Immunity improvement and gut microbiota remodeling of mice by wheat germ globulin

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Abstract
The wheat germ protein (WG) and its proteolytic peptide have a variety of biological activities. Our previous work showed that WG could improve immunity of the immunosuppressive mice established by cyclophosphamide. However, in the healthy condition and normal diet, as a supplementary food, the effects of immunity improvement and gut microbiota remodeling by the wheat germ globulin has not been studied yet. Here, we reported that WG could improve the immunity and remodel the gut microbiota of the mice, as a potentially safe functional supplementary food for the first time. The increase of interleukin-6 (IL-6) and the decrease of tumor necrosis factor α (TNF-α) and interleukin-10 (IL-10) indicated that WG could enhance the levels of activated T cells and monocytes and anti-inflammatory ability, meanwhile, the significant increase of immunoglobulin G (IgG) and the notable decrease of the immunoglobulin M (IgM) and immunoglobulin A (IgA) illustrated that WG could improve immunity by promoting the differentiation and maturation process of B cells, compared with the NC group (normal control group). 16S rRNA sequencing showed WG could remodel the gut microbiota. At the phylum level, the Bacteroidetes were reduced and Firmicutes were increased in WG group, compared with NC group. At the genus level, the SCFA producing genera of unclassified_f_Lachnospiraceae, Blautia and especially the Roseburia (increased more than threefold) increased notably. Further, the level changes of cytokines and immunoglobulins were associated with the gut microbiota. This work showed that WG could improve immunity and has potential application value as an immune-enhancing functional food.

Keywords Gut microbiota · Immunity improvement · Mice · Wheat germ globulin

Introduction
There are more than 500 species of microbes colonized in the intestine and the gut microbiota is heavily involved in the modulating of immunity, inflammation, metabolism of carbohydrates and fats, and in gut-brain neural circuits, which maintains a complex dynamic balance to effectively resist the invasion of pathogens (Dai et al. 2020; Hegazy et al. 2017). Intestinal microbes participate in and regulate the host’s immune response through bile acids, lipoproteins, lipopolysaccharides, unsaturated fatty acids and other metabolites to form an intestinal immune protective barrier (Anh et al. 2019; Castellanos and Longman 2020; Sanna et al. 2019; Vitetta et al. 2018; Wan et al. 2019). Short-chain fatty acids (SCFAs) derived from metabolism of the gut microbiota are crucial for intestinal health and participate in the maintenance of other important physiological functions of the body (Chang et al. 2020; Shuo et al. 2018). It was reported that the SCFAs, in
particular butyrate, exerted important immuno-modulatory functions (Martin-Gallausiaux et al. 2020; Stilling et al. 2018).

The wheat germ is an important by-product of the flour milling industry. After oil extraction, the germ meal has high protein content (over 30%), with globulin about 15% of total protein. And the wheat germ protein presents a well-balanced amino acid profile, which is a potential supplementary food with high nutritional value (Brandomlini and Hidalgo 2012). The wheat germ protein and it’s proteolytic peptide have a variety of biological activities. Wheat germ proteolytic peptide WG-P exerted an effective antioxidant action by across the intestinal epithelium (Zhang et al. 2019a). The Wheat Germ Globulin proteolytic peptide ECFSTA exerted immunomodulatory effects (Wu et al. 2017). Our previous work showed that wheat germ globulin (WG) could improve the immune function of immunosuppressive mice established by intraperitoneal injection of cyclophosphamide (Ji et al. 2018). However, in the healthy condition and normal diet, whether WG as a functional supplementary food could also improve immunity and whether its immune-promoting effect is related to the improvement of intestinal microbiota has not been studied yet.

In this work, the safety of WG as a functional supplementary food was preliminarily verified. Further, the effects of immunity improvement and gut microbiota remodeling by the wheat germ globulin were investigated.

