Effects of a probiotic on the growth performance, intestinal flora, and immune function of chicks infected with *Salmonella pullorum*

Caicai Chen,[1] Jiayi Li,[1] Hongxing Zhang, Yuanhong Xie, Lixia Xiong, Hui Liu,[2] and Fang Wang[2]

Beijing Engineering Laboratory of Probiotics Key Technology Development, Beijing Laboratory of Food Quality and Safety, Beijing Key Laboratory of Agricultural Product Detection and Control of Spoilage Organisms and Pesticide Residue, Food Science and Engineering College, Beijing University of Agriculture, Beijing, China

ABSTRACT This study investigated the effects of a *Lactobacillus paracasei* KL1 and *Lactobacillus plantarum* subsp. *plantarum* Zhang-LL mixed probiotic on *Salmonella*-caused pullorosis in chicks. A total of 120 1-day-old Nongda no.3 dwarf chicks were randomly assigned to 4 treatments, with 6 replicates of 5 birds each. The treatments were blank group, *Salmonella pullorum*–infected group, probiotic treatment group, and probiotic prevention (**PP**) group. All birds (n = 90) except those in the blank group were infected with *S. pullorum* on day 4. On day 14, the BW, ADG, mortality, pathology of tissue, cecum colony count, immune organ indices, cecal mucosa secretory IgA, and cytokines were investigated. The results showed that the chicks infected with *S. pullorum* were depressed and their BW reduced. The PP group had the highest ADG and lowest mortality rate (0%), whereas the *S. pullorum*–infected group had 37.50% mortality rate and lowest ADG. Pathologic sections showed that the probiotic treatment group had minor lesions but the PP group had no lesions in the ileum, cecum, and liver. Cecal *Lactobacillus* counts was the highest ($P < 0.05$) and *Salmonella* and *Escherichia coli* counts were the lowest ($P < 0.05$) in the PP group; Compared with the *S. pullorum*–infected group, the thymus and spleen indexes of the probiotic treatment group increased ($P < 0.05$), but they were unaffected ($P > 0.05$) in the bursa of Fabricius, whereas in the PP group, all the immune organs were increased ($P < 0.05$). Cecal mucosa secretory IgA and IL-4 were the highest ($P < 0.05$) and tumor necrosis factor α and interferon gamma were the lowest ($P < 0.05$) in the PP group; In summary, the *Lactobacillus* KL1 and *L. plantarum* Zhang-LL mixed probiotic effectively reduced the mortality of pullorosis in chicks, promoted the growth performance, regulated the balance of the intestinal flora, improved the immune function, resisted pullorosis disease, completely prevented chicks from pullorosis after infection, and reduced economy loss in the poultry industry.

Key words: probiotic, chick, *Salmonella*, intestinal flora, immunity

INTRODUCTION

Salmonella pullorum is a gram-negative pathogen in the genus *Salmonella*, which causes pullorosis in chicks and can be transmitted vertically from generation to generation by laying hens. Pullorosis in chicks is difficult to eradicate, so it negatively impacts the economics of the world poultry industry (Barrow and Neto, 2011; Allen et al., 2013). Although pullorosis in chicks has been virtually eliminated from commercial chicks in developed countries, it is still a major problem in many developing countries. In recent years, the overuse of antibiotics has led to drug resistance in poultry, and many countries have restricted the use of antibiotics in feed at varying degrees (Pan et al., 2009; Gao et al., 2017), so antibiotics alternatives were developed and a probiotic has gained attention for their safe and efficient. *Lactobacillus* is widely used in animal feeding and has been shown a simulated growth of chicks (Awad et al., 2009), the inhibition of intestinal microbial pathogens (Mookiah et al., 2014; Tayeri et al., 2018), an enhanced immune function (Wang et al., 2018a), and the reduced morbidity (Peng et al., 2016), and it is gradually becoming a major antibiotic substitute (Shivaprasad, 2003; Mountzouris et al., 2007; Gaggìa et al., 2010). Peng et al. (2016) reported that *Lactobacillus plantarum* B1 (10^9 cfu/chick) increased ADG, the number of *Lactobacillus*, and the secretion of secretory

