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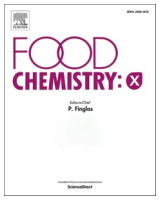

The impact of supplementation with highland barley in different nutrients on weight loss: The nutrients and function relationship

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ABSTRACT

The dietary fiber in highland barley (HB) may be a key nutrient in exerting its physiological functions. How about the effects of other nutrients? In this study, a high-fat diet (HFD)-induced obese mice was constructed to investigate the effects of HB in different nutrients on weight loss. The results showed that peeling treatment had varying effects on HB nutrients. Consumption of HB significantly mitigated weight gain, postprandial blood glucose levels, organ weight, adipose tissue weight, and fat accumulation in obese mice. Consumption of HB also ameliorated hepatic steatosis, hyperlipemia, abnormal liver function, and dysregulation of inflammatory factor expression in mice. Unpeeled and once peeled HB presented the best effect. Furthermore, HB consumption improved the imbalance of gut microbiota in HFD-induced mice, with protein and dietary fiber being the key factors in exerting the improvement effect. This study highlights the potential of protein and dietary fiber in HB for treating obesity.

1. Introduction

Obesity is a global health concern characterized by the excessive accumulation of fat, which can have adverse health effects. Many factors contribute to the prevalence of obesity, including diet and physical activity, with diet playing a primary role. Consumption of high-energy foods, especially in large quantities, is a major contributor to obesity. In recent years, the health benefits of whole grain foods for weight loss and management are reported (Li et al., 2022). Numerous studies have found that whole grains can reduce the incidence of specific diseases. The United States Food and Drug Administration (FDA) recognized the health benefits of whole grains in 1999 and permitted food packaging to state that "consuming whole grain foods can reduce the risk of cardiovascular disease" (Marquart, Wiemer, Jones, & Jacob, 2007; Pauline & Rimm, 2007; Slavin, 2007).

Highland barley (*Hordeum vulgare* L. var. *nudum* Hook. f), a member of the *Poaceae* family and *Hordeum* genus, exhibits strong cold resistance and can thrive in barren lands. With its short growing period and high yield, highland barley is one of the most suitable crops for cultivation in the Qinghai-Tibet Plateau. It is a major staple food in the region, with extensive cultivation and high production (Lyu, Ma, Liu, & Wang, 2022). Highland barley is utilized for various purposes, including food, feed, brewing, and vinegar production. The grains are rich in protein, dietary fiber, and vitamins, while low in fat and sugars, making it a superior cereal. Compared to China's other four major staples-rice, corn, wheat, and potato, highland barley contains higher levels of dietary fiber and trace elements such as calcium, phosphorus, potassium, and sodium. Additionally, it contains numerous bioactive compounds beneficial to health. Studies have shown that highland barley grains are abundant in β-glucans, polyphenol, and essential amino acids. As a whole grain, highland barley retains all its nutrients and has shown great potential in improving conditions such as obesity and regulating cholesterol levels (Li, Qin, et al., 2022; Xia et al., 2017).

Gut bacteria primarily rely on dietary nutrients that cannot be digested by enzymes in the upper digestive tract, such as resistant starch, non-starch polysaccharides, and oligosaccharides, collectively known as dietary fiber, for energy and growth (Teixeira, Prykhodko, Alminger, Fåk Hållenius, & Nyman, 2018). The consumption of barley or β-glucans

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extracted from barley grains in mouse/rat models can lower blood glucose and lipid levels, enhance antioxidant capacity, and mitigate obesity (Garcia-Mazcorro, Mills, Murphy, & Noratto, 2018). These physiological effects are accompanied by the composition changes of gut microbiota. However, what about the effects of other nutrients in highland barley? In this paper, obese mice induced by high-fat diet were used to analyze the effects of highland barley in different nutrients on obesity improvement. Additionally, 16S rRNA sequencing was employed to analyze changes in the gut microbiota. Also, the effect of different nutrients in highland barley on gut microbiota was analyzed by a correlation analysis. By systematically analyzing the impact of highland barley on microbiota alterations, we aim to provide comprehensive insights into its key nutrient against obesity.

2. Materials and methods

2.1. Materials

The highland barley variety used in this study is Kunlun No. 15, purchased from Qinghai Shangkang Biotechnology Co., Ltd. Total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), alanine transaminase (ALT), and aspartate transaminase (AST) assay kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). TNF-α, IL-1β, and IL-6 ELISA kits were purchased from Shanghai Tongwei Biotechnology Co., Ltd. (Shanghai, China). All chemicals used were of chromatographic or analytical quality and were purchased from Sinopharm Chemical Reagent Co., Ltd. in Shanghai, China, unless otherwise stated.

2.2. Sample preparation

Preparation of barley grains with different degrees of dehulling was achieved by processing 10 kg of whole barley grains through a dehulling machine (TM05C, Buhler Machinery Co., Ltd., Changzhou, China). The grains were dehulled once, twice, and three times, respectively. The samples were then ground into powder and stored at −20 °C for later use. The samples were designated as follows: unpeeled barley (PTHB-0), barley peeled once (PTHB-1), barley peeled twice (PTHB-2), and barley peeled three times (PTHB-3).

2.3. Composition analysis of highland barley samples

The nutrients, including moisture, protein, fat, ash, dietary fiber, starch, and total phenolic content, were measured for the four types of highland barley samples. Total starch content was determined using the total starch assay kit from Megazyme (K-TSTA, Germany). Dietary fiber content was determined using dietary fiber assay kit from Megazyme (K-TDFR, Germany). Total phenolic content was measured using the Folin-Ciocalteu method.

2.4. Animal feeding

Six-week-old male C57BL/6 mice, weighing 21–22 g, were obtained from Shanghai Bikai Keyi Biotechnology Co., Ltd. The mice were housed under standard conditions (temperature: 25 ± 2 °C, humidity: 50 ± 5 %, 12-h light/dark cycle). After one week of acclimation, the mice were fed high-fat and high-sugar diet for 12 weeks. Mice were considered successfully modeled when their body weight exceeded the average weight of mice on a normal diet by 20 %. The mice were then randomly divided into six groups (6 per group): control group (NC), unpeeled highland barley group (PTHB-0), once-peeled highland barley group (PTHB-1), twice-peeled highland barley group (PTHB-2), thrice-peeled highland barley group (PTHB-3), and high-fat diet group (HFD). The control group was fed a normal diet, while the high-fat group continued on the high-fat diet for an additional 8 weeks. Blood glucose levels were measured daily, and body weight and food intake were recorded weekly. After 8 weeks, the mice were sacrificed by cervical dislocation, and subcutaneous and visceral fat were collected, weighed, and the fat index (fat weight/body weight) was calculated. All animal protocols adhered to the Guidelines for Care and Use of Laboratory Animals of Shidong Hospital and received approval from the Animal Ethics Committee of Shidong Hospital (Fig. 1A). Also, this study complied with relevant laws and regulations at a national or international level. The diet compositions of all the samples, including NC, HFD, and highland barley diets were shown in detail in Table 1.

2.5. Serum biochemical analysis

At the end of the feeding period (8 weeks), mice were fasted overnight, and blood was collected from the orbital sinus into 1.5 mL EP tubes. Serum was obtained by centrifugation at 3000 rpm for 10 min. Serum biochemical parameters, including TC, TG, LDL-C, HDL-C, ALT, and AST, were measured according to the kit instructions. Inflammatory factors in the liver of the mice, including TNF-α, IL-1β, and IL-6, were measured using ELISA kits following the manufacturer's protocols.

2.6. Histopathological analysis

Liver and epididymal fat were collected for histopathological examination. The fat and liver tissues were fixed in 4 % paraformaldehyde for 4–6 h and then embedded in paraffin. Paraffin sections (approximately 4 μm thick) were stained with hematoxylin and eosin (HE), mounted with neutral resin, and examined under a microscope. Further, the liver tissue was fixed, dehydrated, and embedded to prepare frozen sections. Then, the sections were washed with 60% isopropanol, stained with Oil Red O working solution for a certain period (such as 2-5 minutes or 10-15 minutes), followed by color adjustment with 60% isopropanol. Finally, the nuclei were counterstained with hematoxylin and the sections were mounted for observation. Images were captured and analyzed using KF-Viewer and Image J softwares to measure the long and short diameters of each adipocyte and calculate their area.

2.7. Analysis of gut microbial diversity

At the end of the feeding period (8 weeks), fresh fecal samples (2–3 pellets per mouse) were collected from individually housed mice into sterile 2 mL centrifuge tubes, rapidly frozen in liquid nitrogen, and stored at −80 °C. The total DNA was eluted in 50 μL of elution buffer and stored at −80 °C. PCR amplification of the V3-V4 region of the 16S rRNA gene was performed using the primers 338F (ACTCCTACGGGAGGCAGCAG) and 806R (GGACTACHVGGGTWTCTAAT). The PCR reactions were carried out in a total volume of 25 μL, containing 25 ng of template DNA, 12.5 μL of PCR Premix, 2.5 μL of each primer, and PCR-grade H_2O. The PCR conditions were as follows: initial denaturation at 98 °C for 30 s, followed by 32 cycles of denaturation at 98 °C for 10 s, annealing at 54 °C for 30 s, and extension at 72 °C for 45 s, with a final extension at 72 °C for 10 min. Library construction was performed using a NovaSeq6000 platform and QIIME2 software package.

2.8. Statistical analysis

All the data were analyzed using SPSS software (SPSS for Windows, 16.0, 2007, SPSS Inc., USA) and the results are presented as mean ± standard error (SE, $n \geq 3$).

