

Screening and Characterization of Probiotics Isolated from Traditional Fermented Products of Ethnic-Minorities in Northwest China and Evaluation Replacing Antibiotics Breeding Effect in Broiler

ZE YE^{1*}, BIN JI¹, YINAN PENG¹, JIE SONG¹, TINGWEI ZHAO¹ and ZHIYE WANG^{1,2}

¹ Key Laboratory of Microbial Resources Exploitation and Application of Gansu Province, Institute of Biology, Gansu Academy of Sciences, Lanzhou, China

² School of Life Science, Lanzhou University of Technology, Lanzhou, China

Submitted 26 February 2024, accepted 25 May 2024, published online 25 August 2024

Abstract

In this study, *Lactobacillus fermentum* DM7-6 (DM7-6), *Lactobacillus plantarum* DM9-7 (DM9-7), and *Bacillus subtilis* YF9-4 (YF9-4) were isolated from traditional fermented products. The survival rate of DM7-6, DM9-7, and YF9-4 in simulated intestinal gastric fluid reached 61.29%, 44.82%, and 55.26%, respectively. These strains had inhibition ability against common pathogens, and the inhibition zone diameters were more than 7 mm. Antioxidant tests showed these strains had good scavenging capacity for superoxide anion, hydroxyl radical and DPPH, and the total reduction capacity reached 65%. Then DM7-6, DM9-7 and YF9-4 were fed to broilers to study the effects on antioxidant capacity, immune response, biochemical indices, tissue morphology, and gut microbiota. 180 healthy broilers were allocated randomly into six experimental groups. SOD, GSH-Px, and T-AOC in broilers serum were detected, and the results showed probiotics significantly improve antioxidant capacity compared to CK group, while antibiotics showed the opposite result. Besides, IgA, IgM, IgG, TNF- α , and IL-2 indicated it could significantly improve immunity by adding probiotics in broilers diets. However, antibiotics reduced immunoglobulin levels and enhanced inflammation index. Biochemical indicators and tissue morphology showed probiotics had a protective effect on metabolic organs. Gut microbiota analysis proved antibiotics could significantly decrease microbial community diversity and increase the proportion of opportunistic pathogens, while probiotics could improve the diversity of gut microbiota and promote the colonization of beneficial microorganisms. In summary, probiotics DM7-6, DM9-7, and YF9-4 can improve the broiler's health by improving antioxidant capacity and immune function, regulating gut microbiota, and can be used as alternative probiotics for antibiotics-free breeding of broilers.

Key words: antibiotics, broiler breeding, probiotics, antioxidant, gut microbiota

Introduction

In order to reduce costs and improve economic benefits, the modern breeding industry often adopts intensive farming, which leads to the production of harmful gases (ammonia, hydrogen sulfide), particulates (including dust), and airborne biological components (bioaerosols), as well as complex mixtures of volatile organic compounds. Livestock and poultry exposure to these harmful gases can cause respiratory and digestive diseases (Douglas et al. 2018). In the 1950s and 1960s, farms in Europe and the United States began adding antibiotics to animal feed to prevent the risk of infectious livestock diseases (Al-Khalaifah 2018). In China,

the livestock industry is the largest user of antibiotics. It is reported that more than half of the total antibiotic consumption was used in husbandry in 2013 (Shao et al. 2021). The excessive use of antibiotics in livestock and poultry farming will not be completely metabolized. Among them, antibiotic residues in animal products enter the human body to disrupt the balance of gut microbiota, resulting in the risk of antibiotic resistance. In addition, most antibiotics are released into the natural environment by livestock manure and urine. It will promote the proliferation of some bacteria containing antibiotic resistance genes (Zhong et al. 2018; Chi et al. 2022). A large amount of antibiotics accumulated in the environment also promotes the proliferation

* Corresponding author: Ze Ye, Key Laboratory of Microbial Resources Exploitation and Application of Gansu Province, Institute of Biology, Gansu Academy of Sciences, Lanzhou, China; e-mail: yez16@lzu.edu.cn

© 2024 Ze Ye et al.

This work is licensed under the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 License (<https://creativecommons.org/licenses/by-nc-nd/4.0/>).

of some bacteria containing antibiotic-resistance genes. Developed countries have begun to use antibiotic-free farming methods, and China also banned the addition of growth-promoting antibacterial drugs in feed in 2019 (Tian et al. 2021; Liu et al. 2023). It is urgent to seek alternatives to antibiotics to adapt to the environment and ensure economic benefits and healthy growth of animals.

Currently, the commonly used biological substitutes for antibiotics are probiotics, enzyme preparations, Chinese herbs, and extracts (Jouany and Morgavi 2007; Abdallah et al. 2019; Abd El-Hack et al. 2022). Probiotics' molecular and cellular mechanisms against pathogenic bacteria are mainly involved in four aspects. First, probiotics compete with pathogenic bacteria for intestinal mucosal colonization sites to establish an ecological barrier (Lebeer et al. 2008). This is related to S-layer protein (Horie et al. 2002), lipoteichoic acids (Delcour et al. 1999), and exopolysaccharides (Ruas-Madiedo et al. 2006) on the cell membrane of probiotics. Second, probiotics can produce metabolites such as bacteriocin, H_2O_2 (Otero and Nader-Macías 2006), and organic acids to inhibit the growth and reproduction of intestinal pathogens. Nisin is a natural food preservative recognized as safe worldwide, but Nisin can only inhibit G+ bacteria (Flynn et al. 2002; Yi et al. 2010). Organic acids lower the pH value of the gut, and the difference between the pH value inside and outside the cell membrane of pathogenic bacteria can enhance the cytotoxicity of undissociated organic acids (Ogawa et al. 2001). Third, probiotics can enhance the animal's immune response. The literature reports that probiotics activate the relevant lymphoid tissue in the digestive tract mucosa of animals, increase the production of IgA, and induce T, B cells and macrophages to reduce or eliminate the number of disease cells in animals (Thompson-Chagoyán et al. 2005; Ding et al. 2021). Fourth, probiotics such as *Bacillus subtilis* can quickly consume free oxygen to create a strict anaerobic environment when they invade the gastrointestinal tract of mammals. Thus, aerobic pathogens' reproduction is controlled (Zhang et al. 2021). Because probiotics have good pathogen resistance and non-drug residues, all kinds of lactic acid bacteria are widely used in broiler breeding. It has been reported that adding *Lactobacillus fermentum* to the daily feed of broilers has a positive effect on the growth performance and meat quality of broilers (Racines et al. 2023). In modern farming, chicks are incubated artificially to improve efficiency. The newborn chicks and their mothers lack the necessary contact, making them susceptible to pathogenic bacteria due to a lack of beneficial microorganisms in the gut. This can be compensated by adding probiotics to the chicks' feed or water (Khomayezzi and Adewole 2021). Moreover, supplementation of probiotics can

also promote the intestinal absorption of minerals in different ways, maintain poultry bone health, and produce high-quality eggs (Yaqoob et al. 2022).

The gut microbial ecosystem is the largest and most complex microecosystem of animals. Gut microbiota has an important effect on the digestion and absorption of nutrients. Some lactic acid bacteria in the gut can antagonize heavy metals and organic pesticide residues in chyme and increase the absorption of nutrients (Feng et al. 2018). In addition, microorganisms in the family *Trichospiridae*, *Bifidobacteriaceae*, and some others ferment carbohydrates to produce energy and short-chain fatty acids (Chen et al. 2020). Butyric acid is absorbed by colon epithelial cells for energy, while acetic acid and propionic acid are involved in the metabolism and glucose regulation of the liver and peripheral tissues (Asholu et al. 2021). Intestinal epithelium is the first immune barrier of the animal body, and intestinal epithelial tissue cells transfer various intestinal bacterial metabolites to immune cells to promote local or systemic inflammatory reactions. At the molecular level, multiple signaling pathways work together to coordinate cell-cell interactions to achieve intestinal homeostasis, and dysregulation of these signaling cascades is often associated with intestinal disease. For example, the Wnt gradient (Hou et al. 2017; Guiu et al. 2019), epidermal growth factor (Zhao et al. 2020a), and notch signaling (Hou et al. 2020) at the villi junction from the base of the recess are critical to the proliferation and maintenance of intestinal stem cells. Gut microbiota has an impact on the development of the nervous system, especially the hypothalamic-pituitary-adrenal axis (Sheng et al. 2020). Gut microbiota impacts the nervous system's development, especially the hypothalamic-pituitary-adrenal axis (Sheng et al. 2020). The reason is that gut microbiota influences signaling molecule levels, such as the brain-derived neurotrophic factors norepinephrine and tryptophan in different parts of the central nervous system (Sharon et al. 2016). Disruption of the brain-gut axis may cause abnormal neural stimulation of the enteric nervous system and depression.

In northwest China, Gansu province has all the landforms except the sea, and there is a long tradition of making fermented food. The climate of Hexi Corridor is dry, hot, and cold, alternating violently. Meanwhile, the Qinghai-Tibet Plateau is cold, hypoxic, and has intense ultraviolet radiation. The unique diet, culture, and lifestyle of ethnic minorities and the natural screening make all kinds of excellent strains passed down. Compared with low-altitude probiotics, lactic acid bacteria from traditional fermented yak yogurt on the Qinghai-Tibet Plateau have potent antioxidant activity (Feng et al. 2020). In addition, a variety of *Lactobacillus* strains with special biological functions such

as lowering blood lipids, lowering cholesterol, lowering uric acid (Zhao et al. 2022), improving immunity, and alleviating inflammation have been discovered through animal experiments (Ishaq et al. 2021), which have significant development and utilization value in animal husbandry. Therefore, in this research, we isolated probiotics from traditional fermented products of ethnic minorities in the Gansu Province and investigated biochemical and safety tests. Then, we assessed the effects of these probiotics applied to broiler breeding by analyzing the immune response, antioxidant capacity, and gut microbiota composition.

Experimental

Materials and Methods

Sample collection and strain isolation. Yogurt, sweet fermented grains, fermented glutinous rice, and silage from Dongxiang, Baoan, and Yugur ethnic minorities were collected. 1 ml or 1 g of fresh samples were added into a 10 ml centrifuge tube containing 9 ml sterile saline prepared in advance, mixed by vortex oscillation. Then, all samples were diluted by a 10-fold gradient to obtain 10^{-4} , 10^{-5} , and 10^{-6} bacterial suspensions. 100 μ l bacterial suspension with the above three concentrations was coated on MRS agar medium (Solarbio, China) and incubated at 37°C anaerobically for 72 h (E500 Anaerobic workstation; Gene Science, USA). The clones with noticeable morphological differences were selected, and the bacteria were purified by the streak method. The clones were selected, inoculated in 100 ml MRS liquid medium, cultured under anaerobic conditions for 24 h at 37°C, and stored with 20% glycerol (Rhawn, China) at -80°C .

Strain identification. Total bacterial DNA was extracted using a bacterial genomic DNA extraction kit (TIANGEN Biotech(Beijing)Co.,Ltd., China). The extracts were used as templates to amplify bacterial 16S rDNA. PCR products were sent to Shenzhen Huada Biological for sequencing. The results were compared and analyzed by the NCBI BLAST program.

Bacterial artificial gastroenteric fluid tolerance. Artificial gastric juice: 3.0 g/l pepsin was added to sterilized PBS, the pH value was adjusted to 2.5, and a 0.22 μ m sterile filter membrane was used to remove bacteria. Artificial intestinal fluid: 1.0 g/l trypsin and 1.8% bile salt were added to sterilized PBS, the pH value was adjusted to 8.0, and 0.22 μ m sterile filter membrane was used to remove bacteria (Bao et al. 2010).

The strains DM9-7, YF9-4, DM6-2, DM-10, BM7-6, DM7-6, and YM7-6 were incubated with MRS at 37°C anaerobic for 48 h. The plate count method is used to record 0 h, the original microbial number of the liv-

ing bacterium, as N_0 . The bacteria solution was mixed with artificial gastric juice and cultured at 37°C for three hours. Then, the viable bacteria count N_1 was calculated on a plate after ten times gradient dilution. After being cultured for three hours, artificial gastric fluid containing the test bacteria was transferred into the artificial intestinal fluid. After culturing at 37°C for four hours and eight hours, the number of viable bacteria N_2 was measured.

The survival rate was calculated as follows (Bao et al. 2010; Takeda et al. 2011):

$$\text{Survival rate of artificial gastric juice (\%)} = \frac{N_1}{N_0} \times 100\%$$

$$\text{Survival rate of artificial intestinal fluid (\%)} = \frac{N_2}{N_0} \times 100\%$$

N_0 – 0 h viable bacteria number (CFU/ml),

N_1 – the number of viable bacteria (CFU/ml) inoculated with artificial gastric juice for 3 h,

N_2 – number of viable bacteria (CFU/ml) inoculated with artificial intestinal fluid at 4 h or 8 h.

Determination of bacteriostatic activity. This test uses the filter paper diffusion method (Diguță et al. 2020). Samples of 100 μ l *Escherichia coli* (GSICC30504), *Staphylococcus aureus* (GSICC31902), *Salmonella* (GSICC30503) and *Enterobacter sakazakii* (GSICC30522) were coated on the medium and cultured for 24 h, respectively. Sterile filter paper with a diameter of 4 mm was placed in the medium. 20 μ l probiotic suspensions with a concentration of 1.0×10^8 CFU/ml were dropped on round filter paper, and the test was repeated three times. Culture medium was placed in 37°C constant temperature incubator for 72 h under anaerobic condition, and the diameter of antibacterial zone was measured with vernier caliper.

Determination of drug sensitivity. DM9-7, YF9-4, DM6-2, DM-10, BM7-6, DM7-6, and YM7-6 were cultured in MRS, and the concentration of the bacterial solution was adjusted to 1.0×10^8 CFU/ml. Then 100 μ l of the cultured bacterial suspension was taken and coated on MRS agar medium. Three pieces of each of the 10 kinds of drug-sensitive paper were evenly placed in agar medium and cultured in a biochemical incubator at 37°C for 48 h. The diameter of the antibacterial zone was measured with a vernier caliper. The drug-sensitive results were referred to the American clinical laboratory (Humphries et al. 2021).

Determination of antioxidant capacity. Preparation of bacterial suspension: the inoculated amount was 1%, and the medium was cultured at 37°C for 24 h under anaerobic condition, centrifuged at 6,000 rpm for 10 min. The bacteria were collected. After washing the bacteria three times with sterilized PBS, the concentration of the bacteria solution was adjusted to 1.0×10^9 CFU/ml.

(1) 1,1-Diphenyl-2-picrylhydrazyl (DPPH) scavenging ability

2 ml samples were mixed evenly with 2 ml DPPH anhydrous ethanol solution (0.2 mmol/l), reacted at room temperature for 30 min away from light, and centrifuged at 6,000 rpm for 10 min. The absorbance of the supernatant A_1 was measured at 517 nm.

In the blank group, the DPPH anhydrous ethanol solution was replaced by an equal volume of anhydrous ethanol solution. The absorption value is detected as A_0 . In the control group, the sample solution was replaced by an equal volume of blank solvent. The mixture of equal volumes of distilled water and ethanol was blank-zeroed. The absorption value is detected as A_j (Lin and Chang 2000).

The ability to scavenge DPPH free radicals is calculated as follows:

$$\text{Clearance rate (\%)} = \left[1 - \frac{(A_1 - A_0)}{A_0} \right] \times 100\%$$

A_1 – absorbance value of supernatant,

A_0 – absorbance value of blank group,

A_j – absorbance value of control group.

(2) Hydroxyl radical scavenging ability

0.5 ml of 1,10-phenanthroline monohydrate (6 mmol/l), 0.5 ml of FeSO_4 solution (6 mmol/l) and 1.0 ml of PBS solution (pH 7.2) were mixed. Then, a 0.5 ml sample and 0.5 ml 0.1% hydrogen peroxide were added to the system. The total volume was fixed to 4.0 ml with double steaming water. It was incubated at 37°C for 1 h, and sample absorbance A_s was measured at 536 nm. In addition, the sample was replaced with H_2O , and the absorption value was A_0 . Moreover, the H_2O_2 and sample were replaced with H_2O , and the absorption value was A (Luo et al. 2009).

