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Research Article

Arrowroot (*Maranta arundinacea* L.) Tuber Extract Modulated Gut Microbiota and Improved Glycemic Control in Type 2 Diabetic Mice

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ABSTRACT

Type 2 diabetes mellitus (T2DM) is a metabolic disorder characterized by hyperglycemia and has become a global health concern. Gut microbiota alterations significantly influence diabetes onset, which can be mitigated through prebiotic intake. Arrowroot tubers (*Maranta arundinacea* L.) possess prebiotic potential due to their oligosaccharide and dietary fiber content. This study evaluated the effects of arrowroot tuber extract on blood glucose, glucose tolerance, and gut microbiota composition in a T2DM mouse model. Tubers were macerated in ethanol, and BALB/c mice were fed a high-fat diet for five weeks, followed by streptozotocin injection (35 mg/kg) to induce T2DM. Mice received 150 mg of arrowroot tuber extract orally for 28 days. Blood glucose levels were measured on days 0, 14, and 28, and an oral glucose tolerance test (OGTT) was performed. Cecal samples collected after four weeks underwent 16S rRNA sequencing, and data were analyzed using One-Way ANOVA ($p<0.05$). Treatment with arrowroot tuber extract for 28 days reduced blood glucose levels by 40.5% and improved glucose tolerance. The extract modulated gut microbiota, decreasing harmful bacteria while increasing short-chain fatty acid (SCFA)-producing genera such as Lachnospiraceae NK4A136, *Ligilactobacillus*, and *Alistipes*. These results indicate that arrowroot tuber extract can beneficially alter gut microbiota composition and ameliorate hyperglycemia, suggesting its potential as a prebiotic-based therapeutic strategy for managing T2DM.

1. Introduction

In 2024, the adult world population with diabetes totalled 589 million, which is predicted to rise to 853 million by 2050 (International Diabetes Federation 2025). Type 2 diabetes mellitus (T2DM) is a type of diabetes characterized by pancreatic beta cell failure, which results in decreased insulin secretion. This type of diabetes mellitus is defined by poor glucose, lipid, and protein metabolism, rendering the body unable to maintain glucose homeostasis (Galicia-Garcia *et al.* 2020). This disease is also attributed to insulin resistance, leading to the incapacity of tissues (liver, muscle, and adipose) to respond to insulin. The reduction in insulin production and the development of insulin resistance led to hyperglycaemia, which contributes to the pathophysiology of T2DM. Elevated blood sugar levels, linked to other metabolic disorders, can harm several organs, and heighten the risk of consequences (Barrière *et al.* 2018). Numerous risk factors had been identified for T2DM and recent studies have demonstrated the significant role of gut microbiota in the etiology and progression of T2DM (Chong *et al.* 2025).

The human gut contains millions of bacteria that constitute a complex microbial ecosystem. Healthy gut microbial health is defined by a harmonious balance of microbial abundance and diversity. The beneficial effects of gut microorganisms for host health include digestion, vitamin production, immune system regulation, and

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defence against harmful bacteria (Pircalabioru *et al.* 2021). Dysbiosis refers to an imbalance in the gut microbiota and is linked to various diseases, including diabetes. Alteration in gut microbial composition will influence metabolism, inflammatory conditions, and insulin resistance (Al-Rashidi 2022). Differences in gut microbiota composition exist between T2DM patients and healthy individuals, including a reduction in gut bacterial diversity and dysbiosis in the Firmicutes to Bacteroidetes ratio (Polidori *et al.* 2022). Metabolic endotoxemia in type 2 diabetes mellitus (T2DM) may arise from the accumulation of lipopolysaccharides due to an increase in gram-negative bacteria, along with compromised gut barrier function, leading to low-grade inflammation that induces insulin resistance, obesity, and T2DM (Lu *et al.* 2021).

The modulation of gut microbiota is crucial in the prevention of metabolic illnesses and exerts positive effects on diabetic situations. Prebiotics are one method to alter the gut microbiome. Research indicates that the treatment of the prebiotic inulin to C57BL/6 mice with type 2 diabetes mellitus can equilibrate the ratio of Bacteroidetes and Firmicutes (Li *et al.* 2023). Prebiotics can modify the gut microbiota by supplying substrates that gut bacteria ferment, hence augmenting the population of short-chain fatty acid (SCFAs) generating bacteria. The synthesis of SCFAs such as acetate, propionate, and butyrate might affect metabolic pathways and serve as a mechanism to prevent and regulate type 2 diabetes (T2DM) (Salamone *et al.* 2021).

Prebiotic can be derived from numerous plants, including arrowroot tubers (*Maranta arundinacea* L.), which contain 8.7% oligosaccharide and 5.0% soluble dietary fibre (Harmayani *et al.* 2011; Kumalasari *et al.* 2012). The metagenomic study of murine intestinal microbiota demonstrated the prebiotic potential of arrowroot tubers, revealing an increase in beneficial bacteria, notably *Bifidobacter* and *Lactobacillus*, alongside a reduction in pathogenic bacteria following the administration of arrowroot tuber extract (Waskita *et al.* 2024). Consequently, alterations in gut microbiota composition resulting from the supplementation of arrowroot tuber extract may provide a potential therapeutic approach for the control of T2DM. Nonetheless, no research has investigated the prebiotic potential of arrowroot tuber on gut microbiota in the context of Type 2 Diabetes Mellitus. This study was aimed to evaluate the impact of arrowroot tuber extract on hypoglycaemic effects, glucose tolerance, and the enhancement of caecal bacterial communities in a T2DM Mus musculus BALB/c, induced by a high-fat diet and streptozotocin.

