

Comparison of Intestinal Immune Homeostasis in Aged Mice Fed Diets Containing Inulin or *Hempseed* Dietary Fiber

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How to cite this paper: Zhu, X.T., Lu, S.S., Huang, Q., Ruan, J.J., Chen, Q.Y., Yao, Y., Xie, J.S. and Huang, L.L. (2026) Comparison of Intestinal Immune Homeostasis in Aged Mice Fed Diets Containing Inulin or *Hempseed* Dietary Fiber. *Journal of Biosciences and Medicines*, 14, 504-515.

<https://doi.org/10.4236/jbm.2026.1410033>

Received: August 4, 2026

Accepted: October 24, 2026

Published: October 27, 2026

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Abstract

Objective: To evaluate the regulatory effect of inulin and *hempseed* dietary fiber on intestinal immune homeostasis in aged mice. **Methods:** In this study, a total of 32 male C57BL/6 mice were utilized, with 8 mice aged 20 weeks designated as the young control (YC) group, and the remaining 24 mice aged 84 weeks were divided into the aged control (AC) group, inulin (AIN) group, and *hempseed* dietary fiber (AHS) group. After a 12-week dietary intervention, the changes in the proportion of CD4⁺ T cell subsets (Th1, Th2, Th17, and Treg) of the colon in aged mice were detected by flow cytometry, and immunohistochemistry was used to detect the changes in colonic inflammatory factors. **Results:** When compared with the YC group, the proportion of Th1 and Th17 cells was found to be significantly decreased in the AC group. The proportion of Th1 and Th17 cells in the AIN and AHS groups was significantly increased compared with the AC group. The Th1/Th2 ratio and the Th17/Treg ratio were found to be significantly decreased in the AC group compared with those of the YC group. When compared with the AC group, the Th1/Th2 ratio was significantly improved in the AIN group but not in the AHS group. In addition, the expression of IL-6 was significantly increased in the AC group compared to the YC group. The expression of IL-6 was found to be decreased in the AIN and AHS groups when compared to the AC group. **Conclusion:** Diets containing inulin and *hempseed* dietary fiber components partially improved age-related alterations in intestinal immune responses in aged mice. These findings suggest that both dietary interventions may have potential for mitigating age-related intestinal immune decline.

Keywords

Aging, Intestinal Immune Homeostasis, Inulin, *Hempseed* Dietary Fiber, Th1/Th2 Ratio, Th17/Treg Ratio

1. Introduction

Studies have shown that the immune function of the intestinal mucosa declines with age [1]. Gut immunosenescence is a hallmark of aging and has become a key biological process affecting the health of the aged. Intestinal immune senescence is a key link in the body's aging, and it coordinates systemic immune dysfunction through three mechanisms: cellular autonomy of mucosal immune efficiency, inflammatory cascade caused by dysregulation, and structural disintegration of the intestinal barrier [2].

Initial CD4⁺ T cells can differentiate into one of the lineages of helper cells (Th1, Th2, and Th17) and Treg cells [3]. Th1 cells mainly secrete interferon- γ (IFN- γ) and tumor necrosis factor- β (TNF- β), which mediate cellular immunity, delayed hypersensitivity reactions, and macrophage activation, while Th2 cells secrete interleukin-4 (IL-4), IL-5, IL-9, and IL-13, which are mainly involved in humoral immunity and allergic reactions [4]. Th17 cells secrete IL-17A, IL-17F, IL-21, and IL-22, stimulating the secretion of pro-inflammatory molecules that are further involved in immunity against bacterial or fungal infections and in the pathogenesis of autoimmune or metabolic diseases [5]. Treg cells are a special CD4⁺T cell lineage that has the function of maintaining immune homeostasis and limiting excessive immune responses [6].

Hempseed (Hemp L.) is a traditional medicinal and food-grade plant, and its seeds are rich in dietary fiber, high-quality protein, and a variety of bioactive components, with good nutritional value and potential health-promoting effects [7]. Studies have shown that *hempseed* and its active ingredients have some effects in improving intestinal barrier function and regulating intestinal inflammation [8]. Inulin is a type of dietary fiber, which is a fructan, mainly derived from plants such as chicory, ginger, garlic, onion, and asparagus [9]. A diet rich in inulin has been reported to improve the function of the intestinal barrier and modulate the immune system [10]. However, there are still few studies on the effects of dietary fiber and inulin on regulating intestinal immune function, and the effect of the two interventions is still unclear. Therefore, we used aged mice as a model to compare the effects of diets containing inulin or *hempseed* fiber on intestinal immune function, focusing on CD4⁺ T-cell subsets and inflammatory cytokine expression.

2. Materials and Methods

2.1. Experimental Animals

Thirty-two male C57BL/6 mice were included in the study, comprising eight 20-

week-old mice assigned to the young control (YC) group and twenty-four 84-week-old mice randomly allocated to the aged control (AC), inulin (AIN), and *hempseed* dietary fiber (AHS) groups ($n = 8/\text{group}$). Before randomization, all animals were screened for general health status, and mice exhibiting external injuries or poor general condition were excluded. Mice within the same experimental group were housed together, with four mice per cage, under identical environmental conditions. The investigators were not blinded to group allocation during the animal experiments. The group size was determined with reference to a previous study using aged C57BL/6 mice with a comparable inulin intervention and intestinal and inflammatory endpoints [11]. All mice were raised under the intelligent central exhaust ventilation cage box system of Youjiang Medical University for Nationalities.

