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Effect of xylo-oligosaccharides and β-glucan on gut microbiota and short chain fatty acid in diabetic rats

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ABSTRACT

Obesity and type 2 diabetes mellitus (T2DM) have an impact on an imbalance in the number of microbiota (dysbiosis) which can cause disruption of glucose metabolism by inhibiting short chain fatty acid (SCFA). SCFA is produced by intestinal microbial fermentation of indigestible dietary fibre and it has beneficial role in T2DM. Xylo-oligosaccharides (XOS) and β-glucans can improve the gut the composition of microbiota with prebiotic capabilities. The objective of the study was to investigate the effect of XOS and β-glucan on the *Lactobacillus, Roseburia,* and SCFA in diabetic rats. A total of 28 Wistar rats were divided into 4 groups including control (C), diabetic (D), diabetic+XOS (DX), and diabetic+β-glucan (DBG). D, DX, and DBG groups were provided with a high-fat diet (HFD) for 14 days before receiving an injection of streptozotocin (STZ) (45 mg/kg BW) and nicotinamide (110 mg/kg BW). DX received XOS (450 mg/200 g BW), and DBG received β-glucan (450 mg/200 g BW) for 28 days. The blood glucose levels at DX and DBG showed a significant difference compared with the D gro diabetic rats. XOS did not change the number, while β-glucan increased the number of *Lactobacillus and Roseburia*. The XOS and β-glucan supplementation significantly increased SCFA levels compared to diabetic rats. These results suggest that XOS can improve blood glucose although it does not change the number of *Lactobacillus* and *Roseburia.* Meanwhile, β-glucan increases blood glucose, *Lactobacillus, Roseburia,* and total SCFA in diabetic rats.

INTRODUCTION

The international diabetes federation states that the prevalence of diabetes mellitus worldwide was 9.3% in 2019. It is estimated to increase in 2030 to 10.2%, then in 2045, it is estimated to increase to 10.9% [1]. The results of a case study of diabetes mellitus in Indonesia show a trend of increasing from 6.9% in 2013 to 10.9% in 2018 [2]. Type 2 diabetes mellitus (T2DM) is characterized by hyperglycemia levels with random plasma glucose levels of ≥ 200 mg/dl [3]. There are several risk factors for type 2 diabetes related to changes in lifestyle such as lack of physical activity, high consumption of carbohydrates, and high fat leading to an increase in body mass index (BMI) which then develops into obesity [4].

Short chain fatty acid (SCFA) is the main metabolite of indigestible carbohydrate fermentation. The SCFAs most frequently found in the colon are acetate, propionate, and butyrate [5]. SCFAs produced through fiber fermentation are known to provide positive effects in preventing cardiometabolic disease including T2DM, obesity and cardiovascular disorders [6]. Furthermore, T2DM and obesity can influence an imbalance in the number of microbiota which is called as dysbiosis [7]. Dysbiosis can cause disruption of glucose metabolism by inhibiting the final products of SCFA metabolism which is specifically associated with a decrease in the number of butyrate-producing microbes. A decrease in the amount of butyrate results in modulating the

immune response and it can cause low-grade inflammation which ultimately becomes one of the causes of T2DM [8].

Microbial composition and activity can be influenced by diet, such as the consumption of complex carbohydrates or prebiotics. Administration of prebiotics to experimental HFD-induced diabetic animals has been shown to improve the balance of microbiota [9]. Meanwhile, XOS consists of xylose oligomers which are linked through β-(1-4) linkages. XOS is a group of oligosaccharides which cannot be digested by humans, and it has a prebiotic effect. Prebiotics stimulate the production of activity of one or more bacteria in the colon (*Bifidobacterium* and *Lactobacillus*) by suppressing the activity of entero-decaying organisms and pathogens [10].