2. Materials and Methods

2.1. Extraction of Arrowroot Tubers

The materials used were arrowroot tubers (*Maranta arundinacea* L.) obtained from Boyolali, Central Java. Arrowroot tubers as much as ±7 kg were washed, cut into thin slices, and dried using an oven at 50°C for 72 hours. Next, the arrowroot tubers were pulverized with a dry mill and sieved using a 40-mesh sieve. Extraction was carried out by maceration method using 80% ethanol solvent with a ratio (b/v) of 1:2, for 3 × 24 hours duration with solvent replacement every 24 hours. The filtrate filtered with Whatman filter paper and concentrated using a rotary evaporator at 60°C. Furthermore, the remaining solvent was evaporated using a waterbath at 60°C to obtain a thick paste-shaped extract.

2.2. Animal Modeling of T2DM

The experiment has evaluated and approved by the ethics commission for animal use at the Faculty of Medicine, Sebelas Maret University (No. 50/UN27.06.11/KEP/EC/2024). Male Mus musculus strain BALB/C, aged 6-8 weeks and weighing 20-25 grams, were obtained from CV Dunia Kaca, Karanganyar. Mice were acclimatized for seven days with ad libitum access to drinking water and BR feed (Comfeed, Japfa). T2DM was induced by administering a high-fat diet (HFD) comprising 60% normal feed, 20% pork oil, 15% duck egg yolk, and 5% sucrose. The normal group during the modelling phase was sustained on conventional BR feed. The HFD was supplied for five weeks, and the body weight of the mice were measured weekly (Zhang *et al.* 2018). Streptozotocin/STZ (Bioworld) was administered intraperitoneally at a dosage of 35 mg/kg body weight for a duration of 5 days, using a volume of 0.2 mL. The STZ injectable solution was formulated by dissolving STZ in a 0.1 M citrate buffer at pH 4.5. Following STZ injection, stabilization was conducted for a duration of 7 days (Ramalingam *et al.* 2020). Next, the mice were fasted for approximately 12 hours while still being given water, then their blood sugar levels were checked to ensure that they were diabetic. Mice were considered diabetic if their blood sugar levels were above 200 mg/dL (Gebremeskel *et al.* 2020).

2.3. Experimental Design

Mice were randomly divided into four groups: group 1 (normal mice), group 2 (T2DM control), group 3 (inulin 150 mg/day), and group 4 (arrowroot tuber extract 150 mg/day), with each group comprising 5 duplicates. The experiment on mice was conducted for 28 days with oral administration of the preparation. Normal and control groups of mice were given aquabidest. The inulin group was used to compare the effects of the extract with known prebiotics. The body weight of the test animals was measured weekly during the experiment. Blood glucose levels were measured after STZ injection at weeks 0, 2, and 4 during treatment, as well as an oral glucose tolerance test (OGTT) at the end of treatment. After completion of experiment, DNA extraction and metagenomic analysis of mice cecal bacterial community were performed.

2.4. Measurement of Blood Glucose Levels

Blood was extracted from the tail vein following disinfection with an alcohol swab. The blood was applied to the glucose test strip of the Easy Touch Glucometer (Wang *et al.* 2020).

2.5. Oral Glucose Tolerance Test

Mice were administered feed for 10 hours, and the initial blood glucose level was recorded at the 0-minute mark. Further, mice received an oral glucose injection of 1 g/kg body weight, and blood glucose levels were assessed at 30, 60, and 120-minute intervals using the Easy Touch Glucometer (Lee *et al.* 2021).

2.6. Cecum gDNA Extraction

At the end of the experiment, the mice were dissected, and the cecum was collected for bacterial metagenomic investigation. Cecum from 5 mice per treatment was combined as composite sample. The cecum samples were rinsed with sterile 0.9% NaCl and subsequently sectioned into smaller fragments. Cecum samples were pulverized with a mortar and pestle until homogeneous and weighed 150 mg. The gDNA from the cecum was extracted utilizing the ZymoBIOMICS™ DNA Miniprep Kit (Zymo Research, USA). The quality and quantity of extracted DNA were assessed using a Biophotometer Plus (Eppendorf) at an absorbance of 260/280 nm.

2.7. Library Preparation and Sequencing

A metagenomic investigation of cecal bacteria was conducted by Next Generation Sequencing (NGS) technology using MiSeq Illumina platform at PT Genetics Science Indonesia. The 16S rRNA gene (in regions V3-V4) was amplified using specific primers 341F (5'CCTAYGGGRBGCASCAG'3) and 806R (5'GGACTACNNGGGTATCTAAT'3). All PCR reactions were performed with 15 µL of Phusion® High - Fidelity PCR Master Mix (New England Biolabs); 0.2 µM of forward and reverse primers, and 10 ng template DNA. Thermal cycling consisted of initial denaturation at 98°C for 1 min, followed by 30 cycles of denaturation at 98°C for 10 s, annealing at 50°C for 30 s, and elongation at 72°C for 30 s and 72°C for 5 min. The amplicons were isolated from 2% agarose gel and purified using the Universal DNA Purification Kit (TianGen, China, Catalog #: DP214) following the manufacturer's instructions.

Sequencing libraries were generated using NEB Next® Ultra™ II FS DNA PCR-free Library Prep Kit (New England Biolabs, USA, Catalog #: E7430L) following manufacturer's recommendations, and indexes were added. The library was checked with Qubit and real-time PCR for quantification and bioanalyzer for size distribution detection. Quantified libraries were pooled and sequenced on Illumina platforms, to generate 250 bp paired-end raw reads.