IgA (**sIgA**) in the cecum. Roshanfekr and Mamooee (2009) demonstrated that *Lactobacillus* reduced the number of intestinal pathogens in chicks without developing drug resistance. In the study by Higgins et al. (2008), when chicks were given *Salmonella* at 10^4 cfu/chick and then treated with 11 kinds of *Lactobacillus* at 10^8 cfu/chick, the results showed that *Lactobacillus* can reduce the cecal *Salmonella* counts, improve the balance of intestinal flora, and decrease the mortality of dieased in chicks. Haghighi et al. (2008) administered *Lactobacillus acidophilus* and *Bifidobacterium bifidum* at 10^6 cfu/chick to 1-day-old chicks and infected them with *Salmonella typhimurium* at 10^4 cfu/chick on the following day, with the results showing that the cecal *Salmonella* counts and chick mortality decreased and the interferon gamma mRNA expression decreased by 81.01%. Similarly, Zhang et al. (2012) administered *Lactobacillus reuteri* ATCC 55730 and L22 to 1-day-old chicks and then infected with *S. pullorum* ATCC 9120, proving that the number of *Lactobacillus* increased and chick mortality decreased by 20 to 38%. In a study by Chen et al. (2015), chicks were treated with a probiotic of *Lactobacillus johnsonii* at 10^9 cfu/chick and then infected with *Salmonella sofia* at 10^7 cfu/chick the next day. The number of *Lactobacillus* was higher by 0.6 orders of magnitude than in the positive control group, and the number of *Salmonella* was lower by 3 orders of magnitude. All these studies shown that *Lactobacillus* could promoted the growth performance, regulate the balance of intestinal flora, and improve the immunity of chicks infected with *Salmonella*. However, it has not been reported that the probiotic can completely prevent pullorosis in chick and improve their growth performance.

Lactobacillus paracasei KL1 and *L. plantarum* subsp. *plantarum* Zhang-LL were isolated from a complex bacterial leaven of Tibetan kefir and fermented meat products, respectively (Liu et al., 2013; Sui et al., 2016), and they tolerated the adverse environmental conditions of the gastrointestinal tract. *L. paracasei* KL1 exerts broad-spectrum antibacterial effects and regulates the balance of the intestinal flora (Liu et al., 2011). *L. plantarum* Zhang-LL not only effectively improves the immunity of piglets, reduces the mortality rates, and enhances their digestive capacity and ADG (Xie et al., 2018) but also regulates the balance of the intestinal microflora, improves the immunity function, and prevents and treats chronic gastric ulcer colitis in rats (Wu, 2018a).These 2 strains have many benefits, but their effects on pullorosis in chicks have not yet been studied. This study was designed to examine the growth performance, intestinal flora, and immune function of pullorosis of chicks supplemented with a *Lactobacillus* mixed probiotic to provide a theoretical basis for the development of probiotic feed additives to effectively prevent pullorosis in chicks.

MATERIALS AND METHODS

Preparation of Probiotic

L. paracasei KL1 CGMCC no. 11533 and *L. plantarum* Zhang-LL CGMCC no. 6936 are obtained from the National Strain Preservation Center (Beijing, China). The glycerin storage tubes of the 2 strains were inoculated with 2% inoculate into the modified MRS medium (Liu et al., 2016) and activated for 3 generations. Strains were activated with 2% inoculum to 100 mL modified MRS medium at 37°C for 12 h. Then, 2% of the activated strains were inoculated into 2.5 L modified MRS medium and incubated in a 5-L intelligent bioreactor (DM-V5; Magnesium Biological Technology (Shanghai) Co., Ltd., China, Shanghai) in the high-density fermentation, 37°C for 12 h. The bacterial sludge was centrifuged at 4°C for 20 min at 8 000 rpm and collected. The bacteria pellet was resuspended in 250 mL of sterilized degreased milk powder protectant, mixed, and frozen at −80°C for 12–14 h. The frozen samples were then freeze-dried under vacuum (0.13–0.16 mBar) (Labconco Co., Ltd, Kansas) at −50°C for 48 h. This freeze-dried powder was the final probiotic. The viable counts per g of the probiotic were determined with the plate pouring method, and a serial dilution $(10^{-6}$ to $10^{-8})$ with sterilized saline was processed in triplicate. The 2 *Lactobacilli* were mixed as a probiotic in 1:1 ratio for later use.

Culture of S. pullorum

Glycerol-preserved *S. pullorum* CVCC523 (China Veterinary Microbial Strain Preservation and Management Center, Beijing, China) was inoculated with 2% inoculate into the nutrient broth medium and activated for 3 generations. Some of the bacteria were preserved in 25% glycerol and stored at −80°C. The viable counts were determined with the plate smear method, and a serial dilution $(10^{-5}$ to $10^{-7})$ with sterilized saline was processed in triplicate.

