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Fiber and starch of *Colocasia esculenta* var. Mentawai ameliorate adiposity, dyslipidemia and gut dysbiosis in mice fed high fat diet

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ABSTRACT: Mentawai taro (*Colocasia esculenta* var. Mentawai, Araceae) corm is a staple food for local people in Mentawai islands, West Sumatra, Indonesia. This study aimed to determine whether the incorporation of fiber and starch extracted from Mentawai taro corm could improve adiposity and plasma lipid profiles and intestinal microbiota composition in mice fed a fatty diet. Adult male mice (n = 24) were assigned to four groups of diet treatments: normal diet (ND), high-fat diet (HFD), and HFD supplemented with 20% of fiber or starch from Mentawai taro corm, respectively. After 12-week treatment, body weight, adipose tissues, plasma lipid profiles and intestinal microbiota composition were investigated. The results showed that the incorporation of fiber and starch of Mentawai taro corm was capable of substantially preventing the excessive body weight increase against HFD. Moreover, fiber and starch could significantly suppress the increase of white adipose tissue mass and adipocyte hypertrophy while preventing the reduction of brown adipose tissue mass and adipocyte hypertrophy. The fiber and starch also could effectively reduce total plasma cholesterol, low-density lipoprotein-cholesterol, and triglyceride levels. The result also indicated that the fiber and starch of Mentawai taro corm could modulate the diversity of intestinal microbiota by promoting the health-beneficial taxa while suppressing the pathogenic taxa. Overall, the fiber effectivity in managing the detrimental effects of fatty diet outperformed the starch of Mentawai taro corm. Hence, it could be recommended as a potent supplement to combat diet-induced metabolic problems, particularly obesity, dyslipidemia, and gut dysbiosis.

KEYWORDS: adipose tissue; *Colocasia esculenta;* gut dysbiosis; high-fat diet; plasma lipids; staple food

1. INTRODUCTION

Diet-induced metabolic diseases are recognized as serious global health issues [1,2]. The prevalence of these diseases has significantly increased, not only in developed countries but also in many developing nations [3,4]. One prominent dietary factor contributing to metabolic diseases is a high-fat diet (HFD) [5]. Studies on animal models and human subjects have suggested that excessive consumption of fatty diets can contribute to various metabolic disorders, including obesity, adiposity, dyslipidemia, diabetes mellitus, fatty liver disease, and cardiovascular problems [6-9]. Furthermore, chronic intake of an HFD has been demonstrated to induce gut dysbiosis, an alteration in the composition of intestinal microbiota [10,11]. Gut dysbiosis is closely associated with increased risks of obesity, dyslipidemia, inflammatory dysregulation, and disturbances in blood glucose homeostasis [12,13]. On the other hand, dietary modifications, such as incorporating high-fiber content and resistant starch, are suggested to be effective in preventing and alleviating metabolic diseases and dysbiosis [14-17]. Our previous study also revealed that supplementing extracted fiber from the tuber of *Pachyrhizus erosus* could effectively prevent dysbiosis while improving metabolic profiles, including body weight, adipose tissue mass, and blood glucose levels in HFD-fed mice [18]. Therefore, explorative studies are necessary to define effective diets in combating HFD-induced metabolic diseases and dysbiosis.

Among the diverse natural-based resources for diets, taro from the species *Colocasia esculenta* (Araceae) stands out as a potent functional food with numerous health benefits [19,20]. Traditional practices have employed taro to manage various diseases, including gastrointestinal problems, hemorrhage, and

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alopecia [21]. Taro corm is known for being gluten-free and low in fat, making it suitable for individuals with dietary restrictions, including those allergic to gluten [22]. Moreover, taro corm contains a variety of nutrients, minerals, and vitamins that may support overall bodily health [23]. Taro is rich in polysaccharides, imparting antioxidative, antihyperlipidemic, and anti-inflammatory benefits [24]. These bioactivities may be attributed to potent phytochemical constituents such as tarin, polyphenols, digalactosyldiacylglycerols, A-1/B-2 α-amylase inhibitors, taro-4-1 polysaccharides, and nonphenolic antioxidants [21]. Hence, incorporating taro corm into foods and pharmaceuticals has significant potential in preventing diseases, including those induced by fatty diet.

Among the various cultivars of taro in the species *C. esculenta*, Mentawai taro (*C. esculenta* var. Mentawai) corm is commonly consumed as a staple food by the Mentawainese, indigenous people living in the Mentawai islands in the province of West Sumatra, Indonesia [25]. Recently, Mentawai taro has been promoted as a potent candidate for a national alternative food. With its rich starch and fiber contents, Mentawai taro corm is speculated to exert beneficial effects against diseases, including those induced by diet. However, studies focusing on the beneficial effects of fiber and starch from Mentawai taro corm against diet-induced health issues, particularly metabolic diseases, have been limited until recently. Additionally, its effect on intestinal microbiota composition remains to be elucidated. Therefore, this research was conducted to investigate whether incorporating fiber and starch extracted from Mentawai taro corm could be a preventive measure to manage adiposity, dyslipidemia, and gut dysbiosis caused by an HFD.

2. RESULTS

2.1. Effect of Fiber and Starch of Mentawai taro corm on body weight and adipose tissue profiles

Body weight monitoring conducted periodically for 12 weeks revealed that mice in the HFD group exhibited progressive body weight increments compared to all other groups (Figure 1A). In contrast, mice fed with taro fiber and taro starch had lower body weight at various time points of measurement during the experiments than those fed on HFD. Statistical analysis showed that those fed with taro fiber and starch had significantly lower body weight than that of the HFD-fed group ($P < 0.05$) starting from the fourth week of treatment. Additionally, there was no significant difference in body weight between the normal diet (ND)-fed group and the fiber or starch-fed groups ($P > 0.05$). Moreover, body weight gain was also markedly elevated in HFD mice, significantly differing from those fed ND, fiber, and starch of Mentawai taro corm (Figure 1B; $P < 0.01$). Importantly, there was no significant difference in body weight gain between the fiber or starch-fed group and the ND group ($P > 0.05$).

