



Allergenicity of alcohol-soluble wasp pupal proteins and its impact on the gut microbiota

Yage Ma^{a,b}, Shenglan Zhao^b, Xi Zhang^b, Jingjuan Yang^b, Jiashun Gong^{a,*}

^a College of Food Science and Technology, Yunnan Agricultural University, Heilong Tan, Kunming 650201, Yunnan, China

^b College of Traditional Chinese Medicine, Yunnan University of Traditional Chinese Medicine, Kunming 650500, China

ARTICLE INFO

Keywords:

Allergenicity
BALB/c mice
Alcohol-soluble wasp pupal proteins
Gut microbiota

ABSTRACT

Wasp pupa protein has triggered allergies in certain consumers. In this study, we investigated the allergenicity of alcohol-soluble wasp pupa protein (ACWP) and its effect on the gut microbiota of mice *in vivo*. It was found that ACWP caused skin erythema and diarrhea in mice, the up-regulation of HIS, IgE, IL-4, IL-22, and IL-17A, and down-regulation of IgG2a, IgA, and IL-2 in serum. The results also revealed that the thymus, spleen, and small intestine structures in mice also altered significantly, and the intestinal wall structure was disrupted. Fluorescein isothiocyanate (FITC) labeled dextran intestinal permeability test depicted that the intestinal permeability of mice in the ACWP group increased significantly. The gut microbiota analysis in mice depicted that five bacterial strains in the ACWP group, including *s_Candidatus_Arthromitus_sp._SFB_mice_Japa*, were up-regulated, and nine low-abundant strains, including *s_unclassified_g_norank_f_Ruminococcaceae*, were down-regulated. The association analysis of gut microbiota and serum factors showed that eight serum biochemical factors were significantly correlated with 11 strains. The results revealed that ACWP could cause disturbance of gut microbiota and its metabolism, change the integrity and permeability of the intestinal tract, destroy the intestinal mucosal immune system, and then cause skin erythema and diarrhea as the primary manifestations.

1. Introduction

The Vespidae are members of the order Hymenoptera of the superfamily Vespoidea [1]. The Wasp pupal proteins are high-yielding and high-quality resources, which are highly edible and have medicinal and health benefits [2]. Our preliminary study observed that the amino acid score of wasp pupal proteins was close to 100, much higher than the current model proteins. Many countries eat wasp larvae and pupae worldwide, and Vespidae species have the potential to become the most promising edible insects [3–5]. In Yunnan, China, there are >12 common species of edible Vespidae [4,6,7]. Wasp pupae feed on pests in the forest, and their cultivation promotes positive effects on the ecological cycle [3,5]. Therefore, wasps can be developed into a new edible animal protein source worldwide. However, edible insects may contain allergens, toxic substances, anti-nutritional substances, and pathogens. Many reports exist of food poisoning and even death caused by wasp pupae consumption across the country, which is more frequent in the Yunnan Province [8]. Although most of these are consumed as wild bee pupae, assessing the allergic risk to determine the safety of consuming artificially cultured wasp pupae is necessary. In our previous study, four

proteins, viz., water-soluble apisin (WWP), salt-soluble apisin (SWP), alcohol-soluble (ACWP), and alkaline-soluble apisin (AKWP), were extracted from the wasp pupae. Our study revealed that ACWP could cause an allergic reaction. Alcohol-soluble proteins accounted for only 0.18% of the total protein, with >100-kDa accounting for 19.24%, 50–100-kDa for 11.27, 30–50-kDa for 6.65%, 10–30-kDa for 21.31%, 3–10-kDa for 13.73% and < 3-kDa for 27.8%. However, little is known about its allergic reaction and sensitization mechanism *in vivo* or how ACWP affects allergenicity and gut microbiota.

Clinical symptoms of food allergies (F.A) include slight abdominal discomfort and life-threatening anaphylactic shock [7]. Type II helper T cells (Th2) polarization-related inflammation involves the over-production of antigen (Ag)-specific IgE, Th2 cytokines (e.g., interleukin [IL-4, IL-5, and IL-13]), and profound infiltration of eosinophils and mast cells in tissues. Therefore, they are the fundamental F.A with pathological changes. First, Th2 cytokines, especially IL-4, induce plasma cells to produce IgE [9]. Then, IgE binds to the high-affinity IgE receptors on the mast cell surface, then sensitized. Re-exposure to the specific Ags triggers the sensitized mast cells to release utilized chemical mediators (e.g., histamine and leukotrienes) for evoking allergy attacks

* Corresponding author at: College of Food Science and Technology, Yunnan Agricultural University, China.

E-mail addresses: pigeonma@ynutcm.edu.cn (Y. Ma), gong199@163.com (J. Gong).

<https://doi.org/10.1016/j.clim.2022.109069>

Received 16 March 2022; Received in revised form 21 June 2022; Accepted 22 June 2022

Available online 25 June 2022

1521-6616/© 2022 Elsevier Inc. All rights reserved.

in the tissues [10]. Similarly, allergens can change the abundance of gut microbiota, alter intestinal permeability, destroy mucosal immunity, and cause allergic reactions leading to intestinal discomfort [11].

The current study evaluated the allergenicity of ACWP and its impact on the gut microbiota *in vivo*.

2. Materials and methods

2.1. Materials

Female BALB/c mice were obtained from Hunan Slek Jingda Experimental Animal Co. Ltd. [Experimental animal production license: SCXK(Xiang) 2019-0004] (Hunan, China). The histamine (HIS) ELISA kit was purchased from the Nanjing Jiancheng Biotechnology Co., LTD (Nanjing, Jiangsu, China). Interleukin-2 (IL-2), interleukin-4 (IL-4), Interleukin-22 (IL-22), Interleukin-17A(IL-17A), Immunoglobulin E (IgE), Immunoglobulin G2a (IgG2a), Immunoglobulin A (IgA), and Interferon- γ (IFN- γ) ELISA kits were acquired from the Neobioscience Biotechnology Co., LTD (Shenzhen, China). The cholera toxin B subunit (CTB) and fluorescein isothiocyanate (FITC) labeled dextran (FITC: Glucose = 1:250, 4 k Da) were obtained from Sigma-Aldrich Trading Co., Ltd. (St.Louis MO, USA).

2.2. Preparation of the alcohol-soluble wasp pupal proteins (ACWP)

After the wall of wasp pupae was broken, the 1:5 solid-liquid ratio was added to -20°C for pre-cooling acetone. The wasp pupae were defatted at 4°C for 24 h. Then, the liquid was stirred several times until the acetone was clarified and centrifuged (5424r, Eppendorf Germany) at 3000 rpm for 30 min. Then, the supernatant was discarded, the filtrate residue was collected, acetone was dried in a fume hood, and the total wasp pupae protein (TWP) was prepared. At TWP, 0.5002 g was accurately weighed, and 70% ethanol solution was added at the 1:20 ratio of material to liquid (m/V), heated to 37°C , extracted for 1 h, and then

centrifuged at 3500 rpm for 20 min. Finally, the supernatant was collected to obtain ACWP, and the protein content was evaluated.

2.3. Murine allergenicity model

2.3.1. Mice sensitization and challenge

The sensitization and challenge of BALB/c mice (female, six weeks old) were kept at the animal center of the Yunnan University of Chinese Medicine (Kunming, China). The approval number for animal testing was 2019-09-15. Mice were randomized into four groups (15 per group): PBS negative control [cholera toxin (CTB) in PBS served as an adjuvant], OVA (Chicken Ovalbumin) positive control [7], ACWP high-dose control (HDG-ACWP), and ACWP low-dose control (LDG-ACWP) groups. After one week of acclimation, the PBS-negative control mice were treated with gavage using 0.2 mL of PBS containing CTB (10 $\mu\text{g}/\text{mice}$). In the OVA, HDG-ACWP, and LDG-ACWP groups, the mice were sensitized with gavage using 0.2 mL of PBS containing CTB (10 $\mu\text{g}/\text{mice}$) and OVA (1 mg/mice), ACWP (2 mg/mice) or ACWP (1 mg/mice), on days 6, 7, 8, 13, 14 and 15. The mice were sensitized through gavage of 0.2 mL of PBS containing OVA (10 mg/mice) or ACWP (20 mg/mice) or ACWP (10 mg/mice) on days 20 and 25 (Fig. 1). Finally, the mice were anesthetized and killed through cervical dislocation, and the whole blood was collected [12]. The mice serum was separated from the whole blood through centrifugation at 3000 g for 10 min (4°C) and stored at -80°C (Haier, China).

All the animal experiments complied with the ARRIVE guidelines and were carried out following the U.K. Animals (Scientific Procedures) Act, 1986, along with the associated guidelines of the E.U. Directive 2010/63/E.U.