After 1 week of adaptive feeding of all SPF level mice, they were transferred to the intelligent central exhaust ventilation cage system. The mice were freely fed and watered, and the feeding environment was constant temperature ($24^{\circ}\text{C} \pm 2^{\circ}\text{C}$), constant humidity ($50\% \pm 5\%$), and the light/dark period was 12 hours each. Change the litter every 4 days, observe the mental state of the mice every day, divide the cage and add sterile cotton if necessary.

2.2. Intervention

Under identical feeding conditions, the young control (YC) and aged control (AC) groups received an ordinary maintenance feed, while the AIN and AHS groups were fed experimental diets formulated with 20% inulin or 25% *hempseed* fiber ingredient, respectively. The nutritional components of the feed are shown in **Supplementary Table 1**. Compared with the ordinary maintenance feed, the dietary fiber diet containing inulin and *hempseed* fiber has an increased moisture content, decreased crude protein and crude fat content. The calcium content in each group is similar. Since soluble dietary fiber cannot be detected by the method for crude fiber, no significant difference in crude fiber levels was observed. The mice were fed for a total of 12 weeks. All the feeds were customized by Jiangsu Synergy Pharmaceutical Bioengineering Co., Ltd. and tested by Xi'an Guolian Quality Inspection Technology Co., Ltd.

2.3. Indicator Detection

2.3.1. Flow Cytometric Analysis of Colonic CD4⁺ T-Cell Subsets in Mice

Colonic CD4⁺ T-cell subsets were analyzed by flow cytometry. Briefly, the entire colon was collected, washed, minced, and enzymatically digested with pancreatic enzymes and type II collagenase to obtain a single-cell suspension. For Th1, Th2, and Th17 analysis, cells were stimulated with PMA and ionomycin in the presence of a protein transport inhibitor, followed by surface and intracellular staining. For Treg analysis, cells were stained for CD4 and CD25, followed by fixation, permeabilization, and Foxp3 staining. Flow cytometric data were analyzed using a sequential gating strategy. Lymphocytes were first gated based on FSC/SSC characteristics, followed by doublet exclusion using FSC-A versus FSC-H. CD4⁺ T cells

were then identified within the singlet lymphocyte population. Compensation was performed using single-stained controls, and unstained controls were used to assess background autofluorescence. Th1, Th2, and Th17 cells were defined as CD4⁺IFN- γ ⁺, CD4⁺IL-4⁺, and CD4⁺IL-17A⁺ cells, respectively. Tregs were defined as CD4⁺CD25⁺Foxp3⁺ cells. The proportions of each subset were calculated as percentages of CD4⁺ T cells.

2.3.2. Colonic Immune Factor Immunohistochemistry

Mouse colon tissue was collected, fixed with 4% paraformaldehyde, and covered with conventional paraffin. Antigen repair was performed after dewaxing and rehydration, blocking endogenous peroxidase and blocking. IL-6, IL-10, IFN- γ and TNF- α primary antibodies (Beyotime Biotechnology, China) were added and incubated overnight at 4°C, followed by HRP-labeled secondary antibodies (Beyotime Biotechnology, China), DAB chromogenic, and hematoxylin counterstaining. The field of view was randomly selected to take pictures, and the average optical density (MOD) was used to perform semi-quantitative analysis. At least three representative fields were randomly selected for analysis from each mouse, and all mice from each experimental group were included. Image analysis was performed using ImageJ by an investigator blinded to group allocation. The same thresholding criteria were applied consistently to all images, and the average optical density (AOD) was calculated as the integrated density (IntDen) divided by the stained area (AOD = IntDen/Area).

2.4. Statistical Analysis

GraphPad Prism (version 9.5.1) was used for statistical analysis. Data were expressed as mean $\pm$ SEM. Data were assessed for normality and homogeneity of variance before parametric analyses. For comparisons with the aged control (AC) group, one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test was used, with adjustment for multiple comparisons. A prespecified comparison between the AIN and AHS groups was performed using an unpaired two-tailed Student's t-test. When $P < 0.05$ was considered statistically significant. Exact P values are reported where applicable.

3. Results

3.1. Effects of Diets Containing Inulin or Hempseed Fiber on Body Weight and Colonic CD4⁺ T-Cell Subsets in Aged Mice

At the end of the intervention, no significant differences in body weight were observed among the YC, AC, AIN, and AHS groups (**Figure 1**).

Compared with YC group, the proportion of Th1 cells in the AC group showed a downward trend. Compared with the AC group, the proportion of Th1 cells in the AIN group and AHS group was significantly increased. (AIN: $P = 0.0104$; AHS: $P = 0.0475$). There was no statistical difference in Th2 cells between the groups. Compared with YC group, the Th1/Th2 ratio in the AC group was significantly decreased ($P = 0.0191$). Compared with the AC group, the Th1/Th2 ratio was sig-

nificantly increased in the AIN group ($P = 0.0439$), while there was no statistical difference in the AHS group (Figure 2).