Materials and methods

Animal groups and protein administration

48 BALB/c male mice (age of 6–8 weeks, weight of 18–22 g), purchased from the Experimental Animal Center of Zhengzhou University, were fed under specific pathogen-free conditions at a temperature of 25°C and humidity of 60%. The mice were fed with the standard laboratory diet according to AIN-93G standards (Ji et al. 2018). Then, the 48 mice were divided averagely into four groups at random and fed different protein samples (with the same calorie levels), in addition to the standard diet, as shown in Table 1.

Hematological parameters and hematological biochemical parameters analysis

The blood supernatant were prepared and used to detect blood indexes using the automatic biochemical analyzer Coulter-JT (Beckman Coulter Co., Ltd. USA).

Inflammatory factors and immunoglobulins analysis by ELISA

Peripheral blood samples were collected from the mice eyeball, and were kept at room temperature for 2 h. Then, the samples were centrifuged at 2000 g for 20 min. The serum was collected and stored at −20 °C without repeated freezing and thawing. The levels of IL-β, IL-6, IL-10, TNF-α, IFN-γ, IgG, IgA and IgM of each sample were determined according to the instructions of the ELISA kit (NeoBioScience Co., Ltd. Beijing, China).

Acquisition of intestinal contents and genomic DNA extraction

The ileocecal areas of the mice were cut and washed with PBS. Then, the precipitate was collected by centrifugation, which was freezeed in liquid nitrogen for 0.5 h and transferred to −80°C for storage. Microbial community genomic DNA was extracted by the E.Z.N.A.® soil DNA Kit (Omega Bio-tek, Norcross, GA, U.S.) according to manufacturer’s instructions. The hypervariable region V3-V4 of the bacterial 16S rRNA genes were amplified with primer pairs 338F and 806R (338F: 5′-ACTCCTACGGGAGGCACGAG-3′; 806R: 5′-GGACTACHVGGGTWTCTAAT-3′) by an ABI GeneAmp® 9700 PCR thermocycler (ABI, CA, USA) and performed in triplicate (Wan et al. 2019; Wang et al. 2019). The PCR product was extracted from 2% agarose gel and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to manufacturer’s instructions and quantified using Quantus™ Fluorometer (Promega, USA).

16S rRNA gene sequencing and processing of sequencing data

Purified amplicons were pooled in equimolar and paired-end sequenced (2 × 300) on an Illumina MiSeq platform (Illumina, San Diego, USA) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). The raw sequences of 16S rRNA gene sequencing have been deposited in the NCBI Sequence Read Archive (SRA) database under the BioProject accession number of PRJNA689839 (the specific Accession Number: SAMN17192475, SAMN17192476, SAMN17192477, SAMN17192478, SAMN17192479, SAMN17192480.

Table 1 Animal model grouping

| Group                   | Intragastric administration (30 days) |
|-------------------------|----------------------------------------|
| Normal control group (NC) | Saline                                |
| Wheat germ globulin group (WG) | 100 mg WG/kg BW/day                    |
| Egg white protein group (EG) | 100 mg EG/kg BW/day                    |
| Whey Protein group (RG) | 100 mg DG/kg BW/day                    |

Groups division: NC normal control group, WG wheat germ globulin group, EG egg white protein group, RG whey protein group
SAMN17192481, SAMN17192482, SAMN17192483, SAMN17192484, SAMN17192485, AMN17192486, SAMN17192487, SAMN17192488, SAMN17192489).

The raw sequencing reads were demultiplexed, quality-filtered by Trimmomatic and merged by FLASH with the following criteria: (1) the 300 bp reads were truncated at any site receiving an average quality score of < 20 over a 50 bp sliding window, and the truncated reads shorter than 50 bp were discarded, reads containing ambiguous characters were also discarded; (2) only overlapping sequences longer than 10 bp were assembled according to their overlapped sequence. The maximum mismatch ratio of overlap region is 0.2. Reads that could not be assembled were discarded; (3) Samples were distinguished according to the barcode and primers, and the sequence direction was adjusted, exact barcode matching, 2 nucleotide mismatch in primer matching. Operational taxonomic units (OTUs) with 97% similarity cutoff were clustered using UPARSE (version 7.1, http://drive5.com/uparse/), and chimeric sequences were identified and removed. The taxonomy of each OTU representative sequence was analyzed by RDP Classifier (http://rdp.cme.msu.edu/) against the Silva database (SSU128) using confidence threshold of 0.7.