Consuming prebiotics is an effort to improve microbiota imbalance. β-glucan is a group of polysaccharides which have a prebiotic effect since they can modulate the intestinal microbiota. Consumption of β-glucan from oats or barley increases the growth of *Roseburia* population producing SCFAs such as propionate and butyrate [11]. Research shows that administration of oats and barley containing β-glucan stimulates the growth of *Lactobacillus* and *Bifidobacteria,* which can help to inhibit dysbiosis [12].

Based on the description above, XOS and β-glucan have potential effects on metabolic parameters as well as gut microbiota. Therefore, this study aimed to determine the effect of administering XOS and β-glucan on the number of *Lactobacillus, Roseburia* and SCFA composition in diabetic Wistar rats.

MATERIALS AND METHODS

Animals

This study was a true experimental post-test only with a randomized controlled group design. In this study, 28 male Wistar rats weighing 160 to 220 g aged 8 weeks were used which were divided into 4 groups such as control (C), diabetic (D), diabetic+XOS (DX), and diabetic+β-glucan (DBG). Each rat was acclimatized for 7 days in a cage, humidity ranges from 70-75% while temperature ranges from 25-28^{0}C, the light settings were set to 12 am and 12 pm at the Experimental Animal Laboratory of the Centre for Food and Nutrition Studies, Gadjah Mada University, Yogyakarta, Indonesia. This research was conducted from December 2022 to February 2023. All of the rats were adjusted to room temperature, and they were given Comfeed AD II food standard and water *ad libitum* during the study. After the acclimatization period, the initial body weight of the rats was recorded. The control group was only given standard Comfeed AD II feed and treated as healthy rats. Groups D, DX, and DBG were given high fat diet (HFD) for 14 days and treated with streptozotocin (STZ) 45 mg/kg BW and nicotinamide (NA) 110 mg/kg BW. The DX group received XOS at 450 mg/200g BW. The DBG group received at 450 mg β-glucan/200g BW. XOS and β-glucan were given for 28 days, respectively. XOS was purchased from Shandong Longlive Biotechnology (Shandong, China) and β-glucan from Lake Avenue Nutrition (Madre Labs, Laguna Canyon Rd). At the end of the study, all of the rats were dissected, and their cecum was taken, then placed in a falcon tube. The cecum was weighed 500 mg and then put into a microtube. After collection, the cecum was transported on ice to the Biochemistry Laboratory at the Gadjah Mada University Faculty of Medicine, Public Health, and Nursing, where it was kept at -20°C until it was analyzed.

Animal facilities, their management, and handling during the experiment were done in compliance with the Guidelines for Care and Use of Laboratory Animals of CNFS

Gadjah Mada University and were approved by the Research Ethics Committee of the Faculty of Medicine, Diponegoro University (No.120/EC/H/FK-UNDIP/X/2022).

Blood glucose analysis

Blood glucose levels were checked 3 times such as a) at starting of the study, b) 3 days after the STZ+NA treatment to ensure that the rats were hyperglycemic, and c) at the end of the study. All rats were fasted 8-10 h before taking blood. 2 ml of blood was taken through the plexus retroorbital, then put in a tube containing EDTA anticoagulant. The samples were centrifuged at 3000 rpm for 15 minutes, then the serum was collected. The analysis was carried out using the Enzymatic colorimetric test using GOD-PAP method. Rats are declared diabetic when their blood glucose levels were greater than 250 mg/dl [13].

SCFA analysis

SCFA components were analyzed using Gas Chromatography Mass Spectrophotometry. Briefly, 10 mg of cecum was taken into a 1.5 ml tube and added 400 µL of water and homogenized by vortexing. Then, it was centrifuged at 1400 rpm for 10 min. The supernatants were transferred into 1.5 mL tubes. Then, the supernatants were diluted 1:5 with water for a total volume of 50 µL using a 1.5 mL tube [14]. In addition, the samples were analyzed by Shimadzhu GC-2010 Plus. SCFA levels were analyzed at the Food Technology and Agricultural Products Testing Laboratory, Gadjah Mada University, Yogyakarta, Indonesia.

