

Adzuki Bean Alleviates Obesity

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Adzuki bean consumption has many health benefits, but its effects on obesity and regulating gut microbiota imbalances induced by a high-fat diet (HFD) have not been thoroughly studied. Mice were fed a low-fat diet, a HFD, and a HFD supplemented with 15% adzuki bean (HFD-AB) for 12 weeks. Adzuki bean supplementation significantly reduced obesity, lipid accumulation, and serum lipid and lipopolysaccharide (LPS) levels induced by HFD. It also mitigated liver function damage and hepatic steatosis. In particular, adzuki bean supplementation improved glucose homeostasis by increasing insulin sensitivity. In addition, it significantly reversed HFD-induced gut microbiota imbalances. Adzuki bean significantly reduced the ratio of Firmicutes/Bacteroidetes (F/B); enriched the occurrence of Bifidobacterium, Prevotellaceae, Ruminococcus_1, norank_f_Muribaculaceae, Alloprevotella, Muribaculum, Turicibacter, Lachnospiraceae_NK4A136_group, and Lachnospiraceae; and returned HFD-dependent taxa (Desulfovibrionaceae, Bilophila, Ruminiclostridium_9, Blautia, and Ruminiclostridium) back to normal status. PICRUST2 analysis showed that the changes in gut microbiota induced by adzuki bean supplementation may be associated with the metabolism of carbohydrates, lipids, sulfur, and cysteine and methionine; and LPS biosynthesis; and valine, leucine, and isoleucine degradation.

Keywords: adzuki bean ; obesity ; insulin resistance ; gut microbiota ; PICRUST2 analysis

1. Introduction

At present, public health is threatened by the increasing occurrence of obesity worldwide. It is reported that 20% of the adult population will suffer from obesity by 2030 [1]. Excessive consumption of high-fat diets (HFDs) can easily lead to obesity [2]. The disease is multifactorial, usually accompanied by dyslipidemia, hepatic steatosis, insulin resistance, and gastrointestinal diseases. Current obesity treatment measures include increasing energy expenditure, suppressing appetite, inhibiting fat production, and regulating gut microbiota [3]. A HFD can cause changes in the gut microbiota composition and function [4]. Studies have found that gut microbiota is related to lipid metabolism and energy homeostasis, which implies that gut microbiota is significant in obesity and related complications development [5]. More and more evidence shows that functional food, especially beans, can regulate the composition of gut microbiota, and thereby inhibit metabolic disorders development [6][7].

Among the 12 most important cereal and legume crops, adzuki bean (*Vigna angularis*) is consumed in Asia [8]. It is planted in more than 30 countries and regions, of which China has the largest production [9]. According to the Chinese pharmacopoeia, adzuki bean can be used in the treatment of diuresis, swelling, and abscesses. Since the time of the Tang Dynasty in China, adzuki bean has been used for weight control [10]. In addition, the dietary fiber, polysaccharides, protein, and bioactive substances (e.g., polyphenols and saponins) of adzuki bean were found to have many health benefits, such as anti-diabetes [11], anti-obesity [6][8][10][12], liver protection [13], and antioxidant activity [14]. Although public interest in adzuki bean is increasing, current research mainly focuses on either single components or extracts of adzuki bean. In most cases, adzuki bean is usually consumed in the form of the whole bean. However, the biological benefits of whole adzuki bean seeds are not currently well studied. Previous research has indicated that 30% adzuki bean supplementation can significantly decrease serum glucose, low-density lipoprotein cholesterol (LDL-C), and total cholesterol (TC) levels, and improve the glucose tolerance of mice with diabetes induced by a HFD combined with streptozotocin [15]. However, consumption of such a large amount of adzuki bean in the daily diet would be difficult. Therefore, it is not clear whether whole adzuki bean supplementation has beneficial effects on obesity and its complications induced by HFD, especially in the case of intake of a more practical low dose (15%). Furthermore, the relationship between the health benefits of adzuki bean supplementation and gut microbiota also needs to be clarified.

In order to explore the effects of adzuki bean supplementation to improve obesity and gut microbiota imbalances induced by HFD, changes in histological, physiological, and biochemical parameters, and serum LPS level were measured. The regulatory effect of adzuki bean supplementation on the gut microbiota composition was also studied. This work in a

model system will enhance scientific understanding of adzuki bean as a functional food to prevent obesity, including the influence of gut microbiota.