Statistical analysis

The experimental data were analyzed with software SPSS version 19.0 for Windows. For two groups comparisons, the unpaired two tailed Student’s t-test was applied, while the analysis of variance was conducted by Duncan’s multiple range tests to determine significant differences among the samples for more than two groups comparisons. All data with error bar are represented as mean ± SEM (Standard Error of Mean). The statistical significance was set at P < 0.01 to indicate very significant differences, P < 0.05 to indicate significant difference, and P > 0.05 to indicate absence of significant differences. The data of 16S rRNA gene sequencing were further statistically analyzed by the Majorbio Cloud Platform (https://cloud.majorbio.com) and the specific analysis methods were as follows. The alpha diversity analysis (by the indexes of Chao, Shannon and Simpson) and the beta diversity analysis (by principal coordinate analysis) of intestinal microorganisms were both performed at the operational taxonomic unit (OTU) level. Significance tests of the species abundance differences among the four groups samples of NC, WG, RG (whey protein) and EG (egg white protein) were performed by Kruskal–Wallis H test, both on the phylum level and genus level. Significance test of the species abundance differences between the groups samples of NC and WG was further performed by the unpaired two tailed Student’s t-test on the genus level. The correlation between the relative abundance of the most abundant 50 genera and the biochemical parameters of IL-6, IL-10, TNF-α, lgG, lgA and lgM was analyzed by Spearman correlation analysis.

Results

Wheat germ globulin could be potentially used as a safe supplementary dietary protein

As shown in Table 2, there were no significant differences in the hematological parameters among the four groups of NC, WG, EG and RG on the whole, and they were all within the normal range, although there were some differences in some indexes, such as leukocytes and some indexes of platelet. Specifically, compared with the NC group, the leukocytes (NC: 3.80, WG: 8.68, EG: 5.72, RG: 7.88) and platelet (NC: 667.00, WG: 941.67, EG: 1309.00, RG: 695.50) were significantly higher. For the other indexes, there were no significant differences. The specific data are shown in Table 2.

Table 2 Comparison of hematological parameters on different treatment group mice

| Item                        | NC-GROUP         | WG-GROUP         | EG-GROUP         | RG-GROUP         |
|-----------------------------|------------------|------------------|------------------|------------------|
| Leukocytes (× 10^9/L)       | 3.80 ± 0.48      | 8.68 ± 0.55      | 5.72 ± 0.21      | 7.88 ± 0.35      |
| Red blood cell (× 10^9/L)   | 7.32 ± 0.02      | 8.12 ± 0.13      | 7.17 ± 0.21      | 8.59 ± 0.14      |
| Hematocrit (%)              | 25.26 ± 4.09     | 37.40 ± 1.05     | 33.80 ± 0.70     | 39.10 ± 2.28     |
| Mean hematocrit (fV)        | 45.35 ± 0.15     | 46.07 ± 1.43     | 47.53 ± 1.37     | 45.43 ± 1.97     |
| Red blood cell distribution width (%) | 22.77 ± 5.47 | 17.00 ± 2.40     | 15.47 ± 1.02     | 14.04 ± 0.80     |
| Red blood cell distribution width SD (fL) | 36.00 ± 4.00 | 33.00 ± 5.03     | 31.00 ± 3.00     | 27.33 ± 0.66     |
| Platelet count (× 10^9/L)   | 667.00 ± 9.00    | 941.67 ± 7.49    | 1309.00 ± 9.00   | 695.50 ± 67.50   |
| Plateletcrit (%)            | 0.72 ± 0.22      | 0.54 ± 0.05      | 0.67 ± 0.04      | 0.41 ± 0.04      |
| Platelet volume (fL)        | 8.05 ± 0.35      | 5.70 ± 0.17      | 5.47 ± 0.03      | 5.33 ± 0.07      |
| Platelet distribution width (%) | 15.07 ± 0.20   | 14.70 ± 0.15     | 14.47 ± 0.12     | 14.43 ± 0.09     |
| Hemoglobin (g/L)            | 164.50 ± 0.50    | 136.67 ± 1.45    | 108.00 ± 1.00    | 130.00 ± 8.18    |
| Mean corpuscular hemoglobin (pg) | 19.25 ± 2.25 | 16.80 ± 0.25     | 15.17 ± 0.19     | 15.10 ± 0.70     |
| Mean corpuscular hemoglobin concentration (g/L) | 424.50 ± 5.05   | 379.50 ± 5.00    | 319.00 ± 5.29    | 332.33 ± 1.86    |
of the protein supplement groups increased, while the platelet volume (NC: 8.05, WG: 5.70, EG: 5.47, RG: 5.33) and plateletcrit of the protein supplement groups (NC: 0.72, WG: 0.54, EG: 0.67, RG: 0.41) decreased slightly.