Cecum bacterial DNA extraction

DNA od cecum samples were extracted using Favor PrepTM DNA isolation kit (FavorGen Biotech, Taiwan, China) following the manufacturer's instruction. Total DNA concentration was calculated by using nanodrop (Maestrogen Inc).

Standard curves creation

The *Lactobacillus* inoculum at concentration of 10^9 CFU/g was obtained from the Laboratory of the Center for Food and Nutrition Studies Gadjah Mada University and it was gradually diluted to 10^2 CFU/g. After dilution, it was centrifuged at 5000 rpm for 5 min at 4°C until a precipitate was formed. After that, the samples were rinsed with 10 mL of 0.9% NaCl. Then the centrifugation and vortexing was conducted again until a precipitate was created.

G-blocks Gene Fragment Roseburia with a concentration of 500 ng which had been designed and ordered at (Genetica Science Indonesia). The dilution process began with spin down for 15 seconds to ensure the material is at the bottom of the tube, 50 µ/L nuclease free water (NFW) was added until it reached a final concentration of 10 ng/ µL. Then, it was vortexed briefly, incubated at 50^0 C for 20 min, and again vortexed briefly. Finally, 1 µ/L was taken, put it in a new 2 ml tube which contained 99 µ/L NFW, and then conducted gradual dilution until 10^2 CFU/g.

The logarithmic concentration of bacteria inoculum dilutions and G-Blocks were plotted against a threshold number in order to produce a standard curve. The standard curve obtained was as follows: *Lactobacillus*: $Y = -3.3789x + 47.084$ ($R^2 = 0.9926$) and

Roseburia: Y= -3.468x + 44.944 (R^2 = 0.9782). The plotting results in the dilution range showed a linear relationship in which the line detected is a dilution of 1x102 - 1x109 copies/µL.

Quantification of bacterial by RT-PCR

The primers used to detect *Roseburia* were based on the 16srRNA gene sequence taken from the BLAST database desaigned an ordered from Genetika Science Indonesia. The primer sequences for *Roseburia* were forward-GCCACATTGGGACTGAGACA and reverse-GCTGCTGGCACGTATTTAGC. Meanwhile, the *Lactobacillus* primer sequences as in previous research, was primers forward-AGCCAGTAGGGAATCTTCCA and reverse-CACCGCTACACATGGAG [15]. The mixture composition in the PCR tube contains 10 µl of ExcelTaq 2x Fast Q-PCR SYBR master mix (SMOBIO Technology Inc), 1 µl of reverse primer and 1 µl of forward primer each, the amount of bacterial DNA samples adjusted to the nano drop results, the amount of nuclease free adjusted the bacterial DNA samples to reach a total volume 20 µl. Furthermore, the mixture in the PCR tube was analyzed using Bio Rad CFX96 Real-Time. The PCR temperature setting was as follows: 95^{0}C for 10 min, followed by 40 cycles for 15 seconds at 95^{0}C and 1 min at 60^{0}C for *Lactobacillus* while temperature settings for *Roseburia* was: 95^{0}C for 10 min, followed by 40 cycles for 15 seconds at 95^{0}C and 30 seconds at 54^{0}C. After the running process, melting curve analysis was carried out to confirm to ensure the accuracy of the RT-PCR data. The standard curve of the bacterial strain was used to calculate the cycle thresholds of bacterial DNA. Log CFU/gr was used to express the results.

Statistical analysis

SPSS 23 software (IBM/SPSS Inc.) was used to perform statistical analysis. Meanwhile, the Shapiro-Wilk normality test was used to analyze the research data. Post-intervention data were normally distributed using one way ANOVA on the effect of blood glucose, *Lactobacillus* and SCFA levels. Because the *Roseburia* data were not normally distributed, Kruskal Wallis analysis was used as a non-parametric test. The P value < 0.05 was considered significant, and all results were presented as mean ± SD.

