



## Research article

# Mangrove fruit (*Bruguiera gymnorhiza*) increases circulating GLP-1 and PYY, modulates lipid profiles, and reduces systemic inflammation by improving SCFA levels in obese wistar rats



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## ABSTRACT

*Bruguiera gymnorhiza* (BG) has potential as a functional food because of its dietary fibre content and bioactive components such as flavonoids and phenolic compounds. However, it is not studied in the context of diet-related disease prevention. In the present study, we aimed to investigate the effects of *Bruguiera gymnorhiza* fruit flour (BGF) on satiety hormone, lipid profile, systemic inflammation, body weight, and caecum SCFA levels in diet-induced obese rats. A total of 28 obese male Wistar rats were divided into four groups. Group 1 (K1) was given a standard chow, group 2 (K2) standard chow + orlistat, group 3 (P1) standard chow + BGF 2 g/200 g BW/day, and group 4 (P2) standard chow + BGF 4 g/200 g BW/day for 28 days. The levels of GLP-1, PYY, total cholesterol (TC), triglyceride (TG), HDL, IL-6, TNF- $\alpha$ , and body weight were measured before and after the intervention; meanwhile, the caecum SCFA levels were assessed only after the intervention. In this study, BGF intervention increased the dose-dependent plasma GLP-1 and PYY levels ( $P < 0.000$ ). In addition, BGF intervention also decreased lipid profiles (TC & TG) ( $P < 0.000$ , respectively) and systemic inflammation in a dose-dependent manner. Finally, acetate, propionate, and total SCFA concentrations were higher in the BGF intervention group (P2) compared to the other groups ( $p < 0.05$ ). The SCFA levels were associated with satiety hormones, lipids, and systemic inflammation ( $P < 0.05$ ). The BGF intervention improved satiety hormone, lipid profile, systemic inflammation, and SCFA levels.

## 1. Introduction

Obesity is a significant public health problem, and its prevalence continues to rise worldwide [1]. The fundamental cause of obesity is a chronic energy imbalance, in which the energy from food consumption is higher than the expenditure (through physical activity and exercise) [2]. Furthermore, this energy intake and expenditure imbalance may also be influenced by metabolic, genetic, neurobiological, environmental, and psychological factors [3]. Obesity causes insulin resistance, type 2 diabetes, cardiovascular disease, and several types of cancer [1]. In the current condition of the COVID-19 pandemic, a meta-analysis suggested

that obesity is one of the comorbidities in individuals infected with COVID-19 [4].

The targets for preventing and treating obesity have focused on modifying the dietary intake and increasing physical activity, such as exercise [5]. How diet can affect changes in body weight can be explained through various biological mechanisms, including modulation in composition and diversity of the gut microbiota [6]. A recent comprehensive review indicated that the gut microbiota could produce metabolites, one of which is short-chain fatty acids (SCFA) [7]. Intervention studies have shown that SCFA supplementation can affect the satiety response mediated by digestive hormones such as glucagon-like

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peptide-1 (GLP-1) and peptide YY (PYY) [8], which then have an impact on the regulation of body weight [9]. Furthermore, an *in vitro* study on adipocyte cells of obese individual donors has shown that supplementation of the SCFA mixture can also modulate adipocyte function in lipid metabolism [10]. In addition, a study suggested that SCFA can also modulate adipose function by suppressing levels of interleukine-6 (IL-6) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) as biomarkers of inflammation (low-grade inflammation) in obesity [11]. These provide proof of evidence of the role of SCFA on metabolic health.

However, it should be noted that the above studies used SCFA supplementation as an intervention molecule. Next to that, practically, SCFA supplementation is not always accessible to most people daily. Therefore, food ingredients that can be fermented by the gut microbiota and produce SCFA metabolites are options. It has been shown that the gut microbiota can also ferment dietary fibre and complex carbohydrates to make SCFA [6]. Therefore, consumption patterns containing complex carbohydrates (including dietary fibre) and bioactive components are a dietary modification strategy to improve metabolic health.

Indonesia has various foods classified as sources of complex carbohydrates, one of which is *Bruguiera gymnorhiza* (BG) fruit. BG fruit has a relatively high carbohydrate and fibre content which has been widely consumed by people living in coastal areas [12]. For instance, the Tual community in Southeast Maluku Regency usually uses it to replace rice as a source of carbohydrates. While in other countries (Solomon Islands), this fruit is commonly consumed as a vegetable and is widely sold in the market [13]. *B. gymnorhiza* fruit flour (BGF) contains 86.26 % carbohydrates, 7.5% soluble dietary fibre, and 38.6% soluble dietary fibre [12]. Furthermore, BG fruit contains bioactive components such as flavonoids and phenolic compounds [14]. Allegedly, an elegant review has suggested that phenolic bioactive compounds can also modulate SCFA [15]. To our knowledge, the metabolic effect of BG has not yet been investigated in the obesity model. In this study, we aimed to examine the impact of BGF on satiety hormone, lipid profile, systemic inflammation, caecum SCFA levels, and body weight in obese Wistar rats induced with a high-fat diet.

## 2. Materials and methods

### 2.1. Materials

The fruit of *B. gymnorhiza* (Figure 1) was collected from the mangrove forest in the coastal area of Mangkang, Semarang, Central Java, Indonesia, in April 2021. They were selected purposively without comparing similar species from other regions. Fruits with green/



Figure 1. *B. gymnorhiza* fruit.

brownish-green skin and reddish fruit petals were picked. The *B. gymnorhiza* flour production referred to the previous studies [16]. Fresh *B. gymnorhiza* fruits were washed and peeled, then soaked in 30a % (w/w) solution of rice husk ash for 24 h. The ratio between the fruit and the husk ash solution used was 1:4. The soaked *B. gymnorhiza* fruits were rinsed with tap water and then boiled for 30 min. The cooked fruits were drained and sliced horizontally into equal sizes (0.5–1 cm). Next, the sliced *B. gymnorhiza* fruits were dried using a cabinet dryer at a temperature of 70 °C for 12 h, then ground using a grinder. Finally, the milled result was sieved using a 60 mesh sieve (250  $\mu$ m in diameter).

### 2.2. Biochemical analyses of BGF

Analysis of proximate (moisture, lipid, protein, ash, and carbohydrate content) and dietary fibre content of *B. gymnorhiza* flour (BGF) was carried out in duplicate using the techniques described by AOAC (Association of Official Analytical Chemists) [17]. Total phenolics and flavonoids were analyzed according to the method described by Ghasemzadeh et al. [18].

Tannin analysis was measured using a Folin-Denis reagent, and the absorbance of the solution was measured with a spectrophotometer (Genesys 20, Thermo Scientific., USA) at 730 nm. For HCN content, distilled water was added to 2 g of BGF up to the mark on the measuring flask. The mixture was then centrifuged, and 5 ml of the alkaline picrate solution was added to 1 ml of the clear filtrate. Next, the solution was then heated in a 100 °C water bath for 30 min. After that, let it cool down and add 4 ml of distilled water. Then, shake the solution until homogeneous using Vortex, and read the absorbance using a spectrophotometer (Genesys 20, Thermo Scientific., USA) at 480 nm. Record the data obtained and then calculate using a standard curve with pure KCN.