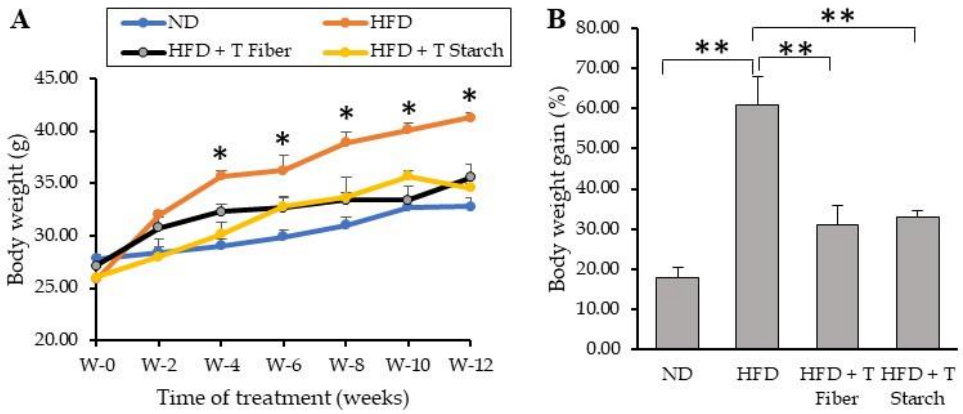

Figure 1. Effects of fiber and starch of Mentawai taro corm on body weight of mice. (A) Body weight measured weekly, (B) Change in body weight after 12-week treatments. ND (normal diet), HFD (high-fat diet), T Fiber (Taro fiber), T Starch (Taro starch). *) and **) indicate significant differences between compared groups based on Bonferroni Test at $P < 0.05$ and $P < 0.01$, respectively.

Investigation on adipose tissue masses (Figure 2) revealed that mice fed a fatty diet had noticeably higher white adipose tissue (WAT) weight compared to the control (ND) group ($P < 0.01$; Figure 2A). Furthermore, mice fed with fiber and starch had significantly lower WAT weight than the HFD group ($P < 0.01$ and $P < 0.05$, respectively). Similarly, the WAT index (the percentage of WAT mass to total body weight) showed a substantial increase in HFD-fed mice compared to the ND group ($P < 0.01$; Figure 2B). Both the fiber-fed and starch-fed groups exhibited a significantly lower WAT index than the HFD group,

with the difference being more apparent in fiber-fed mice ($P < 0.01$) compared to starch-fed mice ($P < 0.05$). Moreover, there was no significant difference in WAT weight and WAT index between the fiber-fed group and the ND group ($P > 0.05$). However, those treated with starch showed a substantial difference compared to the ND group ($P < 0.05$). Brown adipose tissue (BAT) mass data (Figure 2C) demonstrated a significant reduction in the HFD-fed group compared to all other groups, including ND, fiber, and starch-fed groups ($P < 0.05$). However, BAT index (the percentage of BAT weight to total body weight; Figure 2D) showed no significant difference between the HFD group and the ND group ($P > 0.05$), while those fed with fiber and starch showed a substantial increment in the WAT index compared to the HFD and ND group ($P < 0.05$). Additionally, the BAT index of the fiber-fed group and starch-fed group were statistically comparable ($P > 0.05$).