The scavenging rate of hydroxyl radical is calculated as follows:

$$\text{Scavenging rate (\%)} = \frac{(A_s - A_0)}{(A - A_0)} \times 100\%$$

A_s – absorbance value of the sample,

A_0 – replace the sample with H_2O ,

A – Replace H_2O_2 and sample with H_2O .

(3) Superoxide anion scavenging capacity

0.5 ml sample, 1 ml Tris-HCl (150 mmol/l, pH 8.2), 1 ml phloroglucinol (1.2 mmol/l) and 1 ml diethylenetriaminepentaacetic acid (3 mmol/l) were mixed. It was bathed at 25°C for 10 min, and the absorbance A_{11} at 325 nm was measured. In addition, the sample and pyrogallol were replaced with H_2O , and the absorption value was A_{00} . The sample was replaced with H_2O , and the absorption value was A_{01} . Moreover, the pyrogallol was replaced with H_2O , and the absorption value was A_{10} (Zhang et al. 2009).

The calculation formula is as follows:

$$\text{Clearance rate (\%)} = \left[1 - \frac{(A_{11} - A_{10})}{(A_{01} - A_{00})} \right] \times 100\%$$

A_{00} – absorbance value of no sample and pyrogallol,

A_{01} – absorbance value without sample, containing pyrogallol,

A_{10} – absorbance value with sample and no pyrogallol,

A_{11} – absorbance value containing sample and pyrogallol.

(4) Total reducing capacity evaluation

0.5 ml 0.2M PBS solution (pH = 6.6), 0.5 ml 0.1% $\text{K}_3[\text{Fe}(\text{CN})_6]$ solution and 0.5 ml bacteria suspension samples were mixed. It was bathed at 50°C for 20 minutes, then sharply cooled. 0.5 ml 10% trichloroacetic acid was added to the samples, then centrifuged at 3,000 rpm for 10 min to collect supernatant solution. 1 ml 0.1% FeCl_3 solution, 1 ml distilled water, and 1 ml supernatant solution were mixed. It was incubated at room temperature for 10 min, and its absorbance A_s was measured at 700 nm. In addition, the sample was replaced with PBS, and the absorption value was A_b (Wu et al. 2003).

The calculation formula is as follows:

$$\text{Reduction capacity (\%)} = \left[\frac{(A_s - A_b)}{A_b} \right] \times 100\%$$

A_s – absorbance value of sample group,

A_b – absorbance value of the blank group PBS.

Probiotics feed additive. DM7-6, DM9-7, and YF9-4 stored in glycerol tubes were inoculated in an MRS liquid medium for 48 h under anaerobic conditions. MRS bacterial solution was centrifuged at 8,000 rpm for 5 min (Xiangyi, Changsha, China) to get bacterial cells. The precipitate was washed with a sterilized PBS solution. Then, the precipitate was re-suspended with a sterilized skimmed milk powder solution (BD Difco™ Skim Milk; Becton, Dickinson and Company, USA). The re-suspension was freeze-dried in a vacuum (<10 Pa), -45 ~ 65°C for 24 ~ 72 h (Xinbexi Biobase-BK-FD10S; Biobase Biodustry(Shandong), Co., Ltd., China). Moreover, the number of bacteria contained in each gram of bactericide was more than 1×10^8 CFU/g.

Animal experiments. A total of 180 healthy 3-day-old broilers with similar body weight (Zhengda, Lanzhou) were selected for the experiment. After one week of adaptation, they were randomly divided into six treatment groups (Lazic et al. 2018; Šefcová et al. 2021b). CK group: the chicks were fed the basal diet (Keaoxieli, Beijing); AT group: the basal diet supplemented with 100 mg/kg chlortetracycline; BI group: the basal diet supplemented with commercial feed additive (*B. subtilis* 1.5×10^8 CFU/kg); PB group: the basal diet supplemented with 1.5 g/kg *B. subtilis* YF9-4; PL group: the basal diet supplemented with 0.75 g/kg

L. fermentum DM7-6 and 0.75 g/kg *Lactobacillus plantarum* DM9-7; PC group: the basal diet supplemented with 0.5 g/kg *B. subtilis* YF9-4, 0.5 g/kg *L. fermentum* DM7-6 and 0.5 g/kg *L. plantarum* DM9-7.

In this study, every treatment group had a region containing 6 cages. Each cage was a subgroup that owned five broilers independent from the others. The subgroup was considered the experimental unit. For sampling, five subgroups were randomly selected from the six subgroups, and one broiler was randomly selected from each subgroup ($n = 5$). The biochemical results of each broiler were derived from an independent intervention, and therefore, it could not be influenced by other experimental broilers. All broilers were free access to feed and water during the experimental duration. The room temperature was maintained at 33°C for the first 4 d and then reduced by 3°C per week to a final temperature of 24°C.

DNA extraction and processing for sequencing.

The broilers were fed for 42 days. On the day before the end of the experiment, broilers were fasted, and fresh feces were collected in PE tubes. All samples were labeled ($n = 5$ for each experimental group) and transported with dry ice. Fecal bacterial genomic DNA was extracted and subjected to Illumina NovaSeq sequencing (Biomarker Company, China).

In this study, the observed index (species richness index) and Shannon index of α diversity of the community were analyzed with thoroughness. Principal Component Analysis (PCA) was performed for the β diversity of the community. Finally, rigorous statistical methods were used to analyze the differences of intestinal microorganisms in broilers at phylum and genus level, ensuring the validity of the conclusions.

Biochemical analysis of blood. Blood was collected under the right wing of chickens using vacuum blood collection vessels. Then, blood samples were kept at room temperature for 30 min and centrifuged at 3,000 rpm for 10 min to get serum. Serums were stored at -20°C until detection. The broilers were sacrificed by the water drowning method. Immune organs such as the bursa, spleen, and thymus were collected and weighed after dissection. In addition, about 1 cm of liver, kidney, and small intestine was collected. The tissues were rinsed with PBS and fixed with 4% paraformaldehyde immediately. Later, all samples were sent to make physiological sections and stained with hematoxylin and eosin (HE). The morphology of the sections was observed under a microscope (Carl Zeiss Microscopy GmbH, Germany).

Serum biochemical analysis. Alanine transaminase (ALT), aspartate transaminase (AST), creatinine (CR), blood urea nitrogen (BUN), uric acid (UA), malondialdehyde (MDA), and total protein (TP) in serum were determined according to the manufacturer's instructions (Nanjing Jiancheng Nanjing, China). Superoxide

dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), total antioxidant capacity (T-AOC), immunoglobulin A (IgA), immunoglobulin G (IgG), immunoglobulin M (IgM), interleukin-2 (IL-2), interleukin-6 (IL-6), and tumor necrosis factor- α (TNF- α) in serum were measured by commercial kits (Shanghai Enzyme-linked Biotechnology Co., Ltd.) according to the protocols provided. Five samples from each group were selected for independent assay.

Statistical analysis of data and chart drawing.

GraphPad Prism version 8.0 (GraphPad Software, USA, www.graphpad.com) was used to make relevant data graphs. Data were evaluated for normal distribution and homogeneity of variance according to Shapiro-Wilk's and Levene's tests in SPSS® 17.0 software (IBM® SPSS® Statistics for Windows, version 17.0, IBM Corp., USA), respectively. Then, a one-way ANOVA analysis of variance and a Tukey post hoc test was used to analyze the significance between different groups when data were homoscedastic and normally distributed. Welch's ANOVA and Welch's t -test were applied when data were normally distributed and heteroscedastic. The Kruskal-Wallis test and the Mann-Whitney U test were used when data were non-normally distributed (Šefcová et al. 2021b; Šefcová et al. 2023).

Results

Two hundred twenty-eight strains were isolated and purified in this experiment. Based on the growth of the strains, the seven microorganisms were selected for subsequent experiments. DM9-7 (GenBank entry number: MT549680.1) is similar to *L. plantarum* strain MLG5-17, and the similarity reached 100% by blast analysis. YF9-4 (GenBank entry number: MT590663.1) is similar to *B. subtilis* strain IB-22, and the similarity reached 100%. DM6-2 (GenBank entry number: CP049052.1) is similar to *Lactobacillus delbrueckii* subsp. *bulgaricus* strain LJJ, and the similarity reached 100%. DM-10 (GenBank entry number: MT613517.1) is similar to *L. fermentum* strain 2871, and the similarity reached 99.66%. BM7-6 (GenBank entry number: AB559504.2) is similar to *Bifidobacterium saguini*, and the similarity reached 99.89%. DM7-6 (GenBank entry number: MT613608.1) is similar to *L. fermentum* strain 3314, and the similarity reached 100%. YM7-6 (GenBank entry number: NR113173.1) is similar to *B. saguini* DSM 23967, and the similarity reached 99.98%.

As shown in Table I, the survival rate of DM7-6 in artificial gastric fluid (pH 2.5) for 3 h was 79.79%, and that cultured in artificial intestinal fluid (pH 8.0) for 8 h was 61.29%. The survival rate of DM9-7 cultured in artificial gastric fluid for 3 h was 63.22% and cultured in artificial intestinal fluid for 8 h was 44.82%. The survival

Table I
The growth of probiotics in artificial gastric juice and intestinal juice.

	Gastric juice			Intestinal juice			
	0 h (CFU/ml)	3 h (CFU/ml)	Survival rate (%)	7 h (CFU/ml)	Survival rate (%)	11 h (CFU/ml)	Survival rate (%)
BM7-6	$3.0 \pm 0.5 \times 10^8$	$7.8 \pm 0.8 \times 10^7$	26.11	$9.6 \pm 1.0 \times 10^6$	3.2	$8.7 \pm 0.7 \times 10^6$	2.9
DM-10	$2.8 \pm 0.4 \times 10^8$	$1.8 \pm 0.3 \times 10^7$	6.55	$< 10^5$	< 1	$< 10^5$	< 1
DM6-2	$1.7 \pm 0.2 \times 10^9$	$1.4 \pm 0.4 \times 10^8$	7.92	$< 10^5$	< 1	$< 10^5$	< 1
DM7-6	$3.1 \pm 0.4 \times 10^9$	$2.5 \pm 0.6 \times 10^9$	79.79	$1.5 \pm 0.3 \times 10^9$	48.39	$1.9 \pm 0.7 \times 10^9$	61.29
DM9-7	$5.8 \pm 0.9 \times 10^9$	$3.7 \pm 0.8 \times 10^9$	63.22	$2.5 \pm 0.6 \times 10^9$	43.10	$2.6 \pm 0.5 \times 10^9$	44.82
YF9-4	$7.6 \pm 1.0 \times 10^9$	$5.4 \pm 0.7 \times 10^9$	71.05	$4.2 \pm 0.5 \times 10^9$	55.26	$4.2 \pm 1.4 \times 10^9$	55.26
YM7-6	$2.3 \pm 0.5 \times 10^8$	$8.7 \pm 0.8 \times 10^7$	37.14	$2.7 \pm 0.3 \times 10^7$	11.74	$2.3 \pm 0.6 \times 10^7$	10.00

rate of YF9-4 cultured in artificial gastric juice for 3 h was 71.05% and cultured in artificial intestinal fluid for 8 h was 55.26%. The survival rate of the other four strains cultured in artificial gastric fluid for 3 h was 6.55–37.14% and cultured in artificial intestinal fluid for 8 h was 1–10%. Therefore, DM7-6, DM9-7, and YF9-4 strains had good tolerance to artificial gastroenteric fluid.

As shown in Fig. 1, probiotics all had inhibitory effects on different pathogenic bacteria. DM9-7, DM7-6,

and DM-10 had good inhibitory effects on *S. aureus*, *E. coli*, *Salmonella* spp., and *E. sakazakii*, and the diameter of inhibitory zone was over 9 mm. YF9-4 had good inhibition ability against *S. aureus*, *Salmonella* spp., and *E. sakazakii*, and the inhibitory zone diameter was more than 10 mm. BM7-6 had weak inhibition ability against *S. aureus* and *E. sakazakii*, and the inhibitory zone diameter was less than 7 mm. YM7-6 had weak inhibition ability against *E. coli*, *S. aureus* and

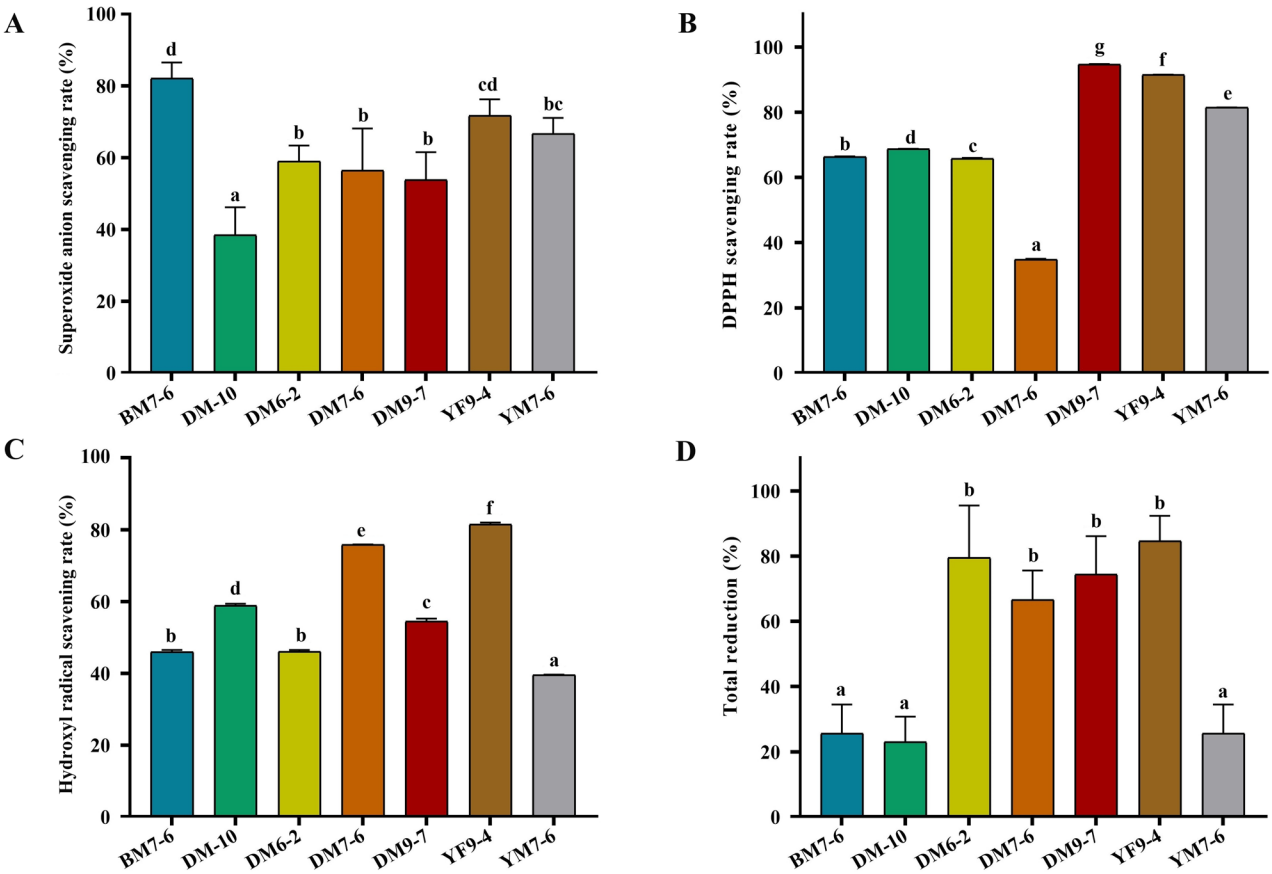


Fig. 1. The antioxidant capacity of strains *in vitro*.

A) Evaluation the scavenging ability of different probiotics on superoxide; B) evaluation the scavenging ability of different probiotics on DPPH; C) evaluation the scavenging capacity of different probiotics on hydroxyl radical; D) evaluation the scavenging capacity of different probiotics on total reduction.