Feeding and Infection Methods

A total of 120 1-day-old Nongda no.3 dwarf chicks, provided by the Animal Genetics and Breeding Laboratory, College of Animal Science and Technology (China Agricultural University, Beijing, China), were randomized into 4 treatments, with 6 replicates of 5 birds each. Dosage of *S. pullorum* and the method of infection was based on those reported by Higgins et al. (2008), and the experimental method is shown in Table 1. Briefly, we gavaged 0.2 mL/chick of *S. pullorum* solution of 8.46×10^8 cfu/mL to 90 chicks except the blank group for infection; probiotic was dissolved in sterile normal saline and make its final concentration reach was 7.50×10^9 cfu/mL and gavaged 0.2 mL/chick after weighing at 7:00 each day. All of chicks were dissected on day 14. The chicks were ad libitum fed in separate silos, where the chicks were kept at a constant temperature of 36°C

Table 1. Probiotic and *Salmonella pullorum* dosage for each treatment.

Treatment groups	Probiotic added	*S. pullorum*[1] infected
Blank group (n = 30)	0	0
S. pullorum[1]–infected group (n = 30)	0	1.69×10^8 cfu/chick on day 4
Probiotic treatment group (n = 30)	1.50×10^9 cfu/chick on day 4–14	1.69×10^8 cfu/chick on day 4
Probiotic prevention group (n = 30)	1.50×10^9 cfu/chick on day 1–14	1.69×10^8 cfu/chick on day 4

[1]*S. pullorum*, *Salmonella pullorum*. (n = number of birds).

and under a fluorescent lamp for 24 h from day 1 to 3 and per day decreased by 1 h from day 4 to 14. The basal diet was formulated as per the NRC (1994) standard. All animal treatment, housing, and feeding were in accordance with the general rules for animal welfare evaluation and the International Cooperation Committee of Animal Welfare (Beijing, China). The protocol was approved by the ethical committee of the experimental animal care of Beijing agricultural of university (Beijing, China).

Clinical Symptoms and Growth Performance

During the experiment, each group of chicks was weighed on an empty stomach at 07:00 each day. The chicks were observed for clinical signs, morbidity, and mortality. Mortality rate (%) = number of dead chicks per group/number of chicks per group × 100%. ADG= (end weight measurement - beginning weight measurement)/measurement day.

Histopathology of the Liver, Ileum, and Cecum

On day 14 of the experiment, 6 chicks close to the average BW from each group were humanely euthanized and dissected. Five-millimeter-thick samples of liver, ileum, and cecum tissues were immersed in 10% neutral formalin solution, and these were subjected to the classical histologic procedure (Awaad et al., 2010), including embedding in paraffin, cutting slices, deparaffinization, hematoxylin-eosin staining, and sealing (Beijing Xinyikang Technology Co. Ltd, Beijing, China). The samples were then surveyed under a microscope and photographed (CX21; Olympus optical Co., Ltd, Tokyo, Japan).

Colony Counts of Lactobacillus, Escherichia coli, and Salmonella

The chicks were dissected, and 1 g of the cecal contents was accurately measured, added to aseptic saline diluent, and completely mixed with beating. After gradient dilution to 10^{-2}–10^{-8}, each sample was used to inoculate eosin methylene blue agar, modified MRS agar, and bismuth sulfite agar (Beijing Road Bridge Technology Co., Ltd, Beijing, China) and was cultured at 37°C for 18–24 h. The colony morphologies were used to calculate the numbers of *Lactobacillus*, *E. coli,* and *Salmonella* in the cecal contents.

Immune Organ Indices

The chicks were dissected, and their thymus, spleen, and bursa of Fabricius tissues were separated individually and weighed. The relative weights of the organs were then determined by calculating the weights of the individual organs with respect to the live weight of the chick.

Determination of sIgA Content and Relative Cytokine mRNA Expression Levels

The chicks were dissected, 1 cm cecal segments were quick frozen in aseptic centrifuge tubes, and stored at −80°C. Cecal sIgA content was detected using Elisa Biotech Chicken sIgA, and the ELISA Calc software was used for the data analysis. The relative mRNA expression level of interferon gamma, tumor necrosis factor α (**TNFα**), and IL-4 in cecum mucosa waa detected by real-time PCR (LineGene 9600 Plus thermocycler, Bioer Technology Co. Ltd., Hangzhou, China). Total RNA was extracted from samples using the Super Pure RNA Extraction Kit (CWbio. Co. Ltd, Beijing, China) and reverse transcribed using the cDNA Reverse Transcription Kit (CWbio. Co. Ltd, Beijing, China) as per the manufacturer's protocol. A relative quantitative analysis of the data was performed with the $2^{-\Delta\Delta CT}$ method.