### 2.3. Animals handling and ethical approval

In the current study, male Wistar rats aged 9–10 weeks and weighing 180–200 g were used. The rats were acclimatized for seven days before the experiment. Furthermore, the cage room temperature was set at 22 °C and 12 h of lighting. Rats were fed a standard chow of 15 g per day and unlimited water access during the adaptation period. The standard feed used was commercial Comfeed AD II (Japfa Comfeed Indonesia Ltd, Indonesia), which contains 55–60% carbohydrate, 12% water, 7% ash, 15% crude protein, 3–7% crude fat, 6% crude fibre, 0.9–1.1% calcium, 0.6–0.9% phosphorus, and vitamins.

The rats were housed individually in stainless-steel cages and fed a high-fat, high-sucrose diet (HFSD) for 28 days to induce obesity [19]. The composition of HFSD used was Comfeed AD II (Japfa Comfeed Indonesia Ltd, Indonesia) 45%, lard 21%, and sucrose 34%. The criteria for obesity in rats were calculated based on the Lee Index [20] and classified as obese

Table 1. Biochemical content of *B. gymnorhiza* flour (BGF).

Type of biochemical content	Mean $\pm$ SD
Moisture (%)	3.17 $\pm$ 0.02
Lipid (%)	0.64 $\pm$ 0.19
Protein (%)	4.86 $\pm$ 0.23
Ash (%)	1.64 $\pm$ 0.05
Carbohydrate (%)	89.71 $\pm$ 0.49
Insoluble dietary fibre (%)	23.07 $\pm$ 0.04
Soluble dietary fibre (%)	0.37 $\pm$ 0.01
Total dietary fibre (%)	23.43 $\pm$ 0.04
Total phenolic (%)	1.12 $\pm$ 0.09
Total flavonoid (%)	9.07 $\pm$ 0.23
Tannin (%)	2.94 $\pm$ 0.04
HCN (ppm)	30.51 $\pm$ 0.10

Values are means  $\pm$  standard deviation (SD); ppm: part per million.

if the results of the Lee Index value were  $> 300$ . The formula for Lee Index is as below:

$$\text{Lee Index} = [\text{bodyweight (g)}^{1/3} / \text{naso-anal length (cm)}] \times 10^3$$

This result is in accordance with normal conditions (not under pressure or stress), referring to Heldrich et al. [21]. The research protocol (use of experimental animals) was based on the Declaration of Helsinki, The Council for International Organizations of Medical Sciences (CIOMS). Experimental procedures were reviewed and approved by The Ethics Commission of the Faculty of Medicine, Universitas Diponegoro, and Dr. Kariadi Hospital Semarang, Indonesia, with the approval number 37/EC/H/FK-UNDIP/III/2021.

#### 2.4. *In vivo studies investigating the effects of BGF on satiety hormones, lipid profiles, systemic inflammation, and caecum SCFA levels*

All *in vivo* study procedures were documented according to the ARRIVE (Animal Research: Reporting of *In vivo* Experiments) guidelines [22] (Supplementary Data).

##### 2.4.1. *Experimental design*

This study calculated the sample based on previous literature, with seven samples per group [23]. Obese rats were then randomly assigned into four groups consisting of seven rats ( $n = 7$ ) for each group. Each rat stayed in a different cage individually. All groups received 15 g/d/rat of normal chow and drank water freely. The K1 group is a positive control group. Group 2 (K2) was given orlistat (Xenical, Roche) at a dosage of 30 mg/kg body weight (BW)/day. Group 3 (P1) received BGF at a dosage of 2 g/200 g BW/day, while group 4 (P2) received doubled dosage (4 g/200 g BW/day). Orlistat and BGF were administered using an oral feeding tube. The intervention was carried out over 28 days. The normal feed used has an equal composition to the feed used during acclimatization.

The bodyweight (BW) was weighed weekly, and naso-anal length was measured before and after the intervention. The body weight of the rats was measured using a digital scale (Dor Yang JA-P, Shanghai, China). A measuring tape was used to measure the naso-anal length from the tip of the nostril to the anus (end of the tail).

##### 2.4.2. *Blood and caecum samples collection*

In this study, the primary outcome was measured from blood and caecum samples. Blood samples were taken before and after the intervention period. Blood was drawn from rats anesthetized with ketamine at a dosage of 60 mg/kg BW in the light of day. Glass capillary tubes were used to collect 3 ml of blood directly from the orbital sinus. Afterw, the

blood sample was centrifuged at 4000 rpm for 15 min at 4 °C. The supernatant was collected and then stored at -80 °C for further analysis.

Luminal contents of the cecum were collected after the intervention by washing out the luminal part of each sample with phosphate-buffered saline (1X PBS (137 mM NaCl, 2.7 mM KCl, 10ard mM Na<sub>2</sub>HPO<sub>4</sub>, 1.8 mM KH<sub>2</sub>PO<sub>4</sub>) pH 7.4).

##### 2.4.3. *Biomedical analysis of blood and caecum samples*

We analyzed glucagon-like peptide-1 (GLP-1), peptide yy (PYY), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF- $\alpha$ ) levels using a sandwich enzyme-linked immunosorbent assay (ELISA) test kit according to the manufacturer's instructions (Elabscience Biotechnology Inc., USA). In addition to that, lipid profiles were analyzed based on the manufacturer's instructions. Lipid profiles were determined using an enzymatic photometric test (DiaSys Diagnostic, Germany).

**2.4.3.1. *Caecum short-chain fatty acids (SCFAs) analysis.*** The analysis of SCFAs levels was carried out with a gas chromatography-flame ionization detector (GC-FID) method refers to a process described elsewhere [24, 25]. We analyzed the SCFA from caecum samples after the intervention. The total SCFA is the sum of the acetate, propionate, and butyrate concentrations.

##### 2.5. *Data management and statistical analysis*

The data were analyzed using SPSS (version 25), and graphical figures were designed using GraphPad Prism (version 9). All the values were expressed as mean  $\pm$  standard deviation (SD). The normality of the data distribution was tested using the Shapiro-Wilk test. Data for bodyweight, GLP-1, lipid profiles, IL6, and TNF- $\alpha$  before and after intervention were normally distributed. Differences between groups (K1, K2, P1, P2) were tested using One-Way ANOVA followed by Post Hoc Bonferroni or Post Hoc Tamhane based on the Levene test (Supplementary Data). Data were analyzed using Paired t-test to determine the effects before and after the intervention. Since the data of PYY was not normally distributed, the Kruskal-Wallis and the Mann-Whitney test were used. The data were tested using a Wilcoxon test to determine the effect before and after intervention on the PYY level. After the intervention, data of acetate, butyrate, propionate, and total SCFA were not normally distributed; therefore, the Kruskal-Wallis test was used, followed by the Mann-Whitney test. Correlation between variables after the intervention was analyzed using rank Spearman's test. P-value  $< 0.05$  was considered significant.

**Table 2.** Baseline data on bodyweight, circulating satiety hormones, lipid profiles, systemic inflammations, SGOT, and SGPT in high fat-high sucrose diet-induced obesity Wistar rats.