2.3.2. Organ index of determination

The mice were weighed every seven days. The thymus, spleen, lung, kidney, liver, and small intestine tissues were harvested in sterile conditions, washed with 0.9% saline, weighed, and determined the organ

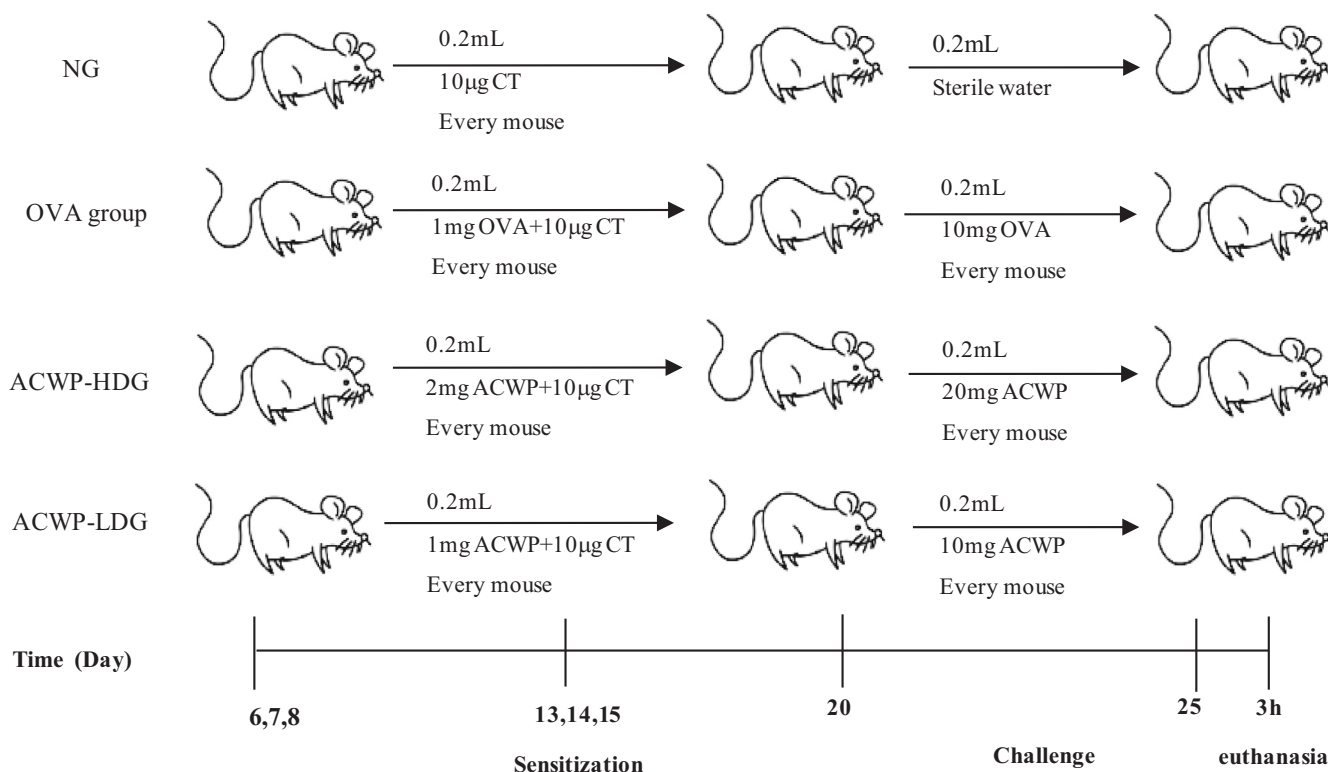


Fig. 1. Experimental design of mouse sensitization and challenge.

index [13].

2.3.3. Allergy symptoms and serum biochemical analysis

Allergy symptoms were evaluated using an established scoring system. The histamine ELISA kit evaluated the serum histamine level using the multi-function enzyme label instrument (Tecanm200 Pro, TECAN, Switzerland). The serum antibodies (IgE, IgA, and IgG) were determined through ELISA. The standard, sample, and blank wells were set up, and the absorbance was measured at 450 nm (Tecanm200 Pro, TECAN, Switzerland) using the ELISA kit instructions. The content of the sample was calculated using the standard curve.

2.3.4. Histopathological analysis

Thymus, spleen, and small intestine tissues were isolated from the mice group, then fixed in 10% paraformaldehyde solution, followed by dehydration, embedding in paraffin, and sectioning into 5 μ m slices. Then, the sections were stained with hematoxylin and eosin [7]. After that, slides were examined under an upright microscope to assess the histopathological damage (DS-U3, NIKON, Japan) [14].

2.3.5. Intestinal permeability analysis

Three mice were randomly selected from each group for the final intragastric administration and were deprived of food and water for 12 h (overnight). During the second day, 0.2 mL of FITC-labeled dextran solution dissolved in PBS was given to the mice through intragastric administration of 40 mg/mL of FITC. Later, blood samples were collected from the eyeballs after 4 h of intragastric administration, and an anticoagulant was introduced into the blood vessels. After standing for 2 h, the blood samples were centrifuged (4 $^{\circ}$ C, 3000 rpm, 20 min), and the upper serum was collected. Each group absorbed a 100 μ L serum sample into the 96-well plates. The blank holes were created using the mice serum fed the same amount of PBS instead of FITC, and the standard curves (1, 2, 4, 8, 16, 32, 64 g/mL) were prepared. The fluorescence values were calculated at 518 nm for excitation at 493 nm (10 M, Tecan SparkTM, Switzerland) [15,16].

2.3.6. Serum cytokine analysis

At the end of the stimulation, after 4 h from the last gavage, the mice were intraperitoneally anesthetized with 5% chloral hydrate, and the eyeballs were removed for blood collection. Later, the blood from the E. P. tube was allowed to stand for 2 h and then spiked for 20 min at 4 $^{\circ}$ C and 3000 rpm. The upper serum layer was frozen at -80° C for storage. IL-2, IL-4, IFN- γ , IL-22, and IL-17A levels were determined using the ELISA kits from the serum.

2.3.7. Small intestine cecal contents DNA extraction and high-throughput sequencing

We collected the small intestine cecal contents of mice from each group using the aseptic method. The cecal contents were kept in sterile centrifuge tubes with sterilized tweezers, and liquid nitrogen was immediately used to quick-freeze them. Finally, they were placed in an 80° C refrigerator to be tested. Based on the manufacturer's instructions, genomic DNA of the microbial community was extracted from the cecal content sample using the E.Z.N.A.[®] soil DNA kit (Omega Bio-Tek, Norcross, Georgia, USA). We amplified the bacterial 16 S rDNA in the highly variable region of the v3-V4 gene using the primer pairs 338 F (5'-ACTCTACGGGAGGCAGCAG-3') and 806 R (5'-GGACTACHVGGG TWTCTAAT-3') with the help of the ABI GeneAmp[®] 9700 PCR thermal cycler (ABI, CA). High-throughput sequencing was undergone following the standard protocol of Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China) [17].

2.4. Statistical analysis

All the data were represented using mean and standard deviation (S. D.). A general linear model and a one-way ANOVA were used to analyze

the data. Duncan's multiple range test evaluated the differences between the groups using the SPSS software (SPSS Inc., Version 21, I.L., USA). The difference was statistically significant with $P < 0.05$ and highly significant at $P < 0.01$.

3. Results

3.1. Effects of ACWP on body weight, water intake, food intake, and organ index in the mice

As shown in Fig. 2, HDG-ACWP reduced the food intake and lung index while improving the thymus, spleen, and liver indexes ($P < 0.05$). LDG-ACWP showed the same trend as HDG-ACWP, but the difference was insignificant. The results revealed that ACWP could cause hyperactivity within the immune-related organs of mice.

3.2. Effects of ACWP on allergy symptoms and immune cytokine in mice

As shown in Fig. 3A-D, ACWP mainly caused diarrhea and skin erythema as phenotypes of food allergic reactions. Compared with the PBS negative group, the mice allergy symptoms in the HDG-ACWP and OVA groups were exacerbated upon subsequent challenges. As depicted in Fig. 3E and G, after ACWP sensitization and challenge, serum histamine and Th2-dependent antibodies (IgE) levels were significantly elevated in the mice of the HDG-ACWP and LDG-ACWP groups than in the N.G. group. In contrast, the IgG2a level was slightly reduced. The HDG-ACWP and LDG-ACWP groups had more severe anaphylactic symptoms than the mice from the OVA group. This data revealed that ACWP could cause a more severe allergic reaction than OVA. Furthermore, the balance between the Th1/Th2 cells and Treg/Th17 balance immunity is crucial in immune stability. IFN- γ and IL-2 are Th1 cytokines, while IL-4 is a Th2 cytokine. Compared with the mice from the N.G. group, the IL-4 level was significantly up-regulated, while the IL-2 level was significantly downregulated in the HDG-ACWP and LDG-ACWP groups (Fig. 3I-J). The Th1 cytokine (IFN- γ) seemed to have no significant participants. These results indicated that ACWP could lead to Th1, Th2-related factor release imbalance and up-regulate the Th2 cytokine release, Th2-dominated status, triggering IgE mediated F.A. Moreover, the IgA secretion promoted immune tolerance in the system, and the clostridium intestine in healthy people stimulated ILC3s to produce IL-22 to maintain the integrity of the epithelial barrier. IL-22 also promoted mucus secretion by goblet cells and antimicrobial peptide secretion by paneth cells, thereby reducing the ability of food allergens in body circulation [18]. The activity of IL-17A is strongly related to the innate immune response cells. This cytokine is essential for protection against numerous extra-cellular pathogens. IL-17A can induce the expression of innumerable inflammatory reaction mediators by participating in the proliferation, maturation, and chemotaxis of neutrophils, essential elements of non-specific immune responses [19,20]. As shown in Fig. 3K-M, the IL-22 level was significantly up-regulated than the N.G. group. In contrast, the IgA level was downregulated significantly within the HDG-ACWP, LDG-ACWP, and OVA groups. The IL-17A level was significantly up-regulated in the HDG-ACWP and LDG-ACWP groups.