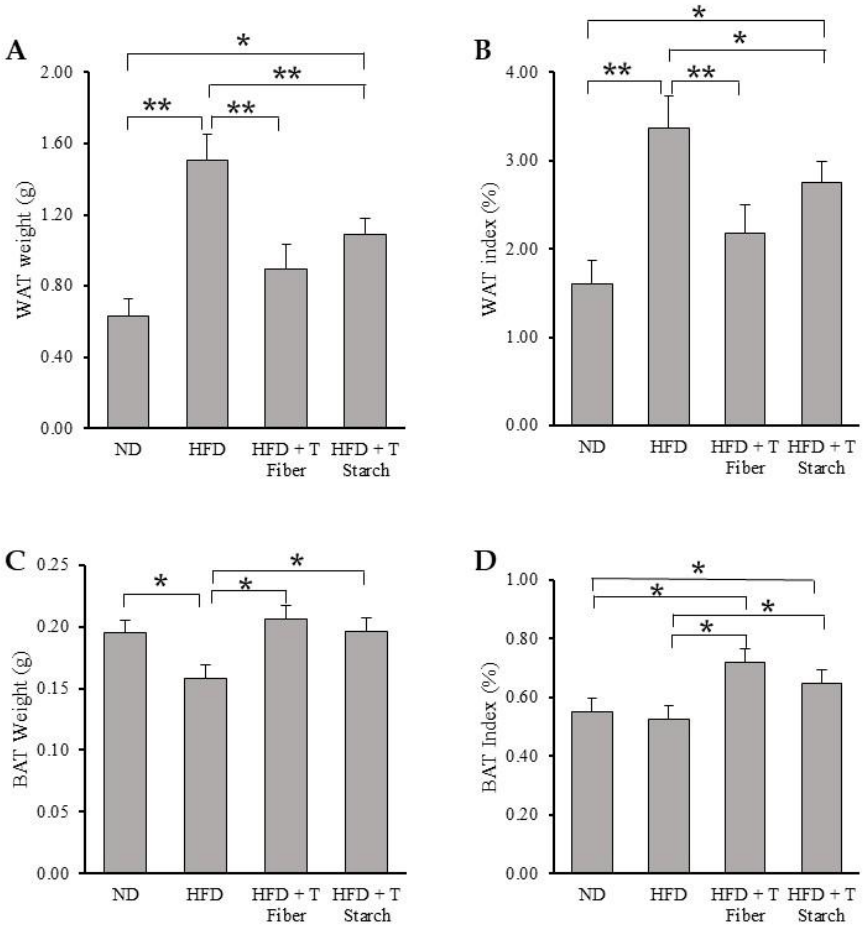

Figure 2. Effects of fiber and starch of Mentawai taro corm on adipose tissue mass in mice. (A) Weight of WAT; (B) WAT index against total body weight, (C) weight of BAT, (D) BAT index against total body weight. WAT (white adipose tissue), BAT (brown adipose tissue), ND (normal diet), HFD (high-fat diet), T Fiber (Taro fiber), T Starch (Taro starch), *) and **) indicate significant differences between compared groups based on Bonferroni Test at $P < 0.05$ and $P < 0.01$, respectively.

Microscopic examinations of WAT tissue (Figure 3A-D) revealed that mice fed with HFD experienced marked enlargement of white adipocytes compared with other groups. Further measurement of white adipocyte size (Figure 3E) demonstrated a marked increment in the HFD group compared to the ND and fiber-treated groups ($P < 0.01$). However, those treated with starch showed no significant difference in white adipocyte size compared to the HFD group ($P > 0.05$), but they were substantially larger than the ND and fiber-treated groups ($P < 0.01$ and $P < 0.05$, respectively). Microscopic observations of BAT tissue (Figure 4A-D) indicated that HFD-treated mice depicted larger brown adipocyte size compared with ND and fiber-fed groups. However, those in the starch-treated group exhibited an enlarged adipocyte size similar to the HFD group. The measurements on brown adipocyte size (Figure 4E) indicated a marked increment in the HFD group compared to the ND and fiber-fed groups ($P < 0.01$). Otherwise, those fed with starch exhibited no

significant difference with the HFD group ($P > 0.05$) while being substantially larger compared to the ND and fiber-fed groups ($P < 0.01$).

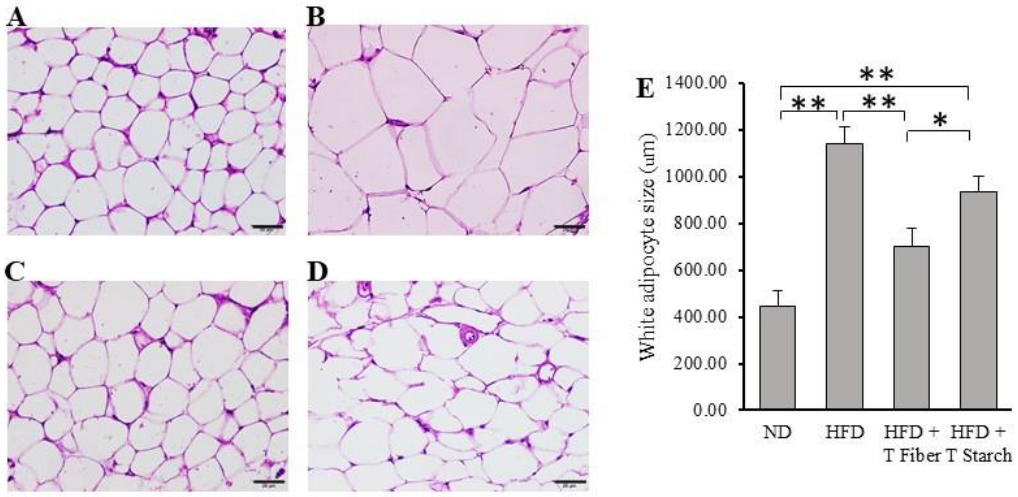

Figure 3. Effects of fiber and starch of Mentawai taro corm on histology of white adipocytes in mice. (A) photomicrograph of white adipocyte in ND group, (B) in HFD group, (C) in HFD + Taro fiber, (D) HFD + Taro starch, (E) Size of white adipocyte. ND (normal diet), HFD (high-fat diet), T Fiber (Taro fiber), T Starch (Taro starch), *) and **) indicate significant differences between compared groups based on Bonferroni Test at $P < 0.05$ and $P < 0.01$, respectively. Scale bars in A-D: 20 µm.

