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Effects of three kinds of Chinese herbs on growth performance, oocysts output and gut microbiota in growing lambs with coccidiosis

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Abstract: Coccidiosis is a protozoan intestinal disease that reduces the production of the sheep industry and causes large economic losses for sheep. Although chemically synthesised drugs are routinely employed to treat coccidiosis in sheep, the anticoccidial drug resistance and drug residues in edible meat have prompted an urgent search for alternatives. Herein, the anticoccidial properties of diclazuril, a conventional anticoccidial drug, and *Allium sativum*, *Houttuynia cordata* and *Portulaca oleracea* were assessed. Forty 45-day-old lambs naturally infected with *Eimeria* spp. were selected and randomly divided into five groups. The results showed that the sheep treated for coccidiosis had considerably decreased average daily gain (ADG) during both administration and withdrawal of the drug compared to the control group. Furthermore, at days 14, 21, 28 and 35, respectively, the three herbs and diclazuril had similar anticoccidial effects, with lower oocysts per gram (OPG) than the control group. On day 78, OPG in the three herbal groups was significantly lower than in the diclazuril group. In addition, the abundance and composition of the gut microbiota were changed in sheep treated with the three herbs and diclazuril compared to the untreated sheep. Moreover, some intestinal microorganisms have a correlation with OPG and ADG when using Spearman correlation analysis. In summary, our results suggest that all three herbs produce anticoccidial effects similar to diclazuril and modulate the balance of gut microbiota in growing lambs.

Keywords: Epizootiology, microbiome, *Eimeria*, prevention and treatment, sheep

Coccidiosis is a common protozoal disease caused by various coccidia in the epithelial cells of the intestinal mucosa of goats or sheep (Taylor and Catchpole 1994, Macrelli and Mitchell 2019, Bangoura et al. 2022). Among parasitic sheep diseases, coccidiosis is the most serious intestinal parasitic disease with the highest rate of transmission and death globally (Bangoura and Bardsley 2020). Clinical manifestations of coccidia infection in lambs are diarrhea, dehydration, loss of appetite, lethargy, anemia and even death, causing potentially huge economic losses to livestock farming (Diao et al. 2022). The infection with species of *Eimeria* Schneider, 1875 can affect sheep of all ages and breeds, but it is more common in those 3 weeks to 5 months of age (Mohamaden et al. 2018).

Nowadays, chemical drugs are extensively used to treat coccidiosis in livestock. However, as long as drugs are continuously abused, drug resistance of coccidiosis and drug residues in animals develop, making coccidiosis treatment and prevention increasingly challenging. Therefore, seeking a safe, efficient and low-toxic anticoccidian drug has become a hot research topic and an urgent problem to be resolved (Nweze and Obiwulu 2009). The use of Chinese herbs for coccidiosis prevention and control can not only achieve the purpose of coccidiosis prevention and control, but also have health, environmental and economic benefits. Of these, the most important is that Chinese herbs can enhance the physique and immunity and promote the growth of animals (Quiroz-Castañeda and Dantán-González 2015).

Allium sativum (AS) (garlic) is an aromatic herb consumed as a food and traditional medicine in treating various diseases all over the world. Traditional medicine has reported that it possesses several biological properties, such as anticancer, antioxidant, antidiabetic, nephroprotective, antiatherosclerotic, antibacterial, antifungal and antihypertensive effects (El-Saber Batiha et al. 2020, Zhang et al. 2020, De Greef et al. 2021, Verma et al. 2023). In

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addition to its antibacterial activity, garlic has been demonstrated to prevent toxins produced by bacterial infections and benefit intestinal health (Magryś et al. 2021). Abad et al. (2020) found that the supplementation of the diet with the extracts of garlic increased the activity of gut microbiota in chickens and improved productivity without affecting the biochemical composition of eggs.

Meanwhile, another study showed that ingested alcohol and allicin in mice resulted in altered gut microbiota and improved the cluster of differentiation 14 and toll-like receptor 4 (CD14-TLR4) pathway to alleviate liver inflammation (Panyod et al. 2020). Importantly, previous studies have indicated that garlic attenuates oxidative liver damage and inflammation and reduces oocyst expulsion in mice infected with coccidia (Dkhil et al. 2011). The above studies suggest that garlic can lessen the negative effects of coccidia on the animal organism and can improve and regulate gut microbiota of animals, which has potential research value for the treatment of coccidia infection in sheep.

Houttuynia cordata (HC) is a perennial herbaceous, rhizomatous plant that is found mainly in China, Korea, Southeast Asia and Japan. Numerous investigations have demonstrated the therapeutic potential of HC extracts for various diseases, including cancer, diabetes, obesity, pulmonary fibrosis, skin diseases and severe acute respiratory syndrome (Chang et al. 2001, Du et al. 2012, Kumar et al. 2014, Kwon and Kim 2014, Shingnaisui et al. 2018, Rafiq et al. 2022). A study has reported that oral administration of HC polysaccharides attenuates lung injury and gut microbiota imbalance caused by H1N1 infection in mice (Chen et al. 2019). Furthermore, it was confirmed that supplementing the diet with 2% powdered HC improved the growth performance of ducks (Wu et al. 2021). The above studies exhibited that HC and its extracts have a wide range of pharmacological effects, such as regulation of gut microbiota and improvement of animal performance. Therefore, HC was selected as one of the research objects for the treatment of coccidial infections in this study.

Portulaca oleracea (PO) is a warm climate succulent annual herb that is distributed throughout the world (Kumar et al. 2021). Furthermore, PO has anti-inflammatory, antioxidant, immunomodulatory and antitumor properties (Rahimi et al. 2019, Kumar et al. 2021), and various compounds such as flavonoids, alkaloids, polysaccharides, fatty acids, terpenoids, sterols, proteins, vitamins and minerals have been isolated from PO (Palaniswamy et al. 2001, Zhou et al. 2015). According to research by Fu et al. (2022), PO could regulate gut microbiota of elderly rats, promote the growth of probiotic bacteria and inhibit the reproduction of pathogenic bacteria. Importantly, previous studies have demonstrated that PO has impacts on the immune system, hypoglycemic and hypolipidemic effects, antioxidants, antitumor properties, and improved gut microbiota balance (Borlinghaus et al. 2014, Zhou et al. 2015).