Data are presented as mean $\pm$ standard deviation (n = 3). Significance was marked as different letters above the bars (p < 0.05) between the groups.

Table II
The susceptibility of candidate lactic acid bacteria (LAB) strains to different antibiotics.

Antibiotics	Dose (µg/disc)	Susceptibility						
		BM7-6	DM-10	DM6-2	DM7-6	DM9-7	YF9-4	YM7-6
Erythromycin	15	S	R	I	S	I	R	S
Amikacin	30	S	N	S	N	N	R	R
Ampicillin	10	I	I	I	S	S	S	I
Penicillin	10	S	N	S	S	N	S	N
Chloramphenicol	30	S	S	S	S	S	I	S
Ceftazidime	30	S	R	R	I	I	S	S
Tetracycline	30	S	N	I	S	N	S	R
Ciprofloxacin	5	S	N	N	N	N	I	S
Clindamycin	2	S	R	R	I	N	S	R
Azithromycin	10	S	S	S	S	S	I	S

E. sakazakii. The diameter of the inhibition zone was less than 7 mm.

As shown in Table II, all strains were sensitive to ampicillin, penicillin, chloramphenicol, and azithromycin. DM-10 and YF9-4 were insensitive to erythromycin. YF9-4 and YM7-6 were not sensitive to

amikacin. DM-10 and DM6-2 were insensitive to ceftazidime. DM-10, DM6-2, and YM7-6 were insensitive to clindamycin.

As can be seen from Fig. 2, BM7-6 had the strongest superoxide anion scavenging ability, reaching 82.05%. DM7-6, DM9-7, and YF9-4 had 56.41%, 53.85%, and

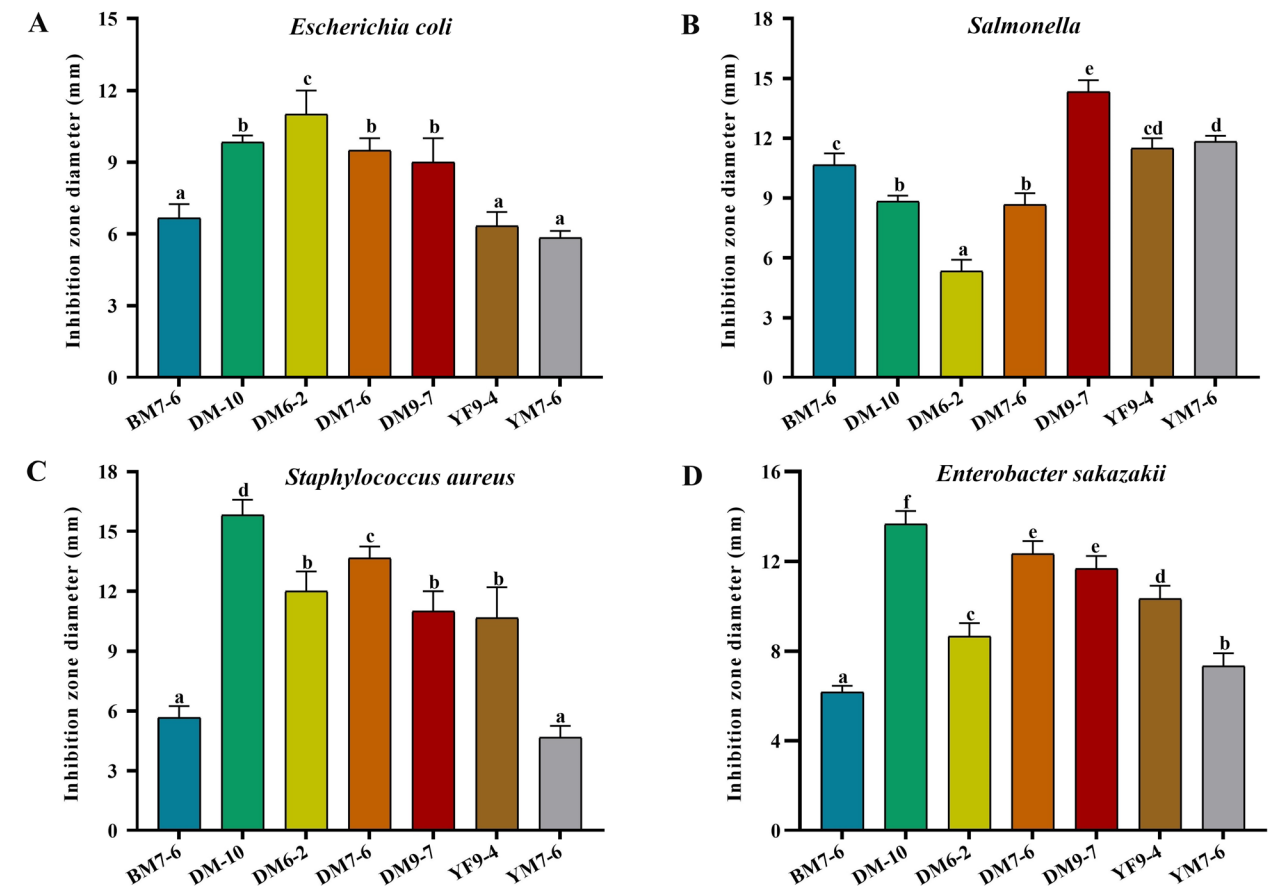


Fig 2. Analysis of antimicrobial ability of the probiotics.

A) Evaluation of antagonistic ability of different probiotics against *Escherichia coli*; B) evaluation of antagonistic ability of different probiotics against *Salmonella* spp.; C) evaluation of antagonistic ability of different probiotics against *Staphylococcus aureus*; D) evaluation of antagonistic ability of different probiotics against *Enterobacter sakazakii*.

Data are presented as mean ± standard deviation (n = 3). Significance was marked as different letters above the bars ($p < 0.05$) between the groups.

71.79%, respectively, and the other strains all had over 40%. DM9-7 had the strongest DPPH scavenging ability (94.70%), DM7-6 had the weakest DPPH scavenging ability (34.92%), and YF9-4 had a scavenging ability of 91.49%. The other strains all reached more than 50%. The hydroxyl radical scavenging ability of YF9-4, DM7-6, and DM9-7 were 81.54%, 75.79%, and 54.45%, respectively, and the other strains reached more than 40%. The total reducing power of YF9-4 was 84.61%, DM7-6, and DM9-7 were 66.67% and 74.36%, respectively.

Oxidative stress is an important physiological activity in animals under stress. As can be seen from Fig. 3,

compared with the control group, the levels of SOD ($p < 0.05$), GSH-Px ($p < 0.05$), and T-AOC ($p < 0.05$) were significantly decreased, and the level of TC ($p < 0.05$) was significantly increased in serum when broilers were fed with antibiotics. Compared to the antibiotic group, the levels of SOD ($p < 0.001$), GSH-Px ($p < 0.05$), and T-AOC ($p < 0.001$) in broilers fed with *B. subtilis* YF9-4 were significantly increased, and the level of TC ($p < 0.01$) was significantly decreased. And compared with the antibiotic group, the addition of *L. fermentum* DM7-6 and *L. plantarum* DM9-7 in broiler feed can increase SOD ($p < 0.05$), and T-AOC ($p < 0.001$) level.

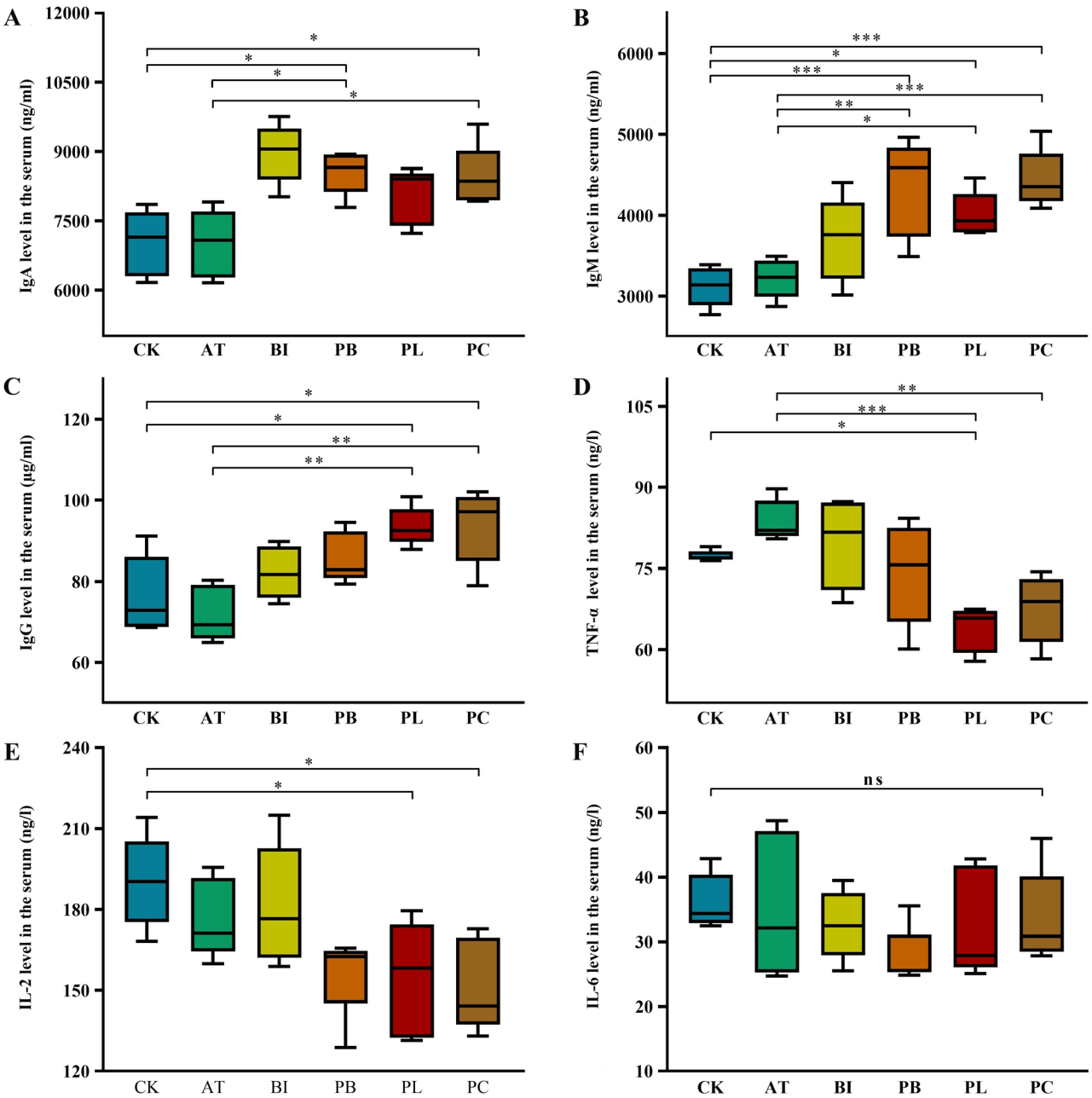


Fig 3. Serum immune indexes of broilers in serum.
A) Levels of IgA in different groups; B) levels of IgG in different groups; C) levels of IgM in different groups; D) levels of TNF-α in different groups; E) levels of IL-2 in different groups; F) levels of IL-6 in different groups.
Data are presented as mean ± standard deviation (n=5). * - $p < 0.05$, ** - $p < 0.01$, *** - $p < 0.001$, ns - non-significance

Compared with the control group and antibiotic group, *B. subtilis* YF9-4 and *L. fermentum* DM7-6 and *L. plantarum* DM9-7 ended to increase serum CAT level, but there was no significant difference ($p>0.05$). Compared with the control group, antibiotic and *B. subtilis* YF9-4 administration had a tendency to reduce serum MDA levels, but there was no significant effect ($p>0.05$). Through the detection of the above indicators, it can be concluded that long-term feeding antibiotics will reduce the body's antioxidant capacity, and probiotics increase the concentration of antioxidant molecules in the blood.

Studies have shown that maintaining an appropriate level of immune activity is essential for the growth

health of broilers. Detecting serum immunoglobulin and immune factors can reflect the body's immune state. It can be seen from Fig. 4 that on the 42nd day, when low concentrations of antibiotics were added to the feed for a long time, compared with the control group, serum levels of IgA, IgG, IgM, TNF- α , IL-2, and IL-6 in broilers serum had no significant changes ($p>0.05$). Compared with the control group and antibiotic group, adding *B. subtilis* YF9-4 in the feed of broilers significantly increased IgA ($p<0.05$), IgM ($p<0.01$), while reducing TNF- α ($p<0.05$), IL-2 ($p<0.05$) level. Similarly, compared with control group and antibiotic group, the addition of *L. fermentum* DM7-6

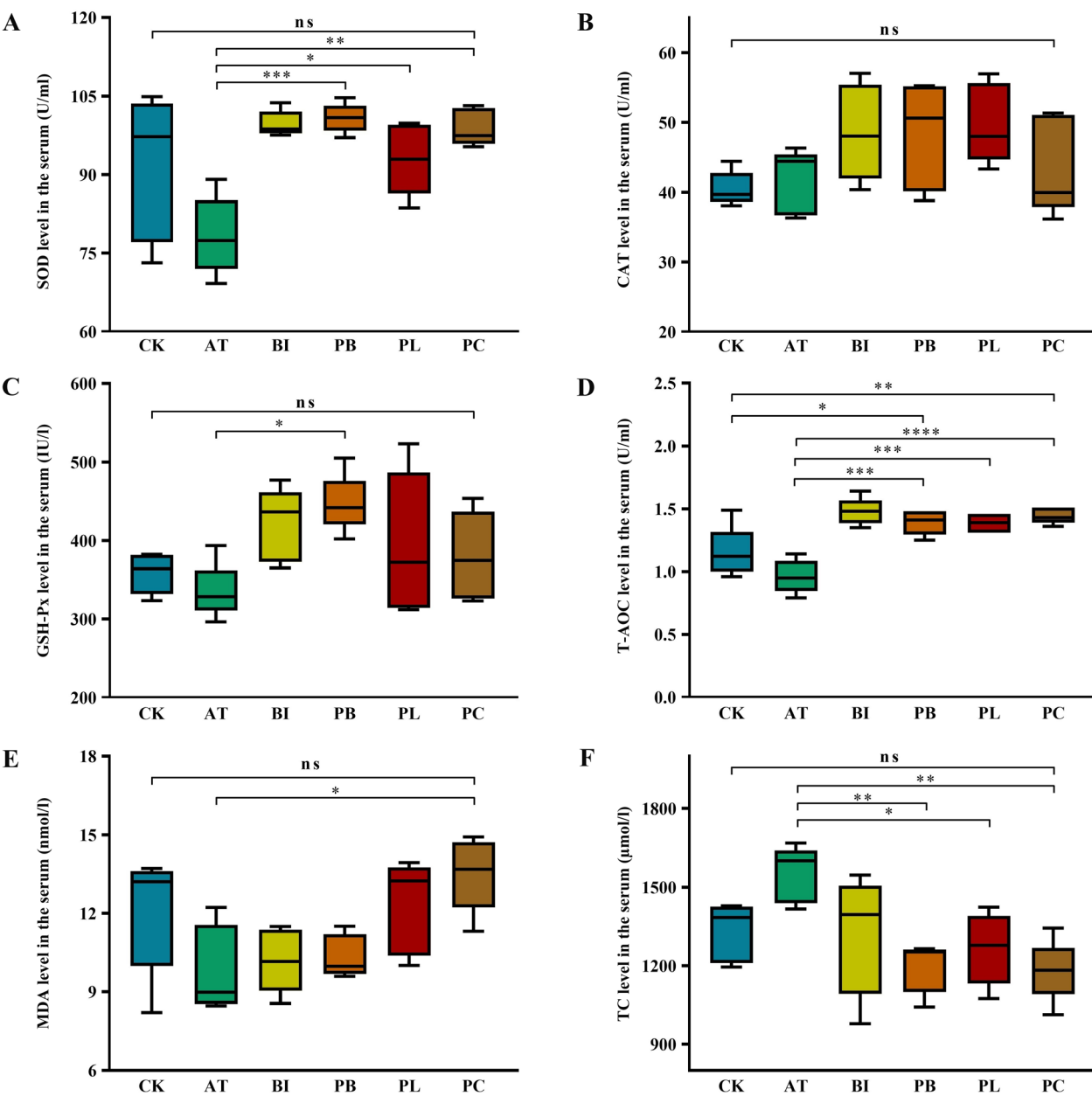


Fig 4. Oxidative stress indexes of broilers in serum.
A) Levels of SOD in different groups; B) levels of CAT in different groups; C) levels of GSH-Px in different groups;
D) levels of T-AOC in different groups; E) levels of MDA in different groups; F) levels of TC in different groups.
Data are presented as mean $\pm$ standard deviation (n=5). * - $p<0.05$, ** - $p<0.01$, *** - $p<0.001$, ns - non-significance

and *L. plantarum* DM9-7 to broiler feed can significantly increase IgA ($p < 0.05$), IgG ($p < 0.01$), and IgM ($p < 0.05$), while reducing TNF- α ($p < 0.05$), IL-2 ($p < 0.05$) level. The supplementation of *B. subtilis* YF9-4 and *L. fermentum* DM7-6 and *L. plantarum* DM9-7 in the diets of broilers had a trend of decreasing serum IL-6 levels, but there was no significant effect ($p > 0.05$). It can be concluded that adding probiotics to feed can increase blood immunoglobulin and decrease the immune factors of broilers.

