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The role of *Lactobacterium plantarum* in solid-state fermentation of *Astragalus membranaceus* for broiler chicken feed

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Abstract
The purpose of this study was to examine the fermentation of *A. membranaceus* by *L. plantarum* and its effects on broiler chickens. *L. plantarum* solid-state fermentation was employed to solve the problem of releasing the active components of *A. membranaceus*. The effect of *L. plantarum* on astragalus was examined by measuring the contents of protein, carbohydrate, calycoflavone, and formononetin in *A. membranaceus* before and after fermentation. The effect of *A. membranaceus* on *L. plantarum* was demonstrated by detecting the changes in galactosidase in *L. plantarum* before and after fermentation. Fermented *A. membranaceus* was fed to broiler chickens, and the indices of growth performance, antioxidants, immune function, and intestinal short-chain fatty acids were recorded. The results showed that the cellulose and pectin on the surface of *A. membranaceus* were decomposed by *L. plantarum* solid-state fermentation, and the macromolecular proteins were degraded into small molecules. *A. membranaceus* increased the content of galactosidase in *L. plantarum*. Solid-state fermentation increased the contents of functional sugars, calycosin, and formononetin in *A. membranaceus*. Fermentation of *A. membranaceus* significantly improved the production performance of broilers as well as the antioxidant index, immune index, intestinal morphology, and intestinal short-chain fatty acid content.

Keywords *Lactobacillus plantarum*, *Astragalus membranaceus*, Solid-state fermentation, Mechanism of interaction, Broiler chickens

Introduction

In 2018, the Ministry of Agriculture and Rural Affairs of China announced that pharmaceutical feed additives would be withdrawn from the livestock industry by 2020. In 2022, the Ministry of Agriculture acted to reduce the use of antibacterial drugs in domestic animals. These policies demonstrated China's determination to curtail the use of antibiotics in animal husbandry. Considering the growing problem of antibiotic resistance, it has become important to search for new, safe, green feed additives in animal husbandry. Under such circumstances, microbial fermentation, traditional Chinese medicine (TCM), and environmental protection have attracted interest

Presided over Henan Province Natural Science Foundation Projects such as 4, 5 authorized National Invention Patents, has published more than 40, SCI article 4, was awarded the first prize for departmental level scientific and technological achievements shall be four, editor in chief of seven works and main fermentation Chinese medicines and animal disease prevention and control.

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in animal husbandry and aquaculture, each with broad application prospects.

Microbial fermentation has a long history. Six thousand years ago, the earliest application of microbial fermentation in China involved wine koji; in the present day, koji is yeast-based. In the Han and Jin Dynasties, fermentation was applied to traditional Chinese medicine to produce medicinal qu, and fermentation became one of the common means of processing TCM. Fermented TCM processing technology uses microorganisms under appropriate inoculum amount, temperature, and other conditions so that secondary metabolites can be produced by the interaction between TCM and microorganisms during the process of fermentation (Yong et al. 2005). Since the composition and properties of TCM vary, the properties and efficacy of TCM can be altered by fermentation, and thus different products can be produced by microbial fermentation technology. This technology has several advantages; it does not require high temperatures; it is convenient to operate, and new metabolites can be produced during the fermentation process. Compared with traditional methods such as cooking and alcohol extraction, microbial fermentation is rapid, effective, and cost-effective. Therefore, fermented processed products are increasingly being used in TCM preparations (Aiello et al. 2023) At present, there are three forms of microbial fermentation technology: solid fermentation, liquid fermentation, and two-way solid fermentation (Li et al. 2009). Solid-state fermentation has a long history as the earliest fermentation technology. Its advantage compared with other fermentation technologies lies in its simple and convenient operation. However, it also has several limitations: under natural conditions, the fermentation rate is often relatively slow, and the evaluation criteria have no scientific basis, making it difficult to realize large-scale production (Xiaoxia et al. 2014). Liquid fermentation has the advantages of a short fermentation time, easy control of the fermentation conditions, and low production cost, and thus it has wide application prospects in practical production. However, liquid fermentation requires strict fermentation conditions; the fermentation process is difficult, and improper operation may easily cause pollution. In addition, gas is generated during the fermentation process due to the metabolism of bacteria, resulting in the phenomenon of bottle expansion, which is not conducive to transportation and preservation and thus affects clinical application (Hyun et al. 2013). Solid-state fermentation has many advantages, including a simple fermentation process, difficulty of contamination, easy long-term storage, and convenient transportation; as a result, it is more suitable for clinical application research than liquid fermentation.

Astragalus membranaceus has a long history of application in China. It was one of the earliest ingredients used in TCM for reinforcing qi. *A. membranaceus* is a perennial grass that is rich in flavonoids, saponins, proteins, amino acids, vitamins, and other nutrients, and it has long been used in China, Southeast Asia, and other countries for the treatment of diseases in humans and animals (Kallon et al. 2013). However, because the cell walls of *A. membranaceus* are composed of cellulose and pectin, it is difficult to extract the active compounds; therefore, the utilization rate of *A. membranaceus* is low, and it is difficult to widely promote its application. At present, there are several technologies for extracting the active ingredients of *A. membranaceus*, but these are not sufficiently cost-effective. Microbial fermentation technology is one possible way to solve this problem (Chen et al. 2008). Microbial decomposition in the process of fermentation produces a variety of enzymes that can effectively degrade the cell wall barrier, thereby increasing the extraction rate of the active compounds. Therefore, fermented processing technology may be a solution to the problem.

Lactobacillus plantarum is a species of lactic acid bacteria that is widely used in the fermentation of dairy products and vegetables. Recent research has shown that *L. plantarum* plays important roles in bowel health, metabolic disorders, and brain health (Xiao et al. 2004). *L. plantarum* is also widely used in the livestock and poultry industries. *L. plantarum* is a safe and multifunctional probiotic, and it can be used as a bacterial strain to prepare fermentation products (Ghabeshi et al. 2023).

In this study, we explored the mechanism of *L. plantarum* fermentation of *A. membranaceus* and added the fermented *A. membranaceus* to the feed of broilers to study their changes in growth performance, antioxidant indexes, immune indexes, and intestinal short-chain fatty acid content to demonstrate the value of *L. plantarum* fermentation of *A. membranaceus* in production (Li et al. 2009).

Materials and methods

Materials

A. membranaceus

A. membranaceus sections for use in decoctions were purchased from the Chinese and Western Medicine Procurement and Supply Station (C318210007) in Yuzhou City, Henan Province. After drying at 60 °C, the sections were crushed through an 80-mesh sieve and stored in a cool and ventilated place for later use.

A. membranaceus was fermented using *L. plantarum* (CGMCC1.557) prepared by Henan University's Laboratory for the Microbial Biotransformation of Traditional Chinese Medicine at the Henan Institute of Animal Husbandry and Economics. The kits used for the antioxidant and immune assays were purchased from Shanghai Yisheng Biological Co., Ltd.

Bacteria

L. plantarum was provided by Henan University's Laboratory for the Microbial Biotransformation of Traditional Chinese Medicine at the Henan Institute of Animal Husbandry and Economics.

Experimental methods

Activation of culture

The *L. plantarum* bacteria stored at – 80° were thawed and then placed in an incubator at 37 °C for 30 min for pre-activation. After recovery, 1 mL of the bacterial solution was added to 100 mL of MRS broth medium under aseptic conditions. After inoculation, the bacterial solution was incubated at a constant temperature of 37 °C for 24 h.

Solid-state fermentation of A. membranaceus

The activated *L. plantarum* solution was evenly mixed with *A. membranaceus* powder in a ratio of 1:1. The mixture was evenly packed into a fermentation bag with a vent valve and cultured in a constant-temperature incubator at 37 °C for 7 days. Multipoint sampling was carried out on a super-clean workbench at 3, 5, and 7 days after fermentation, and the samples were stored at – 20 °C for later use.

Scanning electron microscopy (SEM)

Ten grams of *A. membranaceus* sampled before fermentation and at 3 and 7 days after fermentation was placed in self-sealing bags in an ice box and sent to Zhenjiang Zuobo Detection Technology Co., Ltd., Jiangsu Province, for electron microscopy. The samples were examined under 1000× and 4000× magnification. Micrographs were taken in a high-vacuum mode at 25 kV.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

Proteins were obtained from *A. membranaceus* before fermentation and at 0, 3, 5, and 7 days after fermentation using protein extraction kits. The extracted proteins were mixed with 5× loading buffer at a ratio of 1:4. The diluted sample was denatured at 100 °C in boiling water for 10 min, and then subjected to SDS-PAGE under 150 V. The cells were then stained with Coomassie Brilliant Blue R-250 for 45 min. After staining, the cells were decolorized with methanol + acetic acid and imaged by a gel imager.

Fluorescence quantitative PCR

Primer synthesis

The information for the α-galactosidase gene (AF1897659) and β-galactosidase gene (MN188052) in the GenBank database was used to design primers by Primer5, and the primer specificity was tested by Primer-BLAST. The primers were synthesized by Shanghai Sangon Biological Company.

Total RNA isolation

Before fermentation and at 1, 3, 5, and 7 days after fermentation, RNA of *A. membranaceus* was extracted using a plant nucleic acid extraction kit, and the concentration and purity of RNA were measured by a NanoDrop2000 spectrophotometer (USA). The optical density (OD) value (260/280) was normalized to the range of 1.8–2.0.

cDNA synthesis by reverse transcription

RNA was reverse-transcribed into cDNA (20 μL) using a reverse-transcription kit. The reaction volume contained 3 μL of template RNA, 4 μL of 4× gDNA wiper mix, and 9 μL of double-distilled water (ddH_2O). The PCR reaction program was run for 2 min at 42 °C. After the reaction, a 5× HiScript II qRT SuperMix II 4 μL PCR reverse-transcription program was run (50 °C for 15 min and 85 °C for 5 s). The cDNA products were diluted fivefold with RNANase-Free ddH2O and stored at – 20 °C.

The reaction system (20 μL) of fluorescent quantitative PCR contained the following components: primer 1 (10 μm), 0.4 μL; primer 2 (10 μm), 0.4 μL; template cDNA, 3 mu L; 2xTaq Pro Universal SYBR Master Mix, 10 μL, and ddH_2O, 6.2 μL. The reaction conditions were as follows: 2 min at 95 °C for denaturation, 20 s at 95 °C for annealing, 20 s at 60 °C for further annealing, and 20 s at 72 °C. Forty reaction cycles were carried out. The cDNA samples of each experimental group were used as the reaction templates, and the mRNA expression of β-galactosidase and α-galactosidase genes was measured by real-time quantitative PCR. There were three replicates for each group.