Similarly, there were also no notable differences in the hematological biochemical parameters among the four groups of NC, WG, RG, and EG on the whole, and they were all within the normal range, although there were some differences in some indexes (Table 3). Compared with NC, the high-density lipoprotein index of the WG group increased while the low-density lipoprotein index decreased.

**Wheat germ globulin could improve immunity of mice**

The levels of cytokines involved in inflammation and immunoglobulins in the peripheral blood of four groups of NC, WG, EG, and RG were compared, in order to evaluate the effects of wheat germ globulin in immunity improvement (Fig. 1). There were no significant changes of the levels of IFN-γ and IL-1β for WG and RG groups, except for EG, compared with the NC group (Fig. 1a, c). The change trends of levels of IL-6, TNF-α and IL-10 for WG, EG and RG groups were the same, compared with the NC group. The levels of IL-6 of the WG, EG and RG groups increased significantly, with the WG group the most (increased 93.2%), while the levels of TNF-α and IL-10 of the WG, EG and RG groups decreased slightly, compared with the NC group (Fig. 1b, d, e).

Further, the levels of IgG of WG, EG and RG groups were increased significantly by 17.8%, 5% and 13.4%, respectively, compared with the NC group (Fig. 1g). While the levels of IgM of WG, EG and RG groups were decreased significantly by 37.1%, 41.2% and 36.1%, respectively, compared with the NC group (Fig. 1f). And the levels of IgA of WG and RG groups were decreased significantly by 28.8% and 21.5%, respectively, except for EG, compared with the NC group (Fig. 1h).

**Sampling depth, alpha diversity and the overall gut microbial community structure difference of the four groups samples of NC, WG, RG and EG**

A total of 555,855 high-quality reads, with the average length of 435 bp, were obtained from 15 samples which were assigned to 466 OTUs (Phylum:9, Class:15, Order:20, Family:31, Genus:90 and Species:159), based on ≥ 97% sequence similarity (Supplementary Table 1, Table 2). The rarefaction curves showed that the sequencing data could cover the majority of bacterial diversity (Supplementary Fig. 1). There was no significant difference in alpha diversity among the four groups samples of NC, WG, RG and EG, which indicated that the regulation effect of WG, RG and EG on intestinal organisms were mainly by changing the community structures rather than increasing species abundances (Supplementary Fig. 2). As revealed by principal coordinate analysis (PCA) at operational taxonomic unit (OTU) level, the overall gut microbiota structure of the four groups samples of NC, WG, RG and EG were structurally different from each other and displayed a distinct separation pattern (Supplementary Fig. 3).