The liver is the main detoxification organ. In this study, the activity of AST and ALT in the serum of broilers was detected, and the liver cell structure was analyzed by section to understand the pressure of antibiotics and probiotics on the liver. It can be seen from Fig. 5 that on day 42nd, compared with the control group, low-concentration antibiotics did not cause significant changes

in AST and ALT levels ($p > 0.05$). However, *L. fermentum* DM7-6 and *L. plantarum* DM9-7 significantly reduce the serum AST level ($p < 0.05$). Section analysis showed that antibiotic exposure caused some liver damage, including cytoplasmic vacuolation, nuclear shrinkage, and chromatin aggregation. However, probiotics did not cause significant damage to liver cells.

The kidney is the main detoxification organ. In this study, the levels of Cr, BUN, and UA in broilers serum were detected, and sections analyzed the structure of kidney cells to understand the pressure of antibiotics and probiotics on the kidney. It can be seen from Fig. 6 that on day 42nd, compared with the control group, low-concentration antibiotics did not cause significant changes in Cr, BUN, and UA levels ($p > 0.05$). However, *B. subtilis* YF9-4 could significantly reduce the levels of BUN and UA in serum ($p < 0.05$). Combining *B. subtilis*

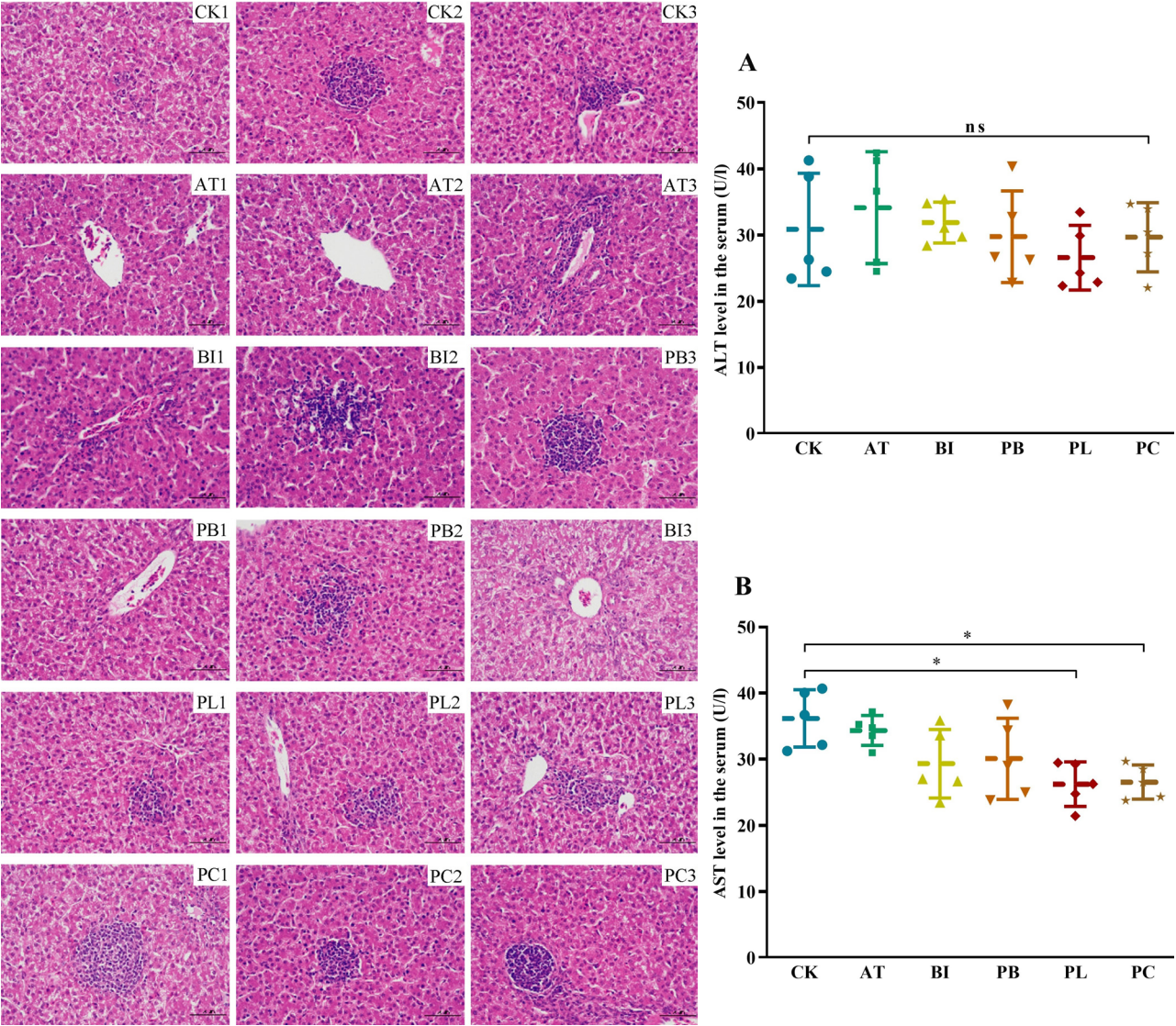


Fig 5. Histopathological sections and biochemical indexes of broilers liver. The liver tissue observed under the following conditions: the eyepiece $\times$ objective = 10×40 , and the scale is $100 \mu\text{m}$. Data are presented as mean $\pm$ standard deviation ($n = 5$). * - $p < 0.05$, ns - non-significance

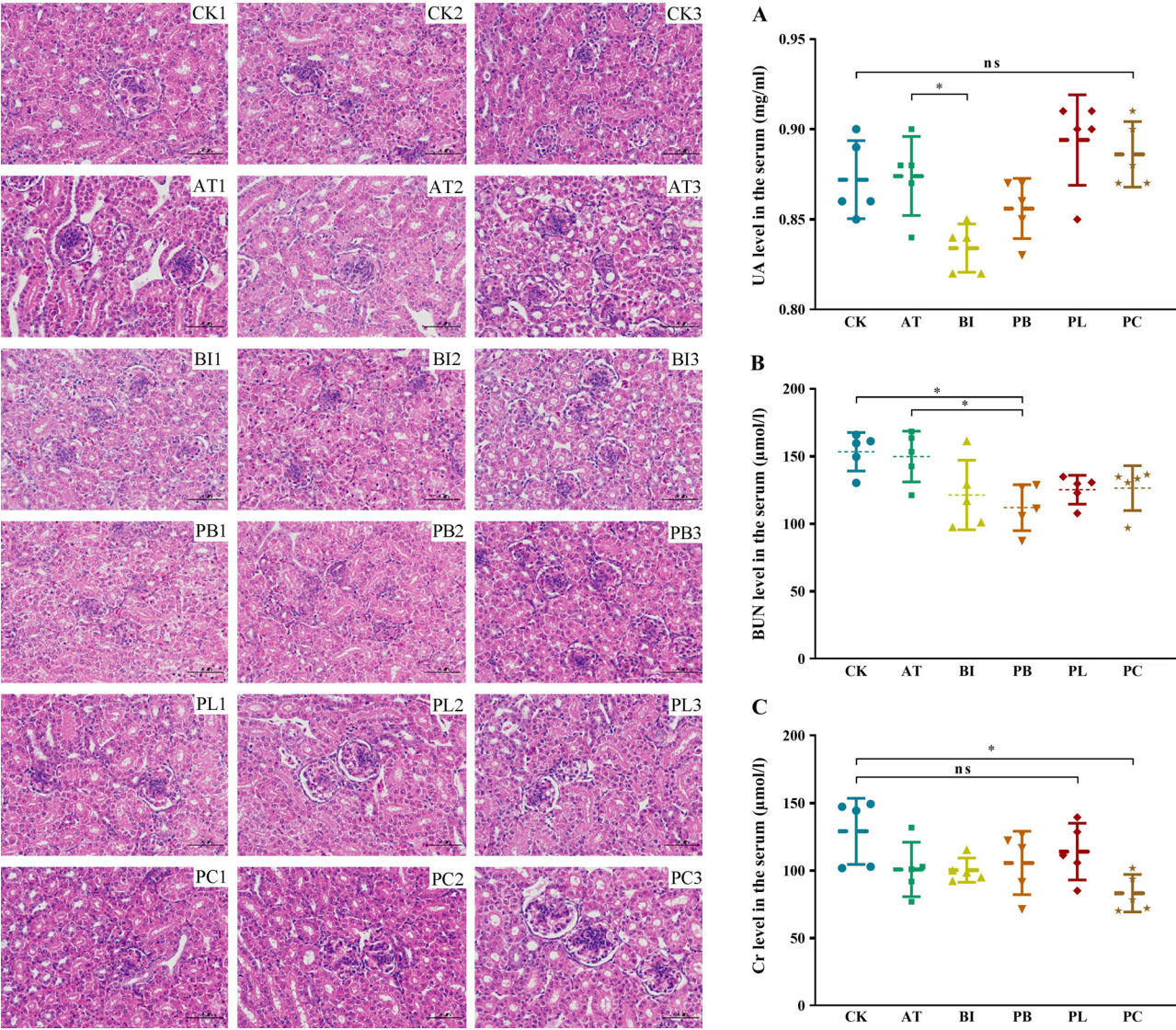


Fig. 6. Histopathological sections and biochemical indexes of broilers kidney. The kidney tissue observed under the following conditions: the eyepiece $\times$ objective = 10×40 , and the scale is $100\mu\text{m}$. Data are presented as mean $\pm$ standard deviation ($n=5$). * - $p<0.05$, ns - non- significance

YF9-4 and *L. fermentum* DM7-6 and *L. plantarum* DM9-7 can significantly reduce serum Cr ($p<0.05$). In section analysis, antibiotic exposure caused kidney damage, including increased cell spacing, tubule contraction, and cell chromatin aggregation. However, probiotics did not cause significant damage to kidney cells, and the kidney tubules were rounded and complete. Antibiotics can be absorbed, and probiotics can colonize the gut. It can affect the morphological structure of intestinal tissue. It can be seen from Fig. 7 that on day 42nd, the intestinal villi of broilers in the antibiotic group were short and thick, the number of intestinal villi per unit area was small and unevenly arranged, and the intestinal glands were separated from the intestinal mucosa. *B. subtilis* YF9-4 and *L. fermentum* DM7-6 and *L. plantarum* DM9-7 can increase the specific surface area of intestinal villi tip. Combined use

of *B. subtilis* YF9-4 and *L. fermentum* DM7-6 and *L. plantarum* DM9-7 can increase the specific surface area of intestinal villi tip. Intestinal villi were fine and long, and the number of intestinal villi per unit area was significantly increased. In this study, Illumina MiSeq sequencing was used to analyze the changes in microbial community structure in the gut of broilers. In Fig. 8A, the Shannon index showed that microbial community diversity decreased significantly after antibiotic intervention ($p<0.0001$), and *L. fermentum* DM7-6 and *L. plantarum* DM9-7 interventions significantly increased microbial community diversity ($p<0.05$). In Fig. 8B, according to the PCA diagram, the intestinal microbial communities of each treatment group were very different, and the microbial communities of the group using probiotics were very similar. Bacteroidetes and Firmicutes

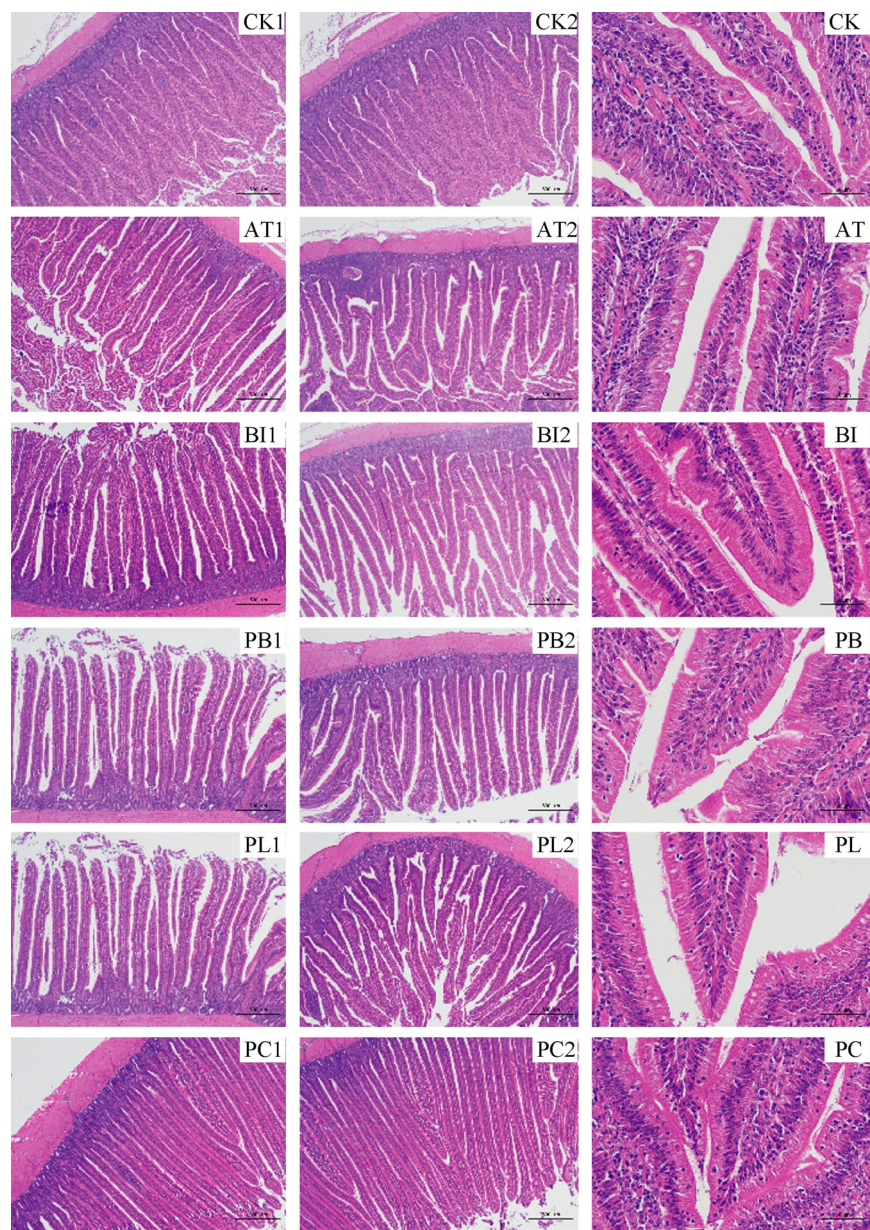


Fig 7. Histopathological sections and biochemical indexes of broilers of the intestinal tissue. The intestinal tissue observed under the following conditions: the eyepiece $\times$ objective = 10×10 , and the scale is $100 \mu\text{m}$.