After the reaction, the specificity of the product was verified using a dissolution curve. If the dissolution curve had a single peak and a good concurrence, there was only one product, making the reaction effect specific. GAPDH and 16 s were used as the internal controls, and the relative gene expression was analyzed by the comparative cycle threshold ($2^{-\Delta\Delta Ct}$) method.

Analysis of sugar metabolomics in astragalus before and after fermentation

Samples of *A. membranaceus* before fermentation and at 0, 3, 5, and 7 days after fermentation were collected and stored at 4 °C. Fifty milligrams of the preserved *A. membranaceus* sample powder was weighed and added with 200 μL of isopropanol, methanol, and water (3:3:2 V/V/V), vortexed for 5 min, ultrasonically fractured on ice for 20 s, and ultrasonically centrifuged for 5 min. Centrifugation was carried out at 15,000 r/min and 4 °C; after centrifugation, 50 μL of the supernatant

was absorbed and 20 μL of ribosol internal standard solution (100 μg/mL) was added, and the mixture was freeze-dried in a lyophilizer after nitrogen drying. Then, 100 mL of methoxyammonium pyridine (15 mg/mL) was added and incubated in a metal bath at 37 °C for 1 h. Subsequently, 150 μL of trichloroacetamide (BSTFA) was added, and the sample was incubated at 37 °C for 60 min to obtain the derivatization solution. Fifty microliters of the derivatization solution was taken, diluted to 1 mL with hexane, and stored in a brown injection bottle in the dark for gas chromatography-mass spectrometry (GC–MS) analysis. The GC–MS analysis was performed by Nanjing Paisen Biotechnology Co., Ltd.

The changes in effective substances of *Astragalus* before and after fermentation were determined by high-performance liquid chromatography (HPLC)

(1) Standard treatment: 10 mg of calycosin and formononetin standard was diluted to 0.1 mg/mL with liquid-grade methanol. Calycosin standard stock solution (0.5, 1, 1.5, 2.0, 2.5, and 3 mL) was added to 10-mL volumetric flasks for use; 1, 1.5, 2, 2.5, 3, and 3.5 mL of formononetin were placed into 10-mL volumetric flasks for later use.
(2) Sample processing: A total of 1 g of *A. membranaceus* before fermentation and 1 g of *A. membranaceus* at 3, 5, and 7 days after fermentation were weighed, added with 100 mL of 50% methanol, and subjected to reflux extraction for 2 h. At the end of the extraction, the sample was filtered through a 0.4-mm filter membrane and concentrated to dryness. Pure methanol was added to a fixed volume of 10 mL to obtain the sample solution.
(3) Standard treatment: 10 mg calycosin and formononetin standard was diluted to 0.1 mg/mL with liquid-grade methanol. Calycosin standard stock solution (0.5, 1, 1.5, 2.0, 2.5, and 3 mL) was placed into 10-mL volumetric flasks for use; 1, 1.5, 2, 2.5, 3, and 3.5 mL formononetin was placed into 10-mL volumetric flasks for later use. The above solutions were injected according to the conditions in (1), and the data were recorded. Calycosin standard curves were plotted by taking the concentration of the standard solution as the abscissa and the peak area as the ordinate; the linear equation was $y = 411.551\ x + 23.0543$ ($R^2 = 0.9914$). The regression equation for the formononetin standard solution was $y = 477.4959x + 57.0753$ ($R^2 = 0.9978$). There was a good linear relationship between the calycosin reference and peak area in the range of 0.5–2.5 μg, and a good linear relationship between the formononetin reference substance and peak area in the range of 1.0–3.0 μg.

Animal experiments

A total of 120 three-day-old broiler chickens were selected and divided into four groups of 10 birds per group, with three replicates per group and 10 birds per replicate. The pretest period was 3 days, and the experimental period lasted for 21 days. The broilers in the control group were fed a basal diet; the *A. membranaceus* group was fed a basal diet + 3% *A. membranaceus;* the fermented *A. membranaceus* group was fed a basal diet + 3% fermented *A. membranaceus;* and the probiotics group was fed a basal diet + 3% *L. plantarum*.

Measurement of indicators

Determination of growth performance

One hundred twenty male white feather broiler chickens were selected from Shandong Haotai Experimental Animal Breeding Co., Ltd. During the experiment, the chickens were weighed at the beginning (at 3 days of age) and then fed their respective diets. The chickens were weighed at weekly intervals, and weighing was performed before feeding on the same day.

Antioxidant and immune indexes

On day 24 of the experiment, each chicken was exposed to excess isoflurane thiophenolate for general anesthesia. Three chickens from each replicate were randomly selected for heart blood collection (5 mL), and the serum was separated in a constant-temperature incubator at 37 °C and stored frozen at – 20 °C for later use. The antioxidant index in serum was detected using an ELISA detection kit, and the immune index was calculated. After blood collection, tissues and organs (the jejunum and liver) were dissected and stored at – 20 °C for intestinal sectioning and SCFA detection.

Histopathology

(1) Sampling: The intestinal tissue of the chickens was aseptically collected, fixed in a 10% formaldehyde solution, and placed in a cool and dark place for two weeks. During the first week, the formaldehyde solution was checked for turbidity. If the solution was cloudy, it was replaced. After the fixation was completed, the sectioning was performed. First, the fixed tissue was removed and cut into 1× 1 cm tissue pieces on an ultra-clean bench.
(2) Deparaffinization: After immersion in 90% ethanol for 3 h, 95% ethanol for 2 h, absolute ethanol for 30 min, absolute ethanol for 30 min, alcohol benzene for 10 min, xylene for 5 min, and xylene II for 10 min, paraffin was melted at 70 °C for 30 min in a drying oven. Paraffin II was melted at 70 °C for 30 min and at 65 °C for 30 min.

(3) Embedding: The samples were embedded in paraffin. The sample tissue was removed from the dehydration box, marked, and placed in an embedding frame containing the melted paraffin (the tissue had to be placed in the embedding frame as soon as possible after removal to prevent the paraffin from solidifying). The samples were cooled at – 20 °C for 30 min and removed for trimming after the wax had completely solidified.
(4) Slicing: After the trimming, the specimens were cooled on a freezing table at – 20 °C and then cut into 4 μm slices using a microtome. The slices were floated in the slicing machine in water at a temperature of 30 °C. Slides were then used to pick up the tissue slices. The slides were baked in an oven at 70 °C until dry and then stored at room temperature for further use.
(5) Staining: The deparaffinized sections were placed in xylene I for 30 min, xylene II for 30 min, absolute ethanol I for 3 min, absolute ethanol II for 3 min, and 75% ethanol for 3 min, and washed with water.
(6) Hematoxylin staining: The sections were stained with a hematoxylin staining solution for 5 min, washed with clean water, differentiated with a differentiation solution, and washed with clean water. Secondary staining was performed with a bluing solution, and the slides were rinsed with running water.
(7) Eosin staining: The sections were placed in 85% ethanol for 3 min, then placed in 95% ethanol for 3 min for decoloring, and finally placed in an eosin staining solution for 3 min.
(8) Dehydration and sealing of the slices: The slices were placed in absolute ethanol I for 3 min, absolute ethanol II for 3 min, absolute ethanol III for 5 min, dimethyl I for 3 min, and xylene II for 3 min for transparency. The slices were then sealed with neutral gum.
(9) Microscopic examination: The sealed sections were placed in an imaging system for image acquisition.

The height of the intestinal villi (VH) and crypt depth (CD) were measured, and the ratio of VH to CD (V/C) was calculated. Three discontinuous sites were observed in each sample, and three VH and corresponding CD were measured in each section. The VH was determined as the vertical height from the opening of the intestinal gland to the top of the villus, and the CD was measured as the vertical height from the muscularis mucosa to the opening of the intestinal gland.

Detection of intestinal short-chain fatty acids in chickens

At the end of the experiment, an appropriate amount of chicken intestinal tissue was taken into a 2-mL centrifuge tube and added to 500 μL of water and 100 mg glass beads. The sample was homogenized for 1 min, then centrifuged at 12,000 rpm and 4 °C for 15 min. A total of 200 μL of the supernatant was taken, and 150 μL of 15% phosphoric acid was added. Then, 20 μL of 375 μg/mL internal standard (4-methylvaleric acid) solution and 300 μL of diethyl ether homogenate were added for 2 min and centrifuged at 12,000 rpm, 4 °C for 15 min. The supernatant was stored on dry ice and sent to Nanjing Paisen Biological Gene Technology Co., Ltd., to complete the analysis of targeted metabolomics.

Data processing

The test data were analyzed by one-way ANOVA with SPSS 26.0. The results were expressed as mean ± standard deviation, and a P value less than 0.05 was considered significant.

Results

SEM

The results of the SEM are displayed in Fig. 1. The surface structure was relatively intact, with no ruptures of unfermented *A. membranaceus* visible at 1000× or 4000× magnification. The surface structure of unfermented *A. membranaceus* at 1000× and 4000× was relatively complete and without rupture. After 3 days of fermentation, there was a small amount of *L. plantarum* attached to the surface of *A. membranaceus,* and the surface membrane structure was broken. After 7 days of fermentation, the structure of *A. membranaceus* appeared severely cracked under 1000× and 4000× magnification, and there was a large number of *L. plantarum* attached to the surface. The breaking of the cell wall of *A. membranaceus* after fermentation with *L. plantarum* was conducive to the precipitation of active substances.

SDS-PAGE

The results of SDS-PAGE are shown in Fig. 2. Before fermentation, the size of the extracted protein fragment of *A. membranaceus* was 40 kDa, and that of the small fragment was 15 kDa. From the first day to the second day of fermentation, the corresponding protein sizes were the same as before fermentation. On the third day of fermentation, the size of the protein fragment was 24 kDa, and that of the small fragment was 15 kDa, while on the fifth day of fermentation, the protein size was 15 kDa. With the increase in the number of days, the macromolecular proteins were gradually degraded; they were initially reduced to 24 kDa on the third day of fermentation, and a fragment of 15 kDa was retained from the fifth to the seventh day of fermentation, indicating that the macromolecular proteins in *A. membranaceus* could be gradually degraded during the fermentation process so that the fermented *A. membranaceus* was more conducive to animal digestion, absorption, and utilization.

