

# Ameliorative effect of yam bean (*Pachyrhizus erosus* L.) fiber on glucagon-like peptide 1 expression, oxidative stress and histopathology of the small intestine in mice fed high-fat diet

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**ABSTRACT:** Yam bean (*Pachyrhizus erosus* L.) is a prominent medicinal plant exerting various health benefits. However, it remains unknown whether yam bean fiber (YB) is also capable of counteracting the deleterious effects of a high-fat diet (HFD) on the small intestine. This present study aimed to investigate the effect of YB supplementation on the glucagon-like peptide 1 (GLP-1) expression, oxidative stress, and histopathological alterations of the small intestine in mice fed an HFD. Twenty-seven mice were assigned equally into three different diet treatment groups: normal diet (ND), HFD, and HFD supplemented with 10% of YB (HFD + YB). After eight weeks, mRNA expression of GLP-1, malondialdehyde (MDA) levels, catalase (CAT) activity in the small intestinal tissue were determined, and the anatomical and histopathological alterations of the intestine were investigated. The results demonstrated that YB supplementation was effective in precluding HFD-induced reduction of GLP-1 mRNA expression in the small intestine. YBF also prevented MDA elevation while ameliorating CAT activity. Furthermore, YBF exerted an ameliorative effect against HFD-induced morphological and histological alterations of the small intestine, particularly in intestinal length, index, and the number of inflammatory cells in the mucosal layer. This study revealed that YB supplementation could effectively prevent the impairment of GLP-1 expression and oxidative stress while ameliorating the histopathological alterations of the small intestine caused by HFD. Hence, YB could be formulated as a supplement to improve the gastrointestinal structure and functions against HFD.

**KEYWORD:** catalase activity; GLP-1, inflammatory cells; malondialdehyde; oxidative stress, small intestine, yam bean.

## 1. INTRODUCTION

As a part of the digestive system, the small intestine plays pivotal roles in bodily functions, including enzymatic digestion, nutritional absorption, defense system against toxins and pathogens, and metabolic regulations [1,2]. The L cells located in the small intestine actively secrete a hormone namely glucagon-like peptide 1 (GLP-1,) in response to the nutrient intake [3]. GLP-1 has been known to regulate meal-related hyperglycemia excursions by increasing insulin and decreasing glucagon secretion, depleting food intake and emptying the stomach while reducing weight gain [4-6]. Hence, sustained protection on the intestine is profoundly implicated in preventing various diseases, including obesity, diabetes mellitus, hepatic steatosis, systematic inflammation, and neuronal disorders [7-9].

Chronic and overconsumption of high-fat diet (HFD) has been shown to promote various detrimental outcomes in the intestine, including inflammatory bowel disease (IBD) and cancer [10-12]. It has been indicated that HFD intake substantially abolished the expression of antimicrobial protein and anti-inflammatory cytokines in the intestinal tissue while accelerating intestinal permeability and lipopolysaccharide infiltration [13-14]. Another study also revealed that HFD impaired the secretory rhythm of the GLP-1 in vivo and disrupted the GLP-1 expression in murine L cells in vitro [15]. Moreover, HFD is also reported to aggravate oxidative stress in the intestinal tissue, thereby promoting inflammatory-induced mucosal degeneration [11].

Mounting evidence has indicated that the incorporation of dietary fiber in the diet could counteract the development of various intestinal disorders [16-17]. A report has suggested that a high dose of cellulose was effective in alleviating the lesion of the intestine and oxidative stress accumulation in mice models [18]. Our previous study also indicated that the incorporation of the dietary fiber extracted from yam bean

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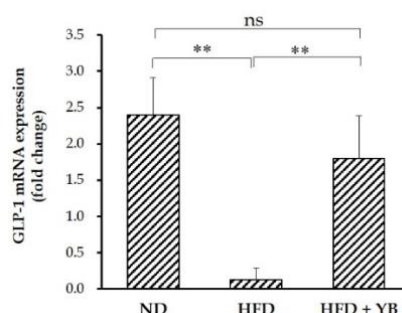
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(*Pachyrhizus erosus*, Fabaceae) tuber reduced the abundance of the pathogenic gut microbiota species, particularly those associated with the intestinal inflammatory response in HFD-fed mice [19]. An in silico study also suggested that some bioactive compounds in the extract of yam bean fiber (YB) might be able to inhibit the activation of toll-like receptor type 4 (TLR-4), an upstream signaling pathway for various inflammatory responses including in the intestine [20]. However, to date, it remains unknown whether YB is also capable of sustaining the expression of GLP-1 in the small intestine. In addition, the anti-oxidative stress and tissue-protecting effects of YB against HFD also remain less explored. Hence, this study was performed to determine it.