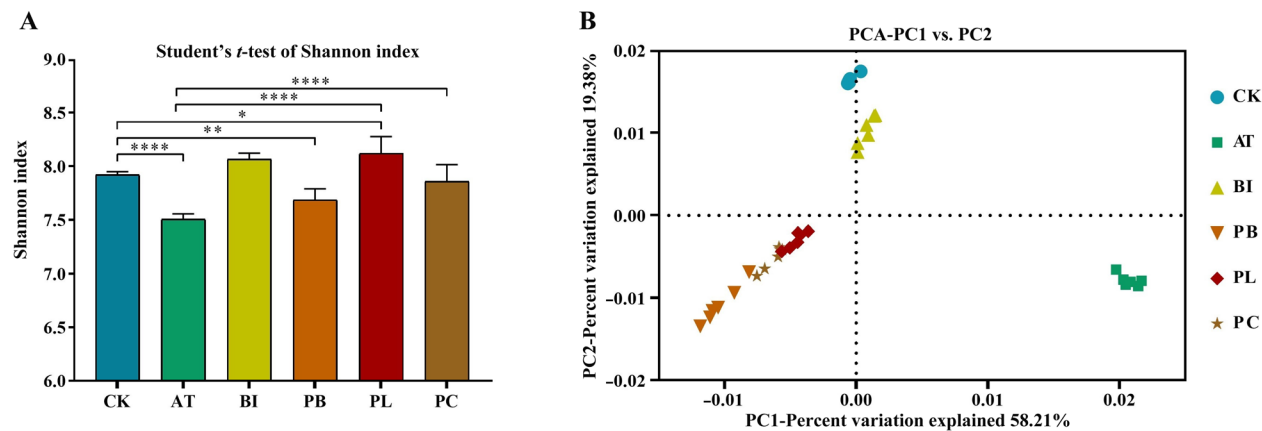


Fig. 8. Effects of daily use of antibiotics and probiotics on gut microbiota of broilers. A) Shannon index in different groups; B) PCA of overall diversity. Data are presented as mean $\pm$ standard deviation ($n=5$). * - $p < 0.05$, ** - $p < 0.01$, *** - $p < 0.001$, **** - $p < 0.0001$

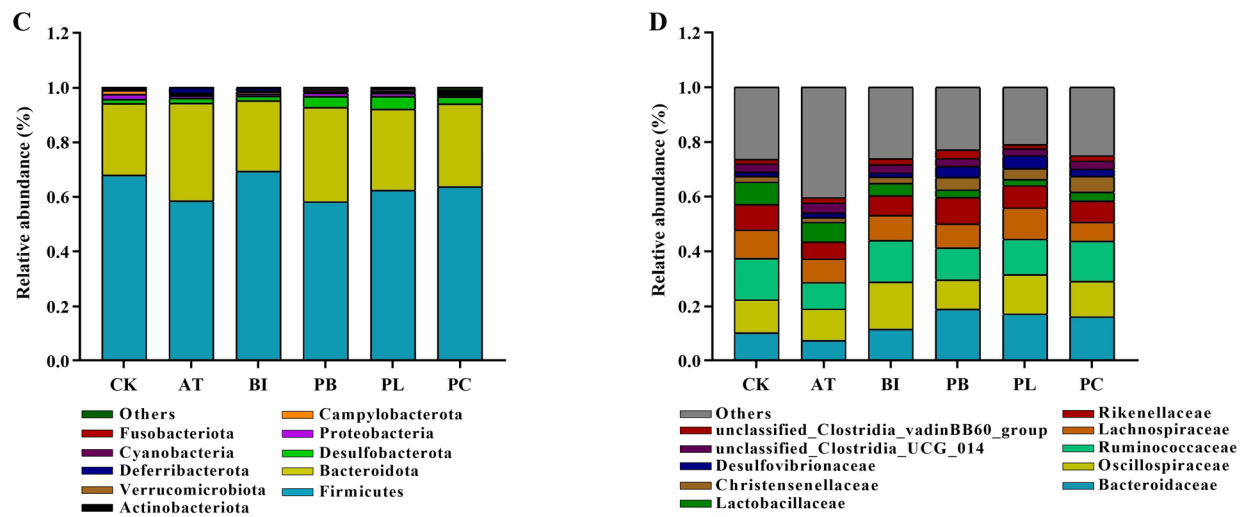


Fig. 8. Effects of daily use of antibiotics and probiotics on gut microbiota of broilers.
C) comparison of phylum relative abundance in different groups; D) comparison of family relative abundance in different groups.
Data are presented as mean $\pm$ standard deviation (n = 5). * - $p < 0.05$, ** - $p < 0.01$, *** - $p < 0.001$, **** - $p < 0.0001$

are the dominant bacterial phyla in the gut of broilers. According to the differences in family levels in Fig. 8C and 8D, it can be concluded that long-term excessive antibiotic intervention can lead to gut microbiota disturbance in broilers.

Fig. 9A shows that compared with the control group and antibiotic group, the abundance of *Lactobacillaceae* in feces of broilers supplemented with probiotics significantly decreased ($p < 0.0001$), and the abundance of *Ruminococcaceae* supplemented with antibiotics also significantly decreased ($p < 0.0001$). Fig. 9C and 9D demonstrate that the proportion of *Desulfovibrionaceae* and *Christensenellaceae* was significantly increased ($p < 0.0001$) when the probiotics DM7-6, DM9-7, and YF9-4 were added to the feed compared with the control group. The proportion of *Alistipes* in broilers feces decreased significantly ($p < 0.0001$) compared to the control diet supplemented with antibiotics as depicted in Fig. 9E.

Discussion

It has been reported that probiotics with different stress resistance functions can be isolated from northwest China. *Limosilactobacillus fermentum* JL-3 isolated from Gansu traditional “Jiangshui” can colonize the intestinal tract of mice to reduce serum uric acid (Wu et al. 2021). *Lactobacillus pentosus* 3-27 from β -cypermethrin (Cyp)-contaminated silage, and the degradation rate of β -Cyp (50 mg/l) in MSM medium after 4 days of culture reached 96% (Liu et al. 2022). In addition, the probiotics isolated from traditional local fermented products also have strong resistance to heavy metals. *Pediococcus acidilactici* BT36 isolated from

Qinghai-Tibet Plateau yogurt, when supplemented in mice for 20 days, can reduce chromate accumulation in liver and tissue damage (Feng et al. 2020). After 12 weeks of human experiments, the yogurt prepared by this strain can reduce the content of copper (34.45%) and nickel (38.34%) in blood higher than that of traditional yogurt (16.41% and 27.57%) (Feng et al. 2022).

The pH of the gastrointestinal of broilers changes dramatically and is rich in various digestive enzymes. In addition, to promote the digestion and absorption of fat (Kudo and Sasaki 2019), the liver secretes a large amount of bile salts into the intestinal lumen. Therefore, the tolerance of probiotics to bile salts and animal intestinal gastric juices is an important indicator to evaluate whether probiotics can be colonized (Monteagudo-Mera et al. 2012). In this study, the survival rates of probiotics DM7-6, DM9-7, and YF9-4 in the artificial digestive system were 61.29%, 44.82%, and 55.26%, respectively. Li et al. (2014) isolated *L. plantarum* DM9218 from Sauerkraut, a traditional fermented food in northeast China, which can survive at pH values as low as 2, could grow well in media supplemented by 0.59 ~ 1.48 mg/ml pepsin and 33.60 ~ 72.32 U/g trypsin. Another research reported that most of the lactic acid bacteria (LAB) isolated from fresh cow and goat milk in Jashore, Bangladesh, could tolerate simulated gastric juices (with and without lysozyme) with pH 2.0 for over 90 min. They can tolerate up to 0.3% bile salts for over 6 h (Reuben et al. 2020). Lactic acid bacteria isolated from Malaysia Kefirs could not survive at pH 2.0, indicating that the samples could not withstand the most acidic conditions of gastric juices. However, most lactic acid bacteria isolated from Kefir samples could tolerate moderate pH levels of pH 3.0 and pH 4.0. Some lactic acid bacteria can tolerate 0.3% and 0.5% bile for 3 h, with survival

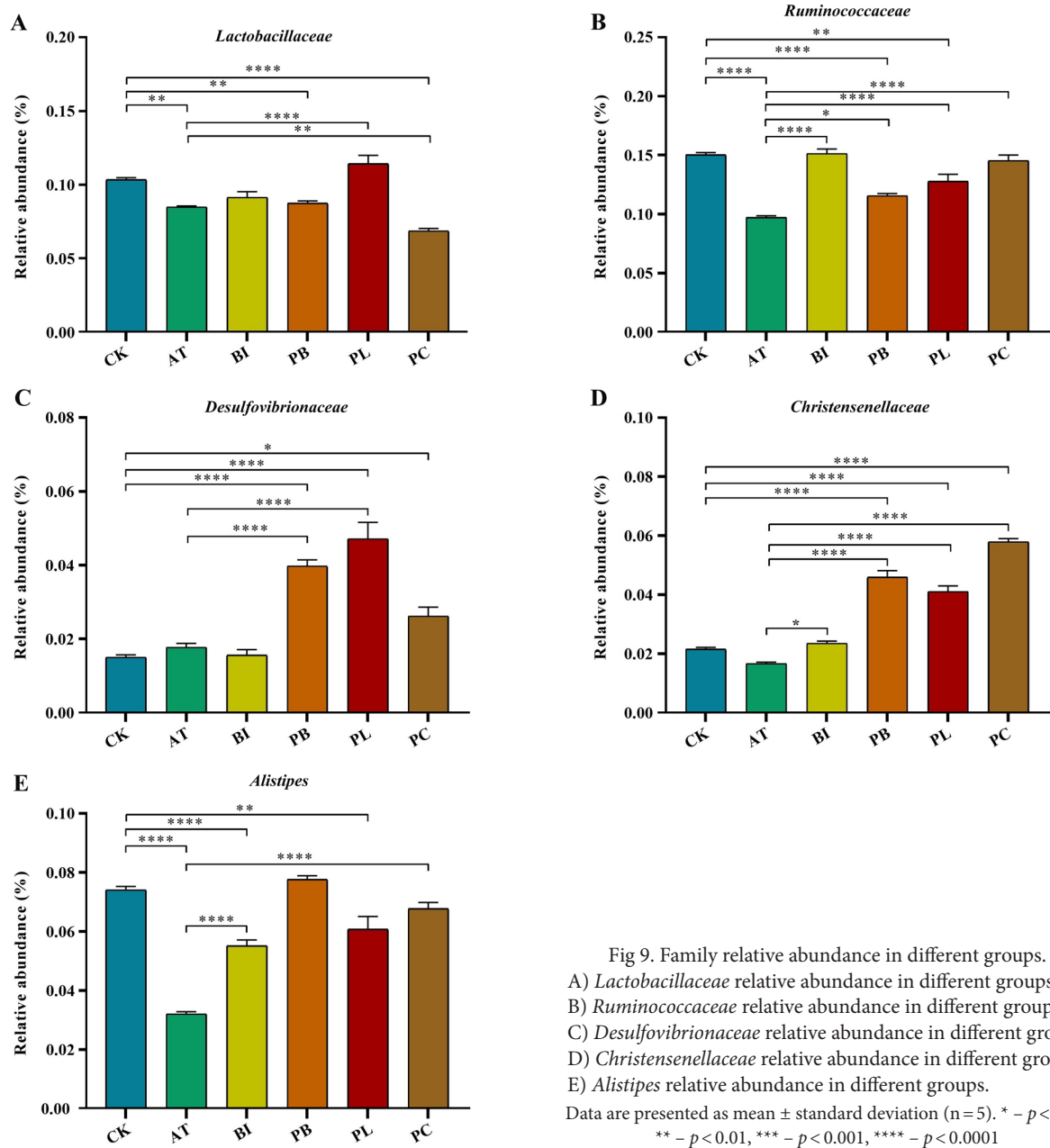


Fig 9. Family relative abundance in different groups. A) *Lactobacillaceae* relative abundance in different groups; B) *Ruminococcaceae* relative abundance in different groups; C) *Desulfovibrionaceae* relative abundance in different groups; D) *Christensenellaceae* relative abundance in different groups; E) *Alistipes* relative abundance in different groups. Data are presented as mean $\pm$ standard deviation (n=5). * - $p < 0.05$, ** - $p < 0.01$, *** - $p < 0.001$, **** - $p < 0.0001$

rates of $96.89 \pm 0.02\%$ and $96.84 \pm 0.02\%$, respectively (Talib et al. 2019). In this study, the digestive system of animals was simulated, which was more in line with the actual situation. The selected probiotics could be used as candidates for the animal breeding industry.

The effect of inhibiting the growth of pathogenic bacteria is often used as an evaluation index of the probiotic properties. Probiotics have auxiliary therapeutic effects on preventing and treating diarrhea, gastrointestinal discomfort, and other diseases (Ren et al. 2022). Studies have reported that *Lactobacillus paracasei* ML07 and *L. paracasei* ML33 isolated and purified from milk and cheese in the Taquari Valley area in southern Brazil have significant inhibitory effects on *Salmonella typhimurium* and *Salmonella enteritidis*. *Lactobacillus parabuchneri* ML58 had a significant inhibitory effect

on *Listeria*, *L. paracasei* CH133 had a significant inhibitory effect on *S. aureus*, *S. typhimurium*, and *E. coli*. *L. parabuchneri* ML58 had a significant inhibitory effect on *S. aureus*, *S. typhimurium*, and *E. coli* (inhibition zone > 10 mm) (Agostini et al. 2018). In another study, *L. reuteri* F03 isolated from feces of breast-fed infants and *L. plantarum* C06 isolated from pickled cabbage showed strong inhibitory ability against *Salmonella* spp. and *E. coli* (inhibition zone > 9 mm). However, most strains isolated from these two materials showed slight inhibitory activity against *Bacillus cereus*, and a few strains showed weak to moderate inhibitory activity against *Streptococcus intestinalis* (Wang et al. 2010). Isolated and purified lactic acid bacteria from Algerian homemade cheeses, 14 strains (B1-B14) were selected based on their anti-*E. coli* and *S. aureus* activi-

ties. These strains also showed antagonism to *Listeria monocytogenes* 161 and *Salmonella* Typhimurium LT2 (Ait Chait et al. 2021). In this study, some of the probiotics showed good antibacterial activity, with the diameter of the antibacterial zone reaching more than 9 mm against the indicator pathogens. However, consistent with previous reports, it was found that the antibacterial effect of a single strain was limited, so different strains could be combined in the later stage to maximize the antibacterial effect of the strain.

There are two views on whether probiotics are sensitive to antibiotics. From the perspective of drug resistance, the application of drug-resistant strains will cause drug-resistant bacteria and affect the prevention and treatment of diseases. From the perspective of stress resistance, probiotics resistant to antibiotics can be used as additives in the feed containing antibiotics (Hashempour-Baltork et al. 2019). The detection of antibiotic sensitivity is the focus of probiotic research, and probiotics for different purposes can be obtained through the analysis of antibiotic sensitivity (Wong et al. 2015). Lactic acid bacteria isolated from north-western Himalaya traditional fermented foods and beverages have all shown resistance to vancomycin, which was due to the presence of D-Ala-D-lactate in their peptidoglycan instead of the normal dipeptide D-Ala-D-Ala (Kumari et al. 2016). The probiotics isolated from Iranian Ahvaz Most Raw milk cheese were sensitive to chloramphenicol and erythromycin (Barzegar et al. 2021). Most of the lactic acid bacteria isolated from traditional Turkish Tulum cheeses were sensitive or intermediate sensitive to the common antibiotic penicillin (Özkan et al. 2021). Most strains of 177 lactic acid bacteria isolated from Ragusano and Pecorino Siciliano cheeses were sensitive to multiple clinical antibiotics (Caggia et al. 2015). Among seven probiotic strains screened in this experiment, YM7-6, DM9-7, DM7-6, and DM6-2 are sensitive to common antibiotics and can be used to develop probiotic additives and other edible products.