RESULTS

Effects of XOS and β-glucan on blood glucose levels in diabetic rats

Administration of HFD and STZ showed an increase in blood glucose levels in rats indicating that the rats had hyperglycemia (Table 1). Supplementation with XOS and β-glucan 450 mg/200 g BW in DX and DBG group showed reduced blood glucose levels, respectively (Figure 1 and Table 1). The Shapiro-Wilk normality test on blood glucose showed significant difference in all groups, indicating that the data is normally distributed (Figure 1 and Table 1). The one-way ANOVA test showed a p value = 0.000 ($p<0.05$), indicating that there is a significant difference in blood glucose levels across all treatment groups. LSD post-hoc test was used to determine which groups were statistically different. The results in Figure 1 showed significant differences in blood glucose levels in D, DX and DBG groups against the C group.

Table 1. Effect of XOS and β-Glucan on blood glucose of HFD-STZ-induced diabetic rats.

Blood Glucose	Group				P value
	C	D	DX	DBG	
Initial Glucose	68.78 ± 1.65	68.78 ± 1.80	68.50 ± 1.82	69.91 ± 1.83	0.470
Pre-treatment glucose	69. 82 ± 1.84	277.93 ± 5.94	282.54 ± 5.73	273.25 ± 9.77	0.000*
Post treatment glucose	71.48 ± 1.63	278.80 ± 5.63	101.87 ± 4.75	112.42 ± 6.74	0.000*
Δ Glucose	1.66 ± 0.72	0.87 ± 0.46	-180.67 ± 1.33	-160.84 ± 3.83	0.000*

Initial glucose level was the level when selecting experimental animals for inclusion criteria; Pre-treatment glucose levels were glucose levels in rats after being induced by STZ and NA; Post-treatment glucose levels were glucose levels after being induced by STZ and NA and after being supplemented with XOS and β-glucan for 28 days; Delta Glucose is the difference between post-treatment glucose levels and pre-treatment glucose levels; C = control group; D = control negative (diabetic) HFD + STZ + NA; DX= Giving HFD + STZ + NA + XOS 450 mg/ 200 g BW; DBG = Giving HFD + STZ + NA + β-glucan 450 mg/200g BW; * = significant ($p < 0.05$).

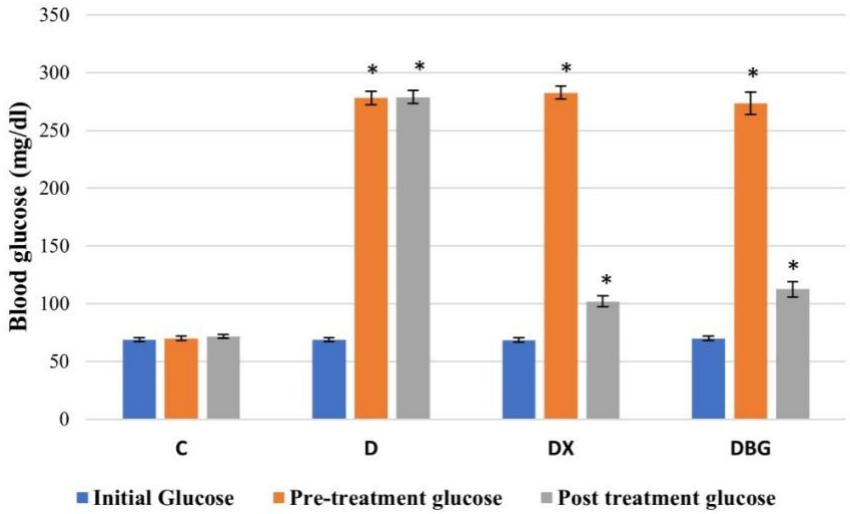

Figure 1. Effect of XOS and β-Glucan on blood glucose of HFD-STZ-induced diabetic rats. Initial glucose level was the level when selecting experimental animals for inclusion criteria; Pre-treatment glucose levels were glucose levels in rats after being induced by STZ and NA; Post-treatment glucose levels were glucose levels after being induced by STZ and NA and after being supplemented with XOS and β-glucan for 28 days. C = control, healthy group; D = control negative (diabetic) HFD + STZ + NA; DX = Diabetic + XOS 450 mg/ 200 g BW; DBG= Giving HFD + STZ + NA + β-glucan 450 mg/200g. The chart shows the results of the post-hoc LSD test, which * indicated significant differences blood glucose between C groups ($p < 0.05$).

