Effects of *Taraxacum* and *Astragalus* extracts combined with probiotic *Bacillus subtilis* and *Lactobacillus* on *Escherichia coli*–infected broiler chickens

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ABSTRACT Diarrhea caused by *Escherichia coli* (***E. coli***) is one of the most common diseases that affects the growth and development of poultry. This study was conducted to investigate the synergistic effects of traditional Chinese medicine (**TCM**) combined with probiotics against *E. coli* infection and its mechanism in broiler chickens. The optimal proportion formula TCM and probiotics was screened by orthogonal test and range analysis method; the in vitro antibacterial activity was based on the Oxford cup method. Isolated pathogenic *E. coli* was injected subcutaneously into the neck of the broilers to establish an *E. coli*–infected model. The broilers were administrated with drugs in drinking water daily for 7 d before and after *E. coli* infection. The diarrhea rate, mortality, body weight (**BW**) gain, feed intake, immune organ index, intestinal and hepatic histopathological changes were monitored. The expression of IL-2, IL-10, and TLR-4 mRNA in the intestinal tissues was measured by RT-PCR. Our results showed that the optimal proportion formula of *Taraxacum* extracts: total flavonoids of *Astragalus*: polysaccharides of *Astragalus*: probiotics was 5: 2: 2: 2; TCM combined with probiotics was highly sensitive to *E. coli*. TCM combined with probiotics synergistically increased BW gain, decreased the diarrhea rate and mortality of broilers, alleviated intestinal and hepatic pathological changes, accompanied by the increase of IL-2 and IL-10 mRNA expression and the inhibition of TLR-4 mRNA expression. It suggests that the combination of TCM and probiotics may produce a synergistic protective effect against *E. coli* infection by improving the indicators of diarrhea and regulating the expression of IL-2, IL-10, and TLR-4 mRNA in broiler chickens. The synergistic interactions between TCM and probiotics represent a promising strategy for the treatment of *E. coli* infection.

Key words: broiler chickens, *Escherichia coli*, diarrhea, traditional Chinese medicine combined with probiotics

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INTRODUCTION

Diarrhea is one of the most common diseases that affects the growth and development of poultry in clinic. Especially, diarrhea caused by *Escherichia coli* (***E. coli***) is widespread, with high morbidity and mortality, low feed conversion rate, difficult control and huge economic losses, which seriously restrict the healthy and rapid development of poultry breeding industry. As we all know, antibiotics have been commonly used to promote growth and control bacterial diarrhea in the breeding industry. However, the abuse or long-term application of antibiotics leads to the low level of immune system, the production of drug-resistant strains, secondary infection and drug residues, which cause a series of food safety and health problems to human beings and the environment (Dibner and Richards, 2005; M'Sadeq et al., 2015; Lekshmi et al., 2017). Such a condition inevitably promotes the demand for valid alternatives with high efficacy, few side effects, low residue, and resistance to prevent and treat bacterial diarrhea.

Probiotics are live microorganisms that confer positive effects and health benefits on the host (Reid et al., 2003). Probiotics can improve growth performance, promote nutrient metabolism, maintain gut integrity, resist pathogen colonization and modulate body immunity (Clavijo and Flórez, 2018). At present, *Lactobacillus* and *Bacillus* are the most commonly used probiotics in livestock and poultry, which play an important role in ensuring the

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health of livestock and poultry (Patterson and Burkholder, 2003; Hossain et al., 2015; Rathnapraba et al., 2018). Traditional Chinese medicine (**TCM**) is another important natural source to replace antibiotics in the control of intestinal diseases. *Taraxacum* and *Astragalus membranaceus* Bunge are the oldest and most widely used crude drugs in traditional Chinese food and medicine for their beneficial effects. Studies have shown that *Taraxacum* possesses multiple pharmacological activities including antiangiogenic, antinociceptive, antitumor, antiseptic, antioxidative, antibacterial, and anti-inflammatory properties (Schütz et al., 2006; Sang et al., 2019; Ge et al., 2020). In particular, antibacterial and anti-inflammatory effects of *Taraxacum* have been in part evaluated in vitro and in animal inflammatory models. Moreover, *Taraxacum* can ameliorate dextran sodium sulfate–induced colitis (Chen et al., 2019). *Astragalus membranaceus* Bunge is a tonic; the total flavonoids and polysaccharides of *Astragalus* are the main and most beneficial active components isolated from *A. membranaceus* Bunge. Series of studies have shown that the total flavonoids and polysaccharides of *Astragalus* have bacteriostatic, anti-inflammatory, cholagogic, hepatoprotective, and immunomodulatory effects (Jin et al., 2014; Liu et al., 2017).