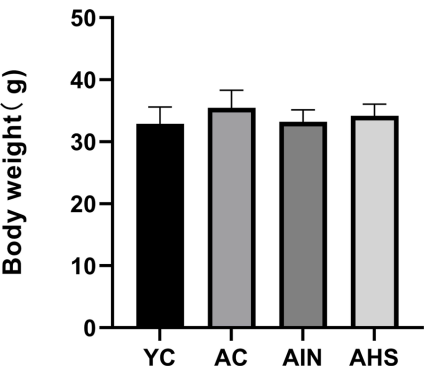


Figure 1. Comparison of body weights among mouse groups after intervention.

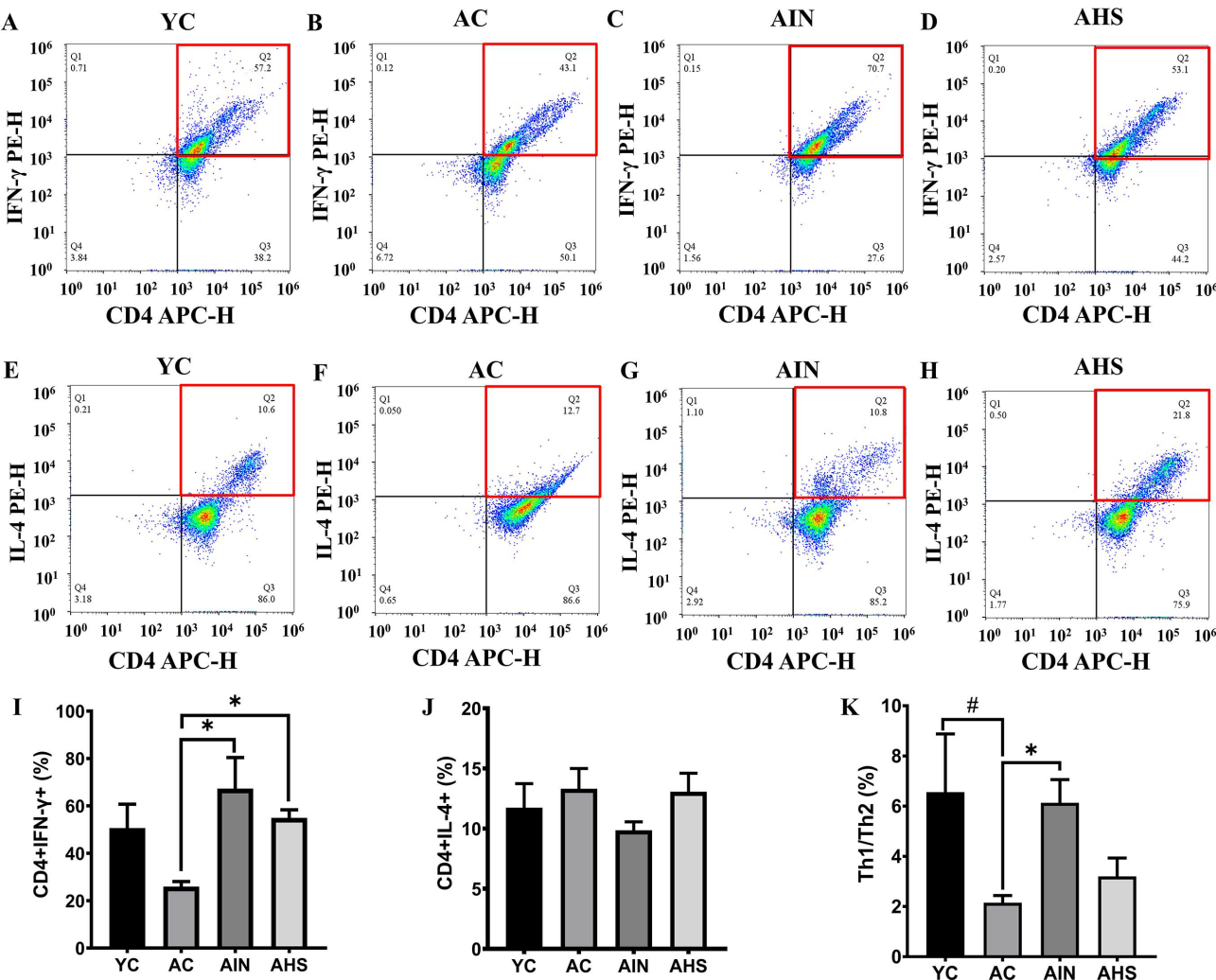


Figure 2. Comparison of Th1, Th2, and Th1/Th2 ratios in colonic tissues of mice in each group. # $P < 0.05$ vs. YC group, * $P < 0.05$ vs. AC group.