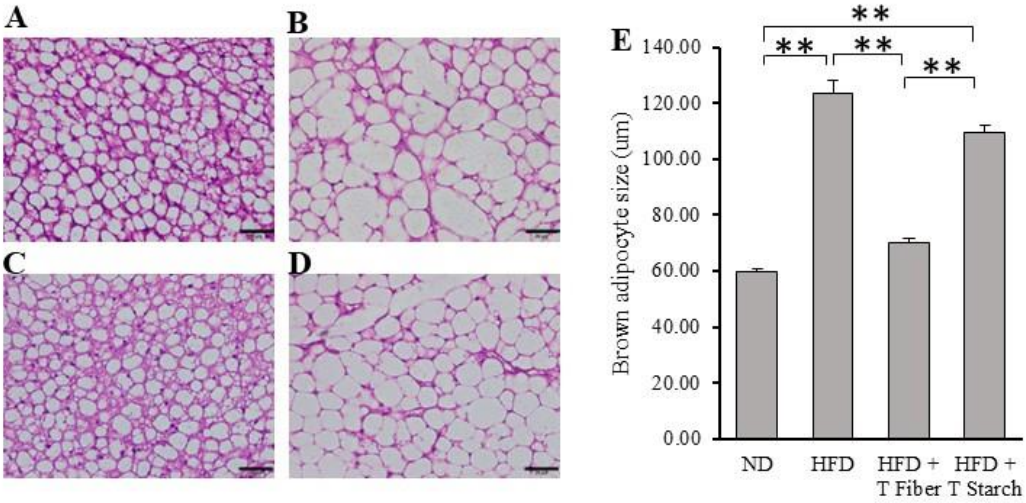

Figure 4. Effects of fiber and starch of Mentawai taro corm on histology of brown adipocytes in mice. (A) photomicrograph of brown adipocyte in ND group, (B) in HFD group, (C) in HFD + Taro fiber, (D) HFD + Taro starch, (E) Size of brown adipocyte. ND (normal diet), HFD (high-fat diet), T Fiber (Taro fiber), T Starch (Taro starch), **) indicate significant differences between compared groups based on Bonferroni Test at $P < 0.01$. Scale bars in A-D: 20 µm

2.2. Effect of Fiber and Starch of Mentawai taro corm on plasma lipid profiles

Measurements of plasma lipid concentration (Figure 5) at the end of the treatment revealed a significant increase in total cholesterol (TC) levels (Figure 5A) in the HFD group compared to the ND group ($P < 0.01$). Furthermore, individuals treated with fiber and starch of Mentawai taro corm exhibited comparable TC levels to the ND group ($P > 0.05$) but significantly different from the HFD-treated group ($P < 0.01$). There was no significant difference in TC levels between the fiber-treated group and the starch-treated group ($P > 0.05$). Additionally, the levels of high-density lipoprotein-cholesterol (HDL-C) (Figure 5B) showed a significant increment in the HFD group compared to the ND and fiber-fed groups ($P < 0.01$) while being comparable to the starch-treated group ($P > 0.05$). Furthermore, HDL-C levels in the starch-fed group were also significantly higher than the ND group and fiber-fed group ($P < 0.05$) while statistically comparable with the HFD group ($P > 0.05$). The levels of low-density lipoprotein-cholesterol (LDL-C) (Figure 5C) and triglycerides (TG) (Figure 5D) followed a similar pattern to TC levels, indicating that the

HFD group had markedly higher LDL-C and TG levels than the other groups ($P < 0.01$). Moreover, there was no statistical difference in LDL-C and TG levels among the ND, fiber-fed, and starch-fed groups ($P > 0.05$).

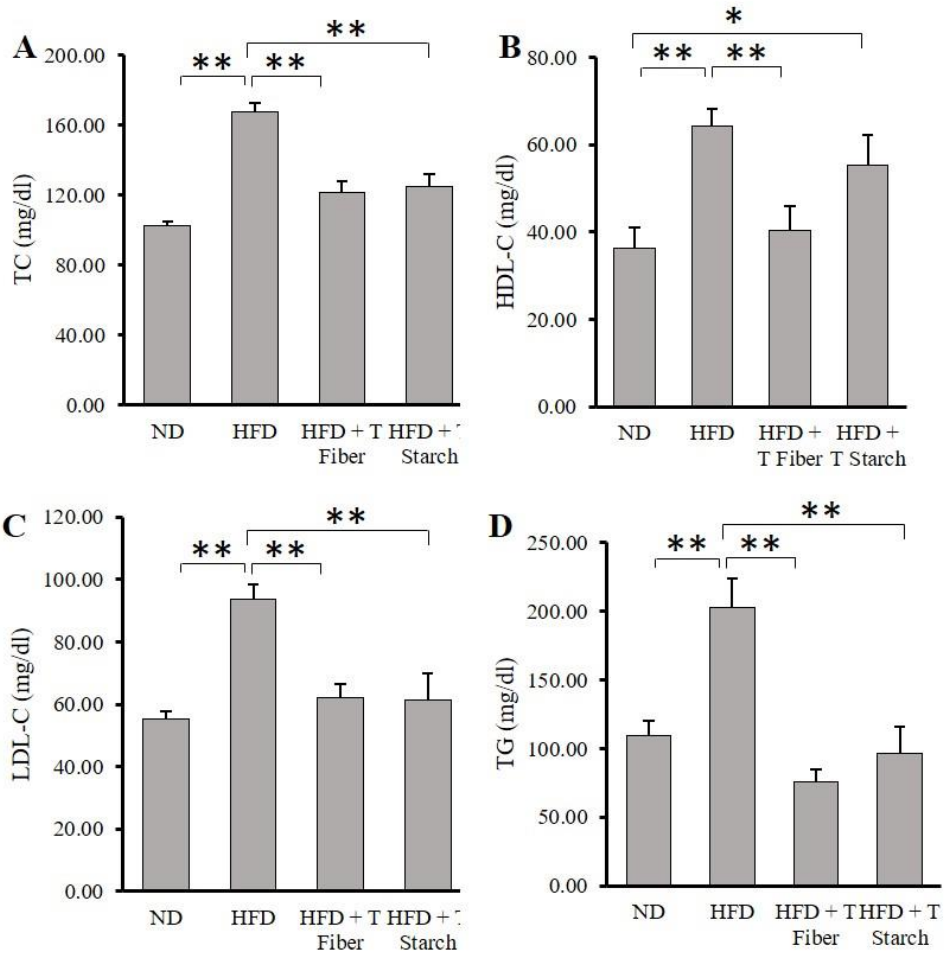

Figure 5. Effects of fiber and starch of Mentawai taro corm on plasma lipid profile in mice. (A) Level of total plasma cholesterol (TC), (B) Level of plasma high-density lipoprotein-cholesterol (HDL-C), (C) Level of plasma low-density lipoprotein-cholesterol (LDL-C), (D) Level of plasma triglyceride (TG). ND (normal diet), HFD (high-fat diet), T Fiber (Taro fiber), T Starch (Taro starch), *) and **) indicate significant differences between compared groups based on Bonferroni Test at $P < 0.05$ and $P < 0.01$, respectively.