## 2. RESULTS

### 2.1. Effect of YB Supplementation on GLP-1 Expression in the Small Intestine

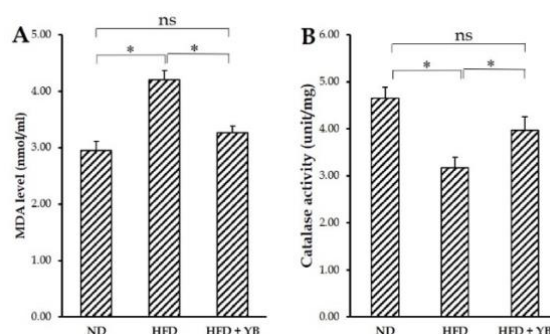
After eight weeks of treatment, mice fed with HFD alone showed a markedly lower GLP-1 mRNA expression (24 times lower) as compared with the normal diet (ND)-fed group ( $0.1 \pm 0.04$  fold change for HFD vs.  $2.4 \pm 0.5$  fold change for ND;  $P < 0.01$ ; Figure 1). In contrast, mice fed with HFD + YB had a higher GLP-1 mRNA expression (18 times higher;  $1.8 \pm 0.6$  fold change) as compared with HFD alone ( $P < 0.01$ ), but statistically comparable with those fed ND ( $P > 0.05$ ).



**Figure 1.** Effect of yam bean fiber supplementation on mRNA expression of GLP-1 in intestinal tissue of HFD-fed mice. GLP-1 mRNA level was expressed as relative expression against  $\beta$ -actin. ND (normal diet), HFD (High-fat diet), YB (yam bean fiber). NS (non significant); \*\* significantly different ( $P < 0.01$ ) based on Bonferroni Post hoc test. Data are presented as mean  $\pm$  SE.

### 2.2 Effect of YB on Malondialdehyde (MDA) and Catalase (CAT) Levels in the Small Intestine

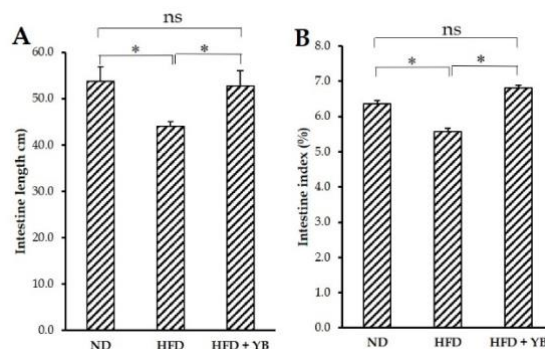
The measurements on MDA levels (marker for oxidative stress; Figure 2A) in the small intestine tissue showed that mice fed with HFD had a significant increase ( $P < 0.05$ ) of MDA ( $4.20 \pm 0.16$  nmol/ml; 1.42 times higher) as compared with ND group ( $2.95 \pm 0.16$  nmol/ml) and HFD + YB group ( $3.27 \pm 0.16$  nmol/ml). Moreover, mice fed with HFD + YB group had a statistically similar ( $P > 0.05$ ) MDA level to ND group. Otherwise, the CAT activity (an endogenous antioxidant; Figure 2B) was substantially decreased ( $P < 0.05$ ) in HFD-fed mice ( $3.17 \pm 0.22$  unit/mg) as compared with the ND group ( $4.65 \pm 0.23$  unit/mg) and HFD + YB group ( $3.97 \pm 0.28$  unit/mg). The CAT activity was lower in YB-fed mice, but it was not significantly different with ND-fed mice ( $P > 0.05$ ).



**Figure 2.** Effect of yam bean fiber supplementation on oxidative stress in intestinal tissue of HFD-fed mice. (A) MDA level in the small intestine, (B) CAT activity level. ND (normal diet), HFD (High-fat diet), YB (yam bean fiber). NS (non significant); \* significantly different ( $P < 0.05$ ) based on Bonferroni Post hoc test. Data are presented as mean  $\pm$  SE.