3.3. Effects of ACWP on thymus spleen and small intestine tissue morphology in mice

The immune function of the thymus is depicted by the width of the medulla region, the boundary between the thymus cortex and the medulla, and the number and structure of the thymus corpuscles in mice. The staining of thymus HE slices (Fig. 4 A) revealed that in mice of the OVA, HDG-ACWP, and LDG-ACWP groups, the thymus medulla region was widened. Moreover, the boundary between the thymus cortex and medulla was clear, the volume of the thymus corpuscle was increased, and the number of lymphocytes in the medulla region was elevated compared with those in the N.G. group. These results suggested that

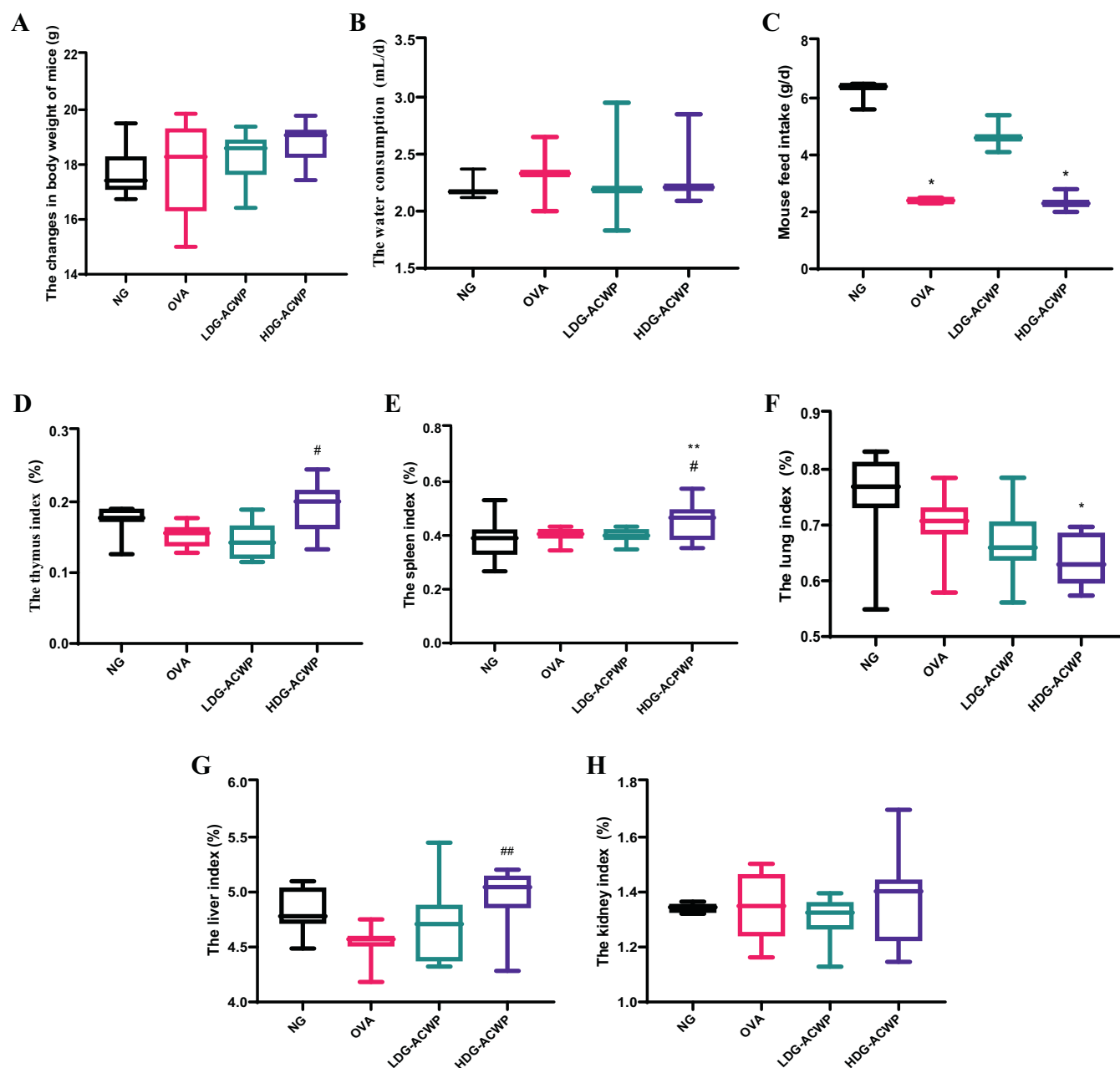


Fig. 2. The effects of wasp pupal proteins on changes in body weight (A) water intake(B), food intake (C) and organ index in mice. (D) thymus index (E) spleen index (F) lung index (G) liver index (H) kidney index. Data were presented as mean ± SD. * indicated the significant difference compared with the NG group, * $P < 0.05$, ** $P < 0.01$; # indicated the significant difference compared with the compared with OVA group, # $P < 0.05$, ## $P < 0.01$.

pupa gliadin induced changes in the thymus tissue and enhanced T cell immunity in mice.

The staining of spleen HE slices (Fig. 4 B) revealed that the area of splenic white pulp, periarterial lymphatic sheath, and splenic nodule in the OVA group enhanced lymphocyte-mediated immune response in OVA mice compared with the N.G. group. In the HDG-ACWP group, the area of the splenic white pulp, periarterial lymphatic sheath, splenic nodule, and lymphocyte enlargement were all elevated. The size and number of cells were greater than those in the OVA group, indicating that the enhanced lymphocyte-mediated immune response within the HDG-ACWP group was more robust than that in the OVA one. In the LDG-ACWP group, no significant change was observed in the size and number of spleen white pulp areas, periarterial lymphocyte sheath, or the splenic nodule, depicting that the food allergic reaction caused by

pupae gliadin was positively associated with dose.

The staining of spleen HE slices (Fig. 4 C) revealed that mice in the N.G. group had a complete intestinal mucosa with transparent layers, orderly villi, precise epithelial cells with goblet cells, orderly intestinal glands, and no significant inflammatory cell infiltration in the lamina propria. In the OVA, HDG-ACWP, and LDG-ACWP groups, the small intestinal wall was thinner, and the mucosa tissue structure was clear and complete compared with the N.G. group. The villi were long and orderly, and the small intestinal glands were orderly. The epithelial cells were clear in contour and rich in cup cells. No obvious inflammatory cell infiltration in the lamina propria was observed.

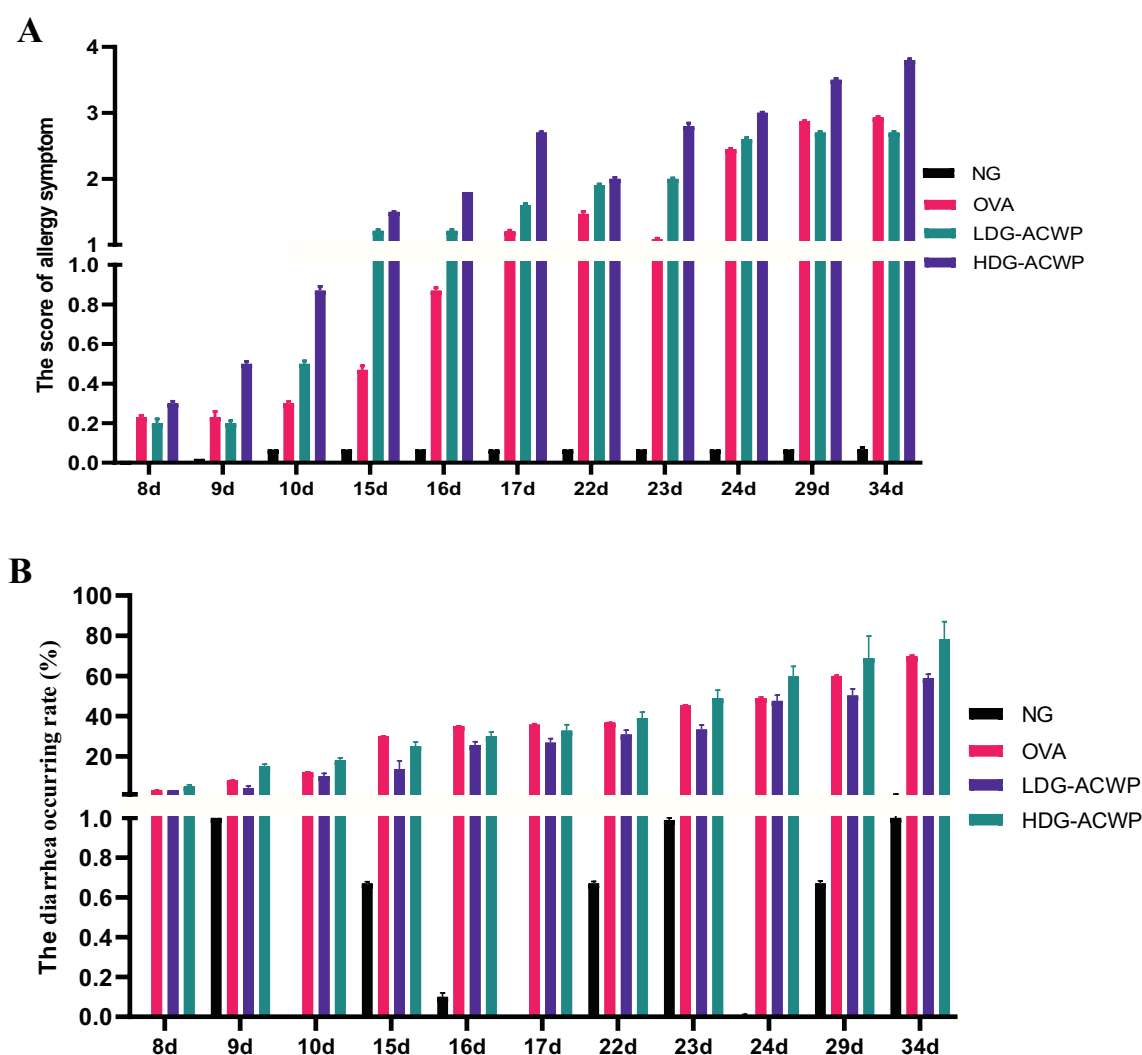


Fig. 3. The effects of wasp pupal proteins in mice. (A) score of allergy symptom, (B) diarrhea occurring rate, (C) Erythema in the tail of HDG-ACWP mice, (D) Erythema in the tail of HDG-ACWP mice, (E) HIS, (F) IgE, (G) IgG, (H) IL-2, (I) IL-4, (J) IFN- γ , (K) IgA I, (L) L-22 and (M) IL-17 A. Data were presented as mean $\pm$ SD. * indicated the significant difference compared with the NG group, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$; # indicated the significant difference compared with the compared with OVA group, # $P < 0.05$, ## $P < 0.01$ ### $P < 0.001$, #### $P < 0.0001$.