**Microbiota composition and difference analysis of the four groups samples of NC, WG, RG and EG**

At the phylum level, Firmicutes and Bacteroidetes were the most abundant in the four groups samples of NC, WG, RG and EG and together they make up more than 95% of the total, as shown in Fig. 2a. Compared with the NC group, the Bacteroidetes in the WG group were significantly reduced and Firmicutes were significantly increased, that is, the ratio of Bacteroidetes to Firmicutes (B/F ratio) was significantly reduced (Fig. 2b).

At the genus level, the microbiota compositions of the four groups of NC, WG, RG and EG were significantly different. The norank_f__Bacteroidales_S24-7, Lachnospiraceae_NK4A136, norank_f__Lachnospiraceae, Lactobacillus, unclassified_f__Lachnospiraceae, Roseburia and

### Table 3: Comparison of hematological biochemical parameters on different treatment group model mice

| Item                          | NC-GROUP   | WG-GROUP   | EG-GROUP   | RG-GROUP   |
|-------------------------------|------------|------------|------------|------------|
| Alanine aminotransferase (ALT) (U/L) | 32.90±2.40 | 31.10±1.70 | 33.40±0.36 | 31.03±1.33 |
| Aspartate aminotransferase (AST) (U/L) | 147.83±5.65 | 158.25±6.75 | 158.37±8.99 | 139.67±7.19 |
| AST/ALT                       | 3.78±1.02  | 5.12±0.70  | 4.73±0.90  | 4.52±0.59  |
| Total protein (g/L)           | 47.17±0.88 | 47.23±1.14 | 45.83±2.04 | 47.43±1.44 |
| Albumin (A) (g/L)             | 28.57±0.58 | 27.40±0.23 | 27.50±1.70 | 28.50±0.95 |
| Globulin (G) (g/L)            | 18.60±0.35 | 19.83±0.96 | 19.50±1.20 | 18.93±0.60 |
| A/G                           | 1.53±0.03  | 1.40±0.58  | 1.40±0.00  | 1.53±0.03  |
| Carboxyldiamide (mmol/L)      | 10.03±0.16 | 10.55±0.14 | 7.54±0.21  | 6.80±0.38  |
| High density protein (mmol/L) | 1.56±0.02  | 1.78±0.14  | 1.83±0.08  | 1.79±0.11  |
| Low density protein (mmol/L)  | 0.20±0.02  | 0.18±0.02  | 0.28±0.03  | 0.20±0.01  |
Fig. 1 Plasma levels of various immune factors in different groups
Alistipes were the most abundant in the four groups of NC, WG, RG and EG (Fig. 3a). Beneficial bacteria of Roseburia (NC: 2.27%, WG: 9.19%, EG: 2.65%, RG: 2.37%), capable of producing butyric acid, was remarkably increased in the WG group, compared with the groups of NC, EG and RG, although the P value was slightly greater than 0.05 (0.05556), as shown in Fig. 3b. The abundance of norank_f_Bacteroidales_S24-7 observably decreased in the

Fig. 2 Community abundance on phylum level. a Microbial community bar plot at the phylum level with the relative abundance higher than 0.01%. b Kruskal–Wallis H test bar plot on phylum level. The asterisk represents significance (*p < 0.05)
Fig. 3  Community abundance on genus level. **a** Microbial community bar plot at the genus level with the relative abundance higher than 1%. **b** Kruskal–Wallis H test bar plot on genus level. The asterisk represents significance (*p < 0.05*)
WG group, while the abundance of unclassified_f__Lachnospiraceae both in groups of NC and WG was significantly higher than that in groups of EG and RG (p < 0.05) (Fig. 3b). It was worth noting that the beneficial bacteria of Blautia was remarkably increased in the WG group, compared with the groups of NC, EG and RG (p < 0.05) (Fig. 3b).

Further, the microbiota composition difference between the groups of NC and WG was analyzed (Fig. 4). Beneficial bacteria of Roseburia was remarkably increased in the WG group (with the p value of 0.0417), while the abundance of norank_f__Bacteroidales_S24-7 observably decreased in the WG group (p < 0.05), compared with the group NC, as shown in Fig. 4.