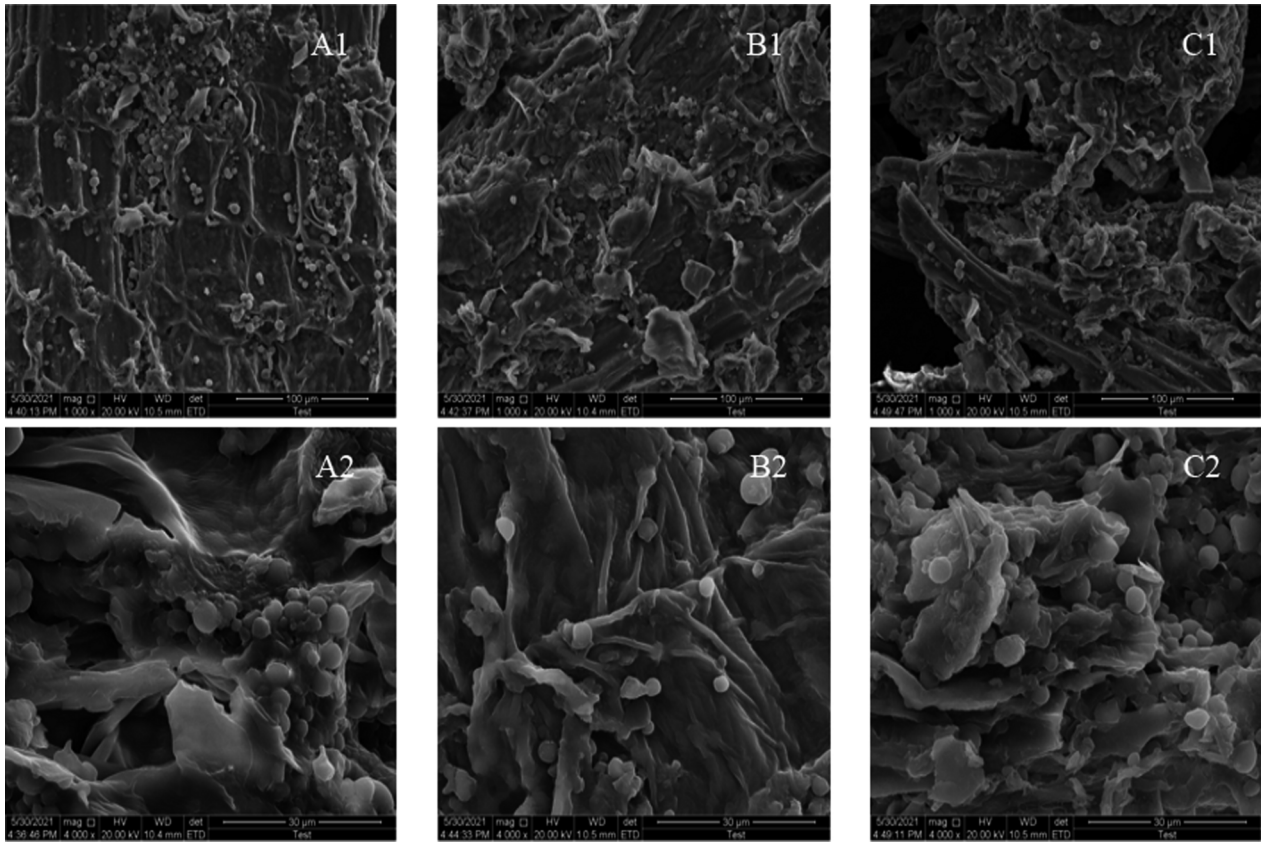

Fig. 1 Scanning electron microscope mages of *A. membranaceus* before and after fermentation. Note: A1: 1000×, unfermented *A. membranaceus;* A2: 4000×, unfermented *A. membranaceus*; B1: 1000×, 3 days of fermentation; B2: 4000×, 3 days of fermentation; C1: 1000×, 7 days of fermentation, C2: 4000×, 7 days of fermentation

Expression of α-galactosidase and β-galactosidase mRNA in *L. plantarum* after fermentation

The expression levels of α-galactosidase (melA-1) and β-galactosidase (lacc-1) mRNA in *L. plantarum* after fermentation were quantified by real-time fluorescence quantitative PCR (Figs. 3 and 4). There was no significant difference in α-galactosidase between before fermentation and at 1 and 3 days after fermentation ($P > 0.05$), but the level was significantly increased by the fifth day of fermentation ($P < 0.05$), and by the seventh day of fermentation was significantly higher than before fermentation and on days 1, 3, and 5 ($P < 0.05$). There was no significant difference in β-galactosidase between before fermentation and the first and third days of fermentation ($P > 0.05$), but the expression level was significantly increased on the fifth day of fermentation ($P < 0.05$), and the level by the seventh day of fermentation was significantly higher than that before fermentation, and days 1, 3, and 5 ($P < 0.05$). The levels of α-galactosidase and β-galactosidase in *L. plantarum* were significantly higher than those in unfermented *L. plantarum* after fermentation and increased gradually with time. There were no significant changes in α-galactosidase or β-galactosidase during the first 3 days of fermentation, but the levels were significantly increased by the fifth day of fermentation, reaching a peak on the seventh day of fermentation. Therefore, the interaction between *L. plantarum* and astragalus fermentation can effectively improve the conversion of galactosidase, thereby reducing the degradation of galactosidase in astragalus and enhancing the absorption of nutrients.

Changes in sugar content in *A. membranaceus* due to fermentation

The gas chromatography-tandem mass spectrometry results indicated that *A. membranaceus* polysaccharides were composed of 13 monosaccharides before and after fermentation, and several monosaccharide contents were altered by fermentation. The results are shown in Figs. 5 and 6. Trehalose, lactose, L-fucose, inositol, L-rhamnose, D-sorbitol, and xylitol were not significantly different after fermentation. The content of maltose gradually decreased with the fermentation time. The content of sucrose was high before fermentation, and then decreased rapidly and remained stable after fermentation. The D-arabinose level increased significantly with the increase in fermentation time. The D-fructose level gradually increased with fermentation time, reaching 61.18

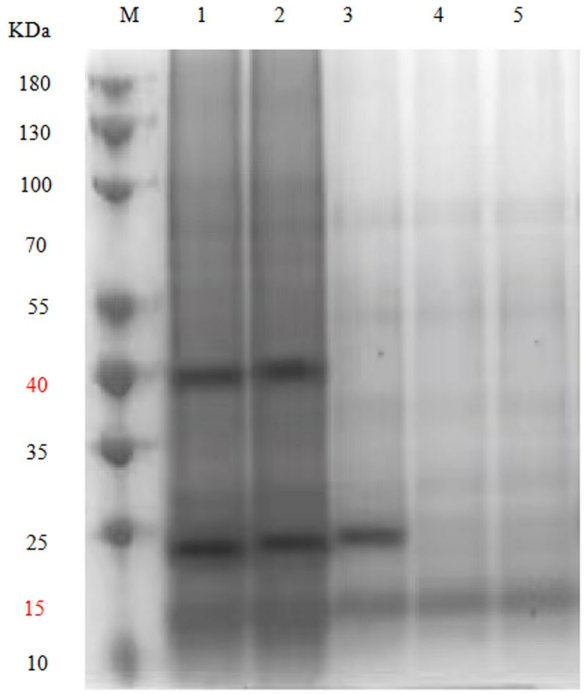

Fig. 2 The results of SDS-PAGE of *A. membranaceus* before and during fermentation. M: Protein molecular weight (10–180 kDa). 1: *A. membranaceus* before fermentation; 2: Fermented *A. membranaceus,* (initial state); 3: *A. membranaceus* fermented for 3 days; 4: *A. membranaceus* fermented for 5 days; 5: *A. membranaceus* fermented for 7 days

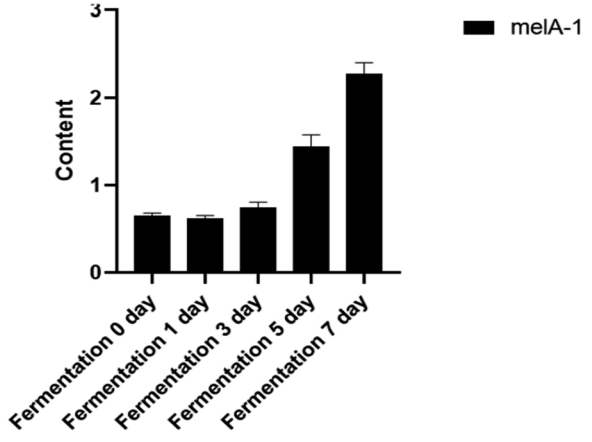

Fig. 3 Expression of melA-1 gene in *Lactobacillus plantarum*

μL/g after 3 days of fermentation, and then decreased. The glucose level gradually increased with fermentation time and reached the highest level of 64.49 μL/g on the third day, and then gradually decreased. The level of D-galactose increased gradually with the fermentation time.

HPLC detection of the changes in active substances after fermentation of *A. membranaceus*

The results of HPLC are shown in Figs. 7, 8 and 9. The calycosin content in *A. membranaceus* after fermentation

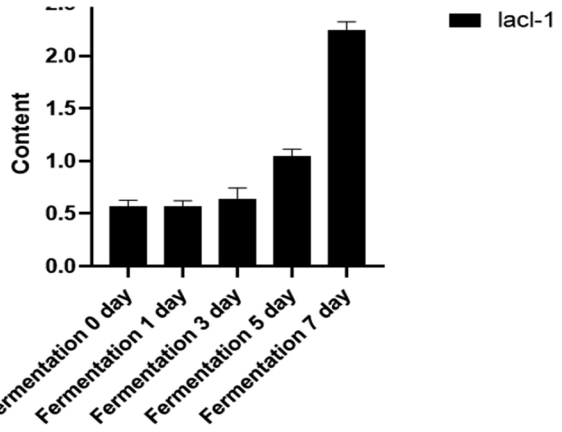

Fig. 4 The expression of the lacl-1 gene in *Lactobacillus plantarum*

was significantly higher than that before fermentation ($P<0.05$). The calycosin content was increased by the third day and gradually increased with the fermentation time; it was significantly higher by day 7 after fermentation than before fermentation and at days 0, 3, and 5 after fermentation ($P<0.05$), reaching the highest value of 9.31 μg/mL. The content of formononetin was significantly altered by fermentation ($P<0.05$), being increased by day 3 and gradually increasing with time, and there was no significant difference between day 5 and day 7 of fermentation ($P>0.05$). However, the content of formononetin was increased after 7 days of fermentation and reached the maximum content of 1.16 μg/mL. Hence, fermentation can significantly increase the contents of the main active compounds in *A. membranaceus* and thereby enhance the medicinal value of fermented *A. membranaceus* preparations for practical clinical application.