3.4. Effect of ACWP on intestinal permeability in mice

The normal intestinal wall vessels of mice in the N.G. group showed a specific permeability to FITC-glucan (Fig. 5). Compared with the N.G. group, the fluorescence value of FITC-glucan in the mice serum in the OVA and HDG-ACWP groups was significantly increased ($P < 0.0001$), indicating an enhancement of the intestinal wall permeability. The fluorescence value of the FITC-glucan in the serum of the LDG-ACWP group was not significantly enhanced ($P > 0.05$). Compared with the OVA group, the fluorescence value of FITC-glucan in the mice serum in the HDG-ACWP group was significantly elevated ($P < 0.0001$), and that in the LDG-ACWP group was significantly reduced ($P < 0.0001$). FITC is a fluorescent dye. After the mice have been gavaged with FITC-labeled dextran (FITC-dextran), it enters the bloodstream through the intestine. The FITC-dextran quantity in the serum (fluorescence value) can be analyzed to reflect the permeability of the intestinal wall. The above data on intestinal wall permeability *in vivo* revealed that the alcohol-soluble wasp pupal proteins could significantly enhance intestinal wall permeability based on proportional dosage.

3.5. Effect of ACWP on the gut microbiota of mice

3.5.1. ACWP decreased the overall diversity of gut microbiota in mice

As shown in Table 1, Sobs, Shannon's, Chao's, and P.D. indexes were significantly decreased ($P < 0.05$) in the HDG-ACWP group than in the N.G. group. The results revealed that pupae gliadin reduced the diversity of fecal flora in mice, indicating that this could be one reason for food allergy. (See Table 2.)

The OTUs samples with a similar level of 97% were selected for Venn diagram analysis to understand better the number of common and unique species between each group (Fig. 6 A). The number of OTUs shared between the N.G. and OVA groups (21) was more than that of OTUs shared between the N.G. and HDG-ACWP groups (11). It depicted that the gut microbiota in the OVA group was similar to that in the N.G. group. The result indicated that ACWP could cause mouse gut microbiota disorders. Consistent with the PCoA chart, a sample-level cluster analysis revealed that the HDG-ACWP and the LDG-ACWP groups clustered together. In contrast, the HDG-ACWP, OVA, and N.G. groups clustered separately (Fig. 6 B, C).

The structure of the gut microbiotas within the N.G. and the HDG-

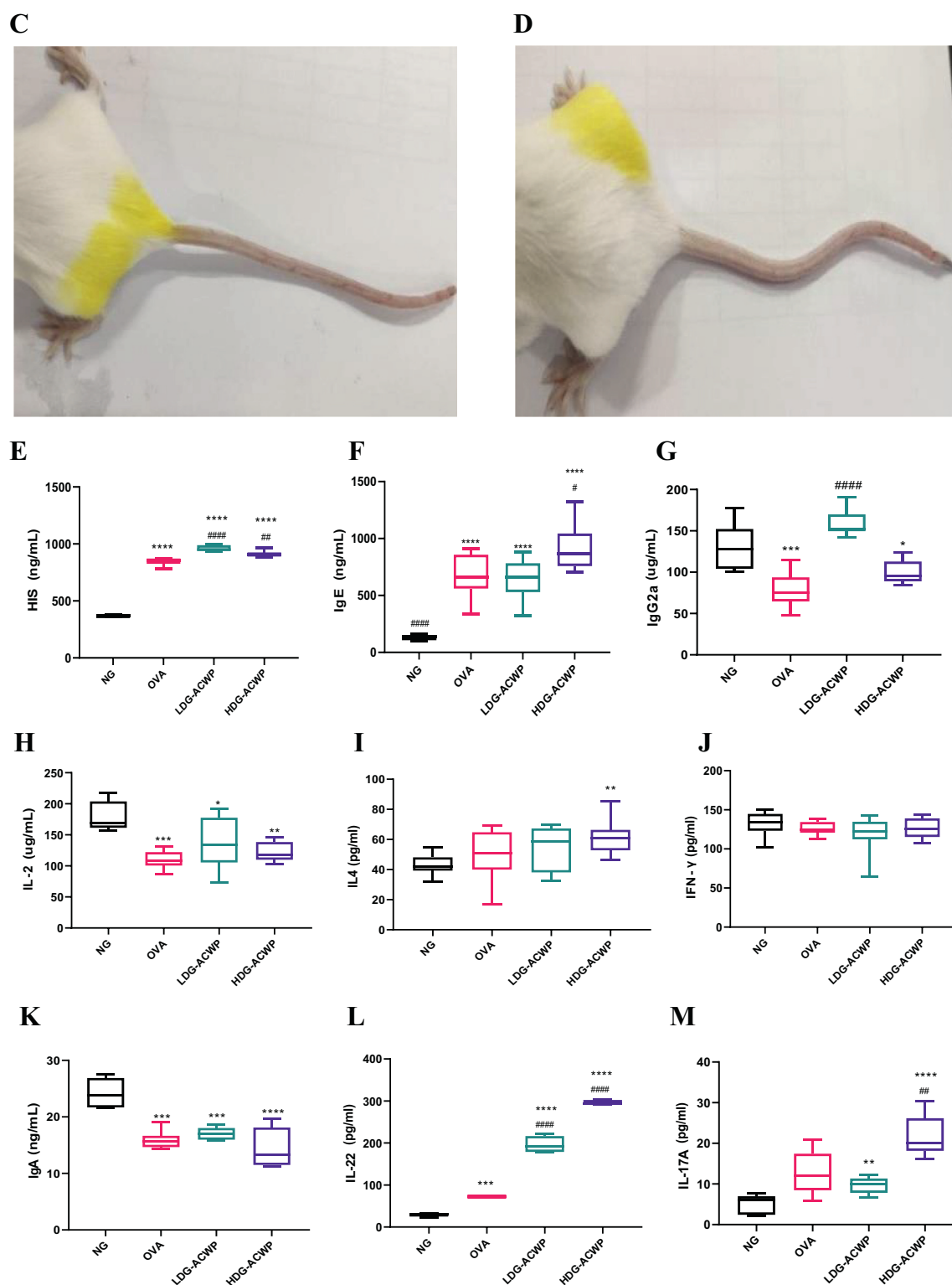


Fig. 3. (continued).

ACWP, LDG-ACWP, and OVA groups were significantly different. The above results indicate that ACWP induced the disorders of the gut microbiota within mice.

3.5.2. ACWP broke the balance of gut microbiota in mice

At the phylum level, the relative abundance of *Verrucomicrobia*, unclassified_k_norank_d_Bacteria, and *Cyanobacteria* inside the gut

microbiota of mice in the HDG-ACWP group increased significantly compared with the N.G. group ($P < 0.05$), while the *Epsilonbacteraeota* decreased.

Food allergy caused by wasp pupa protein may be related to *Firmicutes/Bacteroidetes* ratio imbalance.

At the genus level, the relative abundance of *Candidatus_Arthromitus*, *Clostridium_sensu_stricto_1*, and *Enterococcus* inside the gut microbiota of

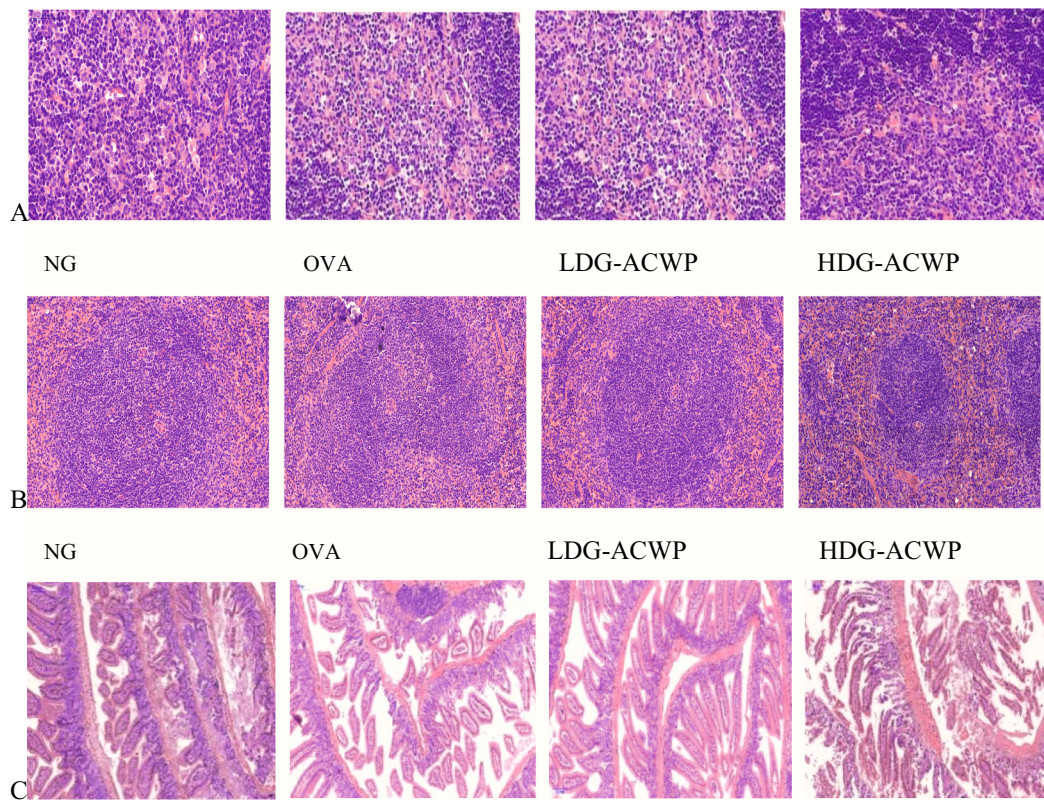


Fig. 4. Pathological analysis of thymus spleen and small intestine. (A) HE staining of thymus; (B) HE staining of spleen; (C) HE staining of small intestine.