Parameter/group	K1	K2	P1	P2	p
Bodyweight (g)	242.57 $\pm$ 5.83	242.86 $\pm$ 3.98	242.86 $\pm$ 3.53	239.14 $\pm$ 4.41	0.357
GLP-1 (pg/ml)	5.93 $\pm$ 0.23	6.09 $\pm$ 0.32	6.17 $\pm$ 0.14	6.12 $\pm$ 0.09	0.205
PYY (pg/ml)	25.90 $\pm$ 1.11	26.15 $\pm$ 0.93	25.16 $\pm$ 1.65	26.40 $\pm$ 0.97	0.273
Total cholesterol (mg/dl)	190.08 $\pm$ 4.56	188.22 $\pm$ 4.09	187.24 $\pm$ 6.63	191.95 $\pm$ 2.62	0.273
Triglyceride (mg/dl)	134.21 $\pm$ 4.49	131.81 $\pm$ 1.92	133.16 $\pm$ 3.65	134.00 $\pm$ 3.04	0.308
HDL (mg/dl)	25.31 $\pm$ 1.82	25.21 $\pm$ 0.68	25.11 $\pm$ 1.94	25.31 $\pm$ 1.96	0.995
TG/HDL ratio	5.33 $\pm$ 0.45	5.23 $\pm$ 0.15	5.32 $\pm$ 0.31	5.32 $\pm$ 0.46	0.950
IL-6 (pg/ml)	169.16 $\pm$ 9.45	169.58 $\pm$ 3.98	172.80 $\pm$ 3.99	171.12 $\pm$ 2.86	0.627
TNF- $\alpha$ (pg/ml)	12.31 $\pm$ 0.36	12.18 $\pm$ 0.39	12.20 $\pm$ 0.30	12.02 $\pm$ 0.43	0.541
SGOT (U/l)	73.86 $\pm$ 2.65	75.73 $\pm$ 0.69	75.23 $\pm$ 1.92	75.67 $\pm$ 0.65	0.167
SGPT (U/l)	37.66 $\pm$ 1.08	37.32 $\pm$ 1.32	37.93 $\pm$ 0.94	37.87 $\pm$ 1.05	0.722

Data were expressed as mean  $\pm$  standard deviation (SD) and measured before the treatment period; K1: positive control group (normal diet); K2: negative control group (normal diet + orlistat 30 mg/kg BW/d); P1: treatment group 1 (normal diet + BGF 2 g/200 g BW/d); P2: treatment group 2 (normal diet + BGF 4 g/200 g BW/d); n: 7; GLP-1: glucagon-like peptide-1; PYY: peptide yy; HDL: high-density lipoprotein; TG: triglyceride; IL-6: interleukin-6; TNF- $\alpha$ : tumor necrosis factor alpha; SGOT: serum glutamic oxaloacetic transaminase; SGPT: serum glutamic pyruvic transaminase; p  $< 0.05$  was considered significant with One Way ANOVA test.

**Table 3.** Effect of BGF on bodyweight, GLP-1, PYY, Lipid Profiles, IL-6, and TNF- $\alpha$ 

Group/Parameter	Bodyweight (g)	GLP-1 ( $\mu\text{g/ml}$ )	PYY ( $\mu\text{g/ml}$ )	Total cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	TG/HDL ratio	IL-6 ( $\mu\text{g/ml}$ )	TNF- $\alpha$ ( $\mu\text{g/ml}$ )	
K1	Pre	242.57 $\pm$ 5.83	5.93 $\pm$ 0.23	25.90 $\pm$ 1.11	190.08 $\pm$ 4.56	134.20 $\pm$ 4.48	25.31 $\pm$ 1.82	5.32 $\pm$ 0.45	169.16 $\pm$ 9.45	12.31 $\pm$ 0.36
	Post	291.71 $\pm$ 7.41	5.90 $\pm$ 0.28	25.01 $\pm$ 1.19	212.18 $\pm$ 3.99	138.38 $\pm$ 5.92	23.19 $\pm$ 2.06	6.01 $\pm$ 0.67	171.54 $\pm$ 9.01	12.53 $\pm$ 0.35
	p	<0.001*	0.778	<0.001*	<0.001*	0.032**	*0.001*	*0.001*	0.004*	<0.001*
K2	Pre	242.86 $\pm$ 3.98	6.09 $\pm$ 0.32	26.15 $\pm$ 0.93	188.21 $\pm$ 4.09	131.80 $\pm$ 1.92	25.21 $\pm$ 0.68	5.23 $\pm$ 0.15	169.58 $\pm$ 3.98	12.18 $\pm$ 0.39
	Post	267.71 $\pm$ 5.69	19.98 $\pm$ 0.29	510.99 $\pm$ 26.20	103.40 $\pm$ 4.08	76.33 $\pm$ 3.52	89.94 $\pm$ 2.34	0.89 $\pm$ 0.04	73.97 $\pm$ 2.53	5.97 $\pm$ 0.24
	p	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
P1	Pre	242.86 $\pm$ 3.53	6.17 $\pm$ 0.14	25.16 $\pm$ 1.65	187.24 $\pm$ 6.62	133.16 $\pm$ 3.64	25.11 $\pm$ 1.94	5.32 $\pm$ 0.31	172.80 $\pm$ 3.99	12.20 $\pm$ 0.30
	Post	275.00 $\pm$ 4.08	12.03 $\pm$ 0.22	344.21 $\pm$ 16.14	146.13 $\pm$ 6.95	110.66 $\pm$ 2.42	46.49 $\pm$ 3.72	2.39 $\pm$ 0.21	103.47 $\pm$ 2.29	9.36 $\pm$ 0.39
	p	<0.001*	<0.001*	0.018 $\square$	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
P2	Pre	239.14 $\pm$ 4.41	6.12 $\pm$ 0.09	26.40 $\pm$ 0.97	191.95 $\pm$ 2.62	133.99 $\pm$ 3.03	25.31 $\pm$ 1.95	5.32 $\pm$ 0.46	171.12 $\pm$ 2.86	12.02 $\pm$ 0.43
	Post	263.00 $\pm$ 4.62	14.67 $\pm$ 0.34	413.72 $\pm$ 23.89	109.13 $\pm$ 3.35	84.86 $\pm$ 2.42	74.06 $\pm$ 4.43	1.15 $\pm$ 0.06	81.52 $\pm$ 4.02	6.19 $\pm$ 0.29
	p	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*

Data were expressed as mean  $\pm$  standard deviation (SD) and measured before and after the treatment period; K1: positive control group (normal diet); K2: negative control group (normal diet + orlistat 30 mg/kg BW/d); P1: treatment group 1 (normal diet + BGF 2 g/200 g BW/d); P2: treatment group 2 (normal diet + BGF 4 g/200 g BW/d); n: 7; GLP-1: glucagon-like peptide-1; PYY: peptide yy; HDL: high-density lipoprotein; TG: triglyceride; IL-6: interleukin-6; TNF- $\alpha$ : tumor necrosis factor alpha; \*p < 0.001, \*\*p < 0.05 were considered significant with Paired t-test,  $\square$ Wilcoxon test.

### 3. Results

#### 3.1. Biochemical content of BGF.

The proximate analysis results showed that BGF contains 3.17% moisture, 0.64% lipid, 4.86% protein, 1.64% ash, and 89.71% carbohydrate (Table 1). BGF also contains 2.94% tannins and 30.51 ppm HCN, which are considered anti-nutrients in excessive amounts. These two components of BGF were within acceptable limits for human consumption since the Acceptable Daily Intake (ADI) guideline for tannin levels in meals is 560 mg/kg BW/day. Meanwhile, the HCN concentration in food should not exceed 50 ppm, as the Food and Agriculture Organization (FAO) suggested. Moreover, the content of dietary fibre, total phenol, and total flavonoid in BGF were 23.43%, 1.12%, and 9.07%, respectively.