2.8. Data Analysis

Blood sugar levels, body weight, and OGTT data were analyzed as mean ± SEM. Statistical analysis employed One Way ANOVA followed by Tukey's post hoc test, with a significance threshold of $p<0.05$. Statistical analysis was conducted utilizing SPSS version 26 software. The Area Under Curve (AUC) computations were evaluated using One Way ANOVA, followed by Dunnett's post hoc test, employing Prism 8.0 software. Adapters and PCR primer sequences from paired-end reads, were eliminated using Cutadapt. DADA2 was employed to rectify sequencing errors, eliminate low-quality sequences, and remove error chimeras. The outcome was Amplicon Sequence Variant (ASV) data. The resulting ASVs data was used for taxonomic classification against SILVA (silva_nr99_v138.1). The rarefaction curve utilized to assess the quality and coverage of 16S rRNA gene metagenomic sequencing (V3-V4 region) in mouse cecum specimens. Bioinformatics analysis of NGS data was performed by calculating diversity indices using Chao1, ACE, Shannon, and Simpson indices. All indices were calculated using the Qiime program version 1.7.0 and displayed using R software version 2.15.3. Beta diversity was calculated using the Qiime program version 1.7.0.

Principal Coordinate Analysis (PCoA) was used as a hierarchical clustering method using the Qiime program.

3. Results

3.1. Animal Modelling of T2DM

Normal and HFD-fed mice had significant body weight differences after 5 weeks, as shown in Table 1. HFD feeding increased test animal body weight by 29-30%. Increased body weight due to HFD suggests obesity in test animals. Five weeks of HFD induction has not induced diabetes, however blood sugar levels are significantly higher than in the normal group (Table 2, $p<0.05$). Based on statistical analysis, there is a difference in blood sugar levels of mice injected with STZ higher than the normal group ($p<05.0$). All test animals that have been induced by HFD and STZ have blood sugar levels ≥200 mg/dL which indicates the success of T2DM modeling in test animals. Test animals that have experienced T2DM conditions are then randomly grouped consisting of normal groups, model control, inulin, and arrowroot tuber extract.

3.2. The Effect of Arrowroot Tuber Extract on Blood Sugar Levels and Body Weight

The effect of arrowroot tuber extract on mice blood sugar can be seen in Table 3. Initially, there was no significant difference in blood sugar levels in the control group, inulin, and arrowroot tuber extract ($p>0.05$), indicating the three treatments groups had homogeneous blood sugar levels and were in a diabetic condition. On day 14, administration of the extract did not cause any difference in blood sugar levels between the arrowroot extract group and the control group, but in the inulin group, blood sugar levels decreased significantly. Furthermore, on day 28, there was a significant difference in blood sugar levels between the arrowroot tuber extract treatments with the control group ($p<0.05$). However, no significant difference was found between the inulin and extract treatments at the end of the treatment. This indicates that the administration of arrowroot tuber extract has the same ability as inulin in reducing blood sugar levels in T2DM mice after 28 days of treatment. The decrease in blood sugar levels in the group given the extract treatment was 40.5%, lower than that given inulin, but the blood sugar levels in both groups were still not within the normal range. Thus, mice are still in hyperglycemia but there is a significant decrease in blood sugar levels when compared to diabetic mice without treatment.

Table 1. Body weight of mice during modeling of T2DM

Weeks	Body weight (gram) ± SEM			
	Normal group	High fat diet induction		
		Group I	Group II	Group III
0	22.96±0.95^a	23.7±0.38^a	22.38±0.72^a	23.86±0.15^a
1	23.68±1.00^a	31.48±1.14^b	31.88±1.10^b	29.06±1.76^b
2	24.44±0.79^a	32.48±1.09^b	33.32±1.49^b	31.2±1.82^b
3	24.68±0.54^a	33.06±0.62^b	33.92±1.20^b	32.34±1.99^b
4	25.18±0.42^a	34 ± 1.00^b	35.12±1.31^b	33.62±2.25^b
5	24.98±0.25^a	34.7±1.21	35.06±1.39^b	33.8±2.12^b
STZ injection	25.7±0.61^a	32.3±2.63^b	33.1±2.46^b	31.66±4.69^b

different letter notation in the same row indicates significant difference with p value <0.05

Table 2. Blood sugar levels of mice during T2DM modeling

Time	Blood glucose levels (mg/dl)			
	Normal group	Injeksi STZ		
		Group I	Group II	Group III
Adaptation	93±1.41^a	88±2.43^a	93.6±2.16^a	93.8±2.96^a
HFD induction	88.2±3.93^a	136±3.82^b	137.4±2.25^b	139.8±4.83^b
STZ injection	89±5.12^a	227.6±14.04^b	225.2±10.24^b	227.4±5.84^b

different letter notation in the same row indicates significant difference with p value <0.05

Table 3. Blood sugar levels in mice on days 0, 14, and 28 of treatment

Day to-	Blood glucose levels (mg/dl) ± SEM		
	Control	Inulin	Arrowroot
0	294.8±23^a	259.8±11.7^a	242.8±8.7^a
14	249.6±19.3^a	179.4±6^{bc}	217.6±15.8^{ab}
28	252.4±18.9^a	139±13.3^b	144.4±9.2^b
Percentage decrease from day 0 to 28	14.4%	46.5%	40.5%

Different letter in the same row indicates significantly different results (p value <0.05)

Body weight measurements were also taken to determine the effect of the extract on changes in body weight of diabetic (Figure 1). At the end of the treatment, the body weight of the control group was significantly lower than that of the inulin-treated group ($p<0.05$). However, the body weight of the arrowroot tuber extract treatment was not significantly different from the control group. These results indicate that inulin treatment can significantly prevent significant weight loss in T2DM mice while the administration of arrowroot tuber extract cannot prevent weight loss.