Statistical Analysis

The test data were analyzed with one-way ANOVA using the SPSS Statistics 22.0 (SPSS Inc., Chicago, IL) statistical software. Duncan's multiple comparison test was performed, and the graphs were generated with Origin 9.0 (Origin Lab). All data are expressed as

Table 2. The mortality (%) in different treatment groups of chicks.

Items	BG[1] (n = 30)	SG[1] (n = 30)	PT[1] (n = 30)	PP[1] (n = 30)
On day 4–14	0.00	37.50	22.22	0.00

[1]Abbreviations: BG, blank group; PP, probiotic prevention group; PT, probiotic treatment group; SG, *Salmonella pullorum*–infected group. (n = number of birds).

Table 3. The BW (g) and ADG (g) in different treatment groups of chicks.

Items	BG[1] (n = 30)	SG[1] (n = 30)	PT[1] (n = 30)	PP[1] (n = 30)
BW on day 1	39.83 ± 2.36^a	39.46 ± 1.98^a	40.95 ± 2.57^a	41.26 ± 1.76^a
BW on day 4	47.85 ± 2.18^a	47.61 ± 2.42^a	46.99 ± 2.42^a	49.64 ± 2.41^a
BW on day 14	73.86 ± 6.35^b	69.64 ± 9.07^a	77.15 ± 8.20^{bc}	82.86 ± 2.93^c
ADG on day 1–14	2.43 ± 0.67^b	2.16 ± 0.15^a	2.59 ± 0.70^b	2.97 ± 0.38^c

Values with different letters (a, b, c) differed significantly within a bird trial ($P < 0.05$).

The data were expressed as $\overline{x} \pm SD$.

[1]Abbreviations: BG, blank group; PP, probiotic prevention group; PT, probiotic treatment group; SG, *Salmonella pullorum*–infected group (n = number of birds).

means $\pm$ SD ($\overline{x} \pm SD$). Differences were considered significant at $P < 0.05$.

RESULTS

Changes in Growth Performance

Clinical Symptoms and Mortality in Chicks The sick chicks infected with *S. pullorum* were depressed, anorexic, and cold susceptible, often huddled together, which meant that they could not defecate. Anatomic examinations showed that the dead chicks had enlarged cecum, malabsorption of a greasy yolk, hepatomegaly and abnormal maculae, thickened pericardium, and increased pericardial fluid and white nodes in the myocardium. The mortality of the chicks is shown in Table 2. From day 1 to 3 of the experiment, the chicks in each group showed normal performance, with no adverse reactions. From day 4 to 14, the mortality rate was 37.5% in the SG group and compared with the SG
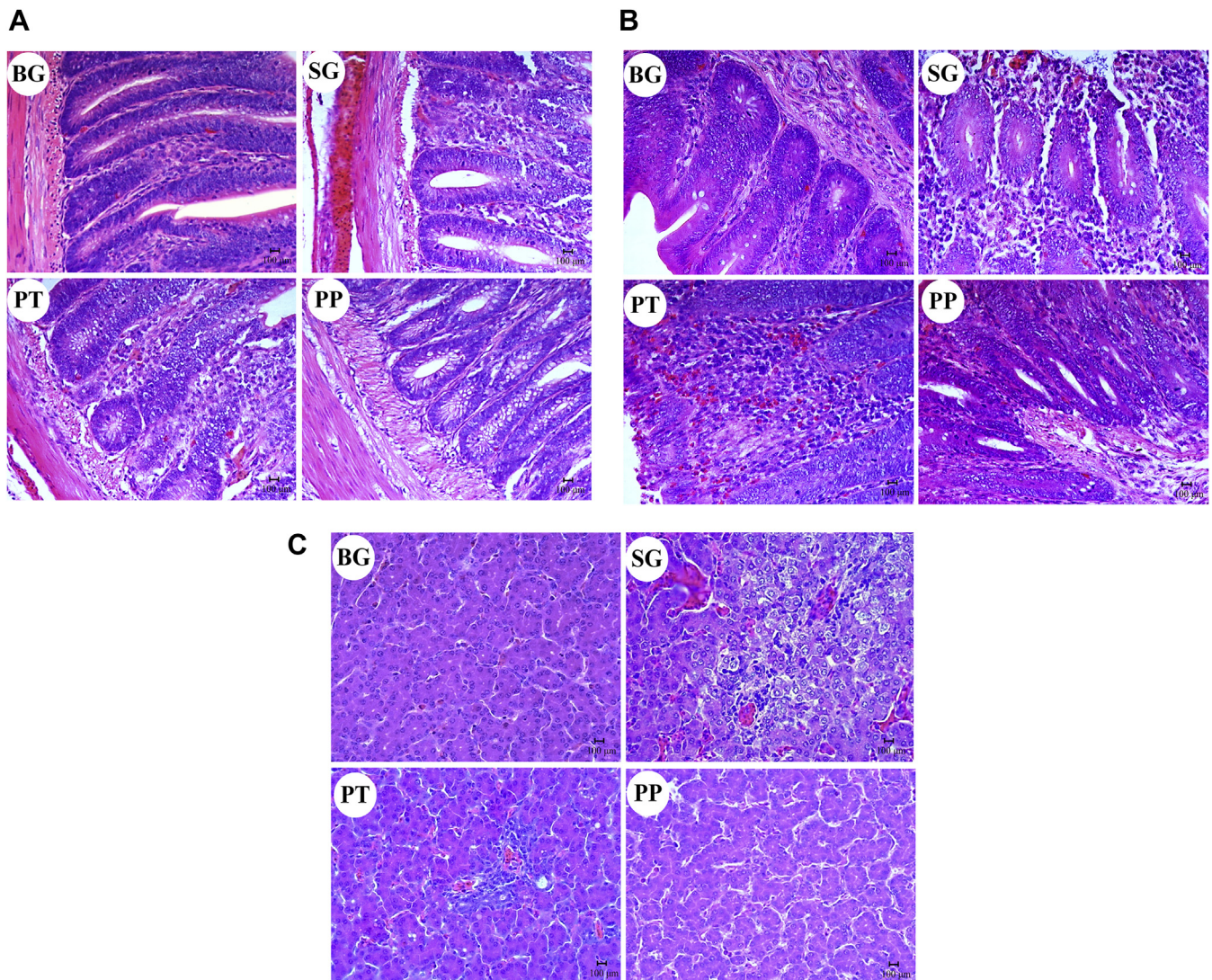