Antioxidant activity can effectively regulate the balance of oxidative stress in the body, and the antioxidant activity of probiotics is a hot field of research (Lombardo et al. 2020). Superoxide anion and hydroxyl group are the most aggressive free radicals in intestinal contents, which can attack all biological macromolecules (Bhattacharyya et al. 2014). The total reducing capacity is a comprehensive index to evaluate the antioxidant capacity of the sample. Antioxidant substances can prefer redox reactions with free radicals to protect macromolecular substances from attack. According to literature reports, lactic acid bacteria have strong antioxidant capacity *in vitro* if their free radical scavenging rate of DPPH is higher than 40% (Alsayadi et al. 2013). *L. plantarum* C88 isolated from traditional Chinese

fermented foods at a dose of 10^{10} CFU/ml showed the highest hydroxyl radical and DPPH scavenging activities, with inhibition rates of 44.31% and 53.05% (Li et al. 2012). *L. plantarum* MA2 isolated from Chinese traditional Tibetan kefir grains can tolerate hydrogen peroxide up to 2.0 mM, and its fermentate (fermented supernatant, intact cell, and cell-free extract) had strong reducing capacities, lipid peroxidation inhibition capacities, Fe^{2+} -chelating abilities (Tang et al. 2016; Tang et al. 2017). *L. plantarum* GXL94 can tolerate hydrogen peroxide up to 22 mM, and it could normally grow in MRS with 5 mM H_2O_2 . Meanwhile, eight antioxidant-related genes were found to up-regulate with varying degrees under the H_2O_2 challenge (Zhou et al. 2022). Lactic acid bacteria were isolated from nine Chinese traditional fermented foods, 70 of which had DPPH scavenging (Xu et al. 2016). DM7-6, DM9-7, and YF9-4 of seven probiotic strains screened in this experiment showed that the clearance rates of superoxide anion, hydroxyl, and DPPH were above 40%, and the total reduction capacity was above 60%. The results were consistent with *Limosilactobacillus fermentum* GR-3 isolated from traditional fermentation “Jiangshui” in north-west China (Han et al. 2022). The clearance rates of DDPH, hydroxyl radical and iron chelate of 15 strains purified from fermented food (“Jiangshui” and pickles) or feces were 28.81–82.75%, 6.54–68.52%, and 9.46–17.92%, respectively, and the scavenging activities of superoxide anion exceeded 10% (Hu et al. 2023). These results indicated that probiotics with better stress resistance were more easily isolated in extreme climates.

Modern intensive broiler breeding is highly likely to induce the outbreak of poultry infectious diseases, so farms generally add antibiotics in feed to prevent and treat diseases (Murakami et al. 2015). In China, the residual concentration of various types of veterinary antibiotics is higher than that of other countries, especially tetracycline antibiotics (Fuller 1989). A significant cause of animal diseases is oxidative stress caused by external environmental stimulation. Excessive reactive oxygen species (ROS) destroy the cell structure of carbohydrates, nucleic acids, lipids, and proteins, so removing ROS produced by cell metabolism is necessary. In this study, the use of antibiotics decreased broilers' antioxidant capacity, but probiotics increased the antioxidant effect and avoided excessive energy waste in broilers. *L. plantarum* As21 isolated from traditional fermented Tibetan yak milk can reduce the production of ROS and MDA damage in *Caenorhabditis elegans*. It promoted the production of antioxidants SOD, CAT, and GSH and finally extended the lifespan of *C. caditis* by 34.5% (Li et al. 2022). 1×10^9 CFU/kg *Clostridium butyricum* was added into the broiler feed and given them 42 days. Compared with the control group, the activities of serum T-AOC, SOD, and GSH-Px were

significantly increased ($p < 0.05$). Another study showed that adding yeast alone to broiler feed could significantly increase the activity of GSH-Px in serum but had little effect on SOD and MDA (Li et al. 2021).

Another important cause of animal disease is decreased immunity, so we further evaluated the effects of antibiotics and probiotics on early immune function in broilers. Bao et al. (2022) studied the probiotic effect of *Bacillus amyloliquefaciens* TL106 instead of antibiotics on broilers. Compared with the control group, probiotics could significantly reduce the levels of IL-1 β , IFN- γ , and IL-6 in serum, jejunum, and ileum ($p < 0.05$), which is consistent with the results of this experiment. The combined effect of bacteria and enzymes is another important research direction for replacing antibiotics (Bao et al. 2022). Wang et al. (2022) studied the combined effect of dietary supplementation of *B. amyloliquefaciens* SC06 and glucose oxidase (GOD) on the immune function of broilers. When compared with the control group, treatment with GOD significantly reduced IL-10 and IF- γ levels.

In contrast, combined treatment significantly down-regulated the level of IF- γ in jejunum mucosa and up-regulated the expression of secretory IgA, transforming growth factor- β (TGF- β) and IL-2 (Wang et al. 2022; Šefcová et al. 2023). He et al. (2022) found that dietary supplementation of 400 mg/kg resveratrol in broilers significantly reduced the elevated concentrations of intestinal TNF- α , IL-6, and IL-1 β induced by LPS stimulation ($p < 0.05$). This study found that probiotics can significantly increase the content of immunoglobulin in blood and reduce the content of immune factors, indicating that probiotics can reduce the immune stress response caused by culture environment.

Antibiotics and anti-nutrients in the diet attack liver cells, resulting in the proliferation and infiltration of inflammatory cells and hepatocyte degeneration. In this study, long-term use of low-dose tetracycline also induced the same liver cell damage, but probiotics did not increase the burden on liver cell metabolism as the control group. Khan et al. (2019) administered a complex probiotic prepared by *S. cerevisiae* and *L. acidophilus* to a day-old male broiler in a 42-day experiment and found that liver analysis showed a decrease in MDA levels. Salem et al. (2018) found that the use of biological mycotoxin binders (Nutritox®) can alleviate the pathological changes in both liver and kidney caused by aflatoxins. *Lactobacillus crispatus* 7-4, *Lactobacillus johnsonii* 3-1, and *P. acidilactici* 20-1 were used to microencapsulation and evaluated its regulatory effect on the health of broilers. Microencapsulation could promote growth performance ($p < 0.05$) in the intestine and liver ($p < 0.05$) (Wu et al. 2024). de Souza et al. (2020) found that lactic acid bacteria can alleviate intestinal and liver oxidative stress induced by the

deoxynivalenol diet, reaching the control group's level. Guo et al. (2022) used aflatoxin B₁-degrading enzyme, montmorillonite, and compound probiotics to make triple-action compound mycotoxin detoxifier and prepare for broiler breeding. The immunoreactivity of caspase-3, TNF- α , and NF- κ B induced by aflatoxin B₁-induced caspase-3 was alleviated by immunohistochemical (IHC) of the intestine, liver, and kidney (Guo et al. 2022). Wu et al. (2019) studied the effects of *L. plantarum* 16 and *Paenibacillus polymyxa* 10 on biochemical indices of broilers. The result found that probiotics could significantly reduce the level of MDA in jejunum mucosa and serum ($p < 0.05$). However, liver GSH-Px and jejunum CAT activities were significantly increased ($p < 0.05$) (Wu et al. 2019). Consistent with previous findings, the probiotics used in this study were also not toxic to the liver and had a specific protective effect.

The kidney is also a significant organ vulnerable to the attack of toxic substances. Toxic substances in feed can cause the thickening of the glomerular basement membrane and the increase of stromal cells, the enlargement of glomeruli, the exfoliation of tubular epithelial cells, glomerular collapse, and structural damage of broilers. In this study, the kidney cells of broilers treated with probiotics were healthy, but the antibiotics caused the glomerulus to shrink. According to a comprehensive evaluation (Dai et al. 2022), probiotics can delay the progression of renal damage in diabetic nephropathy patients, and it is affected by intervention time, probiotic dose, and consumption pattern. Deepthi et al. (2017) modified fumonisin B1-induced liver and kidney injury in broilers using *L. plantarum* MYS6. Result found that creatinine recovered significantly, and renal histopathological data further confirmed the overall protective effect of *L. plantarum* MYS6 against FB1-induced cytotoxicity and organ damage in broilers (Deepthi et al. 2017). Another study found that using probiotics could relieve the kidney enlargement caused by aflatoxin B₁ poisoning in broilers (Śliżewska et al. 2019). These data suggest that probiotics not only do not burden kidney cells but also alleviate kidney damage induced by anti-nutrient substances in the feed.

The gut is the main source of nutrient absorption and the primary target of toxic substances. Anti-nutrients in the feed directly destroy intestinal villi cells and cause inflammation. In this study, probiotics promote the growth and development of intestinal villi cells, but antibiotics reduce the villi height/crypt depth ratio of broilers. Another study found that dietary supplementation of 3‰ compound probiotics significantly increases the ratio of intestinal villus height to crypt depth in broilers (Šefcová et al. 2021a; Wu et al. 2022). 0.1% multi-strain probiotic combined with 0.05% *Gardeniase fructus* increased the villus height/crypt depth ratio in ileum of broilers (Chang et al. 2019). The composite

preparation composed of probiotics (*B. subtilis*) and prebiotics (xylo-oligosaccharide and mannano-oligosaccharide) increased the villus height and villus/crypt ratio ($p < 0.05$) (Singh et al. 2022). de Souza et al. (2020) found that the mycotoxin deoxynivalenol decreased the villus height, increased crypt depth, and increased the number of intraepithelial lymphocytes (IEL) in jejunum and ileum. *Lactobacillus* spp. treatments reduced goblet cell counts (de Souza et al. 2020; Šefcová et al. 2023). Broilers fed *Bacillus licheniformis* H2 could significantly improve the ileum and liver pathological injury. Probiotics enhance intestinal barrier function and epithelial cell renewal, reduce energy consumption, and improve enteric nutrient absorption (Zhao et al. 2020b). Probiotics increase the gut's surface area and help digestion and absorption of nutrients in the feed. However, antibiotics, like anti-nutrients in feed, damage intestinal cells and cannot be used in feed for long.

The gastrointestinal tract of animals is directly involved in digesting and absorbing host nutrients and developing the immune system. The development and maturation of the gut microbiota are the key to the healthy growth of broilers. Firmicutes were the first dominant bacteria in this study in the six treatment groups. According to the Shannon index and principal component analysis, antibiotics can reduce the diversity of the intestinal microbial community, and lactic acid bacteria alone can increase the diversity of the intestinal microbial community. Similarly, *Astragalus membranaceus* and *Glycyrrhiza uralensis* were added to broiler feed with higher Shannon index, Chao1 index, and observed species number (Qiao et al. 2022). In addition, the changes in the relative abundance of *Bacteroidaceae* involved in the degradation of complex carbohydrates were more sensitive to the response of probiotics treatment, which was consistent with the studies using xylo-oligosaccharides or sodium butyrate (Deng et al. 2023).

Long-term addition of antibiotics in animal feed increases the abundance of *Lactobacillaceae* in feces, which leads to the loss of a large number of probiotics in the gut of broilers. On the contrary, the number of *Lactobacillaceae* in probiotics groups significantly decreases. It is consistent with studies using *B. amyloliquefaciens* TL106 (Bao et al. 2021). In addition, when *Taraxacum mongolicum* Hand.-Mazz. was used instead of antibiotics, the relative abundance of *Lactobacillus* in the low-dose dandelion group and the antibiotic group tended to increase ($p < 0.05$) (Mao et al. 2022). In this study, the abundance of *Ruminococcaceae* in the antibiotics group was significantly reduced, while the proportion of *Desulfovibrionaceae* and *Christensenellaceae* in probiotics groups was significantly increased. Both probiotics and antibiotics can expel *Alistipes*, but the effect of antibiotics is more visible. In a similar study, *B. amyloliquefaciens* 40 regulated the

microbiome by increasing the abundance of *Desulfovibrio* and reducing the proliferation of pathogens (Jiang et al. 2023). Xu et al. (2022) reduced the abundance of *Ruminococcus* in cecum by treating it with *Bacillus amyloliticus* SC06. Adding *A. membranaceus* and *G. uralensis* to the feed significantly reduced the abundance of *Desulfovibrio* while increasing the abundance of *Ruminococcus* and *Alistipes* (Qiao et al. 2022). The results of gut microbiota changes provided a strategic basis for dietary probiotics DM7-6, DM9-7, and YF9-4 to improve broilers' health status and performance.

Conclusions

The probiotics DM7-6, DM9-7, and YF9-4 isolated from the traditional fermented products of ethnic minorities in Gansu Province had good gastric and intestinal fluid tolerance, strong antioxidant and inhibition ability against common pathogens. Compared with using antibiotics, probiotics can improve the serum oxidative stress index, enhance immunity, and not cause damage to the liver, kidney, and intestinal tissue of broilers. In addition, probiotics can regulate the structure of the entire gut microbiota, promote the excretion of opportunistic pathogens, and increase the colonization of probiotics. These results indicate that this study has uncovered new probiotics for resistant broiler culture.

ORCID

Ze Ye <https://orcid.org/0009-0009-4179-1404>

Availability of data and material

The data implemented to support the results of the study are presented within the manuscript. All data or strains included in this manuscript are available upon request by contacting the corresponding author.

Ethical statement

The operation of the relevant animals involved in the experiments was approved by the Animal Ethics Committee of Lanzhou University of Technology. Throughout the experiment, we conducted the study in strict compliance with all relevant ethical codes and guidelines.

Acknowledgments

We thank the Gansu Provincial Key Laboratory of Microbial Resources Development and Utilization, and Gansu Branch of China Center of Industrial Culture Collection, Institute of Biology, Gansu Academy of Sciences for providing all necessary equipment.

Author contributions

Ze Ye: conceptualization, investigation, data curation, writing – original draft. Bin Ji: conceptualization, molecular biology, data curation and investigation, writing – review and editing. Yinan Peng: writing – review and editing. Jie Song and Tingwei Zhao: validation and formal analysis. Zhiye Wang: supervision and funding acquisition.

Funding

The work was supported by Science and Technology Program of Gansu Province (Grant ID: 22JR11RA210).

Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

Literature

- Abd El-Hack ME, El-Saadony MT, Salem HM, El-Tahan AM, Soliman MM, Youssef GBA, Taha AE, Soliman SM, Ahmed AE, El-Kott AE, et al. Alternatives to antibiotics for organic poultry production: Types, modes of action and impacts on bird's health and production. *Poult Sci.* 2022;101(4):101696. <https://doi.org/10.1016/j.psj.2022.101696>
- Abdallah A, Zhang P, Zhong Q, Sun Z. Application of traditional Chinese Herbal Medicine by-products as dietary feed supplements and antibiotic replacements in animal production. *Curr Drug Metab.* 2019;20(1):54–64. <https://doi.org/10.2174/1389200219666180523102920>
- Agostini C, Eckert C, Vincenzi A, Machado BL, Jordon BC, Kipper JP, Dullius A, Dullius CH, Lehn DN, Sperotto RA, et al. Characterization of technological and probiotic properties of indigenous *Lactobacillus* spp. from south Brazil. *3 Biotech.* 2018;8(11):451. <https://doi.org/10.1007/s13205-018-1469-7>
- Ait Chait Y, Gunenc A, Hosseinian F, Bendali F. Antipathogenic and probiotic potential of *Lactobacillus brevis* strains newly isolated from Algerian artisanal cheeses. *Folia Microbiol.* 2021;66(3):429–440. <https://doi.org/10.1007/s12223-021-00857-1>
- Al-Khalaifah HS. Benefits of probiotics and/or prebiotics for antibiotic-reduced poultry. *Poult Sci.* 2018;97(11):3807–3815. <https://doi.org/10.3382/ps/pey160>
- Alsayadi MSM, Al Jawfi Y, Belarbi M, Sabri FZ. Antioxidant potency of water kefir. *J Microbiol Biotechnol Food Sci.* 2013; 2(6): 2444–2447. <https://office2.jmbfs.org/index.php/JMBFS/article/view/7101>
- Ashaolu TJ, Ashaolu JO, Adeyeye SAO. Fermentation of prebiotics by human colonic microbiota *in vitro* and short-chain fatty acids production: A critical review. *J Appl Microbiol.* 2021;130(3):677–687. <https://doi.org/10.1111/jam.14843>
- Bao C, Zhang W, Wang J, Liu Y, Cao H, Li F, Liu S, Shang Z, Cao Y, Dong B. The effects of dietary *Bacillus amyloliquefaciens* TL106 supplementation, as an alternative to antibiotics, on growth performance, intestinal immunity, epithelial barrier integrity, and intestinal microbiota in broilers. *Animals.* 2022;12(22):3085. <https://doi.org/10.3390/ani12223085>
- Bao CL, Liu SZ, Shang ZD, Liu YJ, Wang J, Zhang WX, Dong B, Cao YH. 2021. *Bacillus amyloliquefaciens* TL106 protects mice against enterohaemorrhagic *Escherichia coli* O157:H7-induced intestinal disease through improving immune response, intestinal barrier function and gut microbiota. *J Appl Microbiol.* 2021;131(1):470–484. <https://doi.org/10.1111/jam.14952>
- Bao Y, Zhang Y, Zhang Y, Liu Y, Wang S, Dong X, Wang Y, Zhang H. Screening of potential probiotic properties of *Lactobacillus fermentum* isolated from traditional dairy products. *Food Control.* 2010; 21(5):695–701. <https://doi.org/10.1016/j.foodcont.2009.10.010>
- Barzegar H, Alizadeh Behbahani B, Falah F. Safety, probiotic properties, antimicrobial activity, and technological performance of *Lactobacillus* strains isolated from Iranian raw milk cheeses. *Food Sci Nutr.* 2021;9(8):4094–4107. <https://doi.org/10.1002/fsn3.2365>
- Bhattacharyya A, Chattopadhyay R, Mitra S, Crowe SE. Oxidative stress: An essential factor in the pathogenesis of gastrointestinal mucosal diseases. *Physiol Rev.* 2014;94(2):329–354. <https://doi.org/10.1152/physrev.00040.2012>
- Caggia C, De Angelis M, Pitino I, Pino A, Randazzo CL. Probiotic features of *Lactobacillus* strains isolated from Ragusano and Pecorino Siciliano cheeses. *Food Microbiol.* 2015;50:109–117. <https://doi.org/10.1016/j.fm.2015.03.010>
- Chang CH, Teng PY, Lee TT, Yu B. Effects of multi-strain probiotics combined with *Gardeniae fructus* on intestinal microbiota, metabolites, and morphology in broilers. *J Poult Sci.* 2019;56(1):32–43. <https://doi.org/10.2141/jpsa.0170179>
- Chen Y, Chang SKC, Zhang Y, Hsu CY, Nannapaneni R. Gut microbiota and short chain fatty acid composition as affected by legume type and processing methods as assessed by simulated *in vitro* digestion assays. *Food Chem.* 2020;312:126040. <https://doi.org/10.1016/j.foodchem.2019.126040>
- Chi S, Xu W, Han Y. ARGs distribution and high-risk ARGs identification based on continuous application of manure in purple soil. *Sci Total Environ.* 2022;853:158667. <https://doi.org/10.1016/j.scitotenv.2022.158667>
- Dai Y, Quan J, Xiong L, Luo Y, Yi B. Probiotics improve renal function, glucose, lipids, inflammation and oxidative stress in diabetic kidney disease: A systematic review and meta-analysis. *Ren Fail Renal Failure.* 2022;44(1):862–880. <https://doi.org/10.1080/0886022X.2022.2079522>
- de Souza M, Baptista AAS, Valdiviezo MJJ, Justino L, Menck-Costa ME, Ferraz CR, da Gloria EM, Verri WA Jr, Bracarense APFRL. *Lactobacillus* spp. reduces morphological changes and oxidative stress induced by deoxynivalenol on the intestine and liver of broilers. *Toxicon.* 2020;185:203–212. <https://doi.org/10.1016/j.toxicon.2020.07.002>
- Deepthi BV, Somashekaraiah R, Poornachandra Rao K, Deepa N, Dharanesha NK, Girish KS, Sreenivasa MY. *Lactobacillus plantarum* MYS6 ameliorates fumonisins B1-induced hepatorenal damage in broilers. *Front Microbiol.* 2017;8:2317. <https://doi.org/10.3389/fmicb.2017.02317>
- Delcour J, Ferain T, Deghorain M, Palumbo E, Hols P. The biosynthesis and functionality of the cell-wall of lactic acid bacteria. In: Konings WN, Kuipers OP, In 't Veld JHJH, editors. *Lactic acid bacteria: Genetics, metabolism and applications*. Dordrecht (The Netherlands): Springer; 1999. p. 159–184. https://doi.org/10.1007/978-94-017-2027-4_7
- Deng F, Tang S, Zhao H, Zhong R, Liu L, Meng Q, Zhang H, Chen L. Combined effects of sodium butyrate and xylo-oligosaccharide on growth performance, anti-inflammatory and antioxidant capacity, intestinal morphology and microbiota of broilers at early stage. *Poult Sci.* 2023;102(5):102585. <https://doi.org/10.1016/j.psj.2023.102585>
- Diguță CF, Nițoi GD, Matei F, Luță G, Cornea CP. The biotechnological potential of *Pediococcus* spp. isolated from Kombucha Microbial Consortium. *Foods.* 2020;9(12):1780. <https://doi.org/10.3390/foods9121780>
- Ding S, Yan W, Ma Y, Fang J. The impact of probiotics on gut health via alternation of immune status of monogastric animals. *Anim Nutr.* 2021;7(1):24–30. <https://doi.org/10.1016/j.aninu.2020.11.004>
- Douglas P, Robertson S, Gay R, Hansell AL, Gant TW. A systematic review of the public health risks of bioaerosols from intensive farming. *Int J Hyg Environ Health.* 2018;221(2):134–173. <https://doi.org/10.1016/j.ijheh.2017.10.019>
- Feng P, Yang J, Zhao S, Ling Z, Han R, Wu Y, Salama ES, Kakade A, Khan A, Jin W, et al. Human supplementation with *Pediococcus acidilactici* GR-1 decreases heavy metals levels through modifying the gut microbiota and metabolome. *NPJ Biofilms Microbiomes.* 2022;8(1):63. <https://doi.org/10.1038/s41522-022-00326-8>

- Feng P, Ye Z, Han H, Ling Z, Zhou T, Zhao S, Virk AK, Kakade A, Abomohra AE, El-Dalatony MM, et al. Tibet plateau probiotic mitigates chromate toxicity in mice by alleviating oxidative stress in gut microbiota. *Commun Biol*. 2020;3(1):242. <https://doi.org/10.1038/s42003-020-0968-3>
- Feng P, Ye Z, Kakade A, Virk AK, Li X, Liu P. A Review on gut remediation of selected environmental contaminants: Possible roles of probiotics and gut microbiota. *Nutrients*. 2018;11(1):22. <https://doi.org/10.3390/nu11010022>
- Flynn S, van Sinderen D, Thornton GM, Holo H, Nes IF, Collins JK. Characterization of the genetic locus responsible for the production of ABP-118, a novel bacteriocin produced by the probiotic bacterium *Lactobacillus salivarius* subsp. *salivarius* UCC118. *Microbiology*. 2002;148(4):973–984. <https://doi.org/10.1099/00221287-148-4-973>
- Fuller R. Probiotics in man and animals. *J Appl Bacteriol*. 1989; 66(5):365–378. <https://doi.org/10.1111/j.1365-2672.1989.tb05105.x>
- Guiu J, Hannezo E, Yui S, Demharter S, Ulyanchenko S, Maimets M, Jørgensen A, Perlman S, Lundvall L, Mamsen LS, et al. Tracing the origin of adult intestinal stem cells. *Nature*. 2019;570(7759):107–111. <https://doi.org/10.1038/s41586-019-1212-5>
- Guo H, Wang P, Liu C, Zhou T, Chang J, Yin Q, Wang L, Jin S, Zhu Q, Lu F. Effects of compound mycotoxin detoxifier on alleviating aflatoxin B₁-induced inflammatory responses in intestine, liver and kidney of broilers. *Toxins*. 2022;14(10):665. <https://doi.org/10.3390/toxins14100665>
- Han R, Khan A, Ling Z, Wu Y, Feng P, Zhou T, Salama ES, El-Dalatony MM, Tian X, Liu P, et al. Feed-additive *Limosilactobacillus fermentum* GR-3 reduces arsenic accumulation in *Procambarus clarkii*. *Ecotoxicol Environ Saf*. 2022;231:113216. <https://doi.org/10.1016/j.ecoenv.2022.113216>
- Hashempour-Baltork F, Hosseini H, Shojaei-Aliabadi S, Torbati M, Alizadeh AM, Alizadeh M. Drug resistance and the prevention strategies in food borne bacteria: An update review. *Adv Pharm Bull*. 2019;9(3):335–347. <https://doi.org/10.15171/apb.2019.041>
- He Z, Li Y, Xiong T, Nie X, Zhang H, Zhu C. Effect of dietary resveratrol supplementation on growth performance, antioxidant capacity, intestinal immunity and gut microbiota in yellow-feathered broilers challenged with lipopolysaccharide. *Front Microbiol*. 2022;13:977087. <https://doi.org/10.3389/fmicb.2022.977087>
- Horie M, Ishiyama A, Fujihira-Ueki Y, Sillanpää J, Korhonen TK, Toba T. Inhibition of the adherence of *Escherichia coli* strains to basement membrane by *Lactobacillus crispatus* expressing an S-layer. *J Appl Microbiol*. 2002;92(3):396–403. <https://doi.org/10.1046/j.1365-2672.2002.01539.x>
- Hou Q, Huang J, Ayansola H, Masatoshi H, Zhang B. Intestinal stem cells and immune cell relationships: Potential therapeutic targets for inflammatory bowel diseases. *Front Immunol*. 2020;11: 623691. <https://doi.org/10.3389/fimmu.2020.623691>
- Hou Q, Ye L, Huang L, Yu Q. The research progress on intestinal stem cells and its relationship with intestinal microbiota. *Front Immunol*. 2017;8:599. <https://doi.org/10.3389/fimmu.2017.00599>
- Hu Y, Zhao Y, Jia X, Liu D, Huang X, Wang C, Zhu Y, Yue C, Deng S, Lyu Y. Lactic acid bacteria with a strong antioxidant function isolated from “Jiangshui”, pickles, and feces. *Front Microbiol*. 2023;14:1163662. <https://doi.org/10.3389/fmicb.2023.1163662>
- Humphries R, Bobenchik AM, Hindler JA, Schuetz AN. Overview of changes to the clinical and laboratory standards institute performance standards for *Antimicrobial Susceptibility Testing*, M100, 31st Edition. *J Clin Microbiol*. 2021;59(12):e0021321. <https://doi.org/10.1128/jcm.00213-21>
- Ishaq M, Khan A, Bacha AS, Shah T, Hanif A, Ahmad AA, Ke W, Li F, Ud Din A, Ding Z, et al. Microbiota targeted interventions of probiotic *Lactobacillus* as an anti-ageing approach: A review. *Antioxidants*. 2021;10(12):1930. <https://doi.org/10.3390/antiox10121930>
- Jiang Z, Su W, Li W, Wen C, Du S, He H, Zhang Y, Gong T, Wang X, Wang Y, et al. *Bacillus amyloliquefaciens* 40 regulates piglet performance, antioxidant capacity, immune status and gut microbiota. *Anim Nutr*. 2023;12:116–127. <https://doi.org/10.1016/j.aninu.2022.09.006>
- Jouany JP, Morgavi DP. Use of ‘natural’ products as alternatives to antibiotic feed additives in ruminant production. *Animal*. 2007; 1(10):1443–1466. <https://doi.org/10.1017/S1751731107000742>
- Khan AZ, Khan IU, Khan S, Afzal S, Hamid M, Tariq M, Haq IU, Ullah N, Khan MA, Bilal S, et al. Selenium-enriched probiotics improve hepatic protection by regulating pro-inflammatory cytokines and antioxidant capacity in broilers under heat stress conditions. *J Adv Vet Anim Res*. 2019;6(3):355–361. <https://doi.org/10.5455/javar.2019.f354>
- Khomayez R, Adewole D. Probiotics, prebiotics, and synbiotics: An overview of their delivery routes and effects on growth and health of broiler chickens. *Worlds Poult Sci J*. 2021;78(1):57–81. <https://doi.org/10.1080/00439339.2022.1988804>
- Kudo H, Sasaki Y. Intracellular pH determination for the study of acid tolerance of lactic acid bacteria. In: Kanauchi M, editor. *Lactic acid bacteria. Methods in Molecular Biology*, vol 1887. New York (USA): Humana Press; 2019. p. 33–41. https://doi.org/10.1007/978-1-4939-8907-2_4
- Kumari A, Angmo K, Monika, Bhalla TC. Probiotic attributes of indigenous *Lactobacillus* spp. isolated from traditional fermented foods and beverages of north-western Himalayas using *in vitro* screening and principal component analysis. *J Food Sci Technol*. 2016;53(5):2463–2475. <https://doi.org/10.1007/s13197-016-2231-y>
- Lazic SE, Clarke-Williams CJ, Munafò MR. What exactly is ‘N’ in cell culture and animal experiments? *PLoS Biol*. 2018; 16(4): e2005282. <https://doi.org/10.1371/journal.pbio.2005282>
- Lebeer S, Vanderleyden J, De Keersmaecker SC. Genes and molecules of lactobacilli supporting probiotic action. *Microbiol Mol Biol Rev*. 2008;72(4):728–764. <https://doi.org/10.1128/mmb.00017-08>
- Li M, Yang D, Mei L, Yuan L, Xie A, Yuan J. Screening and characterization of purine nucleoside degrading lactic acid bacteria isolated from Chinese sauerkraut and evaluation of the serum uric acid lowering effect in hyperuricemic rats. *PLoS One*. 2014;9(9):e105577. <https://doi.org/10.1371/journal.pone.0105577>
- Li S, Zhao Y, Zhang L, Zhang X, Huang L, Li D, Niu C, Yang Z, Wang Q. Antioxidant activity of *Lactobacillus plantarum* strains isolated from traditional Chinese fermented foods. *Food Chem*. 2012;135(3):1914–1919. <https://doi.org/10.1016/j.foodchem.2012.06.048>
- Li W, Gao L, Huang W, Ma Y, Muhammad I, Hanif A, Ding Z, Guo X. Antioxidant properties of lactic acid bacteria isolated from traditional fermented yak milk and their probiotic effects on the oxidative senescence of *Caenorhabditis elegans*. *Food Funct*. 2022;13(6):3690–3703. <https://doi.org/10.1039/d1fo03538j>
- Li W, Xu B, Wang L, Sun Q, Deng W, Wei F, Ma H, Fu C, Wang G, Li S. Effects of *Clostridium butyricum* on growth performance, gut microbiota and intestinal barrier function of broilers. *Front Microbiol*. 2021;12:777456. <https://doi.org/10.3389/fmicb.2021.777456>
- Lin MY, Chang FJ. Antioxidative effect of intestinal bacteria *Bifidobacterium longum* ATCC 15708 and *Lactobacillus acidophilus* ATCC 4356. *Dig Dis Sci*. 2000;45(8):1617–1622. <https://doi.org/10.1023/a:1005577330695>
- Liu B, Wang W, Deng Z, Ma C, Wang N, Fu C, Lambert H, Yan F. Antibiotic governance and use on commercial and smallholder farms in eastern China. *Front Vet Sci*. 2023;10:1128707. <https://doi.org/10.3389/fvets.2023.1128707>
- Liu F, Bai J, Huang W, Li F, Ke W, Zhang Y, Xie D, Zhang B, Guo X. Characterization of a novel beta-cypermethrin-degrading strain of *Lactobacillus pentosus* 3-27 and its effects on bioremediation