3.2. Effects of Diets Containing Inulin or Hempseed Fiber on Intestinal Inflammatory Cytokines in Aged Mice

Compared with YC group, the proportion of Th17 cells in the AC group was significantly reduced ($P = 0.0388$). Compared with AC group, the proportion of Th17 cells in the AIN group and AHS group was significantly increased ($P=0.0396$, $P=0.0303$, respectively). There was no statistically significant difference in the proportion of Treg cells between groups. Compared with YC group, the Th17/Treg ratio in the AC group was significantly reduced ($P = 0.0208$), and there was no significant change after AIN and AHS intervention, as shown in **Figure 3**.

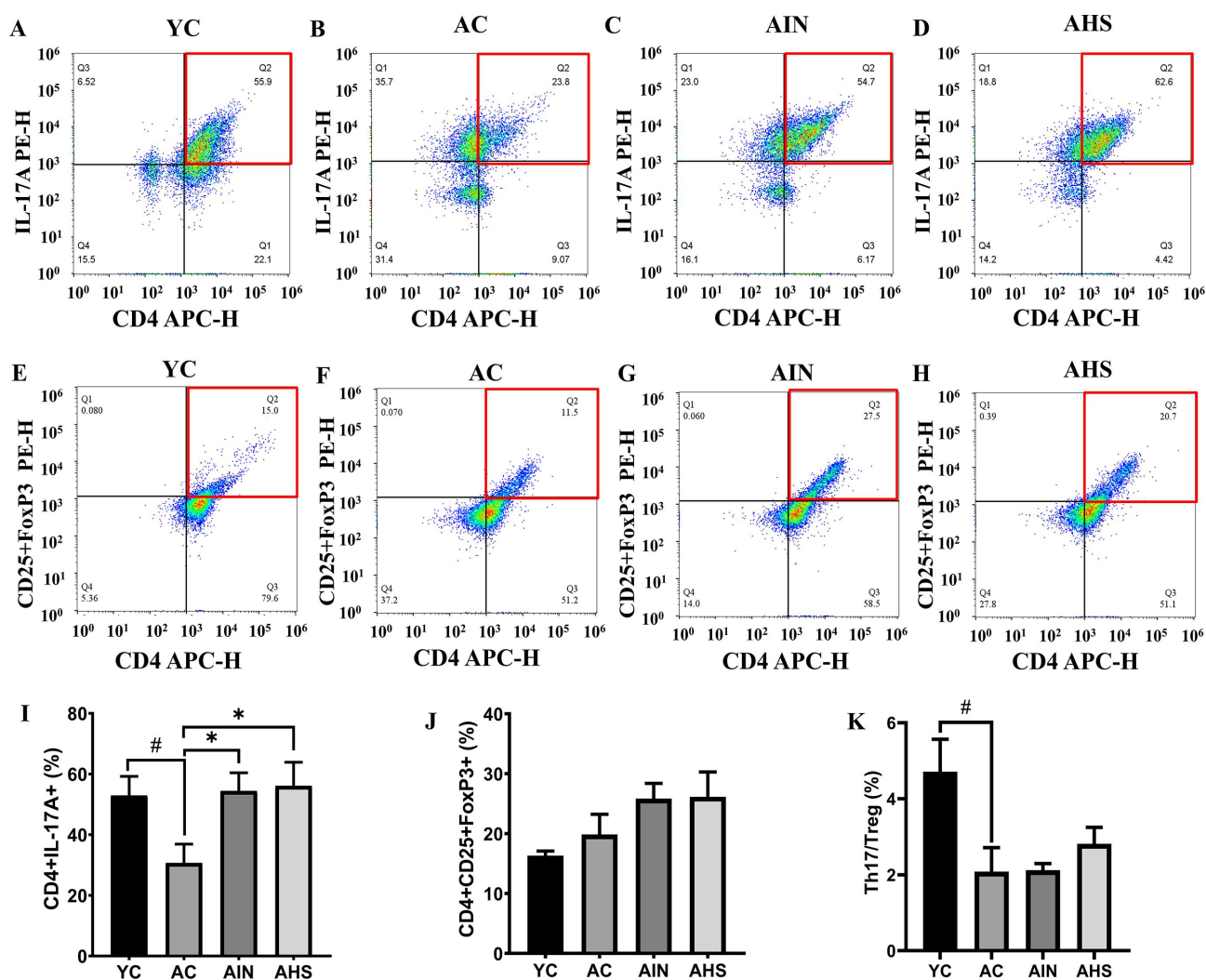


Figure 3. Comparison of Th17, Treg, and Th17/Treg ratios in colonic tissues of mice in each group. # $P < 0.05$ vs. YC group, * $P < 0.05$ vs. AC group.

The results of colonic inflammatory factor immunohistochemistry showed that the expression of IL-6 in the AC group was increased compared with YC group ($P = 0.0151$), and the expression of IL-6 in the AIN group and AHS group decreased compared with the AC group ($P = 0.0269$, $P = 0.0038$, respectively). The IL-10,

IFN- γ and TNF- α were also detected, but there was no significant difference between the groups, as shown in **Figure 4**.