In recent years, TCM and probiotics have been widely used in the prevention and treatment of intestinal diseases because of their advantages of natural, low toxicity, low residue, and no pollution. However, most of them remain in the independent application of TCM or probiotics. Traditional Chinese medicine alone is slow to play its efficacy and some effective ingredients are not easy to be absorbed, which limits its promotion in practice. However, probiotics alone have some problems such as poor stability and limited application scope. In view of these, we explored the combined efficacy of TCM and probiotics on *E. coli*–infected broiler chickens to enhance their synergistic interaction. It will provide the scientific basis for the combined use of drugs in clinic.

MATERIALS AND METHODS

Reagents

Bacterial biochemical kits, indigo matrix, Voges-Proskauer and methyl red reagents were purchased from Hangzhou Binhe Microorganism Reagent Co. Ltd. (Hangzhou, Zhejiang, China); Luria-Bertani (**LB**) broth, agar powder, eosin methylene blue, DeMan-Rogosa-Sharpe, Triple Sugar Iron, citrate (**IMViC**) culture medium were purchased from Qingdao Haibo Biotech Co. Ltd. (Qingdao, Shandong, China); RNAiso Plus and PrimeScript reverse transcription–polymerase chain reaction (**RT-PCR**) kits were purchased from TaKaRa Bio. Inc. (Osaka, Japan); All the other chemical reagents used in this study were analytical grade.

Isolation and Identification of Escherichia coli

Blood, heart, liver, and intestinal tissue samples were collected from chicks with diarrhea in Yanbian area. The samples were isolated and cultured in LB and eosin methylene blue medium. The pathogenic bacteria were identified as *E. coli* by gram staining and biochemical and serological analysis.

Probiotics

Bacillus subtilis and *Lactobacillus acidophilus* are generously donated by Microbiology Laboratory, Agricultural College of Yanbian University. *Bacillus subtilis* was inoculated into LB broth at 37°C for 18–24 h, and the content was $\geq 1 \times 10^6$ CFU/mL. *Lactobacillus acidophilus* was inoculated into DeMan-Rogosa-Sharpe broth at 40°C for 48 h under anaerobic conditions, and the content was $\geq 1 \times 10^6$ CFU/mL.

Preparation of Traditional Chinese Medicine Extracts

Taraxacum and *A. membranaceus* were purchased from Yanji Tongrentang Pharmacy (Yanji, Jilin, China). They were identified macroscopically and microscopically according to the Pharmacopoeia of China, and the voucher specimens were deposited at the Herbarium of Yanbian University (Yanji, Jilin, China). The whole herb of *Taraxacum* was soaked overnight, and extracted 3 times by decocting in distilled water for 3 h, 1 h, and 1 h, respectively. After filtration, the filtrate was concentrated under decompression and merged to obtain 1 g/mL of *Taraxacum* extract. *Astragalus membranaceus* was soaked overnight in distilled water, decocted 3 times, combined with filtrate, and concentrated, and the crude extract of *A. membranaceus* was prepared; the polysaccharides of *Astragalus* from the crude extract of *A. membranaceus* were further prepared by traditional water extraction and ethanol precipitation method. Briefly, the crude extract of *A. membranaceus* was added into ethanol (60% final concentration), left overnight, centrifuged, precipitated, and dissolved with water, then added with ethanol (80% final concentration), centrifuged, precipitated, washed with 100% ethanol, and dried at 60°C to obtain the polysaccharides of *Astragalus*. The total flavonoids of *Astragalus* were prepared by ethanol reflux and petroleum ether extraction method. Briefly, *A. membranaceus* was crushed, sifted, added with 80% ethanol, reflux with water bath, filtered, filtrate was extracted with petroleum ether, overnight at 4°C, and the lower layer liquid was taken to obtain total flavonoids of *Astragalus*. The polysaccharides and total flavonoids of *Astragalus* were prepared at a concentration of 1 g/mL.

Table 1. The primer sequences used for RT-PCR.

Genes	Primer sequence	Product size (bp)
TLR-4	Forward 5′-GATGCACTCTTCTCTTCAGG-3′ Reverse 5′-CAAACTCCTGCATCTGTACC-3′	140
IL-10	Forward 5′-CCCATCCTCACCATCCTTACA-3′ Reverse 5′-CTCGATCTTGCGGTCCCTC-3′	102
IL-2	Forward 5′-TCTGGGACCACTGTATGCTCT-3′ Reverse 5′-ACACCAGTGGGAAACAGTATA-3′	429
β-actin	Forward 5′-TGAAGCCCAGAGCAAAAGAGGTAT-3′ Reverse 5′-TGCTCCTCAGGGCTACTCTC-3′	135

Abbreviation: RT-PCR, reverse transcription–polymerase chain reaction.