Figure 1. Histopathologic changes of the ileum (A), cecum (B), and liver (C) in different treatment groups of chicks. Abbreviations: BG, blank group; PP, probiotic prevention group; PT, probiotic treatment group; SG, *Salmonella pullorum*–infected group. Magnification is 400.
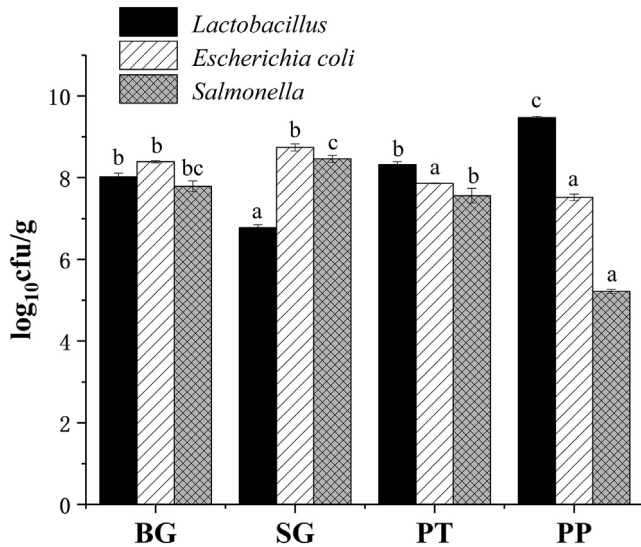
Figure 2. Average logarithm counts of *Lactobacillus*, *Escherichia coli*, and *Salmonella* in the cecum of the different treatment groups of chicks. Abbreviations: BG, blank group; PP, probiotic prevention group; PT, probiotic treatment group; SG, *Salmonella pullorum*–infected group. Values with different letters (a, b, c) differed significantly within a bird trial ($P < 0.05$). All data are in $\log_{10}$ cfu/g contents and expressed as $\overline{x} \pm SD$.

group, the mortality rate of chicks in the PT group reduced by 40.75%, and there were no deaths in the PP group.