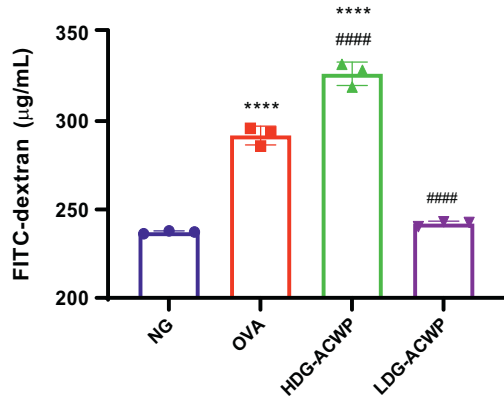


Fig. 5. The effect of ACWP on intestinal permeability in mice. Data were presented as mean $\pm$ SD. * indicated the significant difference compared with the NG group, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$; # indicated the significant difference compared with the OVA group, # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$, #### $P < 0.0001$.

mice in the HDG-ACWP group increased significantly compared with the N.G. group ($P < 0.05$), while the *Parabacteroides*, *Bacteroides*, *Akkermansia*, *Parasutterella*, *unclassified_k_norank_d_Bacteria*, and *unclassified_o_Bacteroidales* decreased (Fig. 6 D).

At the species level, the relative abundance of *Lactobacillus intestinalis*, *Lactobacillus reuteri*, *unclassified_bacterium_g_Desulfovibrio*, *Candidatus_Arthromitus_sp_SFB_mice_Japan* and *unclassified_g_Streptococcus* in the gut microbiota of mice belonging to the HDG-ACWP group increased significantly ($P < 0.01$) compared with the NG group. In contrast, *unclassified_bacterium_g_norank_f_Muribaculaceae*, *unclassified_g_Alistipes*, *unclassified_bacterium_g_Parasutterella*, *gut_metagenome_g_Eubacterium_coprostanoligenes_group*,

Table 1

Comparison of Alpha diversity of gut flora of mice in each group ($X \pm s$, $n = 4, 3, 5, 5$).

	NG	HDG-ACWP	LDG-ACWP	OVA
Sobs	264.25 $\pm$ 28.61	194.33 $\pm$ 32.18*	252.80 $\pm$ 11.35	257.80 $\pm$ 8.15
Chao1	308.09 $\pm$ 16.30	229.97 $\pm$ 15.06*	294.01 $\pm$ 18.86	295.17 $\pm$ 36.21
Shannon's	2.89 $\pm$ 0.30	2.35 $\pm$ 0.37*	3.35 $\pm$ 0.33	3.37 $\pm$ 0.22
PD	21.29 $\pm$ 1.06	17.15 $\pm$ 1.48*	20.18 $\pm$ 0.45	20.49 $\pm$ 2.37
coverage	0.9989 $\pm$ 0.0002	0.9992 $\pm$ 0.0001	0.9989 $\pm$ 0.0002	0.9989 $\pm$ 0.0002

* indicated the significant difference compared with the NG group, * $P < 0.05$.

unclassified_bacterium_g_Ruminococcaceae_UCG-014, *unclassified_g_norank_f_Erysipelotrichaceae*, *unclassified_g_norank_f_Ruminococcaceae*, *unclassified_g_Parabacteroides* and *unclassified_g_Bacteroides* decreased ($P < 0.05$, $P < 0.01$) (Fig. 6 E). However, the impact on the strains in the LDG-ACWP group was similar to the trend in HDG-ACWP but not significantly.

Comprehensive analysis revealed that ACWP-induced food allergies were correlated with the significantly increased abundance of five species and decreased abundance of nine species. The correlation was proportional to dose, which meant that the more significant the change in strain abundance, the more severe the allergic degree.

3.5.3. Correlation between the abundance of gut microbes and biochemical parameters

Spearman correlation analyzed the correlation between changes in serum biochemical factors and changes in gut microbiota. As shown in Fig. 7, it was observed that the relative abundance of multiple species had a very significant correlation with the biochemical parameters. The IL-22, HIS, IgE, IgG, IL-4, and IL-17A levels were positively associated

Table 2The effect of ACWP on the level of phylum, family, genus and species of mice gut flora, $n = 4, 3, 5, 5$.

Microbes	NG	HDG_ACWP	LDG_ACWP	OVA
p_Firmicutes	73.14 ± 21.5	65.55 ± 3.40	54.51 ± 7.07*	20.08 ± 8.93
p_Bacteroidetes	13.46 ± 3.72	17.77 ± 4.73	23.61 ± 2.41	63.09 ± 5.84*
p_Verrucomicrobia	0.0594 ± 0.0020	2.44 ± 0.05*	0.2812 ± 0.07	4.31 ± 0.96
p_unclassified_k_norank_d_Bacteria	0.0041 ± 0.0007	0.0775 ± 0.0047*	0.1333 ± 0.078*	0.0655 ± 0.0011
p_Epsilonbacteraeota	0.0400 ± 0.0056	0.0007 ± 0.0001*	0.0198 ± 0.0081	0.0581 ± 0.0072
p_Cyanobacteria	0.0058 ± 0.0005	0.0109 ± 0.0090*	0.0041 ± 0.0004	0.0092 ± 0.0113
f_Lactobacillaceae	42.17 ± 1.92	59.23 ± 4.78	41.85 ± 2.22	10.51 ± 0.59**
f_Muribaculaceae	9.62 ± 3.71	12.45 ± 2.34	16.02 ± 1.12	36.38 ± 0.21*
f_Erysipelotrichaceae	8.35 ± 0.54	0.57 ± 0.093*	1.37 ± 0.80	1.83 ± 0.19
f_Bacteroidaceae	3.65 ± 0.04	0.0845 ± 0.0072*	2.22 ± 0.18	11.80 ± 0.78
f_Akkermansiaceae	2.44 ± 0.05	0.0593 ± 0.0010*	0.28 ± 0.05	4.31 ± 0.96
f_Burkholderiaceae	0.14 ± 0.06	0.06 ± 0.0030*	0.77 ± 0.02*	1.88 ± 0.37*
f_Tannerellaceae	0.26 ± 0.02	0.0139 ± 0.0019	0.85 ± 0.09	1.65 ± 0.09*
f_Enterobacteriaceae	0.0015 ± 0.0002	0.0029 ± 0.0002	0.0748 ± 0.0016	0.55 ± 0.04*
f_Clostridiaceae_1	0.0088 ± 0.0008	1.013 ± 0.17*	0.1014 ± 0.05**	0.0170 ± 0.0012
f_unclassified_k_norank_d_Bacteria	0.0775 ± 0.0047	0.0041 ± 0.0007*	0.0653 ± 0.0024*	0.29 ± 0.04
f_Enterococcaceae	0.0006 ± 0.0001	0.0015 ± 0.0006*	0.0038 ± 0.0001*	0.0008 ± 0.0001
g_Bacteroides	3.65 ± 0.04	0.08 ± 0.01*	2.22 ± 0.18	11.88 ± 0.78
g_Akkermansia	2.44 ± 0.49	0.06 ± 0.01**	0.28 ± 0.05	4.31 ± 0.96
g_Parasutterella	0.14 ± 0.06	0.0098 ± 0.0009*	0.77 ± 0.02**	1.88 ± 0.37*
g_unclassified_k_norank_d_Bacteria	0.08 ± 0.01	0.0041 ± 0.0007*	0.13 ± 0.03	0.07 ± 0.05
g_unclassified_o_Bacteroidales	0.02 ± 0.0070	0.0014 ± 0.0002**	0.0153 ± 0.0023*	0.29 ± 0.01
g_Candidatus_Arthromitus	0.0067 ± 0.0009	1.00 ± 0.07**	0.0526 ± 0.0028**	0.0040 ± 0.0002
g_Clostridium_sensu_stricto_1	0.0022 ± 0.0009	0.0488 ± 0.0071*	0.0084 ± 0.0002	0.0132 ± 0.0021
g_Enterococcus	0.0006 ± 0.0005	0.0015 ± 0.0003**	0.0038 ± 0.0008**	0.0008 ± 0.0000
g_Parabacteroides	0.26 ± 0.02	0.0139 ± 0.0028**	0.85 ± 0.02	1.65 ± 0.94*
s_Lactobacillus_intestinalis	0.45 ± 0.08	18.00 ± 1.99**	5.58 ± 0.28**	3.00 ± 0.18*
s_Lactobacillus_reuteri	1.29 ± 0.24	16.47 ± 1.65**	8.27 ± 0.32**	2.54 ± 0.13
s_uncultured_bacterium_g_norank_f_Muribaculaceae	5.71 ± 0.16	1.01 ± 0.29**	5.14 ± 0.57	14.05 ± 0.98
s_uncultured_bacterium_g_Desulfovibrio	0.27 ± 0.09	4.08 ± 0.62**	0.88 ± 0.01	0.20 ± 0.03
s_Candidatus_Arthromitus_sp_SFB_mice_Japan	0.0067 ± 0.0006	1.00 ± 0.17**	0.05258 ± 0.0023**	0.0040 ± 0.0002
s_unclassified_g_Alistipes	0.9576 ± 0.08	0.0389 ± 0.0047*	0.57 ± 0.01	2.92 ± 0.29
s_uncultured_bacterium_g_Parasutterella	0.1374 ± 0.06	0.00978 ± 0.0009*	0.77 ± 0.02**	1.88 ± 0.14*
s_gut_metagenome_g_Eubacterium_coprostanoligenes_group	0.0186 ± 0.0030	0.0022 ± 0.0009**	0.03 ± 0.02	0.25 ± 0.07
s_uncultured_bacterium_g_Ruminococcaceae_UCG-014	0.31 ± 0.03	0.0119 ± 0.0037*	0.0247 ± 0.0012*	0.14 ± 0.03
s_unclassified_g_Streptococcus	0.0817 ± 0.0037	0.17 ± 0.04**	0.03625 ± 0.0098*	0.0238 ± 0.0016*
s_unclassified_g_norank_f_Erysipelotrichaceae	0.0150 ± 0.0019	0.0028 ± 0.0004**	0.00831 ± 0.0038*	0.0299 ± 0.0014
s_unclassified_o_Bacteroidales	0.0153 ± 0.0017	0.0014 ± 0.0002**	0.0095 ± 0.0024**	0.29 ± 0.05
s_unclassified_g_norank_f_Ruminococcaceae	0.0565 ± 0.0041	0.0098 ± 0.0008*	0.0480 ± 0.0004	0.0162 ± 0.0025
s_unclassified_g_Parabacteroides	0.22 ± ±0.02	0.0076 ± 0.0002**	0.22 ± 0.02	1.34 ± 0.84*
s_unclassified_g_Bacteroides	1.273 ± 0.21	0.0294 ± 0.0024*	0.48 ± 0.03	3.81 ± 0.26