Orthogonal Test

As per L9 (34) orthogonal table, 4 factors and 3 levels of orthogonal test were designed to analyze the bacteriostatic effect of probiotics combined with TCM on pathogenic bacteria. The optimal proportion formula was screened by range analysis method, namely *Taraxacum* extracts: total flavonoids of *Astragalus*: polysaccharides of *Astragalus*: probiotics = 5: 2: 2: 2.

In Vitro Antibacterial Test

The in vitro antibacterial test was based on the Oxford cup method: the separated *E. coli* solution was evenly coated on LB plate, the Oxford cup was placed on the plate and medicinal liquid was added, and incubated at 37°C for 18–24 h, and the diameter of antibacterial circle was measured. The diameter of bacteriostatic circle: $>$ 20 mm (extremely sensitive), 15–20 mm (high sensitive), 10–15 mm (medium sensitive), $<$ 10 mm (low sensitive), none (insensitive).

Animal Experiments

Chinese yellow broilers were purchased from Yanbian Huadu Agricultural Development Co. Ltd. (Longjing, Jinlin, China). The broilers were domesticated in an age-appropriate temperature, humidity, and light regime, and had free access to food and water. All animal experimental procedures were performed in accordance with the guidelines of the Ethical Committee for the Experimental Use of Animals at Yanbian University (approved no. 20171101, Yanji, Jinlin, China).

A total of 100 one-day-old female broilers were randomly divided into 5 groups as follows (n = 20 each group): normal group, model group, TCM group (*Taraxacum* extracts + total flavonoids of *Astragalus* + polysaccharides of *Astragalus*, TCM), probiotics group (*B. subtilis* and *L. acidophilus*), TCM combined with probiotics group (TCM + probiotics). The broilers were adaptively fed for 7 d. Isolated pathogenic *E. coli* (0.4 mL, 9×10^8 CFU/mL) was injected subcutaneously into the neck of the broilers as previous description (Cai et al., 2006). The broilers were administrated with drugs in drinking water daily for 7 d before and after *E. coli* infection (excepting for normal group). The broilers in TCM group were administered with TCM (9 g/kg BW). The broilers in probiotics group were administered with *B. subtilis* (1×10^6 CFU/kg BW) and *L. acidophilus* (1×10^6 CFU/kg BW). The broilers in TCM + probiotics group were administered with TCM (9 g/kg BW) + probiotics (2×10^6 CFU/kg BW). The broilers in normal and model groups were administered with saline. Stool consistency, diarrhea, mortality, BW gain, feed intake were monitored during the drugs treatment. At the end of this experiment, the broilers were euthanized by exsanguination; the spleen, thymus, bursa of fabricius, liver, and intestine were removed for the determination of the following indexes.

Measurement of Growth Performance and Immune Index

Each index was calculated as follows: BW gain (kg) in each group was individually measured on day 0, 7, 14,

Table 2. Biochemical and serological test results of *Escherichia coli* isolates from diarrhea broilers.

Test items	Indole	MR	KCN	VP	IMViC	H_2S	TSI	Lactose	Mannitol	Serotype
Samples	+	+	+	−	−	−	⊕	⊕	⊕	O78

"+" represents positive, "−" represents negative, "⊕" represents acid and gas production.
Abbreviations: MR, methyl red; TSI, Triple Sugar Iron; VP, Voges-Proskauer.

Table 3. The in vitro antibacterial activity on *Escherichia coli* in each group.

Groups	The diameter of antibacterial circle (mm)	Sensibility
Probiotics	9.81 ± 0.56	Low sensitive
TCM	13.80 ± 1.49	Medium sensitive
TCM + probiotics	16.46 ± 1.08	High sensitive

The values are expressed as means ± SEM.
Abbreviation: TCM, traditional Chinese medicine.

and 21 of age; feed conversion ratio = total feed intake (kg)/BW gain in the same period (kg); diarrhea rate (%) = number of diarrhea broilers/total number of broilers × 100%; mortality (%) = number of dead broilers/total number of broilers × 100%; immune organ index (g/kg) = immune organ weight (g)/BW (kg).

Intestinal and Hepatic Histopathological Evaluation

A small fragment of the cecal tissues and liver tissues were preserved in 10% paraformaldehyde and embedded in paraffin, and stained with hematoxylin and eosin, and then the histopathological changes were observed under a light microscope.