3. Results

3.1. Composition analysis

The chemical compositions of highland barley at different peeling times are listed in Table 2. There were no significant differences in the

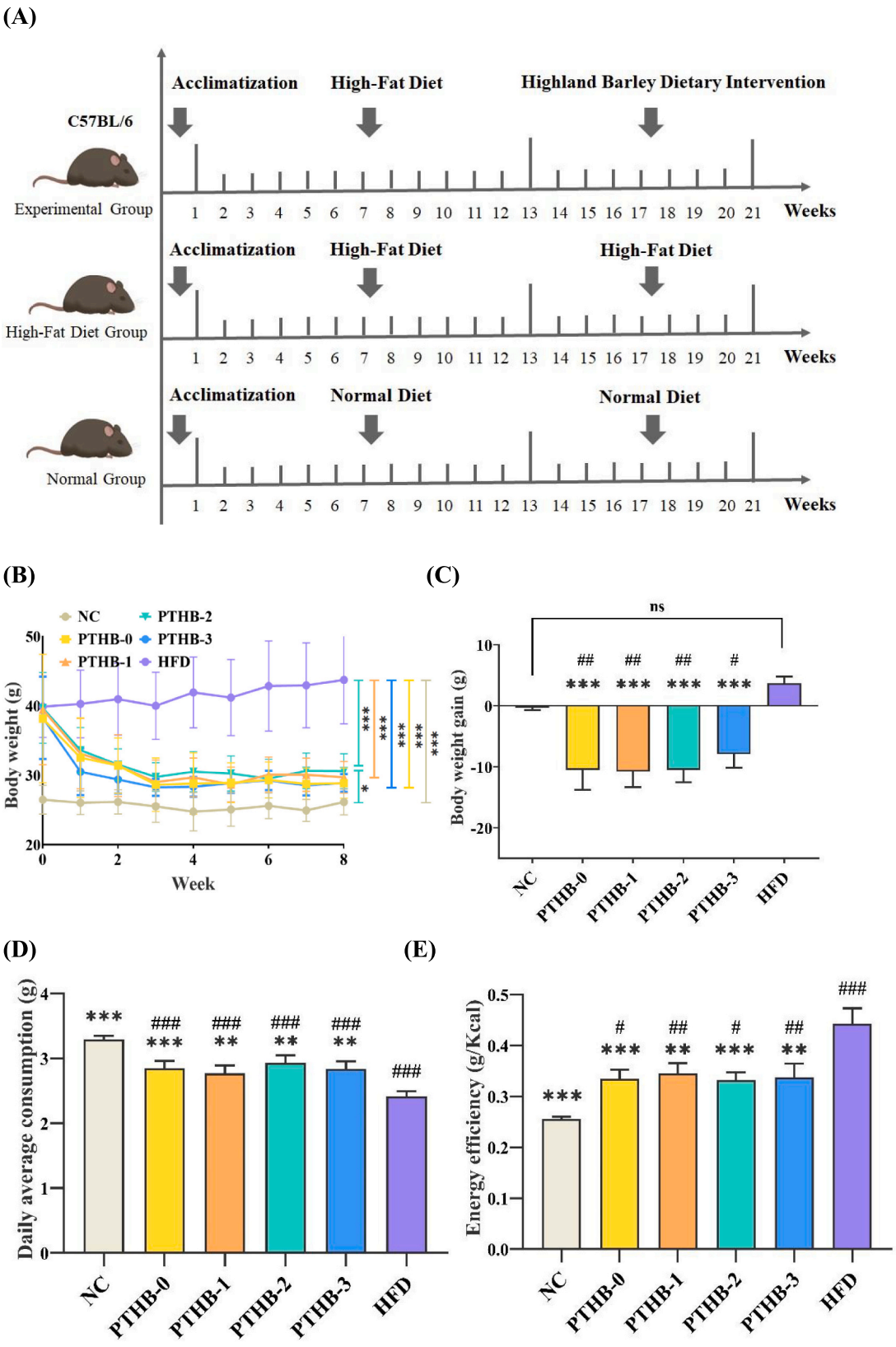


Fig. 1. Effect of highland barley on body weight, food and energy intake, and blood glucose in mice obese induced by HFD. (A) Animal experimental design; (B) Body weight changes over 8 weeks; (C) Body weight gain; (D) Daily average consumption; (E) Energy intake; (F) Blood glucose. The data are expressed as the means ± SE. Different symbols indicated significant difference among the compared groups ($^{*/\#}$ $P < 0.05$, $^{**/\#\#}$ $P < 0.01$, $^{***/\#\#\#}$ $P < 0.001$).

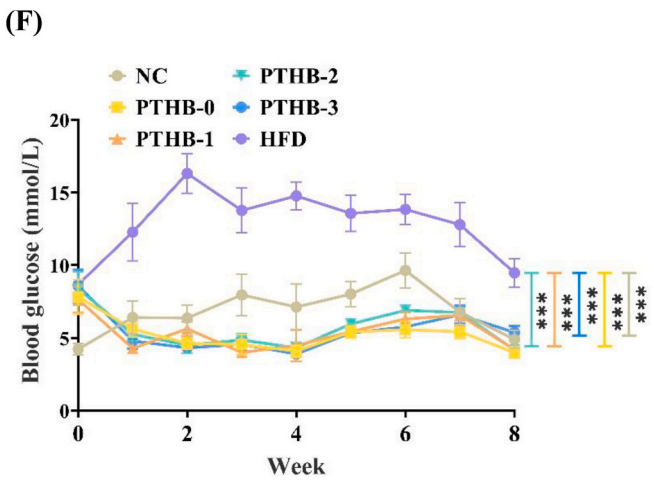


Fig. 1. (*continued*).

Table 1
Composition of the experimental diets (g/100 g).

Ingredient (%)	NC	HFD	PTHB-0	PTHB-1	PTHB-2	PTHB-3
Highland barley	–	–	100.00	100.00	100.00	100.00
Casein, 80 Mesh	–	25.84	–	–	–	–
L-Cystine	–	0.39	–	–	–	–
Starch	59.00	–	–	–	–	–
Maltodextrin	1.00	16.15	–	–	–	–
Sucrose	–	8.89	–	–	–	–
Cellulose	–	6.45	–	–	–	–
Soybean Meal	25.00	–	–	–	–	–
Fish Meal	5.00	–	–	–	–	–
Soybean Oil	2.00	3.23	–	–	–	–
Lard*	–	31.66	–	–	–	–
Vitamin and Mineral Mix	8.00	7.36	–	–	–	–
Carbohydrate	60.00	31.49	52.69	56.31	53.99	58.77
Protein	5.00	25.84	10.66	13.06	10.28	9.27
Fat	2.00	34.89	3.70	3.28	2.93	2.71
Caloric density (kcal/100 g)	278.00	543.33	286.66	306.94	283.45	296.51

moisture and ash content of highland barley with different peeling times. PTHB-3 exhibited the lowest protein content (9.27 %. *w*/w), while PTHB-0 (10.66 %. w/w) and PTHB-2 (10.28 %. w/w) had higher protein content than PTHB-3 ($P < 0.05$). Interestingly, PTHB-1 had the highest protein content (13.06 %, w/w) ($P < 0.05$). The increase in peeling times resulted in a decrease in fat and dietary fiber content in highland barley ($P < 0.05$). In particular, after the second peeling, the dietary fiber content showed the most significant reduction, decreasing by 47.3 %, and then stabilizing after the third peeling, indicating that two peeling sessions resulted in a higher loss of dietary fiber in highland barley. Notably, the increase in peeling times led to an increase in starch content in highland barley ($P < 0.05$). After the third peeling, starch content exhibited the highest increase (11.5 %). Furthermore, the free, bound, and total phenolic of the highland barley samples were also analyzed and listed in Table 2. Compared to the unpeeled highland barley, the phenolic contents decreased substantially with the increasing peeling times, particularly for total and free phenolic contents (total phenolic content decreased by 36.4 % for PTHB-1 and 50.8 % for PTHB-3). This result indicated an imbalanced distribution of phenolics in highland barley between the outer and inner layers. Consequently, it was evident that the peeling treatment of highland barley increased starch content but also resulted in the loss of other nutrients, with the extent of loss varying with the peeling time. The nutrient quality of highland barley tended to stabilize after three peeling sessions.

3.2. *The effect of highland barley on body weight, food and energy intake, and blood glucose in mice*

The body weights of the six groups of mice during the feeding period were shown in Fig. 1. At the end of the modeling, mice in the HFD group presented significantly higher body weight than the NC group ($P < 0.05$) and remained so until the end of the 8-week feeding period, indicating successful modeling of the high-fat diet. From the first week onwards, the PTHB groups had significantly lower body weights than the HFD group ($P < 0.05$), and this difference persisted until the end of the feeding period (8 weeks). For the PTHB groups, the body weights decreased dramatically in the 3-week feeding period. There was no significant difference until the end of the feeding period (8 weeks). Also, there was no significant difference of the body weight variation values in the PTHB groups ($P > 0.05$), but the values were significantly lower in the NC or HFD group ($P < 0.05$) (Fig. 1BC). These results suggested that highland barley could effectively mitigate weight gain induced by a

Table 2
Nutritional composition of highland barley in different peeling times(%, *w*/w).

Different peeling times	Moisture	Ash	Protein	Fat	Starch	Dietary fiber	Total phenolic (mg/100 g)	Free phenolic (mg/100 g)	Conjugated phenolic (mg/100 g)
0	11.21 ± 0.38a	1.35 ± 0.23a	10.66 ± 0.07b	3.70 ± 0.12c	52.69 ± 1.38a	21.15 ± 0.55c	251.98 ± 1.81d	163.66 ± 4.39c	88.32 ± 3.01c
1	11.06 ± 0.13a	1.18 ± 0.46a	13.06 ± 0.20c	3.28 ± 0.17b	53.99 ± 0.31a	18.08 ± 0.64b	160.17 ± 1.67c	81.75 ± 2.59b	78.41 ± 3.32b
2	10.49 ± 0.39a	1.09 ± 0.19a	10.28 ± 0.23b	2.93 ± 0.21ab	56.31 ± 0.36ba	11.14 ± 0.31a	133.35 ± 1.24b	74.76 ± 2.44a	58.60 ± 2.07b
3	9.69 ± 0.09a	0.80 ± 0.27a	9.27 ± 0.38a	2.71 ± 0.27a	58.77 ± 0.95c	9.53 ± 1.47a	123.94 ± 2.69a	56.35 ± 1.83a	67.59 ± 3.68a

Note: Different letters in each column presented a significant difference ($P < 0.05$).

high-fat diet.

The NC group had a higher average food intake than the HFD groups ($P < 0.001$). Furthermore, the NC and PTHB groups had a lower energy intake than the HFD group ($P < 0.01$). Moreover, the blood glucose of NC group mice was within the normal range (4–6 mmol/L at the zero and eight week), while the blood glucose of HFD-fed mice reached 9.1 mmol/L at the end of the feeding period, indicating a severe impact of the high-fat diet on blood glucose levels. The blood glucose levels in the highland barley feeding groups were significantly decreased during 1 to 4 weeks of feeding (from 7.8 mmol/L (PTHB-0) to the normal range), and increased slightly during 5–8 weeks feeding (still in the normal range), indicating the potential of highland barley to lower blood glucose levels in high-fat diet mice (Fig. 1DEF).

3.3. The effect of highland barley on organ weight and fat distribution in mice

The high-fat diet feeding may induce fat accumulation in some organs. Hence, the weights of the liver of mice in the six groups were recorded and listed in Fig. 2. Compared to the HFD group, the weights of the liver were lower in the NC and PTHB groups ($P < 0.001$). The liver weight to body weight (liver index) in the PTHB-2 and PTHB-3 groups were slightly higher, while the weights in the PTHB-0 and PTHB-1 groups were similar to the NC group (Fig. 2AB). These results demonstrated that highland barley with zero and one time of peeling might be more beneficial for reducing organ weight to the normal level in the obese mice caused by a high-fat diet.