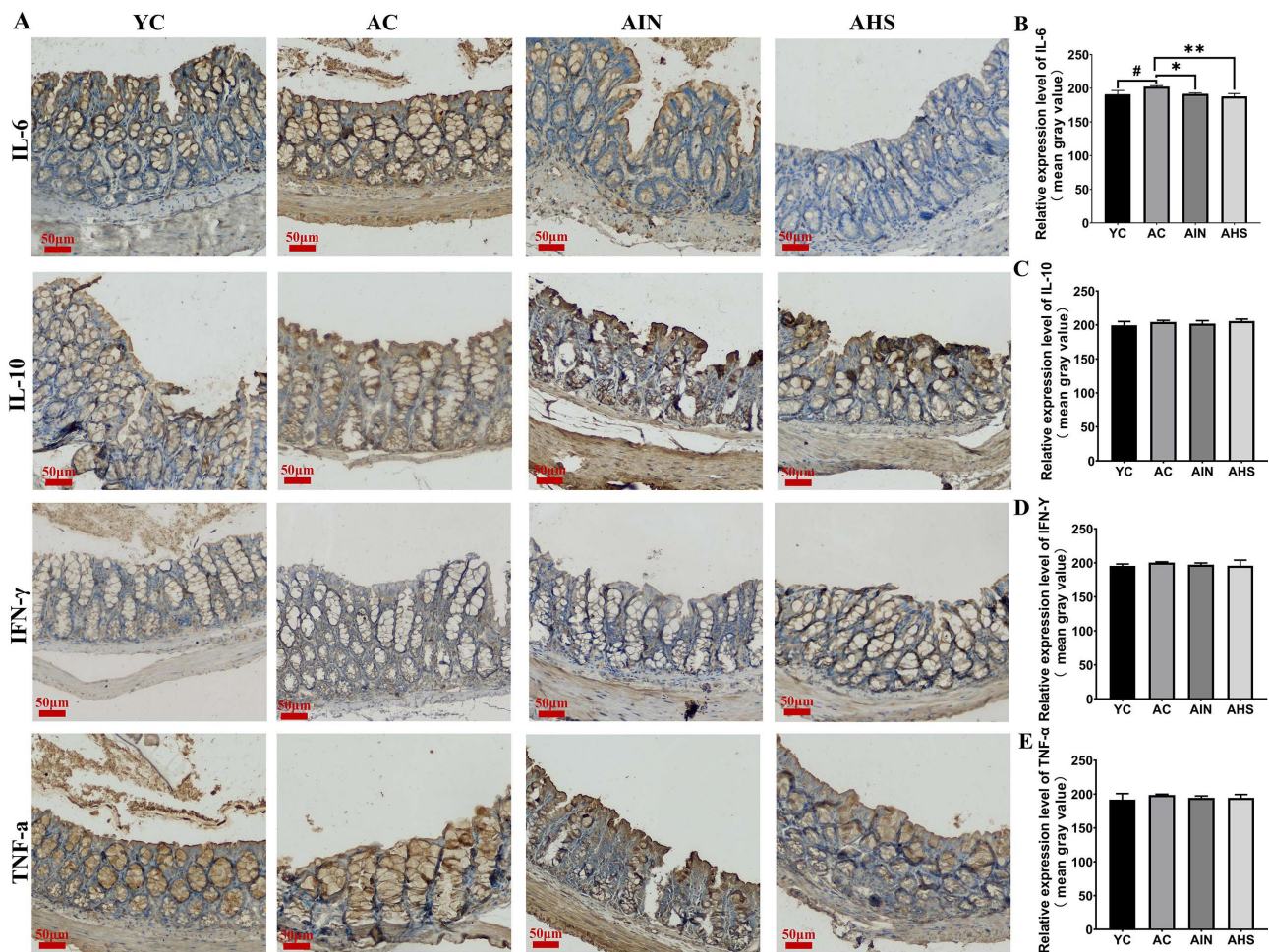


Figure 4. Comparison of IL-6, IL-10, IFN- γ , and TNF- α in colonic tissues of mice in each group. [#]P < 0.05 vs. YC group, ^{*}P < 0.05 vs. AC group, ^{**}P < 0.01 vs. AC group.

4. Discussion

In this study, the regulatory effect of dietary fiber on intestinal immune function in elderly mice from the aspects of CD4⁺ T cell subsets and inflammatory factor levels were evaluated. The results showed that aging led to local immune dysfunction in the colon, which was manifested by a decrease in the proportion of Th1 and Th17 cells, an imbalance of Th1/Th2 and Th17/Treg ratio, and an increase in IL-6 expression.

Interestingly, no significant differences in body weight were observed among the groups at the end of the intervention. This finding suggests that the observed changes in intestinal inflammatory cytokines and CD4⁺ T-cell subsets were not accompanied by detectable changes in overall body weight. Consistent with our findings, a previous study reported that an inulin-based dietary fiber intervention did not significantly affect body weight in aged mice [12].