RT-PCR Assay

Total RNA was extracted from cecal tissues by using Trizol reagent based on the manufacturer's instructions. The cDNA was synthesized from RNA (1 μg) by RT-PCR. β-Actin was used as a reference gene to quantitatively analyze the target genes. The primer sequences (sense and antisense sequences) designed by using Primer Premier 5 (Premier, Canada) were shown in Table 1. All mRNA primers were synthesized by Invitrogen Co. (Shenyang, China). The parameters of PCR reactions were 94°C for 5 min for one cycle, and then 94°C for 30 s, 50°C–60°C for 30 s, 72°C for 1 min, and 72°C for 7 min for 30 cycles. The PCR products were separated on 1% agarose gels and visualized with ethidium bromide staining. Samples were run in duplicate. Gene-specific expression values were normalized against the level of β-actin house keeping gene expression.

Statistical Analysis

All values are expressed as means ± standard error of the mean. Differences between mean values of normally distributed data were assessed with one-way analysis of variance and two-tailed Student *t*-test. A P-value <0.05 was considered significant.

RESULTS

Isolation and Identification Results of Escherichia coli

Gram staining showed that the pathogenic bacteria were characterized by blunt round ends, dense staining, non–spore forming and short rod-shaped gram-negative bacteria. The results of biochemical and serological tests are shown in Table 2. Indole production, methyl red, and KCN tests were positive; Voges-Proskauer, IMViC, and H_2S tests were negative; Triple Sugar Iron, lactose and mannitol tests showed acid and gas production. The isolates belonged to O78 by serological analysis.

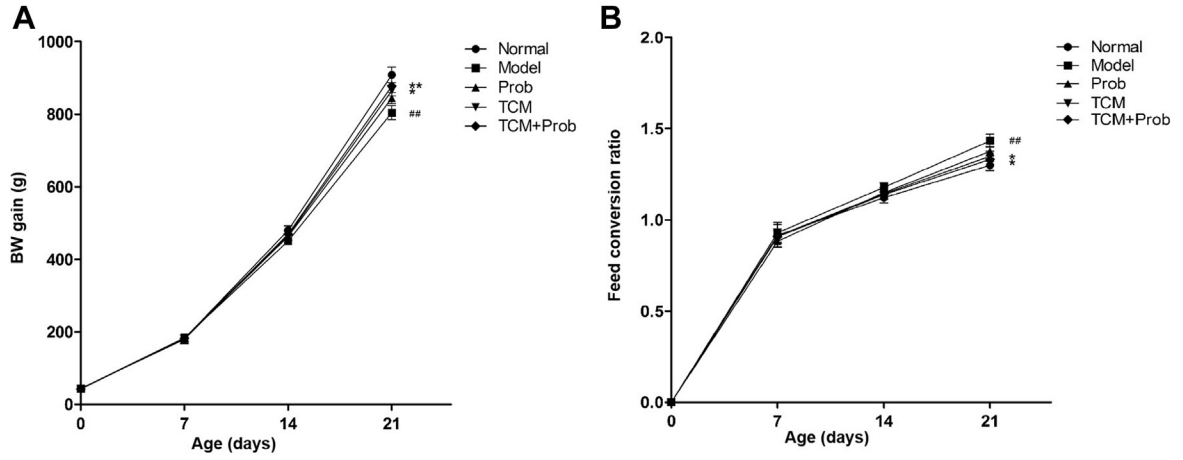

Figure 1. Effects of TCM and probiotics alone or in combination on BW gain and feed conversion ratio of broilers with *Escherichia coli* infection. The values are expressed as means ± SEM. $^{\#\#}P < 0.01$ vs. normal group; $^{*}P < 0.05$, $^{**}P < 0.01$ vs. model group. Abbreviation: TCM, traditional Chinese medicine.

Table 4. Effects of TCM combined with probiotics on diarrhea rate and mortality of broilers.

Groups	Normal	Model	Probiotics	TCM	TCM + probiotics
Number of diarrhea (n)	0	20	20	17	15
Number of deaths (n)	0	16	15	9	4
Total (n)	20	20	20	20	20
Diarrhea rate (%)	0	100	100	85	75
Mortality (%)	0	80	75	45	20

Abbreviation: TCM, traditional Chinese medicine.