Effects of XOS and β-glucan on *Lactobacillus* and *Roseburia* in diabetic rats

In the cecum, the number of *Lactobacillus* in diabetic group was higher than in control group (Figure 2 and Table 2). In contrast, the number of *Lactobacillus* in the cecum of DX group was decreased than baseline. Compared to control group and DX group, the cecum of the DBG group contained more of *Lactobacillus*. According to Shapiro Wilk test for the *Lactobacillus*, the outcomes for all groups were significant ($p>0.05$), indicating that the data is of normal distribution (Figure 2 and Table 2). The one-way ANOVA test showed a $p = 0.313$ ($p>0.05$), indicating there is no significant difference in the number of *Lactobacillus* among all treatment groups (Figure 2 and Table 2). In addition, the number of *Roseburia* in diabetic group decreased compared to the control group (Figure 2 and Table 2). It means that rats with diabetes mellitus had dysbiosis. However, in the DX group total *Roseburia* was slightly decrease than control and diabetic groups. Interestingly, the number of *Roseburia* in DBG group is greater than C, D, DX. The Shapiro-Wilk normality test showed that *Roseburia* were not normally distributed ($p<0.05$) (Figure 2). The Kruskal Wallis test showed a $p = 0.021$ ($p<0.05$), indicating there was significant difference in the number of *Roseburia*. LSD post-hoc test was used to determine which groups were statistically different. The results in Figure 2

showed significant differences in the number of *Roseburia* between diabetic and C groups as well as significant differences between DBG and diabetic groups.

Table 2. The number of *Lactobacillus* and *Roseburia* after the intervention.

	Group				P value
	C	D	DX	DBG	
Lactobacillus	9.41 ± 1.01	9.65 ± .42105	9.35± 1.04	10.0742 ± 0.34	0.313
Roseburia	9.76 ± 0.49	9.03 ± 1.27	7.52 ± 1.33	9.97 ± 0.78	0.002*

C = control, healthy group; D = control negative (diabetic) HFD + STZ + NA; DX = Diabetic + XOS 450 mg/ 200 g BW; DBG= Giving HFD + STZ + NA + β-glucan 450 mg/200g; * = significant ($p < 0.05$)