2.3. Effect of Fiber and Starch of Mentawai taro corm on intestinal microbiota composition

To assess the composition of intestinal microbiota, fecal microbiota in the large intestine were analyzed using next-generation sequencing (NGS). As depicted in Figure 6A, at the phylum level, the HFD group exhibited dominance of Firmicutes, Bacteroidota, and Desulfobacterota. In contrast, the ND group was dominated by Firmicutes and Bacteroidota but not Desulfobacterota. Furthermore, mice in the fiber-fed group showed an increment in Bacteroidota dominance over Firmicutes with a markedly decreased portion of Desulfobacterota. On the other hand, in the starch-fed group, the proportion of Bacteriodota compared with Firmicutes was not as high as the fiber-fed group. Another phylum, namely Protobacteria, increased in the fiber group, while those treated with starch exhibited an increment in Spirochaetota. Furthermore, at the family level (Figure 6B), mice treated with ND showed a higher abundance of *Lachnospiraceae*, *Lactobacillaceae*, *Muribaculaceae*, and *Ruminococcaceae*. In contrast, mice treated with HFD exhibited a higher abundance of *Desulfovibrionaceae*, in addition to *Lachnospiraceae* and *Muribaculaceae*, with an apparent reduction in *Lactobacillaceae*. Furthermore, mice treated with fiber showed an increment of *Lactobacillaceae*, in addition to *Lachnospiraceae* and *Muribaculaceae*, resembling the ND-treated group. Next, those treated with starch had a relatively similar composition as the fiber-treated group but with a reduced portion of *Muribaculaceae* and *Lactobacilaceae*. Additionally, the abundances of *Erysipelotrichaceae* and *Rikenellaceae* increased in the starch-treated group.

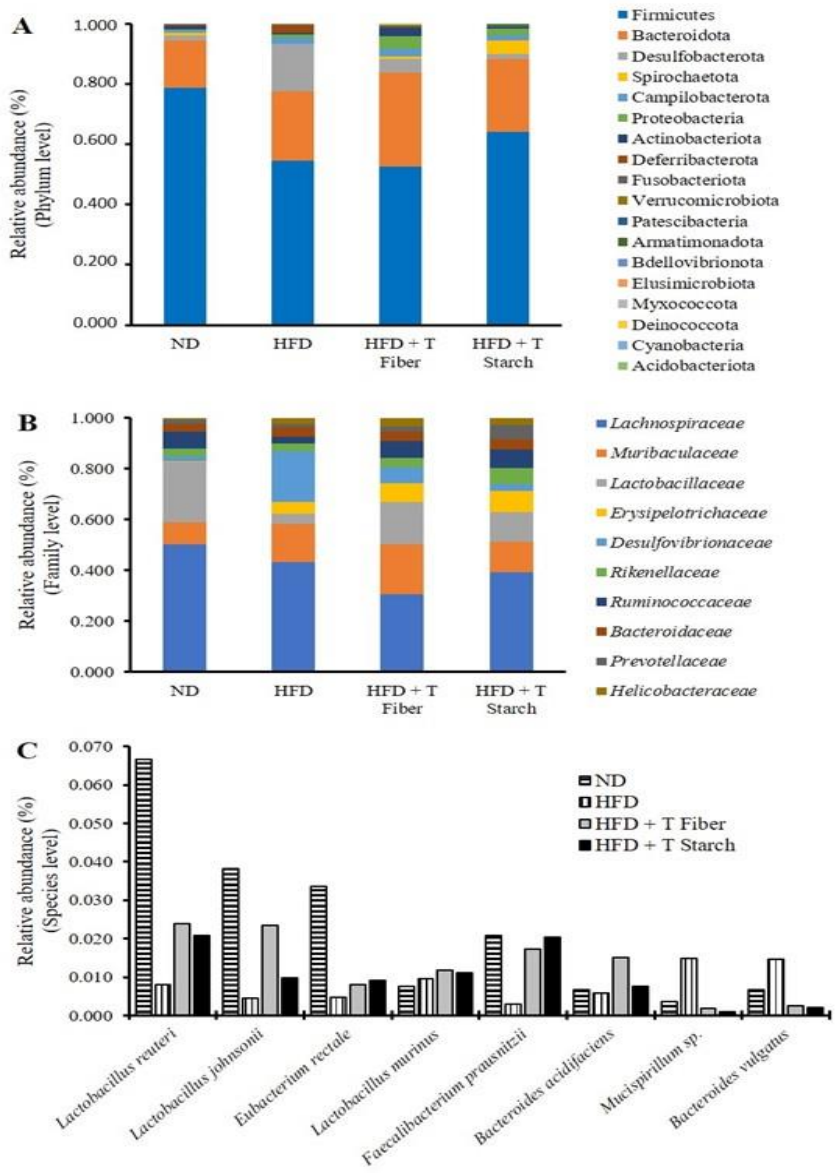

Figure 6. Effects of fiber and starch of Mentawai taro corm on intestinal microbiota composition in mice. (A) Relative abundance of major phylum of intestinal microbiota, (B) Relative abundance of major family of intestinal microbiota, (C) Relative abundance of major species of intestinal microbiota. ND (normal diet), HFD (high-fat diet), T Fiber (Taro fiber), T Starch (Taro starch).