In Vitro Antibacterial Activity of TCM Combined With Probiotics

Inhibitory effects of TCM and probiotics alone or in combination on separated *E. coli* were measured by the Oxford cup method. As shown in Table 3, the in vitro antibacterial activity against *E. coli* was as follows: TCM + probiotics > TCM > probiotics. Traditional Chinese medicine combined with probiotics was high sensitive to *E. coli*, treatment with TCM alone was medium sensitive, and treatment with probiotics alone was low sensitive. It indicated that combined treatment with TCM and probiotics had a significant synergistic antibacterial activity on *E. coli* compared with TCM and probiotics used separately.

Effects of TCM Combined With Probiotics on BW Gain and Feed Conversion Ratio of Broilers With E. coli *Infection*

As shown in Figure 1, there were no significant differences for BW gain and feed conversion ratio between groups on day 7 and 14 ($P > 0.05$). On day 21, BW gain was significantly increased in TCM alone group compared with the single infected group ($P < 0.05$); BW gain was extremely significantly increased in TCM + probiotics group compared with the single infected group ($P < 0.01$). On the other hand, feed conversion ratio was significantly decreased in TCM alone group and TCM + probiotics group compared with the single infected group ($P < 0.05$). It indicated that TCM combined with probiotics synergistically increased BW gain and decreased feed conversion ratio of broilers.

Effects of TCM Combined With Probiotics on Diarrhea Rate and Mortality of Broilers With E. coli *Infection*

As shown in Table 4, the broilers had typical diarrhea and mortality after injecting subcutaneously *E. coli*, the diarrhea rate and mortality were 100 and 80%, respectively. The diarrhea rates decreased from 100 to 85 and 75% in TCM group and TCM + probiotics group, respectively, the diarrhea rate did not decrease in probiotics group. Mortality decreased from 80 to 75, 45, and 20% in the probiotics group, TCM group, and TCM + probiotics group, respectively.

Effects of TCM Combined With Probiotics on Organ Index of Broilers With E. coli *Infection*

As shown in Figure 2, the spleen index, thymus index, and bursa of fabricius index in each group were higher than those of the model group on day 21, among which TCM + probiotics group was the highest. The spleen index, thymus index, and bursa of fabricius index in the TCM + probiotics group were significantly increased compared with the model group ($P < 0.01$ or $P < 0.05$). The spleen index and thymus index in the probiotics group and TCM group was significantly increased compared with the model group ($P < 0.05$), but the bursa of fabricius index had no significant different compared with the model group ($P > 0.05$).

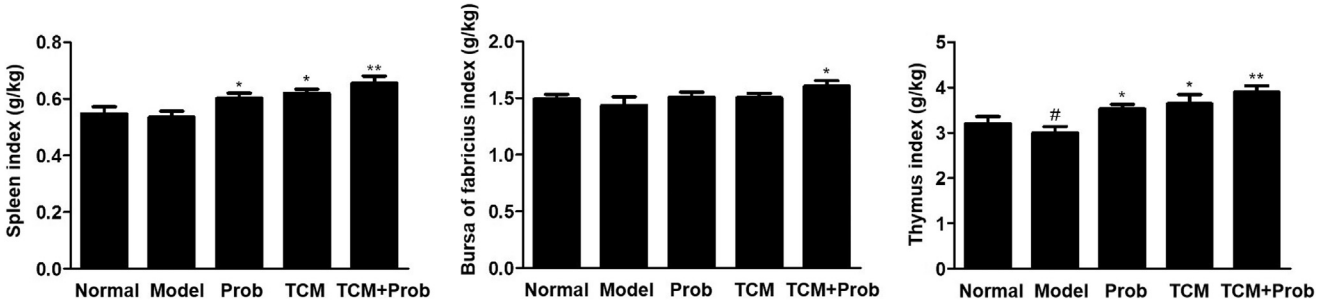

Figure 2. Effects of TCM and probiotics alone or in combination on immune organ (spleen, thymus and bursa of fabricius) index of broilers with *Escherichia coli* infection. The values are expressed as means ± SEM. $^{\#}P < 0.05$ vs. normal group; $^{*}P < 0.05$, $^{**}P < 0.01$ vs. model group. Abbreviation: TCM, traditional Chinese medicine.