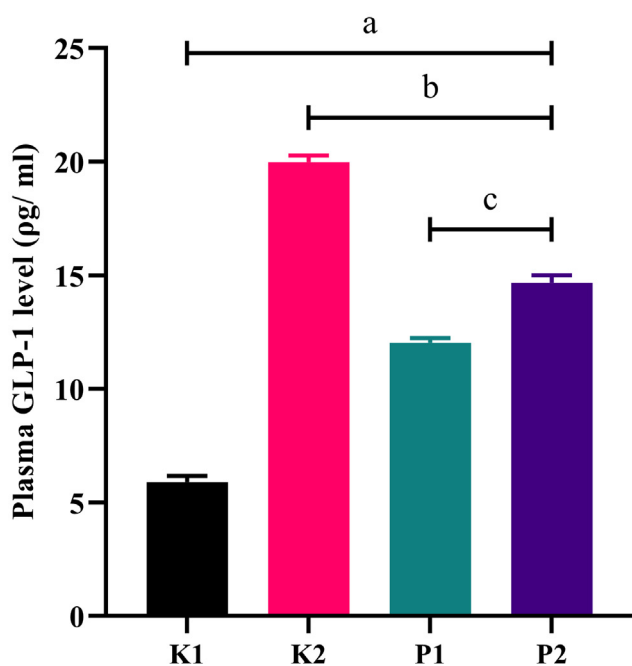
#### 3.2. Circulating GLP-1, PYY, lipid profiles and inflammatory markers before the treatment period.

A total of 28 diet-induced obesity Wistar rats were observed (7 rats for each group). No significant differences were observed in the baseline data for all parameters (Table 2).

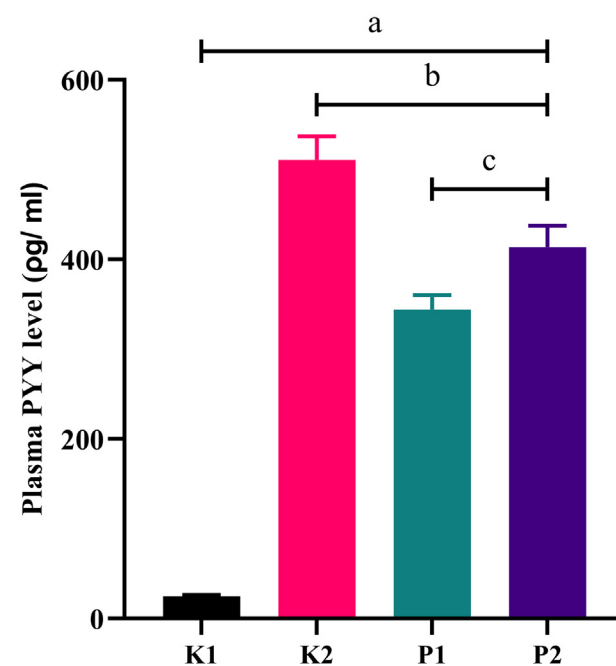
#### 3.3. Effect of BGF intervention on circulating satiety hormones

The BGF intervention significantly increased circulating GLP-1 levels in K2, P1, and P2 groups from baseline, but no effect was observed in K1 group (Table 3). Furthermore, Figure 2 illustrates circulating GLP-1 level (A) which was significantly different between the K2, P1, and P2 groups compared to the K1 group [(K2) 19.98  $\pm$  0.29  $\mu\text{g/ml}$ , (P1) 12.03  $\pm$  0.22

(A) GLP-1 level

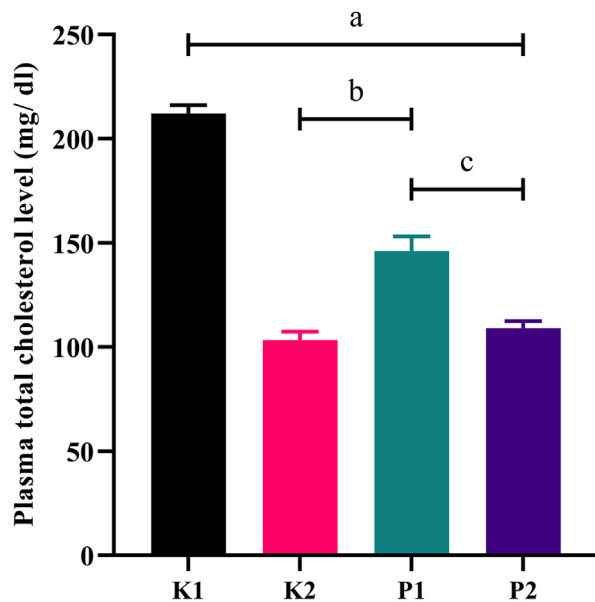


(B) PYY level

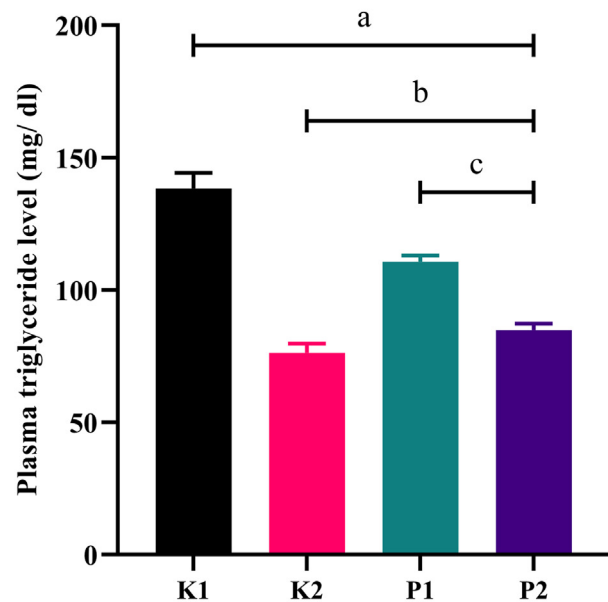


**Figure 2.** Effect of BGF intervention on circulating GLP-1 (A) and PYY (B) level in diet-induced obesity rats. Values were expressed as mean  $\pm$  standard deviation (SD); K1: positive control group (normal diet); K2: negative control group (normal diet + orlistat 30 mg/kg BW/d); P1: treatment group 1 (normal diet + BGF 2 g/200 g BW/d); P2: treatment group 2 (normal diet + BGF 4 g/200 g BW/d); n: 7; a: p < 0.001 vs K1; b: p < 0.001 vs K2; c: p < 0.001 vs P1; p < 0.05 was considered significant with One Way ANOVA test and Bonferroni post hoc test.

