression of weight gain can be influenced by the physical properties of glucomannan which can form a gel and increase the viscosity of the gastrointestinal tract thus reducing the absorption of food by the intestine [31]. Consumption of fiber increases the viscosity through the formation of a gel layer and is impermeable in the gastrointestinal tract [32]. The gel formation is able to block food contact so that it has an impact on reducing energy absorption from fat and does not contribute to weight body gain [33].

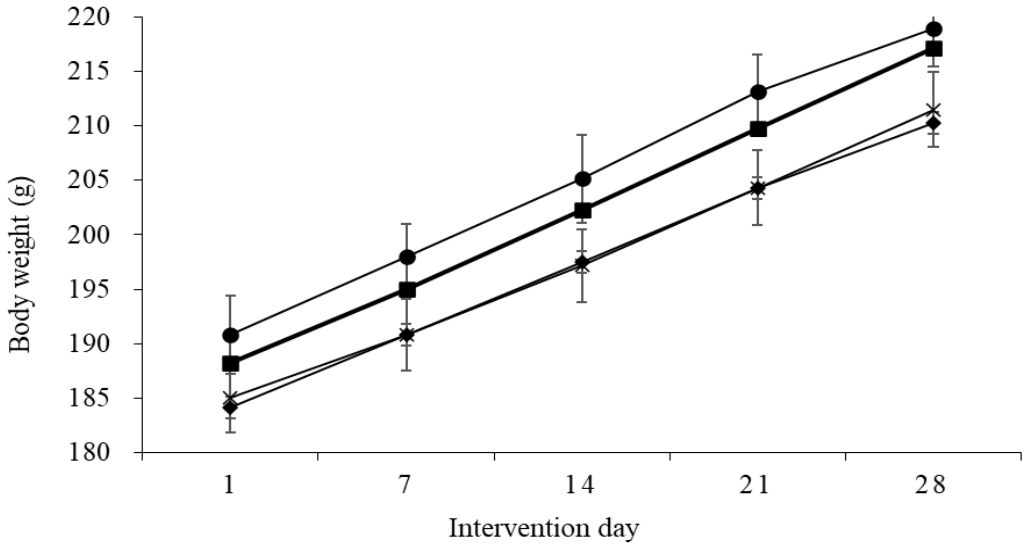

Figure 3 Body weight of Sprague Dawley rats during 28 days of intervention supplemented by aquadest/control (●), inulin (■), GIFD dose 1 (×), and GIFD dose 2 (♦).

Table 2 The average feed intake and body weight gain. Values are means ± SD. The same notation in a column shows no significant difference ($p > 0.05$), KON = control, INL = inulin, GIFD-1 = glucomannan and IMO fiber drink dose 1, GIFD-2 = glucomannan and IMO fiber drink dose 2.

Intervention	Feed intake (g)	Weight gain (g)
KON	17.15 ± 0.37^{ab}	28.17 ± 0.75^{a}
INL	18.17 ± 1.69^{b}	29.00 ± 0.89^{a}
GIFD-1	15.91 ± 1.82^{ab}	26.50 ± 0.55^{b}
GIFD-2	16.81 ± 1.41^{a}	26.17 ± 0.98^{b}
Sig.	0.04	< 0.001

Digesta profile

Digesta profiles can be used to evaluate the effects of dietary fiber consumption. To eliminate the effects of changes in body weight to digesta weight, a ratio of relative organ weight, also known as the 'organ index ratio' is calculated by dividing each animal's organ weight by its body weight [34]. Based on **Table 3**, the organ index of the GIFD-2 group differs significantly from the inulin group. These results could be influenced by the lower feed consumption in the GIFD-2 group compared to the inulin group (**Table 2**). Inulin had the highest organ index although it was not significantly

different from GIFD and KON. Meanwhile, GIFD-1 and GIFD-2 were not significantly different from the control ($p > 0.05$). The results were similar to the study conducted by Harmayani *et al*. [6], which found that different fibers from konjac glucomannan, porang glucomannan, and inulin did not affect the weight of digesta. Similar results were also shown by Wahjuningsih *et al.* [35] that consumption of fiber from soybeans had no effect on the weight of rats digesta. This can be due to an inadequate fiber supply. In general, weight gain is influenced by the amount of fiber consumed. Increasing fiber intake can help to maintain body weight through increasing satiety level and reducing energy intake [36].