3.3. Oral Glucose Tolerance Test (OGTT)

The effect of giving arrowroot tuber extract on glucose tolerance in T2DM model mice was determined by OGTT. This test is used as an index to evaluate pancreatic islet function, if insulin secretion is

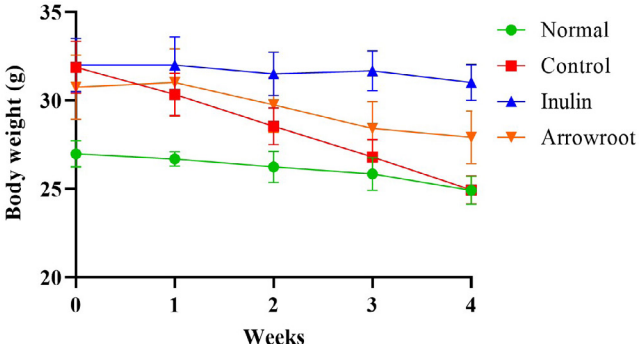

Figure 1. Body weight of diabetic mice following treatment with 150 mg/day of prebiotics. Normal: non-diabetic mice; control: T2DM mice that received no treatment; Inulin: 150 mg/day inulin; Arrowroot: 150 mg/day Arrowroot tuber extract

inadequate then post prandial blood sugar levels will be high. In addition, OGTT is also generally used for the diagnosis of metabolic diseases including diabetes and estimating the body's ability to regulate blood sugar levels. The results indicated that the control group had impaired glucose tolerance, indicated by blood sugar levels that remained consistently high during the test (Figure 2A). However, the inulin and arrowroot tuber extract treatment groups showed significant decreases at all points within 120 minutes after glucose ingestion. At the end of the test, blood sugar level of arrowroot tuber extract group was not significantly different from the normal mice group, which indicated that arrowroot tuber extract could improve impaired glucose tolerance in T2DM mice.

The Area Under Curve (AUC) of the OGTT assesses the impact of prebiotics on postprandial blood glucose levels, quantified by the total blood glucose over 120 minutes. The AUC for each treatment is illustrated in Figure 2B. An increase in AUC signified impaired glucose tolerance in the control group. In the arrowroot tuber extract group, AUC was lower relative to the control group, even to the inulin ($p<0.05$). These findings demonstrate that arrowroot tuber extract effectively inhibit postprandial blood glucose elevation.

3.4. Alfa and Beta Diversity of Mice Cecum Bacterial Community

The rarefaction curve (Figure 3) illustrates that the attained sequencing depth is adequate in all groups, as additional sequences will not uncover many new taxa (species). Consequently, it can be inferred that this work has effectively documented the predominant taxonomic diversity within the mouse cecum bacterial

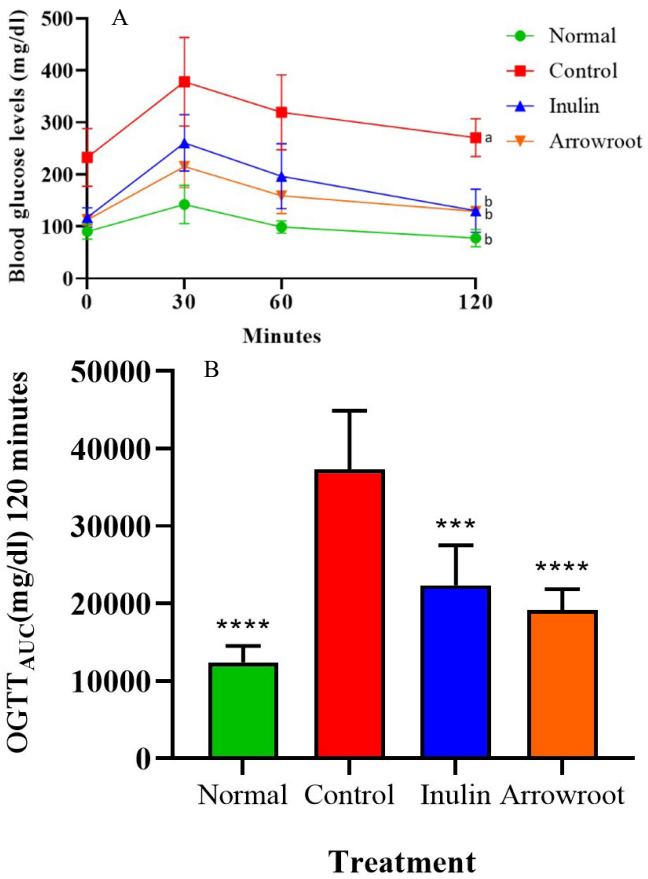

Figure 2. Oral glucose tolerance test of diabetic mice following treatment with 150 mg/day of prebiotics. (A) Post-prandial blood glucose levels, groups sharing the same letter are not significantly different ($p\geq0.05$, Tukey's test). (B) Area under curve, data are expressed as mean ± SEM ($n = 5$). Statistical analysis by one-way ANOVA followed by Dunnett's multiple comparisons test, comparing each treatment with the diabetic control. *$p<0.05$; **$p<0.01$; ***$p<0.001$; ****$p<0.0001$. Normal: non-diabetic mice; control: T2DM mice that received no treatment; Inulin: 150 mg/day inulin; Arrowroot: 150 mg/day Arrowroot tuber extract

population in all groups, thereby ensuring the high validity of the data utilized for subsequent analysis.