2. Adzuki Bean Supplementation Regulated Gut Microbiota Dysbiosis

Gut microbiota affects metabolic function and is important in the development of obesity. α diversity reflects the richness and diversity of a microbial community. Community richness refers to the number of species. Community diversity is a comprehensive index of species richness and evenness. The ACE and Chao indices represent the community richness and are often used to estimate the total number of species. Simpson and Shannon indices represent the community diversity. The greater the Shannon value, the higher the community diversity, while the Simpson index is the opposite [46]. Compared with the NCD group, HFD feeding led to significantly decreased community richness (Figure 1A,B), and a significant increase in the community diversity (Figure 1C,D). Conversely, adzuki bean supplementation showed significantly increased community richness, but no significant change in community diversity. By using β diversity analysis based on PCoA and NMDS, the three groups formed clusters separated from each other, indicating that gut microbiota composition changed significantly in response to adzuki bean supplementation and HFD feeding (Figure 1E,F). At the phylum level, *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Verrucomicrobia*, and *Proteobacteria* were abundant (Figure 2A). Compared with the NCD group, HFD feeding significantly increased the ratio of *F/B*, but this effect was reversed by adzuki bean supplementation (Figure 2B). At the genus level, HFD feeding significantly decreased the abundance of *Bifidobacterium* and *norank_f_Muribaculaceae*, and enriched *Bilophila*, *Blautia*, *Ruminiclostridium_9*, and *Ruminiclostridium* compared with the NCD group (Figure 2C). Adzuki bean supplementation not only reversed these effects, but also significantly enriched *Lachnospiraceae_NK4A136_group*. The cladogram corresponding to five phylogenetic levels (from phylum to genus) generated from LEfSe analysis indicates that different dietary interventions led to specific changes in the bacterial taxa (Figure 3A). Further, the LDA scores from LEfSe analysis show that HFD-fed mice were rich in *Bilophila*, *Blautia*, *Ruminiclostridium*, *Ruminiclostridium_9*, and *Desulfovibrionaceae* compared with the NCD group (Figure 3B). Adzuki bean supplementation not only reversed these effects, but also significantly enriched *Lachnospiraceae_NK4A136_group*, *norank_f_Muribaculaceae*, *Bifidobacterium*, *Turicibacter*, *Lachnoclostridium*, *Prevotellaceae*, *Ruminococcus_1*, *Muribaculum*, and *Alloprevotella* (Figure 3B).