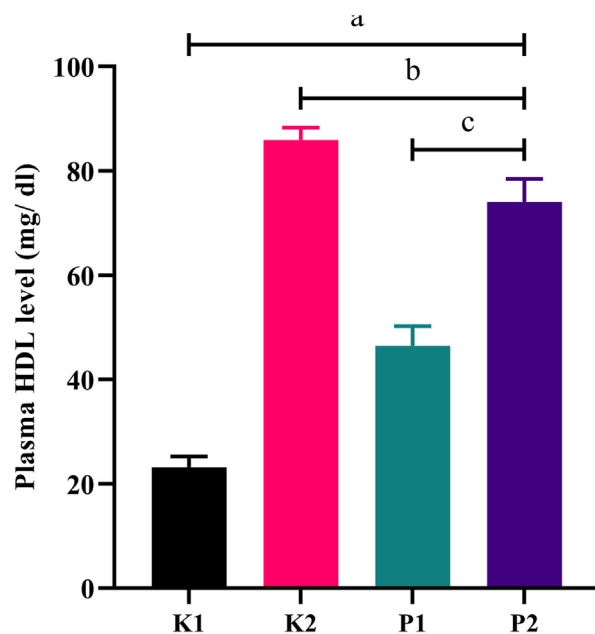
## (A) Total cholesterol level



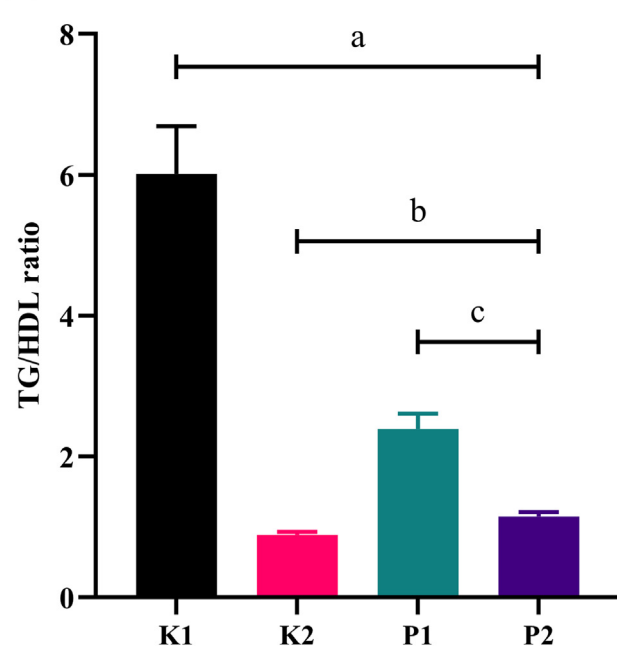
## (B) Triglyceride level



## (C) HDL level



## (D) TG/HDL ratio



**Figure 3.** Effect of BGF intervention on lipid profiles [(A) Total Cholesterol, (B) Triglyceride, (C) HDL Cholesterol, and (D) TG/HDL ratio] in diet-induced obesity rats. Values were expressed as mean  $\pm$  standard deviation (SD); K1: positive control group (normal diet); K2: negative control group (normal diet + orlistat 30 mg/kg BW/d); P1: treatment group 1 (normal diet + BGF 2 g/200 g BW/d); P2: treatment group 2 (normal diet + BGF 4 g/200 g BW/d); n: 7; a:  $p < 0.001$  vs K1; b:  $p < 0.001$  vs K2; c:  $p < 0.001$  vs P1;  $p < 0.05$  was considered significant with One Way ANOVA test and Tamhane post hoc test for all parameters.

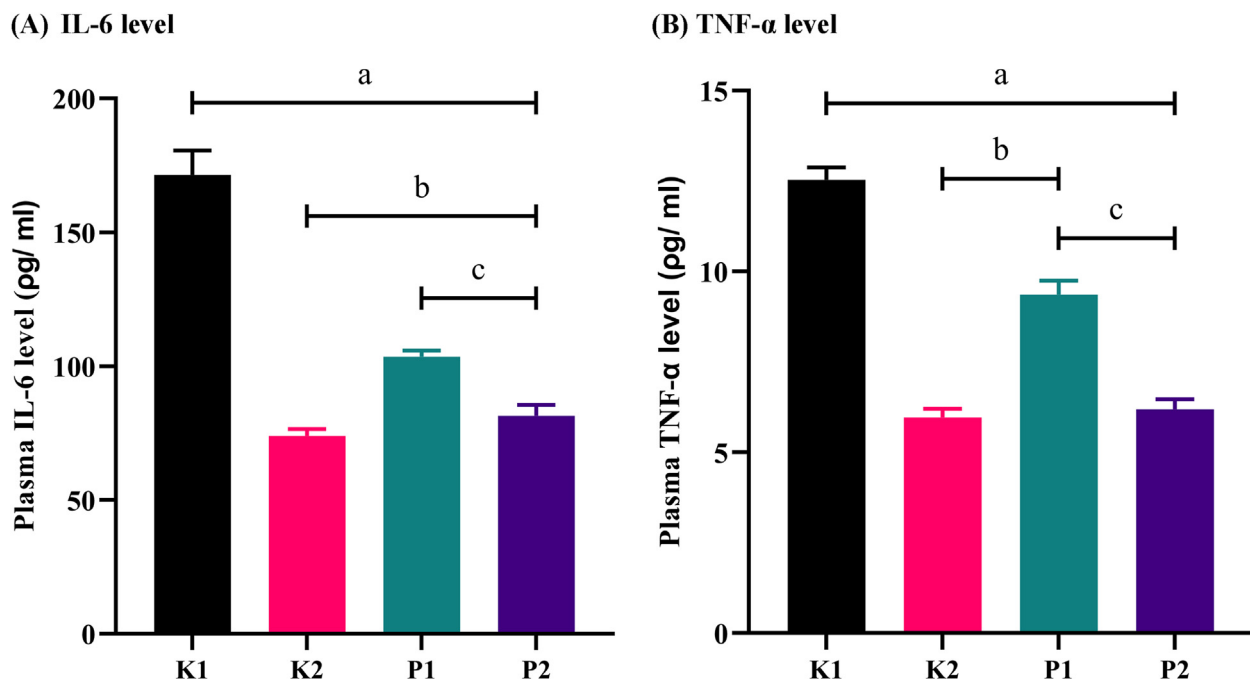
$\mu\text{g/ml}$ , (P2)  $14.67 \pm 0.34 \mu\text{g/ml}$  vs. (K1)  $5.90 \pm 0.28 \mu\text{g/ml}$ ;  $P < 0.000$ ]. As expected, the GLP-1 level from K2 (orlistat group) was the highest compared to the P1 and P2 groups. Of interest, the GLP-1 level from the P2 group (BGF 4g) was significantly higher as compared to the P1 (BGF 2g) ( $P < 0.000$ , Figure 2A).

Similarly, circulating PYY increased significantly in K2, P1, and P2 groups after the BGF intervention. Meanwhile, circulating PYY decreased in the K1 group (Table 3). There was a significant difference in PYY levels after the intervention between (K2)  $510.99 \pm 2.62 \mu\text{g/ml}$ , (P1)  $344.21 \pm 1.61 \mu\text{g/ml}$ , (P2)  $413.72 \pm 2.39 \mu\text{g/ml}$  vs (K1)  $25.02 \pm 1.19 \mu\text{g/ml}$ ,

respectively ( $P < 0.000$ ). The concentration of PYY level in group K2 was significantly higher as compared to P1 and P2 groups, as expected ( $P < 0.000$ , Figure 2B). Similar to the GLP-1 level, the PYY level was significantly higher in the P2 group (BGF 4g) than in the P1 group (BGF 2g), which may suggest a dose-dependent effect on the BGF intervention.