Effects of different treatments on the growth performance of broilers

The weekly weights of the chickens are shown in Fig. 10. The fermented *A. membranaceus* group was significantly different from the control group, the *A. membranaceus* group, and the probiotics group with the increase in feeding time ($P<0.05$). The mean weight of the fermented *A. membranaceus* group increased by 20.60% compared with the control group, by 13.20% compared with the *A. membranaceus* group, and by 13.20% compared with the probiotics group. In general, adding fermented *A. membranaceus* to the diet effectively improved growth performance.

The effects of different treatments on antioxidant indexes

The changes in antioxidant indexes in the serum of chickens were detected by ELISA, as shown in Fig. 11. After 21 days of feeding, the glutathione peroxidase (GSH-Px) activity of the fermented *A. membranaceus* group was

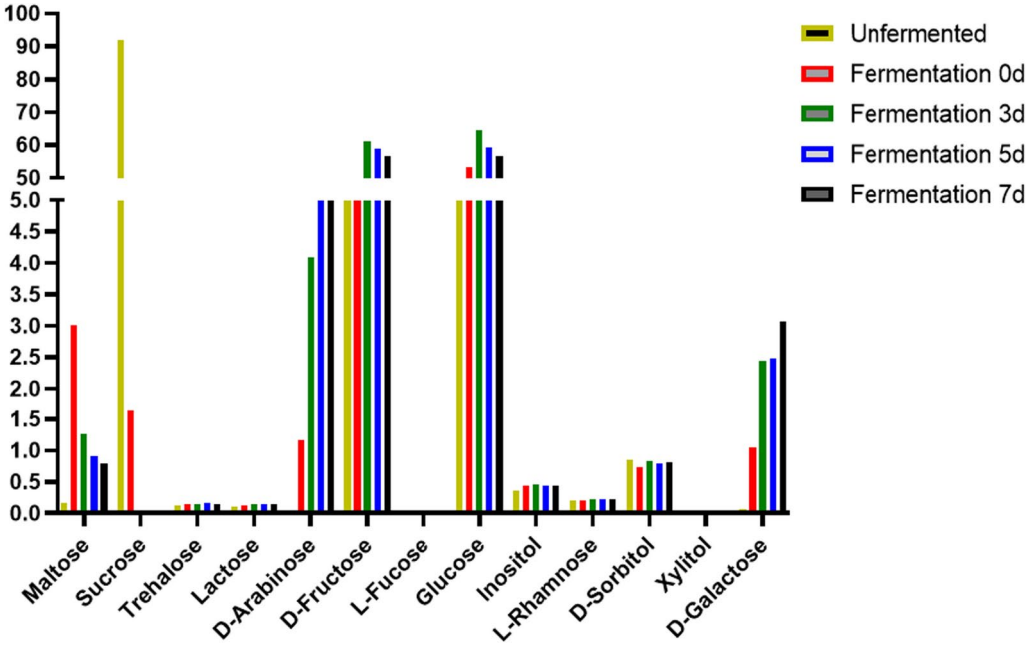

Fig. 5 Changes in sugar contents of *A. membranaceus* due to fermentation (μg/mL)

the highest (916.61 nmol/mL), being significantly different from the control group, the *A. membranaceus* group, and the probiotics group ($P<0.05$). The malondialdehyde (MDA) content of the fermented *A. membranaceus* group was the lowest (7.17 nmol/mL) and was significantly different from those of the control group, the probiotics group, and the *A. membranaceus* group ($P<0.05$). The total antioxidant capacity (T-AOC) of the fermented *A. membranaceus* group was 0.18 nmol/mL, significantly higher than those of the other groups ($P<0.05$). The superoxide dismutase (SOD) activity of the fermentation group was 570.32 nmol/mL, significantly higher than the values of the other groups. In conclusion, solid fermentation of *A. membranaceus* by *L. plantarum* reduced the content of MDA and increased the activities of different antioxidant enzymes in serum, thereby improving the antioxidant capacity of the serum.

Effects of different treatments on immune indexes of broilers

The changes in immune indexes in the serum of broilers treated with different methods were detected by ELISA and are shown in Fig. 12. After 21 days of feeding, the IgA level of the fermentation group was significantly different from that of the control group and the probiotic group ($P<0.05$) but was not significantly different from that of the *A. membranaceus* group ($P>0.05$). The content of IgM in the fermentation group was 405.4 μg/mL, significantly different from the control group ($P<0.05$), but not significantly different from the *A. membranaceus* group or the probiotics group ($P>0.05$). Compared with the control group, the *A. membranaceus* group, and the probiotics group, the IgG fermentation group increased by 41.3%, 45.5%, and 48.6%, respectively ($P<0.05$). There were no significant differences in IFN-γ or IL-12 levels between the fermentation group and the other three groups ($P>0.05$). The level of IL-6 in the fermentation group was 19.9 μg/mL, significantly lower than in the control group, the *A. membranaceus* group, and the probiotics group by 12.3%, 10.2%, and 10.9%, respectively ($P<0.05$). In conclusion, *L. plantarum*–fermented *A. membranaceus* increased the contents of IgM, IgG, and IL-6 in the chickens' serum and enhanced their levels of immunity.

The results of intestinal tissue analysis

The effects of the different treatments on VH and CD are shown in Figs. 13 and 14. The intestinal villi of the control group were sparse, and the intestinal wall was thin, while the density of intestinal villi of the *A. membranaceus* group was increased compared with the control group, and the CD was unchanged (Table 8). Therefore, adding *A. membranaceus*, fermented *A. membranaceus*, or probiotics to the diet achieved better results than the normal diet. The VH of the fermentation group significantly increased by 16.28%, 13.84%, and 14.28% relative to the control group, the *A. membranaceus* group, and the probiotics group, respectively ($P<0.05$). There was no significant difference in CD between the fermentation group and the probiotics group ($P>0.05$), and CD in the fermentation group was reduced by 30.86% and 25.87% compared with the control group and the *A.*

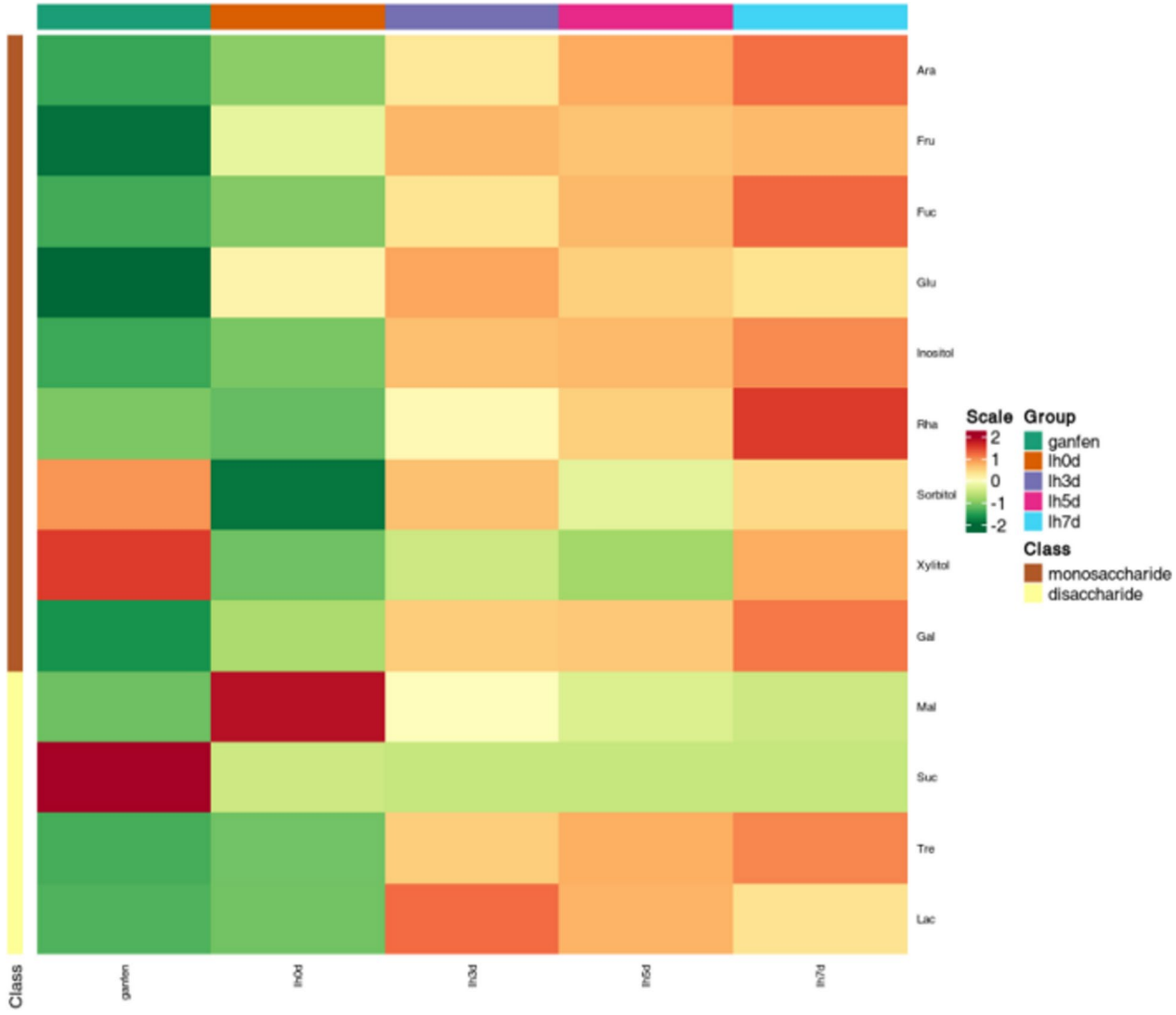

Fig. 6 Cluster analysis of sugar content in *A. membranaceus* before and after fermentation. Note: The horizontal axis refers to the sample name; the vertical axis refers to the metabolite information. Different colors refer to the values obtained after standardized treatment with different relative contents (red represents high content; green represents low content). Group refers to grouping; Class refers to substance classification. Here, all_heatmap_class: heatmap classified by substance, all_heatmap_col-row_cluster: Cluster analysis was carried out for both metabolites and samples. The cluster line on the left side of the figure is the cluster line for metabolites; the cluster line on the upper part of the figure is the cluster line for samples; all_heatmap_row_cluster: Cluster analysis was only conducted for metabolites

membranaceus group, respectively. The V/C (height of the intestinal villi/depth of crypt) ratios in the fermentation group were 45.89%, 38.73%, and 31.28% higher than those in the other three groups ($P < 0.05$). The V/C ratios in the fermentation group were 45.89%, 38.73%, and 31.28% higher than those in the control group, *A. membranaceus* group, and probiotics group, respectively, and the differences were significant ($P < 0.05$). However, the V/C ratio in the probiotics group was significantly higher than those in the control and *A. membranaceus* groups. VH, CD, and V/C are important measures of intestinal digestive and absorptive functions. The results showed that the fermentation of *A. membranaceus* improved VH, CD, and V/C, thereby promoting intestinal nutrient absorption and enhancing growth performance.