Furthermore, the aqueous and ethanolic extracts of PO can be used as folk medicine to treat gastrointestinal disorders (Karimi et al. 2004). Additionally, ethanolic extracts of PO may lessen colonic inflammation by attenuating the immune response and inhibiting the severity of colitis, which could be a treatment option for ulcerative colitis (Alfwuaires et al. 2021). Bao et al. (2022) concluded that extracts from PO improved blood glucose and insulin levels, increased anti-inflammatory cytokine levels, lowered serum levels of branched-chain amino acids, increased serum levels of glutamine, and prevented the formation of duodenal ulcers. In conclusion, PO has multiple pharmacological effects and can regulate gut microbiota, with potential therapeutic effects of enhancing the intestinal immune barrier and physical barrier. Therefore, PO may reduce the damage and functional disruption caused by the invasion of coccidia into the intestinal tract of animals or provide some degree of protection against coccidia infestation.

Herbal homologues provide new possibilities for the treatment of coccidiosis in sheep since they are immediately edible, healthy, residue-free, and drug-free. In this study, homologous herbs were used to treat coccidiosis in sheep, and the reduction rate of coccidial oocysts, body mass growth rate and the effect on gut microbiota were analysed to screen out the homologous herbs with better efficacy and provide a reference for green and healthy breeding in the meat sheep industry.

MATERIALS AND METHODS

Preparation of experimental treatment

Garlic was purchased from Zhengzhou Dennis supermarket. *Houttuynia cordata* was acquired from Hubei Zhangzhongjing pharmacy. *Portulaca oleracea* was purchased from Zhengzhou Zhangzhongjing pharmacy. Diclazuril solution was obtained from Henan Anjin Biotechnology Co., Ltd., each bottle of 100 ml, containing 500 mg diclazuril.

Experimental animals

Forty 45-day-old female lambs infected with coccidia naturally were provided by Henan Zhongyang Animal Husbandry Co., Ltd. Daily diet were coarse fodder (50% maize straw and 50% peanut vine) and concentrated feed (35% corn, 45% soybean meal, 15% bran, 4% baking soda and 1% NaCl).

Experimental design and grouping

The selected forty lambs were evenly divided into five groups based on their body weight and oocysts per gram (OPG, detected by McMaster method – Bauer et al. [2010]), with eight lambs in each group. Every group was housed in an identical semi-open enclosure, the sheep bed was laid with cement leaky floor, and the enclosure was well ventilated. The experimental sheep received the same nutrition and mangement. All sheep experiments followed the animal welfare guidelines of Henan Agricultural University (Zhengzhou, China) (Permit No. 17–0126).

The five experimental groups were as follows: *Allium sativum*-treated group (AS), *Houttuynia cordata*-treated group (HC), *Portulaca oleracea*-treated group (PO), diclazuril solution-treated group (DS) and control group (CON). *Allium sativum*, *H. cordata* and *P. oleracea* were crushed and mixed with sheep feed, respectively, 30 g for each sheep, once daily for 21 days. Diclazuril solution was diluted 5 times and then given 1 ml/kg (1 mg/kg) body weight, twice a week for 3 weeks. Sheep of the CON group was given a normal diet. Faeces were collected from