The cecum microbial community in all groups has high bacterial diversity with the highest diversity was observed in Arrowroot tuber extract group based on observed species, Shannon, Simpson, and inverse Simpson index (Table 4). The diabetes mice demonstrated lower richness but elevated Shannon and Simpson indices relative to the normal group. This may indicate a more equitable distribution of microbial species post-STZ induction, likely resulting from the decline of dominating taxa and the expansion of opportunistic species. Inulin supplementation

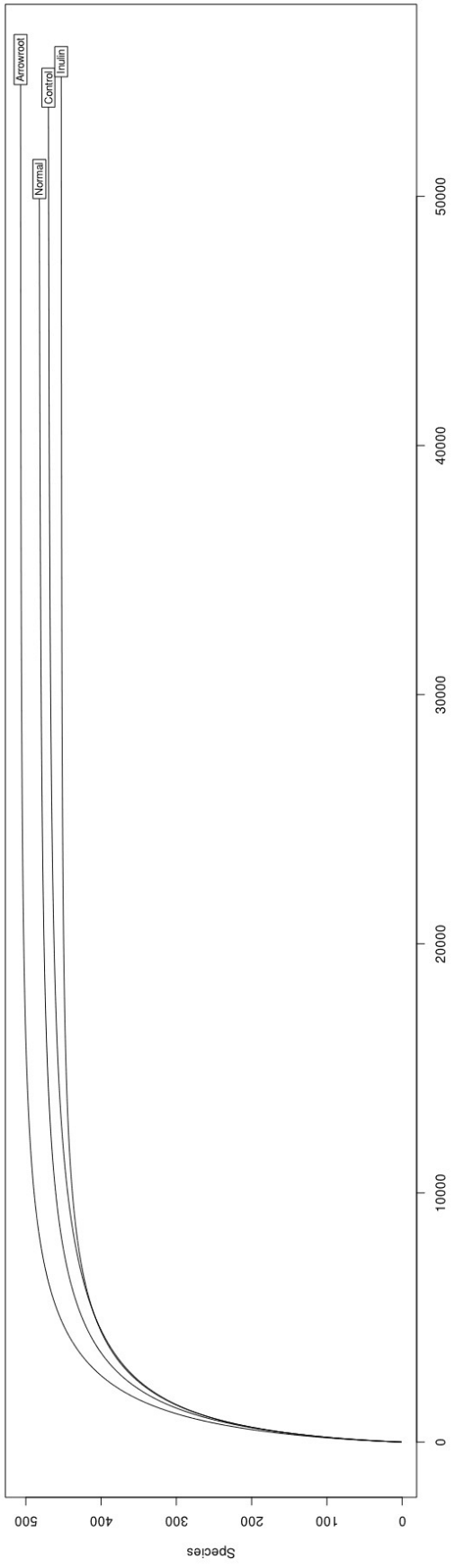

Figure 3. Rarefaction curve plotting the number of species in the 16S rRNA gene library by the number of sequences samples from cecum in all mice groups. Normal: non-diabetic mice; control: T2DM mice that received no treatment; Inulin: 150 mg/day inulin; Arrowroot: 150 mg/day Arrowroot tuber extract

Table 4. Alpha diversity of mice cecum bacterial communities following treatment with 150 mg/day of inulin or arrowroot tuber extract

Diversity index	Normal	Control	Inulin	Arrowroot
Observed Species	482.00	470.00	453.00	507.00
Shannon	4.80	4.93	4.86	5.10
Simpson	0.96	0.98	0.97	0.98
Inverse Simpson	27.46	45.96	34.28	47.33

normal: non-diabetic mice; control: T2DM mice that received no treatment; Inulin: 150 mg/day inulin; Arrowroot: 150 mg/day Arrowroot tuber extract

resulted in diminished species richness, aligning with its established selective prebiotic impact, however preserved comparatively elevated Shannon and Simpson indices, signifying a stable community equilibrium. Conversely, treatment with arrowroot extract yielded the greatest richness and diversity. The data indicate that arrowroot extract promotes a more diverse and equally distributed bacterial community than inulin.

The Venn diagram (Figure 4) illustrates both the shared and unique bacterial taxa across treatment groups. As many as 122 ASVs were common to all mouse groups. The presence of these core ASVs signifies the existence of essential bacterial taxa that are consistently observed irrespective of alterations in dietary treatment. Moreover, unique diversity (species exclusive to a single group) exhibited considerable variance.

The normal group demonstrated 189 distinct species, underscoring the diversity of a healthy gut microbiome that is partially diminished in diabetic settings. The control group maintained 143 unique species, indicating a transition towards a separate dysbiotic community. These taxa are probably enriched with opportunistic or pathogenic bacteria linked to hyperglycemia and metabolic dysregulation.

Supplementation with arrowroot extract yielded the highest diversity of unique species, suggesting that arrowroot enhances a more extensive bacterial repertoire than inulin or the control groups. This corresponds with the alpha diversity findings, wherein arrowroot had the highest indices of richness and diversity. Conversely, inulin supplementation resulted in the lowest number of distinct species, indicating its established capacity to selectively stimulate particular beneficial taxa, like *Bifidobacterium* and *Lactobacillus*. This selectivity improves specific microbial functions but may diminish overall species richness.