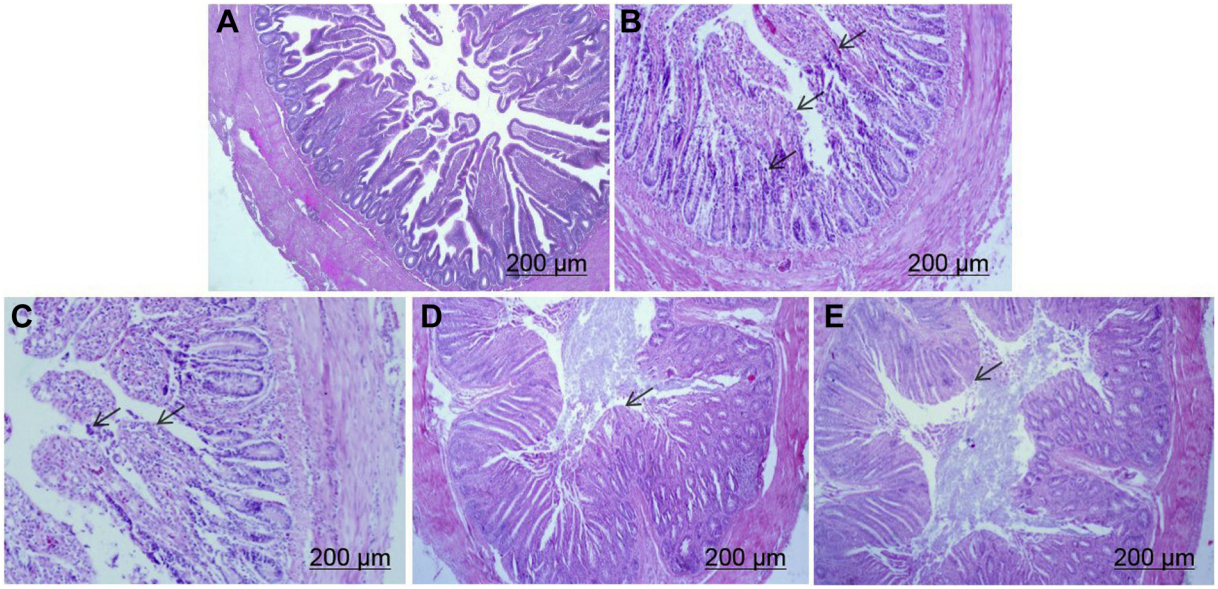

Figure 3. Effects of TCM and probiotics alone or in combination on intestinal histopathological changes of broilers with *Escherichia coli* infection (H&E staining, × 200). (A) Control group; (B) Model group; (C) Probiotics group; (D) TCM group; (E) TCM + probiotics group. Abbreviation: TCM, traditional Chinese medicine.

Effects of TCM Combined With Probiotics on Intestinal and Hepatic Histopathological Changes of Broilers With E. coli *Infection*

As shown in Figures 3 and 4, normal groups exhibited normal intestinal and hepatic structures (Figures 3A and 4A). *Escherichia coli* infection resulted in a series of severe intestinal pathological changes including disordered arrangement, fragile intestinal wall, sloughed mucosa, and congestion in the model group (Figure 3B). Liver sections from *E. coli* infection appeared the disappearance of basic architecture, inflammatory cell infiltration, hyperemia, and necrosis in the model group (Figure 4B). Treatment with probiotics, TCM, and TCM + probiotics, respectively, alleviated those pathological changes to differing extent (Figures 3C–3E and Figures 4C–4E), among which the TCM + probiotics group was the most obvious.

Effects of TCM Combined With Probiotics on TLR-4, IL-2, and IL-10 mRNA Expression of Broilers With E. coli *Infection*

TLR-4, IL-2, and IL-10 mRNA expression in intestine tissues was measured to further explore the protective mechanism of TCM combined with probiotics on *E. coli*–infected diarrhea. As shown in Figure 5, *E. coli* infection significantly upregulated TLR-4 mRNA expression, and downregulated IL-10 and IL-2 mRNA expression in the model group compared with the normal group ($P < 0.01$ or $P < 0.05$). However, TLR-4 mRNA expression was extremely significantly downregulated in the TCM group and TCM + probiotics group compared with the model group ($P < 0.01$), but there was significant difference between the probiotics group and model group ($P > 0.05$). IL-2 mRNA expression was significantly upregulated in the TCM group ($P < 0.05$), extremely significantly upregulated in the probiotics group and TCM + probiotics group compared with the model group ($P < 0.01$). IL-10 mRNA expression was extremely significantly upregulated in the probiotics group, TCM group, and TCM + probiotics group compared with the model group ($P < 0.01$).