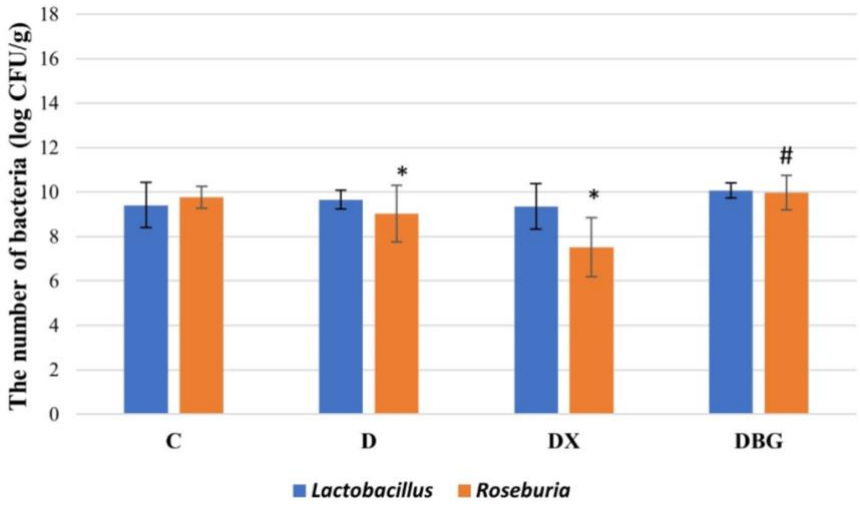

Figure 2. Effect of XOS and *β-glucan* on the number of *Lactobacillus* and *Roseburia* after intervention in rats. According to the results of a one-way ANOVA test, there was no significant difference in *Lactobacillus* between groups. In additions, the number of *Roseburia* according to the result of *Kruskall Wallis* show there is a significant difference, the chart shows the results of post hoc using Mann Whitney U test. C = control, healthy group; D = control negative (diabetic) HFD + STZ + NA; DX = Diabetic + XOS 450 mg/ 200 g BW; DBG= Giving HFD + STZ + NA + β-glucan 450 mg/200g. * = significant differences in the number of *Roseburia* between C groups and # ($p<0.05$) significant differences between DX groups.

Effects of XOS and β-glucan on SCFA in diabetic rats

SCFA in the cecum were measured to verify the presence of the final products after XOS and β-glucan administration. In the DX and DBG groups, propionate, acetate, and butyrate levels were substantially higher after receiving XOS and β-glucan treatment in diabetic rats (Figure 3 and Table 3). The distribution of SCFA in the diabetic group decreased compared to the control group. In the DX group, the amount of propionate is found to be higher while in the DBG group the amount of acetate is higher. The Shapiro-Wilk normality test for on SCFA significance in all group ($p>0.05$), indicating that the data was of normal distribution (Figure 3). The one-way ANOVA test showed a $p = 0.000$ ($p<0.05$), indicating there was a significant difference in SCFA across all treatment groups. LSD post-hoc test was used to determine which groups were statistically different. The results showed significant differences in the SCFA components between groups (Figure 3 and Table 3).

Table 3. SCFAs level after intervention.

Composition of SCFA	Group				P value
	C	D	DX	DBG	
Acetate	51.36 ± 2.18	12.14 ± 1.47	31.86 ± 1.61	30. 27 ± 2.57	0.000*
Propionate	44.38 ± 3.02	12.51 ± 2.76	35.37 ± 3.29	25.87 ± 2.64	0.000*
Butyrate	24.72 ± 2.10	5.11 ± 1.56	19.83 ± 0.53	16.87 ± 2.23	0.000*

C = control, healthy group; D = control negative (diabetic) HFD + STZ + NA; DX = Diabetic + XOS 450 mg/ 200 g BW; DBG= Giving HFD + STZ + NA + β-glucan 450 mg/200g; * = significant ($p < 0.05$)