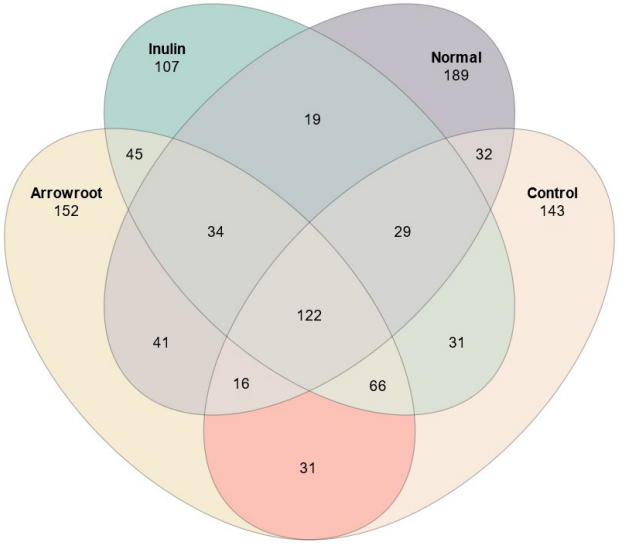

Figure 4. Venn diagram showing the shared and unique bacterial taxa among the cecal microbiota of mice in different treatment groups following supplementation of prebiotics. Note: normal: non-diabetic mice; control: T2DM mice that received no treatment; Inulin: 150 mg/day inulin; Arrowroot: 150 mg/day Arrowroot tuber extract

Taken together, these results suggest that arrowroot extract exerts a broad-spectrum prebiotic effect, increasing microbial richness and supporting diverse community structures beyond those promoted by inulin. Such diversity may contribute to greater metabolic flexibility and resilience of the gut microbiota, which is particularly relevant in the context of T2DM-associated dysbiosis.

The beta diversity of bacteria in the mouse cecum for each group was assessed by Principal Coordinates Analysis (PCoA) and non-metric multidimensional scaling (NMDS) as shown in Figure 5. The PCoA plot clearly separating the diabetic control group from the normal group, while inulin and arrowroot clustered closely together. Similarly, NMDS analysis yielded consistent clustering patterns, further validating the observed group separations. The two ordination methods confirmed that arrowroot and inulin supplementation induced comparable shifts in the gut microbiota, distinct from the diabetic control and closer to the healthy profile.

3.5. Mice Cecum Bacterial Community Composition in Each Treatment

The relative abundance of bacteria at the phylum, family, and genus levels were assessed to enhance the understanding of the unique composition of the cecal bacterial population in all groups. The findings

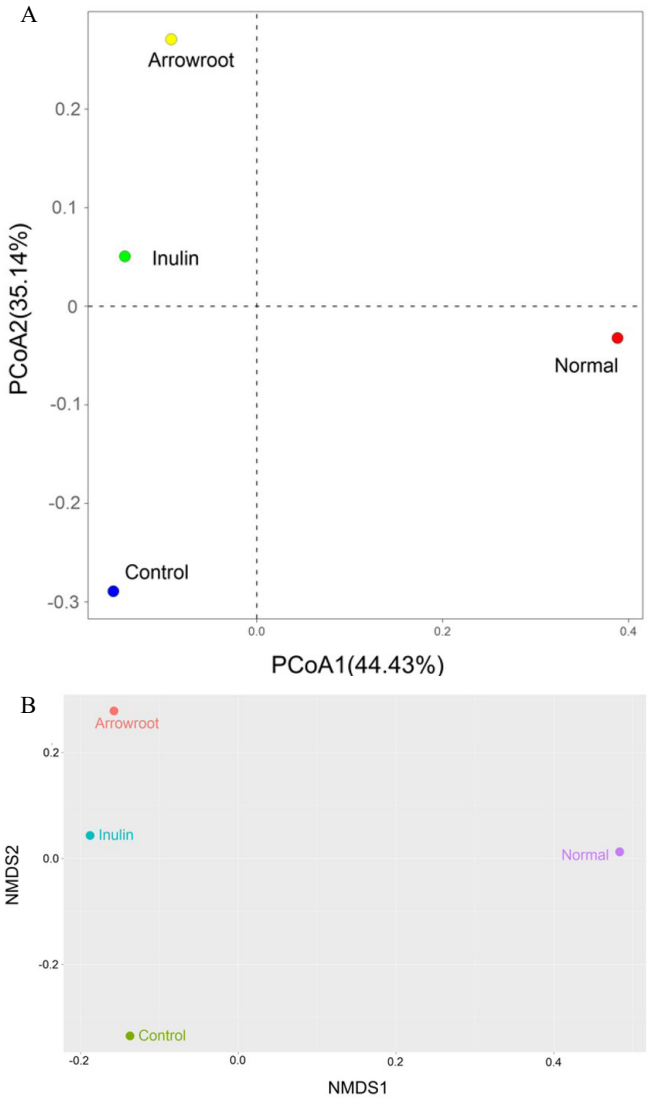

Figure 5. Beta-diversity analysis of cecum microbiota composition among treatment groups (A) Principal Coordinates Analysis (PCoA) Bray-Curtis Plot, (B) Non-metric Multidimensional Scaling (NMDS). Arrowroot and Inulin treatments shift the microbial community structure toward that of the Normal group. Note: normal: non-diabetic mice; control: T2DM mice that received no treatment; Inulin: 150 mg/day inulin; Arrowroot: 150 mg/day Arrowroot tuber extract

revealed the predominant phyla being Firmicutes and Bacteroidota (Figure 6A). The Firmicutes to Bacteroidota (F/B) ratio in the Arrowroot tuber extract treatments was 7:2, which was more similar to the normal group (7:2) and inulin group (6:3) than to the control group (5:4).