### 3.4. Effect of BGF intervention on lipid profiles

We investigated the effect of BGF intervention on total cholesterol, triglyceride (TG), HDL cholesterol, and TG/HDL ratio. Compared to



**Figure 4.** Effect of BGF intervention on lipid profiles (A) IL-6 and (B) TNF- $\alpha$  in diet-induced obesity rats. Values were expressed as mean  $\pm$  standard deviation (SD); K1: positive control group (normal diet); K2: negative control group (normal diet + orlistat 30 mg/kg BW/d); P1: treatment group 1 (normal diet + BGF 2 g/200 g BW/d); P2: treatment group 2 (normal diet + BGF 4 g/200 g BW/d); n: 7; a:  $p < 0.001$  vs K1; b:  $p < 0.001$  vs K2; c:  $p < 0.001$  vs P1;  $p < 0.05$  was considered significant with One Way ANOVA test with Tamhane post hoc test for IL-6 and Bonferroni post hoc for TNF- $\alpha$ .

baseline levels, all lipid parameters significantly declined in K2 (orlistat), P1 (BGF 2g), and P2 (BGF 4g) (Table 3). In contrast, the K1 group showed a significant drop in total cholesterol, TG, TG/HDL ratio, and HDL compared to the baseline.

After the intervention, total cholesterol, TG, and TG/HDL ratio in the K2, P1 and P2 groups were significantly lower from those in the K1 group [total cholesterol  $103.40 \pm 4.08$  mg/dl,  $146.14 \pm 6.95$  mg/dl,  $109.13 \pm 3.36$  mg/dl vs.  $212.18 \pm 4.00$  mg/dl; triglyceride  $76.33 \pm 3.52$  mg/dl,  $110.66 \pm 2.42$  mg/dl,  $84.86 \pm 2.43$  mg/dl vs.  $138.38 \pm 5.92$  mg/dl; TG/HDL ratio  $0.89 \pm 0.04$ ,  $2.39 \pm 0.21$ ,  $1.15 \pm 0.06$  vs.  $6.01 \pm 0.68$ , respectively;  $P < 0.000$ ; Figure 3A,B,D]. In addition, HDL cholesterol was significantly higher in the K2, P1, and P2 vs K1 group [ $85.94 \pm 2.35$  mg/dl,  $46.50 \pm 3.72$  mg/dl,  $74.06 \pm 4.43$  mg/dl vs  $23.19 \pm 2.07$  mg/dl,  $P < 0.001$ , Figure 3C].

Interestingly, the total cholesterol level in the P2 group (BGF 4g) was not different from the K2 group (orlistat) and was significantly lower than in the P1 group (BGF 2g). The TG/HDL ratio of the P2 group was considerably lower than the P1 group and comparable to the K2 group (Figure 3A, D). These findings imply that the BGF reduced total cholesterol level and TG/HDL ratio as effectively as orlistat in a dose-dependent manner on HFSD diet-induced obese rats.

### 3.5. Effect of BGF intervention on systemic inflammations

In this *in vivo* study, the effects of BGF intervention on IL6 and TNF- $\alpha$  levels were determined. The systemic IL6 and TNF- $\alpha$  levels declined after the intervention in the K2, P1, and P2 groups compared to the baseline (Table 3). Meanwhile, the K1 group was inclined compared to baseline levels.

We found that IL-6 and TNF- $\alpha$  levels of the P2 group were significantly lower as compared to the K1 group [IL-6  $81.52 \pm 4.02$  pg/ml vs.  $171.54 \pm 9.01$  pg/ml; TNF- $\alpha$   $6.19 \pm 0.29$  pg/ml vs.  $12.53 \pm 0.35$  pg/ml, respectively; Figure 4A, 4B]. The systemic inflammation of the P2 group was also substantially lower compared to the P1 group (IL-6  $103.47 \pm 2.29$  pg/ml; TNF- $\alpha$   $9.36 \pm 0.39$  pg/ml) despite being slightly higher when compared to the K2 group (IL-6  $73.97 \pm 2.53$  pg/ml; TNF- $\alpha$   $5.97 \pm 0.24$

pg/ml) (Figure 4A, B). These findings suggested that the BGF intervention effect on systemic inflammation is likely dose-dependent.

### 3.6. Effect of BGF intervention on caecum SCFAs concentration

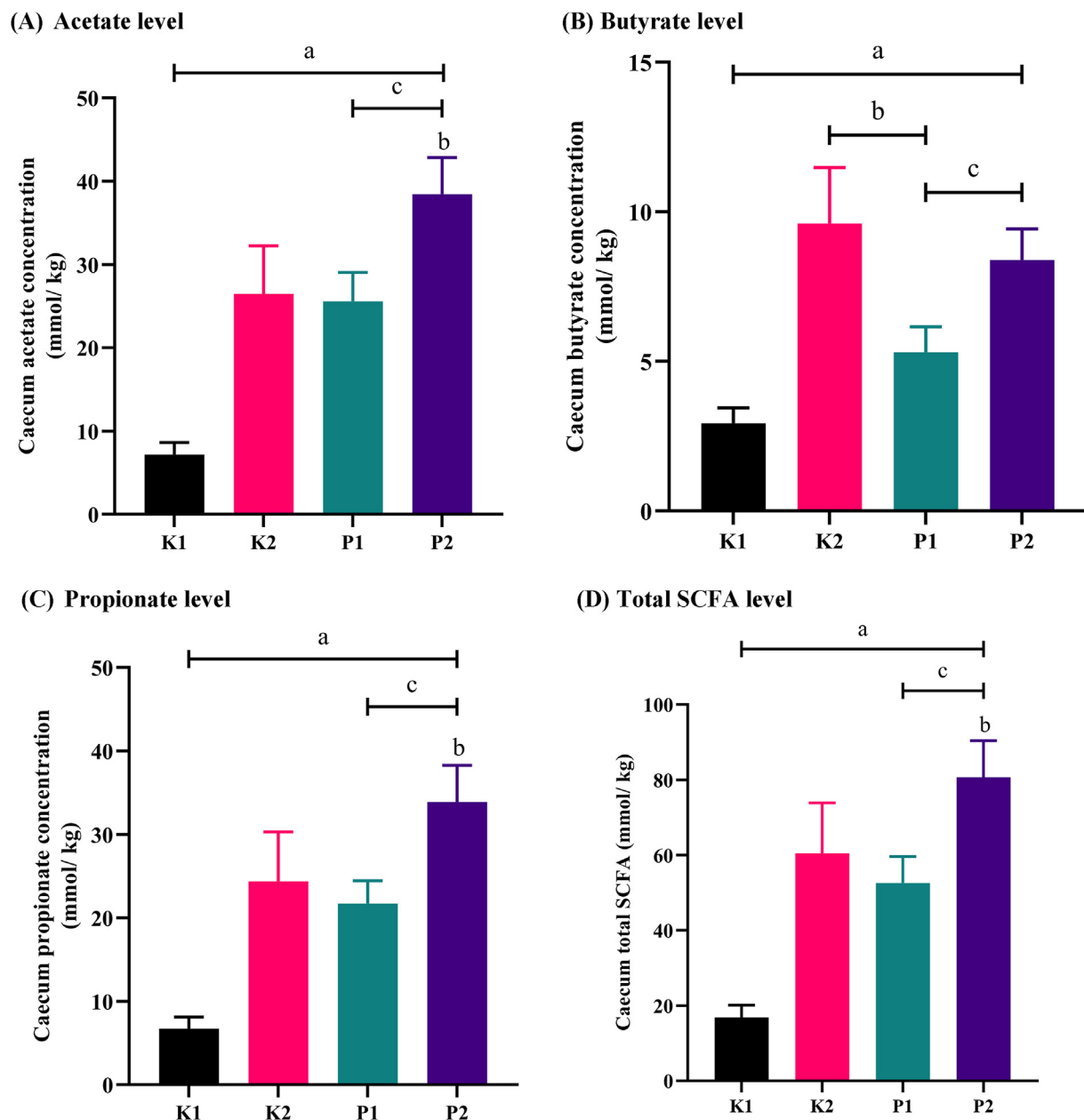
Acetate, propionate, butyrate, and total SCFA concentrations in K2, P1, and P2 groups were significantly higher from those in the K1 group [(A) acetate  $26.49 \pm 5.78$  mmol/kg,  $25.57 \pm 3.49$  mmol/kg,  $38.47 \pm 4.39$  mmol/kg vs.  $7.19 \pm 1.45$  mmol/kg; (B) butyrate  $9.61 \pm 1.87$  mmol/kg,  $5.30 \pm 0.86$  mmol/kg,  $8.39 \pm 1.05$  mmol/kg vs  $2.94 \pm 0.51$  mmol/kg; (C) propionate  $24.38 \pm 5.94$  mmol/kg,  $21.73 \pm 2.73$  mmol/kg,  $33.90 \pm 4.36$  mmol/kg vs  $6.73 \pm 1.38$  mmol/kg; (D) total SCFA  $60.48 \pm 13.44$  mmol/kg,  $52.60 \pm 6.99$  mmol/kg,  $80.75 \pm 9.67$  mmol/kg vs  $16.86 \pm 3.27$  mmol/kg, respectively, Figure 5A–D]. The P2 group had considerably higher acetate, propionate, and total SCFA concentrations than the K2 and P1 groups. Surprisingly, the P2 group caecum butyrate was comparable to the K2 group ( $p = 0.065$ ). These findings suggested that BGF treatment modulates gut microbiota metabolites acetate, butyrate, and propionate.