### 2.3 Effect of YB on Morphology and Histopathology of Small Intestine

An observation on small intestine morphology revealed that after eight weeks of treatment, HFD caused a significant reduction in intestinal length ( $44.0 \pm 1.0$  cm) as compared with ND ( $53.8 \pm 3.1$  cm) and HFD + YB ( $52.8 \pm 3.3$  cm) (Figure 3A;  $P < 0.05$ ). Otherwise, those fed with HFD + YB had a statistically comparable intestinal length as the ND group ( $P > 0.05$ ). The intestinal index (expressed as a ratio of organ weight to total body weight; Figure 3B) also showed a significant decrease in the HFD-fed group ( $5.6 \pm 0.1\%$ ) as compared with the ND group ( $6.4 \pm 0.1\%$ ) and HFD + YB group ( $6.8 \pm 0.1\%$ ) ( $P < 0.05$ ), while it was statistically comparable between ND and HFD + YBF group ( $P > 0.05$ ).



**Figure 3.** Effect of yam bean fiber supplementation on small intestinal length and index of HFD-fed mice. ND (normal diet), HFD (High-fat diet), YB (yam bean fiber). NS (non significant); \*) significantly different ( $P < 0.05$ ) based on Bonferroni Post hoc test. Data are presented as mean  $\pm$  SE.

Histopathological observations on the small intestine (Figure 4) revealed that mice fed with HFD for eight weeks exhibited severe mucosal abrasion and dilatation of the lumen. Such alterations were mildly improved in mice fed with HFD + YB. Otherwise, mice in the ND group depicted the normal mucosal architecture and lack of lumen dilation. Further histomorphometry analysis (Figure 5) revealed that mice fed with HFD had a thinner mucosal height ( $176.07 \pm 20.17$   $\mu$ m) as compared with the ND group ( $226.71 \pm 2.93$   $\mu$ m) and HFD + YB group ( $221.38 \pm 23.0$   $\mu$ m). However, there was no significant difference statistically ( $P > 0.05$ ) in the mucosal height among all groups of treatment (Figure 5A). The length of villi (Figure 5B) also exhibited a similar pattern as mucosal height with no significant difference ( $P > 0.05$ ) between the HFD group ( $108.11 \pm 20.85$   $\mu$ m), ND group ( $133.30 \pm 13.35$   $\mu$ m) and HFD + YB group ( $152.19 \pm 25.48$   $\mu$ m). The crypt depth (Figure 5C) was significantly lower ( $P < 0.05$ ) in the HFD group ( $63.17 \pm 4.23$   $\mu$ m) and HFD + YB group ( $69.92 \pm 6.54$   $\mu$ m) as compared with the ND group ( $93.91 \pm 10.64$   $\mu$ m).

Moreover, the number of inflammatory cells (an indicator of tissue inflammatory response) in the small intestine (Figure 5D) showed a salient elevation ( $P < 0.01$ ) in the HFD-fed group ( $4421 \pm 13.85$  cells/area; 2.6 times higher) as compared with the ND group ( $1702 \pm 6.33$  cells/area). The inflammatory cell count was also lower in HFD + YB group ( $2429 \pm 3.1$  cells/area) than in the HFD group ( $P < 0.05$ ). However, HFD + YB mice had a statistically higher number of inflammatory cells as compared with the ND group ( $P < 0.01$ ).

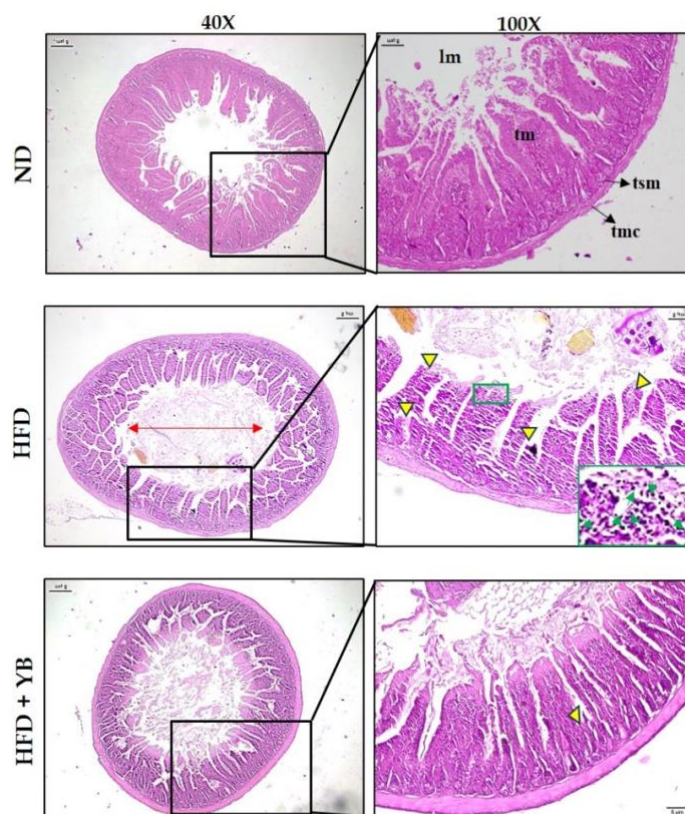
### 2.4 Effect of YB on Body Weight Gain