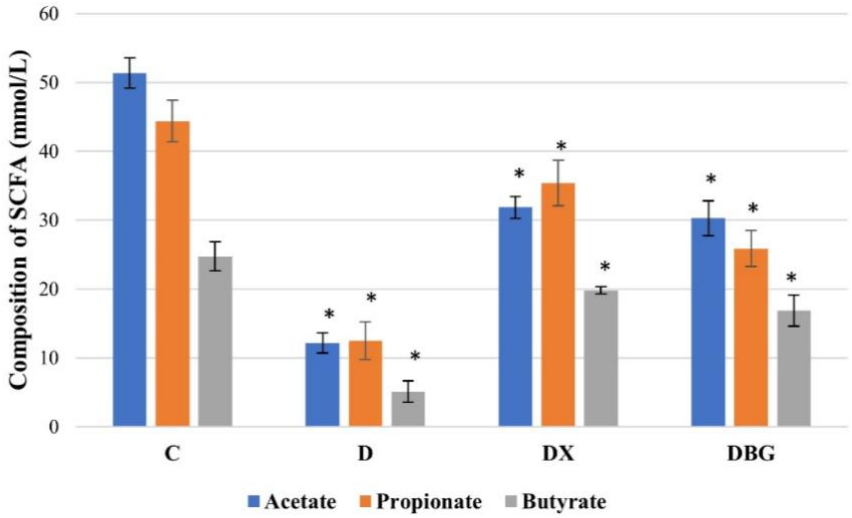

Figure 3. Effect of XOS and *β-glucan* on SCFA compositions after intervention in rats. The chart shows the results of the post-hoc LSD test, which indicated significant differences in SCFA compositions between groups ($p < 0.05$). C = control, healthy group; D = control negative (diabetic) HFD + STZ + NA; DX = Diabetic + XOS 450 mg/ 200 g BW; DBG= Giving HFD + STZ + NA + *β-glucan* 450 mg/200g BW; * = indicates significant differences between C groups.

DISCUSSION

The characteristic of diabetes mellitus is chronic hyperglycemia caused by lack of insulin secretion, and lack of insulin action on target tissues, or both [16]. Recently, great attention has been paid to derivative products made from natural ingredients intended to help people suffering from diabetes. Our study showed that administration of XOS and β-glucan at a dose 450 mg/200 g BW for 28 days showed a beneficial effect in reducing blood glucose levels in rats with T2DM which indicates anti-hyperglycemic outcomes.

Nowadays, various experimental animal models have been developed to study diabetes. For a decade, STZ, a natural glucosamine-nitrosourea, has been used to induce diabetes in rats. After the STZ treatment, pancreatic β cells are destroyed due to the alkylation process of the diabetogenic agent STZ, causing decreased insulin production and uncontrolled plasma glucose levels in diabetic rats [13]. Administration of low doses of STZ and HFD are often used to induce diabetes in rats [17]. The characteristics of diabetic rat include abdominal obesity, insulin resistance, hyperglycemia, hyperinsulinemia, and dyslipidemia, similar to human condition. These conditions are suitable for studying the therapeutic effects on diabetes in our experimental study.

The biological properties of XOS have been demonstrated by numerous reports [18]. Furthermore, commercial administration of XOS to STZ-induced diabetic rat for 5

weeks of intervention showed improvement in several diseases including inhibiting of increases in glucose, cholesterol and triglyceride concentrations. XOS can significantly increase fecal content as well as cecal acetate concentration in STZ-induced diabetic rats [19]. Similarly, our results showed beneficial effects on blood glucose after XOS administration in STZ-induced and HFD-fed in rats. It is in line with previous clinical studies show that administration of XOS (4g/day) for 8 weeks has advantage effects on blood glucose, HbA1C, fructosamine, and lipid profile in diabetic patients [20]. These data contribute to the confirmation that XOS has hypoglycemic and hypolipidemic effects as well as amelioration of insulin resistance. XOS has been reported in various studies to have benefits in diabetes both in vivo and clinically, but the underlying mechanisms are still unclear [21].

The results of our studies showed that administration XOS at 450 mg/ 200 g BW does not increase the number of *Lactobacillus*. It is in line with previous study that XOS supplementation does not increase *Lactobacillus* [22]. *Lactobacillus* in the diabetic rat group increased compared to the control group. The results of research in human show that the gut microbiota associated with obesity has increased levels of *Lactobacillus* [23]. Another study shown in Southern China populations shows that T2DM cause increased numbers of *L. Bulgaricum, L, Rhamnosus* and *L. acidophilus* compared with control group. In type 2 diabetes mellitus, related lactic acid bacteria such as *Enterococcus*, *Streptococcus* and *Lactobacillus* are decreased due to XOS [24]. In addition, this study shows that the number of *Roseburia* does not increase with XOS supplementation.