DISCUSSION

In some drug prescriptions, different types of drugs can be made with a mixture for clinical use. There are many advantages to drug combination including targeting of multiple critical molecular processes, enhanced efficacy, delivery of lower doses of agents with lower toxicity, and increased body tolerance (Lin et al., 2013; An et al., 2015). Therefore, drug combination therapy in further research is an alternative strategy compared with conventional therapy. At present, TCM and probiotics alone have been widely used in livestock and poultry, especially to the prevention and treatment of intestinal diseases, but the effect is not ideal. In this study, we explored the protective effect of TCM (*Taraxacum* extracts, *Astragalus* polysaccharides, and total flavonoids) and probiotics (*Lactobacillus* and *Bacillus*) alone or in combination on experimental model of *E. coli*–infected broiler chicken. The results demonstrated that TCM and probiotics alone or in combination had different degrees of protective effect on *E. coli*–infected chicken by inhibiting *E. coli*, increasing BW gain, decreasing the diarrhea rate and mortality of broilers, alleviating intestinal and hepatic pathological changes. Among them, TCM combined with probiotics had the best effect; this may contribute to the synergistic or additive effect of this combination therapy.

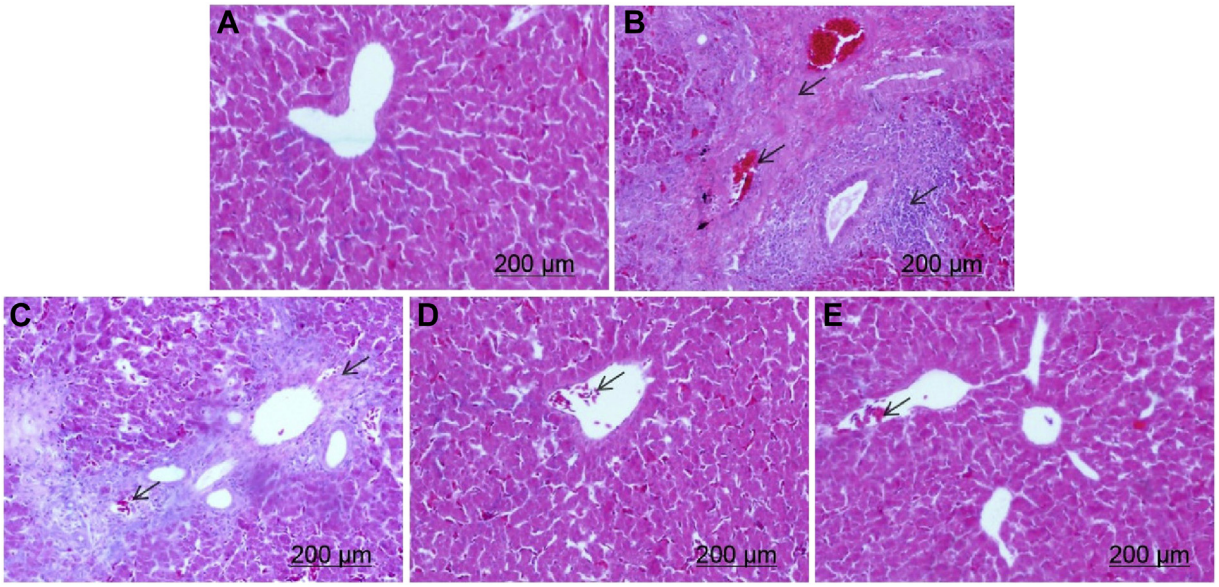

Figure 4. Effects of TCM and probiotics alone or in combination on hepatic histopathological changes of broilers with *Escherichia coli* infection (H&E staining, × 200). (A) Control group; (B) Model group; (C) Probiotics group; (D) TCM group; (E) TCM + probiotics group. Abbreviation: TCM, traditional Chinese medicine.

Immune function is closely related to the development of immune organs, and the development of the thymus, bursa of fabricius, and spleen has a direct impact on immune function (Al-Khalifa et al., 2012; Li et al., 2017). The immune index of these organ can be used to evaluate and measure the immune status of the body. Our study showed that the immune organ index in each experimental group was higher than that of the model group, and TCM + probiotics group had the best effect. It indicates that the combination of TCM and probiotics may produce a synergistic protective effect against *E. coli*–infected chickens by enhancing the immune function of the body.

Anti-inflammatory cytokine IL-10 plays an important role in inflammation and maintaining immunological self-tolerance. IL-10 can inhibit the synthesis and release of proinflammatory cytokines; its deficiency can cause inflammation and infection (Conti et al., 2003; Mocellin et al., 2003; Xu et al., 2020). The studies have shown that pathogenic factors stimulate the release of inflammatory cytokines, leading to apoptosis of intestinal epithelial cells and structural damage (Hackam