Furthermore, there was no significant fat accumulation in the subcutaneous and abdominal fat of mice in the NC group and PTHB groups, while a large amount of fat accumulation could be seen in the subcutaneous and abdominal fat of mice in the HFD group (Fig. 2C). The abdominal fat weight to body weight in HFD mice was significantly higher than that in the NC and PTHB groups ($P < 0.001$). Moreover, epididymal fat weight to body weight in HFD mice was also significantly higher than that in the NC and PTHB groups ($P < 0.001$) (Fig. 2DE). This result further indicated that long-term consumption of highland barley could reduce the accumulation of fat in obese mice and could restore it to the level of mice with a normal diet.

3.4. Histological staining

To further investigate the histological characteristics and lipid

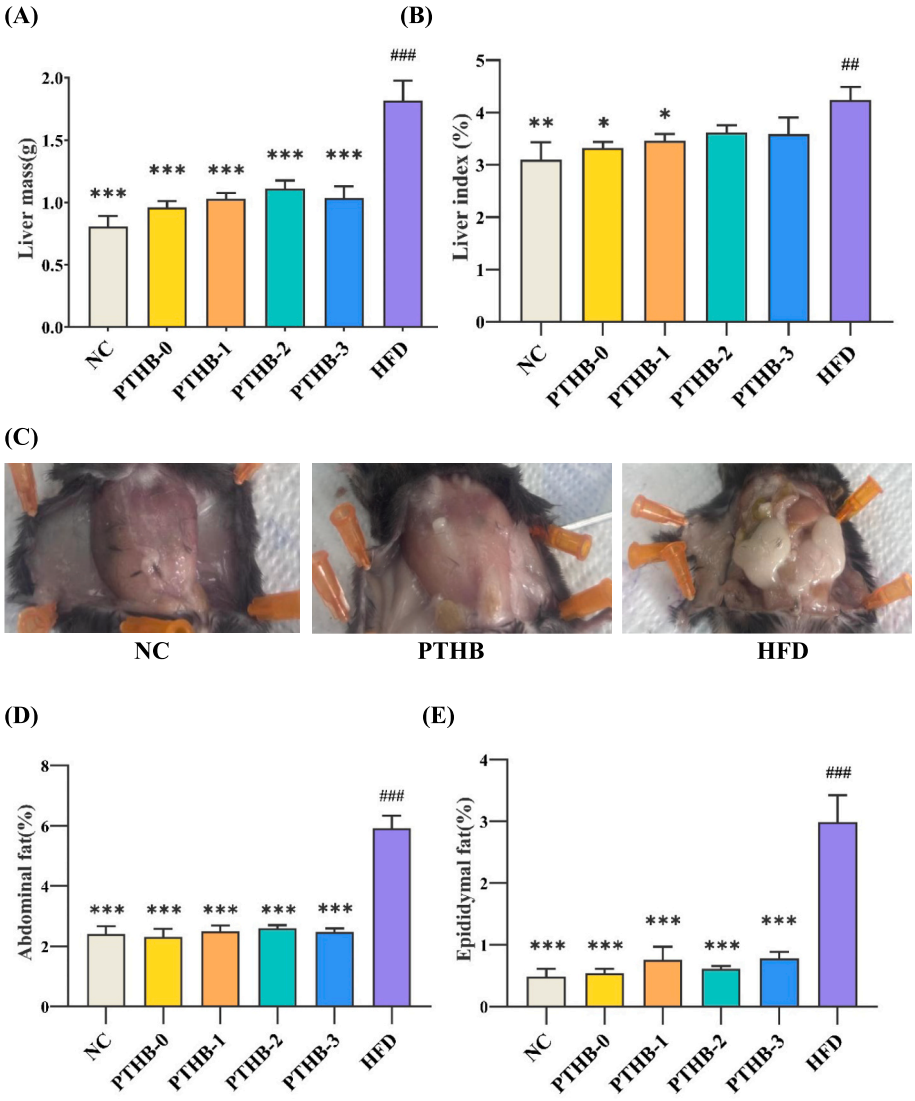


Fig. 2. Effect of highland barley on organ weight and fat distribution in mice. (A) Liver weight; (B) Liver index; (C) Representative image of subcutaneous and abdominal fat; (D) Abdominal fat weight to body weight; (E) Epididymal fat weight to body weight. The data are expressed as the means ± SE. Different symbols indicated significant difference among the compared groups ($^{*/\#}$ $P < 0.05$, $^{**/\#\#}$ $P < 0.01$, $^{***/\#\#\#}$ $P < 0.001$).

accumulation in the liver cells of the mice in each group, H&E and Oil Red O staining were performed on liver tissues (Fig. 3). The control group showed orderly hepatocyte arrangement, clear cell structure, and centrally located nuclei without steatosis, while the HFD group exhibited severe steatosis with numerous fat vacuoles, particularly around the central veins. However, the PTHB groups showed a marked improvement in this condition (Fig. 3A). The Oil Red O staining was same as those of the H&E staining, with the HFD group showing disrupted hepatocyte structures and notable fat accumulation, along with a marked aggregation of macrophages, and PTHB helped mitigate the disruption of liver cells and structures, reduce macrophage infiltration, and decrease fat accumulation in the liver (Fig. 3B). Furthermore, the NC and PTHB groups had a lower relative content of fat droplets than the HFD group ($P < 0.001$) (Fig. 3D). Thus, the lipid deposition symptoms in the vascular endothelium were alleviated in the highland barley consumption groups, demonstrating the effective relief of lipid accumulation in the mice.

In epididymal fat staining, the HFD group had a lower number of fat cells than the NC group, and the fat cells were significantly enlarged, while the NC group had smaller and more uniform fat cells (Fig. 3C). Although the PTHB groups had larger fat cells than the NC group, they were smaller than the HFD group (Fig. 3E, $P < 0.001$). Moreover, the HFD group had larger fat cell area than the NC and PTHB groups. These results indicated that a long-term high-fat diet might increase both body fat deposition and fat cell size in mice, and long-term intake of highland barley could improve the enlargement of epididymal fat cells caused by high-fat diets, especially for unpeeled highland barley.

3.5. The effect of highland barley on blood lipids and liver function

Obesity-related biochemical indicators such as TC, TG, HDL-C, and LDL-C were measured after 8 weeks. The results indicated that the levels of biochemical indicators in the HFD groups were significantly higher ($P < 0.001$). Furthermore, no significant difference was found in TC, HDL-C, and LDL-C levels between the NC and PTHB groups, but the TG value in the PTHB-3 group was significantly higher ($P < 0.05$). These results suggested that highland barley consumption led to a significant reduction in TC, TG, HDL-C, and LDL-C levels compared to the HFD group ($P < 0.05$), and the unpeeled, one, and two times peeled highland barley presented a better effect (Fig. 4ABCD).

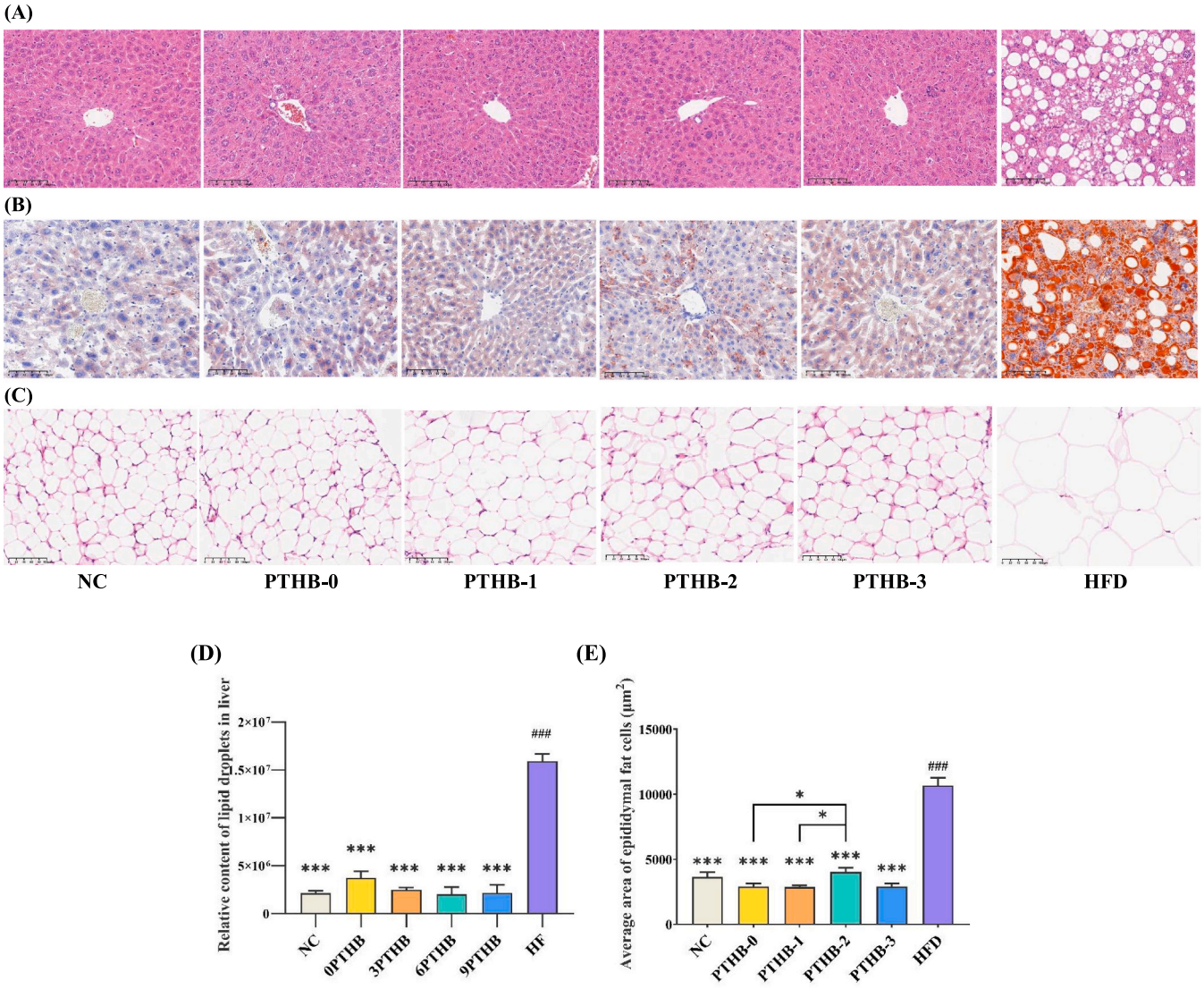


Fig. 3. Histological staining. (A) Representative image of H&*E*-stained liver tissues; (B) Representative image of Oil Red O-stained liver tissues; (C) Representative image of H&E-stained epididymal fat; (D) the relative content of fat droplets in livers; (E) Average area of epididymal fat. The data are expressed as the means ± SE. Different symbols indicated significant difference among the compared groups ($^{*/\#}$ $P < 0.05$, $^{**/\#\#}$ $P < 0.01$, $^{***/\#\#\#}$ $P < 0.001$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