The analysis of microbiota species composition (Figure 6C) demonstrated eight species with apparent abundance across the treatment groups. *Lactobacillus reuteri*, *L. Johnsonii*, *Eubacterium rectale*, and *Faecalibacterium prausnitzii* were highly abundant in mice fed with ND, while they were markedly reduced in the HFD-fed group. Conversely, *Mucispirillum* sp. and *Bacteroides vulgatus* were markedly high in the HFD group. Moreover, mice treated with fiber and starch of Mentawai taro corm exhibited an increment of *L. reuteri, L. johnsonii*, and *F. prausnitzii*, although the increments were not as high as in the ND group. Furthermore, mice fed with fiber and starch had a reduced abundance of *Mucispirillum* sp. and *B. vulgatus*, with those fed with fiber specifically showing an increment in *Bacteroides acidifaciens*.

3. DISCUSSION

The present findings reveal that fiber and starch extracted from the corms of Mentawai taro could prevent excessive body weight gain and improve adipose tissue and plasma lipid profiles in adult male mice subjected to an HFD. Both the fiber and starch from Mentawai taro corms demonstrated comparable beneficial effects on body weight gain, TC, LDL-C, and TG levels. However, the fiber was shown to be more effective in preventing WAT and BAT mass alterations as well as adipocyte hypertrophy, when compared to

the starch extract. Additionally, both fiber and starch modulated intestinal microbiota composition in HFD-fed mice at phylum, family and species levels.

It was found that the fiber and starch from Mentawai taro corm could mitigate excessive body weight gain, an indicator of obesity, resulting from chronic and excessive consumption of fatty diets. This finding aligns with a previous study that demonstrated the effective prevention of excessive body weight and WAT mass gain in diet-induced obese mice through the incorporation of fiber extracted from *P. erosus* tuber [18]. Another study concluded that proper dietary fiber intake in humans could manage obesity and related metabolic disturbances [26]. Likewise, a previous report suggested that a high-starch diet could effectively prevent the development of obesity and adiposity in adult male mice [27]. Additionally, resistant starch is also indicated to prevent the development of obesity and dysregulated carbohydrate metabolism in humans [28]. The marked elevation of body weight gain caused by HFD intake could result from lipid deposition in the WAT, acting as storage for excessive fat [18]. Similarly, in our study, body weight gain markedly elevated in line with increased WAT mass and WAT index in HFD-fed mice, while, in contrast, those fed with fiber and starch from Mentawai taro corm exhibited lower WAT mass and index with smaller white adipocyte sizes. This finding indicates that the incorporation of fiber and starch from Mentawai taro corm prevented fat deposition in WAT, thereby mitigating white adipocyte hypertrophy. However, it was found that the effectiveness of fiber was more pronounced than starch in preventing an increment of WAT mass and white adipocyte hypertrophy. This difference could be attributed to a higher energy content in starch compared with fiber. Hence, the consumption of starch from Mentawai taro might also contribute to the increment of total energy intake, thereby gaining WAT mass when compared with fiber, which has a lower accessible energy resource.

In addition to WAT, our present study also observed changes in BAT mass and its cells (brown adipocytes). BAT is a center of thermogenesis where fat, commonly in the form of TG, is catabolized to produce heat, thereby sustaining body temperature and increasing energy expenditure [29]. Studies in rodents have demonstrated that a HFD could significantly reduce BAT mass and its thermogenesis activity [30]. In our study, BAT mass was substantially reduced in HFD-fed mice, while it remained higher under fiber and starch treatment, indicating preventive effects against HFD. Furthermore, brown adipocyte size was enlarged due to HFD, indicating hypertrophy. In contrast, brown adipocyte size was substantially smaller in fiber-treated mice but larger in the starch-treated group. Changes in BAT mass and cell size under HFD challenge could be due to several factors, including disruption in hormonal signaling involving metabolic hormones (such as adiponectin, insulin, and leptin) [31]. Moreover, BAT could also be reduced due to inflammation as well as adipose tissue remodeling promoted by HFD [32]. Based on the histological observations in our study, it was clear that BAT in the HFD-fed group and starch-fed group underwent tissue remodeling to become white adipose, as indicated by their larger cell size, thereby accommodating more fat deposition. On the other hand, it was effectively inhibited in the fiber-treated group. The effectiveness of fiber from Mentawai taro corm in preventing a reduction in BAT mass and brown adipocyte hypertrophy might reflect its capability in regulating metabolic hormones, inflammation, and adipose tissue remodeling against HFD. As demonstrated by a previous report, fiber supplementation from *P. erosus* is effective in mitigating dysregulated metabolic hormones, inflammatory response, and adiposity [18]. Moreover, it has also been suggested that fiber intake promotes a substantial increase in energy expenditure, thereby improving lipid metabolism and adipose tissue profile [33]. Meanwhile, the starch treatment failed to counteract the HFD-induced BAT hypertrophy. This discrepancy may be due to its higher energy content compared with fiber. Unfortunately, our present study did not investigate metabolic hormones, inflammation, and energy expenditure. Furthermore, the energy content of fiber and starch from Mentawai taro corm was not determined. As a result, the exact mechanism underlying the beneficial effects of fiber in mitigating BAT alterations against HFD remains unelucidated. Future research is needed to define it.

Due to the excessive load of fatty acids from the diet, the levels of plasma lipids could be altered toward dyslipidemia [6,34]. Similarly, in our study, HFD treatment promoted marked increments in TC, HDL-C, LDL-C, and TG, indicating disrupted lipid metabolism. In contrast, the incorporation of fiber and starch from Mentawai taro corm could effectively suppress TC, LDL-C, and TG levels against the HFD challenge. This finding indicates the capability of fiber and starch from Mentawai taro in sustaining lipid metabolism. This capability could be attributed to several mechanisms. Firstly, the fiber and starch might exert a preventive effect against the enzymatic digestion of fat from the diet in the gastrointestinal tract, thereby reducing lipid absorption. Moreover, the fiber and starch could also elevate lipid oxidation, thereby mitigating hyperlipidemia in the circulatory system. Alternatively, the fiber and starch could also enhance short-chain fatty acids (SCFAs) production in the intestinal tract, thereby indirectly regulating lipid

metabolism. It has been demonstrated that fiber and resistant starch are capable of reducing lipase activity [35]. Moreover, fiber has been suggested to increase the metabolic rate, thereby enhancing lipid oxidation [33]. As a result, the lipid loading from the diet to the circulatory system could be reduced, while its utilization for energy production is elevated. Moreover, sufficient intake of fiber and starch (particularly resistant starch) promotes an increment in SCFAs production, thereby preventing adiposity and dyslipidemia [36].