Food fiber will bind water and other organic compounds such as fat, cholesterol, gallic acid, vitamins, and minerals so that digesta weight will increase [37,38]. In addition, increasing the mass of bacteria that ferment fiber also affects the increment of digesta weight [39].

In this research, we also did not observe any significant difference on the moisture content of rat ($p > 0.05$). It indicated that fiber water binding in all groups had the same strength. The moisture content level of digesta is a reflection of the water-holding capacity of dietary components, especially polysaccharides [35]. The moisture content of digesta that was not significantly different resulted in similar organ index in each group.

Based on **Table 3**, the pH value in all groups was significantly lower than the control group ($p < 0.05$). Fermentation of dietary fiber-producing SCFA (short-chain fatty acid) decreased pH value. Other studies found that glucomannan has prebiotic effects such as FOS, MOS, and XOS which are capable of lowering cecal digesta pH value [6,40]. Generally, lactic acid and acetate have the ability to reduce the pH value [41].

Table 3 The profile of digesta cecum. Organ index = organ mass (mg)/animal body mass (g). Values are means ± SD. The same notation in a column shows no significant difference ($p > 0.05$), KON: Control, INL = inulin, GIFD-1 = glucomannan and IMO fiber drink dose 1, GIFD-2 = glucomannan and IMO fiber drink dose 2.

Intervention	Organ index	Moisture content (%)	pH
KON	0.62 ± 0.08^{ab}	84.84 ± 2.17	7.18 ± 0.21^{a}
INL	0.85 ± 0.20^{b}	85.05 ± 6.85	6.72 ± 0.19^{b}
GIFD-1	0.70 ± 0.23^{ab}	83.24 ± 2.33	6.20 ± 0.15^{b}
GIFD-2	0.50 ± 0.18^{a}	80.48 ± 4.12	6.46 ± 0.2^{b}
p-value	0.022	0.276	< 0.001

SCFA

Short chain fatty acids (SCFA) are metabolites produced by the gut microbiota through anaerobic fermentation of non-digestible polysaccharides [38]. Acetate, propionate and butyrate represent the majority of SCFAs in the gastrointestinal tract. Cho and Finocchiaro [42] stated that the ideal molar ratio of SCFA (acetic acid: Propionic acid: Butyric acid) produced by the fermentation of undigested carbohydrates in the intestine is 60:20:20. The molar proportion of SCFA digesta of rats after the intervention can be seen in **Table 4**.

Table 4 The molar ratio of cecal digesta SCFA. Values are means ± SD. The same notation in a column shows no significant difference ($p > 0.05$), KON = control, INL = inulin; GIFD-1 = glucomannan and IMO fiber drink dose 1, GIFD-2 = glucomannan and IMO fiber drink dose 2.

Molar ratio (%)		
Acetic acid	**Propionic acid**	**Butyric acid**
67.14 ± 2.33^{b}	25.42 ± 2.26	7.43 ± 0.37
55.64 ± 3.00^{a}	30.42 ± 4.92	13.93 ± 6.56
60.57 ± 3.56^{a}	31.53 ± 1.73	7.91 ± 4.49
60.38 ± 4.14^{a}	30.89 ± 7.83	8.73 ± 6.18
< 0.001	0.138	0.120

The molar ratio of acetic acid in all groups was higher than that of propionate, followed by butyrate. This result is consistent with a study by Topping and Clifton [43], which reported that the molar ratios of SCFAs were in the order of acetate > propionate ≥ butyrate. Changes in the proportion of one SCFA can shift the proportions of other SCFAs.

Based on **Table 4**, there's a shift in the proportion of propionic acid for GIFD and inulin groups, and of butyric acid for inulin group. Although insignificant, it suggests that ingestion of GIFD and inulin may increase propionic acid in the gut. This is consistent with a study by Harmayani *et al*. [6], that porang glucomannan intervention are increasing propionic acid concentration in the gut.

Additionally, although statistically insignificant, a higher molar proportion of propionate compared to the control may suggest that consumption of fiber drink could help alleviate hypercholesterolemia. Propionic acid is capable of blocking cholesterol synthesis in the heart and has a hypolipidemic effect [42]. Other studies suggest that propionate inhibits the conversion of acetate into fats and sterols, leading to a reduction in fatty acids and cholesterol synthesis [44]. Propionic acid has been identified to lower cholesterol levels by inhibiting the activity of the enzyme β hydroxy-β methyl glutamyl CoA (HMG-CoA) reductase, which plays a role in cholesterol synthesis. Propionic acid inhibits acetate incorporation into plasma cholesterol by competing with acetate transporter toward hepatocyte cells. This results in decreased cholesterol synthesis, as acetate is a precursor in cholesterol formation [45,46].