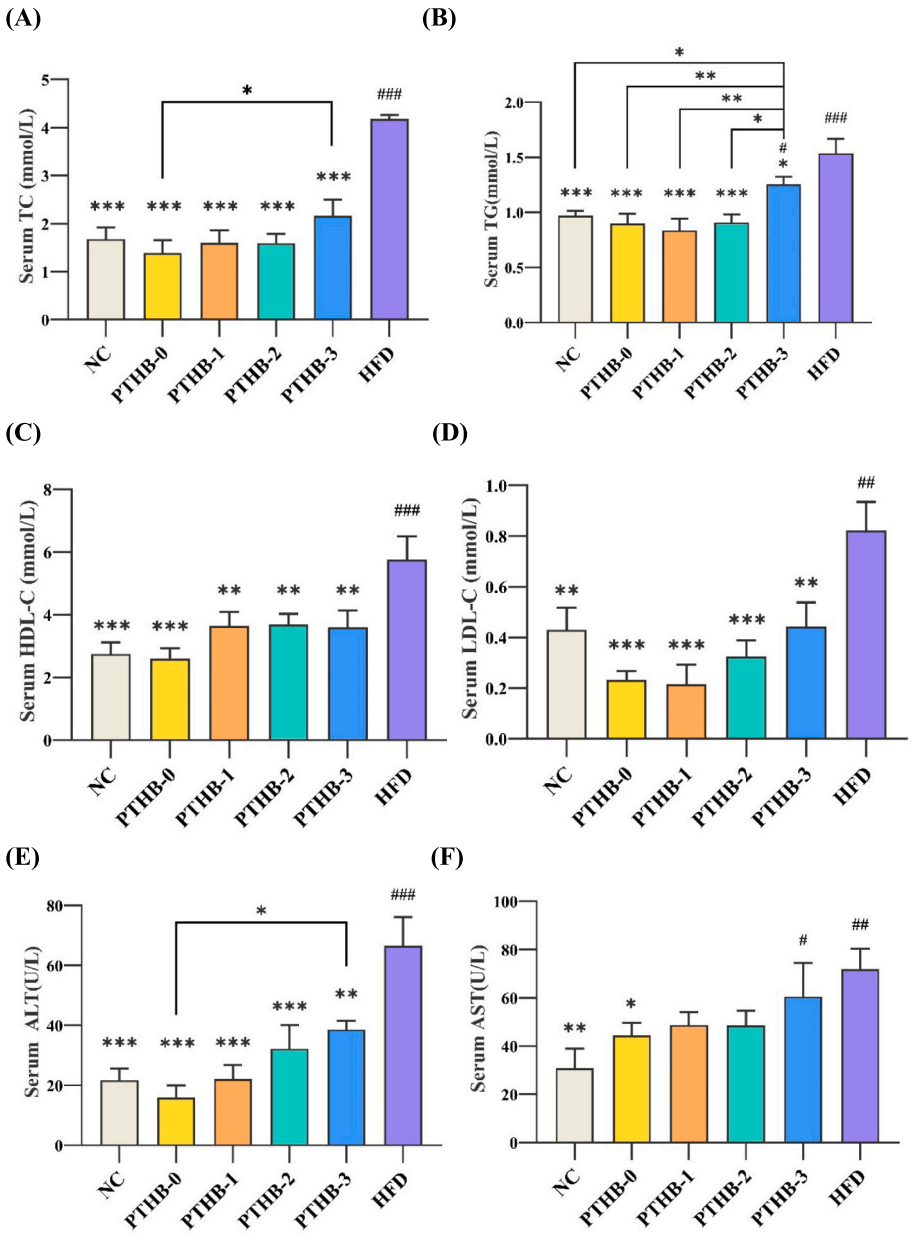


Fig. 4. Effect of highland barley on blood lipids and liver function. (A) TC; (B) TG; (C) HDL-C; (D) LDL-C; (E); ALT (F) AST; (G) TNF-α; (H) IL-1β; (I) IL-6. The data are expressed as the means ± SE. Different symbols indicated significant difference among the compared groups ($^{*/\#}$ $P < 0.05$, $^{**/\#\#}$ $P < 0.01$, $^{***/\#\#\#}$ $P < 0.001$).

Alanine aminotransferase (ALT) is primarily found in liver cells, and its release into the bloodstream indicates liver cell necrosis or damage. Aspartate aminotransferase (AST) levels are distributed in the myocardium, liver, skeletal muscle, and kidneys, with elevated levels in the blood indicating liver cell damage (Shin, Kim, & Nam, 2016). ALT activity was higher in the HFD group than in the NC group and PTHB groups ($P < 0.001$), and there was no significant difference between the NC and PTHB groups (Fig. 4EF). AST activity was higher in the HFD group compared to the NC group, with a slight reduction in the PTHB groups, but without significant differences from the HFD group ($P > 0.05$). These results suggested that highland barley consumption partially alleviated liver damage induced by a high-fat diet.

Obesity and its associated metabolic syndromes are often accompanied by abnormal inflammatory responses. These responses are characterized by a decrease in the expression of anti-inflammatory factors and an increase in the expression of pro-inflammatory factors. On the other hand, IL-1β is a prototypical pro-inflammatory cytokine that can induce the expression of intercellular adhesion molecule 1 (ICAM-1), leading to increased adhesion of leukocytes and endothelial cells and exacerbating inflammatory damage (Valenti et al., 2008). Moreover, the serum levels of TNF-α are often significantly elevated in obese patients. No significant difference was found in the levels of IL-1β and TNF-α among the six groups. Furthermore, the IL-6 level in the HFD group was significantly higher, and the level in the groups consuming highland barley without peeling and with one peeling was lower than that in the groups with two peeling and three peeling (Fig. 4GHI). These results indicated that consumption of highland barley effectively alleviated the dysregulation of inflammatory factor expression induced by the HFD, thus mitigating systemic inflammatory responses. This effect was particularly prominent in highland barley with zero and one peeling.

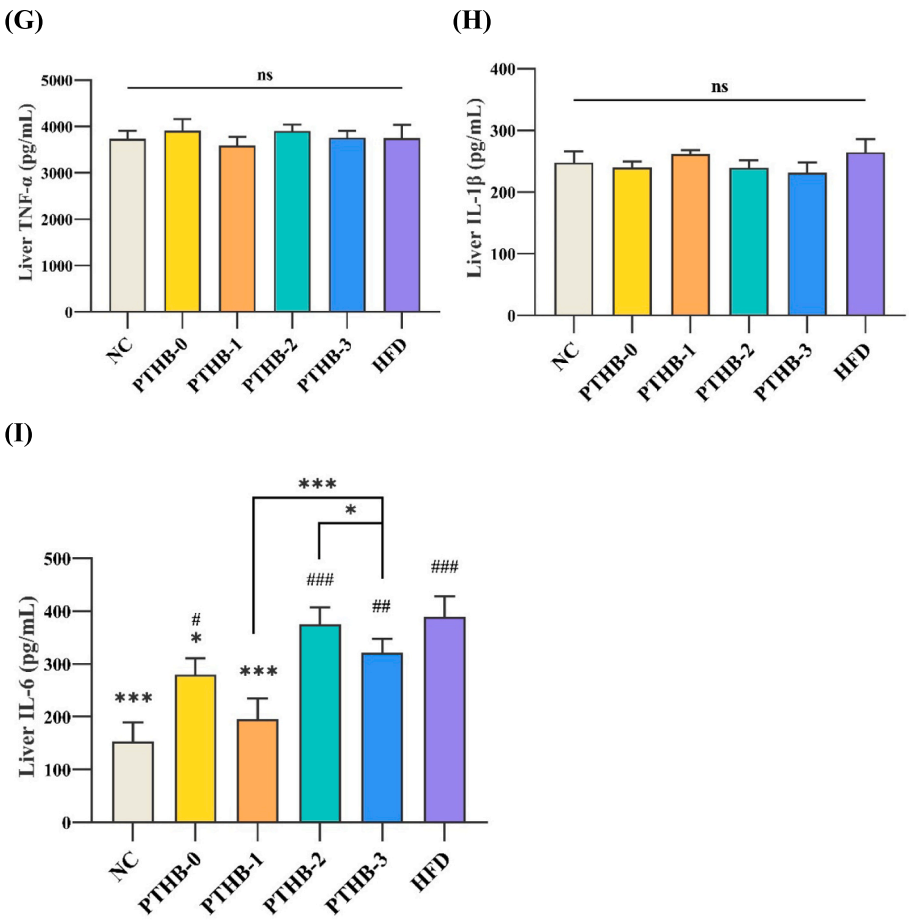


Fig. 4. (*continued*).

3.6. The effects of highland barley on gut microbiota in mice

3.6.1. Gut microbiota diversity

Alpha diversity reflects species richness, evenness, and sequencing depth. This diversity can be measured using indices such as Chao and Shannon. The Chao index primarily reflects species richness, while Shannon comprehensively reflects both richness and evenness. A higher Shannon index indicates greater uncertainty and higher diversity (Lozupone & Knight, 2008). Beta diversity, on the other hand, refers to the differences in species composition between different environmental communities. It represents the overall diversity or biological heterogeneity of a specific environmental community, along with alpha diversity. The diversity indices of gut microbiota in the three groups of mice are presented in Fig. 5ABC. The analysis of alpha diversity indicated that the Shannon, ACE, and Chao indices of the HFD group were significantly reduced. This suggested that the alpha diversity of gut microbiota would be severely affected by high-fat diet. In contrast, the groups consuming highland barley showed increased alpha diversity compared to the HFD group. There were significant increases in the ACE and Shannon indices for the PTHB-0 ($P < 0.05$) and PTHB-1 group ($P < 0.01$), as well as in the Chao index for the PTHB-0 ($P < 0.05$), PTHB-1 ($P < 0.01$), and PTHB-2 group ($P < 0.05$).