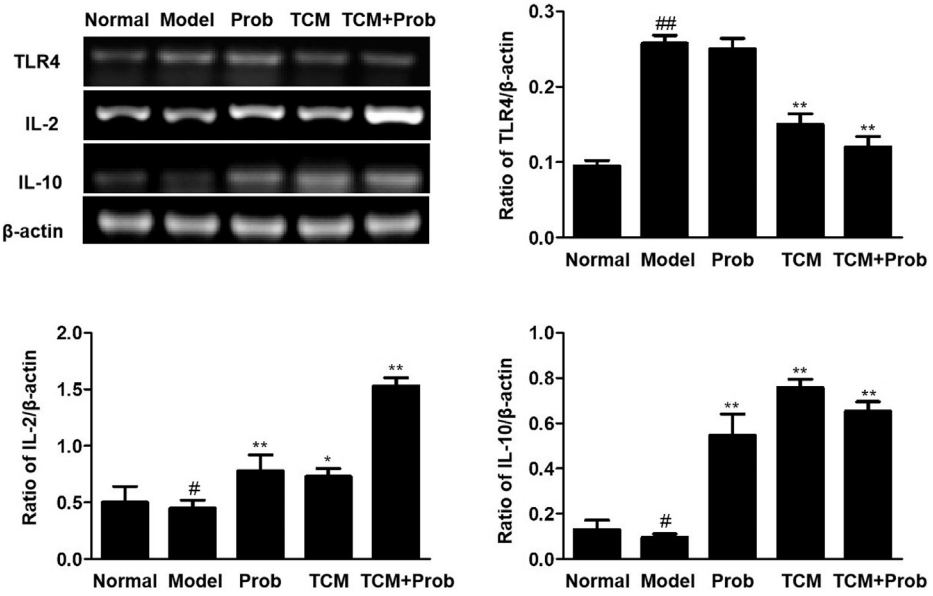

Figure 5. Effects of TCM and probiotics alone or in combination on intestinal TLR-4, IL-2 and IL-10 mRNA expression of broilers with *Escherichia coli* infection. The values are expressed as means ± SEM. $\#P < 0.05$, $\#\#P < 0.01$ vs. normal group; $^{*}P < 0.05$, $^{**}P < 0.01$ vs. model group. Abbreviations: TCM, traditional Chinese medicine; TLR, Toll-like receptors.

et al., 2005). When inflammation occurs due to the invasion of pathogenic microorganisms, neutrophils are activated to release myeloperoxidase (MPO) (Witkosarsa et al., 2000), and the activation signal is transmitted from the cytoplasm to the nucleus through the cell signal transduction pathway, and the expression of inflammation-related genes is induced (Naskalski et al., 2002). As another important immunomodulatory cytokine, IL-2 is closely related to the occurrence and immunity of avian diseases. The study has shown that the level of IL-2 secretion is significantly reduced in some avian infectious diseases (Hong et al., 2006). The present study showed that *E. coli* infection significantly decreased IL-10 and IL-2 mRNA expression in intestinal tissues, TCM and probiotics alone or in combination increased the expression of intestinal IL-10 and IL-2 mRNA, and TCM combined with probiotics had the best effect. It indicates the combination of TCM and probiotics could synergistically inhibit intestinal inflammation infected by *E. coli* via increasing IL-10 and IL-2 mRNA expression.

Furthermore, the mechanism of protective effect of TCM and probiotics alone or in combination on *E. coli*–infected broiler chickens may be involved to the inhibition of Toll-like receptors (**TLR**). Toll-like receptors are a group of immune receptors and widely distributed in various tissues and cells of the body (Takeda and Akira, 2015). TLR-4 is an important member of TLR family, which is mainly distributed and expressed in the intestinal epithelial cells, dendritic cells, and other cells, and plays an important role in inflammatory response (Peng et al., 2020). TLR-4 is normally expressed at low levels in the intestinal mucosa, while it is upregulated in patients with inflammatory bowel disease (Hausmann et al., 2002; Fukata et al., 2007). Therefore, TLR-4 can promote the occurrence of inflammation and aggravate the injury of tissues and organs. In the present study, the expression of TLR-4 mRNA was significantly increased in intestinal tissues of diarrhea chickens, which aggravated the inflammatory reaction, while TCM combined with probiotics significantly decreased the expression of TLR-4 mRNA, indicating the combination of TCM and probiotics could synergistically inhibit the expression of intestinal TLR-4 mRNA infected by *E. coli*.

In summary, TCM and probiotics may produce a synergistic protective effect against *E. coli*–infected broiler chickens, accompany by the improvement of diarrhea indicators, immune organ index, hepatic and intestinal histopathological changes through the regulation of IL-2, IL-10, and TLR-4 mRNA expression. The synergistic interactions between TCM and probiotics represent a promising strategy for the treatment of *E. coli*–infected broiler chickens in clinic.

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DISCLOSURES

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

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