* indicated the significant difference compared with the NG group, * $P < 0.05$; ** $P < 0.01$.

with *Lactobacillus reuteri*, *Lactobacillus intestinalis*, uncultured_bacterium_g_Desulfovibrio, unclassified_g_norank_f_Erysipelotrichaceae, and *Candidatus_Arthromitus* sp_SFB mouse Japan. On the contrary, a significantly negative correlation was observed between the biochemical parameters and uncultured_bacterium_g_Ruminococcaceae_UCG-014, unclassified_g_norank_f_Erysipelotrichaceae, unclassified_g_Parabacteroides, unclassified_g_Bacteroides and unclassified_g_Alistipes. The IL-2 level was positively correlated with the unclassified_g_norank_f_Ruminococcaceae, while negatively associated with unclassified_g_Parabacteroides. The IFN-gamma level was positively correlated with uncultured_bacterium_g_Desulfovibrio, whereas it was negatively correlated with *Lactobacillus reuteri* and *Lactobacillus intestinalis*.

4. Discussion

The current study proved that ACWP caused diarrhea and skin erythema as phenotypes of food allergic reactions for the first time. Moreover, ACWP could cause hyperactivity within the immune-related organs of mice and induce gut microbiota disorders simultaneously. The ACWP-induced food allergies were correlated with the significantly increased abundance of five species and decreased abundance of nine species. The data on intestinal wall permeability *in vivo* revealed that ACWP could significantly enhance intestinal wall permeability depending on the proportional dosage.

We found erythema and necrosis of varying degrees in the tail of the ACWP-interfered mice through animal experiments, accompanied by

adverse intestinal diarrhea. Therefore, the main target organs of the mice are the skin and intestinal tract without causing severe systemic sensitization. Based on the allergy and diarrhea degrees scores, the allergy degree score of ACWP was 2–3, belonging to moderate and mild food allergies and diarrhea, and 60% to 80% of the sample presented with diarrhea symptoms. The degree of allergy was proportional to the dose.

The mechanism of food allergy could be divided into IgE-mediated and non-IgE-mediated F.A.

Food allergies are adverse immune responses to food proteins resulting in typical clinical symptoms of the dermatological, respiratory, gastrointestinal, cardiovascular, and neurological systems. The IgE-mediated food-allergic disease differs from non-IgE-mediated disease because the pathophysiology activates the immune system, causing a T helper 2 response. Thus, IgE binding to Fcε receptors on effector cells causes mast cells and basophils. The activation of these cells releases the histamine and other mediators and rapid symptom onset [20]. The process was divided into two stages. During the allergic sensitization phase, the first contact with the allergen occurs with an initial immunological response, breaking the tolerance, followed by specific IgE production [21]. After that, IgE anchors to the high-affinity Fc fragment receptor on the membrane of tissue mast cells and circulating basophils [22]. The cytoplasm of these cells contains vasoactive substances and anaphylaxis mediators like histamine. Therefore, these cells become sensitized and activate and degranulate on the second contact with that allergen, releasing anaphylactic mediators in the tissues and the blood

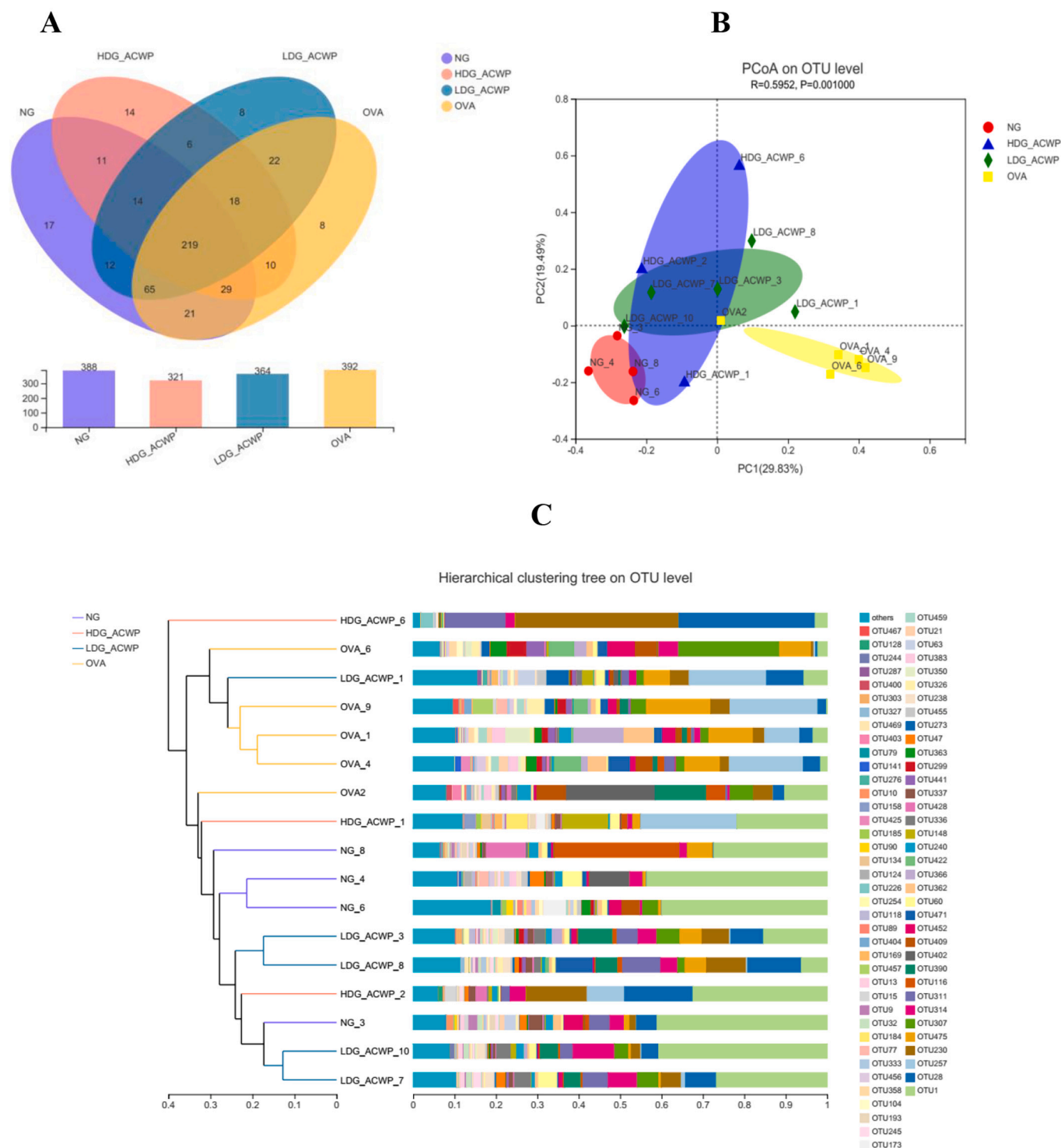


Fig. 6. ACWP improved the overall structure of gut bacteria in mice. (A) Venn diagram based on OTU level; (B) PCoA on OTU level; (C) Sample-level cluster analysis. Compared with the NG group; (D) Genus-level cluster analysis. Compared with the NG group; (E) Species-level cluster analysis. Compared with the NG group, * $P < 0.05$, ** $P < 0.01$; vs OVA group, # $P < 0.05$, ## $P < 0.01$.

flow. The “*de novo*” production of other immunological mediators also occurs following effector cell degranulation, including the platelet activation factor (PAF), leukotrienes, and cytokines like IL-4, IL-5, and others, causing allergic inflammation [23].

Our study discovered that ACWP significantly elevated histamine, IgE, IL-4, IL-22, and IL-17A, slightly reduced IgG2a, significantly decreased IgA and IL-2, and had no effect on IFN-gamma. IgA

downregulation facilitated the breaking of immune tolerance, causing IgE release, which binds to the effector cells and releases histamine and IL4, leading to allergic inflammation. Particularly, IL-17A, an effector factor of Th17 cells, was released abundantly in the early stages of allergic inflammation. IL-17 effectively mediates the excitatory process of neutrophil mobilization, thus controlling the tissue-specific inflammatory response. The Th1-dependent antibody IgG2a was significantly