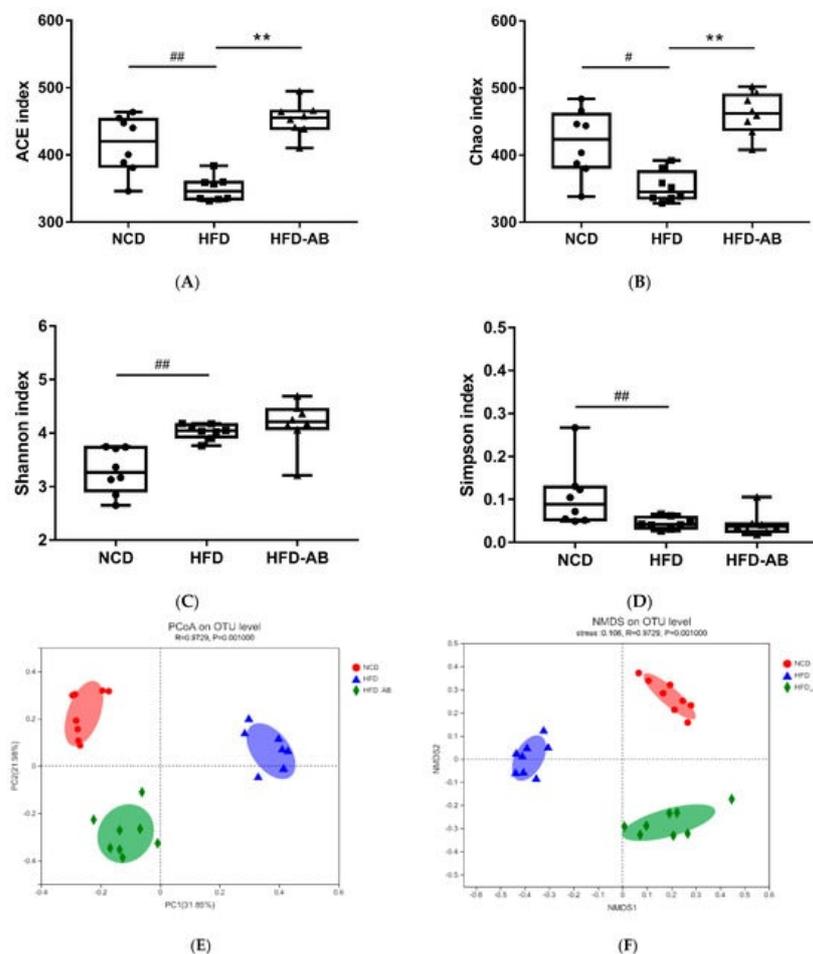


Figure 1. Effects of adzuki bean supplementation on the α and β diversity of gut microbiota in mice ($n = 8$ per group). (A,B) The community richness accessed by the ACE and Chao indices, (C,D) the community diversity accessed by the Shannon and Simpson indices, and (E,F) Principal coordinate analysis (PcoA) score plot and nonmetric multidimensional

scaling (NMDS) score plot based on Bray–Curtis. # $p < 0.05$ and ## $p < 0.01$, HFD compared with NCD mice. ** $p < 0.01$, HFD-AB compared with HFD mice. NCD, normal control diet; HFD, high-fat diet; HFD-AB, high-fat diet supplemented with adzuki bean.