In addition to improved physiological profiles, our present study also demonstrated the modulatory effect of fiber and starch from Mentawai taro corm on the intestinal microbiota community. It has been shown that a fatty diet profoundly promotes the increment of Firmicutes, a phylum that is commonly dominated by pathogenic species and attributed to the development of obesity, inflammation, and diabetes mellitus [37,38]. In contrast, an HFD is known to reduce Bacteroidota, a phylum associated with well-sustained metabolic regulation [39]. In our study, the fiber and starch of Mentawai taro corm reversely reduced the abundance of Firmicutes while elevating the composition of Bacteroidota, suggesting a modulatory beneficial effect. Additionally, fiber and starch supplementation also markedly reduced the abundance of Desulfobacterota. The increment of Desulfobacterota is also linked with obesity and metabolic disturbances [38,39]. Our current findings are also in line with a previous study demonstrating that supplementation of fiber from P. erosus modulated the composition of Firmicutes, Desulfobacterota, and Bacteroidota in HFD-fed mice [18]. Moreover, another study showed the ability of the flour and starch of taro in the species of *Xanthosoma sagittifolium* to modulate the microbiota composition in the gastrointestinal tract, including the dominance of Bacteroidota over Firmicutes [40]. It has also been reported that in type-2 diabetes mice models, taro starch was capable of altering gut microbiota composition, particularly by promoting the growth of health-beneficial taxa and enhancing SCFAs production [41]. Since SCFAs are beneficial to prevent metabolic diseases [36,42], their increment could be attributed to the improvement of health status against HFD.

Further investigation at the family level indicated that fiber and starch from Mentawai taro corm promoted the growth of *Lactobacillaceae* while suppressing *Desulfovibrionaceae*. In contrast, an HFD enhanced the abundance of *Desulfovibrinaceae* while reducing *Lactobacillaceae*, confirming the findings of a previous study [18]. *Lactobacillaceae* is known to be associated with improved lipid profiles against dyslipidemia [43]. On the other hand, *Desulfovibronaceae* is linked with obesity and hepatic steatosis in both rodents and humans [44,45]. It has also been reported that *Lactobacillaceae* could counteract the detrimental effect of *Desulfovibronaceae* under HFD treatment [39]. Next, fiber and starch from Mentawai taro corm substantially enhanced the growth of *L. reuteri*, *L. Johnsonii*, and *F. prausnitzii*. These beneficial species are important for sustaining the function of the gut barrier in the intestinal tract to prevent the development of diet-induced diseases, including obesity and dyslipidemia [43, 45-47]. In addition, fiber and starch from Mentawai taro corm also substantially suppressed the abundance of *Mucispirillum* sp., a species that is closely linked to metabolic dysregulation and related health issues [48].

It was found that fiber from Mentawai taro corm was more favorable than the starch in promoting the growth of beneficial taxa of intestinal microbiota against HFD. This difference may be attributed to the fact that fiber is completely undigestible, thereby increasing the fermentative activity of the beneficial taxa. In contrast, starch is composed of both digestible and undigestible (resistant) portions. Hence, the capability of fiber in promoting the fermentative activity of the beneficial microbiota could be higher than that of starch. As a consequence, the beneficial taxa will be richer in those fed with fiber compared to those treated with starch. This could also explain why, in some cases (WAT and BAT profiles), mice treated with fiber exhibited a better condition compared to starch-treated mice.

In summary, our current findings demonstrate that fiber and starch extracted from Mentawai taro corm exerted ameliorative effects against adiposity, dyslipidemia and gut dysbiosis caused by HFD. However, some limitations should also be properly considered. Firstly, the physicochemical characteristics of fiber and starch extracted from Mentawai taro corm were not elucidated in this study. Moreover, the dose of fiber and starch from Mentawai taro corm was only a single dose (20%), which precluded us from defining whether a higher dose also exerts different effects against HFD. Additionally, we did not investigate the molecular mechanisms involved in metabolic regulations, such as the expression of proteins for adipogenesis, due to fiber and starch treatments. Furthermore, we did not determine the levels of SCFAs in the fecal and blood plasma. As a result, the contribution of fiber and starch from Mentawai taro corm to the production of SCFAs remains unknown.

4. CONCLUSION

This present study demonstrates that the incorporation of extracted fiber and starch from Mentawai taro corm could beneficially improve body weight and white and brown adipose tissue profiles against HFD. Furthermore, the fiber and starch could also ameliorate plasma lipid profiles, particularly TC, LDL-C, and TG levels. Additionally, the fiber and starch of Mentawai taro corm could modulate the composition of intestinal microbiota community by promoting health-beneficial taxa while suppressing pathogenic taxa. Overall, the effectiveness of fiber in managing the detrimental effects of a fatty diet surpassed that of the starch from Mentawai taro corm. Hence, fiber of Mentawai taro corm could be recommended as a potent supplement to combat diet-induced metabolic problems, particularly obesity, dyslipidemia, and dysbiosis.