Our results showed that the proportion of Th17 cells in the elderly group decreased significantly, while the dietary fiber intervention of inulin and *hempseed* recovered significantly. One previous study also report low dietary fiber intake impairs intestinal Th17 cell development in mice, and fiber supplementation can restore T cell development [13]. Consistent with the results of this study, it is suggested that aging may lead to a decrease in Th17 cell development or recruitment ability, and supplementation with *hempseed* dietary fiber and inulin may improve the intestinal microenvironment by regulating the intestinal flora and its metabolites, thereby promoting the differentiation of Th17 cells or the restoration of recruitment ability. Although there was no statistical difference in the proportion of Treg cells between the groups, and there was no significant change after AIN and AHS intervention, Th17 cell recovery could still indicate an improvement in intestinal immune function in aged mice. The Th17/Treg ratio in the AC group decreased significantly. This result is consistent with previous studies that show the ratio of effector T cells to regulatory T cells changes during aging, leading to a decrease in immune response capacity [14]. There was no significant change in the Th17/Treg ratio after AIN and AHS intervention, suggesting that dietary fiber could play an immunomodulatory role by maintaining the balance between Th17 and Treg and avoiding the risk of excessive immune activation.

Th1 cells are responsible for driving cytotoxic effector mechanisms, and Th2 cells drive humoral immunomodulation [15]. Among them, the lineage-specific cytokines responsible for driving Th1 and Th2 differentiation are interferons γ /IL-12 and IL-4, respectively, which exert antagonistic effects [16]. Studies have found that restoring Th1/Th2 balance can regulate the intestinal mucosa and the systemic immune system [17]. We found that the proportion of Th1 cells in the elderly group showed a downward trend, and the proportion of Th2 cells did not change between groups. It suggests that aging has a more significant effect on Th1-related cellular immune function, while the effect on Th2-mediated humoral immunity is relatively limited. Previous studies have shown that there is a significant shift in helper T cell immunity during aging, manifested by a decrease in Th1-related cytokines (e.g., IFN- γ) and an increase in Th2-related cytokines (e.g., IL-4 and IL-5), resulting in an imbalance of Th1/Th2 imbalance. In this study, a decrease in the proportion of Th1 cells and the Th1/Th2 ratio in the AC group was observed, which is consistent with the above immunosenescence characteristics [18].

The proportion of Th1 cells was significantly increased in both the AIN and AHS groups, indicating that both dietary interventions may partially improve the age-related decline in cellular immune function. The Th1/Th2 ratio was significantly increased in the AIN group, whereas no significant change was observed in the AHS group. However, these findings alone do not establish a difference between the two diets.

Aging is accompanied by a chronic low-grade inflammatory state, manifested by increased expression of pro-inflammatory factors such as IL-6 [19]. Previous

studies have shown that IL-6 can hinder Th1 cell differentiation and reduce Th1 cell function by inhibiting the IFN- γ signaling pathway [20]. In this study, the increase in IL-6 expression in the elderly group was accompanied by a decrease in the proportion of Th1 cells, which further supports the important role of IL-6-mediated abnormal T cell differentiation in intestinal immunosenescence. The combination of inulin with other prebiotics has been found to be effective in treating intestinal inflammation [21]. These findings suggest that diets containing inulin or *hempseed* fiber may exert immunomodulatory effects by modulating intestinal inflammatory responses and CD4⁺ T-cell profiles.

It is worth noting that the two dietary interventions show certain differences in immunomodulation. This study showed that both interventions could increase the ratio of Th1 and Th17 cells and reduce IL-6 expression, but only the AIN intervention significantly improved the Th1/Th2 ratio. These findings suggest that the two dietary interventions may differentially affect specific aspects of intestinal immune responses. Previous studies have suggested that inulin may influence T-cell differentiation through modulation of the gut microbiota and its metabolites [22]. Whereas *hempseed* may exert immunomodulatory effects through anti-inflammatory pathways [23].

Studies have shown that maintaining a balanced diet and intake of dietary components can support healthy aging by reducing inflammation, improving the gut microbiome, and overall immune function. Choosing the right dietary fiber and developing personalized nutrition strategies to improve health outcomes and ultimately extend healthy life expectancy in the elderly population is crucial. Dietary fiber from inulin and *hempseed* can improve intestinal immune aging and reduce inflammation, so reasonable intake of dietary fiber may become an important nutritional intervention strategy to delay intestinal immune aging and improve the health status of the elderly.

This study has several limitations. Intestinal immune function was assessed primarily through CD4⁺ T-cell subsets and inflammatory cytokines, without investigating potential mechanisms involving the gut microbiota or microbial metabolites. Moreover, dietary energy density was not systematically determined, and daily food intake was not recorded during the intervention, so potential differences in energy intake among the dietary groups cannot be completely excluded. Further studies are needed to determine whether these findings are applicable to humans.

5. Conclusion

Aging disrupted colonic immunity in mice, as evidenced by reduced Th17 cells and a lower Th17/Treg ratio, a Th1/Th2 imbalance, and elevated IL-6. Inulin and *hempseed* dietary fiber partially reversed these alterations by increasing Th1 and Th17 proportions and reducing IL-6 expression. These results indicate that both dietary interventions may help mitigate age-related intestinal immune decline.