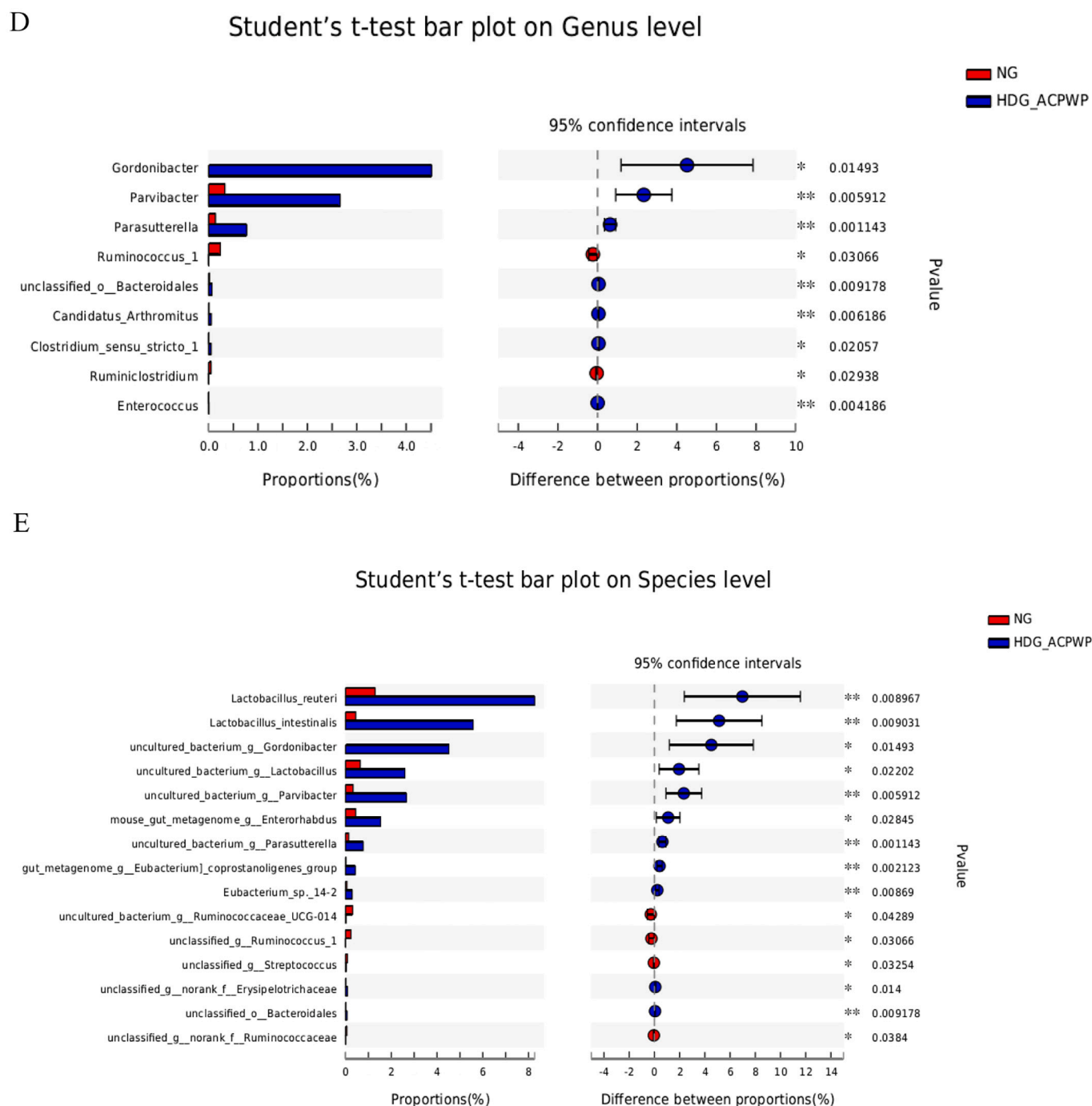


Fig. 6. (continued).

down-regulated in the late stage of allergic inflammation, and the Th1-related factor IL-2. IFN-gamma was not significantly involved. The Th2-related factor IL-4 was significantly up-regulated, depicting disruption in the Th1/Th2 cell balance and the status developed toward Th2-dominated, with a slow and long-term allergic inflammatory response. However, IL-22 was significantly up-regulated to maintain the integrity of the tissue barrier. As a result, the entry of allergens into the systemic circulation was reduced, and severe systemic allergic reactions caused by ACWP were avoided.

Furthermore, ACWP reduced food intake and lung index and elevated the thymus, spleen, and liver indexes ($P < 0.05$). In addition, we also observed the pathological morphology of the thymus, spleen, and small intestine in mice. It was found that ACWP led to a clear

boundary between the thymus cortex and medulla, a significant increase in thymus corpuscle volume and number, causing a close distribution and increasing number of lymphocytes within the medulla. In mice, there was an increase in the area of spleen white pulp, the size and thickness of the periarterial lymphatic sheath, the size and number of splenic nodules, and the number of T lymphocytes, accompanied by the T lymphocyte differentiation. These results depicted that ACWP elevated the immune response regulated by T lymphocytes in the thymus and spleen of mice.

Emerging evidence indicates that the increasing prevalence of food allergies is associated with compositional and functional changes within the gut microbiota. The gut barrier dysfunction can contribute to the development of F.A. Both innate and adaptive immune cells, and

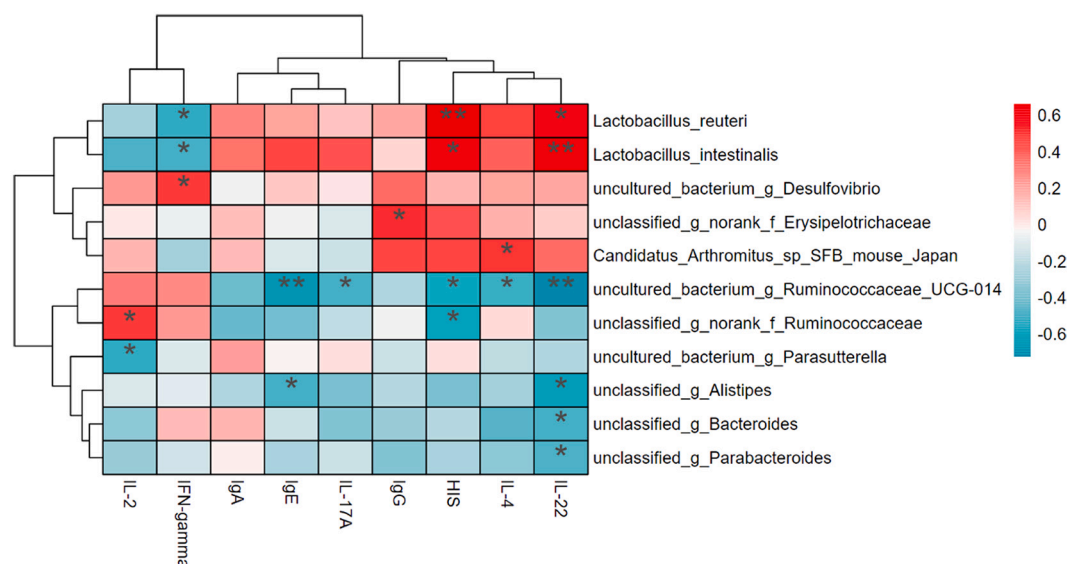


Fig. 7. The correlation between the abundance of the main species of the intestine feces and biochemical indicators in different groups. The bottom is the biochemical indicators, and the right is the flora.

epithelial cells combine to maintain immunological homeostasis at the mucosal barriers. Thus, the role of the microbiota in establishing and maintaining food tolerance is substantially emerging in this complex signaling network [24].

The intestinal microbiota affects the delicate balance between immunological tolerance and allergic sensitization within the gut. The microbiota is crucial in maintaining a tolerance toward food antigens [25]. The microorganisms colonizing the intestine impact the maturation of the immune system and influence food tolerance. Intestinal composition changes are associated with F.A, and a protective effect of probiotics has been demonstrated [26]. Clostridia species derived from the human gut microbiota attenuate allergic responses and inhibit F.A. development by promoting Treg cell generation or inhibiting systemic absorption of major food allergens. The B regulatory cells Breg promotion and tolerance induction by *Clostridium butyricum* in association with immunotherapy has also been described. Allergic infants exhibit a gut microbial community led by the Firmicutes phylum, Ruminococcaceae, and Lachnospiraceae. *Bacteroidales* and *Clostridiales* strains inhibit F.A. by inducing ROR-gammat1 Treg lymphocytes, enhancing IgA, and suppressing GATA-31 Treg cells and IgE. Therefore, certain bacterial strains and the microbial diversity support Treg lymphocyte maturation and facilitate food antigen tolerance. In contrast, antibiotic overuse could destroy the diversity of the microbiota, thereby promoting allergic occurrence [9,24].

Therefore, we investigated the effect of ACWP on intestinal barrier function and observed the pathological sections of the small intestine in mice. It was observed that ACWP would change the small intestine structure in mice. The mucosal layer of the small intestine would be thinned or even destroyed, thus increasing the permeability and easing the entry of foreign substances into the small intestine. With the small intestinal villi being damaged, both the gland of lamina propria and the pan's cells in the small intestinal gland maintaining the intestinal mucosal barrier function were significantly reduced, thereby breaking the intestinal mucosal barrier. The intestinal permeability of mice was detected using the FITC-Dextran tracer method. The results also revealed that ACWP could enhance the intestinal permeability of mice, suggesting that the protein of the wasp's pupa could break the intestinal mucosal barrier by elevating the intestinal mucosal permeability and releasing inflammatory factors causing food allergy.

Based on the α -diversity analysis, SobS, Shannon's, Chao's, and P.D. indexes in the HDG-ACWP group were significantly decreased.

Similarly, the indexes in the LDG-ACWP group were reduced. However, a significant level was not achieved, indicating that ACWP can reduce the microbial richness and diversity in mice. In addition, the microbial richness and diversity become lower with a higher dose.

At the phylum level, ACWP caused food allergy associated with elevated Verrucomicrobia, ran to fied_k_norank_d_bacteria, Cyanobacteria, and decreased Epsilonbacteraeota abundance. Comprehensive analysis of species differences revealed that F.A induced by ACWP was related to the abundance of *Lactobacillus intestinalis*, *Lactobacillus reuteri*, *uncultured_bacterium_g_Desulfovibrio*, *Candidatus_Arthromitus_sp_SFB_mice_Japan*, and *unclassified_g_Streptococcus*. The abundance of nine species reduced significantly, and the correlation was proportional to dosage. The severity of allergy degree depended on the significant change in strain abundance. These results indicated that food allergies induced by ACWP could be closely associated with reduced low-abundance strains.