A monitoring of body weight (Figure 6A) found that mice fed with HFD exhibited significant body weight gain ( $P < 0.05$ ) starting from the second week ( $6.13 \pm 1.0$  g) until the end of the treatment ( $13.03 \pm 1.2$  g) as compared with the ND group ( $0.63 \pm 0.2$  g at second week and  $3.17 \pm 0.9$  g at the end of treatment) and HFD + YB group ( $2.82 \pm 0.5$  g at the second week and  $6.25 \pm 0.8$  g at the end of treatment). Unlikely, the body weight gain in HFD + YB group remained comparable statistically with the ND group ( $P > 0.05$ ). At the end of treatment, mice in the HFD-fed group had a noticeable body weight gain ( $51.61 \pm 6.7\%$  of increase against their initial body weight; Figure 6B), while the gain was lower in the ND group ( $11.50 \pm 3.3\%$ ) and HFD + YB group ( $22.47 \pm 2.8\%$ ). Statistical tests revealed that body weight gain was significantly different between HFD and other groups ( $P < 0.01$ ).

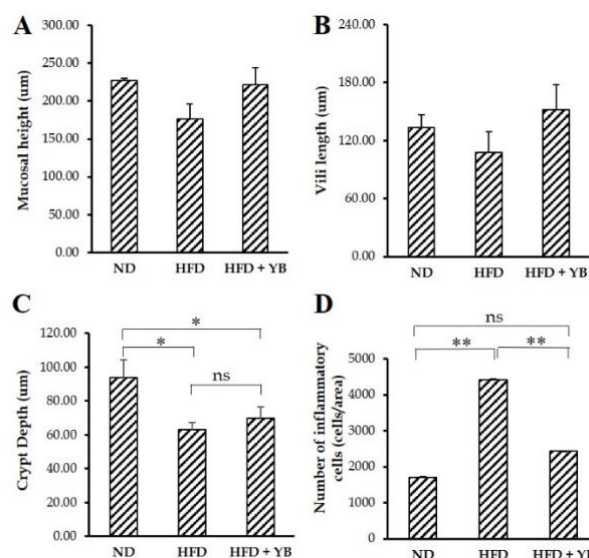
## 3. DISCUSSION

This study revealed the beneficial effect of dietary fiber extracted from yam bean tuber in ameliorating GLP-1 expression while preventing the elevation of MDA and partially improving CAT activity level in the

small intestine of HFD-fed mice. YB also ameliorated the structural changes in the small intestine, especially its length and index, as well as the number of mucosal inflammatory cells. In addition, YB effectively precluded HFD-induced excessive body weight gain toward obesity. YB exerted an ameliorative effect on the small intestine against HFD might be by modulating oxidative stress and inflammatory response.

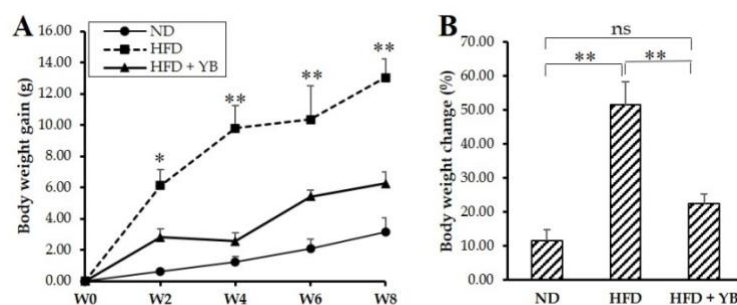


**Figure 4.** Effect of yam bean fiber supplementation on histopathology of small intestine of HFD-fed mice. ND (normal diet), HFD (High-fat diet), YB (yam bean fiber), lm (lumen), tm (tunica mucosa), tsm (tunica submucosa), tmc (tunica muscularis). Yellow arrowheads indicate mucosal abrasion, green arrows indicate aggregated inflammatory cells in the green marked area, red line with two arrowheads indicate lumen dilatation. Scale bars = 5  $\mu$ m.