Changes in Chick BW and ADG The changes in the chick BW and ADG are shown in Table 3. On day 1, there were no significant differences in the chick BW among the groups ($P > 0.05$). On day 4, the BW of the chicks in the PP group increased slightly, but there were no significant differences among the groups ($P > 0.05$). On day 14, compared with the SG group, the BW and ADG were significantly higher in the PT
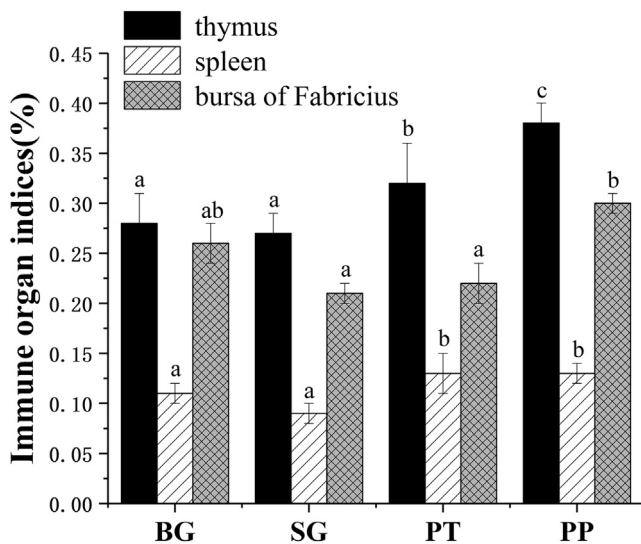

Figure 3. Immune organ indices in different treatment groups of chicks. Abbreviations: BG, blank group; PP, probiotic prevention group; PT, probiotic treatment group; SG, *Salmonella pullorum*–infected group. Values with different letters (a, b, c) differed significantly within a bird trial ($P < 0.05$). The data are expressed as $\overline{x} \pm SD$.
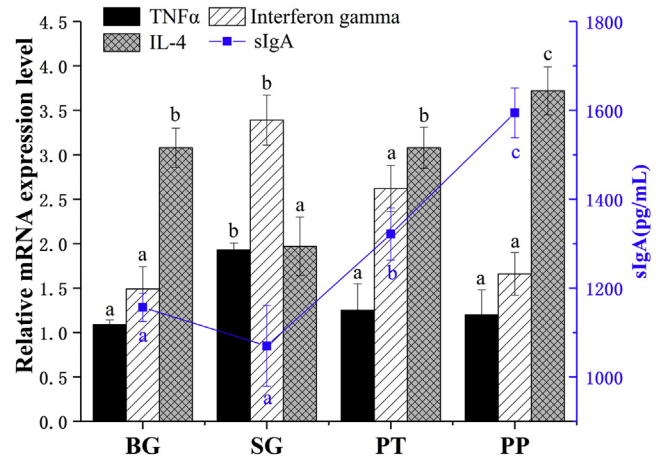

Figure 4. Comparison of sIgA content and relative cytokine mRNA expression levels of cecum mucosa in different treatment groups of chicks. Abbreviations: BG, blank group; PP, probiotic prevention group; PT, probiotic treatment group; SG, *Salmonella pullorum*–infected group; sIgA, secretory IgA; TNFα, tumor necrosis factor α. Values with different letters (a, b, c) differed significantly within a bird trial ($P < 0.05$). The data are expressed as $\overline{x} \pm SD$.

($P < 0.05$) and highest in the PP group. Surprisingly, on day 14, the BW and ADG were also higher in the PP group than in the BG group.

Histologic Changes in the Ileum, Cecum, and Liver

The histologic changes in the ileum, cecum, and liver of the chicks observed on day 14 are shown in Figures 1A–1C. In the ileum (A) and cecum (B) of the SG group, the intestinal villi were the shortest, arranged irregularly, and a large number of lymphocytes infiltrated the villous space, and in the submucosa, the connective tissue was loosened (by a transudate). The liver (C) of the SG group showed focal necrosis, with vacuoles in the cytoplasm of hepatocytes, the disintegration of the liver cell nuclei, and the infiltration of inflammatory cells in necrotic foci. In the ileum (A) and cecum (B) of the PT group, the ileal villi were shorter, with a large number of lymphocytes infiltrating the lamina propria of the mucosa and a large number of infiltrating lymphocytes; The liver (C) of the PT group had a small number of infiltrating lymphocytes. The ileum (A) and cecum (B) of the BG and PP groups showed a regular arrangement of villi and normal tissue and no pathologic changes in the liver (C).

Colony Counts of Lactobacillus, E. coli, and Salmonella

As shown in Figure 2, Compared with the SG group, the *Salmonella* and *E. coli* counts were decreased by 10.64 and 10.07% ($P < 0.05$), respectively, and *Lactobacillus* counts were increased by 22.53% ($P < 0.05$) in the PT group. The *Salmonella* and *E. coli* counts in the PP group were markedly lower by 38.18 and 13.84%, respectively, than in the SG group ($P < 0.05$), and

Lactobacillus counts were 39.47% higher ($P < 0.05$). In the PP group, the *Lactobacillus* counts were 13.82% higher than in the PT group ($P < 0.05$), the *Salmonella* counts were 28.39% lower ($P < 0.05$), whereas the *E. coli* counts did not differ significantly ($P > 0.05$).