### 3.7. Effect of BGF intervention on body weight

There was a statistically significant difference in body weight of the K2, P1, and P2 groups compared to the K1 group ( $267.71 \pm 5.69$  g,  $275.00 \pm 4.08$  g,  $263.00 \pm 4.62$  g vs.  $291.71 \pm 7.41$  g, respectively). The average body weight in the P2 group differed considerably from those in the P1 group. A slope trend could be seen in the K2 and P2 groups after a week of BGF treatment despite a rise in body weight. In comparison to the other groups, the P2 group gained the least weight (Figure 6A and B).

### 3.8. Correlation between variables after BGF intervention

The Spearman test revealed that following the BGF intervention, SCFA statistically correlates with all other variables. SCFA levels were positively associated with GLP-1, PYY, and HDL levels. On the other hand, there was a negative relationship between SCFA levels and total



**Figure 5.** Effect of BGF intervention on caecum SCFAs concentration [(A) Acetate, (B) Butyrate, (C) Propionate, and (D) Total SCFA in diet-induced obesity rats. Values were expressed as mean  $\pm$  standard deviation (SD); K1: positive control group (normal diet); K2: negative control group (normal diet + orlistat 30 mg/kg BW/d); P1: treatment group 1 (normal diet + BGF 2 g/200 g BW/d); P2: treatment group 2 (normal diet + BGF 4 g/200 g BW/d); n: 7; a:  $p < 0.001$  vs K1; b:  $p < 0.001$  vs K2; c:  $p < 0.001$  vs P1;  $p < 0.05$  was considered significant with Kruskal Wallis test, followed with Mann Whitney test for all parameters.

cholesterol, triglycerides, TG/HDL ratio, IL-6, TNF- $\alpha$ , and body weight (Table 4). These findings suggested SCFAs may play a role in metabolic health regulation of obesity.

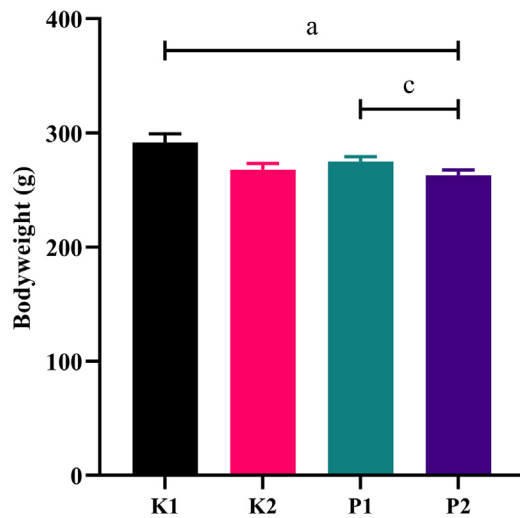
#### 4. Discussion

In this study, administering BGF containing dietary fibre, flavonoid, and phenolic compounds increased circulating satiety hormone levels and reduced body weight. Furthermore, we demonstrated a dose-dependent manner of decreased lipid profiles (total cholesterol, TG, and TG/HDL ratio) and inflammatory markers (IL6 and TNF- $\alpha$ ) after the BGF intervention. Moreover, the BGF intervention increased total SCFA levels, acetate, butyrate, and propionate levels. These SCFA levels substantially correlated with those metabolic parameters after the intervention. Thus, the present results revealed that probably, BGF affects

metabolic health in obesity by modulating the gut microbiota induce-SCFA production.

This study determined that the BGF product has a higher fibre and flavonoid content. Some studies have reported that dietary fibre, flavonoids, and phenols impact metabolic health and obesity [15, 26, 27, 28]. Dietary fibres are essential for intestinal health. They increase stool bulk and decrease transit time, enhancing digestion and absorption. The indigenous microbiota also ferments fibre and nondigestible carbohydrates such as resistant starch, which results in short-chain fatty acids (SCFAs) production, mainly acetate, propionate, and butyrate [6]. According to the findings of this study, SCFA concentrations were higher in the group that received the BGF in a dose-dependent manner. This finding is consistent with previous research indicating that dietary fibre from various food sources or supplements might increase SCFA synthesis in rats and humans [29, 30, 31, 32].

(A) Bodyweight change after intervention



(B) Bodyweight trend during intervention

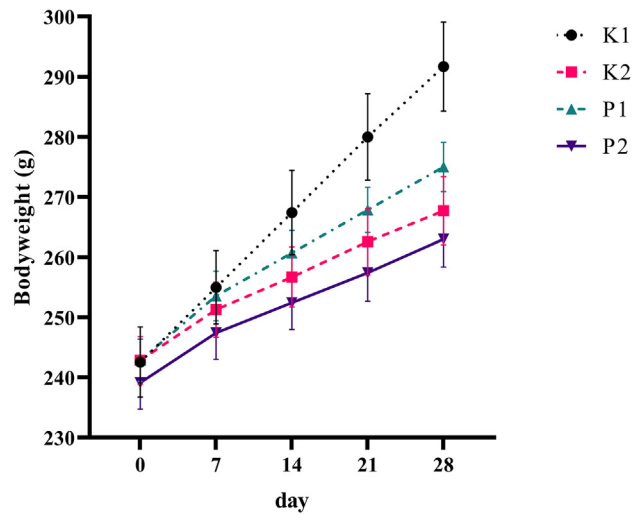


Figure 6. Effect of BGF intervention on bodyweight in diet-induced obesity rats. Values were expressed as mean ± standard deviation (SD); K1: positive control group (normal diet); K2: negative control group (normal diet + orlistat 30 mg/kg BW/d); P1: treatment group 1 (normal diet + BGF 2 g/200 g BW/d); P2: treatment group 2 (normal diet + BGF 4 g/200 g BW/d); n: 7; a: p < 0.05 vs K1; b: p < 0.05 vs K2; c: p < 0.05 vs P1; p < 0.05 was considered significant with One Way ANOVA test, followed with Bonferroni post hoc test.