**Figure 5.** Effect of yam bean fiber supplementation on histomorphometry of small intestine in HFD-fed mice. ND (normal diet), HFD (High-fat diet), YB (yam bean fiber). NS (non significant); \*\*) significantly different ( $P < 0.01$ ); \*) significantly different ( $P < 0.05$ ) based on Bonferroni Post hoc test. Data are presented as mean  $\pm$  SE.





**Figure 6.** Effect of yam bean fiber supplementation on body weight gain of HFD-fed mice. ND (normal diet), HFD (High-fat diet), YB (yam bean fiber). NS (non significant); \*\*\*) significantly different ( $P < 0.01$ ); \*) significantly different ( $P < 0.05$ ) based on Bonferroni Post hoc test. Data are presented as mean  $\pm$  SE.

A previous report indicated that HFD reduced GLP-1 expression in L cells of the intestine and impaired the responsiveness of GLP-1 secretion to nutrient stimuli [21]. The GLP-1 disruptions could lead to metabolic dysregulation, thereby developing obesity [22]. Likewise, in our present study, it was found that 8-week-HFD treatment caused a noticeable reduction of GLP-1 mRNA expression in the small intestine along with excessive body weight gain. In contrast, YB supplementation in HFD precluded the reduction of GLP-1 expression while sustaining normal body weight change to be comparable to those fed with ND. The capability of YB in sustaining GLP-1 expression might be attributed to some plausible mechanisms. The presence of YB and its by-products of fermentation by gut microbiota (namely short-chain fatty acids, SCFAs) is thought to mitigate the HFD-induced inflammatory response and L cell (GLP-1 producer) dysfunction. As previously indicated that HFD could directly stimulate the proinflammatory signaling pathway in the intestinal cells leading to substantial tissue degeneration in the intestinal wall [13], while the YB has been shown to exert an immunomodulatory effect that might counteract inflammatory responses [19,23]. In addition, as previously demonstrated by in silico simulation that a bioactive compound in YB (cycloarthenol) could deactivate the Toll-like receptor 4 (TLR4), thereby reducing the inflammatory response [20]. This is also supported by our present data indicating that YB supplementation significantly dampened HFD-induced increment of inflammatory cell number in the mucosal layer of the small intestine.

HFD might also impair GLP-1 expression by increasing the abundance of pathogenic microbiota species in the gut, particularly those associated with the inflammation. It has been demonstrated by a study in piglets that the microbial-induced inflammation of intestinal L cells profoundly impaired the GLP-1 secretion leading to metabolic dysregulation [24]. Otherwise, a study found that the gut microbiota, namely *Akkermansia muciniphila* secreted protein that was capable of inducing GLP-1 production both in vitro and in vivo [25]. Importantly, it has been indicated that dietary fiber intake could increase the abundance of *Akkermansia* [26]. Hence, it could be suggested that another plausible mechanism of YB in sustaining GLP-1 expression is by promoting the gut microbiota that act as GLP-1 inducer. However, in this present study, we did not determine the modulatory effect of YB supplementation on the abundance of *Akkermansia* and other gut microbiota species. Thus, further investigation is needed to clarify such speculation.

The suppression of oxidative stress and the sustained endogenous antioxidant level are crucial for intestinal tissue protection against pathological alterations. Previous studies demonstrated that excessive intake of HFD could elevate the oxidative stress in the intestine leading to detrimental outcomes, including apparent inflammation and degeneration [27,28]. Free fatty acids derived from HFD are known to promote oxidative stress and disrupt the intestinal immune system, thereby promoting cellular damage and tissue degeneration [12]. Accordingly, our present study revealed that HFD profoundly increased MDA levels in the intestinal tissue, indicating oxidative stress. At the same time, HFD substantially reduced CAT activity in the small intestine, thereby weakening the protective function of endogenous antioxidants against oxidative stress. In contrast, the YB supplementation effectively precluded the MDA elevation while ameliorating the CAT activity in the small intestinal tissue. A previous study also showed that a high-fiber diet could counteract lipid peroxidation, thereby alleviating oxidative stress [18]. Moreover, supplementation of fibers originating from pea, sweet potato, and wheat bran has been shown to significantly elevate endogenous antioxidant levels, including CAT, superoxide dismutase (SOD), glutathione (GSH) and total antioxidant capacity in the jejunum, plasma, liver, and spleen of rats [29]. In addition, SCFAs, including butyrate, propionate, and acetate, as fermentative products of fiber by gut microbiota, also have been indicated to reduce reactive oxygen species while increasing endogenous antioxidants, including SOD and GSH [30,31].