The family-level bacterial abundance was predominantly comprised of the Lactobacillaceae, Lachnospiraceae, Muribaculaceae, Bacteroidaceae, and

Ruminococcaceae groups (Figure 6B). In T2DM mice, the prevalence of Lactobacillaceae diminished, but the treatment of arrowroot tuber extract and inulin restored this family. Alongside the Lactobacillaceae family, the prevalence of Lachnospiraceae and Ruminococcaceae also rose in the arrowroot tuber extract group relative to the control group, akin to the inulin group. Conversely, the prevalence of Desulfovibrionaceae and Prevotellaceae diminished in the arrowroot tuber extract treatment (4%) and inulin treatment (2%) relative to the control group.

At the genus level, within the arrowroot tuber group, the genus Lachnospiraceae NK4A136 constituted the predominant genus, representing 24.5% of the total (Figure 6C). Simultaneously, the genus exhibiting a notable rise in abundance in both the arrowroot tuber extract and inulin groups was *Ligilactobacillus*, with 22.6% and 27.2%, respectively, in contrast to

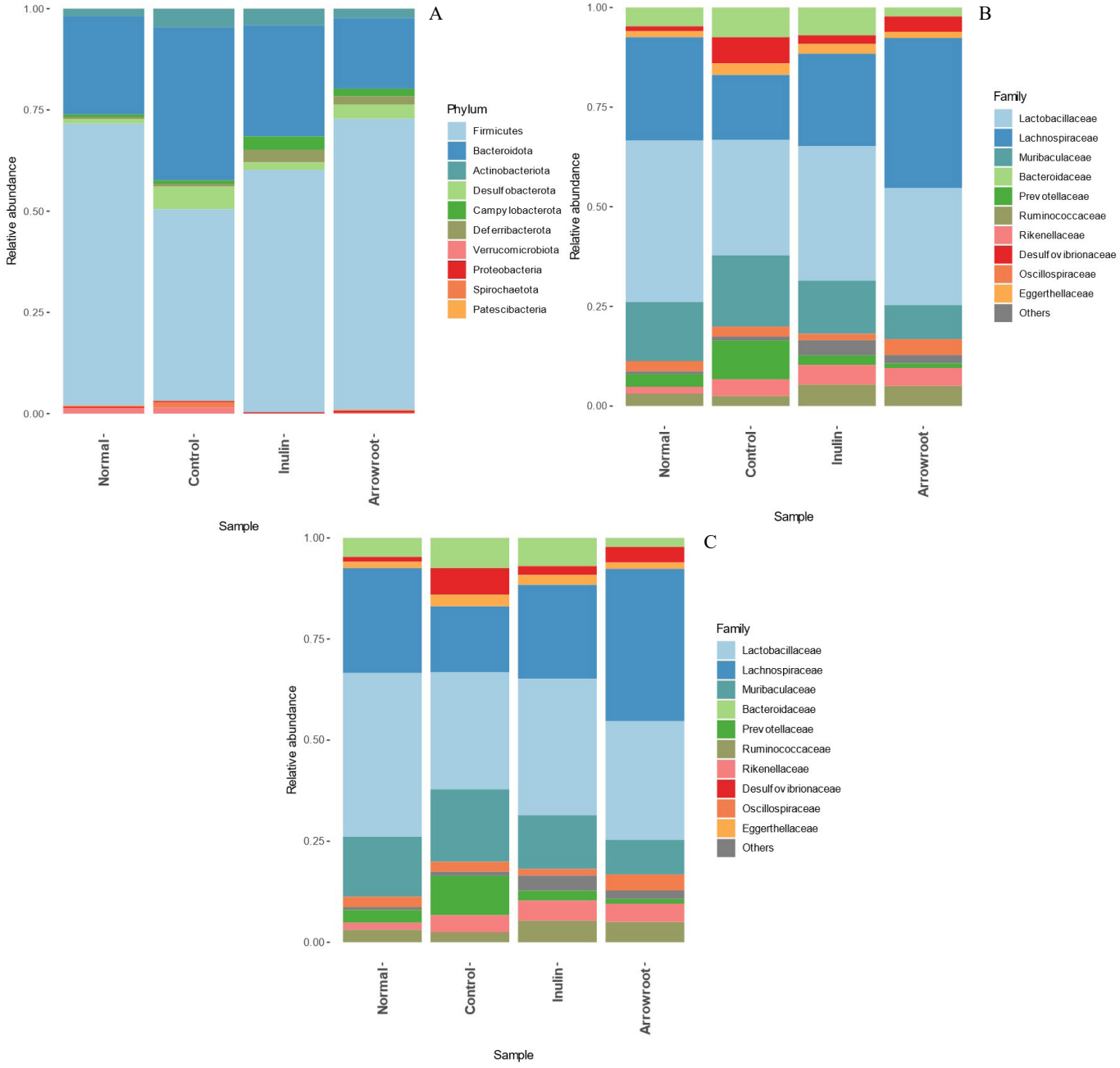

Figure 6. Relative abundance of bacterial communities in the cecum of mice following supplementation with prebiotics compared to normal and diabetic control groups at the phylum (A), family (B), and genus (C) levels. Normal: non-diabetic mice; control: T2DM mice that received no treatment; Inulin: 150 mg/day inulin; Arrowroot: 150 mg/day Arrowroot tuber extract

the control group, which registered merely 8%. The genus *Alistipes* exhibited a notable abundance in the arrowroot tuber extract and inulin groups, at 6.5% and 6%, respectively. Simultaneously, the prevalence of the genus *Prevotella* UCG-001 diminished with the administration of tuber extract relative to the control group, and its prevalence could be further decreased compared to the inulin group.