Interestingly, bioactive components such as flavonoids and polyphenols have been shown to promote the synthesis of SCFAs by colonic bacteria. This mechanism either accelerates the development of SCFA-producing bacteria (prebiotic effect) or stimulates their metabolic processes [15]. A study utilizing flavonoid-rich herbal plant extracts demonstrated that it is possible to increase the concentration of cecum SCFA in mice fed a high-fat, high-sugar (HFSD) diet [33].

It is suggested that the SCFA might interact with G-protein-coupled receptors (GPR41/FFAR3 and GPR43/FFAR2) to alter tissue-specific lipid metabolism and energy harvesting. Several in vitro experiments using human and rodent intestinal cell lines found that SCFA can promote GLP-1 and PYY production via G-protein-coupled receptor activation (GPR41 and GPR43) [34]. In addition, an increased adipose tissue function by SCFA metabolites has been linked to various pathways, including metabolic flux regulation and satiety signalling. SCFA signalling via FFAR2 and FFAR3 promotes GLP-1 release from intestinal cells and initiates gluconeogenesis in the first pathway [35]. Intestinal gluconeogenesis is a metabolic mechanism that helps to double insulin sensitivity and reduce appetite. These mechanistic findings support research in humans and rats that has demonstrated modulation of PYY production, improved satiety, and decreased energy intake by gut microbiota-derived metabolites SCFA [36].

The SCFA, acetate, and butyrate increase intestinal GLP-1 secretion and, to a minor extent, increase intestinal PYY secretion [37]. In particular, propionate can bind to receptors on the surface of colonic epithelial cells. The anorexigenic peptides GLP-1 and PYY are secreted when these receptors are activated. GLP-1 and PYY releases will enhance satiety in the host [38]. Previous animal studies have shown that dietary fibre consumption can promote enteroendocrine L cell differentiation, increasing luminal and plasma PYY and GLP-1 levels [39, 40]. As shown in humans, prebiotic supplementation (16 g/day) has enhanced plasma PYY and GLP-1 levels, decreased appetite, and improved glycemic sensitivity [41]. Similarly, dietary supplementation with inulin-type fructan up to 21 g/day in obese adults might increase plasma PYY and suppress ghrelin production, a marker related to reduced food consumption, fat accumulation, and weight gain [42].

Furthermore, we found that the BGF intervention has decreased TG/HDL ratio in this study. Recently it has been demonstrated that TG/HDL ratio was positively correlated with insulin resistance in humans [43]. SCFA may affect the adipose tissue lipid buffering capacity by affecting intracellular lipolysis, the hydrolysis process of stored triacylglycerol into glycerol and fatty acids, and may thereby affect circulating lipid concentrations [10]. An increased level of SCFA may partly explain the

Table 4. Spearman's correlation between blood biomarkers and caecum SCFA after BGF intervention.

Parameter	Acetate		Propionate		Butyrate		Total SCFA	
	r	p	r	p	r	p	r	p
GLP-1	0.558	0.006*	0.576	0.004*	0.826	<0.001*	0.626	0.001*
PYY	0.548	0.007*	0.575	0.004*	0.850	<0.001*	0.617	0.002*
Total cholesterol	-0.674	<0.001*	-0.681	<0.001*	-0.909	<0.001*	-0.727	<0.001*
Triglyceride	-0.590	0.003*	-0.623	0.002*	-0.882	<0.001*	-0.665	0.001*
HDL	0.601	0.002*	0.628	0.001*	0.859	<0.001*	0.665	0.001*
TG/HDL ratio	-0.608	0.002*	-0.631	0.001*	-0.874	<0.001*	-0.671	<0.001*
IL-6	-0.586	0.003*	-0.618	0.002*	-0.852	<0.001*	-0.651	0.001*
TNF-α	-0.652	0.001*	-0.662	0.001*	-0.838	<0.001*	-0.698	<0.001*
Bodyweight	-0.694	<0.001*	-0.694	<0.001*	-0.718	<0.001*	-0.714	<0.001*

GLP-1: glucagon-like peptide-1; PYY: peptide yy; HDL: high-density lipoprotein; TG: triglyceride; IL-6: interleukin-6; TNF-α: tumor necrosis factor-alpha; SCFA: short-chain fatty acid; \*: statistically significant at p < 0.05.



TG/HDL ratio decrease. This finding supports a previous study showing an improvement in insulin sensitivity following dietary fibre intervention in humans. The SCFA, particularly acetate and propionate, may improve adipose tissue lipid buffering by inhibiting intracellular lipolysis and increasing adipogenesis [44].

Obesity is characterized by low-grade chronic inflammation. Inflammatory markers such as IL-6 and TNF- $\alpha$  are increased in obesity [45]. An increased fat accumulation, followed by adipocyte remodelling, is often accompanied by elevation of IL6 and TNF- $\alpha$  secreted by adipose tissue [46]. As markedly by both IL6 and TNF- $\alpha$  levels, chronic low-grade inflammation is often associated with obesity and insulin resistance [47]. In a review, it was found that SCFA interacts with G protein-coupled receptors (GPCR; GPR41, GPR43, and GPR109A) to enhance immunity [48]. *In vivo* data show that acute rectal and intravenous administration of sodium acetate (compared to saline) reduces plasma TNF- $\alpha$  levels in obese women [49]. Consistent with these results, *in vitro* studies show that SCFA can also decrease IL-6 and TNF- $\alpha$  by suppressing local inflammation in intestinal tissues [50, 51]. In a human observational study, dietary fibre intake was associated with gut microbiota composition and lower inflammation. That cohort was conducted in relatively healthy men [52]. Despite the beneficial effects of SCFA on the regulation of immune function, the role of dietary fibre on inflammation remains to be investigated in a well-controlled clinical trial study.

This present study has certain limitations. First, we did not analyze the type of dietary fibre in BGF. Next, we did not perform the transcriptomic analysis at tissue levels and the gut microbiota, which may benefit from observing the underlying mechanisms. Finally, the high dosage of flour used to transpose into human study may need to be re-calculated due to the significant amount of flour they will consume. Nevertheless, we have performed extensive analysis concerning systemic metabolic biomarkers and gut-derived metabolite caecum SCFAs.

## 5. Conclusions

In this *in vivo* study in Wistar rats induced obesity, the BGF intervention increases circulating GLP-1 and PYY, decreases lipid profiles, reduces systemic inflammations, and increases SCFA levels in a dose-dependent manner. Furthermore, BGF intervention slightly decreases body weight gain in diet-induced obesity Wistar rats. A further mechanistic study is needed to unravel underlying mechanisms. These results suggest that BGF might be considered a dietary fibre source (a prebiotic functional food) to prevent and control obesity; therefore, more well-controlled human studies may warrant investigation.

## Declarations

### Author contribution statement

Rinta Amalia; Adriyan Pramono: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Diana Nur Afifah: Analyzed and interpreted the data.

Etika Ratna Noer; Muflihatul Muniroh; Andri Cahyo Kumoro: Conceived and designed the experiments; Analyzed and interpreted the data.

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### Data availability statement

Data included in article/supp. material/referenced in article.

## Declaration of interest's statement

The authors declare no conflict of interest.

## Additional information

No additional information is available for this paper.

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