The effects of fermented *A. membranaceus* on the intestinal content of short-chain fatty acids

The expression levels of differential metabolites in seven ion modes after dimensionality reduction were analyzed by cluster analysis. Each metabolite was represented by one value according to the expression values of differentially expressed substances among the four groups of samples, and the intensity of each metabolite was scaled and displayed in a heat map. The results showed that the expression profiles of the metabolites in

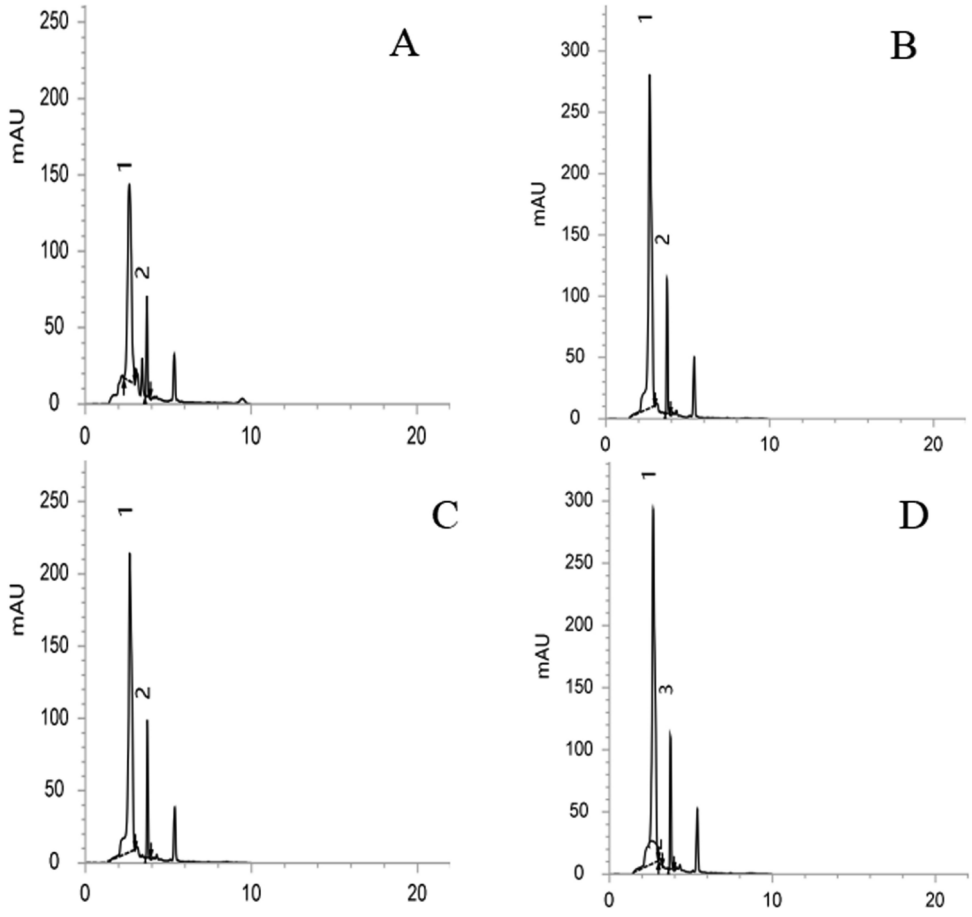

Fig. 7 Dissolution rates of calycosin and formononetin in *A. membranaceus* before and after fermentation. Note: (**A**) *A. membranaceus* before fermentation; (**B**) *A. membranaceus* after 3 days of fermentation; (**C**) *A. membranaceus* after 5 days of fermentation; (**D**) *A. membranaceus* after 7 days of fermentation; 1: calynoisoflavone glycosides; 2, 3: formononetin

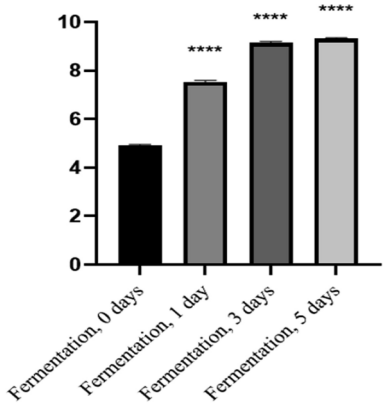

Fig. 8 Changes in the content of isoflavone after fermentation (μg/mL)

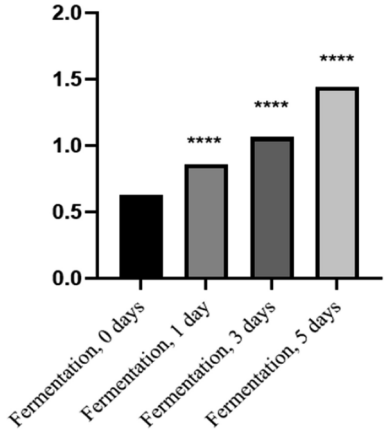

Fig. 9 Changes in the content of formononetin after fermentation (μg/mL)

the control group, the *A. membranaceus* group, the fermentation group, and the probiotics group were essentially the same; the aggregation effect was good, and the highly expressed and least expressed metabolites could be distinguished. The effects of fermented *A. membranaceus* on intestinal SCFAs are shown in Figs. 15 and 16.

Propionic acid in the fermentation group was increased by 68.1%, 54.7%, and 74.3% compared with the control group, the *A. membranaceus* group, and the probiotics group, respectively ($P<0.05$). Acetic acid in the fermentation group was increased by 53.2%, 49.3%, and 60.6%

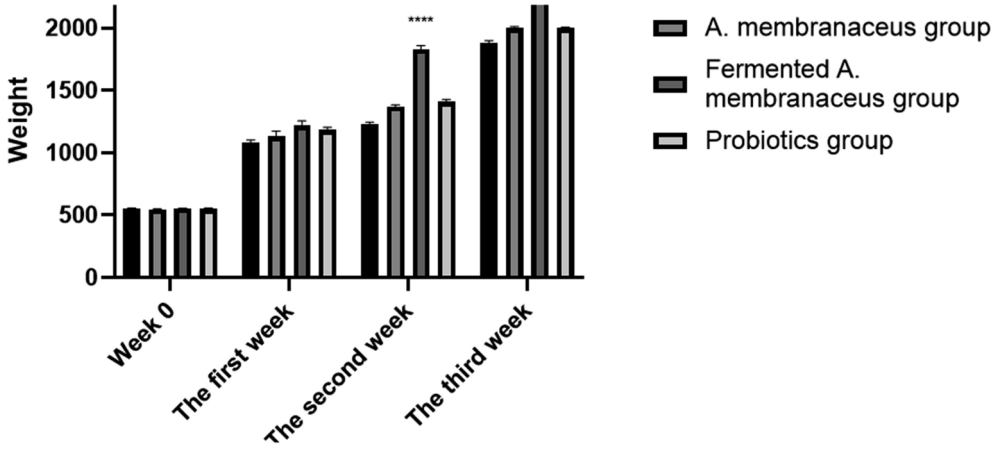

Fig. 10 Growth performance of each group at different ages (g)

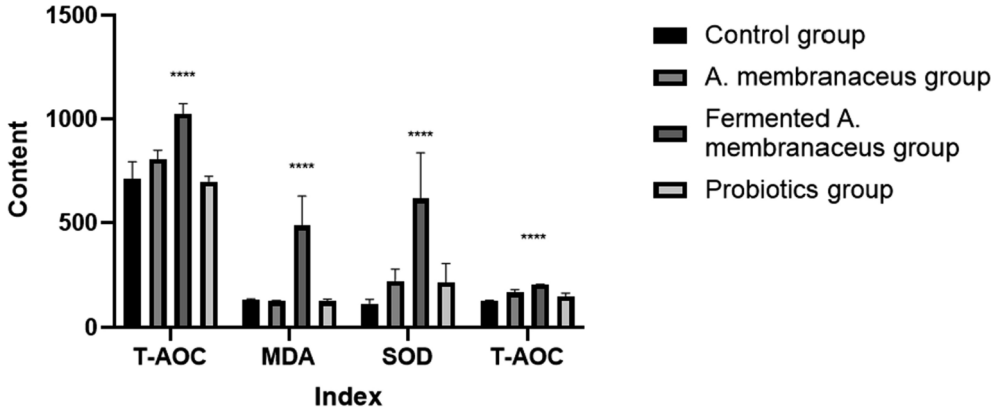

Fig. 11 Intestinal index between different groups

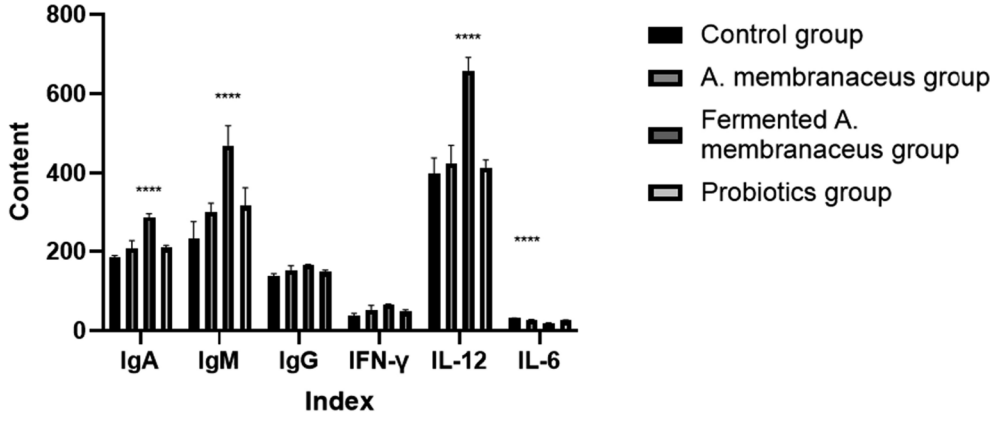

Fig. 12 Effects of different treatments on immune indexes of broilers (μg/mL)