In the principal coordinates analysis (PCoA) (beta diversity) plots, the samples primarily clustered around the central region, with PC1 accounting for 38.29 % and PC2 for 18.35 % of the variation (Fig. 5D). The NC or HFD group was distinctly separated from the PTHB groups. The PTHB groups clustered together, with some overlap and slight differences in distribution. Alpha and beta diversity analyses of the gut microbiota indicated that a high-fat diet affected the structure of the gut microbiota in mice, with a significant reduction in diversity compared to normal diet mice. The PTHB groups exhibited smaller differences in microbiota diversity and distribution compared to the HFD and NC groups, indicating that gut microbiota structure in HFD mice could be altered by long-term highland barley consumption.

3.6.2. Gut microbiota composition

The major bacterial phyla present in the gut microbiota of mice were *Bacteroidetes, Firmicutes*, *Verrucomicrobiota*, and *Actinobacteria*. Compared to the NC group, the HFD group exhibited a significant increase in *Firmicutes* and a decrease in *Bacteroidetes*, resulting in an increased *Firmicutes/Bacteroidetes* ratio, which is characteristic of obese model mice (Fig. 6ABC). Highland barley consumption significantly reduced the *Firmicutes/Bacteroidetes* ratio, and no significant difference was found between the PTHB and NC group, suggesting that highland barley consumption alleviated the obesity symptoms induced by a high-fat diet. The HFD group exhibited a significant decrease in the relative abundance of *norank_f_Muribaculaceae*, while the relative abundance of *Faecalibaculum*, *Dubosiella*, *Akkermansia*, *Bacillus*, and *Lactobacillus* significantly increased. Following highland barley consumption, the relative abundance of *Dubosiella*, *Bacillus*, and *Faecalibaculum* visibly decreased, indicating the effective consumption of highland barley in combating obesity.

In Fig. 6D, the average relative abundance of *norank_f_Muribaculaceae* and *norank_f_Clostridia_UCG-014* was higher in the NC or PTHB groups. Moreover, compared with the NC group, the differences in the relative abundance of different genera in the HFD group were more pronounced, while the differences in the distribution of different genera in the highland barley group were relatively small.

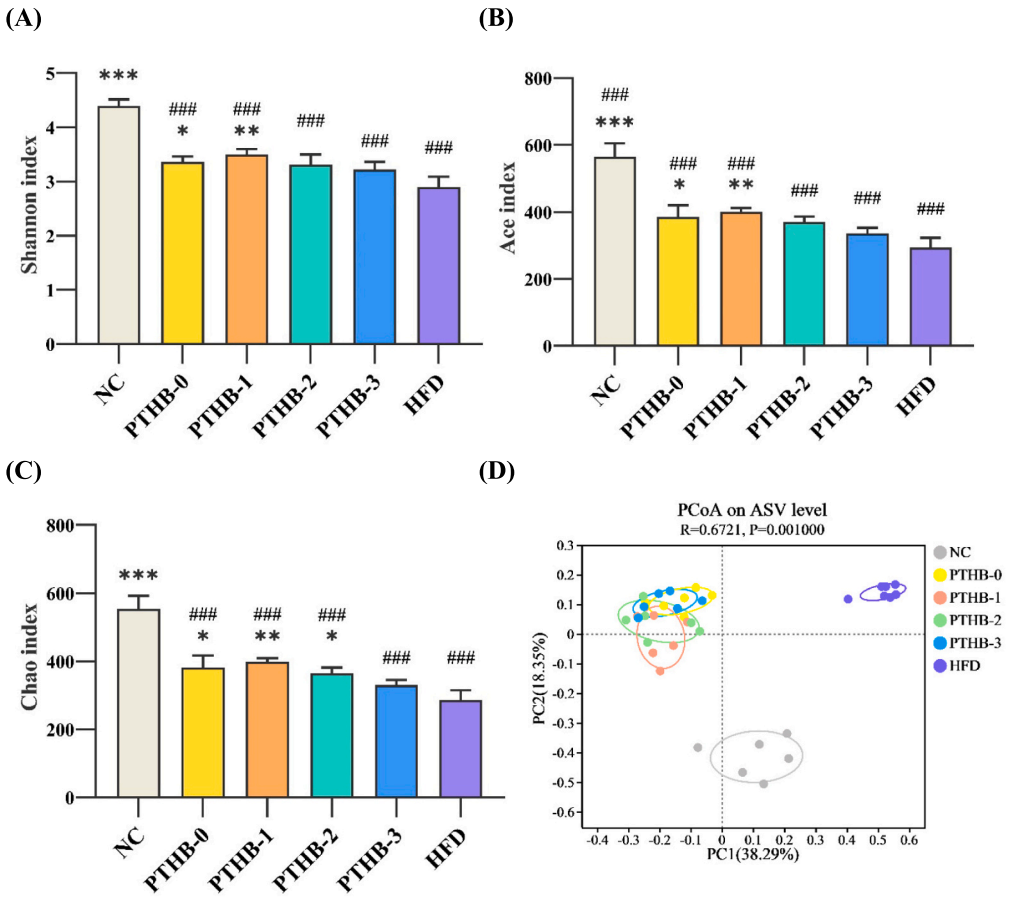


Fig. 5. Effects of highland barley on the diversities of the gut microbiota. (A) Shannon index; (B) Ace index; (C) Chao index; (D) PCoA analysis. The data are expressed as the means ± SE. Different symbols indicated significant difference among the compared groups ($^{*/\#}$ $P < 0.05$, $^{**/\#\#}$ $P < 0.01$, $^{***/\#\#\#}$ $P < 0.001$).

3.6.3. Gut microbiota difference

As shown in Fig. 6E, the ralative abundance of gut microbiota in fifteen genera were highly significant ($P < 0.01$), including 9 species with high significance ($P < 0.001$). By analyzing representative genera, it can be seen that the relative abundance of *norank_f_Muribaculaceae* in the HFD group was the lowest, while the relative abundance of *norank_f_Muribaculaceae* in the barley group was higher than 50 %. The relative abundance of *Lactobacillus* in the HFD group was the highest. In addition, the relative abundance of *Faecalibaculum*, *Dubosiella*, and *Bacillus* in the HFD group was significantly higher than that in the normal group and highland barley group. The relative abundance of *Bacteroides*, *Muribaculum*, and *Coriobacteriaceae_*UCG-002 in the barley group was significantly higher than that in the NC and HFD group.

3.7. Correlation analysis between highland barley nutrients and gut microbiota in mice

To analyze the relationship between the nutrients and gut microbiota, the redundancy analysis (RDA) and Spearman correlation heatmap analysis were conducted. As shown in Fig. 6FG, protein (P), fat (F), and dietary fiber (D) contents in highland barley were the major environmental factors impacting the gut microbiota in mice. Additionally, protein in highland barley was positively correlated with *Muribaculum* ($P < 0.001$), *norank_f_norank_o_Costridia_*UCG-014 ($P < 0.05$), *Coriobacteriaceae_*UCG-002 ($P < 0.05$), *Prevotellaceae_*UCG-001 ($P < 0.001$), *Ruminococcus* ($P < 0.05$), *Enterorhabdus* ($P < 0.01$), *Lachnospiraceae_*CG-001 ($P < 0.05$), and *unclassified_c_Clostridia* ($P < 0.01$), and negatively correlated with *Faecalbaculum* ($P < 0.05$), *Eubacterium_siraeum_group* ($P < 0.01$), and *Lachnospiraceae_*UCG-006 ($P < 0.01$). Fat, dietary fiber, and total phenolic (TP) contents were positively correlated with *Prevotellaceae_*UCG-001 ($P < 0.001$), *Roseburia* ($P < 0.05$), *Ruminococcus* ($P < 0.05$), *Enterorhabdus* ($P < 0.001$), *Lachnospiraceae_*UCG-001 ($P < 0.05$), and *unclassified_c_Clostridia* ($P < 0.05$), and negatively correlated with *Eubacterium_siraeum_group* ($P < 0.001$). Starch (S) content was positively correlated with *Eubacterium_siraeum_group* ($P < 0.01$), and negatively correlated with *Rikenellaceae_RC9_gut_group* ($P < 0.01$), *Prevotellaceae_*UCG-001 ($P < 0.05$), *Roseburia* ($P < 0.01$), *norank_f_Oscillospiraceae* ($P < 0.05$), *Odoribacter* ($P < 0.05$), *Lachnospiraceae_*CG-001 ($P < 0.01$), and *Lachnoclostridium* ($P < 0.05$).

4. Discussion

In this study, obese mice induced by a high-fat diet were used to explore the health effects of highland barley in different nutrients on abnormal glucose and lipid metabolism and preliminarily clarified the underlying mechanism based on gut microbiota. The results demonstrated that peeling treatment had varying effects on the nutrients of highland barley. This could be attributed to the fact that protein was mainly distributed in the endosperm of highland barley, and only a small amount of endosperm was peeled off during the first peeling process (Zheng, Wang, Xiong, Song, & Zhang, 2023; Zong, Tian, Zhang, Liu, & Chen, 2022). These findings are consistent with those reported by Zong et al. (2022). Approximately 75 % of β-glucan was distributed in the endosperm, while about 26 % was found in the cell walls of the aleurone layer. Additionally, fat was mainly distributed in the cortex and embryo of highland barley. The removal of the peel and seed coat resulted in the enrichment of starch in HBF. Furthermore, free phenolic compounds were concentrated in the bran and aleurone layers (Xia et al., 2022;