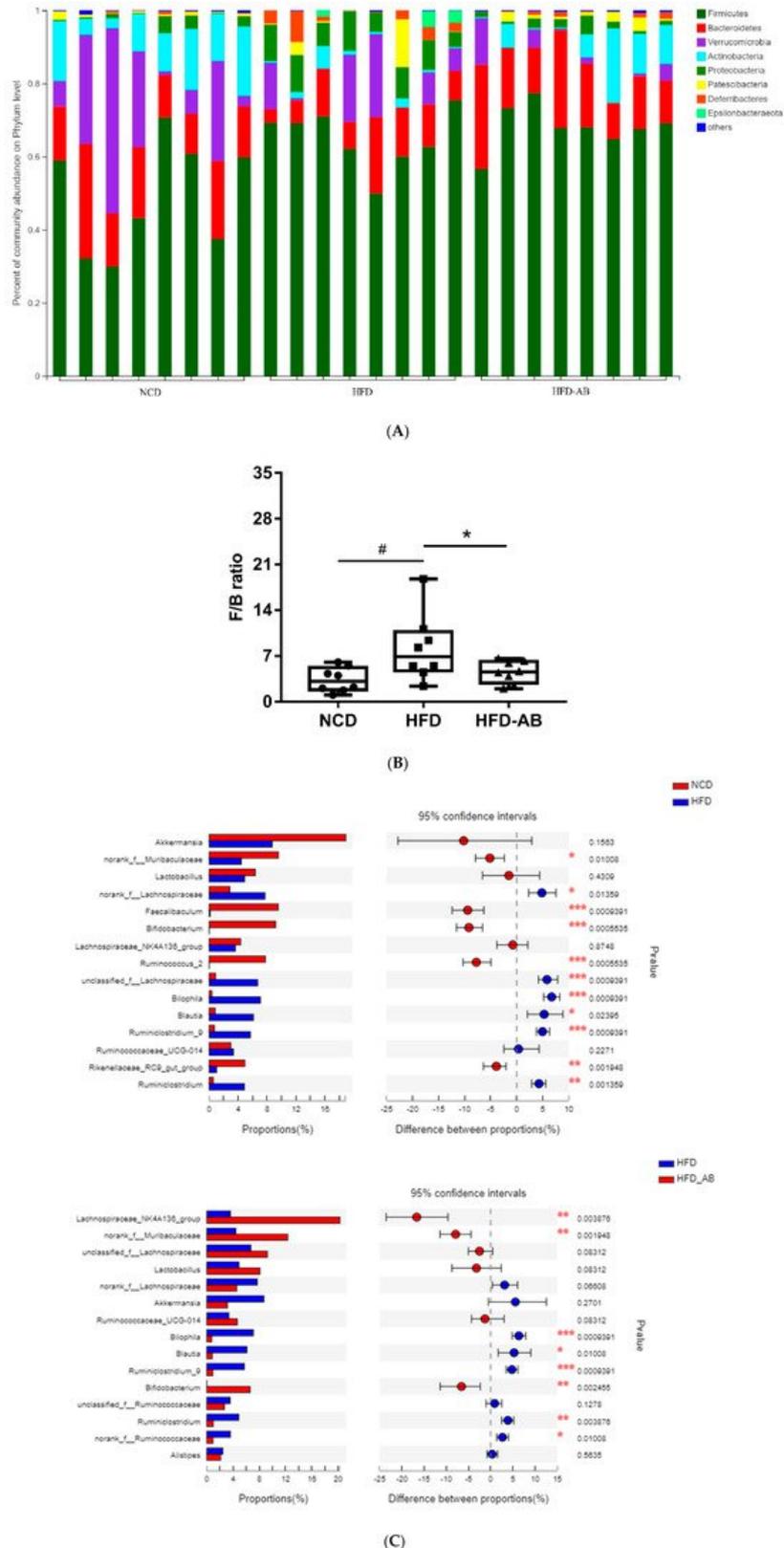


Figure 2. Adzuki bean supplementation modulated gut microbiota composition ($n = 8$ per group). **(A)** The abundances of gut microbiota at the phylum level, **(B)** the *F/B* ratio, and **(C)** mean proportions of key genera in different groups. # $p < 0.05$, HFD compared with NCD mice. * $p < 0.05$, HFD-AB compared with HFD mice (in **Figure 2B**). ** $p < 0.01$, *** $p < 0.001$ (in **Figure 2C**). NCD, normal control diet; HFD, high-fat diet; HFD-AB, high-fat diet supplemented with adzuki bean.