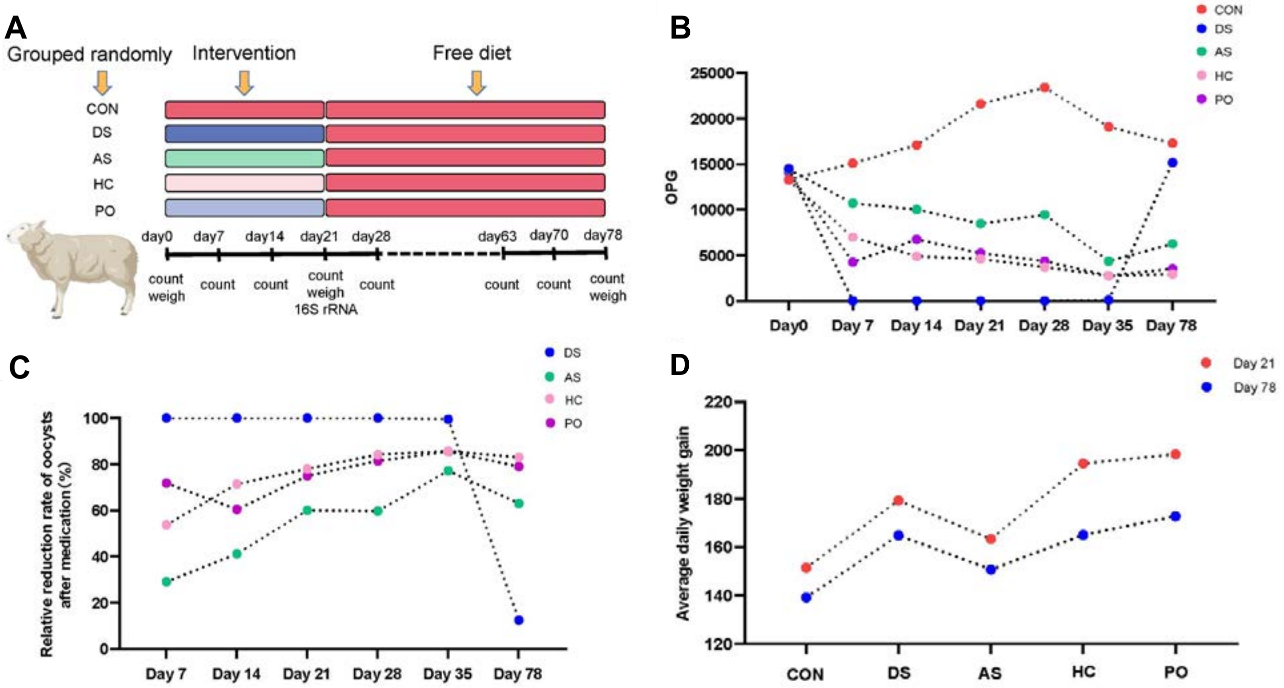

Fig. 1. Experimental groupings and line graphs of OPG and ADG. **A** – experiment grouping and processing diagram; **B** – trend chart of oocysts number from day 0 to 78 days; **C** – relative reduction rate of oocysts after medication; computational formula: Relative reduction rate of oocysts after medication = (oocyst production in CON group – oocyst production in drug group)/oocyst production in CON group × 100%; **D** – average daily gain of lambs in different periods. The ordinate is in grams. OPG – oocysts per gram; ADG – average daily gain.

Table 1. Usage and dosage of experimental drugs.

Group[1]	Dosage	Usage
AS	30 g/sheep/day	Mix it with feed after crushing and put it in the trough, lasting for 21 days
HC		
PO		
DS	1 mg/kg	At 1 mg/kg by oral infusion for 2 d per week for 3 weeks
CON	——	Free diet and water

[1] There are five groups mentioned in this table, including *Allium sativum* (AS) group, *Houttuynia cordata* (HC) group, *Portulaca oleracea* (PO) group, diclazuril solution (DS) group and control (CON) group.

the lambs on days 0, 7, 14, 21, 28, 35 and 78, and lambs were weighed on days 0, 21, 35 and 78 (Table 1, Fig. 1A).

Collection of faecal samples

Faecal samples were collected from the rectum of all sheep and then were immediately placed in a sterile 2 ml centrifuge tube, stored in a dry ice box, and the ear tag number of the sheep at the time of collection was recorded. One part of the faeces was tested for OPG and the other part was tested for microbiota characteristics in the faeces by high-throughput sequencing.

16S rRNA gene sequencing

Day 21 of the experiment, faecal samples for high-throughput sequencing were collected from the rectum and placed on dry ice. Eventually, samples were sent to Shanghai Majorbio Bio-pharm Technology Co., Ltd. (Shanghai, China) for high-throughput sequencing. Following the manufactures' instructions, total microbial genomic DNA was extracted from faecal samples using a E.Z.N.A.soil DNA extraction kit (Omega Bio-tek, Norcross, Georgia, USA). The quality of DNA samples was assessed by 1% agarose gel electrophoresis. The V3-V4 hypervariable region of the bacterial 16S rRNA gene was amplified using the primer pair 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). Then, the amplicons were purified by using the AxyPrep DNA gel extraction kit (Axygen Biosciences, Union City, California, USA). The purified amplicons were used to generate paired-end reads (2 × 300 bp) on the Illumina MiSeq PE300 platform (Illumina, San Diego, California, USA). The bacterial 16S rRNA sequencing data obtained from the faecal samples have been deposited in the NCBI Sequence Read Archive (SRA) database under the accession number (PRJNA1037301).

Sequencing data analysis

The raw paired-end sequences obtained from Illumina sequencing were spliced using FLASH software (v1.2.7) to obtain original tags. Subsequently, the original tags were filtred using UPARSE software (version 7.0.1090) with removal of chimeras to obtain high-quality sequences for OTU clustering based on 97% similarity. Meanwhile, the QIIME (1.9.1) was used for taxonomic analysis of the OTU representative sequences to obtain the species classification information corresponding to each OUT. The alpha diversity and beta diversity were calculated and analysed using MOTHUR (1.30.2) and QIIME (1.9.1) software, respectively. Alpha diversity indices (including Chao1, Shannon, Simpson and Ace index) were calculated to evaluate microbial species evenness. Beta diversity was evaluated by principal component analysis (PCA) based on the analysis of similarities (ANOSIM). Microbiota abundances with each group were statistically compared at the phylum, family and genus levels. Microbiological differences across groups were determined using linear discriminant analysis (LDA) coupled with effect size (LEfSe) and