Correlation analysis between gut microbiota and cytokines and immunoglobulin

To determine whether there were potential associations between the alteration of the gut microbiota and biochemical parameters of cytokines and immunoglobulin, we analyzed the correlation between the relative abundance of the most abundant 50 genera and the biochemical parameters by Spearman correlation analysis. As shown in Fig. 5, IL-6 was positively correlated with Anaeroplasma (p < 0.05) and negatively correlated with Parasutterella (p < 0.05) and Bifidobacterium (p < 0.05). IL-10 showed positive correlation with Blautia (p < 0.05), Bifidobacterium (p < 0.05), Rikenella (p < 0.05) and norank_f__Clostridiales_vadinBB60_group (p < 0.05), while negative correlation with Ruminococcaceae_UCG-009 (p < 0.05). TNF-α was positively correlated with Ruminiclostridium_5 (p < 0.05) and negatively correlated with Bacteroides (p < 0.05).

As shown in Fig. 6, IgG showed positive correlation with Anaeroplasma (p < 0.05), Anaerotruncus (p < 0.05) and Ruminococcaceae_UCG-009 (p < 0.05), while negative correlation with norank_o_Mollicutes_RF9 (p < 0.05), Parasutterella (p < 0.05), unclassified_f_Ruminococcaceae (p < 0.05) and Bifidobacterium (p < 0.01). While, IgM was positively correlated with Rikenella (p < 0.05). The IgA was notably positively correlated with unclassified_f_Ruminococcaceae (p < 0.01) and Ruminococcaceae_UCG-014 (p < 0.05), while negatively correlated with Lachnospiraceae_UCG-006 (p < 0.05) and Anaeroplasma (p < 0.05). The above research results indicated that the alteration of the gut microbiota was one of the important reasons for the level changes of cytokines and immunoglobulin.

Discussion

There were no significant differences in the hematological parameters and hematological biochemical parameters among the WG, NC, RG, and EG groups on the whole

![Fig. 4](image-url) The microbiota composition difference between the groups of NC and WG. The asterisk represents significance (*p < 0.05)
and they were all within the normal range, which indicated that WG could be potentially used as a safe supplementary dietary protein, except for the people with specific allergies (further related researches on safety are performing in our group). Further, the high-density lipoprotein index increased while the low-density lipoprotein index decreased, in WG group, compared with NC group, indicating that the high-protein diet supplemented with WG could be beneficial for reducing blood lipids.

The levels of IL-6 increased significantly, with the WG group the most (increased 93.2%), while the levels of TNF-α and IL-10 decreased slightly, in the groups of WG, EG and RG, compared with the NC group. IL-6 is mainly synthesized and secreted by activated T lymphocytes and peripheral