Furthermore, propionic acid is also associated with weight control functions. Propionate can modulate energy intake and body weight through appetite regulation. In a randomized crossover study involving healthy volunteers, 10 g of inulin-propionate esterification treatment increased satiety and reduced appetite compared to the administration of 10 g of inulin alone, as measured by a visual analog scale for hunger and satiety. This is related to the function of SCFAs in activating free fatty acid receptors to stimulate satiety hormone secretion [47,48]. These results are consistent with the lower weight gain observed in rats given fiber drink compared to other groups.

Bacterial population in digesta profile

The gut microbiota plays a role in maintaining the existence and barrier function of the intestinal mucosa, participating in the absorption and digestion of nutrients, the transportation and metabolism of substances, regulating human immunity, growth, and development [49]. Gut microbiota analysis by calculating the bacterial abundance was conducted to determine the effect of dietary fiber consumption on the composition of healthy and pathogenic microflora. Prebiotics can be selectively fermented by specific bacteria, especially *bifidobacteria* and *lactobacilli* in the colon, potentially

providing health-promoting effects on the host and maintaining the balance of the gut microbiota [50]. As shown on the **Figure 4**, the administration GIFD did not significantly increase the bacterial abundance percentage of *Bifidobacterium* compared to the control treatment. One of the reasons underlying the results of this study is the use of doses that are not yet optimal in influencing the abundance of *Bifidobacterium* bacteria. *Bifidobacterium* is one of the most common probiotics which metabolizes dietary fibers and produces SCFA. SCFA are beneficial for gut health and help prevent constipation. It holds several functions since it has anti-inflammatory effects and nourish colonocytes. In addition, SCFA influences gastrointestinal epithelial cell integrity, glucose homeostasis, lipid metabolism, appetite regulation, and immune function. *Bifidobacterium* has been reported to maintain a healthy gut flora, which is crucial for proper digestion and the synthesis of essential vitamins [51].

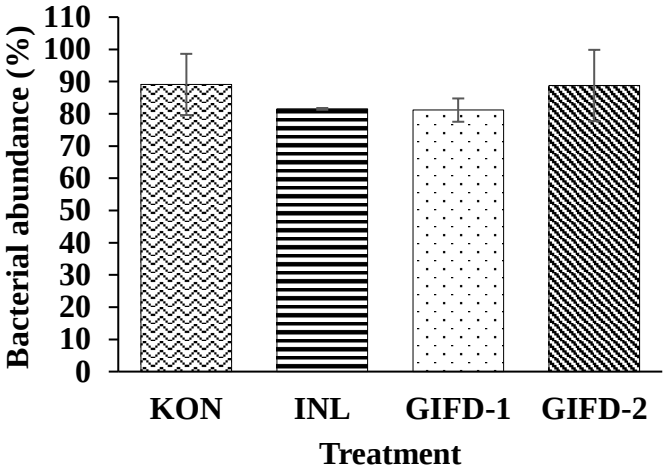

Figure 4 Bacterial Abundance (%) of *Bifidobacterium* in digesta Sprague Dawley rats. KON = control, INL = inulin; GIFD-1 = glucomannan and IMO fiber drink dose 1, GIFD-2 = glucomannan and IMO fiber drink dose 2.

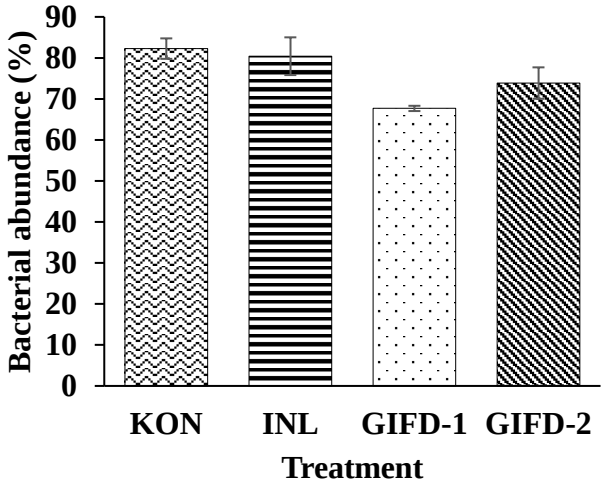

Figure 5 Bacterial Abundance (%) of *E. coli* in digesta Sprague Dawley rats. KON = control, INL = inulin, GIFD-1 = glucomannan and IMO fiber drink dose 1, GIFD-2 = glucomannan and IMO fiber drink dose 2.