4. Discussion

This study found that giving T2DM mice arrowroot tuber extract (150 mg/day for 28 days) significantly decreased their blood sugar levels and improved glucose tolerance. The hypoglycemic effect observed is likely mediated through the modulation of the gut microbiota, as indicated by the shifts in microbial diversity and community composition. The T2DM control group showed the expected metabolic impairments, characterized by chronic hyperglycemia and diminished glucose tolerance, hence validating the efficacy of the HFD–STZ model in disease induction. Dysbiosis in this group was characterized by a reduced Firmicutes/Bacteroidota (F/B) ratio, decreased species richness, and the proliferation of potentially proinflammatory groups, including Desulfovibrionaceae and Prevotellaceae. These alterations align with prior studies associating T2DM with increased levels of endotoxin-producing Gram-negative bacteria and compromised intestinal barrier function (Larsen *et al.* 2010; Magne *et al.* 2020). Conversely, the Normal group exhibited equilibrated microbial communities characterized by elevated α-diversity and a predominant presence of the Firmicutes and Bacteroidota phyla (ratio ≈ 7:2), signifying a healthy and physiologically stable gastrointestinal environment.

Administration of inulin, a well-established prebiotic, was given as a positive control. Inulin treatment markedly decreased blood glucose levels and enhanced oral glucose tolerance, in line with prior findings that inulin supplementation enhances insulin sensitivity and enriches beneficial bacteria including *Lactobacillus, Bifidobacterium*, and *Lachnospiraceae* (Hughes *et al.* 2022; Riva *et al.* 2023; Corrêa *et al.* 2023). The inulin group exhibited a moderate increase in microbial diversity and clustered close to the arrowroot group in both the PCoA and NMDS analyses, indicating similar prebiotic actions. Inulin, on the other hand, tends to promote selective enrichment of a few species rather than broad microbial diversification, which is in line with what is known about its substrate specificity.

Interestingly, the arrowroot tuber extract group had the most diverse and even microbial population of all the treatments. Alpha-diversity indices (Shannon = 5.10; Observed species = 507) exceeded those of both the inulin and control groups. Beta-diversity analyses (PCoA and NMDS) demonstrated that arrowroot-treated mice clustered more closely with the Normal group than with the T2DM control, suggesting a partial restoration of microbiota composition towards a healthy condition. This indicates that arrowroot polysaccharides may exhibit a broader-spectrum prebiotic effect compared to inulin, potentially attributable to their more intricate carbohydrate composition, which encompasses raffinose, lactulose, and stachyose, along with both soluble and insoluble fibers (Harmayani *et al.* 2011; Senavirathna *et al.* 2014). Waskita *et al.* (2024) reported that the Arrowroot tuber contained the highest level of indigestible polysaccharides compared to other native Indonesian tubers and shown prebiotic activity both *in vitro* and *in vivo*.

At the phylum level, arrowroot supplementation restored the F/B ratio (7:2) to near-normal values, while decreasing Desulfobacterota and Proteobacteria, groups linked with sulfur metabolism and lipopolysaccharide release (Hao *et al.* 2020; Huang *et al.* 2021). Lactobacillaceae, Lachnospiraceae, and Ruminococcaceae rose significantly at the family level in the arrowroot and inulin groups compared to the diabetic control. These taxa are known to make SCFAs that help keep the intestinal barrier strong, control the immune system, and improve glucose metabolism (Zhao *et al.* 2020; Li *et al.* 2023).

At the genus level, *Ligilactobacillus*, Lachnospiraceae NK4A136 group, and *Alistipes* were more common in both prebiotic treatments, but they were slightly more prevalent in the arrowroot group. *Ligilactobacillus* species are recognized probiotics that exhibit antioxidant properties and glucose-lowering benefit (Hsieh *et al.* 2020; Zhou *et al.* 2024). Lachnospiraceae NK4A136 group is an important butyrate producer that has been connected to better insulin sensitivity (Galié *et al.* 2021; Wang *et al.* 2023). *Alistipes*, on the other hand, has been linked to positive metabolic benefits and lower insulin resistance (Takeuchi *et al.* 2023). Conversely, the prevalence of Prevotellaceae UCG-001, linked to inflammation and glucose dysregulation (Leite *et al.* 2017; Hong *et al.*

2022), diminished in both the arrowroot and inulin groups, nearing baseline levels.

The favorable metabolic results, characterized by reduced fasting glucose levels and enhanced glucose tolerance, are likely facilitated by elevated synthesis of short-chain fatty acids (SCFAs), particularly butyrate and propionate. SCFAs influence host metabolism by increasing GLP-1 production, decreasing intestinal permeability, and augmenting insulin sensitivity (Du *et al.* 2024). The intricate polysaccharide composition of arrowroot, featuring both soluble and insoluble fibers, offers a variety of fermentation substrates that facilitate the proliferation of numerous bacterial taxa. This wide range of fermentation profiles may help explain why there are more types of microbes than inulin, which has a simpler β-(2→1)-fructan structure that only allows a few types of fermenters (Riva *et al.* 2023).

These results demonstrate that arrowroot tuber extract provides metabolic and microbiological advantages similar to inulin, but with a more extensive enrichment of bacteria. The extract successfully lowered blood glucose levels, improved glucose tolerance, and reverted the microbial community composition to resemble that of healthy mice. These findings endorse the potential of arrowroot as an innovative functional food component possessing prebiotic and anti-diabetic characteristics. However, since the evidence on arrowroot tuber is still insufficient compared to traditional prebiotics, additional research incorporating metagenomic, metabolomic, and clinical methodologies is necessary to elucidate its processes and validate its usefulness in humans.

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