5. MATERIALS AND METHODS

5.1. Sample collection and processing of Mentawai taro corm

Mentawai taro corms (7 months old at harvesting time) were obtained from local farmer in Sipora (Mentawai Islands, West Sumatra). The taxonomical identity of species was determined by botanist in Herbarium Andalas (Biology Department, Andalas University). Upon arrival in lab, the corms were washed using tap water and peeled using electric peeler. Furthermore, the corms were sliced and grinded to achieve porridge-like constituents. Subsequently, the separation of fiber fraction and starch fraction was deployed by following the previous procedure [18] with slight modifications. Briefly, the sample was soaked overnight in the distilled water (1:4) at 8^{o}C to allow the starch as precipitate to separate with fiber fraction (as supernatant). Afterwards, the fractions were separated gently by firstly collecting the supernatant then the precipitate. Then, both fractions were subjected to centrifugation at 2500 RPM for 30 min (DLab, Beijing) and steaming at 100^{o}C for 30 min. Subsequently, the samples were dried using dehydrator at 70^{o}C for 18 h to achieve the chips-like texture before being grinded to be powder. The fine powder of each fraction (fiber and starch) was stored in sealed plastic bags and kept in dry container before being use in the experiment.

5.2. Animal models and Treatments

The adult male mice (n = 24; aged 2 months old, body weight 25-27.1 g, BALB/c strain) were procured from Balai Vetereiner Baso (Bukttinggi, West Sumatra). In animal room, mice were reared for a week with free access to food of standard rodent chow diet (RATBIO, Jakarta) and tap water drink. The room was regulated for its temperature (26.0-27.1^{o}C), humidity (67.4-68.0%), and light/dark cycle (12 h dark/12 h light). After one week of acclimatization, mice were equally grouped into 4 diet types randomly, such as:

- Group 1 (fed with normal diet; standard rodent chow diet; ND)
- Group 2 (fed with fatty diet; 21% of milkfat in standard diet; HFD)
- Group 3 (fed with HFD supplemented with 20% of Mentawai taro corm fiber; HFD + T Fiber)
- Group 4 (fed with HFD supplemented with 20% of Mentawai taro corm starch; HFD + T Starch)

The diet paradigms were deployed continuously for 12 weeks. The foods and drink were freshly provided on daily basis. The dose of fiber and starch (20%) was justified based on a preliminary study showing that such dose was the proper dose to preclude obesity development in mice. The animal handlings and experimental protocols of this study has been approved by the Research Etic Committee of Andalas University (No.432/UNAND/REC/2023).

5.3. Measurements of Body Weight

The weights of mice were measured using analytical balance (Ohaus SJX622N/E; Michigan) every two weeks. The measurements were conducted in the morning (09.00-10.00 a.m.). Afterwards, body weight gain was calculated using the following formula:

$$\text{Body weight change (\%)} = \frac{\text{Body weight at the end of treatment} - \text{Body weight at the begining of treatment}}{\text{Body weight at the begining of treatment}} \text{ x } 100\%$$

5.4. Measurements of Plasma Lipids

The mice were terminated at the end of treatment followed by blood sampling via cardiac puncture. The blood plasma was separated by centrifugation at 5000 RPM for 20 min (DM0506, DLab) and kept in -80^{0}C until use further analysis. Furthermore, the levels of TC, HDL-C, LDL-C, and TG were determined

using mouse lipid plasma assay kits (cholesterol assay kit Abcam- ab65390; triglyceride assay kit Abcam – ab65336; Biovision, San Francisco).

5.5. Assessments of Adipose Tissue Profiles

After 12-week diet treatments, mice were sacrificed and the epidydimal white adipose tissue (eWAT) and interscapular brown adipose tissue were gently collected. Subsequently, the weights of adipose tissues were measured using an analytical balance. Then, adipose tissue samples were fixed in 10% of neutral-buffered formalin solution (Sigma-Aldrich, St. Louis) overnight followed by graded dehydration in ethanol (Sigma-Aldrich) and clearance using xylene (Sigma-Aldrich) before being embedded in paraffin block (Surgipath Paraplast, Leica, Wetzlar). The embedded tissues were sliced using a rotary microtome (Leica) with 5 mm of thickness. Subsequently, the slices were stained with hematoxylin-eosin staining kit (Vectorlab, California). The procedures for histological slide preparations were as per protocols described previously [49]. The slides were photographed using a microscope (Olympus CX23, Tokyo) integrated with camera (Leica Microsystem). Afterwards, the adipocyte sizes were determined using Image J software (National Institute of Health, USA) based on the microscopic photographs.

5.6. Assessments of Intestinal Microbiota by Next-Generation Sequencing

Fecal samples were freshly collected from the large intestine upon animal scarification and immediately put into microtube filled with RNA latter solution (Thermo Fisher Scientific, Waltham). Next, the samples were kept in the -80^{o}C until further analysis. The further processing steps were according to a previously described workflow [18]. Briefly, the microbial DNA isolation was conducted as per protocol in DNAeasy Powersoil Pro Kit (Qiagen, Hilden), and the amplification of hypervariable regions of 16S rRNA of bacterial gene was performed using polymerase chain reaction (PCR) with 341F (forward) and 806R (reverse) specific primers purchased from Sigma-Aldrich. The purified PCR products were subjected to NGS (Novaseq 6000 Illumina, Novogene; Singapore). Eventually, the 16S Metagemonic software GAIA (Sequentia Biotech, Barcelona) was deployed to analyze the NGS data.

5.7. Statistical Analysis

Quantitative data were firstly tested for the normality by Shapiro-Wilk test. The test revealed that the data were distributed normally. Therefore, the data were subsequently analyzed using analysis of variance followed by Bonferroni post hoc test using SPSS Ver. 26 software (IBM). The $P < 0.05$ was set as significant value.

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