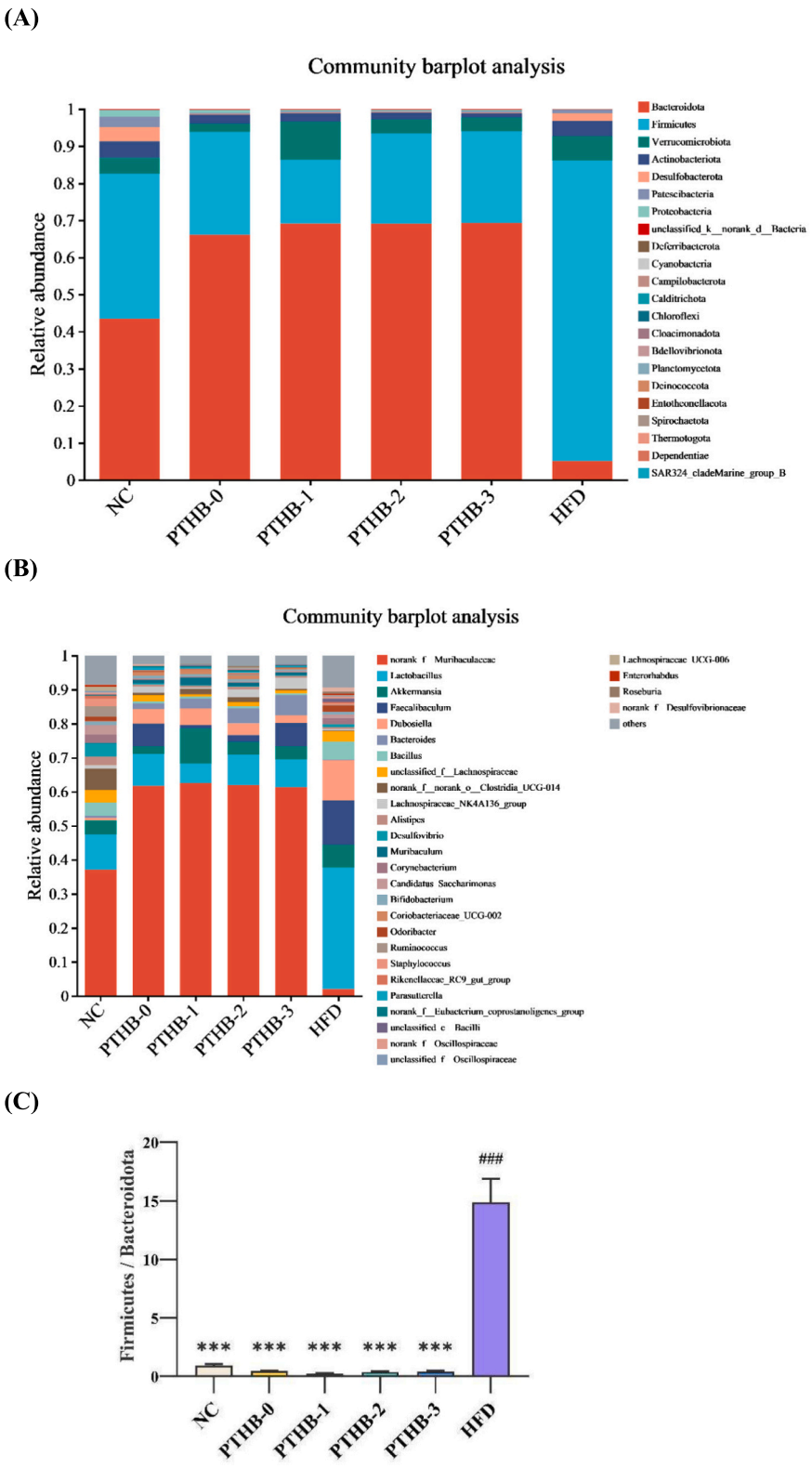


Fig. 6. Effects of highland barley on gut microbiota composition and species difference and correlation analysis between highland barley components and gut microbiota in mice. (AB) gut microbiota structure at the phylum and genus levels in each experimental group; (C) *Firmicutes/Bacteroidetes* ratio in each experimental group; (D) Species-relative abundance of gut microbiota; (E) Kruskal-Wallis test; (F) RDA on Genus level; (G) Spearman correlation heatmap analysis. The data are expressed as the means ± SE. Different symbols indicated significant difference among the compared groups ($^{*/\#}$ $P < 0.05$, $^{**/\#\#}$ $P < 0.01$, $^{***/\#\#\#}$ $P < 0.001$).

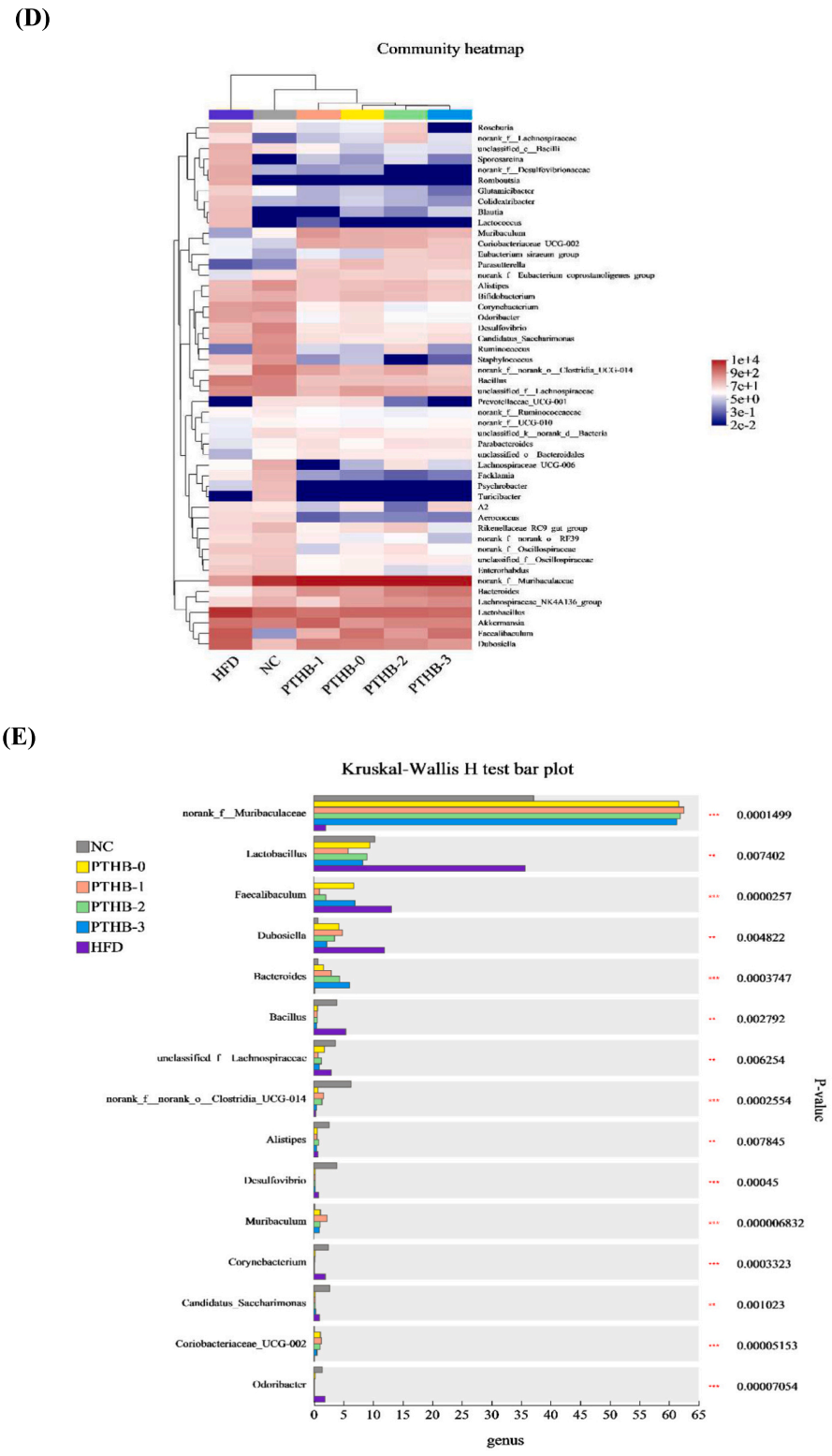
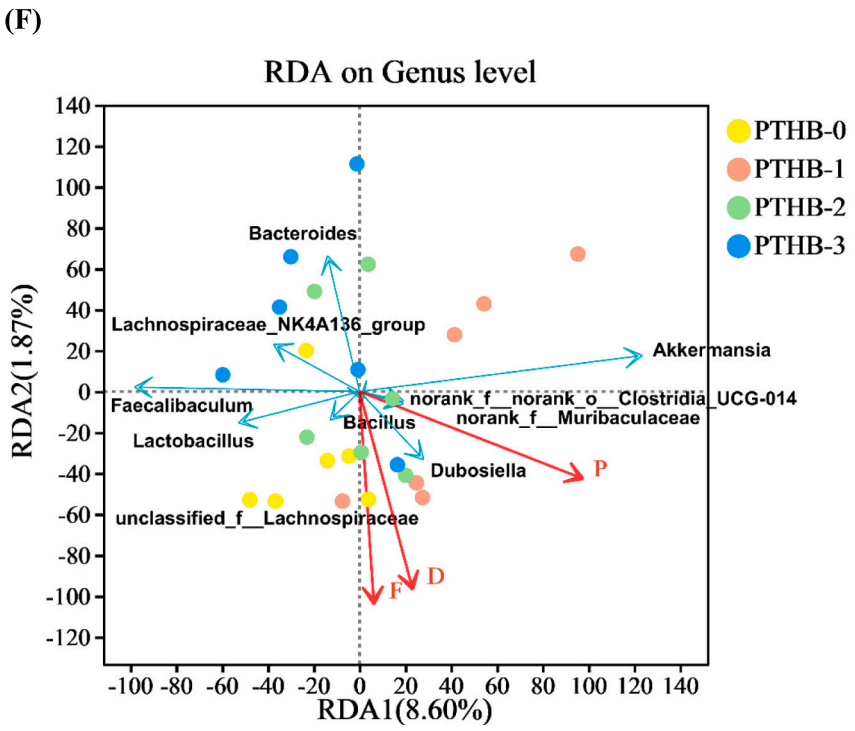


Fig. 6. (*continued*).

Zheng et al., 2023; Zong et al., 2022).

Obesity is an inflammatory disease accompanied by numerous metabolic complications. Previous studies have shown that highland barley could alleviate obesity induced by HFD and related metabolic diseases in animal models (Sun et al., 2020; Zheng et al., 2021). In this study, the consumption of highland barley significantly reduced HFD-induced weight gain, postprandial blood glucose levels, organ weight, adipose tissue weight, and fat accumulation. It also ameliorated hepatic steatosis, hyperlipemia, abnormal liver function, and dysregulation of inflammatory factor expression in mice. Long-term high-fat diets could exacerbate lipid deposition and steatosis in the liver, potentially resulting in or worsening liver damage. The probiotic effects of highland barley were often associated with reduced fat accumulation in organs such as the liver, subcutaneous tissues, muscles, and epididymal fat, as