Changes in Immune Organ Indices

The changes in the thymus, spleen, and bursa of Fabricius are shown in Figure 3. Compared with the SG group, the thymus and spleen indices were significantly ($P < 0.05$) increased by 18.52 and 41.58%, respectively in the PT group, whereas the bursa of Fabricius index was unaffected ($P > 0.05$). The thymus, spleen, and bursa of Fabricius indices were markedly increased in the PP group compared with the SG group by 40.74, 44.44, and 42.86% respectively ($P < 0.05$). The thymus and bursa of Fabricius indices in the PP group were 18.75 and 25.00% higher than those in the PT group ($P < 0.05$).

Changes in sIgA Content and Relative Cytokine mRNA Expression Levels

The changes in mucosal sIgA content and cytokine expression are shown in Figure 4. Compared with SG group, sIgA content in cecal mucosa, TNFα, interferon gamma, and IL-4 in the PP and PT groups were significantly increased by 49.01 and 23.52% ($P < 0.05$), reduced by 37.82 and 35.23% ($P < 0.05$), reduced by 51.03 and 22.71% ($P < 0.05$), and increased by 107.82 and 72.06% ($P < 0.05$), respectively.

DISCUSSION

Numerous studies have shown that the mortality of chicks infected with $S \cdot pullorum$ was between 10 and 100% (Barrow and Neto, 2011; Zhang et al, 2012; Allen et al., 2013). In this study, all the chicks in each group were healthy before infection, and the mortality after infected with *S. pullorum* was 37.5%. The clinical symptoms of chick death after infection were consistent with the study of Wu et al. (2018b). This indicates that the model of pullorosis in chicks was successfully established. In this study, adding probiotic to the diet improved the mental state of depression and increased the survival rate of pullorosis in chicks. The BW and ADG of the 2 groups of chicks fed the probiotic was better than those of the SG group. It is notable that the liver, cecum, and ileal tissues of chicks in the PP group are dense and intact, which was identical to the condition of the healthy chicks. The effect was best in the PP group, indicating that the *Lactobacillus* mixed probiotic improved the clinical symptoms of the *S. pullorum*–infected chicks by effectively preventing clinical disease and promoted their growth by reducing intestinal lesions. Zhang et al. (2012) showed that the survival rate of chicks increased from 40 to 78% after the intragastric administration of *Lactobacillus* every day from 1 to 5 D of age, with a *Salmonella* infection on day 5. Feeding

Lactobacillus to chicks before *Salmonella* infection effectively reduced their dysentery and increased their survival rate, which was consistent with the effects of the probiotic in our study.

Previous studies have shown that the balance of the intestinal flora is destroyed in chicks infected with *S. pullorum* (Van der Wielen et al., 2002; Revolledo et al., 2009). Therefore, it was likely that the balance of the intestinal flora in chicks infected with *S. pullorum* was destroyed at the age of 4 D in this study. *Lactobacillus* reduces the colonization of pathogenic *Salmonella* and regulates the balance of the intestinal microecosystem (Watkins and Miller, 1983; Jin et al., 1998; Higgins et al., 2008). *L. paracasei* KL1 and *L. plantarum* Zhang-LL strains have a strong tolerance for the adverse gastrointestinal environment, colonizing the intestinal tracts and improving the intestinal health of chicks (Sui et al., 2016). In this study, feeding chicks with the *Lactobacillus* mixed probiotic reduced intestinal infection by reducing the numbers of *Salmonella* and *E. coli* and increasing the numbers of *Lactobacillus*. The intestinal flora of freshly hatched chicks is incomplete, so the early intake of *Lactobacillus* can promote the formation of a dominant intestinal flora and increase the chick's resistance to colonization by nonhost specific *Salmonella* (Schokker et al., 2012; Peng et al., 2016), which implied that the preventive effect of the probiotic is better than using it for treatment. Van der Wielen et al. (2002) have reported that the number of *Salmonella* decreased in the cecum and the number of *Lactobacillus* increased when the *Lactobacillus crispatus* were administered before infected with *Salmonella*. Higgins et al. (2008) reported that the 9 kinds of *Lactobacillus* mixed probiotics effectively reduced intestinal *Salmonella* infections in chicks. Wang et al. (2018b) found that *L. plantarum* reduced the number of *Salmonella* in their livers and cecum and reduced their future colonization by *Salmonella*, protecting the host from the destruction of the intestinal barrier.