Acknowledgements

This research was supported by the Guangxi Natural Science Foundation under Grant No.2025JJH140107 (L.H.); The 2023 Baise City Scientific Research and Technological Development Program Project (BAIKE 20233650) (L.H.); Open Competition Project of Guangxi Plateau Discipline (2026), Youjiang Medical University for Nationalities (No.YY2026GY38) (J.X.); National College Students' Innovative Entrepreneurial Training Plan Program (No.202310599006) (S.L.); Guangxi College Students' Innovative Entrepreneurial Training Plan Program (No.S202310599057) (Q.H.)

Author Contributions

X.Z.: Design of the work, Acquisition, Analysis, Interpretation of data, Draft the manuscript; S.L., Q.H. and J.R.: Sample collection, Experimental operation; Y.Y. and Q.C.: Animal experimentation, Sample collection; J.X.: Interpretation of data, Revise the manuscript; L.H.: Conception, Design of the work, Interpretation of data, Revise the manuscript, Final approval of the manuscript to be published.

Statement

This study was approved by the animal ethics review of the Experimental Animal Ethics Committee of Youjiang Medical University for Nationalities (Approval No.: 2024032901).

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

- [1] Zheng, H., Zhang, C., Wang, Q., Feng, S., Fang, Y. and Zhang, S. (2022) The Impact of Aging on Intestinal Mucosal Immune Function and Clinical Applications. *Frontiers in Immunology*, **13**, Article ID: 1029948. <https://doi.org/10.3389/fimmu.2022.1029948>
- [2] Shen, X., Gao, X. and Wang, L. (2026) Intestinal Aging-Related Immune Dysfunction: Mechanisms and Interventions. *Acta Biochimica et Biophysica Sinica*, **58**, 183-200. <https://doi.org/10.3724/abbs.2025157>
- [3] Bensussen, A., Torres-Magallanes, J.A., Álvarez-Buylla, E.R. and de Álvarez-Buylla, E.R. (2024) Hybrid Lineages of CD4⁺ T Cells: A Handbook Update. *Frontiers in Immunology*, **15**, Article ID: 1344078. <https://doi.org/10.3389/fimmu.2024.1344078>
- [4] Liu, W., Fan, M., Lu, W., Zhu, W., Meng, L. and Lu, S. (2022) Emerging Roles of T Helper Cells in Non-Infectious Neuroinflammation: Savior or Sinner. *Frontiers in Immunology*, **13**, Article ID: 872167. <https://doi.org/10.3389/fimmu.2022.872167>
- [5] Mills, K.H.G. (2023) IL-17 and IL-17-Producing Cells in Protection versus Pathology. *Nature Reviews Immunology*, **23**, 38-54. <https://doi.org/10.1038/s41577-022-00746-9>
- [6] Grover, P., Goel, P.N. and Greene, M.I. (2021) Regulatory T Cells: Regulation of Identi-