Spearman correlation analysis revealed that the levels of IL-22, HIS, IgE, IgG, IL-4, and IL-17A were positively associated with *Lactobacillus reuteri*, *Lactobacillus intestinalis*, and *Candidatus_Arthromitus_sp_SFB_mouse_Japan*. On the contrary, the above biochemical parameters were negatively associated with *uncultured_bacterium_g_Ruminococcaceae_UCG-014*, *unclassified_g_norank_f_Erysipelotrichaceae*, *unclassified_g_Parabacteroides*, *unclassified_g_Bacteroides*, and *unclassified_g_Alistipes*. IL-2 levels in *unclassified_g_norank_f_Ruminococcaceae* were positively correlated, whereas the *unclassified_g_Parabacteroides* had a significantly negative correlation. The IFN-gamma levels were positively correlated with *uncultured_bacterium_g_Desulfovibrio*. In contrast, they were significantly negatively correlated with *Lactobacillus reuteri*, and *Lactobacillus intestinalis*.

It was further confirmed that gut microflora could be involved in activating and degranulating host immune effector cells. Moreover, they help differentiate T cells and release cytokines, thus playing an essential role in developing ACWP-induced food allergies. Therefore, the plan is to use metabolomics and microbial diversity association analysis to establish that the gut microbiota is involved in activating and degranulating host immune effector cells.

5. Conclusions

ACWP led to allergic symptoms of skin erythema and diarrhea in mice. ACWP prompted plasma cells to secrete IgE by breaking the

immune tolerance of the body, which combines with mast cells and basophils to degranulate them. Thus, histamine and other substances were released, inducing allergic inflammatory reactions. Further differentiation of T cells toward Th2 broke the Th1/Th2 balance and released corresponding cytokines, including the high expression of IL-17A, IL-4, and IL-22. The factors released by allergic inflammatory reactions could destroy the intestinal barrier function and the small intestinal wall structure, increase intestinal permeability, and contribute to gut microbiota structural changes, thus resulting in intestinal allergic reactions such as diarrhea.

Declaration of Competing Interest

The authors declare no conflicts of interest.

Acknowledgments

The present study was supported by the Project of Yunnan Provincial Science and Technology Department Applied Basic Research grant (No. 202101AT070242, 2021), Yunnan Provincial Science and Technology Department-Applied Basic Research Joint Special Funds of the Yunnan University of Chinese Medicine (No. 202001AZ070001-031, 2020), and the Yunling Mountains Scholars Program (YNWR-YLXZ-2018-026).

References

- [1] C. Ozdemir, U.C. Kucuksezer, M. Akdis, C.A. Akdis, Mechanisms of immunotherapy to wasp and bee venom, *Clin. Exp. Allergy* 41 (2011) 1226–1234.
- [2] C.L. de Souza, J.R.A. Dos Santos-Pinto, F.G. Esteves, A. Perez-Riverol, L.G. R. Fernandes, R. de Lima Zollner, M.S. Palma, Revisiting *Polybia paulista* wasp venom using shotgun proteomics - insights into the N-linked glycosylated venom proteins, *J. Proteome* 200 (2019) 60–73.
- [3] T.K. Kim, H.I. Yong, Y.B. Kim, H.W. Kim, Y.S. Choi, Edible insects as a protein source: a review of public perception, processing technology, and research trends, *Food Sci Anim Resour* 39 (2019) 521–540.
- [4] M.M. Ulaszewska, C.H. Weinert, A. Trimigno, R. Portmann, C. Andres Lacueva, R. Badertscher, L. Brennan, C. Brunius, A. Bub, F. Capozzi, M. Cialliè Rosso, C. E. Cordero, H. Daniel, S. Durand, B. Egert, P.G. Ferrario, E.J.M. Feskens, P. Franceschi, M. Garcia-Aloy, F. Giacomoni, P. Giesbertz, R. González-Domínguez, K. Hanhineva, L.Y. Hemeryck, J. Kopka, S.E. Kulling, R. Llorach, C. Manach, F. Mattivi, C. Migné, L.H. Münger, B. Ott, G. Picone, G. Pimentel, E. Pujos-Guillot, S. Riccadonna, M.J. Rist, C. Rombouts, J. Rubert, T. Skurk, P.S.C. Sri Harsha, L. Van Meulebroek, L. Vanhaecke, R. Vázquez-Fresno, D. Wishart, G. Vergères, *Nutrimetabolomics: an integrative action for metabolomic analyses in human nutritional studies*, *Mol. Nutr. Food Res.* 63 (2019) e1800384.
- [5] B.A. Rumpold, O.K. Schlüter, Nutritional composition and safety aspects of edible insects, *Mol. Nutr. Food Res.* 57 (2013) 802–823.
- [6] Y. Feng, X.M. Chen, M. Zhao, Z. He, L. Sun, C.Y. Wang, W.F. Ding, Edible insects in China: utilization and prospects, *Insect Sci* 25 (2018) 184–198.
- [7] W. Zhao, H.E. Ho, S. Bunyavanich, The gut microbiome in food allergy, *Ann. Allergy Asthma Immunol.* 122 (2019) 276–282.
- [8] A. Abdel-Gadir, E. Stephen-Victor, G.K. Gerber, M. Noval Rivas, S. Wang, H. Harb, L. Wang, N. Li, E. Crestani, S. Spielman, W. Secor, H. Biehl, N. DiBenedetto, X. Dong, D.T. Umetsu, L. Bry, R. Rachid, T.A. Chatila, Microbiota therapy acts via a regulatory T cell MyD88/ROR γ t pathway to suppress food allergy, *Nat. Med.* 25 (2019) 1164–1174.
- [9] S. Bunyavanich, Food allergy: could the gut microbiota hold the key? *Nat. Rev. Gastroenterol. Hepatol.* 16 (2019) 201–202.
- [10] K.V. Barros, V.L. Flor Silveira, M.S. Laranjeira, N.F. Wandalsen, S. Passeti, R. de Oliveira, R.V. Munkata, P.S. Noakes, E.A. Miles, P.C. Calder, Evidence for involvement of IL-9 and IL-22 in Cows' milk allergy in infants, *Nutrients* 9 (2017).
- [11] H.E. Ho, S. Bunyavanich, Microbial adjuncts for food allergen immunotherapy, *Curr Allergy Asthma Rep* 19 (2019) 25.
- [12] Z. Zhang, H. Xiao, P. Zhou, Glycation by saccharides of different molecular sizes affected the allergenicity of shrimp tropomyosin via epitope loss and the generation of advanced glycation end products, *Food Funct.* 10 (2019) 7042–7051.
- [13] Z. Zhang, X.M. Li, H. Xiao, A. Nowak-Węgrzyn, P. Zhou, Insight into the allergenicity of shrimp tropomyosin glycated by functional oligosaccharides containing advanced glycation end products, *Food Chem.* 302 (2020), 125348.
- [14] Y. Li, D. Chen, C. Xu, Q. Zhao, Y. Ma, S. Zhao, C. Chen, Glycolipid metabolism and liver transcriptomic analysis of the therapeutic effects of pressed degreased walnut meal extracts on type 2 diabetes mellitus rats, *Food Funct.* 11 (2020) 5538–5552.
- [15] Wang Xiaoshuang, Chen Dan, Li Yulan, Zhao Shenglan, Chen Chaoyin, Alleviating effects of walnut green husk extract on disorders of lipid levels and gut bacteria flora in high fat diet-induced obesity rats, *J. Funct. Foods* 52 (2019) 576–586.
- [16] F. Zhang, D. Chen, L. Zhang, Q. Zhao, Y. Ma, X. Zhang, S. Zhao, C. Chen, *Diaphragma juglandis* extracts modifies the gut microbiota during prevention of type 2 diabetes in rats, *J. Ethnopharmacol.* 283 (2022), 114484.
- [17] K. Hosoki, A. Chakraborty, S. Sur, Molecular mechanisms and epidemiology of COVID-19 from an allergist's perspective, *J. Allergy Clin. Immunol.* 146 (2020) 285–299.
- [18] A. Hugh Sampson, Utility of food-specific IgE concentrations in predicting symptomatic food allergy, *J. Allergy Clin. Immunol.* 107 (2001) 891–896.
- [19] S. Barni, G. Liccioli, L. Sarti, M. Giovannini, E. Novembre, F. Mori, Immunoglobulin E (IgE)-mediated food allergy in children: epidemiology, pathogenesis, diagnosis, prevention, and management, *Medicina (Kaunas)* 56 (2020).
- [20] W. Yu, D.M.H. Freeland, K.C. Nadeau, Food allergy: immune mechanisms, diagnosis and immunotherapy, *Nat. Rev. Immunol.* 16 (2016) 751–765.
- [21] S. Anvari, J. Miller, C.Y. Yeh, C.M. Davis, IgE-mediated food allergy, *Clin. Rev. Allergy Immunol.* 57 (2019) 244–260.
- [22] S.A. Shu, A.W.T. Yuen, E. Woo, K.H. Chu, H.S. Kwan, G.X. Yang, Y. Yang, P.S. C. Leung, Microbiota and food allergy, *Clin. Rev. Allergy Immunol.* 57 (2019) 83–97.
- [23] M.V. Khodoun, S. Tomar, J.E. Tocker, Y.H. Wang, F.D. Finkelman, Prevention of food allergy development and suppression of established food allergy by neutralization of thymic stromal lymphopoietin, IL-25, and IL-33, *J. Allergy Clin. Immunol.* 141 (2018), 171–179.e171.
- [24] K.N. Jungles, K.M. Jungles, L. Greenfield, M. Mahdavinia, The infant microbiome and its impact on development of food allergy, *Immunol. Allergy Clin. N. Am.* 41 (2021) 285–299.
- [25] L.Z. Xu, R.D. Xie, H. Xie, J.Y. Ju, X.Y. Fu, D.L. Di, M.Y. Peng, W. Gao, Y.Y. Zhang, D. Yu, J.Q. Liu, G. Yang, Z.Q. Liu, Z.G. Liu, P.C. Yang, Chimeric specific antigen epitope-carrying dendritic cells induce interleukin-17(+) regulatory T cells to suppress food allergy, *Clin. Exp. Allergy* 50 (2020) 231–243.
- [26] S. de Gier, K. Verhoeckx, Insect (food) allergy and allergens, *Mol. Immunol.* 100 (2018) 82–106.