After *S. pullorum* invades the intestines of chicks, it weakens the intestinal mucosal immune barrier, attaches to mucosal epithelial cells, and invades the submucosal tissue, from where it is transferred to other organs to cause the systemic infection of the host (Schokker et al., 2012), so maintaining the immune activity of chicks is vital for their growth. The immune organ index is an important marker of the immune status of poultry (Heckert et al., 2002). The bursa of Fabricius is a unique immune organ of nestlings, which is essential for B cell development and systemic humoral immunity, as confirmed by Glick et al. (1956), who showed that when the bursa of Fabricius was excised from chicks, they lost the ability to produce an antibody response. Rivas and Fabricant (1988) demonstrated that the weight of the thymus, spleen, and bursa of Fabricius could be used to evaluate the immune status of chicks, as greater absolute and relative weight indicated stronger cellular and humoral immune functions. In this study, the thymus, spleen, and bursa of Fabricius indices of the PT and PP groups were higher than those of the

SG group, and the effect of adding before the disease was better than that of adding after the disease. This indicated that the *Lactobacillus* mixed probiotic improved the immune function of chicks and enhanced their disease resistance. Awad et al. (2009) showed that *Lactobacillus* promoted the growth and development of immune organs such as the thymus, spleen, and bursa of Fabricius in chicks. Olnood et al. (2015) treated 1-day-old chicks with *L. johnsonii* at 10^9 cfu/chick and infected them with *S. sofia* at 10^7 cfu/chick the next day. After 2 wk, the spleen and bursa of Fabricius indices were higher than in the sick chick and mortality rate was reduced. The aforementioned studies are consistent with the results of this study.

The intestinal mucosa recognizes and excludes harmful microorganisms and plays a crucial role in immunity (Luo et al., 2013). Immunoglobulin (SIgA) and immunoactive cytokines in the form of lymphocytes in the intestinal mucosa constitute the main part of the intestinal immune barrier and collaborate on local immune function of the intestinal tract (Zhang, 2018). Secretory IgA serves as the first immune defense for intestinal epithelium and maintains homeostasis in the gut (Schroeder and Cavacini, 2010; Mantis et al., 2011). IL-4 promotes Th2 type immune response, which in turn stimulate the proliferation and differentiation activates B cells into plasma cells and induces the secretion of sIgA (De Mon et al., 1986; Externest et al., 2000; Wils-Plotz and Klasing, 2017). At the same time, the Th1 cytokines TNFα and interferon gamma inhibit the secretion of IgA (Mosmann and Coffman, 1989; Ward et al., 1991; Cameron et al., 1997). Cheeseman (2007) demonstrated that the expression of TNFα and interferon gamma increased in the cecum of chicks infected with *Salmonella*. This study showed that the *Lactobacillus* mixed probiotic promoted the expression of the anti-inflammatory factor IL-4 and reduced the expression of proinflammatory factors TNFα and interferon gamma, promoting sIgA content in the cecal mucosa, enhancing the intestinal mucosal immune barrier, slowing the inflammatory reaction, improving the immunity of the chicks, and reducing the mortality of the chicks. Mao et al. (1996) reported that *L. plantarum* 299v increased intestinal sIgA content and improved immune function. Revolledo et al. (2009) reported that 12 kinds of *Lactobacillus* mixed probiotics promoted the sIgA content and reduced the mortality in chicks infected with *Salmonella*. Haghighi et al. (2008) illustrated that 3 kinds of *Lactobacillus* mixed probiotics induced the host expression of IL-4, resulting in the inhibiting of the expression of TNFα and interferon gamma and enhancing the immunity of chicks. All these results are consistent with the results of this study.

CONCLUSION

In summary, the *L. paracasei* KL1 and *L. plantarum* Zhang-LL mixed probiotic effectively promoted the growth performance, improved the immune function, resisted pullorosis disease, and reduced the mortality

rate of pullorosis in chicks. This result is remarkable that it completely prevented chicks from pullorosis after infection. Because antibiotics are forbidden in most of the countries, the *L. paracasei* KL1 and *L. plantarum* Zhang-LL mixed probiotic is appropriately used for preventing *Salmonella*-caused pullorosis in chicks, and it potentially reduce economy loss in the poultry industry, while also building a strategy for future feeding additives research and development.

Conflicts of Interest Statement: The authors declare no conflicts of interest.

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