In vitro and in vivo studies show that SCFAs stimulate the secretion of glucagon-like-peptide-1 (GLP-1) and peptide YY (PYY) in the intestine by activating GPR41 and GPR43 [25]. Moreover, GLP-1 and PYY have a very good role in increasing satiety through the gut-brain-axis. This process can indirectly reduce appetite and food intake so that it inhibits weight gain, which is considered a risk factor for T2DM. In addition, SCFAs can regulate blood glucose through increasing GLP-1-mediated insulin secretion [26]. Additionally, acetate and butyrate affect skeletal muscle glucose metabolism in an AMPK-dependent manner that in turn increases glucose uptake (via GLUT4) and glycogen storage via a GPR41/GPR43-mediated mechanism. SCFA indirectly influences muscle insulin sensitivity and glucose metabolism through increasing gut-derived PYY and GLP-1 levels so that it affects skeletal muscle insulin action and glucose absorption that contribute to increased muscle insulin and glucose sensitivity [27].

The result of this study shows that XOS is able to increase the amount of SCFA, especially propionate. Propionate enhances glucose-stimulated insulin release by pancreatic β-cell in vivo via activating FFAR2 and protecting β cells from apoptotic stimuli [28]. Another advantage of propionate in terms of health benefits is due to its anti-lipogenic, cholesterol-lowering, anti-inflammatory, and anti-cancer effects [29]. Furthermore, in the β-glucan group, we find higher acetate. It has been suggested that acetate plays a direct role in central appetite regulation [30]. Based on the results of previous research, it was shown that giving high fat to diabetic rats can reduce total SCFA; especially, butyrate. Total butyrate is associated with reduced glucose, insulin resistance and body weight in diabetic rats fed a high-fat diet. Butyrate deficiency can alter glucose metabolism and worsen the pathogenesis of diabetes by inducing inflammation and oxidative stress [31].

β-glucan is a soluble fiber polysaccharide with a mixture of β-(1-3) and β-(1-4) glycosidic bonds. β-glucan has health benefits, including lowering blood glucose and cholesterol in obesity and cardiovascular disease. The study shows that low oat β-glucan intake significantly lowered blood glucose and insulin levels. The positive hypoglycemic effect of β-glucan is due to its viscosity effect [32]. The interaction of β-

glucan with intestinal mucus is considered as another potential mechanism of cereal β-glucan in the regulation of postprandial blood glucose and insulin levels [33]. Moreover, studies report that oat β-glucan particles, which are part of the cell wall, exist in a natural tissue-like structure which can encapsulate protein and starch in order to form a complex cell matrix in the cell wall. This matrix can reduce access enzyme which could slow down leads to reduced starch digestion and decreases postprandial glycemic response [34].

Based on studies, the indigestible form of β-glucan found in cereals can pass through the digestive tract. As a result, it can serve as a substrate for microbial fermentation and specifically promotes the growth and activity of a number of small beneficial microorganism, such as *Bifidobacteria* and *Lactobacillus*, which occur during the formation of SCFA [35]. The prebiotic impact of cereal β-glucan has now been linked to the viscosity of β-glucan and it has been shown to be influenced by the volume of non-fermentable polysaccharides in the colon of rats [36]. According to previous studies, the effects of oat β-glucan on intestinal flora depend on molecular weight and dose. The better the effect so that the lower the molecular weight and the larger the dose. In addition, recent studies have shown that administration of cereals β-glucan increase the number of *Lactobacillus* in rats [37].

CONCLUSIONS

Based on this study, it can be concluded that XOS can improve blood glucose and increase SCFA in diabetic rats. However, XOS did not increase the number of *Lactobacillus* and *Roseburia*. It is important to be paid more attention to increase the dose and duration of the treatment by using XOS and environmental factors, such as measuring the pH in cecum. In conclusion, β-glucan can improve the blood glucose in diabetic rats so that it can increase the number of *Lactobacillus* and *Roseburia* as well as SCFA.

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AUTHOR CONTRIBUTIONS

IEZ, the main researcher on the study, had designed and conceived, written the manuscript, and performed data collection. AZJ and EM checked the text and contributed suggestions on its interpretation and data analysis. Meanwhile, MS and AP were reviewers of the manuscript. The final version of the manuscript was approved by all authors.

CONFLICTS OF INTEREST

There is no conflict of interest among the authors.

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