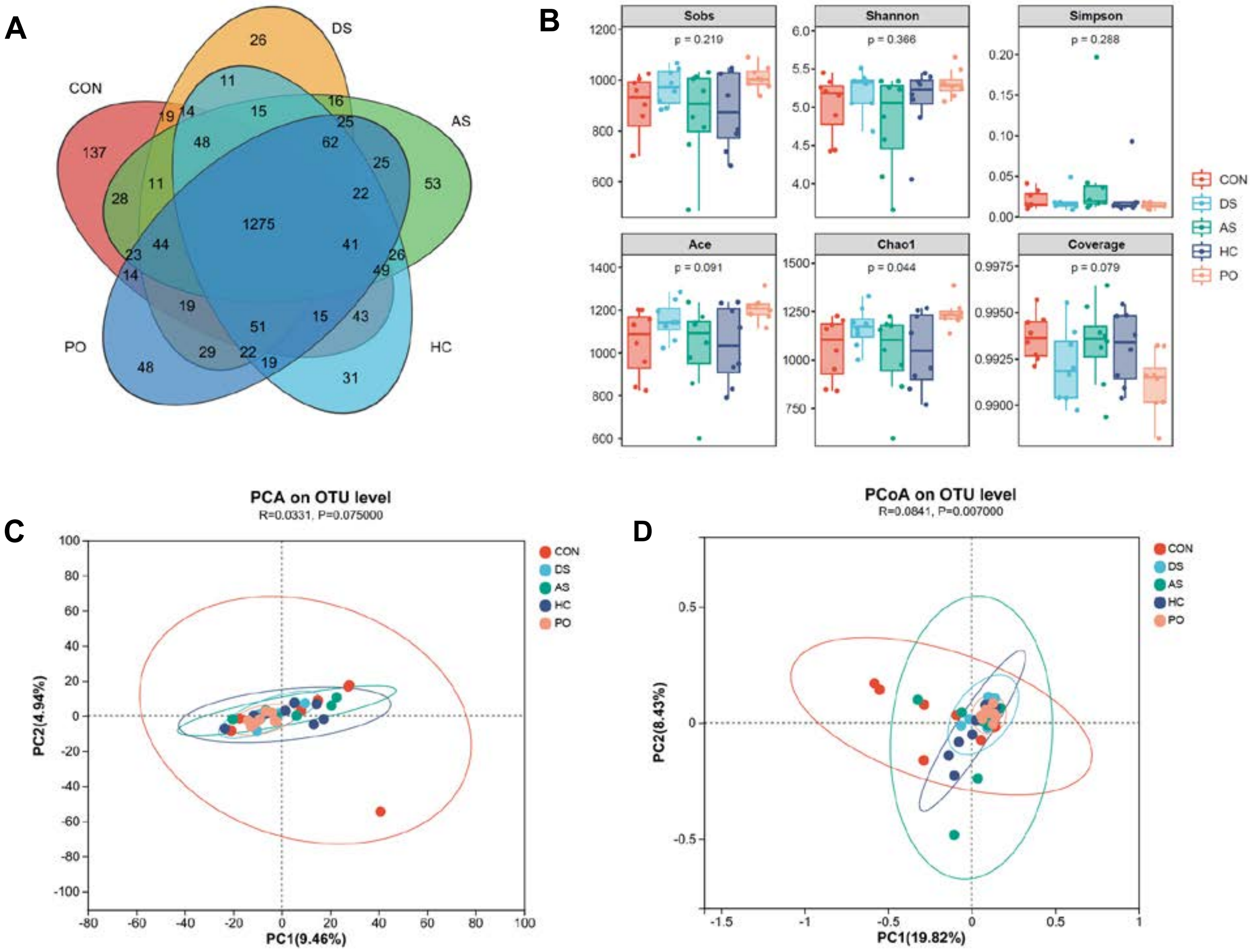

Fig. 2. The operational taxonomic units (OTUs) shared by gut microbiota and analysis of alpha diversity and beta diversity of gut microbiota; **A** – Venn diagram statistics of the number of common and unique species OTUs in each group of samples; **B** – Alpha diversity index, including species diversity index (Sobs), Shannon, Simpson, Abundance-based Coverage Estimator (Ace), Chao1 and Coverage indexes, was used to detect whether there was a significant difference between the index values of each two groups by statistical students t-test. Beta diversity analysis, including; **C** – PCA (principal component analysis); and **D** – PCoA (principal co-ordinates analysis), reflected the similarity or difference of community composition among samples of different groups (*p* < 0.05 was statistically significant).

LDA > 2. The most prevalent genus was compared between using the Wilcoxon rank-sum test.

Statistical analysis

One-way analysis of variance (ANOVA) was performed for the data following the normal-distribution and expressed as mean ± standard deviation (SD) using GraphPad Prism 8.0.2 (GraphPad Software, La Jolla, California, USA). The Welch test was used if variances were heterogeneous. Non-parametric test was conducted for data that did not conform to normal distribution. The Kruskal-Wallis test was used to compare the differences in OPG between groups. The Wilcoxon rank-sum test was used to identify differences in the most abundant genus between groups. When a value of *p* < 0.05 was reached, differences between groups were considered statistically significant.

Table 2. Effect of different treatment groups on OPG (oocysts per gram), expressed as mean, with range in parentheses.

Time[1] (day)	Group CON	DS	AS	HC	PO	*P*-value
0	9,700 (6,300–17,300)	9,800 (6,800–17,400)	9,800 (6,800–17,300)	9,900 (6,700–16,500)	10,200 (6,800–17,700)	1
7	11,400 (15,600–17,400)	0 (0–0)**	12,700 (4,800–15,700)	6,300 (4,300–9,200)	2,000 (1,300–7,300)	< 0.01
14	16,800 (12,800–19,700)	0 (0–0)**	9,500 (3,100–17,500)	4,200 (3,000–6,100)	2,900 (1,900–7,100)	< 0.01
21	19,300 (16,600–24,300)	0 (0–0)**	6,900 (4,700–10,600)	3,800 (3,200–6,400)*	3,900 (2,700–5,600)*	< 0.01
28	20,700 (18,900–29,100)	0 (0–0)**	9,600 (5,800–14,000)	3,400 (1,100–5,000)*	3,400 (1,700–5,500)*	< 0.01
35	20,400 (16,600–22,800)	0 (0–100)**	7,800 (4,100–18,600)	2,100 (800–4,100)*	2,200 (1,200–7,900)	< 0.01
78	13,200 (6,000–28,300)	2,400 (1,000–16,300)	7,200 (5,800–8,800)	2,100 (800–4,700)	3,100 (2,100–5,600)	0.025

[1] Statistical analysis was performed by Kruskal–Willis test. * $p < 0.05$, ** $p < 0.01$ indicates a significant difference between the treatment group and the CON group. There are five groups mentioned in this table, including *Allium sativum* (AS) group, *Houttuynia cordata* (HC) group, *Portulaca oleracea* (PO) group, diclazuril solution (DS) group and control (CON) group.

Table 3. Relative reduction rate of oocysts of coccidia in different groups after medication.

Group[1]	Relative reduction rate of oocysts (%)					
	Day 7	Day 14	Day 21	Day 28	Day 35	Day 78
AS	29.0	41.2	60.9	59.7	77.2	63.8
HC	53.7	71.4	78.7	84.2	85.7	83.1
PO	71.8	60.4	75.8	81.4	85.5	79.6
DS	100	100	10.0	100	99.5	12.4

[1] Calculation method of relative reduction rate of oocysts: (oocysts of CON group – oocysts of treatment group)/ Oocysts of CON group *100%. There are four groups mentioned in this table, including *Allium sativum* (AS) group, *Houttuynia cordata* (HC) group, *Portulaca oleracea* (PO) group and diclazuril solution (DS) group.