compared with the control group, the *A. membranaceus* group, and the probiotics group, respectively ($P<0.05$). Butyric acid in the fermentation group was increased by 20.83%, 29.2%, and 18.6 ($P<0.05$). There were no significant differences in the levels of isobutyric acid or isovaleric acid between the groups ($P>0.05$). There was a significant difference in valeric acid content between the control group and the other three groups ($P<0.05$). In conclusion, dietary supplementation of fermented *A. membranaceus* could increase the production of SCFAs in the intestine of broilers, especially the contents of the three main SCFAs.

Note: The relative content in the figure is shown by the color difference. The redder the color, the higher the

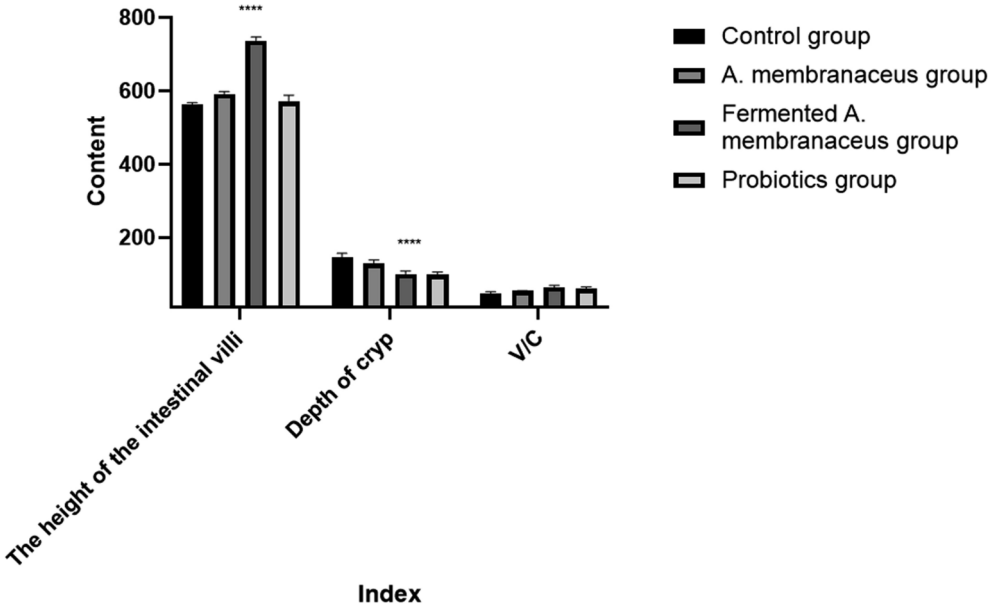

Fig. 13 Effects of different treatments on chicken intestinal tract (μm)

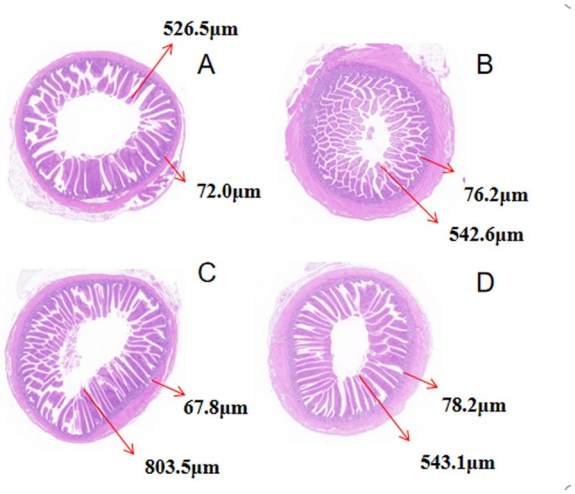

Fig. 14 Effects of different treatments on chicken intestinal tract (μm)

expression level; the bluer the color, the lower the expression level. The columns represent samples; the rows represent metabolite names, and the cluster tree on the left of the figure is the differential metabolite cluster tree. A: control group; B: *A. membranaceus* group; C: Fermented *A. membranaceus* group; D: Probiotics group.

Discussion

Changes in the apparent structure and composition of *Astragalus* before and after fermentation by *Lactobacillus plantarum*

In this study, we used *L. plantarum* to ferment *A. membranaceus* to ensure more effective use of the nutrient components and the release of medicinally active compounds. The apparent structure of *A. membranaceus* before and after fermentation was analyzed by scanning electron microscopy and SDS-PAGE (Zhang et al. 2023). The results showed that the plant fibers of *A. membranaceus* were gradually decomposed after fermentation by *L. plantarum*, thereby making the nutrients in the fibers more easily absorbable by animals. Because *L. plantarum* are lactic acid bacteria, there is a large amount of acid produced during the fermentation process, resulting in a weak acidic fermentation environment (pH 4.4) that causes the decomposition of the plant fiber structure on the surface (Shang et al. 2018). In addition, during the fermentation process, cellulose hydrolase is produced to accelerate the degradation of cellulose and pectin in the cell walls of *A. membranaceus* (Cui et al. 2018). The nutrient release in *A. membranaceus* was higher than that in unfermented *A. membranaceus* (Hsu et al. 2009). Therefore, this study shows that solid-state fermentation of *A. membranaceus* by *L. plantarum* can facilitate the release of nutrients and promote their absorption; thus, Astragalus can be employed in production.

A. membranaceus is rich in nutrients; its protein content is 15%, but it is difficult to be absorbed and utilized by animals (Upadhaya et al. 2016). To promote the absorption and utilization of the protein from *A. membranaceus*, we used *L. plantarum* to ferment *A. membranaceus* and analyzed the changes in protein after fermentation. The results showed that after fermentation with *L. plantarum*, the macromolecular proteins in *A. membranaceus* were gradually degraded, and only the small molecular proteins that could be absorbed by animals were retained (Li et al. 2022). The fermentation temperature was closely related to the alterations of proteins of *A. membranaceus*. An appropriate

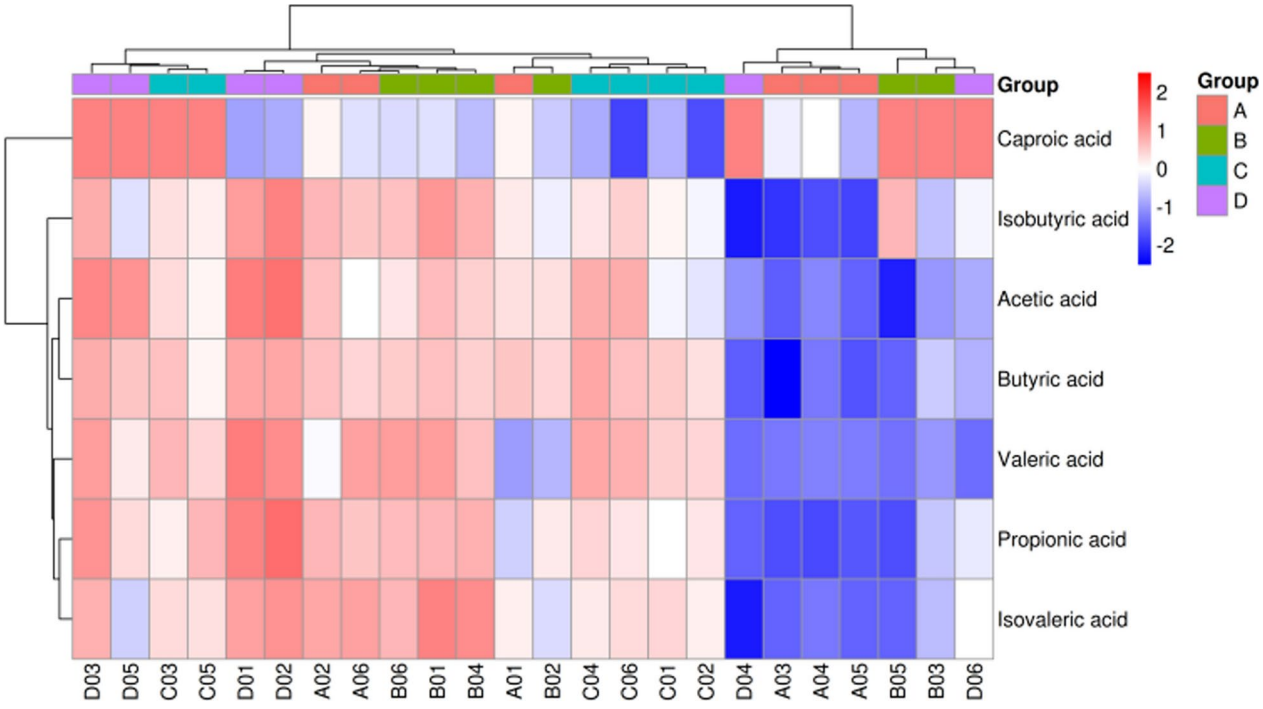

Fig. 15 Overall heat map of cluster analysis

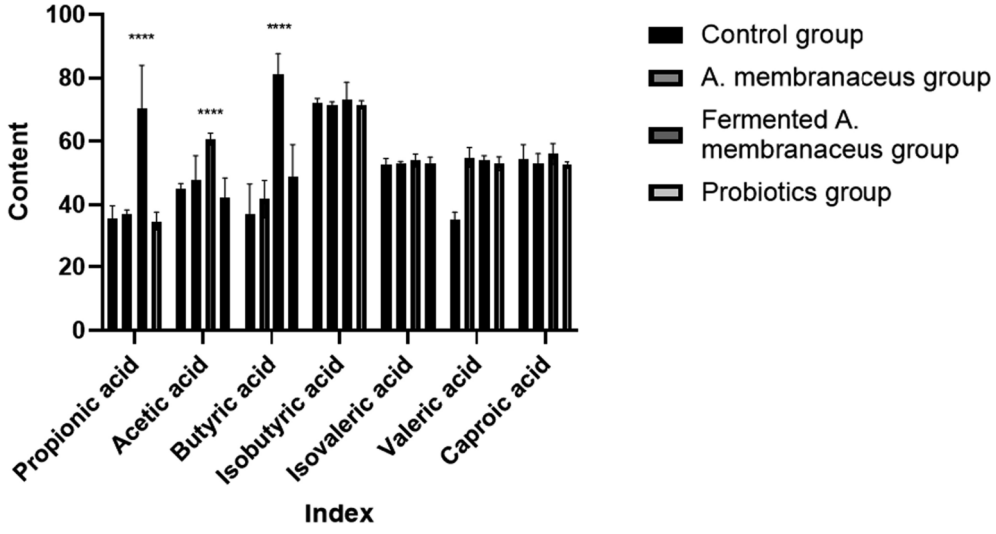

Fig. 16 The effects of fermented *A. membranaceus* on intestinal short-chain fatty acids (μg/g)