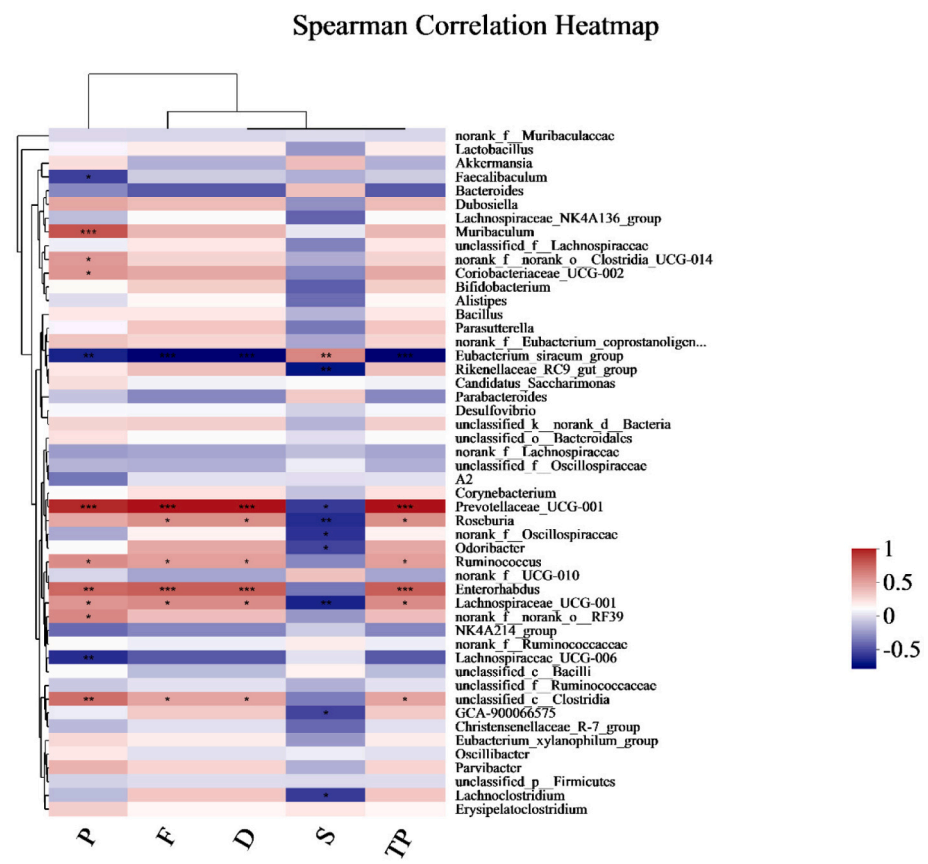


Fig. 6. (*continued*).

well as alleviated inflammation (Li et al., 2021; Zhou & Wu, 2022).

This effect could be attributed to the high content of dietary fiber, polyphenols, and protein content of highland barley. Similar findings were reported by Deng et al. (2020) and Ren et al. (2024). Accordingly, dietary fiber, β-glucan, polyphenols, and protein in highland barley might have potential hypolipidemic effects (Deng et al., 2020; Li et al.,

2022). Dietary fiber, which contains β-glucan and resistant starch, could promote the secretion of bile acids and the concentration of short-chain fatty acids (SCFAs) to exert hypolipidemic effects, slow down the absorption of dietary cholesterol and fats, and reduce appetite and calorie intake (Gan et al., 2023; Ren et al., 2024). Moreover, protein in whole grains contained various bioactive peptides, which played a crucial role in improving lipolytic enzyme activity, inhibiting fatty acid synthase activity, and increasing adiponectin levels. Further, protein could increase thermogenesis and energy expenditure (Gong, Wang, Sun, Wang, & Sun, 2019; Ren et al., 2024). Also, procyanidins and catechins from highland barley were active phenolic compounds that alleviate hyperlipidemia by activating the expression of peroxisome proliferator-activated receptors, resulting in an increase in serum HDL-C levels and a decrease in TC, TG, and LDL-C levels. Phenolic compounds in highland barley could reduce inflammation associated with metabolic disorders (Liu, Liu, Tang, Zhang, & Tian, 2024; Omagari et al., 2024). However, further investigation and verification were needed to determine which nutrient in highland barley played a major role in alleviating the symptoms of HFD-induced obese mice.

Excessive HFD intake could lead to various body metabolism disorders, including liver inflammation, lipid metabolism issues, and gut microbiota disorders (Li et al., 2019; Navar-Boggan et al., 2015). The gut microbiota played a crucial role in maintaining health status and was influenced by daily diet. Analysis of gut microbiota had shown that PTHB consumption partially restored the disruption of gut microbiota caused by the HFD. In particularly, PTHB consumption significantly reduced the *Firmicutes/Bacteroidetes* ratio (no significant difference with NC group), which was often associated with improved metabolic outcomes and decreased calorie extraction (Liu et al., 2024). Similar findings have been reported by Li, Wang, et al. (2022). Moreover, numerous studies have demonstrated significant differences in gut microbiota composition and structure between obese individuals and normal individuals, with obese individuals showing an increased *Firmicutes/Bacteroidetes* ratio (Navar-Boggan et al., 2015).

Additionally, the PTHB groups exhibited a significantly higher relative abundance of *Akkermansia* compared to the HFD group. *Akkermansia* is a bacterium that degrades mucin, and its abundance is often lower in pathological conditions such as alcoholic liver disease, hypertension, and obesity compared to normal controls (Navar-Boggan et al., 2015). Highland barley consumption significantly restored the relative abundance of *Akkermansia*, suggesting that barley consumption might reduce the risk of obesity and related metabolic diseases. This finding was consistent with previous studies demonstrating that barley effectively mitigates weight gain in HFD-induced obese mice (Meng et al., 2023). Additionally, several studies have shown a significant positive correlation between the relative abundance of *Romboutsia* and obesity symptoms, and highland barley consumption alleviated these changes in bacterial abundance (Wang et al., 2019).

Further, *Actinobacteriota* was significantly upregulated in the HFD group, and their abundance decreased following highland barley consumption, indicating the beneficial effects of highland barley consumption on health (Barczynska et al., 2015). Additionally, in line with the findings of Zeng et al. (2019), *Muribaculaceae* play vital roles in energy metabolism, blood glucose, and lipid levels in the gut and are regarded as beneficial bacteria. The higher abundance of *norank_f_Muribaculaceae* in the PTHB groups indicated that highland barley could impact obesity-related bacterial abundance and thereby influence obesity and related diseases in mice. Moreover, *norank_f_Muribaculaceae* not only acted as an intestinal probiotic, increasing levels of acetic acid and propionic acid, but also upregulated the expression of intestinal TJs, including occludin, claudins, and ZO-1 (Chen et al., 2023; Fang et al., 2024). Also, the relative abundance of *Faecalibaculum* and *Dubosiella* was higher in the HFD mice. *Dubosiella* strains have been recently identified as novel members of the family *Erysipelotrichaceae*, which thrives particularly in the gut of HFD-fed obese mice (Qiu et al., 2021). *Faecalibaculum* strains are also significant genera leading to obesity (Sun et al., 2020). Furthermore, *Ruminococcus* abundance is higher in obese patients and is positively correlated with type II diabetes and non-alcoholic fatty liver disease, identified as one of the causes of Crohn's disease symptoms, which was found in the HFD group and did not appear in the PTHB groups (Alam et al., 2014; Liu et al., 2024). The results indicated that PTHB consumption could inhibit the growth of *Faecalibaculum*, *Dubosiella*, and *Ruminococcus* strains, which were positively correlated with obesity, type II diabetes, liver disease, or Crohn's disease, thus alleviating the obese symptoms in HFD-induced mice (Alam et al., 2014; Liu et al., 2024).

The Spearman correlation heatmap analysis also suggested that protein, dietary fiber, and polyphenolics were positively related to the abundance of the strains that were beneficial to energy metabolism, blood glucose, and lipid levels in the gut, including *Muribaculum, norank_f_norank_o_Costridia*_UCG-014, and *Prevotellaceae*_UCG-001. According to the RDA and Spearman correlation heatmap analysis results, protein and dietary fiber might be the two major factors in highland barley that alleviate obesity. *Lachnospiraceae* is positively correlated with the following functions related to amino acid metabolism, such as alanine degradation, methionine degradation, and aspartic acid degradation (Teixeira et al., 2018). Also, *Costridia* genes have the ability to metabolize aromatic amino acids (Madsen, Myrmel, Fjære, Liaset, & Kristiansen, 2017). The increase in the abundance of these bacteria helped alleviate certain intestinal diseases. The results suggested that protein in highland barley was a key factor in alleviating the obese symptoms in HFD-induced mice.

Furthermore, dietary fiber was positively correlated with *Prevotellaceae*_UCG-001, *Roseburia*, *Ruminococcus*, *Enterorhabdus*, *Lachnospiraceae*_UCG-001, and *unclassified_c_Clostridia*. Reportedly, *Prevotellaceae*_UCG-001, *Roseburia*, *Enterorhabdus*, and *Ruminococcus* are the genera with saccharolytic activity, capable of hydrolyzing β-glucan in highland barley to produce SCFAs (Chen, Huang, Wang, Geng, & Nie, 2022; Okouchi et al., 2019). *Lachnospiraceae_UCG-001* can also utilize polysaccharides. These bacteria play a key role in maintaining gut homeostasis and regulating various metabolic processes. This result was consistent with that reported by Gan et al. (2023) and Zheng et al. (2024). Additionally, polyphenolics showed a similar relationship with dietary fiber and gut microbiota. This could be attributed to the regulation of metabolic syndrome in HFD-fed mice by polyphenolics in highland barley, promoting the abundance of bacteria that produce SCFAs (Omagari et al., 2024). Also, starch content was found to be positively correlated with *Eubacterium_siraeum_group*, which has been identified as a pivotal genus in the HFD fecal microecosystem (Li, Qin, et al., 2022).

In conclusion, peeling treatment increased the total starch content of highland barley but also led to a loss of other nutrients, the extent of which varies with the peeling time. Moreover, consumption of highland barley alleviated weight gain, postprandial blood glucose levels, organ weight, adipose tissue weight, and fat accumulation in HFD-induced mice. It also ameliorated hepatic steatosis, hyperlipemia, abnormal liver function, and dysregulation of inflammatory factor expression. Furthermore, highland barley consumption improved the imbalance of gut microbiota in HFD-induced mice, with protein and dietary fiber being key factors in exerting this beneficial effect. Future research using gnotobiotic mice is needed to provide causal evidence and mechanistic insights.

CRediT authorship contribution statement

Fan Xie: Writing – original draft, Funding acquisition, Data curation. **Yuting Yan:** Data curation. **Xin Gao:** Data curation. **Haocheng She:** Data curation. **Jingyi Wang:** Data curation. **Jie Li:** Data curation. **Yi Zhang:** Data curation. **Jun Zhang:** Data curation. **Zhou Zhang:** Data curation. **Lianzhong Ai:** Visualization, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

References

Alam, M. A., Subhan, N., Rahman, M. M., Uddin, S. J., Reza, H. M., & Sarker, S. D. (2014). Effect of citrus flavonoids, naringin and naringenin, on metabolic syndrome and their mechanisms of action. *Advances in Nutrition, 5*(4), 404–417.

Barczynska, R., Slizewska, K., Litwin, M., Szalecki, M., Zarski, A., & Kapusniak, J. (2015). The effect of dietary fibre preparations from potato starch on the growth and activity of bacterial strains belonging to the phyla Firmicutes, Bacteroidetes, and Actinobacteria. *Journal of Functional Foods, 19*, 661–668.