It has been suggested that the incorporation of the fibers in the diet could exert a protective effect on the intestinal tissue both in animals and humans [30,32,33]. A study in piglets also showed that fiber in the form of pectin effectively alleviated gut injury, including in the small intestine [17]. Accordingly, the structural and functional ameliorations of small intestinal tissue by the YB, as found by our recent study, could be attributed to several plausible mechanisms. Firstly, sustained CAT level and reduction of MDA promoted by YB supplementation might prevent substantial inflammatory response and subsequent tissue degeneration in the small intestine. Secondly, the elevation of SCFAs resulting from the YB fermentation by gut microbiota might also exert a protective effect on intestinal tissue. Thus, structural alterations of the intestine could be minimized by YB supplementation. Thirdly, physicochemical constituents of the YB could also limit the interaction between fatty acids in HFD and the mucosal layer of the small intestine. As a result, HFD-induced inflammation and subsequent tissue degeneration could be reduced by YB supplementation. However, our present data demonstrated that YB supplementation for eight weeks of treatment could not fully protect the small intestinal tissue against HFD-induced degeneration and inflammation. This discrepancy may indicate that the dose of YB used in this study (10%) was not effective enough to overcome the detrimental effects of HFD. Alternatively, it requires a longer time (more than eight weeks) to achieve the optimum protective effect of YB against HFD-induced intestinal degeneration. Future experiment using higher doses of YB and a longer period of treatment is expected to find out a reasonable explanation.

There are some limitations of our present study. Firstly, the plasma GLP-1 levels were not measured. Thus, it remains unknown whether the impaired mRNA GLP-1 expression in the intestine could also contribute to a reduction in circulated GLP-1 level. Secondly, other enteroendocrine peptides, such as ghrelin and peptide Y, that might also be affected by the HFD and YB treatment, were not also determined. Next, the expression and protein levels of proinflammatory cytokines in the intestinal tissue were not also investigated. In addition, the level of SCFAs and the microbial diversity in the gut were not observed. Future studies emphasizing the abovementioned aspects are required to deepen our understanding of the mechanisms of YB in exerting its beneficial effects on health.

#### 4. CONCLUSION

This study revealed that YB supplementation could effectively prevent the impairment of GLP-1 expression and oxidative stress while ameliorating the histopathological alterations of the small intestine in HFD-fed mice. These findings suggest that YB could be formulated as a supplement to improve the gastrointestinal structure and functions against HFD-induced degeneration.

#### 5. MATERIALS AND METHODS

##### 5.1. Yam Bean Fiber Extraction

Fresh yam bean tubers were obtained from a local farmer in the Kuranji sub-district, Padang, West Sumatra. Species identity was validated by a certified taxonomist in the Herbarium ANDA (Biology Department, Andalas University). The samples were peeled and washed five times with distilled water before being proceeded for fiber extraction. The extraction procedures for the fiber were performed as per the protocol previously described elsewhere [34].

##### 5.2. Animal Provision and Experimental Treatment

The adult male Deutschland, Denken, and Yoken (DDY) mice strain (n = 27; 2 months old; 23-25 grams of body weight) were provided by the Baso Veterinary Center, West Sumatra, Indonesia. Before the experiment, the mice were acclimatized for a week in the individual polycarbonate cages in a regulated animal house (temperature 25.5-26.5°C, humidity 66-67.5%, and 12 hours light/dark cycle) and fed standard rodent chows (Citra Ina Fedmill, Jakarta, Indonesia) and tap water ad libitum. Thereafter, the animals were randomly assigned into three different groups of diet treatment as follows:

- Group 1: Mice fed with normal diet (ND)
- Group 2: Mice fed with high-fat diet (HFD)
- Group 3: Mice fed HFD supplemented with 10% of yam bean fiber (HFD + YB)

The diet treatment was carried out for eight weeks continuously. The ND was a standard commercial diet for rodents (Rat Bio, PT Citra Ina Feedmill, Jakarta) containing 4% fat (w/w), while the HFD was composed of 21% milkfat (w/w). The diet composition and the dose of YB (10%) were decided according to a previous study showing that 10% was the most effective dose of YB in preventing metabolic diseases and gut dysbiosis [20]. The protocols in this study have been approved by the committee of research ethic, Faculty of Medicine Andalas University (NO-528-UN.16.2/ KEP-FK-2021).