Fig. 5 The correlation analysis between gut microbiota and cytokines. The asterisk represents significance (*p < 0.05)
blood mononuclear cells, and has a positive regulatory effect on immunity (Farsakoglu et al. 2019; Muhsin et al. 2018). While IL-10 has a negative regulatory effect on immunity (Matsumoto et al. 2009; Neumann et al. 2019). Thus, the increase of IL-6 levels and the decrease of the IL-10 levels indicated the improve of immunity by enhancing the levels of activated T cells and monocytes. TNF-α is an important pro-inflammatory cytokine, and the decrease of its level indicating the increase in the body’s anti-inflammatory ability (Decourt et al. 2017; Kemanetzoglu and Andreoudou 2017). The level of IgG increased significantly by 17.8%, while the levels of IgM and IgA decreased significantly by 37.1%
and 28.8%, respectively, compared with the NC group. The change trends of levels of IgG in both the RG and EG groups were as same as the WG group, but with smaller change levels. The IgG is the main component of the antibodies in serum and body fluids, accounting for about 80% of the total serum immunoglobulins. Serum IgG can neutralize pathogens or toxins and activate the classical or alternative pathway of complement, forming a membrane attack complex (MAC) on the surface of the pathogen’s membrane and turning on complement-dependent cytotoxicity (CDC) (Xie et al. 2020; Żabczyńska et al. 2020). The differentiation and maturation process of B cells is divided into five stages: immature B cells (im-B), mature B cells (ma-B), activated B cells (ac-B), memory B cell and plasma cells (Wang et al. 2020). The IgM was synthesized by im-B, ma-B and ac-B. And the memory B cell can synthesize both IgG and IgA, while plasma cells only synthesize and secrete IgG to mediate humoral immunity (Martin-Subero and Oakes 2018; Sigvardsson 2018). Therefore, the increased levels of high-affinity IgG and the decreased levels of low-affinity IgM and IgA indicated that WG were beneficial to promote the differentiation and maturation of B cells and improve humoral immunity. In summary, protein supplementation could improve the body’s immunity and the effect of WG is better than that of EG and RG. Our previous research work found that WG could reduce the immunosuppression caused by cyclophosphamide by enhancing the levels of IL-2 and IL-4 and the ratio of CD4+/CD8+ and restoring Th1/Th2 imbalance (Ji et al. 2018). The results of this work could prove that WG could improve the body’s immunity, from another perspective and has potential application value as an immune-enhancing functional food.

16S rRNA sequencing showed WG could remodel the gut microbiota. At the phylum level, the Bacteroidetes were reduced and Firmicutes were increased in WG group, compared with NC group. Although studies have shown that a lower B/F ratio is good for gut health (Xu et al. 2020), there have been cases where the opposite results have been found (Fabrizio and Yehuda 2018; He et al. 2016). There is growing consensus that the B/F ratio cannot be used as a biomarker for obesity, diabetes and cardiovascular disease (Magne et al. 2020). It was reported that walnuts could increase the abundance of Firmicutes and reduced the abundance of Bacteroidetes (Byerley et al. 2017). Thus, the decrease of B/F ratio in The WG should not be considered as an adverse change in the intestinal microecological structure. At the genus level, the SCFA producing genera of unclassified_f_Lachnospiraceae, Blautia and especially the Roseburia increased notably in the WG group, compared with the group of NC. The butyrate-producing bacteria of unclassified_f_Lachnospiraceae was necessary for the health of colonic epithelial tissue and maturation of the immune system, which was also negatively correlated with the inflammatory bowel disease (IBD) (Steinmeyer et al. 2015; Sun et al. 2017). The lower-fat diet resulted in a significant increase in relative abundance of Blautia, while the higher-fat diet led to a significant decrease in Blautia. And the Blautia was negatively associated with the changes in serum total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and non-high-density lipoprotein cholesterol (non-HDL-C) (Wan et al. 2019). Further, the Blautia was inversely associated with visceral fat accumulation in adults 20–76 years of age, both in males and females (Ozato et al. 2019). In summary, as one of the main SCFA producers Blautia showed notable negative correlation with obesity and insulin resistance and exerted anti-inflammatory effects (Benítez-Páez et al. 2020; Zhang et al. 2019b). Roseburia, producing short-chain fatty acids, especially butyrate, could sustain colonic motility, immunity maintenance and anti-inflammatory properties and the decrease in Roseburia abundance associated with several diseases (Tamanai-Shacoori et al. 2017). It was reported that the decrease in Roseburia abundance was positively correlated with IBD and the Roseburia abundance significantly increased in IBD-patients, after treatment (Imhann et al. 2018; Walujkar et al. 2018). The Roseburia abundance significantly increased in diabetic rats, after treatment of baicalein and Roseburia was notably positively correlated with SCFAs content and negatively correlated with the levels of triglyceride (TG), TC, blood glucose, lipopolysaccharide (LPS) and inflammation (Zhang et al. 2018). The reduction of Roseburia abundance was also associated with chronic kidney disease progression and Roseburia was negatively associated with C-reactive protein in plasma (Jiang et al. 2016). Studies have shown that the composition of the gut microbiota could be influenced by dietary composition and animal-based diets and plant-based diets could shape different types of gut microbiota (Dahl et al. 2020; Jia et al. 2020). The plant-based diet appears to be beneficial for human health by promoting gut microbiota (Tomova et al. 2019). The animal-based diet increased the abundance of bile-tolerant microorganisms of Bacteroides and decreased the levels of Firmicutes, such as Roseburia and Lachnospiraceae (David et al. 2014; Barrett et al. 2018), which were inherently consistent with the reduced B/F ratio of plant-derived WG. In summary, the gut microbiota of mice could be improved by WG, by the way of enhancing the levels of SCFAs producing bacteria of Roseburia, unclassified_f_Lachnospiraceae and Blautia.