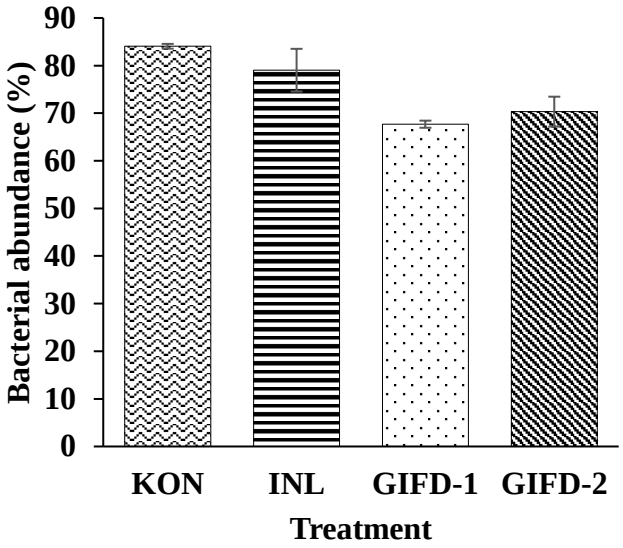

Figure 6 Bacterial Abundance (%) of *Clostridium* in digesta Sprague Dawley rats. KON = control, INL = inulin, GIFD-1 = glucomannan and isomalto-oligosaccharide fiber drink dose 1, GIFD-2 = glucomannan and IMO fiber drink dose 2.

Functional foods, including prebiotics have been shown to enhance the immune system of mucous membranes in the intestinal wall, help preventing infection by modifying the composition and/or metabolic activity of intestinal microflora in order to better combat pathogens [52]. As a dietary fiber, glucomannan is able to stimulate and increase the population of beneficial bacteria, including lactic acid bacteria (LAB). LAB in the gastrointestinal tract has the potential to produce metabolite compounds in the form of organic acids and carbon dioxide. These organic acids produced include lactic acid and acetic acid, are known to lower the pH of the gastrointestinal tract, increasing the production of antimicrobial or bacteriocin substances, creating a competitive exclusion environment that inhibits the growth of pathogenic bacteria such as *Bacteroides*, *Coliform*, *Escherichia coli* and *Clostridia* while improves beneficial bacteria in the small intestine [53,54].

The result of this study indicates that in comparison to controls, samples that had GIFD treatment have a lower percentage of the abundance of *E. coli* bacteria (**Figure 5**) and *Clostridium* bacteria (**Figure 6**) compared to controls. Compared to inulin, GIFD treated groups performed better in suppressing enteric bacteria. This may be due to the higher fiber content in GIFD as fiber drink contain 2 sources of fiber i.e. glucomannan and IMO. The content of these 2 sources of fiber will affect the increase in SCFA production. Increased production of SCFAs will lead to a smaller population of enteric bacteria. In acidic condition, SCFA do not dissociate which enable them to act as antibacterial agents [55], which inhibit microbial growth. It is also believed that the glucomannan content in GIFD lowers the abundance of enteric bacteria. The presence of IMO as the main ingredient in making GIFD products can increase SCFA production and can inhibit the growth of *Clostridium* [56]. While glucomannan can increase SCFA production, especially in butyric acid concentrations. Butyric acid can stimulate the expression of mucin genes (MUC2, MUC3, and MUC4) in colonic epithelial cells. Mucin is the main constituent in the lining of the digestive tract to protect against pathogenic microorganisms. This layer can reduce the adherence of *E. coli* [57]. Porang glucomannan supplementation can inhibit the growth of *E. coli* by increasing SCFA production through lowering pH to suppress pathogens. SCFAs are metabolites of

carbohydrate fermentation by microbes. SCFA production provides energy for epithelial cells and improves colon health. SCFAs that play an important role in intestinal health are butyric acid which has been reported as the main energy source for colonocytes and is produced by *Faecalibacterium sp.* and *Eubacterium sp.* Previous studies have shown that glucomannan fermentation both *in vitro* and *in vivo* can produce SCFAs (acetic acid, propionic acid and butyric acid) [58].