fermentation temperature allows *L. plantarum* to grow and improves the enzyme activity in *A. membranaceus*, thereby increasing the protein quality (Lv et al. 2021). The extension of fermentation time increased the enzyme metabolites and degraded the macromolecular proteins in *A. membranaceus*, results that were consistent with those of Shen et al. (2014a, b) who found that microbial fermentation degraded the proteins in *Moringa* leaves to small molecular proteins with the extension of fermentation time. Our experiment shows that solid-state fermentation by *L. plantarum* can degrade the proteins in *A. membranaceus* to small molecular proteins that can be more easily absorbed by the body, thereby further improving the application effect of fermented *A. membranaceus* in clinical practice.

Effect of *A. membranaceus* on galactosidase of *L. plantarum*

L. plantorum can regulate the intestinal flora, promote nutrient absorption, alleviate lactose intolerance, and reduce antinutritional factors (Shi et al. 2020a, b). The alleviation of lactose intolerance and the degradation of antinutritional factors by *L. plantorum* rely on the

hydrolysis of polysaccharides by galactosidase. Galactosidase is divided into α-galactosidase and β-galactosidase, both of which can hydrolyze lactose (Tseng et al. 2015; Hyemee et al. 2022). The content of galactosidase in *L. plantarum* is low, and methods of increasing the content of galactosidase have not been reported. In this study, we used *L. plantarum* for solid-state fermentation of *A. membranaceus* and detected the effect on the expression of galactosidase genes by real-time PCR. The results showed that the levels of α-galactosidase and β-galactosidase in *L. plantarum* were significantly increased after mixing with *A. membranaceus*. During the fermentation process, the cellulose structure on the surface of *A. membranaceus* was decomposed so that the nutrients could be fully released, thereby providing an effective carbon source for the growth of *L. plantarum*. An appropriate fermentation temperature, a sufficient carbon source, and the increased enzyme activity in *L. plantarum* accelerated the substrate conversion rates of the enzyme reactions (Jamshidparvar et al. 2017). This led to a significant increase in the galactosidase level in *L. plantarum*. Combining *L. plantarum* and *A. membranaceus* increased the content of galactosidase in *L. plantarum* after fermentation, and the galactosidase accelerated the degradation of galactoside antinutrients in *A. membranaceus*, thereby increasing the content of galactose (Ibrahim et al. 2013).

The effect of *L. plantarum* on sugars in *A. membranaceus*

Polysaccharides are the main substances used for energy storage and structural support of *A. membranaceus*. Polysaccharides provide both energy and nutrients (Cao F L et al., 2012). In this study, the changes in *A. membranaceus* polysaccharides after fermentation and the reasons for the changes were analyzed by gas chromatography-tandem mass spectrometry. The results showed that solid-state fermentation by *L. plantarum* significantly improved the functional *A. membranaceus* polysaccharides. This may be because the maltose in the *Astragalus* polysaccharides gradually decreased with the fermentation time, and *Lactobacillus plantarum* was likely consumed during the proliferation process. The content of sucrose was high before fermentation and decreased rapidly and remained stable after fermentation. This may have been due to the production of sucrose hydrolase by *L. plantarum* during the fermentation process. Some polysaccharides provide energy for microbial metabolism, while others accumulate to increase the contents of D-fructose and glucose (Wang X J et al., 2021). As a result, the fructose and glucose levels gradually increased during the fermentation process, reached the highest values on the third day of fermentation, and then gradually decreased, primarily because with the extension of fermentation time, the content of sucrose decreased sharply, and the sucrose in *A. membranaceus* could not meet the requirement of *L. plantarum*, thereby reducing the activities of sucrose hydrolase and other enzymes. However, the levels of fructose and glucose in the fermented *A. membranaceus* were still significantly higher than those before fermentation. Therefore, the antifatigue effect of fermented *A. membranaceus* was better than that of unfermented *A. membranaceus*, and the main reason was the increases in the fructose and glucose contents. D-arabinose showed a significant increase with fermentation time due to *L. plantarum* containing an arabinose isomerase (Wu et al. 2010). Due to the acidic environment produced by *L. plantarum* during the fermentation process, the apparent cellulose and pectin substances of *A. membranaceus* were cleaved, and large amounts of polysaccharides were released. The polysaccharide precipitates in *A. membranaceus* reacted with arabinose isomerase in *L. plantarum*, resulting in an increased arabinose content after fermentation. This increase in arabinose promoted the regulation of carbohydrate degradation. The D-galactose content gradually increased with the fermentation time due to the galactosidase in *L. plantarum* decomposing the galactosides in *A. membranaceus*; hence, the dissolution rate of galactose in *A. membranaceus* increased so that the contents of antinutritional factors in the fermented *A. membranaceus* extract decreased. This ultimately increased the content of galactose. The increased galactose content can improve the anti-aging effect of *A. membranaceus* (Gao et al. 2017). The increase in functional monosaccharide content influences the medicinal effects of the fermented *A. membranaceus*, including its anti-stress and antioxidant effects.

The effect of *L. plantarum* on calycosin and formononetin contents

Isoflavones such as calycosin and formononetin are natural active substances in *A. membranaceus* that have antioxidant and hypoglycemic effects. However, such macromolecular substances are difficult to extract (Shen et al. 2014a, b). In this study, the contents of calycosin and formononetin in *A. membranaceus* before and after fermentation were detected by HPLC. The contents of calycosin and formononetin in *A. membranaceus* after fermentation were significantly higher than those before fermentation and gradually increased with the fermentation time, reaching the highest value at 7 days of fermentation. During the fermentation process, *L. plantarum* contains a variety of enzymes (such as b-glucosidase) that promote the hydrolysis of glycosidic bonds, allowing the deglycosylation reaction to occur and increasing the contents of calycosin and formononetin in *A. membranaceus* after fermentation. The activity of *L. plantarum* increased with the fermentation time, and the contents

of enzymes increased to accelerate the glycosylation reaction. Thus, calycosin and formononetin content increase with the extension of fermentation time (Choe et al. 2012). The results showed that solid-state fermentation of *A. membranaceus* by *L. plantarum* could increase the contents of calycosin and formononetin, thereby increasing the efficacy of *A. membranaceus*.

The effects of *L. plantarum* fermentation on the growth performance of broilers

As a TCM, *A. membranaceus* can enhance immunity, lower blood glucose, reduce tumors, and decelerate aging. In this study, the addition of 3% *L. plantarum*–fermented *A. membranaceus* to the basal diet of broiler chickens significantly improved their growth performance. There were two reasons for this. First, the cell walls of *A. membranaceus* cannot be completely decomposed in vivo, which seriously hinders the release of its active components. *L. plantarum,* a commonly used probiotic, produces acids during fermentation. The fermentation environment and acid production can effectively decompose the fibers and pectin structure of the *A. membranaceus* cell wall (Zhou et al. 2023) so that the active ingredients can be released and absorbed. *A. membranaceus* can also produce a unique sweet taste after fermentation, thereby improving the palatability of feed and increasing the feed intake (Dong et al. 2023). In this study, when *L. plantarum* was used to ferment *A. membranaceus*, cellulase produced by the interaction effectively degraded pectin, hemicellulose, and other substances so that cellulose in the diet could be decomposed into glucose and volatile fatty acids, and the absorption and utilization of such nutrients in the feed could be increased (Song et al. 2023). After the fermentation of *A. membranaceus* by *L. plantarum*, substances released such as amylase can promote the microorganisms in the intestine, thereby benefiting the health of livestock and poultry (Thi et al. 2017). After fermentation, the cell walls of *A. membranaceus* are cleaved, and the active components of *A. membranaceus* can be fully released. The macromolecular proteins, polysaccharides, and saponins in *A. membranaceus* can be degraded into small molecules that are easily absorbed and utilized. The metabolites produced by lactic acid bacteria fermentation can improve the digestive function of the gastrointestinal tract and hence the growth performance of broilers.