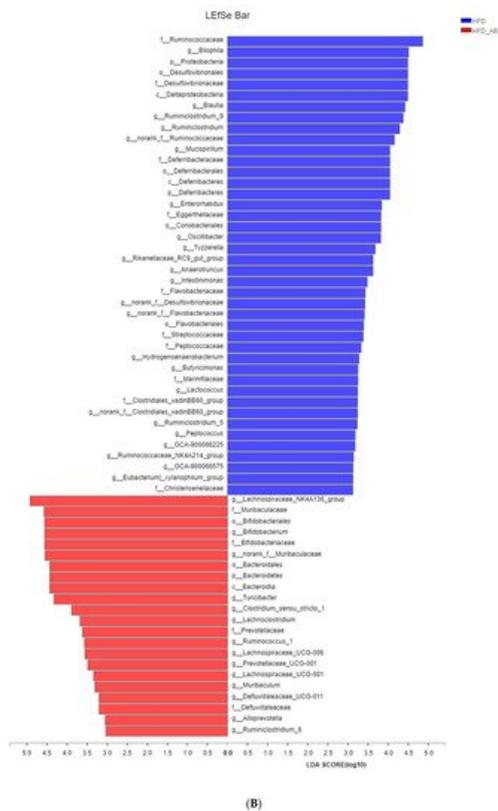
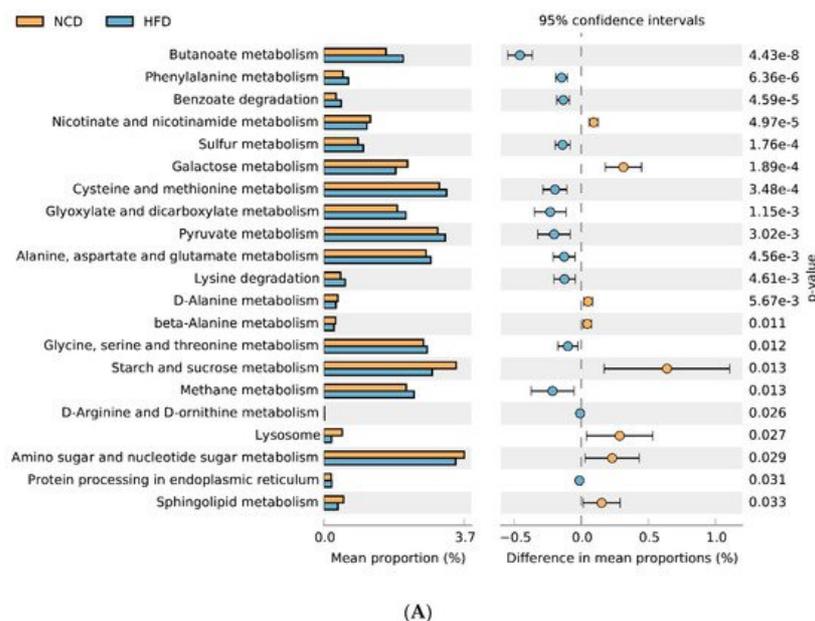
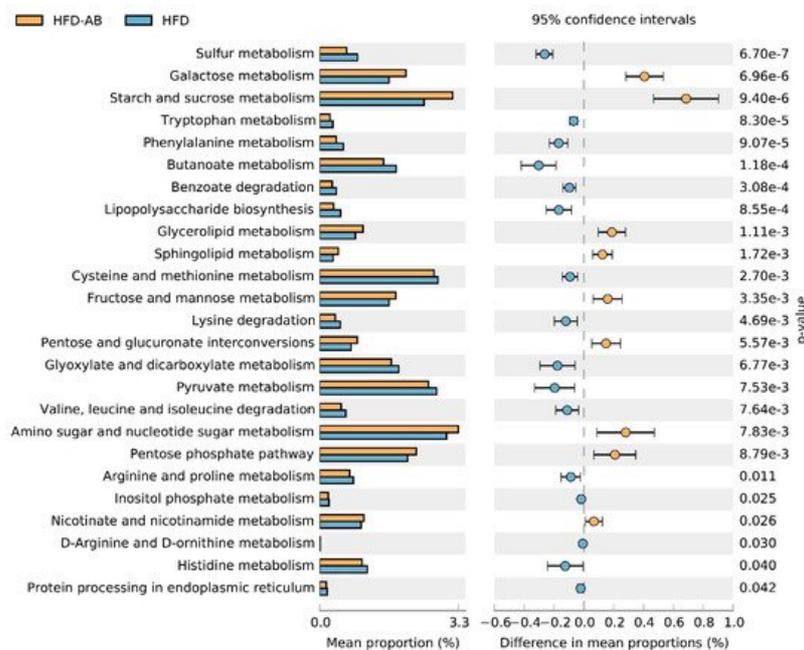


Figure 3. Key phylotypes of gut microbiota. (A) Cladogram generated from LEfSe analysis showing the relationship among taxons, and (B) linear discriminant analysis (LDA > 3) scores derived from LEfSe analysis.

3. Gut Microbial Metabolic Functions

The 16S rRNA data were mapped to the KEGG pathway, and the metabolic characteristics of gut microbiota under different dietary interventions were predicted based on the abundance distribution of each metabolic pathway in each sample. Compared with the NCD group, HFD feeding significantly reduced the metabolism of sphingolipids, starch and sucrose, amino sugar and nucleotide sugars, galactose, nicotinate, and nicotinamide; and enhanced sulfur metabolism and cysteine and methionine metabolism (Figure 4A). Adzuki bean supplementation not only reversed the effects of HFD feeding, but it also reduced lipopolysaccharide biosynthesis and valine, leucine, and isoleucine degradation. It also enhanced the pentose phosphate pathway, glycerolipid metabolism, pentose and glucuronate interconversions, and fructose and mannose metabolism (Figure 4B).





(B)

Figure 4. Predicted metabolic profile of the fecal microbiome after different treatments. (A) HFD vs. NCD; (B) HFD-AB vs. HFD. 16S rRNA data were analyzed as indicated by the PICRUST2. Statistical significance difference among treatment groups based on Welch's *t*-test ($p < 0.05$) in STAMP. The colored circles represent 95% confidence intervals calculated using Welch's inverted method.

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