GIFD products also contain cocoa powder. Cocoa powder can also have a good impact on colon health. Chocolate or cocoa is considered prebiotic rich in polyphenol content. Most polyphenols cannot be absorbed by the gut and will interact with the gastrointestinal microbiota. The diversity and specificity of microorganisms in the colon are essential in polyphenol metabolism to produce bioactive secondary metabolites that interact with human biochemical synthesis pathways. Cocoa consumption can significantly reduce *Clostridium* bacteria since cocoa contains triglycerides and C-reactive protein which can affect the condition of the colon and change the population of microorganisms [59].

sIgA concentration of intestinal fluid

The role of prebiotics in stimulating immunity can be studied by testing the parameters of the mucosal immune response, one of which is the concentration of sIgA in rat duodenal intestinal fluid using ELISA. The results of the sIgA concentration test in the control, inulin, and FD treatment groups were not significantly different (**Figure 7**).

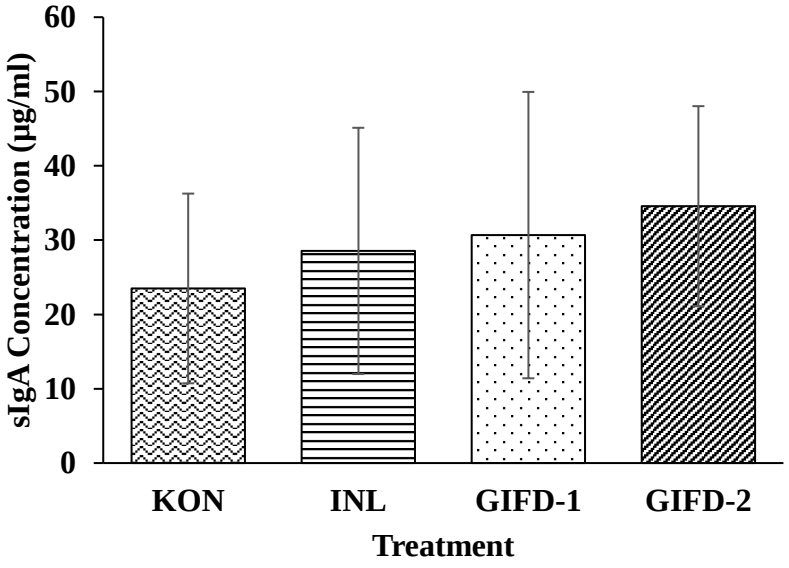

Figure 7 The concentration of sIgA in duodenum intestinal fluid. Values are means ± SD, n = 6. The same notation on the graph shows no significant difference ($p > 0.05$). KON = control, INL = inulin, GIFD-1 = glucomannan and IMO fiber drink dose 1, GIFD-2 = glucomannan and IMO fiber drink dose 2.

Secretory immunoglobulin A (sIgA) is the best effector site produced by the intestinal mucosa. sIgA with innate immune factors such as mucus in the intestinal lumen will achieve immune exclusion so as to protect the mucosal surface. The immune response will be active when an allergen enters the body due to the formation of globulin proteins in the body so that the body will issue a response to capture the allergen [60]. Addition of fermentable fiber to the diet alters gut function and structure and modifies gut-derived

hormone production so as to enhance whole-body glucose homeostasis. The concentration of sIgA in the group of rats given the fiber drink was not significantly different from all treatment groups. This indicates that a fiber drink containing glucomannan and IMO at a dose of 0.54 and 1.08 g cannot increase intestinal fluid sIgA. The results of this study are consistent with work of other researchers [61]. Rats that were given cellulose, inulin, oligofructose + cellulose had the same faecal IgA concentrations and were not statistically significant. In addition, another study using a combination of XOS and inulin also showed IgA concentrations that were not significantly different from controls [62].

Based on **Figure 7**, the trend of adding prebiotics to the sIgA concentration is an increase in sIgA in GIFD-2. Prebiotics can be recognized by immune cells in the gastrointestinal tract and affect cytokines in the gastrointestinal mucosa. TGF-β is a key regulator of IgA production among all other IgA-modulating immune cell factors and cytokines, with B cell-specific TGFβR deletion resulting in the loss of IgA-producing B cells [63].

Conclusions

This study reveals that glucomannan IMO fiber drink (GIFD) is potential to be developed as weight management product. Consumption of GIFD could lowering the abundance percentages of pathogenic bacteria such as *E. coli* and *Clostridium* indicating its positive effect on gut health. For further research, clinical trials of this product are of interest.

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