Conclusion

In summary, WG could be potentially used as a safe supplementary dietary protein (except for the people with specific allergies) and the safety of WG was verified by analysis of the hematological parameters and hematological
biochemical parameters, after a WG administration of 30 days (further related researches on safety are performing in our group). The WG supplementation significantly increased the level of IL-6 and decreased the levels of TNF-α and IL-10 slightly, which indicated WG could enhance the levels of activated T cells and monocytes and anti-inflammatory ability. Further, the level of IgG increased significantly and the levels of the IgM and IgA decreased notably in the WG group, compared with the NC group, which illustrated that WG could improve immunity by promoting the differentiation and maturation process of B cells. The 16S rRNA sequencing showed that WG could improve the gut microbiota of the mice. At the phylum level, the Bacteriodes were reduced and Firmicutes were increased, in the WG group, compared with the NC group. At the genus level, the SCFA producing genera of unclassified_f_Lachnospiraceae, Blautia and Roseburia increased remarkably. It is notable that the percentage of Roseburia increased more than threefold (NC 2.27%, WG 9.19%). And the level changes of cytokines and immunoglobulin were associated with the gut microbiota.

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Author contributions GY: Analyzed data and wrote the paper. XJ: Designed the study and performed the research. WG: Participating in analyzed data. JH: Conceived of and designed study. AL: Participating in analyzed data. PL: Participating in analyzed data. MH: Participating in designed study. WG: Participating in analyzed data.

Data availability The raw sequences of 16S rRNA gene sequencing have been deposited in the NCBI Sequence Read Archive (SRA) database under Accession Number: (SAMN17192475,SAMN17192477,SAMN17192478,SAMN17192479,SAMN17192480,SAMN17192481,SAMN17192482,SAMN17192483,SAMN17192484,SAMN17192485,SAMN17192486,SAMN17192487,SAMN17192488,SAMN17192489). The web link to the datasets: https://www.ncbi.nlm.nih.gov/biosample/17192475, https://www.ncbi.nlm.nih.gov/biosample/17192476, https://www.ncbi.nlm.nih.gov/biosample/17192477, https://www.ncbi.nlm.nih.gov/biosample/17192478, https://www.ncbi.nlm.nih.gov/biosample/17192479, https://www.ncbi.nlm.nih.gov/biosample/17192480, https://www.ncbi.nlm.nih.gov/biosample/17192481, https://www.ncbi.nlm.nih.gov/biosample/17192482, https://www.ncbi.nlm.nih.gov/biosample/17192483, https://www.ncbi.nlm.nih.gov/biosample/17192484, https://www.ncbi.nlm.nih.gov/biosample/17192485, https://www.ncbi.nlm.nih.gov/biosample/17192486, https://www.ncbi.nlm.nih.gov/biosample/17192487, https://www.ncbi.nlm.nih.gov/biosample/17192488, https://www.ncbi.nlm.nih.gov/biosample/17192489.

Declarations

Conflict of interest There are no conflicts of interest to declare.

Ethical approval Institutional review board.

Informed consent All authors agree to participate in this study. All authors have seen the manuscript and approved to submit the manuscript to World Journal of Microbiology and Biotechnology.

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