RESULTS

Effect of Chinese herbs on infection with coccidia and production performance of lambs

Before the experiment, faecal examination of experimental animals in each group showed that the total infection rate of coccidia was 100%, and all of them were naturally infected. On days 7, 14 and 21 following treatment, the OPG values of each treatment group decreased to different degrees, and the difference between DS and CON groups was statistically significant ($p < 0.01$). The difference between the mean OPG values of AS, HC, PO and DS groups was statistically significant on day 28 (1 week post-discontinuation) ($p < 0.05$).

The mean OPG values of HC group and PO group decreased after day 28 (1 week post-discontinuation), while the mean OPG values of AS, HC and PO groups remained at the lowest level after day 35 (2 weeks after discontinuation of medication), and were lower than those of CON group. The mean OPG values of the DS group were lower than those of CON group. In contrast, the difference in OPG was highly significant in the CON group compared to the situation when the herbs were administered before ($p < 0.01$). The mean OPG values of AS, HC and PO groups remained at the lowest level after day 78 (6 weeks after medication discontinuation). The OPG before and after herbs treatment are shown in Table 2A and Fig. 1B.

In addition, to assess the effect of Chinese herbs on the reduction of coccidia oocyst, we counted the relative reduction rate of oocysts (Table 3, Fig. 1C) for each group. Although the oocyst clearance rate was 0 in each treatment group on days 7, 14, 21, 28, 35 and 78 after treatment, it can keep coccidia at the lowest level, while the oocyst clearance rate was 100% on days 7, 14, 21 and 28 in the DS group, which was significant when compared to the treatment and CON groups ($p < 0.01$). Oocyst reduction rates were higher in all treatment groups than in CON group at days 7, 14 and 21 after treatment, with significant differences in the AS, HC and PO group compared with the CON group ($p < 0 .05$) and extremely differences in the DS group compared with the CON group ($p < 0.01$).

On day 28 (1 week after discontinuation) and day 35 (2 weeks after discontinuation), the relative reduction rates of oocysts were significantly different in the AS, HC and PO group compared with the CON group ($p < 0.05$) and substantially different in the DS group compared with the CON group ($p < 0.01$). At the 78th day after treatment (the second week after discontinuation), there were significant differences between the AS, HC and PO groups in comparison to the CON group ($p < 0.05$). The above results showed that all three Chinese herbs had significant inhibitory effects on coccidial infections, but this inhibition was not as effective as that of the chemical drugs.

Chinese herbs promote lamb growth performance

In addition, we recorded the body weight of all lambs at different periods to assess the effect of the Chinese herbs on lamb production performance (Table 4, Fig. 1D). By analysing the ADG and relative weight gain rate of weaned lambs in each group after 3 weeks of treatment and 8 weeks of cessation of treatment, we found that the ADG of the HC, PO and DS groups was significantly higher than that of CON group sheep after 3 weeks of treatment ($p < 0.05$), and that the daily weight gain of AS group was higher than that of CON group after 3 weeks of herbs treatment, but the difference was not significant ($p > 0.05$).

After 8 weeks of drug discontinuation, the HC, PO and DS group sheep had higher ADG than the CON group, with significant differences ($p < 0.05$), and the AS group had higher ADG than the CON group, but the differences were not significant ($p > 0.05$). The ADG of the treatment groups (except AS group) was significantly higher than that of CON group on day 21 ($p < 0.05$), and the most significant weight gains were in the HC and PO groups. The ADG of these two groups remained significantly higher than that of the CON group after stopping the treatment, indicating that it can be used as a feed additive to enhance the ADG of lambs and has a certain promotion effect on the growth of lambs.

Table 4. Average daily gain and relative gain rate of lambs in different periods.

Group[1]	Day 21		Day 78	
	Average daily gain (gram/day)	Relative weight gain rate (%)	Average daily gain (gram/day)	Relative weight gain rate (%)
AS	165.5 ± 17.5	9.7	148.9 ± 3.8	8.3
HC	192.6 ± 6.5*	27.6	163.0 ± 3.3*	18.5
PO	199.4 ± 9.3*	32.2	170.7 ± 4.1*	24.1
DS	178.3 ± 14.1	18.2	162.8 ± 4.4*	18.4
CON	150.9 ± 17.6	–	137.5 ± 4.7	–
P-value	0.107	–	< 0.01	–

[1] One-way analysis of variance was used for statistical analysis. * $p < 0.05$, ** $p < 0.01$ indicates a significant difference between the treatment group and the CON group. Expression of results: Mean ± Standard Error of the Mean. Calculation method of relative weight gain rate: (ADG of Treatment group – ADG of CON group)/ ADG of CON group *100%. ADG: average daily gain. There are five groups mentioned in this table, including *Allium sativum* (AS) group, *Houttuynia cordata* (HC) group, *Portulaca oleracea* (PO) group, diclazuril solution (DS) group and control (CON) group.