- tity and Function. *Frontiers in Immunology*, **12**, Article ID: 750542. <https://doi.org/10.3389/fimmu.2021.750542>
- [7] Tănase Apetroaei, V., Pricop, E.M., Istrati, D.I. and Vizireanu, C. (2024) Hemp Seeds (*Cannabis sativa* L.) as a Valuable Source of Natural Ingredients for Functional Foods—A Review. *Molecules*, **29**, Article No. 2097. <https://doi.org/10.3390/molecules29092097>
- [8] Cocetta, V., Governa, P., Borgonetti, V., Tinazzi, M., Peron, G., Catanzaro, D., *et al.* (2021) Cannabidiol Isolated from *Cannabis sativa* L. Protects Intestinal Barrier from *in Vitro* Inflammation and Oxidative Stress. *Frontiers in Pharmacology*, **12**, Article ID: 641210. <https://doi.org/10.3389/fphar.2021.641210>
- [9] Hughes, R.L., Alvarado, D.A., Swanson, K.S. and Holscher, H.D. (2022) The Prebiotic Potential of Inulin-Type Fructans: A Systematic Review. *Advances in Nutrition*, **13**, 492-529. <https://doi.org/10.1093/advances/nmab119>
- [10] Yuan, C., Wang, S., Gebeyew, K., Yang, X., Tang, S., Zhou, C., *et al.* (2023) A Low-Carbon High Inulin Diet Improves Intestinal Mucosal Barrier Function and Immunity against Infectious Diseases in Goats. *Frontiers in Veterinary Science*, **9**, Article ID: 1098651. <https://doi.org/10.3389/fvets.2022.1098651>
- [11] Hutchinson, N.T., Wang, S.S., Rund, L.A., Caetano-Silva, M.E., Allen, J.M., Johnson, R.W., *et al.* (2023) Effects of an Inulin Fiber Diet on the Gut Microbiome, Colon, and Inflammatory Biomarkers in Aged Mice. *Experimental Gerontology*, **176**, Article ID: 112164. <https://doi.org/10.1016/j.exger.2023.112164>
- [12] Vailati-Riboni, M., Rund, L., Caetano-Silva, M.E., Hutchinson, N.T., Wang, S.S., Soto-Díaz, K., *et al.* (2022) Dietary Fiber as a Counterbalance to Age-Related Microglial Cell Dysfunction. *Frontiers in Nutrition*, **9**, Article ID: 835824. <https://doi.org/10.3389/fnut.2022.835824>
- [13] Royer, C.J., Rodriguez-Marino, N., Yaceczko, M.D., Rivera-Rodriguez, D.E., Ziegler, T.R. and Cervantes-Barragan, L. (2023) Low Dietary Fiber Intake Impairs Small Intestinal Th17 and Intraepithelial T Cell Development over Generations. *Cell Reports*, **42**, Article ID: 113140. <https://doi.org/10.1016/j.celrep.2023.113140>
- [14] Elyahu, Y., Hekselman, I., Eizenberg-Magar, I., Berner, O., Strominger, I., Schiller, M., *et al.* (2019) Aging Promotes Reorganization of the CD4 T Cell Landscape toward Extreme Regulatory and Effector Phenotypes. *Science Advances*, **5**, eaaw8330. <https://doi.org/10.1126/sciadv.aaw8330>
- [15] Verma, S.K., Ana-Sosa-Batiz, F., Timis, J., Shafee, N., Maule, E., Pinto, P.B.A., *et al.* (2024) Influence of Th1 versus Th2 Immune Bias on Viral, Pathological, and Immunological Dynamics in SARS-CoV-2 Variant-Infected Human ACE2 Knock-In Mice. *eBioMedicine*, **108**, Article ID: 105361. <https://doi.org/10.1016/j.ebiom.2024.105361>
- [16] Rogozynski, N.P. and Dixon, B. (2024) The Th1/Th2 Paradigm: A Misrepresentation of Helper T Cell Plasticity. *Immunology Letters*, **268**, Article ID: 106870. <https://doi.org/10.1016/j.imlet.2024.106870>
- [17] Liu, Z., Wu, S., Zhang, W., Cui, H., Zhang, J., Yin, X., *et al.* (2024) Cordycepin Mitigates Dextran Sulfate Sodium-Induced Colitis through Improving Gut Microbiota Composition and Modulating Th1/Th2 and Th17/Treg Balance. *Biomedicine & Pharmacotherapy*, **180**, Article ID: 117394. <https://doi.org/10.1016/j.biopha.2024.117394>
- [18] Park, H. and Jo, S. (2011) Lasting Effects of an Impairment of Th1-Like Immune Response in γ -Irradiated Mice: A Resemblance between Irradiated Mice and Aged Mice. *Cellular Immunology*, **267**, 1-8. <https://doi.org/10.1016/j.cellimm.2010.10.004>

- [19] Babakhani, K., Kucinskas, A.L., Ye, X., Giles, E.D. and Sun, Y. (2025) Aging Immunity: Unraveling the Complex Nexus of Diet, Gut Microbiome, and Immune Function. *Immunometabolism*, **7**, e00061. <https://doi.org/10.1097/in9.0000000000000061>
- [20] Diehl, S., Anguita, J., Hoffmeyer, A., Zapton, T., Ihle, J.N., Fikrig, E., *et al.* (2000) Inhibition of Th1 Differentiation by IL-6 Is Mediated by SOCS1. *Immunity*, **13**, 805-815. [https://doi.org/10.1016/s1074-7613\(00\)00078-9](https://doi.org/10.1016/s1074-7613(00)00078-9)
- [21] Limketkai, B.N., Godoy-Brewer, G., Shah, N.D., Maas, L., White, J., Parian, A.M., *et al.* (2025) Prebiotics for Induction and Maintenance of Remission in Inflammatory Bowel Disease: Systematic Review and Meta-Analysis. *Inflammatory Bowel Diseases*, **31**, 1220-1230. <https://doi.org/10.1093/ibd/izae115>
- [22] Tawfick, M.M., Xie, H., Zhao, C., Shao, P. and Farag, M.A. (2022) Inulin Fructans in Diet: Role in Gut Homeostasis, Immunity, Health Outcomes and Potential Therapeutics. *International Journal of Biological Macromolecules*, **208**, 948-961. <https://doi.org/10.1016/j.ijbiomac.2022.03.218>
- [23] Sławińska, N. and Olas, B. (2023) Selected Seeds as Sources of Bioactive Compounds with Diverse Biological Activities. *Nutrients*, **15**, Article No. 187. <https://doi.org/10.3390/nu15010187>

Supplementary Table S1. Nutritional composition of the experimental diets.

Nutritional parameter	Ordinary maintenance feed	Inulin dietary fiber feed	Hempseed dietary fiber feed
Moisture (%)	8.50	10.80	11.80
Crude protein (%)	18.78	15.68	14.77
Crude fat (%)	4.40	3.20	2.40
Crude fiber (%)	3.90	3.10	3.20
Crude ash (%)	6.10	5.70	5.50
Calcium (%)	1.19	1.20	1.31
Total phosphorus (%)	0.85	0.83	0.75

The inspection report was issued by Xi'an Guo Lian Quality Detection Technology Co., Ltd. This table only presents the conventional feed nutritional components, and does not separately measure the total dietary fiber and soluble dietary fiber content.