- and the bacterial community of contaminated alfalfa silage. *J Hazard Mater.* 2022;423(Pt_A):127101. <https://doi.org/10.1016/j.jhazmat.2021.127101>
- Lombardo SM, Vindedahl AM, Arnold WA. Determination of hydroxyl radical production from sulfide oxidation relevant to sulfidic porewaters. *ACS Earth Space Chem.* 2020;4(2):261–271. <https://doi.org/10.1021/acsearthspacechem.9b00297>
- Luo Y, Chen G, Li B, Ji B, Guo Y, Tian F. Evaluation of antioxidative and hypolipidemic properties of a novel functional diet formulation of *Auricularia auricula* and Hawthorn. *Innovative Food Sci Emerging Technol.* 2009;10(2):215–221. <https://doi.org/10.1016/j.ifset.2008.06.004>
- Mao J, Wang Y, Wang W, Duan T, Yin N, Guo T, Guo H, Liu N, An X, Qi J. Effects of *Taraxacum mongolicum* Hand.-Mazz. (dandelion) on growth performance, expression of genes coding for tight junction protein and mucin, microbiota composition and short chain fatty acids in ileum of broiler chickens. *BMC Vet Res.* 2022; 18(1):180. <https://doi.org/10.1186/s12917-022-03278-5>
- Monteagudo-Mera A, Rodríguez-Aparicio L, Rúa J, Martínez-Blanco H, Navasa N, García-Armesto MR, Ferrero MÁ. *In vitro* evaluation of physiological probiotic properties of different lactic acid bacteria strains of dairy and human origin. *J Funct Foods.* 2012; 4(2):531–541. <https://doi.org/10.1016/j.jff.2012.02.014>
- Murakami Y, Kawata A, Katayama T, Fujisawa S. Anti-inflammatory activity of the artificial antioxidants 2-*tert*-butyl-4-methoxyphenol (BHA), 2,6-di-*tert*-butyl-4-methylphenol (BHT) and 2,4,6-tri-*tert*-butylphenol (TBP), and their various combinations. *In Vivo.* 2015;29(2):197–206.
- Ogawa M, Shimizu K, Nomoto K, Tanaka R, Hamabata T, Yamasaki S, Takeda T, Takeda Y. Inhibition of *in vitro* growth of Shiga toxin-producing *Escherichia coli* O157:H7 by probiotic *Lactobacillus* strains due to production of lactic acid. *Int J Food Microbiol.* 2001;68(1):135–140. [https://doi.org/10.1016/s0168-1605\(01\)00465-2](https://doi.org/10.1016/s0168-1605(01)00465-2)
- Otero MC, Nader-Macías ME. Inhibition of *Staphylococcus aureus* by H₂O₂-producing *Lactobacillus gasseri* isolated from the vaginal tract of cattle. *Anim Reprod Sci.* 2006;96(1):35–46. <https://doi.org/10.1016/j.anireprosci.2005.11.004>
- Özkan ER, Demirci T, Öztürk H, Akın N. Screening *Lactobacillus* strains from artisanal Turkish goatskin casing Tulum cheeses produced by nomads via molecular and *in vitro* probiotic characteristics. *J Sci Food Agric.* 2020;101(7):2799–2808. <https://doi.org/10.1002/jsfa.10909>
- Qiao Y, Liu C, Guo Y, Zhang W, Guo W, Oleksandr K, Wang Z. Polysaccharides derived from *Astragalus membranaceus* and *Glycyrrhiza uralensis* improve growth performance of broilers by enhancing intestinal health and modulating gut microbiota. *Poult Sci.* 2022;101(7):101905. <https://doi.org/10.1016/j.psj.2022.101905>
- Racines MP, Solis MN, Šefcová MA, Herich R, Larrea-Álvarez M, Revajová V. An overview of the use and applications of *Limosilactobacillus fermentum* in broiler chickens. *Microorganisms.* 2023;11(8). <https://doi.org/10.3390/microorganisms11081944>
- Ren Z, Hong Y, Huo Y, Peng L, Lv H, Chen J, Wu Z, Wan C. Prospects of probiotic adjuvant drugs in clinical treatment. *Nutrients.* 2022;14(22):4723. <https://doi.org/10.3390/nu14224723>
- Reuben RC, Roy PC, Sarkar SL, Rubayet UI Alam ASM, Jahid IK. Characterization and evaluation of lactic acid bacteria from indigenous raw milk for potential probiotic properties. *J Dairy Sci.* 2020; 103(2):1223–1237. <https://doi.org/10.3168/jds.2019-17092>
- Ruas-Madiedo P, Gueimonde M, Margolles A, de los Reyes-Gavilán CG, Salminen S. Exopolysaccharides produced by probiotic strains modify the adhesion of probiotics and enteropathogens to human intestinal mucus. *J Food Prot.* 2006;69(8):2011–2015. <https://doi.org/10.4315/0362-028X-69.8.2011>
- Salem R, El-Habashi N, Fadl SE, Sakr OA, Elbially ZI. Effect of probiotic supplement on aflatoxicosis and gene expression in the liver of broiler chicken. *Environ Toxicol Pharmacol.* 2018;60:118–127. <https://doi.org/10.1016/j.etap.2018.04.015>
- Šefcová MA, Larrea-Álvarez M, Larrea-Álvarez CM, Karařfiová V, Ortega-Paredes D, Vinueza-Burgos C, Ševčíková Z, Levkut M, Herich R, Revajová V. The probiotic *Lactobacillus fermentum* Bio-cenol CCM 7514 moderates *Campylobacter jejuni*-induced body weight impairment by improving gut morphometry and regulating cecal cytokine abundance in broiler chickens. *Animals.* 2021a; 11(1):235. <https://doi.org/10.3390/ani11010235>
- Šefcová MA, Ortega-Paredes D, Larrea-Álvarez CM, Mina I, Guapás V, Ayala-Velasteguí D, Leoro-Garzón P, Molina-Cuasapaz G, Vinueza-Burgos C, Revajová V, et al. Effects of *Lactobacillus fermentum* administration on intestinal morphometry and antibody serum levels in *Salmonella*-infantis-challenged chickens. *Microorganisms.* 2023;11(2):256. <https://doi.org/10.3390/microorganisms11020256>
- Šefcová MA, Santacruz F, Larrea-Álvarez CM, Vinueza-Burgos C, Ortega-Paredes D, Molina-Cuasapaz G, Rodríguez J, Calero-Cáceres W, Revajová V, Fernández-Moreira E, et al. Administration of dietary microalgae ameliorates intestinal parameters, improves body weight, and reduces thawing loss of fillets in broiler chickens: A pilot study. *Animals.* 2021b;11(12):3601. <https://doi.org/10.3390/ani11123601>
- Shao Y, Wang Y, Yuan Y, Xie Y. A systematic review on antibiotics misuse in livestock and aquaculture and regulation implications in China. *Sci Total Environ.* 2021;798:149205. <https://doi.org/10.1016/j.scitotenv.2021.149205>
- Sharon G, Sampson TR, Geschwind DH, Mazmanian SK. The central nervous system and the gut microbiome. *Cell.* 2016; 167(4): 915–932. <https://doi.org/10.1016/j.cell.2016.10.027>
- Sheng JA, Bales NJ, Myers SA, Bautista AI, Roueinfar M, Hale TM, Handa RJ. The hypothalamic-pituitary-adrenal axis: Development, programming actions of hormones, and maternal-fetal interactions. *Front Behav Neurosci.* 2020;14:601939. <https://doi.org/10.3389/fnbeh.2020.601939>
- Singh AK, Tiwari UP, Mishra B, Jha R. Effects of *in ovo* delivered xylo- and mannan- oligosaccharides on growth performance, intestinal immunity, cecal short-chain fatty acids, and cecal microbiota of broilers. *J Anim Sci Biotechnol.* 2022;13(1):13. <https://doi.org/10.1186/s40104-021-00666-z>
- Śliżewska K, Cukrowska B, Smulikowska S, Cielecka-Kuszyk J. The effect of probiotic supplementation on performance and the histopathological changes in liver and kidneys in broiler chickens fed diets with aflatoxin B₁. *Toxins.* 2019;11(2):112. <https://doi.org/10.3390/toxins11020112>
- Takeda S, Yamasaki K, Takeshita M, Kikuchi Y, Tsend-Ayush C, Dashnyam B, Ahhmed AM, Kawahara S, Muguruma M. The investigation of probiotic potential of lactic acid bacteria isolated from traditional Mongolian dairy products. *Anim Sci J.* 2011;82(4):571–579. <https://doi.org/10.1111/j.1740-0929.2011.00874.x>
- Talib N, Mohamad NE, Yeap SK, Hussin Y, Aziz MNM, Masarudin MJ, Sharifuddin SA, Hui YW, Ho CL, Alitheen NB. Isolation and characterization of *Lactobacillus* spp. from kefir samples in Malaysia. *Molecules.* 2019;24(14):2606. <https://doi.org/10.3390/molecules24142606>
- Tang W, Xing Z, Hu W, Li C, Wang J, Wang Y. Antioxidative effects *in vivo* and colonization of *Lactobacillus plantarum* MA2 in the murine intestinal tract. *Appl Microbiol Biotechnol.* 2016; 100(16): 7193–71202. <https://doi.org/10.1007/s00253-016-7581-x>
- Tang W, Xing Z, Li C, Wang J, Wang Y. Molecular mechanisms and *in vitro* antioxidant effects of *Lactobacillus plantarum* MA2. *Food Chem.* 2017;221:1642–1649. <https://doi.org/10.1016/j.foodchem.2016.10.124>

- Thompson-Chagoyán OC, Maldonado J, Gil A. Aetiology of inflammatory bowel disease (IBD): Role of intestinal microbiota and gut-associated lymphoid tissue immune response. *Clin Nutr*. 2005;24(3):339–352. <https://doi.org/10.1016/j.clnu.2005.02.009>
- Tian M, He X, Feng Y, Wang W, Chen H, Gong M, Liu D, Clarke JL, van Eerde A. Pollution by antibiotics and antimicrobial resistance in livestock and poultry manure in China, and counter-measures. *Antibiotics*. 2021;10(5):539. <https://doi.org/10.3390/antibiotics10050539>
- Wang CY, Lin PR, Ng CC, Shyu YT. Probiotic properties of *Lactobacillus* strains isolated from the feces of breast-fed infants and Taiwanese pickled cabbage. *Anaerobe*. 2010;16(6):578–85. <https://doi.org/10.1016/j.anaerobe.2010.10.003>
- Wang Y, Wang B, Zhan X, Wang Y, Li W. Effects of glucose oxidase and its combination with *B. amyloliquefaciens* SC06 on intestinal microbiota, immune response and antioxidative capacity in broilers. *Animal*. 2022;16(3):100473. <https://doi.org/10.1016/j.animal.2022.100473>
- Wong A, Ngu DY, Dan LA, Ooi A, Lim RL. Detection of antibiotic resistance in probiotics of dietary supplements. *Nutr J*. 2015;14:95. <https://doi.org/10.1186/s12937-015-0084-2>
- Wu H, Ding C, Ma X, Gao Z, Liu S, Liu B, Song S. Microencapsulate probiotics (MP) promote growth performance and inhibit inflammatory response in broilers challenged with *Salmonella typhimurium*. *Probiotics Antimicrob Proteins*. 2024;16(2):623–635. <https://doi.org/10.1007/s12602-023-10074-6>
- Wu HC, Chen HM, Shiau CY. Free amino acids and peptides as related to antioxidant properties in protein hydrolysates of mackerel (*Scomber austriasicus*). *Food Res Int*. 2003;36(9):949–957. [https://doi.org/10.1016/S0963-9969\(03\)00104-2](https://doi.org/10.1016/S0963-9969(03)00104-2)
- Wu Y, Wang B, Zeng Z, Liu R, Tang L, Gong L, Li W. Effects of probiotics *Lactobacillus plantarum* 16 and *Paenibacillus polymyxa* 10 on intestinal barrier function, antioxidative capacity, apoptosis, immune response, and biochemical parameters in broilers. *Poult Sci*. 2019;98(10):5028–5039. <https://doi.org/10.3382/ps/pez226>
- Wu Y, Yang F, Jiang W, Hu A, Xiong Z, Yang S, Cao P, Cao Z, Xiong Z, Cao H. Effects of compound probiotics on intestinal barrier function and caecum microbiota composition of broilers. *Avian Pathol*. 2022;51(5):465–475. <https://doi.org/10.1080/03079457.2022.2100740>
- Wu Y, Ye Z, Feng P, Li R, Chen X, Tian X, Han R, Kakade A, Liu P, Li X. *Limosilactobacillus fermentum* JL-3 isolated from “Jiangshui” ameliorates hyperuricemia by degrading uric acid. *Gut Microbes*. 2021;13(1):1–18. <https://doi.org/10.1080/19490976.2021.1897211>
- Xu S, Liu T, Radji CA, Yang J, Chen L. Isolation, identification, and evaluation of new lactic acid bacteria strains with both cellular antioxidant and bile salt hydrolase activities *in vitro*. *J Food Prot*. 2016;79(11):1919–1928. <https://doi.org/10.4315/0362-028X.JFP-16-096>
- Xu S, Wang F, Zou P, Li X, Jin Q, Wang Q, Wang B, Zhou Y, Tang L, Yu D, et al. *Bacillus amyloliquefaciens* SC06 in the diet improves egg quality of hens by altering intestinal microbiota and the effect is diminished by antimicrobial peptide. *Front Nutr*. 2022;9:999998. <https://doi.org/10.3389/fnut.2022.999998>
- Yaqoob MU, Wang G, Wang M. An updated review on probiotics as an alternative of antibiotics in poultry – A review. *Anim Biosci*. 2022;35(8):1109–1120. <https://doi.org/10.5713/ab.21.0485>
- Yi H, Zhang L, Tuo Y, Han X, Du MJFc. A novel method for rapid detection of class IIa bacteriocin-producing lactic acid bacteria. *Food Control*. 2010;21(4):426–430. <https://doi.org/10.1016/j.foodcont.2009.07.002>
- Zhang S, Lv J, Menghe B, Zhang H, Zhang L, Song J, Wang Z. [Resistance of *Lactobacillus casei* subsp. *casei* SY13 and *Lactobacillus delbrueckii* subsp. *bulgaricus* LJ] to reactive oxygen species] (in Chinese). *Acta Microbiol Sin*. 2009;49(2):257–261. <https://doi.org/10.13343/j.cnki.wsxb.2009.02.017>
- Zhang Y, Choi SH, Nogoy KM, Liang S. Review: The development of the gastrointestinal tract microbiota and intervention in neonatal ruminants. *Animal*. 2021;15(8):100316. <https://doi.org/10.1016/j.animal.2021.100316>
- Zhao Q, Guan J, Wang X. Intestinal stem cells and intestinal organoids. *J Genet Genomics*. 2020a;47(6):289–299. <https://doi.org/10.1016/j.jgg.2020.06.005>
- Zhao S, Feng P, Hu X, Cao W, Liu P, Han H, Jin W, Li X. Probiotic *Limosilactobacillus fermentum* GR-3 ameliorates human hyperuricemia via degrading and promoting excretion of uric acid. *iScience*. 2022;25(10):105198. <https://doi.org/10.1016/j.isci.2022.105198>
- Zhao Y, Zeng D, Wang H, Qing X, Sun N, Xin J, Luo M, Khalique A, Pan K, Shu G, et al. Dietary probiotic *Bacillus licheniformis* H2 enhanced growth performance, morphology of small intestine and liver, and antioxidant capacity of broiler chickens against *Clostridium perfringens*-induced subclinical necrotic enteritis. *Probiotics Antimicrob Proteins*. 2020b;12(3):883–895. <https://doi.org/10.1007/s12602-019-09597-8>
- Zhong Y, Chen ZF, Dai X, Liu SS, Zheng G, Zhu X, Liu S, Yin Y, Liu G, Cai Z. Investigation of the interaction between the fate of antibiotics in aquafarms and their level in the environment. *J Environ Manage*. 2018;207:219–229. <https://doi.org/10.1016/j.jenvman.2017.11.030>
- Zhou Y, Gong W, Xu C, Zhu Z, Peng Y, Xie C. Probiotic assessment and antioxidant characterization of *Lactobacillus plantarum* GXL94 isolated from fermented chili. *Front Microbiol*. 2022;13:997940. <https://doi.org/10.3389/fmicb.2022.997940>