Effects of different treatment groups on antioxidant indexes in broilers

The antioxidant enzymes include glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD). These enzymes regulate the balance between oxidation and antioxidant processes. Total antioxidant capacity (T-AOC) is one of the most important indicators of the ability of the body to remove free radicals. Free radicals can lead to the production of reactive oxygen species between cells and cause damage to cells and organs. Antioxidant substances can protect the body from free-radical damage, a process that relies on the induction of antioxidant enzymes to protect against the oxidation of proteins and lipids, thereby maintaining the stability of cell functions (Choe et al. 2012). MDA is the final harmful metabolite of lipid peroxidation. The level of MDA in the body is an important index of the degree of lipid peroxidation (Ke et al. 2018). A study of the active components of *A. membranaceus* from Inner Mongolia determined that the flavonoids could scavenge superoxide anion free radicals. Pharmacological studies have shown that the saponins in *A. membranaceus* can improve the activity of the free-radical scavenging enzyme SOD, reduce the content of lipid peroxide LPO, and significantly improve ischemia caused by free radicals. At present, the research on fermented *A. membranaceus* mainly focuses on the extraction of *A. membranaceus* polysaccharides and their effects on the immune system, while there are few reports on the immune and antioxidant properties of other active ingredients of *A. membranaceus* such as saponins and flavonoids. In this study, we found that the functional polysaccharides, calycosin glycosides, and formononetin glycosides in *A. membranaceus* were increased after fermentation with *L. plantarum*. By adding *A. membranaceus*, probiotics, and fermented *A. membranaceus* to the diet, the activity of GSH-Px in the fermented *A. membranaceus* group was the highest, being significantly different from the activities of the *A. membranaceus* group, the control group, and the probiotics group. The content of MDA in the fermented *A. membranaceus* group was the lowest and was significantly different from that in the control group, the probiotics group, and the *A. membranaceus* group. The activities of TAOC and SOD in the fermented *A. membranaceus* group were significantly higher than in the other groups. The contents of D-fructose, D-arabinose, and galactose in *A. membranaceus* polysaccharides were significantly higher than those in the unfermented *A. membranaceus*, and their increase could improve the animal body's hypoglycemic functions, thereby enhancing the clearance of free radicals. The increases in calycosin and formononetin after fermentation could improve the antioxidant capacity of the body. The results of this study showed that adding an appropriate amount of fermented *A. membranaceus* increased the T-AOC, GSH-Px, and T-SOD activities in the serum of broilers, demonstrating that the effect of *A. membranaceus* fermentation on the antioxidant function of broilers was enhanced. There was no direct relationship between the changes in antioxidant indexes and the amount of *A. membranaceus* added.

Considering all these results, adding 3% of *A. membranaceus* was the optimal fermentation method.

Effects of different treatments on immune indexes of broilers

The level of antibodies in the serum of livestock and poultry is an important indicator of their immune status. In the existing research reports, the effects of feed additives on the immune system are generally tested by detecting the changes in relevant immune indicators. IgA can promote intestinal mucosal immunity, and it can form a biological barrier in the intestinal mucosa to block the invasion of pathogens. A higher IgA content in serum is linked to healthier intestinal tissue. IgG is an important element of the body's immunity and has a role in immune protection. A higher serum IgG content is associated with greater disease resistance. IL-6 is a proinflammatory factor, and lower levels indicate a healthier body. Adding TCM to feed can improve the body's immunity. In this study, *A. membranaceus*, fermented *A. membranaceus*, and probiotics were added to the diet. We found that IgA in the fermentation group was significantly different from that in the control group and the probiotics group, and there was no significant difference between the fermentation group and the *A. membranaceus* group. IgM of the fermentation group was significantly different from that of the control group, but there was no significant difference between the fermentation group, the *A. membranaceus* group, and the probiotics group. There was a significant difference in IgG content between the fermentation group and the other three groups. There was no significant difference in IFN-γ content between the fermentation group and the other three groups. There was no significant difference in IL-12 content between the fermentation group and the other three groups. IL-6 content in the fermentation group was significantly different from that in the other three groups. As a TCM, *A. membranaceus* improves the body's immunity via anti-inflammatory and antioxidant effects. These effects rely on the astragaloside and flavonoids contained in *A. membranaceus* (Niu et al. 2019), and these were significantly increased after fermentation. Therefore, adding fermented *A. membranaceus* to feed can improve the body's immunity and anti-inflammatory ability better than unfermented *A. membranaceus* or probiotics. *L. plantarum* can produce cellulase, amylase, and other enzymes in the fermentation of *A. membranaceus*. These endogenous enzymes can reduce the incidence of disease, affect the balance of intestinal microorganisms, and strengthen the body's disease resistance.

The effects of different treatments on intestinal villi and crypts of broilers

The intestinal tract is the main site of nutrient digestion and absorption, and the normal morphology and structure of the small intestinal mucosa are responsible for nutrient absorption. VH, CD, V/C, and villus surface area are important indicators of intestinal digestive and absorptive functions. V/C is commonly used to measure the functional status of the intestine. When V/C is decreased, the capacity of the intestine for digestion and absorption is reduced, and this can slow the growth and development of animals. Conversely, a high V/C ratio indicates a better condition of the intestinal mucosa; in that case, the digestion and absorption capacities of the intestine are stronger, and this facilitates animal growth and development. In this study, adding a certain amount of *A. membranaceus*, fermented *A. membranaceus*, and probiotics to the diet resulted in the VH in the fermented *A. membranaceus* group being significantly different from those of the control group, the *A. membranaceus* group, and the probiotics group. There was a significant difference in CD between the fermentation group and the control group and the *A. membranaceus* group, but no significant difference between the fermentation group and the probiotics group. The V/C of the fermentation group was significantly different from that of the other three groups, and the reason was that *A. membranaceus* polysaccharides are the main components of the TCM made from *A. membranaceus.* These compounds can increase the height and width of the chicken small intestine villi, the mucosal density, and the villus surface area, and improve the ability of the small intestine to absorb nutrients. We showed that adding *A. membranaceus* polysaccharides to the diet could markedly improve the chickens' duodenum VH, CD, and V/C relative to the control group. *L. plantarum* had a significant promoting effect on the growth and development of the intestinal tract of broilers. The bacteria can adhere to the intestinal epithelial cells, prevent the invasion of harmful bacteria, protect the gastrointestinal mucosa, and improve the intestinal structure. *Lactobacillus* complex added to the drinking water of broilers significantly increased the VH of the duodenum and jejunum and reduced CD. The fermentation of *A. membranaceus* by *L. plantarum* can combine the advantages of the two to produce the effect of "1 + 1 > 2" so that the fermented *A. membranaceus* improves the intestinal flora, and *A. membranaceus* can promote immune regulation, reduce stress, and prevent oxidation. In the process of fermentation of *A. membranaceus*, *L. plantarum* also produces amylase, hydrolase, organic acids, and other substances that have beneficial effects. Studies have shown that the combination of *A. membranaceus* polysaccharides and lactic acid bacteria can make the ileum villi of chickens significantly longer

and thicker; this is beneficial for digestion and absorption. Here, we studied the effect of *L. plantarum* solid-state fermentation of *A. membranaceus* on intestinal tissue (Bartkiene Elena et al., 2018). The results showed that VH was significantly increased; CD was decreased, and V/C was significantly increased in the fermented *A. membranaceus* group compared with the other experimental groups. The fermented *A. membranaceus* may directly or indirectly stimulate intestinal flora and improve intestinal tissue morphology (Thi et al. 2017).

The effects of different treatments on intestinal SCfAs in broilers

Short-chain fatty acids (SCFAs), also known as volatile fatty acids, are one of the most important markers of intestinal flora metabolites. SCFAs are present in the intestinal tract of animals and are the main products of animal intestinal bacterial fermentation. This group includes acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, valeric acid, isocaproic acid, and caproic acid (Gao et al. 2018). Acetic acid, propionic acid, and butyric acid are the most abundant. SCFAs can supply energy, participate in intestinal blood circulation, and inhibit intestinal pathogenic microorganisms. Research has shown that the SCFAs produced by microorganisms in the intestine have important physiological effects. Therefore, the detection of and research on short-chain fatty acids in the intestine are significant. However, there are few studies on the effects of fermented TCM on intestinal SCFAs in chickens. In this study, *A. membranaceus*, fermented *A. membranaceus*, and probiotics were added to the broilers' diet (Shi et al. 2020). We found that propionic acid, acetic acid, and butyric acid in the fermentation group were significantly different from those in the other three groups. The reason is that SCFAs are the product of the metabolism of intestinal flora, and the sources in the body are the direct ingestion of food and the fermentation by intestinal flora. Anaerobic microorganisms are the main flora producing SCFAs in the gut (Tomita et al. 2014). The type and quantity of intestinal flora can affect the fermentation-derived SCFAs. Different kinds of intestinal flora produce various types and quantities of SCFAs. For the animal body, SCFAs are primarily from intestinal microbial fermentation, but the animals generally lack cellulose digestive enzymes and thus depend on intestinal flora for fermentation. However, *A. membranaceus* fermented by *L. plantarum* produces cellulose digestive enzymes that can strengthen the fermentation of animal intestinal contents, increase the content of cellulose digestive enzymes in the intestine, and promote the production of short-chain fatty acids. As a facultative anaerobe, *L. plantarum* can increase the diversity of intestinal flora. It can produce a large amount of acid in the intestine and maintain the stability of pH in the intestine and the stability of the intestinal flora, and this in turn can accelerate the activity of intestinal microorganisms so that they can ferment and decompose intestinal contents more quickly and effectively. Thus, *L. plantarum* promotes the contents of short-chain fatty acids in the intestine. While both *A. membranaceus* and *L. plantarum* can promote the production of short-chain fatty acids in the intestine, each by itself cannot achieve maximum efficiency. Thus, fermentation of *A. membranaceus* by *L. plantarum* has the advantages of both. On the one hand, fermentation can produce cellulose digestive and other enzymes. The rapid decomposition of cellulose in the feed will accelerate the absorption and utilization of nutrients in the intestine, and then the remaining substrate can be decomposed to produce short-chain fatty acids. On the other hand, under the action of *L. plantarum*, the pH value in the intestine becomes stable, allowing the growth of the intestinal flora and promoting the fermentation and decomposition of the substrate by intestinal microorganisms. Therefore, fermentation of *A. membranaceus* can significantly enhance the content of short-chain fatty acids in the intestine. As an important indicator of intestinal health, the improvement of short-chain fatty acids can provide energy sources for the growth of anaerobic bacteria, reduce the pH in the intestine, form an acidic environment, and inhibit the growth of harmful bacteria, thereby mitigating intestinal dysfunction (Gu et al. 2019). Therefore, adding fermented *A. membranaceus* to the diet can improve the intestinal tract and enhance the disease resistance of broilers.

Supplementary Information

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Additional file 1 (DOC 1300 KB)

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Author contributions

L.: Project development, data collection, experiment, manuscript writing. K.S.: Statistical analysis, data collection. F.Z.: Data collection, experiment. S.Z.: Data collection, experiment. Q.B.: Project development.

Data availability

All authors declare that the data supporting the findings of this study are available within the article.

Declarations

Competing interests

The authors declare no competing interests.

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