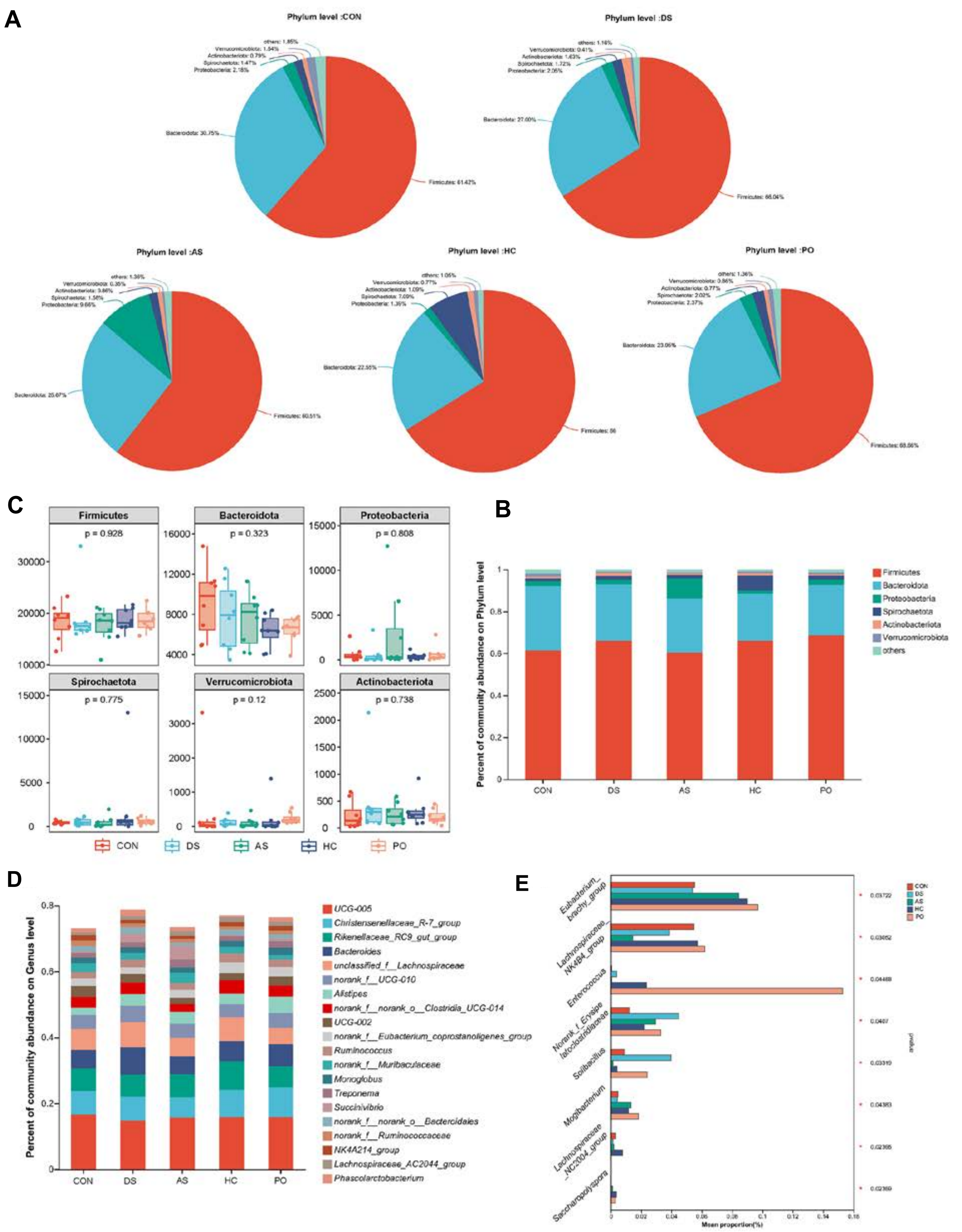

Fig. 3. Community composition of gut microbiota as a percentage and variability analysis at the phylum and genus levels. **A** – the pie chart of horizontal composition of gut microbiota revealed the proportion of horizontal abundance of phylum in each group; **B** – the box diagram shows the difference in the horizontal composition of the door between groups; **C** – percent of community abundance on phylum level; **D** – composition of the top twenty species abundance in samples from different groups at the genus level; **E** – test of significant difference between groups. According to the community abundance data obtained at the genus level, Kruskal-Wallis H test was used to detect the significant difference between groups to screen out the different genus.