### 5.3. Measurements of Body Weight

Body weight was measured biweekly in the morning (09.00-10.00 a.m.) using a digital balance (SF-400C, Zhezong Weighing Apparatus Factory, China). The body weight gain was calculated as the percentage of the final body weight increase against the initial experiment.

### 5.4. Morphological and Histopathological Examinations of Intestine

At the end of treatment, animals were sacrificed by means of dislocation of cervical vertebrae. Subsequently, the animals were dissected, and the intestines were removed, weighed, and the total length was measured. Next, the samples were fixed in 10% formalin and proceeded for histological preparation using the paraffin method and Hematoxylin-Eosin staining. Then photomicrographs and intestinal histology observations were carried out using an Olympus CX43 light microscope with 40x and 100x magnifications. The intestinal histomorphologic analysis was performed using ImageJ software (NIH, USA; <https://imagej.nih.gov/ij/download.html>). In addition, the inflammatory cells were also counted using the grid and cell counter features in the ImageJ.

### 5.5. Measurement of GLP-1 mRNA Expression

A distal part of small intestine tissue (weighs 30–35 mg) was sampled and immediately stored in the RNA lather at -80°C. Purification of total RNA from the intestine tissue was performed as per procedures described in RNeasy Mini Kit Handbook 2019 protocol (Qiagen, US). Homogenization was performed using a mini and held homogenizer. DNase treatment was done on columns, RNA concentration was measured by NanoDrop-1000 (Thermo Scientific), and purity was accessed from OD260/280. For each RNA sample, cDNA was made from 500 ng RNA using iScript™ cDNA Synthesis Kit (Bio-Rad), containing a blend of oligo (dT) and random hexamer primers with a final reaction volume of 20 µl. The reactions were incubated for 5 min at 25°C, 30 min at 42°C, and 5 min at 85°C. The cDNA was stored at -80°C until use. The set primers for mouse GLP-1 were as follows:

- 5'-CAAACCAAGATCACTGACAAGAAAT-3' (forward) and 5'-GGGTACACAATGCTAGAGGGA-3' (reverse),
- For  $\beta$ -actin were as follows: 5'-GGCCAACCGTGAAAAGATGA-3' (forward), and 5'-CAGCCTGGATGGCTACGTACA-3' (reverse).

The values of the Cycle Threshold (CT) were determined and the mRNA expression of GLP-1 was subsequently calculated as a fold change against  $\beta$ -actin [35].

### 5.6. Measurement of MDA and CAT Activity Levels

The intestine tissue of 0.50 g was collected immediately after animal termination followed by homogenization in PBS and centrifugation at 2000 rpm and 4°C for 10 mins. The supernatant was subsequently used for the measurements. The MDA measurement was performed using a lipid peroxidation assay kit (Sigma-Aldrich, USA), and the absorbance of samples was determined by using a spectrophotometer (SmartSpec™ Plus Spectrophotometer, BioRad Laboratories, USA). Measurement of CAT activity was carried out as per the procedures described in Catalase Assay kit protocol (#707002, Cayman).

### 5.7. Statistical Analysis

Data are presented as mean  $\pm$  SE. Quantitative data were analyzed using ANOVA followed by Bonferroni Post hoc test ( $P < 0.05$ ). The ANOVA was chosen because the data were normally distributed (based on the Shapiro-Wilk test of normality).

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**Author contributions:** Concept – P.S.; Design – P.S., R.M.; Supervision – P.S., R.R.; Resources – R.R., R.M.; Materials – W.R., R.M.; Data Collection and/or Processing – P.S., W.R., R.R., R.M.; Analysis and/or Interpretation – P.S., W.R.; Literature Search – P.S., W.R., R.R., RM; Writing – P.S., WR; Critical Reviews – P.S.

**Conflict of interest statement:** The authors declared no conflict of interest.

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