Chen, C., Huang, X., Wang, H., Geng, F., & Nie, S. (2022). Effect of β-glucan on metabolic diseases: A review from the gut microbiota perspective. *Current Opinion in Food Science, 47*, Article 100907.

Chen, L., Jiang, Q., Jiang, C., Lu, H., Hu, W., Yu, S., … Shen, G. (2023). Sciadonic acid attenuates high-fat diet-induced obesity in mice with alterations in the gut microbiota. *Food Function, 14*(6), 2870–2880.

Deng, N., He, Z., Guo, R., Zheng, B., Li, T., & Liu, R. H. (2020). Highland barley whole grain (*Hordeum vulgare* L.) ameliorates hyperlipidemia by modulating cecal microbiota, miRNAs, and AMPK pathways in leptin receptor-deficient db/db mice. *Journal of Agricultural and Food Chemistry, 68*(42), 11735–11746.

Fang, W., Peng, W., Qi, W., Zhang, J., Song, G., Pang, S., & Wang, Y. (2024). Ferulic acid combined with different dietary fibers improve glucose metabolism and intestinal barrier function by regulating gut microbiota in high-fat diet-fed mice. *Journal of Functional Foods, 112*, Article 105919.

Gan, L., Han, J., Li, C., Tang, J., Wang, X., Ma, Y., Chen, Y., Xiao, D., & Guo, X. (2023). Tibetan highland barley fiber improves obesity and regulates gut microbiota in high-fat diet-fed mice. *Food Bioscience, 53*, Article 102620.

Garcia-Mazcorro, J. F., Mills, D. A., Murphy, K., & Noratto, G. (2018). Effect of barley supplementation on the fecal microbiota, caecal biochemistry, and key biomarkers of obesity and inflammation in obese db/db mice. *European Journal of Nutrition, 57*(7), 2513–2528.

Gong, L., Wang, T., Sun, C., Wang, J., & Sun, B. (2019). Whole barley prevents obesity and dyslipidemia without the involvement of the gut microbiota in germ free C57BL/6J obese mice. *Food Function, 10*(11), 7498–7508.

Li, S., Wang, M., Li, C., Meng, Q., Meng, Y., Ying, J., … Xue, Y. (2022). Beneficial effects of partly milled Highland barley on the prevention of high-fat diet-induced glycometabolic disorder and the modulation of gut microbiota in mice. *Nutrients, 14*, 762.

Li, S., Yu, W., Guan, X., Huang, K., Liu, J., Liu, D., & Duan, R. (2019). Effects of millet whole grain supplementation on the lipid profile and gut bacteria in rats fed with high-fat diet. *Journal of Functional Foods, 59*, 49–59.

Li, X., Suo, J., Huang, X., Dai, H., Bian, H., Zhu, M., … Han, N. (2021). Whole grain Qingke attenuates high-fat diet-induced obesity in mice with alterations in gut microbiota and metabolite profile. *Frontiers in Nutrition, 2021*(8), Article 761727.

Li, Y., Qin, C., Dong, L., Zhang, X., Wu, Z., Liu, L., … Liu, L. (2022). Whole grain benefit: Synergistic effect of oat phenolic compounds and β-glucan on hyperlipidemia via gut microbiota in high-fat-diet mice. *Food Function, 13*(24), 12686–12696.

Liu, Z., Liu, J., Tang, R., Zhang, Z., & Tian, S. (2024). Procyanidin B1 and coumaric acid from Highland barley alleviated high-fat-diet-induced hyperlipidemia by regulating PPARα-mediated hepatic lipid metabolism and gut microbiota in diabetic C57BL/6J mice. *Foods, 2024*(13), 1843.

Lozupone, C. A., & Knight, R. (2008). Species divergence and the measurement of microbial diversity. *FEMS Microbiology Reviews, 32*(4), 557–578.

Lyu, Y., Ma, S., Liu, J., & Wang, X. (2022). A systematic review of highland barley: Ingredients, health functions and applications. *Grain & Oil Science and Technology, 5*(1), 35–43.

Madsen, L., Myrmel, L. S., Fjære, E., Liaset, B., & Kristiansen, K. (2017). Links between dietary protein sources, the gut microbiota, and obesity. *Frontiers in Physiology, 8*.

Marquart, L., Wiemer, K. L., Jones, J. M., & Jacob, B. (2007). Whole grain health claims in the USA and other efforts to increase whole-grain consumption. *Proceedings of the Nutrition Society, 62*(1), 151–160.

Meng, Y., Meng, Q., Li, C., Wang, M., Li, S., Ying, J., … Shen, Q. (2023). A comparison between partially peeled hulless barley and whole grain hulless barley: Beneficial effects on the regulation of serum glucose and the gut microbiota in high-fat diet-induced obese mice. *Food Function, 14*(2), 886–898.

Navar-Boggan, A. M., Peterson, E. D., D'Agostino, R. B., Neely, B., Sniderman, A. D., & Pencina, M. J. (2015). Hyperlipidemia in early adulthood increases long-term risk of coronary heart disease. *Circulation, 131*(5), 451–458.

Okouchi, R. E. S., Yamamoto, K., Ota, T., Seki, K., Imai, M., Ota, R., … Tsuduki, T. (2019). Simultaneous intake of Euglena gracilis and vegetables exerts synergistic anti-obesity and anti-inflammatory effects by modulating the gut microbiota in diet-induced obese mice. *Nutrients, 11*, 204.

Omagari, K., Ishida, J., Murata, K., Araki, R., Yogo, M., Shirouchi, B., Suruga, K., Sera, N., Koba, K., Ichimura-Shimizu, M., & Tsuneyama, K. (2024). The effect of barley bran polyphenol-rich extracts on the development of nonalcoholic steatohepatitis in Sprague–Dawley rats fed a high-fat and high-cholesterol diet. *Livers, 4*, 193–208.

Pauline, K. B., & Rimm, E. B. (2007). Whole grain consumption and weight gain: A review of the epidemiological evidence, potential mechanisms and opportunities for future research. *Proceedings of the Nutrition Society, 62*(1), 25–29.

Qiu, X., Macchietto, M. G., Liu, X., Lu, Y., Ma, Y., Guo, H., … Chen, X. (2021). Identification of gut microbiota and microbial metabolites regulated by an antimicrobial peptide lipocalin 2 in high fat diet-induced obesity. *International Journal of Obesity, 45*(1), 143–154.

Ren, X., Zhang, F., Zhang, M., Fang, Y., Chen, Z., & Huan, M. (2024). Fecal microbiota transplantation: Whole grain highland barley improves glucose metabolism by changing gut microbiota. *Food Science and Human Wellness, 13*(4), 2014–2024.

Shin, J. H., Kim, K., & Nam, H. J. (2016). Influence of combined therapy with conventional and herbal medicines on liver function in 138 inpatients with abnormal liver transaminase levels. *BMC Complementary and Alternative Medicine, 16*(1), 496.

Slavin, J. (2007). Why whole grains are protective: Biological mechanisms. *Proceedings of the Nutrition Society, 62*(1), 129–134.

Sun, X., Zhao, H., Liu, Z., Sun, X., Zhang, D., Wang, S., … Wang, D. (2020). Modulation of gut microbiota by fucoxanthin during alleviation of obesity in high-fat diet-fed mice. *Journal of Agricultural and Food Chemistry, 68*(18), 5118–5128.

Teixeira, C., Prykhodko, O., Alminger, M., Fåk Hållenius, F., & Nyman, M. (2018). Barley products of different fiber composition selectively change microbiota composition in rats. *Molecular Nutrition & Food Research, 62*(19), 1701023.

Valenti, L., Rametta, R., Dongiovanni, P., Maggioni, M., Ludovica Fracanzani, A., Zappa, M., … Fargion, S. (2008). Increased expression and activity of the transcription factor FOXO1 in nonalcoholic steatohepatitis. *Diabetes, 57*(5), 1355–1362.

Wang, W., Pan, Y., Wang, L., Zhou, H., Song, G., Wang, Y., … Li, A. (2019). Optimal dietary ferulic acid for suppressing the obesity-related disorders in leptin-deficient obese C57BL/6J-ob/ob mice. *Journal of Agricultural and Food Chemistry, 67*(15), 4250–4258.

Xia, C., Yang, K., Zhu, Y., Liu, T., Chen, J., Deng, J., Zhu, B., Shi, Z., & Xiang, Z. (2022). Distribution of free and bound phenolic compounds, β-glucan, and araboxylan in fractions of milled hulless barley. *LWT, 169*, Article 113935.

Xia, X., Li, G., Ding, Y., Ren, T., Zheng, J., & Kan, J. (2017). Effect of whole grain Qingke (Tibetan *Hordeum vulgare* L. Zangqing 320) on the serum lipid levels and intestinal microbiota of rats under high-fat diet. *Journal of Agricultural and Food Chemistry, 65*(13), 2686–2693.

Zeng, T., Cui, H., Tang, D., Garside, G. B., Wang, Y., Wu, J., … Tao, S. (2019). Short-term dietary restriction in old mice rejuvenates the aging-induced structural imbalance of gut microbiota. *Biogerontology, 20*(6), 837–848.

Zheng, B., Ao, T., Zhao, X., Chen, Y., Xie, J., Gao, X., Liu, L., Hu, X., & Yu, Q. (2024). Comprehensive assessment of the anti-obesity effects of highland barley total, insoluble, and soluble dietary fiber through multi-omics analysis. *Food Research International, 189*, Article 114535.

Zheng, Q., Wang, Z., Xiong, F., Song, Y., & Zhang, G. (2023). Effect of pearling on nutritional value of highland barley flour and processing characteristics of noodles. *Food Chemistry: X, 17*, Article 100596.

Zheng, S., Wang, Y., Fang, J., Geng, R., Li, M., Zhao, Y., … Tong, T. (2021). Oleuropein ameliorates advanced stage of type 2 diabetes in db/db mice by regulating gut microbiota. *Nutrients, 13*(7), 2131.

Zhou, J., & Wu, L. (2022). Modified Highland barley regulates lipid metabolism and liver injury in high fat and cholesterol diet ICR mice. *Foods, 11*, 4067.

Zong, Y., Tian, S., Zhang, Y., Liu, Z., & Chen, Z. (2022). Effects of highland barley powders with different peeling rates on the rheological properties of dough and sensory, volatile flavor evaluation of bread. *Journal of Food Processing and Preservation, 46*(11), Article e16971.

Further reading

Raj, R., Shams, R., Pandey, V. K., Dash, K. K., Singh, P., & Bashir, O. (2023). Barley phytochemicals and health promoting benefits: A comprehensive review. *Journal of Agriculture and Food Research, 14*, Article 100677.