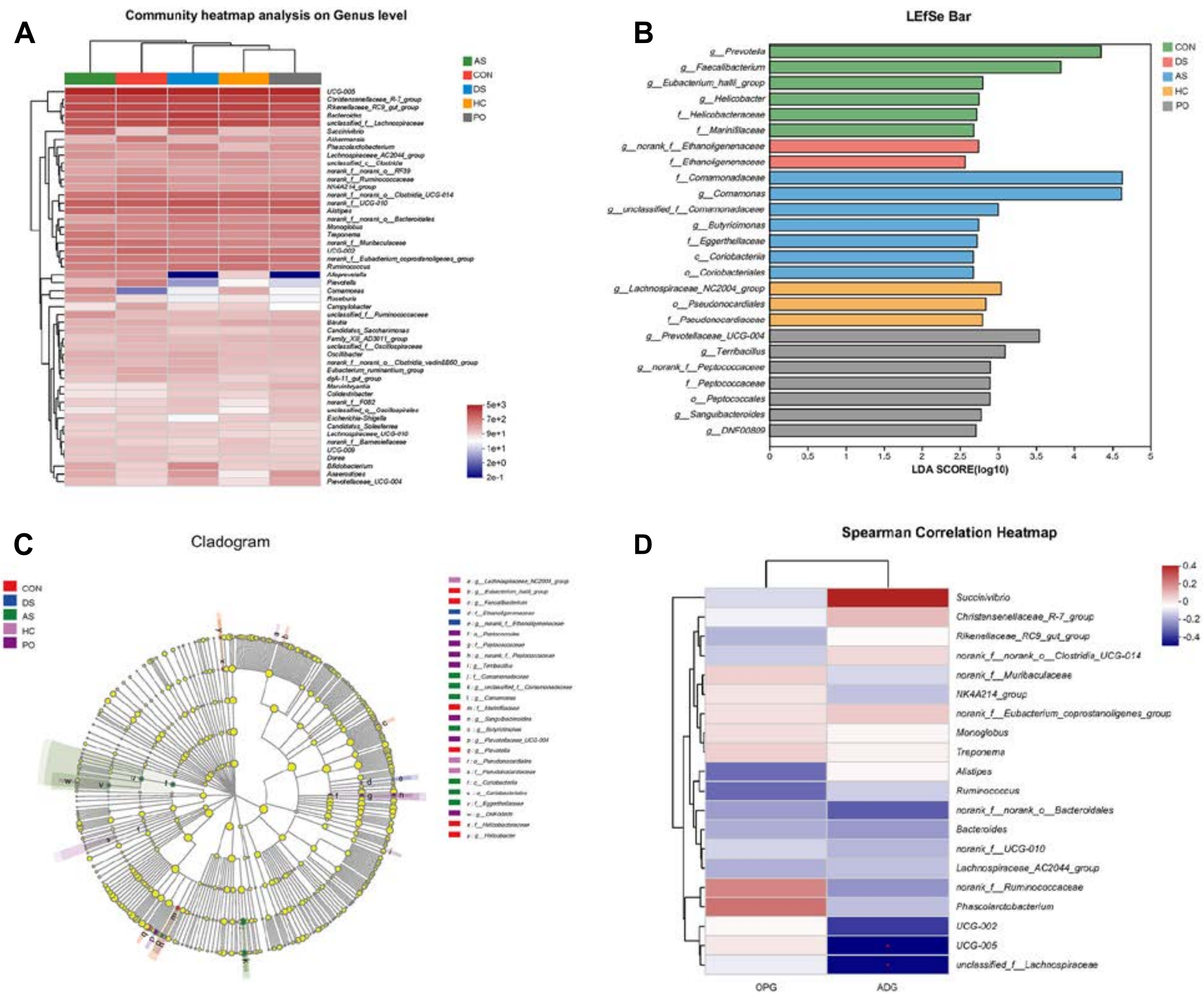

Fig. 4. Screening and analysis of critical differential genus. **A** – the species abundance of each sample was counted at the genus level, and the community composition was visually studied by heat map visualisation method; **B** – the length of the bar column represents the linear discriminant analysis (LDA) score. The microbiome groups with significant differences among groups (LDA score > 2.0) are shown; **C** – microbial species with significant differences in abundance among the five groups of samples. Different colors indicate different groups, from the inside out are phyla, class, order, family, and genus; **D** – spearman Correlation Heatmap. At the genus level, correlations between OPG and ADG are analysed for different microbial species in the samples; ADG – average daily gain; OPG – oocysts per gram.

Effect of Chinese herbs on gut microbiota of sheep

To investigate the changes in the gut microbiota of lambs caused by different herbal treatments, we obtained 40 faecal samples from lambs on the 21st day of the experiment by the rectal collection of faeces and performed 16S rRNA sequencing analysis. The results exhibited 1,275 OTUs in all groups, 137 OTUs unique in the CON group, 26 OTUs unique in the DS group, 53 OTUs unique in the AS group, 31 OTUs unique in the HC group, and 48 OTUs in the PO group (Fig. 2A). Meanwhile, the result of alpha diversity analysis of gut microbiota at OTU level (Fig. 2B) showed Sobs index ($p = 0.219$), Ace index ($p = 0.091$) and Chao1 index ($p = 0.044$), which indicated no significant difference in species diversity.

Furthermore, Shannon index ($p = 0.366$) and Simpson index ($p = 0.288$) indicated similarity in species diversity among groups. Although there was no significant difference in alpha diversity analysis among groups, the composition and diversity of gut microbiota changed compared with the CON group. The Coverage index of each group was greater than 0.99, indicating that the sequencing results represented the real situation of microorganisms in the samples.

Thereafter, faecal samples from every group were subjected to beta diversity analyses. At the OTU level, between-group difference tests (Fig. 2C) were performed using the ANOSIM (a non-parametric test based on the permutation and rank sum tests) method. The analysis displayed ($R = 0.0331$, $p = 0.0750$) that $R > 0$ indicates that the between-group differences are greater than the within-group differences. The differences in faecal samples within the groups, although different, were not significant. This indicates that the between-group differences were greater than the within-group differences between the different subgroups.

Scatter plots were generated based on the first and second principal component values of the Bray Curtis distance algorithm, and PCoA plots were also presented (Fig. 2D). The results showed (R = 0.0841, $p = 0.0070$) significant differences in the gut microbial composition of lambs from different groups. These results indicate variability in the composition of the gut microbiota of lambs from different groups. In short, the composition of the gut microbiota of the samples between the different groups was influenced by the treatment with Chinese herbs.

The treatment with Chinese herbs affected the composition of gut microbiota at the phylum level (Fig. 3A,B). Although there was no significant difference in abundance between the groups at the phylum level, the ratio between the abundance of the components changed. For example, the abundance of *Spirochaetota* in the AS group increased substantially (Fig. 3C), suggesting that the treatment of Chinese herbs could affect the composition of the gut microbiota. At the genus level, the composition of the gut microbiota was quantified and the microbial composition community bar chart (Fig. 3D) shows the top twenty genera in terms of bacterial abundance.

Among the various subgroups, *UCG-005* (CON = 16.69%, DS = 14.84%, AS = 15.70%, HC = 15.76%, PO = 15.84%, respectively) was the most dominant bacteria in the faeces, followed by *Christensenellaceae_R-7_group* (CON = 7.13%, DS = 7.23%, AS = 6.25%, HC = 8.38%, PO = 9.10%, respectively), *Rikenellaceae_RC9_gut_group* (CON = 6.77%, DS = 6.64%, AS = 6.93%, HC = 8.64%, PO = 6.43%, respectively) and *Bacteroides* (CON = 5.68%, DS = 8.37%, AS = 5.45%, HC = 6.14%, PO = 6.60%, respectively). In addition, we focused on the variation in the percentage of *Alistipes* abundance in different groups (CON = 2.25%, DS = 3.64%, AS = 3.56%, HC = 3.14%, PO = 4.86%, respectively). The abundance of genus in each group can be seen more visually from the community Heatmap plot (Fig. 4A).

After that, the Kruskal-Wallis H test was used to test for significant differences between groups (at the genus level), and eight genera with significant differences were screened, namely *Eubacterium_brachy_group*, *Lachnospiraceae_NK4B4_group*, *norank_f__Erysipelatoclostridiaceae*, *Solibacillus*, *Mogibacterium*, *Lachnospiraceae_NC2004_group* and *Saccharopolyspora* (Fig. 3E). The LEfSe analysis was performed to reveal the significance ranking of the differences in the abundance of the bacterial groups, with a total of 25 groups having differences in the five groups (Fig. 4C). Graphs from the LEfSe analysis (Fig. 4B) showed significant differences in LDA scores of microbiota between the five different groups (LDA score > 2.0).

Subsequently, the OPG and ADG of lambs were analysed as environmental factors, for correlation with gut microorganisms at the genus level, and a total of 20 gut microbes with a strong correlation with these two species were explored (Fig. 4D). Among them, ADG showed a significant negative correlation with *UCG-005* ($r = -0.412$, $p \leq 0.05$) and *unclassified_f__Lachnospiraceae* ($r = -0.404$, $p \leq 0.05$). In addition, ADG and *Succinivibrio* abundance depicted a strong positive correlation ($r = 0.343$, $p \geq 0.05$). Although these genera are not significantly associated with OPG, *Alistipes*, *Ruminococcus*, *norank_f__Ruminococcaceae* and *Phascolarctobacterium* are closely related to OPG.

DISCUSSION

Recently, researchers have been working to find environmentally friendly, non-resistant drugs to treat animal diseases. Chinese herbs have received more and more attention from researchers because of their wide range of action, low resistance, safety and greenness (Bauer et al. 2010, Christaki et al. 2020). Sheep coccidiosis, as a global animal disease, has caused continuous and huge economic losses to the animal farming industry. The search for environmentally friendly, safe and effective drugs for treating coccidiosis in sheep is an urgent need of the current era. Based on the physiological characteristics of the intestinal tract colonised by coccidia, several herbs with regulatory and protective effects on intestinal function were selected. Therefore, we selected AS, HC and PO from a wide range of herbs. In the preparation phase of the experiment, we conducted a preliminary trial to analyse the effects of various herbs on sheep with coccidiosis and selected the above three herbs with significant therapeutic effects as the treatment herbs for this experiment.

In the experiment, the herbs AS, HC and PO were used to improve the resistance of lambs to coccidiosis, and the effect of adding the herbs to the diet on the growth performance of the lambs was closely monitored. As we expected, the data in this paper showed that AS, HC and PO significantly reduced the number of coccidia in lambs and had a beneficial effect on the growth of lambs. Among them, the most effective herbs for treatment were HC and PO, and HC and PO had a better effect on ADG. Although AS is not as effective as HC and PO, it has a significant therapeutic effect on sheep coccidiosis and improves performance. Although these three herbs are less effective than DS (chemical drugs), HC and PO have a better effect on weight gain than DS. The results indicate that these three herbs have good therapeutic efficacy and they may have promising applications in the treatment of coccidiosis in various animals.

Then, to explore whether AS, HC and PO exert their pharmacological effects by influencing the gut microbiota, we collected faecal samples from lambs and used 16S rRNA gut microbiota sequencing to investigate the changes in the composition of the gut microbiota. We found that one of the dominant genera of gut microbiota, *Christensenellaceae_R-7_group* (belonging to the phylum ‘Thick-walled Bacteria’, is widespread in the human and animal intestines and mucosa and is important for host health) (Whitty 2019). *Christensenellaceae_R-7_group* was significantly negatively associated with metabolic diseases such as body mass index (BMI) and inflammation, fat deposition, inflammatory bowel disease and metabolic syndrome (Waters et al. 2019).

In our study, the addition of AS and HC to the diet enhanced the proportion of *Christensenellaceae_R-7_group* in the composition of the gut microbiota and contributed to the alleviation of the inflammatory response to coccidian infestation of the intestine. However, our study lacked the detection of certain inflammatory factors and the observation of intestinal damage and the validation of herbal medicines to alleviate intestinal damage and inflammatory reactions. It was shown that *Ruminococcacea-UCG-005* (genus level) was associated with the degradation of starch and fibre in ruminants (Tavella et al. 2021). As the most dominant bacteria in the treatment group, its abundance was decreased compared to the CON group, probably due to the alteration of microbial community diversity under the treatment effect.

We found that the abundance of *Bacteroides* was upregulated in the treatment group (except AS) compared to the CON group. It has been shown (Lee et al. 2020) that *Bacteroides* have a symbiotic relationship with the animal organism in that they help to break down food and produce the nutrients and energy required by the organism. Therefore, this may be one of the reasons why the treatment group helps to improve the growth performance of animals.

In addition, we focused on the changes in the abundance of *Alistipes*, a Gram-negative bacterium of the phylum *Bacteroides*, which was upregulated in the treatment group. It has been confirmed that *Alistipes* may have a protective effect against certain diseases, including liver fibrosis, cancer immunotherapy and cardiovascular disease (Zafar and Saier 2021). However, *Alistipes* been shown to be pathogenic in colorectal cancer and associated with depression (Parker et al. 2020). This bacterium thrives under high-fat dietary conditions and is significantly enriched in the gut microbiota of obese patients, suggesting that *Alistipes* is associated with obesity.

In conclusion, the addition of herbs to the diet significantly reduced the number of coccidium oocysts in lambs in this study and had a preventive effect on coccidia infection. Moreover, the herbs had a stimulating effect on the growth performance of weaned lambs and could improve the gut microbiota of lambs and enhance the resistance of lambs. Therefore, Chinese herbs can be promoted as a natural and green feed additive in animal production.

Authors's contribution. S.C.H and F.C.J contributed to the conceptualisation, resources, funding acquisition, and revision and editing of the manuscript. Y.F.H. performed the analysis of the data, compiled the graphs and drafted the manuscript. Z.M.W and A.S. contributed to the revision and editing of the manuscript. P.C, K.L.L, S.Y.L and M.A collected the literature and reviewed the text. M.M.L and Y.M.W provided assistance for animal experiment. All authors contributed to the article and approved the submitted version.

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